Association Scientifique
Internationale
du Café

DIX-SEPTIÈME COLLOQUE SCIENTIFIQUE
INTERNATIONAL SUR LE CAFÉ
Nairobi, 20-25 juillet 1997

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Génie alimentaire

Communications

Poster

Transfert de technologie en milieu rural

Communications

Posters

Agronomie

Communications

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OPENING SPEECH

by

Wilson R. Opile, chairman of ASIC

Your Excellency Sir,
Honourable Ministers,
Ladies and Gentlemen,

I wish to take this opportunity on behalf of the Association Scientifique Internationale du Café (ASIC) Board and the Kenya National Organizing Committee to welcome you all to the 17th International Conference on Coffee Science in Kenya.

As many of you are aware, ASIC is a voluntary non-profit organization which has its headquarter in Paris, France, and organizes this kind of conference every two years.

The conference will be held here from monday to friday of this week. The theme of the 1997 conference is « Transfer of technology to improve coffee quality ».

It is the first time that ASIC conference has been held in this country and Kenya is the third African country to host this prestigious conference. The first was Côte d'Ivoire, in 1977, and the second was Togo, in 1985.

In this year's conference, the delegates will be discussing the latest trends and ideas on coffee science in terms of production, quality, income generation, safe use of chemicals, health of coffee consumers but with emphasis on technology transfer to producers. The programme will cover the most recent results in many key areas including agronomy, biotechnology, physiology (effects of coffee on health), chemistry (flavour, other products, analytical methodology) and food engineering (processing techniques and packaging). ASIC is honoured to have this conference in Nairobi. It is our hope that the delegates will find their participation interesting and productive.

With these few remarks, Your Excellency Sir, I call upon the next speaker in the programme to address the gathering.

Thank you.

RESEARCH AND DEVELOPMENT IN COFFEE (KENYA)

P. K. Michori [1], D. M. Masaba [2]

1, Director of Research ; 2, Deputy Director of Research, Coffee Research Foundation, Ruiru, Kenya

INTRODUCTION

Kenya's economy depends largely on agriculture. Since its introduction as a cash crop in 1900's, coffee has remained one of the most important product of Kenya's agriculture. The coffee sector is an important production and employment sector. As at 1987, about 70 % of the national labour force was employed in the agricultural sector, out of which about one third was absorbed by the coffee industry. Coffee contributes about 25 % of the gross farm revenue in Kenya, and about 30 % of the revenue from total domestic exports. Furthermore, the coffee sector establishes important demand and supply linkages with the rest of the economy.

The important role played by research in all sectors of national development, may it be agriculture, health, commerce and industry, planning, etc. is recognized worldwide. Indeed, the present upward trend in Government budgetary provisions for research funding in many countries and the ever increasing financial support for research by industries and trade world-wide are by themselves a manifestation of the critical role research plays in development.

Coffee research has been the driving force behind coffee development in Kenya and will continue to play a pivotal role as the era of industrialization takes root in the country coupled with the current trend towards liberalization of the agricultural sector under the terms of Structural Adjustment Programme (SAP). These new developments will undoubtedly be accompanied by new challenges in coffee research services in such areas as relevance of research programmes to be undertaken in relation to customer (coffee farmers, consumers and industrialists) needs, research service costs and benefits.

HISTORICAL DEVELOPMENT

The need for the contribution of research in coffee development in Kenya was recognized immediately : coffee was introduced in this country in early 1900's. The coffee research services in Kenya commenced with the appointment of the first Government entomologist in 1908 and a mycologist in 1913 while a coffee plant inspector was appointed in 1914. In the period 1910-12, a few coffee field trials were established in central Kenya (Kabete) and western Kenya (Kibos) experimental farms and by the period 1945 to 1947, many field trials had been initiated in all parts of the country.

In 1944, the Kenya Government bought the Jacaranda Estate near Ruiru in central Kenya, as a centre for coffee research, and the construction work of the laboratories and buildings was completed by the end of 1949. Three sub-stations were later established in principal coffee growing areas ;

these being Kisii in the wet areas of South West Kenya (1957) ; Mariene on the slopes of Mt Kenya (1958) and Koru on the western slopes of Rift Valley near lake Victoria (1959). The Agricultural Experimental Station at Kitale on the slopes of Mt Elgon was also used for coffee experiments between 1938 and 1967.

Responding to the demands by coffee farmers that they should pay for coffee research, the Government handed over direct responsibility for coffee research to the industry in October 1963. In August 1964, the Coffee Research Foundation (CRF) was incorporated as a registered company limited by guarantee under the Companies Act (Cap 486 Laws of Kenya). The guarantors are the Coffee Board of Kenya (CBK) and the Ministry of Agriculture, Livestock Development and Marketing.

COFFEE RESEARCH ORGANIZATION AND FINANCES

The Foundation has its own Board of Directors drawn from farmers' representatives, Coffee Board of Kenya, Ministry of Agriculture, Kenya Agricultural Research Institute (KARI) and University of Nairobi. The Board has four standing committees namely : staff, finance/tender, coffee research advisory committee (CRAC) and technical evaluation committee (TEC). The coffee research advisory committee advises the Board on matters concerning research planning, execution and evaluation. The committee's membership is drawn from the Board, farmers' representatives from all coffee growing areas, leading scientists from the University of Nairobi, KARI and the Ministry of Agriculture, Livestock Development and Marketing. The second committee, the technical evaluation committee guides the Board on the priority projects to be undertaken and monitors the progress of the approved research. The unique set-up of clients' representatives (coffee growers) and practising scientists has resulted into farmer-oriented research programmes on coffee.

The Foundation is funded by coffee farmers through the Coffee Board of Kenya which levies a 3 % cess on all coffee produced in the country. The levy amount is shared between the CRF and the CBK, the research taking a bigger proportion of the levy.

RESEARCH PROGRAMMES AND ACHIEVEMENTS

The principal objectives of the Foundation are **« to promote research into and investigate all problems relating to coffee and such other crops and systems of husbandry as are associated with coffee throughout Kenya, including the productivity, quality and suitability of land in relation to coffee planting and on matters ancilliary thereto ».** In pursuance of the set goals, the Foundation has over years expanded its areas of research disciplines from the initial entomological and mycological studies to cover areas of coffee agronomy, physiology, pathology, entomology, breeding, soil fertility and nutrition, agricultural engineering (coffee processing and coffee machinery testing), pesticide chemistry, quality and agricultural economics.

Breeding

Following the introduction of commercial coffee in early 1900's, intensive selection within the introduced French Mission cultivars resulted into the high yielding and fine quality cultivars SL 28

and SL 34. The SL cultivars have since become a trade mark of high quality for Kenya coffee. A special breeding programme aimed at combining resistance to two major coffee diseases, namely coffee berry disease (CBD) and coffee leaf rust, with the high yields and the fine quality typical of the traditional coffee cultivars was initiated in 1971. The project was a collaborative effort between the Kenyan and Netherlands Governments. Within a period of 15 years of concerted research by our devoted scientists, a breakthrough was made when an Arabica coffee cultivar known as Ruiru 11 was commercially launched. Ruiru 11 is a mixed population of hybrids which are genetically distinct from each other in the characteristics of resistance to CBD and leaf rust, yield and quality, offering a wide scope for further selection, but which are morphologically similar especially in the compact growth character. It is a high yielding coffee cultivar and its quality is equivalent to that of the SL cultivars. Apart from the savings from fungicide use, the compact variety can be planted at double the plant population of the traditional varieties resulting in higher coffee yields per unit area.

The conventional coffee breeding methodology, which the CRF has been using has limitations due to the long generation time of coffee trees (five years) in addition to the high cost of field trials. For example a minimum of twenty-five years after hybridization (five backcross generations) is required to restore the genetic background of the recipient cultivar thereby ensuring good cup quality of the improved variety. The CRF is therefore developing molecular marker techniques as a selection tool to enhance the future breeding programmes.

Field establishment practices

Using the traditional varieties, research led to the zoning of the Arabica coffee growing areas based on soil and climatic factors. Arabica coffee in Kenya is grown in areas between 1 200 and 2 000 m above sea level. These occupy the broad gentle slopes of Mt Kenya and the Eastern Aberdare Range in the central province ; the slopes of Mount Elgon bordering Uganda ; parts of the Great Rift Valley and some small holdings on the Taita Hills, a short distance from Tanzania.

Coffee nursery management and field establishment practices have also been worked out. In addition, the optimal density of the plantings has been established to be 1 330 and 2 500 trees per hectare for traditional and Ruiru 11 respectively.

Pruning

Further investigations established two systems of pruning based on the tree framework on which the bearing branches are borne and on the method of replacement of stems. The first, the single stem system, has permanent stems restricted in height with a system of primary branches on which the bearing sub-laterals are borne. In the second, the multiple stem system, the top of the tree is not cut and the stems are renewed periodically.

Nutrition

Organic manures, mainly cattle manure and sludge from methane gas plants were used in the early days of coffee growing. With the advent of inorganic fertilizers, evaluations on the types (straight, compound and foliar), rates and time of application led to the best combinations under different eco-

logical zones. A leaf and soil analysis facility was established to assist in determining the critical levels of nutrients needed, leading to more efficient use of fertilizers by farmers.

However, inorganic fertilizers have become expensive over time necessitating the CRF to go back to evaluating the readily available manures and coffee pulp for substitution of inorganic fertilizers. Several mulching materials, napier grass, maize and banana stover and coffee prunings have also been recommended to preserve soil moisture, control soil erosion, improve soil structure, supply mineral nutrients on decomposition, regulate soil surface temperature, suppress weeds and in some cases reduce the incidence of thrips. However, due to the declining land sizes, the area for growing mulch materials has become smaller limiting the use of mulch to large coffee estates.

Water requirements

Kenya coffee is normally grown in high rainfall (above 1 150 mm annually) zones. However, the rainfall in most places occurs in two seasons interspaced with dry periods. Studies on the plant-water relations have revealed that internal water balance could serve as a useful index in monitoring the irrigation requirements of the coffee trees. Based on the studies, the period, rates and frequency of irrigation have been worked out for areas which experience long dry periods.

Coffee protection

The major *Coffea arabica* diseases in Africa include coffee berry disease (CBD) caused by *Colletotrichum kahawae,* coffee leaf rust (CLR) caused by *Hemileia vastatrix* Berk et Br. and to a limited extent, bacterial blight of coffee (BBC) caused by *Pseudomonas syringae* Van Hall mainly in Kenya. There are other diseases such as *Fusarium* root and bark diseases which have tended to disappear with improved cultural practices on coffee farms. All the major diseases are controlled effectively by use of fungicides and bactericides. The new arabica cultivar developed in Kenya, Ruiru 11, is resistant to CBD and CLR. The introduction of this disease resistant coffee variety has contributed significantly in reducing coffee production costs.

There are a number of coffee insect pests and most of these pests are controlled only when they exceed economic threshold levels. The control methods range from sanitary, cultural, use of insecticides and biological control. Use of insecticides to control coffee insect pests is not only expensive, but also destroys natural enemies of some of the coffee pests. Attention has now been focussed on biological control for many insect pests. The control of coffee mealybug (*Planococcus kenyae*) by rearing and dissemination of its natural parasite, *Anagyrus* spp., is one of the classical examples of biological control in Africa. Other examples include control of coffee scales by use of ladybird bugs, and control of giant looper (*Ascotis selenaria reciprocaria*) by *Macroraphis acuta* and control of Antestia (*Antestiopsis* spp.) by a parasitoid wasp (*Asolerus seychellensis*) that parasites the eggs of the pest. More work is still in progress to find natural enemies of the common coffee pests.

The weed species in coffee have been classified into annuals and perennials. Weeds have been shown to reduce coffee yields by over 50 % in addition to reducing the coffee quality. Due to this reduction of yield and quality by weeds, various weed control methods have been recommended. The most common methods used are digging using forked hoes, slashing, mulching and the use of

herbicides. There is a wide range of recommended herbicides including contact, systemic and soil acting for use in coffee plantations.

In an effort to reduce the costs of chemical pest control, and the harmful effects to health and environment resulting from pesticide use, current and future research programmes are geared towards integrated pest management (IPM). The programme involves conscious integration of all available pest control measures (mechanical, cultural, chemical, resistant cultivars and biological), and studies on inter-relations between methods used for control of the total coffee pest complex (insects, diseases and weeds).

Intercropping and shade

Coffee is mainly grown as a monocrop in Kenya. However, due to the fact that coffee occupies a substantial amount of high potential land, smallholder coffee farmers have been intercropping their coffee with various food crops. This has led to initiation of investigations into the types of intercrops and how they can be intercropped to maximize on economic returns without affecting both the yield and quality of coffee. Preliminary results have indicated that it is possible to intercrop young (up to two years) Arabica coffee or during the change of cycle phase with dry beans. Evaluation of other food and tree crops is still in progress.

The original introduction of shade trees in coffee was based on the assumption that being an understorey tree in the forest where it originated, coffee needed to be shaded. Several tree species have been grown in coffee mainly as shade trees or as wind breaks such as *Cordia* spp., *Grevillea robusta, Aibizzia* spp., *Leucaena leucocephala,* and *Cypress* spp. Use of shade trees has been shown to help even out erratic yields caused by periodic overbearing, reduce crinkling of coffee leaves commonly known as « hot and cold » disease and reduce hail damage. Shade has also been shown to reduce infection of bacterial blight of coffee (BBC) in Kenya due to reduced hail damage which predisposes the coffee trees to BBC infection. Further evaluation of shade trees of economic value and the effect of shade on coffee trees is still in progress.

Coffee processing and quality

Majority of Kenya coffee is wet-processed to produce high quality coffee. Studies on coffee processing ranging from pulping, pre-grading, fermentation, washing, final drying and storage have been conducted in the past by the CRF and recommendations are well documented. The processing starts in the field where only the ripe cherry is harvested. The harvested coffee is then sorted into ripes, over-ripes, under-ripes and other foreign bodies before pulping. The bad berries removed are dry-processed. After pulping, the parchment coffee undergoes fermentation, soaking, washing and drying before storage. The whole process has been worked out to an exacting detail. Parchment coffee is delivered to millers for hulling to clean coffee, graded and eventually taken for cup liquoring and marketing.

Methods of measuring the moisture content in parchment and green coffee have been developed and are used during processing and storage to ensure that coffee is fully dried to a moisture content of 10.5 %. Factors which contribute to bean pigments as well as off-flavours continue to be worked out.

Agricultural economics

The introduction of an agricultural economics section at the CRF in 1979 has greatly assisted in monitoring the profitability of coffee as an enterprise. Studies cover the socio-economic factors influencing the adoption of the technical recommendations by farmers and the effects of coffee market prices on coffee production, profitability and farm incomes.

Technology transfer

The CRF has established a research liaison, training and advisory section whose role is to provide, encourage and maintain a continuous contact between coffee farmers, researchers, coffee agencies and other people interested either individually or as a group in the research, production and processing aspects within the coffee industry.

To effectively reach the farmers, the section liaises with coffee extension staff in the Ministry of Agriculture, Livestock Development and Marketing, Ministry of Co-operative Development, the Co-operative Movement managing agencies and the Coffee Board of Kenya field services personnel in disseminating research information and receiving feedback from the field.

Information flow is effected through training, provision of publications including technical circulars on recommended coffee production practices. The circulars are prepared upon successful completion of research trials. The section also disseminates research information through participation in agricultural shows and coffee farmers' field days, making advisory visits to farms, arranging visits by farmers to the station and demonstration sites located in all major coffee growing areas and through audio visual media. There is also a weekly radio programme on coffee production and sale.

The CRF training programmes are conducted for coffee extension workers, factory managers, nursery managers and farm managers. The training facilities have expanded to the extent of forming a full-fledged Kenya coffee college at the CRF.

RESEARCH SERVICES

In addition to research activities, the Foundation offers specialized routine services to growers as a back-up to the technical recommendations. A leaf and soil advisory service which serves as a diagnostic tool for fertilizer use and soil amendment enables fertilizer recommendations to be based on individual farmers' soil and plant condition circumstances. The Foundation has established a specialized laboratory for analysis of the quality of pesticide formulations used on coffee as well as to establish the pesticide residue in soils and plant parts. Furthermore, the production and distribution of certified planting materials to growers ensure that only quality seed and seedlings are planted by farmers.

CONCLUSION

As the scientific arm of the Coffee Board of Kenya, the Foundation was called upon to co-ordinate and participate in the organization of ASIC'97. The experience was involving, challenging and interesting. The consolation is that many of the Foundation scientists will be able to interact with their counterparts from other parts of the world and share experiences and knowledge to keep abreast of new technologies in the quest for the production of better quality coffee while at home. We are indeed proud to be associated with ASIC'97.

HOW SCIENCE CAN HELP TO IMPROVE COFFEE QUALITY

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INTRODUCTION

Coffee scientists often ask me what will the future of coffee research be, what topics should they explore, or even what should their mission be.

As a clairvoyant I am afraid I perform pretty poorly, but at least I have a positive answer to offer as a coffee industrialist :

> « the scientific community can assist coffee economy in supporting and contributing to increase consumption of the highest quality product, by integrating advances in science and technology with practical applications in agriculture and industry, in order to foster continuing improvements in all aspects of coffee quality, from seed to cup ».

This is a very ambitious assignment indeed, involving very many scientific disciplines and a broad compass of human experience and knowledge in a great range of countries, climates, and mentalities.

I will try in this paper to describe some of the improvements we can expect in the different domains. This review is far from being exhaustive : we must remember that science is a dynamic process and some fields, like for instance genetics, are extremely dynamic.

PROSPECT FOR RESEARCH TARGETS : ON GREEN COFFEE

The quality of a green coffee bean depends from the inherited characters of the plant, from the environmental conditions where the plant is grown, and from the processing after the harvest.

The genetic of the plant

a. Genus *Coffea* includes many complex plants, among which *Coffea arabica* is one of the most interesting, with its 44 chromosomes, low caffeine, high lipids and sugars content. Its genoma is close to be decoded, as is happening with the *robusta* species, and genetically modified plants with several benefits will possibly be developed in the near future.
b. In the meantime, assisted natural field selection will help to find peculiar plants. For instance varieties with specific agronomic characters like stature, bean size, adaptation to varied climatic conditions (cold, draught resistant, etc.), or with useful chemical characters like low and high caffeine, oil or sugars will be made available.

c. *Arabica* is prone to many diseases, like leaf rust and CBD, by pests like coffee borer, nematodes and others. Big efforts have been made to find resistant plants : some very successful hybrids like *variedad Colombia* in Colombia and *Ruiru 11* in Kenya make every agronomist happy, but sadly they happen to deliver a mediocre coffee cup. It is not enough to create a great plant, we must have a great bean : geneticists, do not only ask for the agronomist advice, call also an expert cupper.

The environmental conditions

d. The correct ratio of quality versus yield, by the proper selection of the coffee varieties and their planting density, is one of the main problems that should be addressed by the producers. The chemical composition of the soil, as it influences plant growth, micro-flora population and eventually cup merit, must be better clarified. This could allow to control better the moulds responsible for mycotoxin contamination and off-flavours (like Rio taste). Biological warfare too, along with selection of resistant plants, may be foreseen for fighting this enemy.
e. Coffee cultivation under full sun versus shading is also a dilemma. Improving the yield of shaded plantations (to-day with yield limits around 500 kg/hectare) is very important : as the labour cost will likely increase in the next years (from to-day's 1.50-2.75 US$ per day), yields must also increase if coffee has not to become prohibitively expensive to produce. The same problem pertains to the mountain plantations with steep declivity, where mechanization is impractical, if not imagining small handhold tools.
f. High yield and quality, in the full sun farms, seem to be positively correlated. Costs in these areas are controlled by mechanization and automation, and plant death due to overbearing can be reduced by agronomic practices like enhanced fertilization (2-3 times more). In order to simplify harvesting operations and improve product uniformity, also methods for concentrating the flowering in some periods or for allowing only the main blossom fruits to develop will be investigated.

The after-harvest processing

All steps of the process that transform a cherry into a bean should be monitored.

g. In the sun-drying process, influence of the dynamics of water content reduction and of temperature profile must be studied. The optimal level of moisture to be removed by the sun must be understood, to assist in determining the right procedure to finish desiccation by hot air dryer. A critical study on the different machines to turn round the coffee cherries on the patio is of some importance, as is the exploration of the use of solar collectors as an energy source.
h. A similar approach can be planned for the washed coffee. A study on the influence, both on just pulped coffee and on the totally submerged one, of temperature, time, pH, bacterial flora during fermentation may clarify its impact on quality. A thorough check of the drying process of parchment coffee, like the one being suggested for the sun-dried coffees is highly recommended. Also the changes induced by any accidental re-wetting during the drying process are to be investigated, ascertaining their influence on harmful mould and bacterial flora growth.

i. The transformation of a coffee cherry into a perfect bean will also be improved by better machines. The cost factor must be second to the gain attainable preserving quality, to avoid the negative results of Costa Rica where the total time from harvesting to bagging was reduced down to 83 hours : a very efficient system that unfortunately tends to produce a scanty coffee, probably due to the excessive temperature of the drying hot air. The ideal temperature profile of hot air drying is not, to my knowledge, fully understood : what is for instance the outcome of large temperature fluctuations, like those sometimes found in vertical dryers, compared to the more stable temperatures found in drum-type machines ?

PROSPECT FOR RESEARCH TARGETS : ON COFFEE QUALITY

Quality must end to be an empty word, that can be attached to anything.

j. Science must help in finding a delineation that transcends the mere count of defects : a pure quantitative evaluation, easy to use but devoid of significance in term of « pleasure in the cup ». In Brazil, a committee is currently trying to give a content to the « Positive Characteristic of Coffee Quality ». Let us hope that their effort will be successful, and that other definitions for coffee of different origins will emerge. Only if we will be able to define and accept a commonly agreed description of the positive side of quality, it will be possible to have a frame of reference to use for judging the influence of the variables previously mentioned on the cup value.

k. Non destructive analytical methods, correlating chemical, sensorial and morphological characteristics with cup quality will become popular.
This initial phase involves many disciplines, like physics, chemistry, molecular biology, sensorial physiology and semantics along with genetics, botany and agronomy. We covered so far only the initial steps of the long way from a seed to a cup.

l. Quality control means also taking care of the changes induced by shipment. Some effort must be put in investigating how bags can avoid to impart coffee a taint, frequently encountered in some producing countries, caused by the stinking lubricant oil.

PROSPECT FOR RESEARCH TARGETS : ON TRANSFORMATION TECHNOLOGY

m. Nowadays morphologically imperfect beans are effectively rejected, as are colorimetric outlayers by the new powerful polychromatic sorting machines that are becoming more popular, even in the consuming countries. Hopefully, optical sorting will help even more in the future, by separating beans with slight microorganism-induced defects.

n. The main phase still remains the roasting process. A scientific approach should be followed, linking the thermal energy balance and other quantitative factors like temperature dynamics, air flow and time with shrinkage and cup quality. Such studies should consider both the heating and the cooling phase, including the influence of the quenching medium (air or water) on gas and aroma retention and product shelf life. Unfortunately, to-day roasting machine producers get from their market a somehow simplistic message : « give us a machine producing a maximum quantity of roasted coffee in a minimum time and weight loss ».

o. The chemistry of pyrolysis and the influence of different precursors and roasting conditions should be studied again, in view of the multiform aspects of the final consumption such as filter coffee, sitting there waiting for a customer, or espresso coffee, which lets the consumer wait for itself.

p. The transformation of roasted coffee in a cup remains an area of many mysteries. One is grinding : the influence of particle size distribution for espresso or filter coffee optimization must be investigated.
Grinding in protective atmospheres is also an interesting area. Techniques for increasing shelf life of roasted coffee deserve to be critically considered, under the perspective of quality optimization.

q. The thermodynamics of extraction for filter or espresso should be deepened, and changes affecting cup quality after the preparation, like hydrolysis, polycondensations and other reactions in solution are inadequately studied. Even the influence of pH and the mineral content of water is still not totally understood.

CONCLUSION

The relationship between coffee and science is a well established one : coffee uses long since to encourage scientists by brightening their mind, and by making some refreshing breaks during their demanding intellectual activity a customary must.

Scientists should gratefully return this help, doing their best to make the virtues of coffee evident. This encompasses both scientific guidance to prevent that poor handling translates into any bad effect on consumer's health, and an effort in highlighting coffee's beneficial effects on the quality of life. In this view, I may mention some exciting news coming from medical research :

– behavioural studies keep clarifying the role of caffeine in enhancing our mental power, concentration capabilities and long-term memory ;

– progress in brain neurochemistry is about to explain the competition between caffeine and adenosine, showing that caffeine is a non-addictive drug, and can even prevent addiction to other drugs of abuse ;

– several free-radical scavenging substances are being discovered in coffee, meaning that our beverage may have a protective potential in neoplastic diseases, and perhaps aid against liver cirrhosis. Also coffee's aroma helps : an anti-cancer action has been hypothesized.

My final advice : keep on searching, with creativity but with scientific rigor. This way coffee and science will make a real winner pair, for the benefit of all those growing it and those enjoying it as a drink !

SUMMARIES

The scientific community is called to assist coffee economy by integrating advances in science and technology with practical applications in agriculture and industry, to foster regular progress in all aspects of coffee quality, from seed to cup. The main areas expected to undergo major improvements

in the near future are : green coffee production, where plant genetic, agronomic practices and post-harvest processing may be steered towards better yields, without forgetting better tasting beans ; quality assurance, to support by a definition of positive quality ; and transforming technology, where roasting and packaging deserve further attention. Coffee scientists will be also active in explicating the health benefits deriving from the cup that all the people enjoy so much.

La communauté scientifique peut aider l'économie du café en associant les développements de la science et de la technologie à des applications pratiques à l'agriculture et à l'industrie, pour encourager des progrès réguliers dans tous les aspects de la qualité du café, de la fève à la tasse. Les domaines principaux où on attend des améliorations importantes dans le futur proche sont : la production de café vert, où on peut orienter génétique, agronomie et procédés après-récolte vers une meilleure rentabilité, sans oublier des grains au goût plus agréable ; le contrôle de la qualité, qui devrait commencer par une définition de la qualité positive et les technologies de transformation, avec plus d'attention à la torréfaction et à l'emballage. Les savants du café s'activeront aussi pour expliquer les avantages que présente, pour notre santé, la tasse que tout le monde apprécie.

COFFEE AND CAFFEINE DEPENDENCE : WHAT DO WE KNOW NOW ?

A. NEHLIG

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INTRODUCTION

Caffeine is the most widely used psychoactive substance in the world (15). Most of the caffeine consumed comes from dietary sources such as coffee, tea, cola drinks and chocolate. The most notable behavioral effects of caffeine occur after low to moderate doses (50-300 mg) and are increased alertness, energy and ability to concentrate. Higher doses of caffeine rather induce negative effects such as anxiety, restlessness, insomnia and tachychardia. Moderate caffeine consumption leads very rarely to health risks (4,9,40). However, since caffeine was considered in one study as a potential drug of abuse (16) and more recently described as "a model drug of abuse" (34), the possibility that caffeine abuse, dependence and withdrawal should be added to diagnostic manuals has been considered in the United States (38). The present paper will review the available data on caffeine dependence, withdrawal and reinforcement, and try to assess in which respect caffeine differs from drugs of abuse such as amphetamine, cocaine and morphine. It will also consider the reasons why caffeine could be considered a potential drug of abuse.

ADDICTION AND DRUG DEPENDENCE

Drug dependence has been defined as "a pattern of behavior focused on the repetitive and compulsive seeking and taking a psychoactive drug" (30). However, the term addiction has been applied indistinctly to the use not only of all illicit drugs but also of alcohol, tobacco, coffee and even chocolate.

Criteria for dependence

The old definitions for "addiction" and "habit" proposed by the World Health Organization were separate ones (WHO, 71). Drug addiction was characterized by four criteria: (i) an overpowering desire or compulsive need to obtain the substance by any means, (ii) a tendency to increase the dose progressively, (iii) a psychic and generally physical dependence on the effects of the drug, and (iv) detrimental effects on the individual and the society. This concept of addiction could be applied to opiates and alcoholism but not necessarily to cocaine, which does not create any clear physiological withdrawal.

Drug habit consisting of the repeated (not intoxicating) consumption of a substance was characterized by the four following criteria: (i) a strong but not compulsive desire to take the substance for improved well being, (ii) a moderate or no tendency to increase the dose, (iii) a psychic but no physiological abstinence

syndrome, and (iv) detrimental effects, if any, primarily on the individual but not on the society. These criteria were considered to apply to coffee or moderate alcohol consumption, and smoking.

The recent diagnostic manuals from the WHO (72) and the American Psychiatric Association (APA, 1,2) replaced the previous criteria used for addiction or habit by a single set of criteria for "dependence". They combine the old criteria of habit and addiction into a single list, which widely neglects quantitative aspects in favor of qualitative "Yes or No" statements. The diagnosis of dependence requires only three (non specified) of the six (WHO, 71) or seven (APA, 1,2) criteria to be fulfilled conversely to the old definitions of addiction and habit which required the fulfilment of all four criteria.

The seven criteria of dependence as proposed by the APA (2) in DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders*, 4th edn) are: (i) tolerance (not specified for severity), (ii) substance specific withdrawal syndrome (psychic or physiological, not specified for severity), (iii) substance is taken in greater amounts or over longer periods than intended, (iv) persistent desire or unsuccessfulness to cut down or control use, (v) a great deal of the activities and time spent in order to obtain the substance or recover from its effects, (vi) important social, occupational or recreational activities given up or reduced because of substance use, (vii) use continued despite knowledge of persistent or recurrent physical or psychological problems likely to be caused or exacerbated by the substance. The six criteria proposed by the WHO (72) differ only slightly from those of the APA, mainly by a different sequence, slightly different formulations and the combination of criteria (v) and (vi) into a single one According to those criteria, the more or less regular use of any psychoactive substance can be considered as a dependence. The only possibility to differentiate between substances is to classify them according to the number of criteria met and specify for severity of symptoms and frequency of occurrence. DSM-IV does not consider caffeine a substance of dependence on the basis of such evaluations. However, recently, among 99 subjects 16 fulfilled 4 out of the 7 criteria cited above and were thus considered dependent (69). In the study of drug dependence, among the seven criteria cited, the four main factors to consider are withdrawal, tolerance, reinforcement and dependence on a drug.

CAFFEINE WITHDRAWAL

Characterization

Caffeine withdrawal translates into typical symptoms that are feelings of weariness, apathy, weakness and drowsiness, headaches, anxiety, increased muscle tension, occasionally tremor, nausea, vomiting as well as withdrawal feeling (24,51,64,69). Withdrawal symptoms generally begin about 12-24 hrs after cessation of caffeine consumption and reach a peak after 20-48 hrs. However, in some individuals, these symptoms can appear within only 3-6 hrs and last for one week (3,39,51). Withdrawal symptoms do not relate to the quantity of daily caffeine ingestion (26-28,32,33,38,69). They were even reported in newborns whose mothers were heavy coffee drinkers during pregnancy. The infants displayed irritability, high emotivity and eventually vomiting. Symptoms begun at birth but spontaneously disappeared after a few days (49).

Relief of abstinence symptoms by caffeine

Caffeine withdrawal symptoms disappear soon after absorption of caffeine. This effect is strongly linked to the psychological satisfaction related to the ingestion of caffeine; especially true the first cup of the day. The potential reversal of caffeine withdrawal-induced headache has been known for over 50 years (11) and the reversal of withdrawal syndroms induced by caffeinated coffee cessation by caffeine alone has been shown repeatedly (17,18,26). Caffeine content influences coffee consumption (26,27,44) and the beneficial effects of caffeine consumption on mood or alertness seem to incite people to drink coffee or caffeine-containing beverages (45,60). Heavy consumers of coffee show a preference for coffee containing caffeine, while those who use to drink decaffeinated coffee will choose either decaffeinated or caffeine-containing coffee (20,68).

TOLERANCE TO THE EFFECTS OF CAFFEINE

Tolerance to a drug can be considered on two sides. First, tolerance might indicate that the dose necessary to achieve the desired euphoric or reinforcing effects could increase the consumption of the drug

and thus the dependence on the drug. Second, tolerance to the aversive effects of high doses of the drug may occur, thus leading people to consume higher doses of the drug.

The tolerance to the negative effects of caffeine on blood pressure and heart rate may develop within a few days (10,61,63). Recently, a tolerance to several psychoactive negative effects of caffeine, including tension, anxiety, nervousness, and magnitude of drug effect was shown to occur in some individuals (14). Conversely, there is only limited evidence for tolerance to caffeine-induced alertness and wakefulness (8,19,32,33) and tolerance to clearheadedness, happiness, calmness and decreases in tension do not occur (70). Likewise, there is a lack of tolerance of cerebral energy metabolism to caffeine (50).

In humans, sleep seems to be the physiological function most sensitive to the effects of caffeine, as documented in a recent review of 37 studies (66). Generally more than 200 mg caffeine are needed to affect sleep significantly. It is not clearly established yet whether or not the difference in the sensitivity to the effects of coffee on sleep could be attributable to tolerance. According to some studies, this difference could rather reflect the interindividual sensitivity to caffeine, possibly related to differences in the rate of metabolism of caffeine (46) as well as the variability in the subjects response from one night to the next (19,32,33,47) while other studies show the development of tolerance to the effects of caffeine on sleep (6,8,9,73).

Thus, tolerance to the subjective effects of coffee might develop partially, but more data are still necessary to reach a better understanding of the phenomenon of tolerance.

DISCRIMINATION AND REINFORCEMENT OF CAFFEINE IN HUMANS

Discrimination of caffeine

Human subjects are able to discriminate caffeine against placebo both when offered in capsules or in coffee. Doses of 300 mg or higher are usually more easily detected and mainly recognized by their negative effects of jitteriness, anxiety or nervousness whereas the lower doses are detected by their lack of effect or by caffeine withdrawal symptoms. However, doses in the range of 100 mg, which closely approach the caffeine content of a normal serving are detected poorly or at chance level only. Such doses, which neither induce feelings of withdrawal or overdose are preferred by moderate coffee drinkers (36).

Reinforcing effects of caffeine

Reinforcing efficacy of a drug refers to the relative efficacy in establishing or maintaining behavior on which the delivery of the drug is dependent. In animals, intravenous self-administration of caffeine has been studied after the implantation of venous catheters allowing them by pressing a lever to self-administer the drug and assess behavioral reinforcement (25). Caffeine can act as a reinforcer in animals in some conditions but is not self-administered after exposure to a psychoactive drug (7,12,25). Thus there is a marked difference between caffeine and classical drugs of abuse such as amphetamine and cocaine that are maintaining self-administration across species and conditions (23,56). However, animal studies use intravenous self-administration while human caffeine consumption is always by oral route and it is known that the former mode of administration is by far more addictive than the latter one (55).

In humans, the widely recognized behavioral stimulant and mildly reinforcing properties of caffeine are probably responsible for the maintenance of caffeine self-administration, primarily in the form of caffeinated beverages, such as coffee, tea and cola (25,51). Most studies showed that caffeine reinforcement occurs in 100% of heavy caffeine consumers (1020-1530 mg/day) that had also histories of alcohol or drug abuse (20-22). For moderate caffeine users (128-595 mg/day) caffeine reinforcement occurs in about 45% (28,35,36,53,54) to 80-100% (13,65).

Caffeine reinforcement varies with the dose. Doses of caffeine encountered in tea and coffee are high enough to act as reinforcers, since people look for them in case of withdrawal symptoms (35). Indeed, a dose of 25 to 50 mg caffeine per cup of coffee acts as a reinforcer while increasing doses beyond 50 or 100 mg tends to decrease choice of caffeine or frequency of caffeine self-administration (25), and high doses of

caffeine (400-600 mg in a single dose) are avoided (26). Caffeine reinforcement relates also to withdrawal syndroms occurring after coffee cessation. Indeed, subjects that consistently suffer from caffeine withdrawal headache increase their chance to select caffeinated coffee (containing 100 mg caffeine) by 2.6 (37).

Recently, Bickel et al. (5) reviewed 16 studies dealing with the behavioral economics paradigm for the study of drug abuse. Increasing consumption of a fixed price item when another one becomes more expensive indicating thus a substitutive function was particularly apparent for opiates, cocaine and phencyclidine, but not for caffeine. This method should help in the future to further elucidate quantitatively the relative reinforcement value of different psychoactive substances and to assess more clearly the reinforcing properties of caffeine compared to those of the common drugs of abuse.

The conditions under which caffeine functions as a reinforcer are still not clearly understood. However, the possible reinforcing effects of coffee unrelated to caffeine, but related to its smell, taste and social environment usually accompanying coffee consumption should not be totally neglected in the everyday motivations for caffeine-containing or caffeine-free coffee consumption. Indeed, in subjects consuming 50 mg caffeine in tablets or decaffeinated instant coffee for three days, the desire for coffee in the next three days largely increased in the group given caffeine tablets but remained unchanged in the group given decaffeinated instant coffee, although the latter group experienced marked symptoms of caffeine withdrawal.

EFFECTS OF DRUGS OF DEPENDENCE AND CAFFEINE ON CEREBRAL FUNCTIONAL ACTIVITY

The molecular mechanisms underlying reinforcement and drug dependence were recently reviewed (62) and the critical role of the mesolimbic dopamine system emphasized. The mesolimbic dopamine system consists in the dopaminergic neurons originating in the ventral tegmental area and ending in the nucleus accumbens. Rats self-administer amphetamine and dopamine directly into the nucleus accumbens and the ventral tegmental area as well as in other brain regions connected to the mesolimbic dopaminergic system such as the cerebral cortex, hippocampus and lateral hypothalamus (41,62).

The nucleus accumbens that plays a central role in the mechanism of drug dependence is functionally and morphologically divided into a core and a shell part. The medioventral shell part is related to the limbic "extended amygdala" assumed to play a role in emotional and motivational functions, whereas the laterodorsal core part regulates somatomotor functions (31). The specificity of cocaine, amphetamine, morphine and also nicotine is to activate dopaminergic neurotransmission in the shell of the nucleus accumbens (56,57), a property that has been related to their strong addictive properties (42) while caffeine has no effect on dopaminergic neurotransmission in the nucleus accumbens (Fredholm, unpublished data).

Likewise, amphetamine, cocaine and also nicotine at quite low doses increase rates of cerebral glucose utilization in the shell of the nucleus accumbens (57-59,67). Conversely, the acute administration of caffeine leads to widespread increases in the rates of cerebral glucose utilization in the nucleus accumbens, both the shell and the core plus in most of the structures of the extrapyramidal motor system, as well as in many limbic regions and cortices (50,52). With cocaine and amphetamine, increases in cerebral glucose utilization in the dorsal caudate-putamen appear only at high doses (58,59) while they are already present after the injection of low doses (1 mg/kg) of caffeine (52). Taken together, these data show that caffeine has rather widespread effects on cerebral functional activity compared to the specific effects of amphetamine and cocaine on the neural substrates underlying addiction. In fact, caffeine primarily acts on the extrapyramidal motor system and on cerebral structures related to sleep-awake cycle such as the reticular formation, raphe nuclei and locus coeruleus (50,52). These data are in good accordance with the facilitated motor output (39,48) and the increase in wakefulness reported in humans after caffeine ingestion (39).

CONCLUSION

The effect of caffeine on glucose utilization in the shell of the nucleus accumbens, the key structure for reward, motivation and addiction, appears only at rather high doses that are not specific since they activate the whole brain and are usually avoided by the human population. Conversely, in the shell of the nucleus

accumbens, the stimulating effects of classical drugs of abuse appear already after low doses. However, as classical drugs of abuse, caffeine induces withdrawal symptoms but they are moderate and quite transient. Moreover, withdrawal alone is not a sufficient criterion for assuming dependence according to the criteria of the APA (2). Caffeine has also reinforcing properties but only at low doses while high doses are usually rather aversive. Conversely to classical drugs of abuse, caffeine induces only a very limited tolerance at the level of the central nervous sytem.

Thus, compared to other addictive compounds, it is generally admitted that, in spite of the important variations in individual sensitivity to the effects of caffeine, abuse of the methylxanthine represents a minimal risk (20). The great majority of consumers drink caffeinated beverages in a controlled manner, and there are very few reports in the literature of individuals who have great difficulties in reducing or stopping caffeine intake (31). Finally, the fact that the consumption of caffeine occurs by oral route and gradually over the day results in a delay in the absorption and reduces the likelihood to induce a strong dependence conversely to drugs of abuse that are most often administered via the intravenous or inhaled form.

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SUMMARY

Caffeine is the most widely used psychoactive substance in the world. Although it is usually consumed in rather modest amounts that induce mostly positive effects, caffeine has been considered incidentely as a drug of abuse or even a model of drug abuse. Therefore, the possibility that caffeine abuse, dependence and withdrawal should be added to diagnostic manuals has been considered in the United States. The present paper reviews the available data on caffeine dependence, reinforcement and withdrawal to try to assess in which respect caffeine differs from the classical drugs of abuse. After caffeine cessation, withdrawal symptoms develop in a large portion of the population but they are moderate and last only for a few days. Tolerance to the effects of caffeine on the central nervous system is very limited. Caffeine shows reinforcing properties but only at low doses while high doses induce dysphoric effects and are usually avoided. Conversely to the classical drugs of abuse which lead to quite specific increases in cerebral functional activity and dopamine release in the shell of the nucleus accumbens, the key structure for reward, motivation and addiction, caffeine does not primarily act at the level of that structure and increases glucose utilization in the shell of the nucleus accumbens only at rather high doses that stimulate most brain structures and are already somewhat aversive. In conclusion, although caffeine does share some features of dependence with classical drugs of abuse, they are not sufficient to consider that there is a strong dependence to caffeine in the general population.

RESUME

La caféine est la substance psychoactive la plus utilisée dans le monde. Bien qu'elle soit en général consommée en faibles quantités qui ont plutôt des effets positifs, la caféine a été considérée par de rares auteurs comme une drogue de dépendance et même un modèle de drogue de dépendance. Donc la possibilité que l'abus, la dépendance et le sevrage de caféine doivent être ajoutés aux traités de diagnostic a été prise en considération aux Etats-Unis. La présente revue détaille les données disponibles sur la dépendance, le renforcement et le sevrage de caféine pour tenter de déterminer dans quelle mesure la caféine diffère des drogues dures classiques. Après l'arrêt de caféine, les symptômes de sevrage se développent dans un pourcentage élevé de la population mais ils sont modérés et ne durent que quelques jours. La tolérance aux effets de la caféine sur le système nerveux central est très limitée. La caféine a des propriétés renforçatrices mais seulement aux doses faibles alors que les doses élevées induisent des effets dysphoriques et sont en général évitées. Contrairement aux drogues dures classiques qui induisent des augmentations spécifiques de l'activité fonctionnelle cérébrale et de la libération de dopamine dans l'écorce du noyau accumbens, qui est la structure clé des circuits de récompense, de motivation et d'addiction, la caféine n'agit pas primairement sur cette structure mais augmente l'utilisation de glucose dans l'écorce du noyau accumbens uniquement à des doses relativement élevées qui activent également la plupart des autres structures cérébrales et repésentent déjà des dooses quelque peu aversives. En conclusion, bien que la caféine partage un certain nombre des critères de dépendance avec les drogues dures, ceux-ci ne sont pas suffisants pour considérer qu'il y ait une dépendance marquée de la population générale vis-à-vis de la caféine.

EFFECT OF COFFEE COMPONENTS ON GLUTATHIONE S-TRANSFERASES : A POTENTIAL MECHANISM FOR ANTICARCINOGENIC EFFECTS

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Introduction

The diet is believed to play a major role in the aetiology of human cancer (1). An increasing body of evidence indicates that in addition to carcinogens, the human diet contains a variety of chemical compounds that possess protective, anticarcinogenic properties (2,3). These dietary anticarcinogens are very diverse in chemical structure, their mode of action is generally poorly understood and they may intervene at one or several steps of the cancer process (4). Several dietary anticarcinogenic compounds have been shown to act primarily on the initial event of this process through an inhibition of the formation and/or the stimulation of the detoxification of carcinogenic intermediates, resulting in decreased DNA damage (2,4).

The potential for a relationship between coffee and cancer has attracted considerable attention. In a review in 1991, the International Agency for Research on Cancer (5) reported an inverse relationship between coffee consumption and the risk of colon cancer and concluded that, "the collective evidence is compatible with a protective effect". Several epidemiological studies published since have provided additional evidence supporting such an effect (6,7). However, there is insufficient data available to determine whether the chemoprotective effects of coffee are limited to colon cancer or if cancers at other sites are similarly affected, and also whether these effects are influenced by the method used for brewing coffee.

Animal studies have provided evidence for a potentially more general chemoprotective effect of coffee and have led to the identification of some of the coffee components that may be responsible for these effects. For example, long-term exposure of rodents to instant coffee resulted in a decreased incidence of spontaneous tumours at different organ sites (8,9). Several studies have demonstrated that green as well as roasted coffees inhibit the development of 7,12-dimethylbenz[*a*]anthracene-induced carcinogenesis at various tissue sites in different animal cancer models (2,10-12). Although it is likely that several coffee constituents are involved in its chemoprotective effects, a number of studies have consistently

demonstrated that the diterpenes cafestol and kahweol (C+K) protect against tumour formation in different animal models (13-15).

The mechanisms responsible for the chemoprotective effects of C+K are not fully understood, although recent work in a number of laboratories have identified some putative targets including both phase I and phase II xenobiotic metabolizing enzymes (13,14,16). Our most recent studies indicate that certain members of the glutathione S-transferase family of phase II enzymes may represent specific and sensitive targets for C+K (17). The glutathione S-transferases (GSTs) are a family of multifunctional enzymes which catalyse the reaction of glutathione with electrophilic compounds (6,7). GSTs may be involved in cancer chemoprevention by inactivating electrophilic carcinogenic intermediates and compounds that induce GSTs are generally considered as protective agents against cancer (2,4,19).

In this report we review our most recent studies in which we have used a combination of molecular, biochemical and enzymatic tools to analyse the isoform-specific expression of GSTs in rats fed a diet containing C+K. We have focused on the liver, the most important organ involved in carcinogen detoxification. It has been shown for a number of diverse carcinogens that an increase in hepatic GST activity may induce a general protection state, leading to an inhibition of cancer initiation in the liver as well in extra-hepatic sites (20,21). Our results led us to predict that C+K would serve as specific protective agents against two particular carcinogens, benzo[*a*]pyrene and aflatoxin B1 (Figure 1). Preliminary investigations to examine this hypothesis are presented.

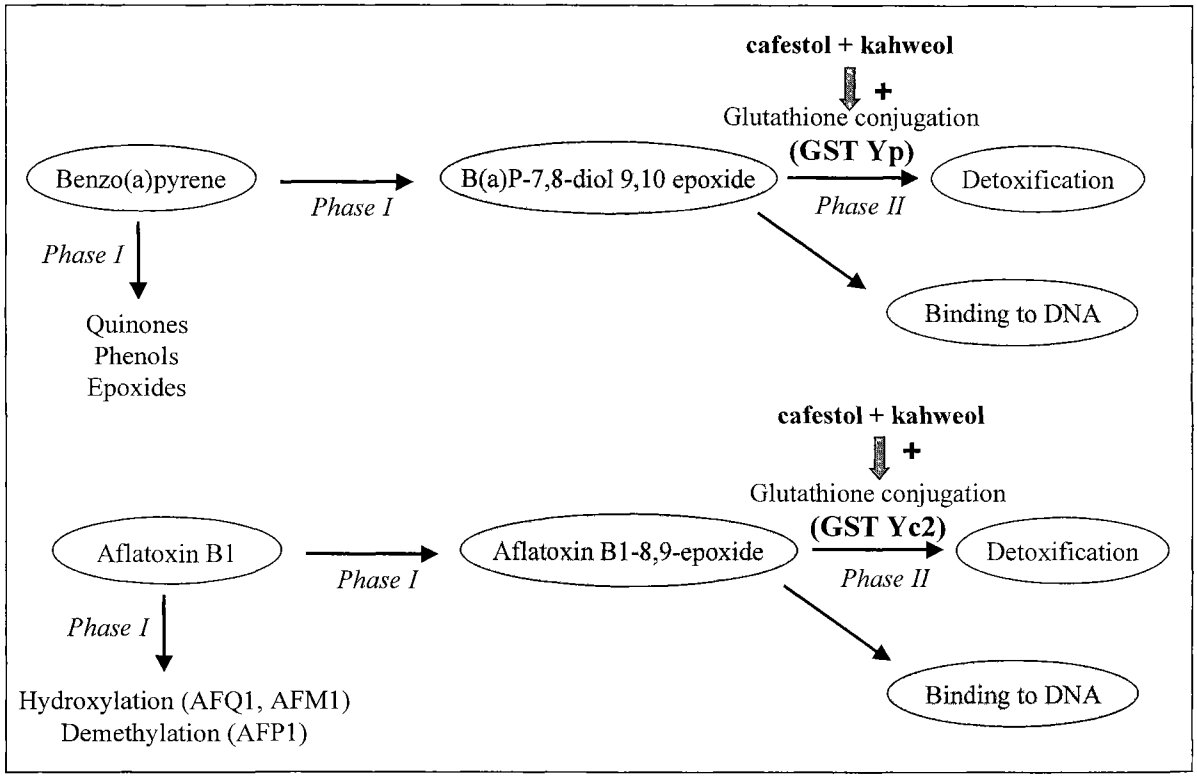

Figure 1. Predicted effects of cafestol and kahweol against benzo[*a*]pyrene and aflatoxin B1.

Materials and Methods

Five-week-old male Sprague Dawley rats (Iffa Credo S.A.) were used. They were provided with Nafag 890 laboratory chow (Nähr- and Futtermittel A.G.) and water *ad libitum* throughout the study. Following acclimatisation on basal diet, the animals were randomly assigned to five treatment groups. A mixture of cafestol and kahweol (53.5:47.5) was solubilized at different concentrations in the vehicle, a 50:50 mixture of corn and palm oils. Animals in the control group received the control diet that comprised basal diet with 2.5% of the vehicle. The remaining groups received basal diet with 2.5% of the vehicle containing increasing amounts of C+K up to 6200 ppm. Subsets of animals were sacrificed after 5, 15, 28 and 90 days of continual exposure to dietary C+K. Additional subsets were subjected to a one-month recovery period in which animals were fed control diet after the test period in order to examine the reversibility of any effects. Each group or group-subset comprised at least 4 animals. Livers were stored at -80°C prior to analysis.

Optimised long-term cultures of differentiated primary hepatocytes from Sprague Dawley rats were prepared as previously described (22). Serum-free conditions were used and a matrigel (Collaborative Biomedical Products) overlay was added to maintain functionality. Hepatocytes were cultured for 48 hours prior to the addition of any test compounds. The inducibility of GST subunits was demonstrated using established inducers, lead nitrate for Yp and coumarin for Yc2. Stock solutions of C+K in dimethylsulphoxide were diluted with media prior to treatment of hepatocytes.

SDS polyacrylamide gel electrophoresis and Western blotting were performed on the liver or hepatocyte cytosolic or S9 fractions according to standard procedures (23). Polyclonal rabbit anti-rat GST Yp, Ya, Yc, Yb1, Yb2 (Biotrin International) and Yc2 (J. Hayes, Dundee University) were used. Detection was by enhanced chemiluminescence (ECL detection kit, Amersham Life Science).

Tritiated aflatoxin B1 (10 nM) or benzo[*a*]pyrene (0.5 μM) (Amersham Life Science) was added to hepatocytes which had been previously incubated with C+K for 24 hours. The cultures were washed prior to their incubation with aflatoxin B1 or benzo[*a*]pyrene and incubated for 1.5 and 3 hours respectively. They were then washed, DNA was extracted according to standard methods (24), and covalently bound aflatoxin B1 or benzo[*a*]pyrene was determined by scintillation counting.

Results

The test diets produced no abnormal behaviour and all animals remained in good health throughout the study period. Cafestol and kahweol produced a dose-dependent increase in the expression of hepatic GST Yp and Yc2 subunits. The data obtained following 28 days feeding with C+K are shown in Figure 2 as a representative example. In control animals little GST Yp protein was detectable but levels increased substantially with increasing dose of C+K. A similar dramatic dose-dependent effect of C+K on the expression of GST Yc2 was also observed. Time-course experiments demonstrated these effects to be observable within 5 days of administration of C+K and to be sustained throughout a three month treatment period. The effects were completely reversible on removal of dietary C+K and immunohistochemical studies demonstrated that at high doses C+K induced a homogenous expression of GST Yp throughout the whole liver acinus (data not shown).

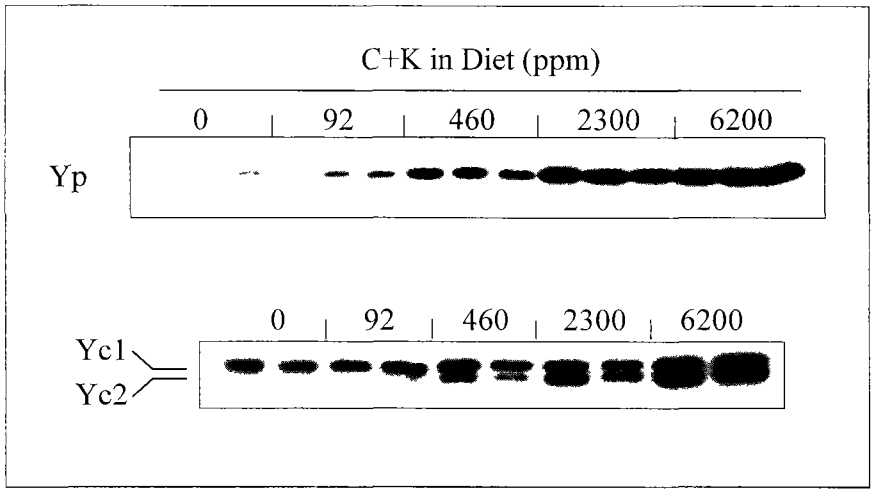

Figure 2. Effect of dietary cafestol and kahweol (0-6200 ppm) on the expression of liver GST subunit proteins after 28 days treatment. The antibody used to recognise GST Yc2 also recognised GST Yc1 whose (upper band) expression is not significantly modulated by C+K.

The ability of the *in vitro* system to respond to GST Yp and Yc2 inducers was demonstrated using lead nitate and coumarin (data not shown). The effects of C+K on the expression of hepatic GST Yp and Yc2 previously observed *in vivo* were accurately reproduced in cultures of primary rat hepatocytes as shown in Figure 3. A marked dose-dependent induction in the expression of both enzymes was observed following incubation of the cells with C+K for 24 hours.

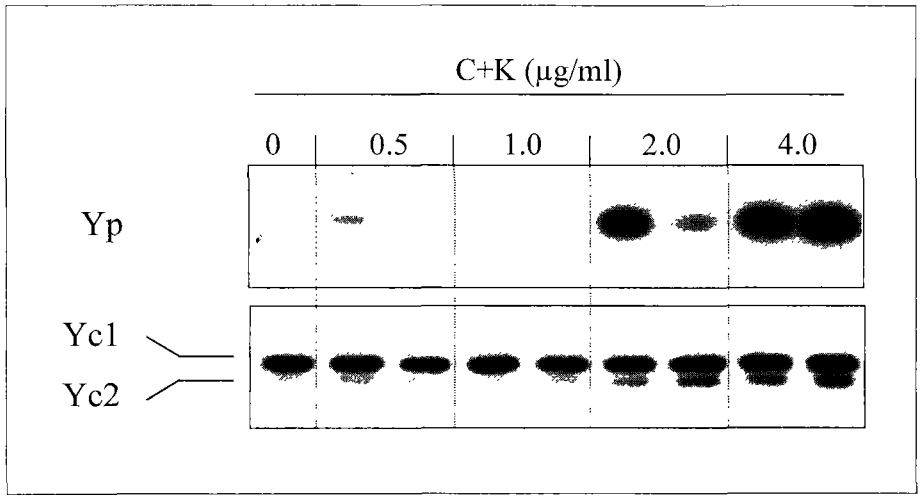

Figure 3. Effect of cafestol and kahweol (0-4 µg/ml) on the expression of GST subunit proteins in primary hepatocytes after 24 hours treatment.

Since GST Yp and Yc2 are involved in the detoxification of the carcinogens benzo[*a*]pyrene and aflatoxin B1 respectively, this finding provided the possibility of utilizing this *in vitro* system to investigate the hypothesis that C+K could prevent the genotoxicity of these carcinogens. The results of studies examining the effects following a 24 hour pretreatment of primary rat hepatocytes with C+K on the subsequent covalent binding of radiolabelled benzo[*a*]pyrene and aflatoxin to cellular DNA are shown in Figure 4.

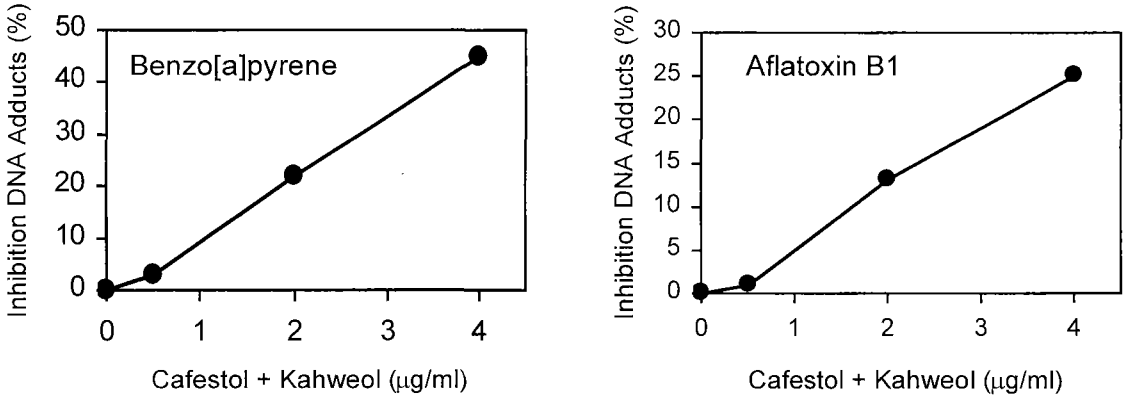

Figure 4. Effect of cafestol and kahweol on the formation of benzo[*a*]pyrene and aflatoxin B1 DNA adducts in primary hepatocyte cultures.

Discussion

The coffee specific diterpenes cafestol and kahweol (C+K) have been reported to be anti-carcinogenic in several animal models (13-15). Recent experimental evidence strongly suggests that this chemoprotective activity may be related to the ability of C+K to induce glutathione S-transferases (17). In the present investigation, studies were performed to characterise the potential effects of cafestol and kahweol on hepatic GST isoenzyme expression and to investigate the hypothesis that the effects observed might provide a protection against the genotoxicity of specific carcinogens whose detoxification is mediated through certain GSTs.

The major effects of C+K observed in rats in the present study with regard to hepatic GSTs were a marked and selective induction of the subunits Yp and Yc2. Interestingly, both of these isoforms are barely detectable in the livers of untreated adult animals but are highly expressed in foetal, neonatal and transformed hepatocytes. Furthermore they have been shown to respond to common inducers (18) suggesting a similar pathway of their induction. The rapid increase in the expression of these enzymes by C+K and the reversibility of this effect demonstrate that the effect of C+K on these GSTs is a transient induction which is unrelated to the permanent deregulation which may occur consequent to neoplastic transformation.

The demonstration of a specific effect of C+K on certain GST isoenzymes allows the prediction of specific carcinogens against which these coffee components may be particularly effective. Several lines of evidence indicate that GST Yp may play a significant role in chemoprotection and human as well as rat GST Yp has been reported to detoxify specific carcinogenic compounds. For example, GST Yp is the major GST in the rat involved in the detoxification of benzo[*a*]pyrene -7,8-diol 9,10 epoxide, the major carcinogenic metabolite of benzo[*a*]pyrene (25). Consequently an induction in the production of GST Yp

enzyme would be expected to reduce the carcinogenic potential of benzo[*a*]pyrene. Similarly, GST Yc2 is the major rat hepatic GST involved in the detoxification of aflatoxin B1-8,9-epoxide (26), and accordingly its induction should protect against the carcinogenic potential of aflatoxin B1. Since both of these carcinogens act through the formation of DNA adducts, i.e. covalent binding to DNA, we put forward the hypothesis that C+K should protect against the covalent binding of benzo[*a*]pyrene and aflatoxin B1 to DNA in the rat model (Figure 1). In order to avoid performing a potentially complex *in vivo* study we devised an *in vitro* methodology for addressing this hypothesis.

The first step in assessing the validity of the *in vitro* methodology selected for the study of the specific chemoprotective effects of C+K was a demonstration that the induction of GST Yp and Yc2 observed *in vivo* could be reproduced *in vitro*. This was achieved by the careful selection and optimisation of a primary rat hepatocyte system utilising defined media supplements to maintain differentiation. Such a system is essential since it assures a very low basal expression of GST Yp and Yc2 in untreated cultures which is a true model of the real life situation. Treatment of cells with C+K at doses below those causing cytotoxicity produced a marked dose-dependent increase in both GST Yp and GST Yc2 that parallelled the effects previously observed *in vivo*. A single time-point of 24 hours incubation with C+K was examined. Further experiments demonstrate that a greater induction of these enzymes may be achieved with longer treatment times (unpublished data).

Having established the efficacy of the *in vitro* system we then tested the hypothesis that C+K would reduce the covalent binding of benzo[*a*]pyrene and aflatoxin B1 to hepatocyte DNA. At the same time point and concentrations at which C+K induced GST Yp and Yc2, these coffee components produced a dose-dependent inhibition in the covalent binding of both benzo[*a*]pyrene and aflatoxin B1 to DNA. The close correlation between the concentration of C+K and the respective effects on GST Yp and Yc2 with the DNA binding of benzo[*a*]pyrene and aflatoxin B1 are highly suggestive of a causal link between GST induction and the inhibition of DNA adduct formation. This however does not rule out a role of C+K in the modulation of the phase I enzymes involved in the activation of these carcinogens. Our previous *in vivo* studies have shown that C+K modulate the expression of certain cytochrome P450s (27). Consequently, we are currently investigating the exact mechanisms responsible for the C+K mediated protection against benzo[*a*]pyrene and aflatoxin B1 toxicity.

In conclusion, the coffee components cafestol and kahweol produce a specific induction in the expression of glutathione S-transferases Yp and Yc2 in rat hepatocytes. This effect of C+K appears to protect the cells against the genotoxicity of certain carcinogens such as benzo[*a*]pyrene and aflatoxin B1. Although the relevance of these data to the human situation remains to be demonstrated, they support previous findings demonstrating that these natural coffee-specific components may possess various promising chemoprotective properties.

Acknowledgements

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Summary

There is increasing evidence that coffee and some of its major components may protect against certain cancers. We have previously proposed that the anticarcinogenic activity of the coffee-specific diterpenes cafestol and kahweol may involve a modulation of the enzymes which activate and detoxify carcinogens. The glutathione S-transferases constitute a family of enzymes whose induction has been linked to the anticarcinogenic activity of several chemoprotective agents. We have investigated the effects of coffee and its components cafestol and kahweol on the expression of different glutathione subunits in the liver, the organ most involved in the detoxification of carcinogens. Dietary administration of cafestol and kahweol to rats resulted in a rapid and reversible, dose-dependent increase in the expression and/or activity of certain specific glutathione S-transferase enzymes. The most notable were an augmentation in glutathione S-transferase Yp and Yc2 subunits. These effects were confirmed *in vitro* using primary hepatocytes. The detoxification of carcinogens such as benzo[*a*]pyrene and aflatoxin B1 is catalysed by glutathione S-transferase Yp and Yc2 respectively. *In vitro* studies demonstrated the efficacy of C+K as protective agents against DNA adduct formation by these carcinogens. These findings demonstrate that the study of the effects of coffee and it components on the modulation of particular glutathione S-transferases may allow the prediction of anticarcinogenic effects of these dietary constituents against specific carcinogens.

QUALITY AND SAFETY OF COFFEE

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Introduction

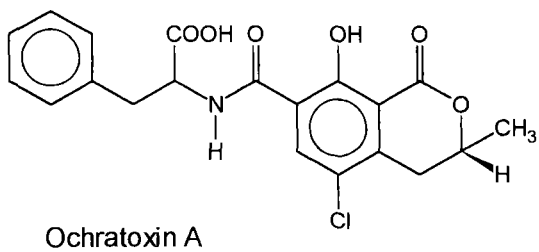
This paper will concentrate on a specific quality and safety issue that is currently causing concern among regulatory agencies viz: the occurrence of the mould contaminant, ochratoxin A (OTA), on green coffee beans and processed products. The questions to be dealt with include:

- What is the nature, source and stability of OTA?
- What is known of the occurrence of OTA in coffee?
- What are the toxicological properties of OTA?
- What are the risks posed by OTA in coffee?
- What regulatory limits are being considered and are they justified?
- What alternative control measures might be needed?

Nature, source and stability of OTA

The chemical structure of OTA is shown in Figure 1.

Figure 1. Structure of Ochratoxin A



Ochratoxin A

This mycotoxin is produced on crops by *Penicillium verrucosum* in temperate climates and by several species of *Aspergillus* in tropical and subtropical climates The best known species of OTA-producing *Aspergillus* is *A.ochraceus* (from which the name of the toxin derives) but others include *A.sulphureus* and *A.sclerotiorum*. Contamination with ochratoxin is principally found in cereals and some pulses but can also occur with coffee, cocoa, nuts and dried fruits. *A.ochraceus* is thought to be the most important OTA-producing mould in relation to coffee beans (Kuiper-Goodman & Scott, 1989; Moss 1996).

Ochratoxin A is a stable compound and does not appear to be removed readily during processing of coffee. However, experimental studies on the fate of ochratoxin during processing of naturally or experimentally contaminated coffee beans have given conflicting and inconclusive results. This situation arises because mould contamination and OTA distribution in naturally contaminated beans is extremely inhomogeneous (see below) and the extent of decomposition during processing cannot be assessed separately from apparent reduction due to mixing (Viani, 1996). Some removal (up to 60%) has been reported during decaffeination (Micco *et al.* 1989) but the losses reported during roasting vary widely from virtually no loss to almost complete destruction. It appears that superficial contamination is usually higher and more readily removed with chaff during roasting than is deep-seated contamination (Pittet *et al.* 1996; Viani, 1996)

Occurrence of OTA in coffee

A few studies have been conducted on green coffee beans which revealed that ochratoxin is distributed very inhomogeneously, even within one sack. Studer-Rohr (1994, 1995) reported levels of <0.5 to 64ng/g and the coefficient of variation between samples was 79-268%. So there are insufficient data to assess what might be an adequate sampling plan for green beans.

The most comprehensive investigation of the occurrence of OTA in roast coffee finished products was conducted by van der Stegen *et al.* (1997) (see table 1). In a survey of 633 samples of coffee (484 roast and ground, and 149 instant) from eight European countries, the overall mean concentration of OTA, corrected for recoveries, was 0.8μg/kg for roast and ground coffee and 1.3μg/kg for instant coffee. More than 50% of the samples were below the detection limit of 0.2-1.0μg and the mean concentration was calculated assuming that these samples contained half the detection limit (probably overestimating the mean). Essentially similar results were obtained in a more limited survey of retail coffees in the U.K. (Patel *et al.* 1997, see table 2).

As with most mycotoxin contamination of foods, the distribution was highly skewed to low

levels and in the study by van der Stegen *et al.* (1997) more than 75% of the samples were in the range 0-1.0μg OTA/kg (Figure 2). Occasional samples were found with OTA levels considerably higher than the mean but only 3 samples were in the range 10-20μg/kg and only 1 sample exceeded 20μg/kg.

Toxicological properties of OTA

OTA is carcinogenic in the rat and mouse (Table 3), producing an increase in tumours in the kidney, but it is not clear whether a genotoxic mechanism is involved or whether it is consequential on chronic nephropathy. Most tests for genotoxicity have been negative up to cytotoxic concentrations. In evaluating these data, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) noted that tumours were only seen at doses above those which caused nephropathy i.e. when the maximum tolerated dose was exceeded (WHO 1991).

OTA has been shown to be nephrotoxic in several species, including the pig, laboratory rats and male mice (see Table 4). It is teratogenic, producing craniofacial anomalies in mice, and is immunosuppressive in chicks and laboratory animals. The most sensitive index of toxicity is nephrotoxicity and the pig appears to be the most sensitive species with the lowest observed effctive dose at 8μg/kg b.wt./day.

Risk assessment of OTA in coffee

In reviewing the toxicological data in 1991 (WHO 1991), the JECFA used the Lowest Observed Effect Level in the pig to calculate a Provisional Tolerable Weekly Intake (PTWI) for man and applied a safety factor of 500, giving a PTWI of 112ng/kg b.wt. (See figure 5 for derivation of the PTWI). In 1995, the JECFA confirmed the basis of their risk assessment and rounded the PTWI to 100ng/kg b.wt./week (WHO 1995). Thus for a 60kg adult human (the WHO assumed mean body weight) the PTWI would be 6000ng/week.

A number of other risk assessments for OTA have been attempted based on nephrotoxicity in the pig or on the rodent carcinogenicity data. These have led to proposed maximum Tolerable Daily Intakes ranging from 0.2-12 ng/kg b.wt. (Creppy *et al.* 1993; Castegnaro *et al.* 1991; Hall & Batuman 1991) i.e. equivalent to a PTWI of 1.4-84ng/kg b.wt.. A working group of the Nordic countries estimated a Virtually Safe Dose for OTA of 5 ng/kg b.wt./day i.e. 35ng/kg b.wt./week or about a third of the JECFA figure (Nordic Working Group on Food

Toxicology and Risk Evaluation, 1991) on the basis of the carcinogenicity data and assuming that OTA might be genotoxic.

With regard to human epidemiological data, OTA has long been suspected of being one of the causal factors of a human tubulo-interstitial nephropathy ("Balkan Endemic Nephropathy") which leads to chronic renal failure in middle age, but a considerable amount of research in this area has failed to corroborate this hypothesis unequivocally. Similarly, attempts to demonstrate a causal link between OTA and urinary tract tumours in South-Eastern Europe have been unsuccessful. In view of the absence of substantive human data suitable for risk assessment, this has had to be based on the animal data mentioned above.

If the JECFA PTWI is taken as the most authoritative basis for a risk assessment, the mean intakes from consuming 4 cups of coffee daily, estimated from the most recent surveys of van der Stegen *et al.* and Patel *et al.*, would be about 2.4ng/kg b.wt./week i.e. only about 2% of the PTWI for OTA. Note that this estimate is for consumers only, not the mean of the whole population. Even extreme consumers are unlikely to exceed 10 cups a day i.e. <10% of the PTWI. (Table 6).

Even if the more stringent conclusion of the Nordic Council of Ministers is taken as a basis for risk assessment, mean intakes of OTA from coffee are less than 18% of the virtually safe dose.

The overall conclusion is that OTA in coffee does not present a significant risk to health.

Regulatory limits for OTA in green coffee; are they justified?

The European Commission is currently considering proposals to introduce limits for OTA in green coffee beans and some member states have indicated that they might take unilateral action if the decision is delayed. However, at present, quite apart from the fact that the evidence presented above indicates that there is no appreciable health risk from OTA in finished coffee consumer products, there are a number of problems with introducing regulatory limits on the green beans.

Firstly, as indicated above, the distribution of OTA in green beans is sporadic and very heterogeneous (Studer-Rohr, 1994; 1995). This makes it difficult to define levels of contamination in consignments.

Secondly, there is no validated sampling plan for OTA in green coffee beans so there are no means of knowing what the consumer risk and producer risk are for any arbitrary system adopted i.e. the risks of accepting defective batches compared with rejecting acceptable material. In the absence of such and agreed sampling procedures, the potential for international trade disputes would appear great.

Thirdly, the limit being proposed in Europe is 5ppb (although figures as low as 3ppb have been discussed). This is similar to the limits for aflatoxins in cereals although aflatoxin B_1 is a much more potent, genotoxic carcinogen and the justification for this is difficult to rationalise on the basis of risk considerations.

Finally, and most tellingly, such a limit would be costly, wasteful, and have little impact on intakes of OTA. This is because of the sporadic incidence and highly skewed distribution of levels of contamination. The costs of sampling and analysis falling both on the market and on the authorities responsible for policing the limits would not be insignificant but the effect on intake levels from coffee would be minimal. As mentioned previously, the data for OTA incidence and levels in green beans are too few to make a realistic assessment of cost and effect but the few existing data do indicate that the effect on intakes of imposing even stringent limits on green beans is likely to be small. However, one can consider such a cost-benefit analysis of the finished products. The recent comprehensive study (van der Stegen *et al.* 1997) revealed that exposure of consumers to OTA via coffee is small even in the absence of regulatory limits. On the basis of the distribution of OTA contamination found in that survey, the impact on intakes of limits on the finished product can be calculated (see Table 7) and it can be seen that introduction of a limit of 5ppb would have the effect only of reducing intakes of consumers of 4 cups of coffee from 0.37 to 0.29**ng**/kg body weight i.e. by 0.08ng/kg body weight - an insignificant gain for a substantial effort and cost.

From this analysis of the data, it would appear that limits for OTA on green beans or finished coffee products are an ineffective way of reducing the risk to to consumers. But this raises the question of what are the alternatives?

Alternative control measures

If stringent regulatory limits for OTA in coffee are to be avoided, it will be necessary to demonstrate that adequate quality assurance measures are in place to limit contamination at

source. However, in order to determine how OTA contamination arises during the stages of production, processing and storage of green beans, what measures might be effective at reducing contamination of coffee and how HACCP-type procedures might be helpful, we need a good deal of fundamental information.This includes a better knowledge of the normal fungal flora found on the coffee plant/fruit, of the ecology and interactions of toxin and non-toxin producing components, and of the environmental conditions that favour or inhibit the production of OTA. A preliminary programme of research on this topic has been initiated and should prove helpful in optimising measures to limit contamination. This approach is likely to be more cost effective and beneficial in reducing overall risks than ill-judged and ill-founded measures to introduce limits without the necessary sound scientific foundation.

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Table 1. Occurrence of Ochratoxin A in Coffee finished products 1.

	Number of samples	Range (ng/g)	Mean (ng/g)
R & G Regular	**445**	**n.d. - 8.2**	**0.8**
R & G Decaffeinated	**39**	**n.d. - 2.8**	**0.7**
Instant Regular	**119**	**n.d. - 27.2**	**1.4**
Instant Decaffeinated	**20**	**n.d. - 5.5**	**1.1**
Instant Mixed	**10**	**n.d. - 1.0**	**0.5**

(van der Stegen et al. 1997)

Table 2. Occurrence of Ochratoxin A in Coffee finished products 2.

	Number of samples	Range (ng/g)	Mean (ng/g)
Instant Powder	12	0.2 - 8.0	2.2
Instant Granules	36	0.1 - 4.9	0.9
Instant Freeze-dried	32	0.2 - 3.0	0.6 - 0.7
Instant Overall	80	0.1 - 8.0	1.0
R&G Regular	20	0.2 - 2.1	0.6

(Patel et al. 1997)

Table 3. Toxicity of Ochratoxin A: 1. Carcinogenicity

- Rat: Renal carcinomas at 70 or 210μg/kg b.w./d **but not at 21μg/kg b.w./d** (male most sensitive)
- Mouse: Renal carcinomas at 40μg/kg b.w./d **but not at 1μg/kg b.w./d** (male most sensitive)
- N.B. **Tumours only seen at above the maximum tolerated dose**

Table 4. Toxicity of Ochratoxin A: 2. Most Significant Effects for Safety Evaluation

Effect	Species	Duration	Lowest observed effect level mg/kg b.w./day	No observed effect level mg/kg b.w./day
Lowered renal function	**Pig**	**90 days**	**0.008**	**-**
Karyomegaly of proximal tubular cells	**Rat**	**90 days**	**0.015**	**-**
Progressive nephropathy	**Pig**	**2 years**	**0.04**	**0.008**
Teratogenicity (craniofacial)	**Mouse**	**(day 9 of gestation)**	**1**	**-**
Necrosis of lymph tissue	**Dog**	**14 days**	**0.1**	**-**

Table 5. Risk Assessment of Ochratoxin A: 1. The JECFA Provisional Tolerable Weekly Intake

$$\text{PTWI} = \frac{\text{Lowest Observed Effect Level in the Pig*}}{500^{+}}$$

$$= \frac{0.008 \text{ mg/kg b.w./day}}{500} = \frac{56{,}000\text{ng/kg b.w./}\mathbf{week}}{500} = 112\text{ng/kg b.wt/week}$$

$$= 0 - 100\text{ng/kg b.w./week (rounded down in 1995)}$$

* The most sensitive species
\+ Larger safety factor because based on a LOEL rather than NOEL

Table 6. Overall Risk Assessment for OTA in Coffee

- **Total intakes of OTA (mainly from cereals) = 8 - 20ng/kg b.wt./week (data from Sweden, Germany & Canada)**
- **Contribution to intakes of OTA by coffee in Europe is much lower than cereals (ca 2.4ng/kg b.wt./week)**
- **Consumers of 4 cups of coffee per day would ingest *only 2% of the JECFA Provisional Tolerable Weekly Intake***
- **Extreme consumers (10 cups per day) would ingest *only 8% of the JECFA Provisional Tolerable Weekly Intake***
- **By the JECFA evaluation:**

  Drinking up to 10 cups of coffee per day does not present an appreciable health risk from Ochratoxin A

- **By the Nordic Council of Ministers evaluation:**

  Mean intakes of Ochratoxin A from coffee are <18% of the Virtually Safe Dose

Conclusion:

- **At the levels currently found in coffee products, Ochratoxin A would not cause a significant increase in cancer risk**

Table 7. Effect of Alternative Limits on Finished Coffee

Limit (ppb)	Remaining No. of Samples	Mean OTA Content (ppb) of Remaining Samples	Intake in 4 cups of Coffee (ng/kg b.w.)
None	633	0.92	0.37
20	632	0.88	0.35
15	631	0.85	0.34
10	629	0.81	0.32
5	621	0.73	0.29
4	618	0.71	0.28

TOWARD THE PREVENTION OF MOULD MEDIATED QUALITY LOSS. HACCP ANALYSIS OF AN OUTDOOR PROCESS

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Introduction

In the food industry Hazard Analysis of Critical Control Points (HACCP) has become the preferred method of quality and safety assurance (1). In its pure form the development of a HACCP programme follows several well defined steps. The risk organisms must be identified. Their physiological and ecological features must be studied with an aim to understand occurrence and dispersal on the one hand and the physiological limits relating to growth, reproduction and survival on the other. The production process must then be modelled in relation to what is understood about the features of the risk organisms. By considering the process and the potential problems together in this way and taking into account practical considerations as well, the so-called critical control points are identified. These are the points in the process which present the best opportunity for control - for preventing the risk organisms from producing spoilage or a hazard. Thus a control strategy emerges and operational parameters are developed for each critical control point. Once a strategy is adopted, resources are committed to monitoring the critical control points because once the parameters are shown to be adequate in exerting the required level of control, adherence to the prescribed operational parameters is equivalent to assurance that the risk is controlled.

Milk Pasteurization provides a straightforward example. The organisms associated with milk, which present a risk to health, are non-sporeforming bacteria such as *Listeria, Salmonella, Staphalococcus, Brucella* and *Campylobacter. Pseudomonas*, also a non-sporeformer and the spore-forming genus *Bacillus* are the most significant spoilage

organisms of this commodity (2). Heat can be used to control these organisms. To control the non-sporeformers a moderate heat treatment is required: 71.7° C for 15 seconds – the product is standard pasteurized milk. A harsher treatment is required to prevent spoilage since spores are relatively resistant to heat: 135°C for 1 second – the product is UHT or Long-life milk with a shelf life of months or years. To assure the product then, and this defines the critical control points, first the quality of the raw milk must be above a certain minimum standard. The prescribed heat treatment must, in actuality, be applied. And lastly the packaging must be conducted such that post control-step contamination is prevented particularly by contact with unprocessed milk. Documented adherence to the process parameters replaces end-product analysis as the best form of quality assurance. Safe milk is assured by the application of a validated control process with strict process monitoring which renders end product analysis superfluous.

Of particular relevance to coffee processing is the way in which the two products mentioned above differ. The harsher treatment causes an alteration of the taste which makes UHT milk unsuitable for some uses. This highlights the importance of evaluating a process with respect to practical and organoleptic considerations and making these an integral part of any HACCP programme.

It must be said that the greater the potential for controlling conditions a process possesses the better it lends itself to the HACCP approach. On this basis, HACCP is particularly well suited for application to relatively high technology activities. Fundamentally, however, the method of process analysis used in HAACP has a potentially much wider application. In this paper we develop a process model and apply the results of field work toward the development of a fully fledged HACCP plan for preventing mould-mediated quality loss of coffee.

Salient Features of the Coffee Production Chain

Coffee is perhaps unique amongst commodities in that its value and desirability is entirely attributable to organoleptic features. It has no use other than as a beverage or flavourant and there is no single formula that can describe the various forms in which people enjoy the coffee beverage around the world. The implication of these features on the development of a process model is that different production methods must be accommodated and that any potential control features must not infringe organoleptic integrity.

Coffee production begins, of course, with the development of the crop. The layout of the orchard (density, shade, inter-cropping), the prevalent cultivars used in production, pruning conventions, soil care (mulching, fertilization, irrigation), and pest control strategies

are aspects which vary greatly between producing countries. At some point the crop is harvested and the methodology employed is dictated by the cost and availability of labour, the preferred type of processing (wet/dry method) in the region and other considerations such as the size of the farm and slope of the land under coffee.

Post-harvest processing of the fruit to the dried bean takes a form that is influenced by many factors. Perhaps most fundamental is the availability of water during the dry (harvest) season. The type of coffee preferred by the market, the willingness to make capital investment, aspects of transport and storage and access to market or market information all figure in the decision to practice wet or dry processing. Of course, the tradition of the coffee-growing region is also of immense importance. Both the plant and the product of *Coffea arabica* are very distinct from those of *C. canephora* and this is important to keep in mind.

Against these factors which lead to diversity in the production methodology is the fact that coffee can be commercially grown in only a relatively narrow geo-climatic zone so the main coffee producing regions have many features in common.

After drying/processing there is a brief period of storage, resting the coffee, before the characteristics of the beverage can be accurately assessed. Initially, storage may be at the farm or estate, at the co-operative or at the curing works depending on the structure of the industry in the producer country. Following sale of the lot, it may undergo a further period of storage in a lowlands area before undergoing transoceanic carriage to consuming countries with all the consequent changes in humidity and temperature. Alternatively it may be processed and consumed in the producing country, possibly never leaving the highlands.

Developing a Process Model

By its nature a model is a generalization or a simplification of a more complex situation. To be successful, which means reliable in a predictive sense, the model must represent the principles that underlie the detail. The veracity of the principles is critical and usually cannot be understood by assessing one variation of a system. For this reason we have closely studied a broad spectrum of coffee production systems in three important producing nations representing three continents:

Brasil: the Triangulo and Zona de Mata regions of Minas Gerais; Pinhal and central regions of Sao Paulo state.

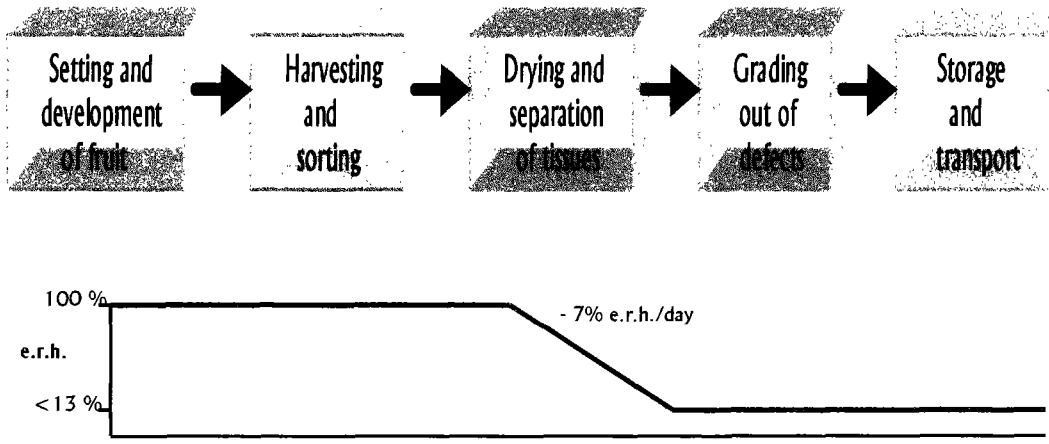
India: Chikmagalur district and Coorg district in Karnataka state; eastern Kerala state.

Indonesia: Lampung province in southern Sumatra; East Java; Enrekang and Kalosi districts in South Sulawesi.

These sites provided a great variety of coffee production methods, farm sizes and industry structures to inform our work. In coming months we will add Venezuela and Kenya to our list of sites.

The model proposed below represents any and all coffee production chains. It does so by focusing on the purpose behind each identifiable stage – the stages may be technologically different from chain to chain but the objectives are very similar in each. So in reality the 'stages' of the model are concepts developed from an evaluation of the functionality of the analogous "links" of several production chains in contributing to the common goal of all coffee producing chains: a healthful, good-tasting beverage free from defects.

FIGURE 1. Proposed model for coffee production starting from the crop and including transportation with water availability shown throughout the production chain.



e.r.h. = equilibrium relative humidity; the rate of -7% /day follows an initial period of 1 day for parchment and 2-3 days for cherry during which there is no change; data collected on-site in India

Critical Control Features

The aim of HACCP analysis is to find the points in a process at which effective control can be exercised. In the development of the analysis, critical control features represent points which appear to have potential as fully fledged critical control points based on current understanding. By implication, further research will lead either to their acceptance as critical control points or their rejection as such. Critical control features may be rejected either because no practical strategy can be devised or because further research shows them not to be critical after all.

1) Setting and development of fruit

Whether the orchard is high density or low density, under shade or not the objective of any farmer is to produce sound fruit in good yields. Fungi are prevented from exploiting the nutrients contained in the coffee fruit by physical exclusion and by the specific response of the plant to microbial activity which may be likened to the immune response of animals although much less well understood. At this stage water is never limiting.

Fungi can enter fruit through the vascular system, at the time of flowering through the flower, through breaks in the cuticle of the fruit or by the activity of specialist organisms that have evolved the means to breach these barriers.

The critical control features at this stage can be characterized as access of the fungi to the fruit and vigour of the plants. There is a need to establish the source of fungal inocula in the orchard and the relative importance of the possible infection routes. A method for assessing plant vigour as a predictive and monitoring tool is also indicated.

2) Harvesting and sorting

Internationally, the methods employed at this stage vary a great deal. Differences in the type of processing, the evenness of ripening, cost of labour and of the cost of mechanization can be taken as the principle factors leading to this diversity. Regardless of the methods, the goal of this stage is to select sound fruit at the correct stage of ripeness for further processing. Although it must be said that little or none of the rejected produce is destroyed, the main product is effectively improved by the removal of certain classes of defective fruit.

Fungi can cause these defects and they can grow rapidly on fruit damaged by other means. On the other hand fungi can grow without producing any physical defect and will develop as a matter of course through the stages of fruit ripening. Once again, water is not limiting here.

The critical control feature of this stage is clearly the utilization of sound fruit. The meaning of "sound fruit", however, requires close scrutiny; the association of defects with fungi; the association of fungi with good quality coffee and its stages of ripeness and the effect of accidental mixing of moulded with sound fruit also require clarification.

3) Drying and separation of tissues

This stage unifies several apparently different technologies based on two shared objectives: the rapid removal of water to stabilize the commodity and the removal of the fruit tissues that are undesirable in the eventual product - green coffee. The order in which drying and the separation of the tissues of the fruit is accomplished and the duration of these

processes depend on the technology employed. Generically refered to as wet processing and natural processing, in reality there are many variations in this area of post harvest technology making it the most complex stage of the production chain to model. All countries and most regions use both methods to some extent regardless of whether the coffee is robusta or arabica. Parchment coffee and cherry coffee have different organoleptic qualities that are essential to the market and many coffees are organoleptically sensitive to the methods employed.

The critical control features can be identified as the drying rate of coffee and this is clearly a critical control point based on current knowledge. Hygeine of the drying facilities, the microbiological quality of the initial product and an assessment of the effect of current processing techniques on the fungal population are the other critical control features. Like the previous two stages this one is also exposed to the vagueries of the weather. It must be emphasized that best practice at this stage can only preserve the existing quality of the product and has no means of restoring quality previously lost by poor practice.

4) Grading out of defects

Whether accomplished by hand, mechanical or electronic means, sorting holds the possibility of eliminating defective beans from the bulk product. Only defects that are visible or produce physical changes can currently be sorted out. With commodities that have no alternative use such as coffee, the quality of the best grades are improved at the expense of the low grades by sorting.

Fungi can cause visible defects or induce changes in the physiologically active green coffee which are visible. It is also probable that in coffee as in other commodities, fungal spoilage usually takes the form of more intense degradation of a small proportion of beans, not as a modest, uniform degradation of the whole lot. On the other hand, certain defects such as the production of off-tastes or of mycotoxins require little growth of fungi and may not be well correlated with a detectable change in the bean.

The critical control feature at this stage is the elimination of defects. The evaluation of the control potential of this elimination relates to the correlation of detectable defects with the problems produced by fungal growth, the fate of the defects once sorted out and practical matters of implementation (high-tech solutions can not be widely implemented). This is essentially a remediation strategy rather than one of prevention that can return a better price to the grower for a proportion of his crop. If effective it could also protect the consumer or at least some consumers, from tainted product.

5) Storage and transport

These apparently different activities are considered together because control is achieved in both by the maintainence of dry conditions in facilities that, in theory, are controllable. The length of the storage period of coffee, unlike with cereals, is limited by loss of organoleptic quality which occurs much more quickly than does loss of microbiological quality. In practice coffee is stored in different facilities as it progresses to the market. Its passage whether to the coast for export or not varies within and between producer countries. This depends on the organization of the market, size of the farm of origin, transport infrastructure and often access to market information. Exported coffee always faces problems of a change in ambient temperature and exposure to moist conditions while on the sea.

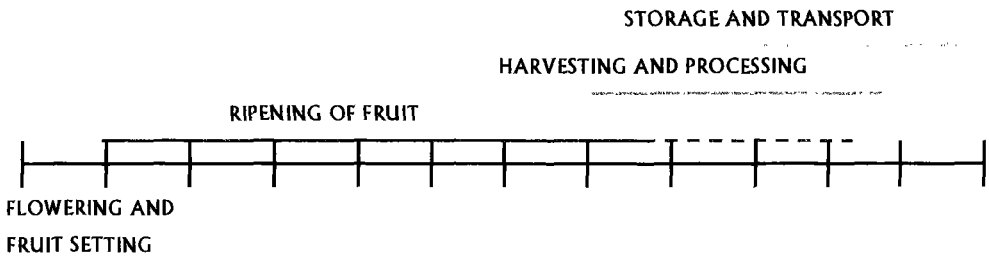
Fungi are not different to other organisms in requiring water for growth and it is quite straightforward to establish water content levels at which a commodity is stable. Drying does not, however, kill fungi initially but rather stimulates it to produce resting structures which can quickly resume growth should water become available. When storage goes wrong, typically it is due to a localized fault, condensation, a leak or insect activity, to mention a few examples, that sets in train a loop where the products of respiration, free-water and heat, stimulate further growth which produces more water, and so on. This process produces what is called a "hot spot".

The water content of coffee at storage and throughout transport is another critical control feature that can undoubtedly be accepted as a critical control point based on current knowlege. A maximum water content of 11 to 13% (3) is already well accepted throughout the coffee world. One difficulty arises in measuring and maintaining the target values as the dried crop proceeds through the transport chain to its destination market. HACCP analysis in this area will focus on monitoring protocols. There are, however, several technical areas that are yet to be fully understood. The effect of high fungal load on storability and the role of specific fungal species in storage conditions; the distribution of water in lots and the implications of uneven water distribution for measuring techniques; an evaluation of requirements for sea-borne transport facilities all require systematic evaluation.

Summary

Hazard Analysis of Critical Control Points (HACCP) is an approach to food quality assurance that is replacing end product analysis. A HACCP analysis directs attention to the physical and biological aspects of a food process and how they interact to produce a safe and high quality product. A model of coffee production applicable to all methodologies is proposed based on processing objectives. Five stages are identified: Setting and development of fruit; Harvesting and sorting; Drying and separation of tissues; Grading out of defects; Storage and transport. Critical control features are developed from this production model by considering fungal activity in light of the conditions and objectives of the individual processes. New field data on the occurrence fungi in the production chain are presented.

FIGURE 2. Significant fungi associated with coffee during a year of production.



FUNGI COMMONLY ISOLATED FROM SURFACE-STERILIZED BEANS:

BEFORE HARVEST	DURING DRYING	DURING STORAGE
Fusarium stilboides	*Fusarium stilboides*	*Eurotium rubrum*
Penicillium brevicompactum	*Penicillium brevicompactum*	*Aspergillus penicilloides*
Colletotrichum spp.	*Candida edax} wet processing*	*Wallemia sebi*
Aspergillus tamarii	*Candida spp. } wet processing*	
Candida edax	*Aspergillus: flavus, niger* and ochraceous grps.	

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"Food business operators shall identify any step in their activities which is critical to ensuring food safety and ensure that adequate safety procedures are identified, implemented, maintained and reviewed using the principles underlying the system of HACCP....."

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CURRENT STATUS AND STRATEGIES FOR MANAGEMENT OF MYCOTOXINS IN INDIAN COFFEES

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INDIAN COFFEE INDUSTRY

Coffee is a lucrative commercial crop and is the second important commodity in world trade, after petroleum products. Coffee cultivation is confined to the semi-tropical areas of the world covering over 80 developing countries including India and coffee trade is instrumental for cultural and economic development in several of these third world countries.

In India, coffee occupies prime position among the plantation crops and is mainly cultivated in southern hilly tracts in a total area of 300 thousand ha. covering the states of Karnataka, Kerala and Tamilnadu. The annual average production is around 200 thousand MT with an average productivity of 860 kg/ha. As an agro based rural enterprise primarily, coffee industry provides employment for over 0.4 million people in the cultivation, processing and trade sectors. India is one of the world's few producers of both arabicas and robustas which is well facilitated by the vast diversity in agro-climates.

Indian coffee industry is characterized by marked cultural dualism between subsistence oriented small growers and market oriented large and corporate producers. The small growers with less than 10 ha. land form the 98% of total holdings covering nearly 65% of area under coffee and approximately 60% to the total production. The large holdings constitute the remaining 2% of the holdings with a share of 35% in area and

40% in total production. Out of the total produce, 60-70% is processed by wet method while the remaining 40-30% is by dry method to produce cherry coffee. Although coffee is thus classified in to two major groups based on processing method, it is opined that at small grower level, little quantities of coffee are produced following his own way of processing protocol which includes harvesting, processing, drying and storing resulting in possible inhomogeneity of pooled commercial lots.

India's share in world coffee trade is only 2.5 to 3.0% and nearly 70% of Indian Coffee is exported. With this meager share, the role of Indian Coffee industry in world coffee market is negligible. The industry's gross turn over touched 20 Billion Rupees during 1996-97 of which the export earnings are .. 400 US $ Million. The recent developments of liberalization in Indian Coffee sector enabling free trade has posed newer challenges to the industry especially on quality front. The expectations of liberalization of Indian coffee market are very high and the coffee trade in general expected marked improvement in the quality.

II. INTERNATIONAL QUALITY CONCERNS

Both the intrinsic and extrinsic parameters of coffee beans/products need not be accepted as the final quality of the produce by the consumer. The awareness about the food safety, and environment concerns in the most of the developed countries have paved the way for stringent quality regulations. The contaminants such as pesticide residues, mycotoxins, heavy metals are of great concern to food industry, as the very presence of these contaminants makes the food unsafe and hence tolerance limits have been fixed for a variety of agricultural products by the importing countries. Oflate, the sporadic presence of mycotoxin especially Ochratoxin-A (OTA) in some trade coffee samples has attracted inquisite interest of both media and Governmental agencies in consuming countries as mycotoxins are reported to be nephrotoxic and nephro carcinogenic.

III. MYCOTOXINS IN COFFEE

Different filamentous fungi are reported to grow on green coffee beans under certain conditions and cause a general loss of quality through production of off - flavours as well as tastes and in some cases mycotoxins. The most common mycotoxin present in coffee, is Ochratoxin-A (OTA) which is found in small quantities. Aflatoxins and Sterigmatocystin are less frequently reported in coffee. The OTA derives its name from *Aspergillus*

ochraceus, (redisgnated *A. aluteeus*), the mould from which it was first isolated. It is now well established that OTA is also produced by other ubiquitously found moulds such as various other strains of *Aspergillus* and *Penicillium*. The natural occurrence of OTA in coffee was first reported by Levi et al. (1974). Since then, a number of researchers have detected its presence in green and roasted coffee samples (Contafora et al., 1983; Toubachi et al. 1984 and 1988, Micco et al., 1989, Studer-Rohr et al., 1995).

The most recent comprehensive investigations of the occurrence of OTA in Coffee in Europe has been reported by van der Stegan, 1995 (cf. Walker, 1996 - personal communication). In a survey of 633 samples of coffee (484 roast & ground and 149 instant) from eight European countries, the overall mean concentration of OTA was 0.8 ug/kg for roast as well as ground coffee and 1.3 ug/kg for instant coffee. More than 75% of the samples were within a range of 0.2-1.0 ug/kg, which is below the detection level. Occasionally a few samples were found with considerably higher levels of OTA .

Another survey carried out by the Ministry of Agriculture, Fisheries and Food (MAFF) in the UK revealed that coffee is not a major source of OTA in the diet. A total of 291 samples of green (unroasted) arabica and robusta beans from over 27 countries of origin entering the UK were analysed. A total of 181 samples were found to contain less than the detectable amounts of OTA. Of the known robusta samples 70% were with in a range of 0.26 to 27.3 ug/kg while 20% of the arabica coffee samples had concentrations of OTA at or above the limit of quantification (0.2 ppm) (MAFF, 1996).

IV. CURRENT STATUS OF RESEARCH ON MYCOTOXINS IN INDIA

A few trade samples of Indian origin analysed at random by the ISIC and also MAFF of UK did not contain the detectable levels of OTA (Personal communication). However, due to the seriousness of the OTA problem and the possible impact of OTA on export trade, Indian Coffee Board has initiated detailed studies on the status of mycotoxins and strategies for management during various stages of coffee production.

1) Random Survey / Analysis Of Commercial Coffee lots

To know the extent of coffee contamination by OTA in India, random survey was undertaken and some samples where the possibility of mould growth can be expected viz., monsooned coffee, berry borer infested coffee, blacks, estate pounded coffee, improperly stored robusta cherry with mould growth etc., were collected. These samples were analysed by M/S Illycafe, S.P.A., Italy for OTA content (Table 1).

The OTA content in all the six samples ranged from < 0.2 to 0.5 ppb, which is well with in the tolerence level prescribed by WHO/FAO. Thus, the present survey / analysis has supported the earlier findings that the extent of OTA contamination was found less in Indian coffees.

2) Studies on Mycoflora Associated with Coffee fruits

In order to understand the association of different fungi with coffee fruits under Indian conditions, studies have been initiated at Central Coffee Research Institute. The preliminary information generated from these studies is presented here. Like any other fruits, ripe coffee cherries too have a complex association of fungal flora, which may likely to change during the process of drying and storage. Hence, fungal populations were studied on external surface of the fruits, mesophyll (pulp) and bean surface (freshly pulped with out drying). The fungal populations associated with the over ripe fruits, sun scorched fruits and split fruits were also studied. Besides, the coffee beans at different stages of drying were also subjected to analysis for studying the changes in fungal populations if any, during the process of drying. The data recorded from various experiments were statistically analysed and presented (Tables 2 - 4). The external mycoflora of the fruits was found dominated by yeast, followed by *Fusarium* and *Cladosporium* (Table 2). The mesophyll and bean mycoflora also found asssociated with similar fungal colonies but the populations were significantly less and further decreased during the process of drying (Table 2). Interestingly, the toxin producing moulds like *Aspergillus* and *Penicillium* sp. were very poorly represented either on the fruit surface or on the bean surface. The studies conducted on the mycoflora of over ripe, sun scorched and split fruits (due to un-seasonal rains) on the plant indicated that the funagl populations were comparatively high in split fruits whereas the frequency was found almost similar in sun scorched and over ripe fruits (Table 3). As regards to the changes in fungal colonies during the process of drying, significant differences could not be observed (Table 4). However, reduction in the quantity of different type of fungal colonies were noticed on 7th day of drying. In addition, quantification of mycoflora was also undertaken in the soil samples collected from different coffee tracts and data is presented in Table 5. From the data it is apparent that ocurrence of *Cladosporium* is predominant followed by *Penicillium, Fusarium,* yeast and *Aspergillus* spp.

V. MANAGEMENT STRATEGIES TO MINIMISE MOULD GROWTH AND OTA CONTAMINATION

In general it is opined that the best way to reduce the occurrence of OTA contamination in coffee is the prevention of the mould infection/growth in raw produce at field level and also during processing, storage and transporting stages.

1) Agronomic Approaches

The agronomic approaches to minimise the conditions mainly involves the practice for checking on tree infection of the moulds. As per the hypothetical considerations already advanced by the IISC, on tree infection occurs due to air borne spores penetrating in to the pulp and seed through skin lesions such as drought lacerations or insect damages. However, it is pertinent to mention that under Indian condition, the chances of experiencing dry spell during bean formation, maturation and fruit ripening stages are less. Hence the chances of on tree infection through drought lacerations are remote. However, the splitting of beans is usually observed due to unseasonal rains received during fruit ripening stage. Hence, separate harvesting and processing of sun scorched fruits and split fruits without mixing with commercial lots is advocated among the growers.

As regard to insect damage, till recently India is fortunate to be free of berry borer (*Hypothenemus hampii*) which infests the berry by direct penetration and causes severe damages to the beans. The first appearance of coffee berry borer in some pockets of India during 1990 and the gradual spread of the pest to the other areas warrants more detailed studies on the possibilities of on tree infection through the berry/bean damage caused by the borer. Although the OTA contamination was found below 0.2 ppb in berry borer infested beans, extensive education programmes for growers have already been taken up for the integrated management of this pest so as to avoid possible infection of OTA producing moulds.

2) Harvesting Process

It is opined that, improper harvest results in berry drop which facilitates on-soil infection due to soil borne spores penetrating in to the fruits through petiole aperture or mechanical rupture during manual picking. In India, these fallen berries are called 'gleanings' and are usually collected at the end of the harvest. The presence of berries on the soil for a long time enables the infection process. Hence to avoid risk from this angle, spreading of polythene sheet or jute mats below the tree/bushes while harvest is being encouraged to minimise the gleanings.

3) Post harvest processing and Handling

In general, the chances of infection of coffee bean with OTA producing moulds are rather high at post harvest processing level especially during drying stage and storage than on-tree or on-soil infection. The mould growth requires a minimum moisture level of 14% and hence improper drying of coffee beans above the 14% moisture level favour the mould infection/growth. The research department of Indian Coffee Board had conducted extensive studies on the methods of processing and drying (types of drying yard, spreading thickness, acceptable moisture level in the dried produce) and the information generated has been transfered to the growers for adoption. In India, washed parchment as well as cherry coffee are sun dried to the recommended moisture level (wet washed coffee to 10% moisture level and un washed Arabica and Robusta cherry to 10.5 and 11% moisture, respectively). Both under drying and over drying leads to poor quality coffee. Under dried coffee turns mouldy and gets bleached during storage and subsequent curing operations.

In addition, usage of trays with wire mesh bottom mounted on wooden poles at a height of 75 to 90 cm. above floor level for surface drying, followed by spread of coffee on clean tiled or concrete floor to a maximum thickness of 7 cm with repeated stirrings (raking) at least once in an hour for uniform drying. The drying coffee lot will be covered in evenings with water proof material to prevent rehydration of coffee from mist or rain. These are some of the important operations counts for minimising the mould infections.

4) Storage of Coffee

Similarly, in order to minimise the infection at storage level, stores should be kept well ventilated and dry without letting in moisture or rain water. Well dried gunny bags should be used and bags containing parchment/cherry coffee should be stored on raised wooden platform to ensure circulation of air and to prevent dampness of the bags. It has also been advised to avoid stacking the parchment and cherry coffee together.

VI. EXTENSION EDUCATION

In India, 98% of the coffee growers come under small grower sector (each holding < 10 ha.). Hence, transfer of technology plays important role not only in achieving the economic yields but also in producing good quality coffee. Since its inception, Indian Coffee Board is doing yeomen services to the growers of the country in transfer of technology developed by the Research Department from time to time. However, with the changing market scenario due to liberalization of coffee industry, increased attention has been given to extension education especialy on the quality front. Organizing training to growers, curers and traders level is

being taken up with the support of extension literature and audio-visual aids to educate on different aspects of quality management in coffee. The concept of master trainees and trend setters at village level for transfer of identified technologies has also been undertaken.

VII INTERNATIONAL COLLABORATION - FUTURE PROSPECTS

Thus, keeping in view the importance of coffee quality in international trade, India has taken up the problem of mycotoxins on top priority. Although it is possible to minimise the mycotoxin producing mould infection with the available information, either at production or processing stages of coffee, still there are several basic aspects such as method of mould infection, congenial conditions for mould growth on coffee, production of mycotoxins, agro-techniques to be adopted to avoid mould growth, post harvest handling, processing methods and storage method to be followed, fate of mycotoxins during processing like roasting and admisible limits of consumption etc. to be studied in depth. Hence, these studies are proposed to be undertaken under a collaborative project of International Coffee Organization, involving few coffee producing countries like Brazil, Kenya, India and renowned laboratories for mycotoxin research from University of Surrey, U.K and Technical University, Denmark. The technical programmes under this project have already been initiated and it is hoped that the information generated under this project will be of immense use in tackling the problem of mycotoxins not only in India but also in other coffee producing countries.

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Table 1. OTA content in some commercial coffee beans

Sample No	Details of the sample	OTA content(ppb)
1	Rb. Prch. Monsooned	0.4
2	Rb. Prch. Berry borer infested	< 0.2
3	Rb. Prch. Blacks	0.3
4	Rb. Che. With Moulds	< 0.2
5	Rb. Che. Estate pounded	< 0.2
6	Rb. Che. Clean coffee	0.5

Table 2. Mycoflora of arabica coffee fruits [surface (external), mesophyll (pulp) and bean]

Fungus	Surface	Mesophyll	Bean	Mean
	Number of c.f.u. (in thousands) per fruit		c.f.u. per Bean	
Yeast	1350.00	8.70	0.26	452.98
Cladosporium	20.50	2.25	0.38	7.71
Fusarium	50.00	0.34	0.07	16.80
Aspergillus	1.23	0.36	0.03	0.54
Penicillium	0.53	0.37	0.02	0.31
Mean	284.45	2.41	0.16	

To compare the difference of means

C. D. at	5%	1%
Between fruit components	21.14	35.06
Between fungus	20.67	28.01
Between fungus in a particular fruit component	35.80	48.52
Between fruit component to a particular fungus	36.13	51.73

c.f.u. -- colony forming unit

Table 3. Fungal population at different stages of fruit ripening in arabica coffee

Fungus	over ripe fruits	sun scorched fruits	split fruits	Mean
	Number of c.f.u. (in thousands) per fruit			
Cladosporium	8.77	55.70	25.87	30.11
Fusarium	1.23	1.23	1.33	1.27
Yeast	32.43	53.00	26.60	37.34
Penicillium	3.60	3.20	22.43	9.74
Aspergillus	3.27	3.30	22.43	9.67
Mean	9.86	23.29	19.73	

To compare the difference of means

C. D. at	5%	1%
Between fruit stages	3.57	5.91
Between fungus	3.47	4.70
Between fungus in a particular fruit stage	6.00	8.14
Between fruit to a particular fungus	6.06	8.68

Table 4. Change in fungal population on coffee bean during the process of drying (solar drying on tiled floor)

Fungus	Wet parchment	On 3rd day of drying	On 7th day of drying	Mean
	Number of c.f.u. (in thousands) per bean			
Yeast	2.37	2.23	1.10	1.90
Cladosporium	3.70	4.03	2.50	3.41
Fusarium	0.07	0.05	0.02	0.05
Aspergillus	0.03	0.02	0.01	0.02
Penicillium	0.03	0.05	0.02	0.03
Mean	1.24	1.28	0.73	

To compare the difference of means

C. D. at	5%	1%
Between days of bean drying	0.09	0.16
Between fungus	0.16	0.22
Between fungus in a particular stage of dry bean	0.28	0.38
Between stage of dry bean to a particular fungus	0.26	0.37

Table 5. Soil mycoflora of coffee plantations

Fungus	Population (in thousands) per gram of soil
Cladosporium	2.0
Fusarium	0.5
Yeast	0.3
Penicillium	0.6
Aspergillus	0.2
C. D. at 5%	0.41
1%	0.60

THE NATURE OF CHLOROGENIC ACIDS. ARE THEY ADVANTAGEOUS COMPOUNDS IN COFFEE ?

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INTRODUCTION

Possibly the first report referring to chlorogenic acids was that published in 1837 by Robiquet and Boutron [1], but the term itself does not seem to have been introduced until 1846 by Payen [2; 3]. In the following one and a half centuries a vast literature has developed. Many aspects have been dealt with in a previous review [4] and while a few basic points will be repeated briefly, this paper will focus on papers published since 1985.

Chlorogenic acids (CGA) are a family of esters formed between certain cinnamic acids and quinic acid. Green coffee beans contain the following:

- three mono-esters of caffeic acid = caffeoylquinic acids (CQA);
- three mono-esters of *p*-coumaric acid = *p*-coumaroylquinic acids (*p*CoQA);
- three mono-esters of ferulic acid = feruloylquinic acids (FQA);
- three di-esters of caffeic acid = dicaffeoylquinic acids (diCQA); and
- six mixed di-esters of caffeic and ferulic acid = caffeoylferuloylquinic acids (CFQA).

A specimen structure is given in Fig. 1 which also illustrates the **preferred IUPAC numbering system** [5]. This numbering system will be used throughout this paper. **When citing other authors their numbering has, if necessary, been changed for consistency.** The mono-esters in coffee are at positions 3, 4 and 5 with that at position 5 dominating: the di-esters in coffee consist of all possible permutations involving those three positions with either the 3,5-di-esters dominating (typical arabicas) or the isomers occurring in similar proportions (typical robustas).

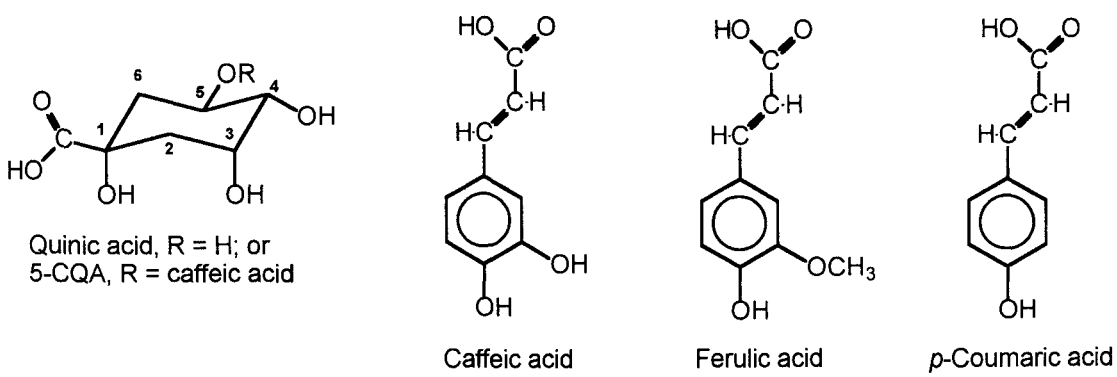


Figure 1. Structures and IUPAC numbering system for Chlorogenic Acid.

CGA are widespread in nature. Simple esters with gallic or sinapic acid also occur. In addition, the three tri-CQA and the single tetra-CQA are known, as are mixed esters involving various permutations of between one and three residues of caffeic acid with one or two residues of a dibasic aliphatic acid (e.g. glutaric, succinic, oxalic) [e.g. references 6–13]. With reference to dietary components, the CQA and diCQA are the most widespread occurring commonly in many fruits and vegetables, and some other beverages. However, of the commodities commonly consumed, probably only maté and globe artichoke have CGA contents approaching those found in coffee. In contrast to coffee, maté has significantly higher 3-CQA and diCQA contents [14], and globe artichoke contains a significant amount of the relatively rare 1,5-diCQA which yields also 1,3-diCQA (cynarin) and 1-CQA after processing [15–17]. Despite some reports to the contrary, 1,3- and 1,5-diCQA are not found in coffee [18]. Coffee beans are the richest common source of FQA [19; 20] and the only known source of CFQA. This latter group is absent from arabicas [21–24]. CGA are only minor components of the common coffee substitutes [25–28].

It has long been known that 5-CQA can form a crystalline potassium 5-caffeoylquinate–caffeine complex, the structure of which has now been described [29]. In coffee, maté and tea (green and black) CGA are accompanied by caffeine, and theobromine in the case of maté. Whether *in vivo* caffeine complexes occur, either in the plant or the consumer, is still a matter of some debate.

Many other cinnamoyl derivatives are produced by plants, and several have been found in coffee. These include N_{β}-caffeoyl-L-tryptophan (Caf–Try) [30] and N_{β}-*p*-coumaroyl-L-tryptophan (*p*Co–Try) [31; 32], N_{β}-caffeoyl-L-tyrosine (Caf–Tyr) and Angola II (probably related but incompletely characterised) which are restricted to robustas. So far, the last two have been found only in Angolan robustas [33–35]. Robusta coffees may contain others as yet unidentified [36]. Representative quantitative data for commercial green coffee beans are given in Table 1.

Table 1. Typical content of CGA and CGA-like components in commercial green coffee beans (%d.b.)

Component	Arabicas	Robustas
CQA	5·2–6·5	5·5–8·0
p-CoQA	0·03–0·07	0·05–0·06
FQA	0·3–0·5	0·7–1·5
diCQA	0·7–1·0	1·4–2·5
CFQA	n.d.	0·2–0·3
Caf–Try	n.d.	0·45–0·67
p-Co-Try	n.d.	0·14–0·17
Caf–Tyr[a]	n.d.	0·13–0·48
Angola II[a]	n.d.	0·19–0·37

a = found only in Angola robustas. n.d. = not detected.

The CGA content of coffee seeds varies with maturity [33; 35] and species (wild and commercial), and the profiles and contents have been used for chemotaxonomy of *Coffea* and *Psilanthus* [37; 38]. Mature coffee seeds have a large content of CGA. Immediately after germination 5-CQA was found primarily in the cotyledons and the surrounding endosperm and hardly at all in the hypocotyl and root. During the next ten weeks the CGA content of the developing cotyledons declined to one-third of the original and was not recovered in other organs of the seedling. Since phenylalanine ammonia lyase activity was barely detectable it was concluded that the CGA had been transformed to lignin [39].

It has been reported that dry processing robusta cherries resulted in beans with the lowest CGA content. Enzyme-accelerated wet processed beans had the highest content and traditionally wet processed beans were intermediate [40]. Monsooned coffee contains more free caffeic acid [24; 41]. CGA profiles in arabicas vary relatively little with geographic origin: robustas are less consistent [36], those from Angola being unique [33; 35]. In the case of Angolan robustas this variation is more likely to be due to genetic factors rather than the location at which grown and processed. Using CGA contents as one criterion in principal component analysis an attempt has been made to distinguish the origins of green and roasted arabicas and robustas [42]. Chlorogenic acids are found also in leaves of coffee (although there are no modern data) and in pulp where they are accompanied by protocatechuic acid. Although robusta beans invariably have greater CGA contents than arabica beans this seems to be reversed for the pulps [43].

Decaffeination has little effect on the CGA content but along with dewaxing or similar steam treatments causes significant acyl migration, some hydrolysis of diCQA [44; 45] and slight subsequent degradation [46]. During roasting there is a progressive destruction and transformation of CGA with some 8–10% being lost for every 1% loss of dry matter [4] and the CGA content relative to the caffeine content has been used as an index of the severity of roasting [47]. In the early stages of roasting (up to ≈4% pyrolysis loss) 3-CQA, 4-CQA, 3-FQA, 4-FQA and free quinic acid contents tend to increase. Hydrolysis of diCQA has been proposed to explain the increase in 3-CQA and 4-CQA [48]. Similar hydrolysis of CFQA might explain the increase in 3-FQA and 4-FQA in robustas, but this increase is also seen in arabicas [45; 48; 49], clearly indicating another mechanism, most likely the well known and facile acyl migration [50]. This migration involves inversion of the quinic acid chair to the carboxy axial conformation.

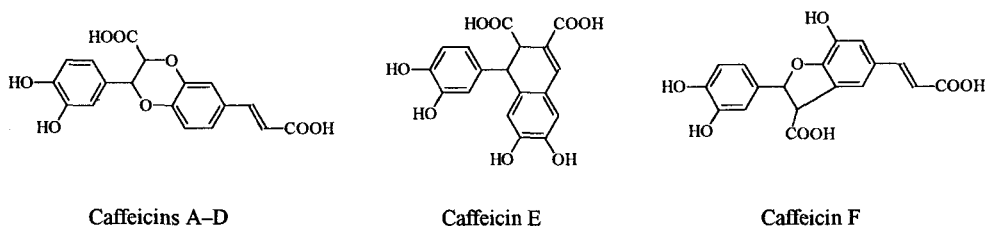
When free quinic acid adopts the carboxy axial conformation, lactonisation readily occurs under the dehydrating conditions associated with the later stages of roasting. Chlorogenic acids in which position 5 is not substituted behave similarly yielding the caffeoyl and feruloyl-1,5-γ-quinides referred to collectively as chlorogenic lactones (CGL) [45; Clifford *et al.*, unpublished]. It is now known that

during roasting free quinic acid epimerises extensively giving rise to all eight possible forms of quinic acid, eight 1,5-γ-quinides and four 1,4-δ-quinides, with several exceeding 0·1% in dark roast coffees [51–53]. It is not known whether roasted coffee contains any epimeric CGA, or CGL other than the 1,5-γ-quinides referred to above.

Green coffee beans always contain a small amount of free L-(−)-quinic acid. The total quinic acid(s) content increases early in roasting, but the content of the associated free cinnamic acids does not. It has been suggested that caffeic, ferulic and a portion of the free quinic acid(s) are converted to novel esters, possibly with polysaccharide. Some of the novel cinnamoyl esters may subsequently decarboxylate. Late in roasting some of the free quinic acid(s) also decarboxylate and dehydrate to simple phenols [48]. For a similar degree of roast (dry matter loss) robustas lose a greater absolute amount of CGA yielding a greater amount of small mass volatile phenols and guaiacols which contribute to a 'smokier' and less attractive aroma.

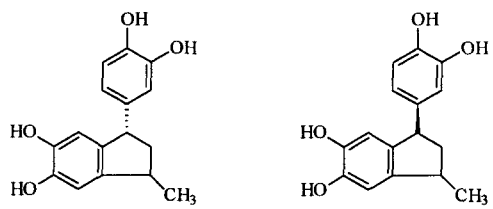
Domestic brewing and commercial instantisation substantially extract the CGA [54] and CGL [45; 55; 56]. Holding the brew hot causes rapid hydrolysis of 4-CQL and particularly 3-CQL and may induce some acyl migration [45]. Commercial instantisation is likely to have a similar effect. Commercial instant coffees vary extensively in their CGA contents [57].

The fate of all the CGA lost during coffee roasting has yet to be elucidated. Model system studies of caffeic acid have identified three types of lignan-like acidic dimers and trimers (caffeicins) as products of autoxidation formed in aqueous solution via the phenolate anion. These include (Fig. 2) four 2,3-dihydro-1,4-benzo-dioxan neolignans (caffeicins A–D), one 2,3-dihydrobenzofuran type neolignan (caffeicin F) and one 1,2-dihydro-naphthalene type compound (caffeicin E) [58; 59]. Oxidation of caffeic acid under acidic conditions or pyrolysis produces two stereoisomers of 2,5-(3',4'-dihydroxy-phenyl) tetrahydrofuran-3,4-dicarboxylic acid [60] and a range of phenylindans probably via decarboxylation and cyclisation of the vinylcatechol intermediate [61]. Two of these rather unstable compounds (Fig. 3) have been found in roasted and instant coffee at 10–15 mg/kg. It has been suggested [61] that such compounds might redox cycle producing superoxide anion radical from molecular oxygen and then hydrogen peroxide through dismutation.



Caffeicins A–D | Caffeicin E | Caffeicin F

Figure 2. Structures of Caffeicins A–F



1,3-*trans* isomer | 1,3-*cis* isomer

Figure 3. The tetra-oxygenated phenylindans

CHLOROGENIC ACID BURDEN

With reference to possible biological effects it is important to consider all dietary sources of CGA, not just coffee, and to consider other cinnamoyl conjugates which might be metabolised similarly. From an extensive search of the literature [Clifford, unpublished] it has been quite easy to identify over 50 fruits and vegetables (and associated products) that contain between 10 and 100 mg/kg (or mg/litre) of CGA or related cinnamoyl conjugates (e.g. caffeoyl-tartaric acid, caffeoyl-malic acid, etc.). Coffee drinkers will derive most of their cinnamoyl conjugates from coffee.

The CGA are efficiently extracted (85–100%) from roasted coffee by commercial instantisation and domestic brewing. Domestic brews commonly extract some 25–30% of the original roast solids depending on method, grind size, solid : water ratio, etc. A typical mid-strength brew would use a 1 : 40–50 coffee : water ratio, i.e. 4–5 g per 200 ml cup. The extreme soluble solids contents of the brews are in the range 1–3% and about 10% of the brew volume will remain with the grounds. Soluble powders will be reconstituted at about the same rate [62; 63].

It has been estimated [64] that a 200 ml cup of roast and ground coffee might supply from 20 mg (weak brew, very dark roast) up to 675 mg CGA (strong brew, very pale roast robusta). More recent studies (Clifford and Balyaya, unpublished) suggest that 70–200 mg per 200 ml cup of arabica and 70–300 (350) mg per 200 ml cup of robusta are more representative. Soluble coffee powder as sold in the UK at that date (2 g per cup) might supply from 70 to 220 mg [4; 57; 65]. Coffee beverage is therefore somewhere in the region of 1 to 3 mM with respect to CGA. Maté beverage might supply between 16 (roast maté) and 133 mg (green maté) per cup [66] but with considerably more diCQA (perhaps ×10) than would be obtained from a cup of coffee. According to IARC [67] the average consumption of coffee world-wide ranges from one to three cups per person per day. It is not possible to make precise predictions of the daily burden of CGA from this limited information: hopefully, the following 'guesstimates' are not unreasonable:

1. people who abstain from coffee and the other rich sources: 10–100 mg CGA per day;
2. people who partake modestly of coffee (up to three cups): 100–1000 mg CGA per day;
3. heavy coffee drinkers: 500–2000 mg CGA per day.

ABSORPTION AND METABOLISM

There do not seem to have been any recent studies of the absorption and metabolism of CGA in animals or man. The early studies have been reviewed [68–70]. It has been established that 5-CQA has low oral toxicity, and rapid metabolism (hydrolysis and methylation) yielding quinic acid, ferulic acid and isoferulic acid. Contrary to previous reports hydrolysis does not seem to occur rapidly in the human stomach since appreciable amounts have been detected in stomach contents *post mortem* (Clifford, unpublished). Although it has been shown that various diCQA enter cultured T-lymphoblasts [12; 71], and that 5-CQA enters cultured human hepatoma cells [72], there are no reports of intact CGA in plasma or urine, suggesting hydrolysis by cytosolic esterases in the gut mucosa or by the gut flora prior to absorption of the released quinic and cinnamic acid(s).

Caffeic acid has been detected in the plasma of rats, rabbits and volunteers after p.o. doses of 40 or 120 mg/kg b.w., 10 mg/kg b.w. and ≈14–17 mg/kg b.w. (1 g per adult) respectively. In rats the half-life of absorption was about 4 min and the half-life for plasma clearance just over 3 hours [73]. In rabbits given 10 mg/kg b.w., maximal plasma levels of ≈1 μg/ml were achieved within 30 minutes and declined steadily to about half that value over four hours [74], whereas rats given 100 mg/kg b.w. achieved 85 μg/ml within 30 min [73]. With volunteers about 10% of the 1 g dose was accounted for as vanillic (22%), caffeic (27%), ferulic (13%) and isoferulic acid (38%) over 48 hours, but with maximal excretion within the first four hours [75]. Such methylations occur in the liver and the methylated metabolites are more extensively excreted in bile (20%) than is caffeic acid (5%). There was at least one other metabolite, a non-phenolic glycerol derivative. The failure to account for a greater proportion of the dose is a limitation of this study.

This failure may reflect transformation of caffeic acid by the gut flora to unrecognisable products. Gut flora metabolism can become of disproportionate significance at high doses compared with small doses, especially if the mechanism(s) of absorption becomes saturated. Dihydrocaffeic acid (3'-(3,4-dihydroxyphenyl)-propionic acid), a gut flora-generated metabolite [76], has been clearly associated with coffee drinking by other investigators [77]. Other gut flora metabolites, at least in animals are 3'-(3-hydroxyphenyl)-propionic acid and 3'-(4-hydroxyphenyl)-propionic acid (*m*- and *p*-hydroxydihydrocinnamic acids respectively). Some metabolites may be conjugated to glycine (hippurates) or glucuronic acid, but glutathione conjugates have not been reported.

When ferulic acid (1 g) was given, caffeic and ferulic acids were recovered from urine along with two unidentified products. Isoferulic and dihydroferulic acids were not detected. Vanillic acid and the non-phenolic glycerol derivative were detected in urine of volunteers who had consumed one litre of coffee beverage [75].

Cytosolic glutathione *S*-transferase (GST) is an important and widely distributed detoxifying enzyme system which metabolises caffeic acid to 2-*S*-glutathionyl-caffeic acid (GSCA). *In vitro* studies [78] have demonstrated that caffeic acid inhibits GST, especially isoenzyme 7-7, in the absence of glutathione (GSH). In the presence of GSH caffeic acid reversibly inhibits GST, isoenzyme 4-4 being the most sensitive (IC_{50} 50 μM). GSCA is a more potent reversible inhibitor. Isoenzyme 2-2 is the least affected (IC_{50} >125 μM) and isoenzyme 3-3 the most sensitive (IC_{50} 7·1 μM). Dietary caffeic acid at doses in the range 50–500 mg/kg b.w. suppressed GST activity in rat liver (isoenzyme 4) but had no effect on kidney enzymes. A slight increase in activity was observed for intestinal GST at 50 mg/kg b.w. but higher doses produced no further increase. GSCA does not seem to have been reported in urine, but a similar compound, 5-cysteinyl-3,4-dihydroxyphenylalanine, has been [79].

When mice were given diets containing either 0·2% caffeic acid or 0·2% 5-CQA it was observed that intestinal arylhydrocarbon hydroxylase (AHH) and UDP-glucuronyl-transferase (UDPGT) activities were significantly suppressed whereas the activity of GST was significantly increased [80]. Such effects were more pronounced after pre-treatment with benz(*a*)pyrene. The equivalent enzymes in the liver were not affected. Food consumption was not recorded, but intakes of caffeic acid and 5-CQA were probably of the order of 200 mg/kg b.w. per day. The equivalent human doses are 14 g per day (mouse study) and 3·5 to 35 g per day (rat study) and such levels are unlikely to be achieved by normal diets. Many GST substrates, including some cinnamic acids (caffeic and ferulic acid were not tested), are potent inducers of NAD(P)H : (quinone-acceptor) oxidoreductase, another important detoxifying enzyme found in mammalian liver [81].

In vivo studies have demonstrated that caffeic acid binds to serum albumins extensively [73], and that *in vitro* caffeic acid binds more extensively (60–90%) than ferulic, *m*-coumaric or *p*-coumaric acids (<10%) [82]. Similar studies using human serum albumin have shown that caffeic acid binds more strongly than 5-CQA and that binding is endothermic and entropically driven [83]. Ferulic acid also associates with the plasma phase rather than the lipid phase [84]. For a large series of benzoic acids and phenylacetic acids it has been reported that the more hydrophobic bind more strongly to human serum albumin and as a consequence enter human erythrocytes less readily [85; 86].

In vitro studies of xanthine oxidase activity have established that *m*-coumaric acid and caffeic acid are inhibitory (IC_{50} 63 μM and 75 μM respectively) whereas dihydrocaffeic acid has no effect [87]. This value for caffeic acid is approximately 10-fold greater than the maximum plasma concentration seen in rabbits given 10 mg/kg p.o. and it is unlikely that such levels would be achieved by coffee drinking. This observation is probably of greater significance with reference to the use of xanthine oxidase and xanthine (or hypoxanthine) as a means of generating hydroxyl radical and superoxide anion so as subsequently to assess whether a particular substance can scavenge the radical(s). The results of the model system are difficult to interpret, if not totally invalid, if the substance inhibits radical generation (for further discussion see reference 88). It has been reported [89; 90] that coffee brew has the ability to scavenge such radicals, but it seems likely that the brew used might easily have resulted in the test system containing CGA at levels well above (perhaps ×10) the IC_{50} quoted for caffeic acid. Since the authors do not appear to have checked for this possible interference, the results are better discounted until the situation can be clarified.

Much of the recent interest in chlorogenic acids and related compounds stems from their antioxidant properties. These should be considered from two quite distinct viewpoints. These are:

1. antioxidant potential in the coffee (green or roasted) and derived soluble powder; and
2. antioxidant potential *in vivo*, i.e. in the tissues and organs of the consumer.

The latter possibility has attracted a great deal of attention in the last decade because there is a perceived benefit for the consumer. This is based upon the premise that:

1. 'phenols are antioxidants';
2. the incorporation of such antioxidant molecules in the diet can diminish the effects of oxidative stress associated with various reactive oxygen species (ROS) [91]; and because
3. the effects of ROS have been implicated [92; 93] in the causation of many age-related degenerative diseases including rheumatoid arthritis, cardiovascular/cerebrovascular disease and cancer at different specific sites [94; 95].

Generally, whichever viewpoint is of interest, the antioxidant potential is investigated using model systems. Model systems never fully duplicate real life and great care is needed both in their design and in the interpretation of the results generated. Over-extrapolation must be resisted: effects that may be significant during the storage of coffee do not necessarily have the same relevance for the consumer and his wellbeing. The review by Halliwell *et al.* [88] critically examining the capabilities of different *in vitro* assay systems is essential reading. One must in addition consider whether the test substance has an intrinsic radical scavenging/quenching reaction rate sufficiently high to compete with the normal endogenous substrates and whether it reaches the tissue at a sufficient concentration to be biologically significant. In many cases it would be more relevant to study the metabolites rather than the unmetabolised compound, but this is rarely done even where the metabolites have been identified. Finally, one must consider whether there might also be injurious effects associated with such dietary phenols at the doses of interest.

CONSUMER-ORIENTED STUDIES—STUDIES USING PURIFIED COMPOUNDS

Lipid oxidation

Many studies have investigated the ability of test phenols to protect cholesterol, a fatty acid or human low-density lipoprotein (LDL) from oxidation when challenged oxidatively. When a free transition metal (e.g. Cu^{2+}/H_2O_2 or Fe^{3+}/H_2O_2) challenge is used the 1,2-dihydroxyphenyl compounds (e.g. caffeic acid) are more effective inhibitors than when a metal-free radical generator is used (e.g. 2,2'-azobis-(2-aminopropane)-hydrochloride (AAPH)) simply because the test substance is able to chelate and inactivate the metal ion.

There is some controversy about whether free metal ion-driven challenges are relevant clinically. It has been argued that metal ions are so strongly bound *in vivo* that they are not able to redox cycle (take part in radical-generating Fenton reactions), and that in any case the oxygen tension existing *in vivo* is much lower than required for efficient promotion of Fenton chemistry [88; 96]. This seems to be true with regard to iron except in particular and somewhat atypical circumstances, e.g. when iron overload has occurred [97], in bone marrow transplant patients [32], or in other pathological conditions, when such phenolic compounds might protect against the effects of excess **unbound** iron. The situation might be different with copper. Some investigators suggest that caeruloplasmin, the major copper-binding protein in blood, contains in its native form one atom of redox-active bound copper [98] and that this can damage LDL unless protective phenols are present [99]. Alternatively, it might be that *in vivo* the caeruloplasmin is acting in a sacrificial manner as has been described for blood plasma albumin and hydroxyl radical ($OH^{\bullet}$)[88]. A review of this topic [100] states 'Free, catalytically-active copper has been found in human atherosclerotic plaque tissue, and has been shown to be capable of oxidising human LDL' but concludes that the role of copper *in vivo* is still unproven and may be biphasic, i.e. both deficiency and excess (through different mechanisms) contributing to development of atherosclerotic lesions. If this assessment ultimately is proven to be correct, then dietary phenols and their metabolites may be advantageous if they are able to inactivate the excess but of no benefit when copper is just adequate or deficient.

Inhibition of metal ion-driven oxidation has been observed with 0·5–1·5 μM (0·09–0·27 μg/ml) caffeic acid [96; 99; 101–103]. This is considerably below the maximal plasma concentration (≈1 μg/ml) observed in rabbits given 10 mg/kg caffeic acid p.o. [74]. **A simple extrapolation** would suggest that a similar effect in humans would require an intake of at least 70 mg caffeic acid (or at least 140 mg CQA) in a relatively short period. CGA consumption appreciably above this minimum could be achieved by many coffee drinkers and might produce such plasma concentrations. However, this requires confirmation, and even if that is the case, the clinical significance is open to debate so far as a benefit to the general public is concerned. 5-CQA is as effective as caffeic acid but ferulic acid is significantly less effective (IC_{50} 1·5 μM vs 2·5 μM) [102; 103] clearly indicating that the 1,2-dihydroxyphenyl structure is required for chelation.

The same study showed that caffeic acid was only about one tenth as effective at inhibiting an oxidative challenge derived from AAPH or macrophages [103]. While such challenges might be more biologically relevant, there is much less likelihood of achieving such concentrations *in vivo* and even if that were the case, it is unlikely that caffeic acid would react sufficiently rapidly to scavenge $OH^{\bullet}$ directly. However, in a different study using the same AAPH-driven model system, caffeic acid and 5-CQA (0·5–1 μM) have been observed to delay lipid peroxidation in human LDL *in vitro* and to protect sacrificially the destruction of β-carotene and α-tocopherol (vitamin E), which are its natural antioxidants [99; 101; 103]. To function efficiently α-tocopherol must 'export' its unpaired electron (radical) from the LDL particle into the aqueous phase and *in vivo,* this requires ubiquinone and acorbate. In their absence, α-tocopherol may be prooxidant. The protection afforded by 5-CQA was attributed to peroxyl radical scavenging and the production of phenoxyl radicals with additional stability because of the conjugated side chain [101] but it may only have been replacing ubiquinone and/or ascorbate and may be less effective *in vivo.*

Caffeic acid and 5-CQA (10 μM) have been shown to protect retinoic acid (vitamin A) from oxidation by haematin or oxyhaemoglobin. Vanillic acid was less potent [104]. In a similar metmyoglobin-driven model system caffeic acid, 5-CQA and ferulic acid (IC_{50} 0·3–0·8 μM) were effective in retarding lipid peroxidation and damage to apoprotein B_{100} whereas *p*-coumaric acid was much less so [102]. Cholesterol oxidation was also retarded although less efficiently (IC_{50} 1·5–2·5 μM). Peroxyl radical scavenging was considered to be a more important mechanism than reduction of ferryl myoglobin [102]. The results of another study [105] suggest that the mechanism of inhibition varies with the structure of the phenol and that 1,2-dihydroxyphenols do inhibit the reduction of ferryl myoglobin. Studies with radio-labelled ferulic acid have established that it does not associate significantly with the lipid phase of LDL but is associated primarily with the albumin phase of plasma where it appears to be a more potent antioxidant than ascorbic acid [84].

These might be observations of some biological significance. Caffeic and ferulic acids are known mammalian metabolites of 5-CQA and presumably also of the other major CGA, together accounting for some 4% of a 1 g oral dose of caffeic acid. Both are of similar antioxidant potency *in vitro* (IC_{50} ≈1 μM) and thus the intake of CGA required to reach a significant concentration of metabolites *in vivo* is correspondingly lower than if only one were effective. Finally, by extrapolating from animal studies, it seems as though such plasma concentrations might be achieved by regular coffee drinkers.

However, this is a facile extrapolation to the *in vivo* human situation and should not be overstated. For example, even if such effects did occur *in vivo* they might only be relevant when subjects were deficient in β-carotene (α-tocopherol deficiency is rare in humans). In addition, the fate of the transformed caffeic acid is not known and should also be considered before reaching a judgement.

Scavenging of nitric oxide

There has been much concern expressed about the undesirable effects of dietary *N*-nitrosamines which are animal carcinogens. Such nitrosamines might also be generated endogenously, either intragastrically (acid-catalysed nitrosation in the stomach) or extragastrically via nitric oxide ($NO^{\bullet}$) generated by neutrophils and macrophages. Model system studies have shown that both caffeic acid and 5-CQA will inhibit the *N*-nitrosation of 2,3-diaminonaphthalene by scavenging nitrogen sesquioxide [106]. In view of the relatively high (mM) concentrations of 5-CQA used in these studies the *in vivo* biological significance is not clear. It should also be noted that nitric oxide has important *in vivo* roles and that it may not necessarily be desirable that it is immediately scavenged.

Prostaglandin and leukotriene synthesis

Prostaglandins (including thromboxanes and prostacyclins) and leukotrienes are synthesised in and discharged from the many mammalian tissues. Along with their metabolites, they collectively influence numerous physiological functions, including smooth muscle contraction, blood pressure, inflammation and lipid metabolism. All are derived *in vivo* from PUFA released from cellular phospholipids by Phospholiapse A, and modulation of their synthesis and/or potency by CGA could therefore be of interest. Studies using cultured cytomastoma cells have shown that caffeic acid inhibits 5-lipoxygenase (IC_{50} 3·7 μM) but stimulates prostaglandin synthase, probably through cyclooxygenase, in a dose-dependent manner at concentrations above 50 μM. The net effect even at low μM concentrations would be a diversion of PUFA away from leukotrienes towards prostaglandins [107]. Stimulation of cylooxygenase activity has been confirmed *in vitro* at mM concentrations which could only be achieved *in vivo* if tissue concentration were to occur [108]. The biological consequences of such effects are at present impossible to predict since individual tissues differ in the compounds synthesised and their responses to them.

Protein oxidation

As referred to above, caffeic acid, 5-CQA and ferulic acid (IC_{50} 0·3–0·8 μM) were effective in preventing apoprotein B_{100} fragmentation in a metmyoglobin-driven model system, but *p*-coumaric acid was much less so [102]. Caffeic acid, 5-CQA, 1,3-diCQA and dicaffeoyltartaric acid protected collagen *in vitro* from oxidative degradation driven by superoxide anion and hydroxyl radical generated

in a xanthine/xanthine oxidase/Fe^{2+}/EDTA model system [109]. The IC_{50} values ranged from 15 to 90 μM which are in marked contrast to those for protection of apoprotein B_{100}. However, since coffee beverage may be 3 mM with respect to CGA it is conceivable that effective concentrations might be achieved at least transiently in the stomach and possibly lower down the GI tract following a bolus dose and therefore such activity might be of some biological significance.

Glucose homeostasis

Hexoses such as glucose and galactose, and some pentoses (e.g. xylose) enter the cell via an Na^+-mediated flux down a concentration gradient. Although intracellular Na^+ concentrations are maintained by an Na^+/K^+-ATPase system which ejects Na^+ from the cell, the resulting low [Na^+] in the cell encourages the entry of more Na^+ from the outside, e.g. the gut lumen. Na^+ entry occurs in connection with certain sugars and a carrier protein, SGLT1 [110].

It has been shown *in vitro* using the rat intestinal brush border membrane that caffeic acid and 5-CQA (IC_{50} ≈0·6 mM) dissipate the Na^+ electrochemical gradient which provides the driving force for active absorption of glucose [44]. It has been suggested [111] that there is an Na^+-dependant carrier-mediated saturable transport mechanism in the rat for the uptake of cinnamic acid and ferulic acid across the jejunal brush border membrane. Caffeic acid was not investigated in this study [111] but its relatively rapid excretion [75] is consistent with absorption from the small intestine.

A range of other phenolic compounds apparently interact with the same carrier protein. The ability of phloridzin to inhibit active transport is well known and consumption may result in glucosuria. It is thought that quercetin-4'-glucoside, quercetin-3-glucoside, quercetin-3-galactoside and quercetin-3-xyloside may be transferred from the small intestine to the hepatic portal vein by this carrier, whereas quercetin-3-rutinoside (rutin) with a distal rhamnose residue is not [112–114]. Preliminary evidence has been presented to suggest that quercetin-3,4'-diglucoside also has a high affinity for the SGLT1 carrier [115] although whether it is transported as the glycoside or after hydrolysis is not clear.

It has been reported that 5-CQA can inhibit liver glucose-6-phosphatase and thus gluconeogenesis and glycogenolysis in rat liver microsomes but not in perfused whole liver. Caffeic acid was not investigated [116].

The physiological significance of these findings with reference both to the active transport of sugars and to the absorption of cinnamic acids and other dietary phenols has yet to be established in man. Whether there are effects also on the passive glucose transport mechanism is unknown.

Opiate receptor binding

It has been reported that the *cis* and *trans* isomers of 1-feruloyl-1,5-γ-quinide (1-FQL), 3-FQL and 4-FQL are opiate antagonists [117–119]. Other studies (Clifford, Kitchen and Scholz, unpublished) observed both agonist and antagonist behaviour from fractions known to contain caffeoylquinides or feruloylquinides. (+)-Catechin-3-gallate has been reported to bind *in vitro* to an opiate receptor (IC_{50} 36 nM). The practical significance of these observations for man is unclear. There are no data to indicate whether or not CGL are absorbed unchanged, but access to GI tract receptors remains a possibility.

CONSUMER-ORIENTED STUDIES—STUDIES USING DIETARY MODIFICATION

There are a number of studies where the diet has been modified by introducing beverages such as wine or coffee. While these protocols may generate data that better reflect what might happen to the general population pursuing their favoured diets, it is very difficult to identify the mechanisms and substances responsible for any effect observed.

Lipid oxidation

It has been shown that the modest consumption of red wine (but not white wine) protects human LDL from oxidation when subsequently tested *in vitro* [120]. Although this observation tends to reinforce the results of the model system studies reported in the previous section, it is not possible to attribute this effect to the presence in red wine of any particular substance(s). However, it is unlikely to be due to caffeic acid or its conjugates since these occur at very low concentrations in matured red wine.

Anticarcinogenicity and antigenotoxicity

Hamsters fed caffeic acid or 5-CQA were shown to be less susceptible to the effects of methylazoxymethanol (MAM), a potent initiator of colon carcinogenesis. The doses were not stated explicitly, but were probably of the order of 25 mg 5-CQA per kg b.w. per day [72] which would correspond to some 1·75 g per day for an adult. This observation is of interest since there is some epidemiological evidence that coffee drinkers might be at less risk of developing colon cancer [67]. The same study also demonstrated protection against 4-nitroquinoline-1-oxide-induced tongue neoplasms. This observation is of interest since the CGA-rich beverage maté has been associated with an increased incidence of oesophageal cancer and CGA (or tannins) have been blamed by some investigators [121; 122]. It should be noted, however, that IARC [67] rejected Morton's hypothesis and attributed the increased incidence of oesophageal cancer unequivocally to repeated scalding of the tissue with boiling water associated with the traditional method of preparation and consumption of maté.

It should be noted that in a two year (life-time) rat study caffeic acid given as 2% of the diet has been shown to cause forestomach cancer [123]. The authors conclude that the caffeic acid probably operates through the same non-genotoxic mechanism as BHA, e.g. depletion of endogenous glutathione from direct binding of quinone metabolites, direct attack on tissues by reactive metabolites, and

possibly also from oxygen radicals generated by redox cycling [124; 125]. When carcinogenesis is initiated by repeated local irritation, long term exposure to doses large enough to overcome efficient detoxification and repair mechanisms is a prerequisite. This dose of caffeic acid would correspond to some 140 g per day for an adult, or an even greater level of CGA, and is in no way representative of normal dietary consumption. While the validity of these studies using very large doses are not in question *per se*, they do not provide a basis for extrapolating to the effect of much lower doses in man, especially when a no-effect threshold can be demonstrated.

Abraham [126], using the mouse bone marrow micronucleus test as the endpoint, has reported that certain combinations of soluble coffee solids and dietary phenols and/or antioxidant vitamins can protect mice from the deleterious effects of certain genotoxins. This procedure has been shown in a ring test [127] to be an accurate and reproducible means of qualitative discrimination between carcinogenic and non-carcinogenic chemicals. However, certain liver carcinogens do not produce detectable chromosomal effects in the bone marrow, and a negative result in the micronucleus test requires supplementary testing for liver carcinogenicity. This test has also been used to monitor the risk to humans of workplace exposure to potentially-hazardous substances.

The results of this study [126] are interesting, but the experimental design makes useful interpretation impossible. A thorough critique of the shortcomings of this study is not possible here due to constraints on paper length, but selected points are discussed to highlight the difficulties associated when uncontrolled changes are made simultaneously to more than one variable.

Coffee solids were prepared by freeze drying a domestic preparation of commercial roasted coffee. No information was given about the origin (arabica, robusta, degree of roast) or about its composition, e.g. CGA content, all of which are pertinent. The dose adopted (100 mg/kg b.w.) corresponds to some 2–3 200 ml cups containing 2% w/v solids and is thus not unreasonable for an adult. Coffee soluble solids (100 mg/kg b.w.) gave no protection against *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) (40 mg/kg b.w.) but, coffee in combination with two of the test mixtures were more effective than the test mixtures alone.

Test mixture D contained 5-CQA (20 mg/kg), caffeic acid (20 mg/kg), ellagic acid (15 mg/kg) and ferulic acid (15 mg/kg) and was given orally in water (10 ml/kg) one hour before the genotoxin was given i.p. Test mixture E contained β-carotene (5 mg/kg), curcumin (5 mg/kg), α-tocopherol (10 mg/kg) and was given orally in corn oil (5 ml/kg) followed by water (5 ml/kg) one hour before the genotoxin given i.p. Ellagic acid taken *per os* is very poorly absorbed [128]. It would appear therefore that the effect of mixture D might reside primarily in 5-CQA and/or its metabolites (caffeic and ferulic acids) and that the same effect might have been achieved by a greater consumption of coffee. The results for test mixture E might seem generally consistent with the results from *in vitro* studies which suggest that CGA metabolites might act *in vivo* by sparing endogenous antioxidant vitamins.

Test mixture F gave some protection against MNNG (40 mg/kg) but this was not increased when given with the coffee solids. Test mixture F contained curcumin (2·5 mg/kg), 5-CQA (20 mg/kg), eugenol (5 mg/kg), anethole (2·5 mg/kg) and α-tocopherol (5 mg/kg) given orally in corn oil (5 ml/kg) followed by water (5 ml/kg) before the genotoxin given i.p. By comparison with the results obtained with test mixture D it would seem as though the effects of D must in some way be associated with the caffeic and ferulic acids (since 5-CQA is common to both and ellagic acid has been discounted due to low availability). By comparison with the results for test mixture E it would seem as though the effects of D must in some way be associated with the β-carotene or the greater doses of curcumin and α-tocopherol.

In summary, it would seem as though the widespread practice of consuming moderate amounts of 5-CQA, caffeic and ferulic acids in association with antioxidant vitamins might offer some, but not complete, protection against MNNG and possibly other genotoxins. One is left with a tantalising suspicion that toxicological and nutritional insights of real value are almost within one's grasp but, due to the imperfect control of variables, they are destined to remain out of reach.

Hypocholesterolaemia

There is a long standing belief that extracts prepared from artichoke and rich in 1,3-diCQA (cynarin) exert in man a mild hypocholesterolaemic effect, generally at doses of 500–1500 mg per day. The literature in support of this belief is extensive and difficult to discount: it has been reviewed [68] and will not be repeated here. The mechanism has never been established. If all CGA are hydrolysed to their constituent cinnamic acid(s) and quinic acid then one might expect coffee CGA, and coffee beverage, also to be hypocholesterolaemic at equivalent doses. That this is not obviously the case is intriguing. Possibly in the case of coffee beverage the effect has been masked by the hypercholesterolaemic effect of coffee diterpenes [129–131].

Absorption of metal ions

Studies in animals and man indicate that 5-CQA and caffeic acid can impair the uptake of zinc and that coffee can impair the uptake of non-haem iron. Although in this latter case the active principle has not been identified, it may well be CGA or associated substances [132–134]. Classically, one would expect 1,2-dihydroxyphenols to be the most likely candidates, but some Maillard reaction products [135] and acidic polysaccharides also may be involved.

Antiviral properties

Reports that certain diCQA (2–8 μM) interfere with the metabolism of HIV-1 in cultured cells [12; 71] have attracted a great deal of attention and prompted claims that coffee drinking may offer the consumer protection from AIDS. Caffeic acid and 5-CQA were inactive. Since there is no evidence that dietary diCQA are absorbed intact *in vivo*, **such inferences are unsound and potentially dangerous.**

COFFEE PRODUCT-ORIENTED STUDIES

During roasting coffee beans are heated in a stream of hot air and by contact with hot surfaces and the bean surface may reach temperatures up to about 250 °C. The temperature achieved at the centre of the bean is uncertain. With short roasting times and limiting gas film heat transfer the centre temperature will lag behind the surface but this lag may to some extent be offset by the exothermic nature of the roasting process. The exothermic reactions commence at about 160 °C, peak at about 210 °C and decline to about 250 °C. Internal pressures of some 5–7 atmospheres, largely due to the production and partial entrapment of carbon dioxide, cause significant bean expansion and provide an essentially oxygen-free atmosphere both within the bean and around the bean during roasting. The roasting process melts the lipids and this internal pressure expels them producing a characteristically shiny appearance in dark roasts [136].

This rather unsaturated lipid, in which oleic and linoleic acids respectively account for some 10% and 40% of total triacylglycerols [137], would normally be quite susceptible to oxidation. However, the associated carbon dioxide and natural antioxidants limit true rancidity in whole roasted coffee to the extent that it is not a serious commercial problem with vacuum packing. Roast and ground coffee is less stable, and an uptake of some 150 ml of oxygen per kg of roasted coffee leads to distinct staling. In pack oxygen contents of less than 0·5% by volume are required for a shelf-life of one year [138].

It seems reasonable to assume that surviving carotenoids and tocopherols, and possibly CGA, will provide some protection from oxidative deterioration whether true rancidity or loss of desirable volatiles. For example, caffeic acid and 5-CQA were on a molar basis as effective as α-tocopherol in preventing peroxidation of emulsified methyl linoleate [9] and various diCQA have been shown to protect β-carotene in emulsified linoleic acid with about one tenth the efficiency of butylated hydroxyanisole (BHA) [11]. At 20 μM, 3,5-diCQA is almost as efficient as α-tocopherol in preventing conjugated diene formation in emulsified linoleic acid. Ferulic acid was appreciably less potent: 5-CQA and caffeic acid were intermediate [139]. It has been reported that caffeic acid is oxidised through a semiquinone radical to an *o*-quinone whereas ferulic acid and *p*-coumaric acid form radicals which decay through radical–radical coupling [140]. However, if the CGA were indeed important, one might expect lightly roasted robustas to stale more slowly than dark roast arabicas. It is not obvious from the literature whether this is indeed the case.

Very little of this lipid is extracted into instant coffee and thus soluble powders are not susceptible to rancidity despite having lost the protective inert atmosphere provided by entrapped carbon dioxide. Their odour and flavour do change rapidly however if headspace oxygen exceeds 4% by volume or moisture content rises above 4% by mass. The practice of coating some instant coffee granules with coffee oil bearing odour impact compounds may exacerbate this susceptibility. Again, there is no evidence of a link between CGA content and stability.

Low concentrations of indoles in general, and 5-hydroxytryptophan in particular, have been reported to protect linoleic acid from peroxidation at 38 °C [141]. It might be therefore that the N_{β}-(acyl)-5-hydroxytryptamines associated with the coffee wax, and therefore on the bean surface at the site of greatest risk, are also important provided that they have not been removed by dewaxing procedures. Several transformation products, including kynurenines, were observed for tryptophan.

It is possible that the ether-soluble tetra-oxygenated phenylindans produced by caffeic acid pyrolysis are important since in model systems they have been shown at low concentrations to be as potent as vitamin E in preventing peroxidation of emulsified ethyl linoleate. At higher concentrations they were pro-oxidative through Fenton-type redox cycling reactions which generate hydrogen peroxide and hydroxyl radical through subsequent homolytic fission [142]. While this was a peculiarity of the Fe^{3+}/H_2O_2-initiated radical generation used in this model system such a mechanism could operate on the bean surface if appropriate transition metal ions were present.

The sugars and amino acids of green coffee beans undergo profound transformations during coffee roasting. In certain foods, such as roasted meat and fish, a range of heterocyclic amines have been reported, some of which are potent mutagens *in vitro* and carcinogens at least in rodents. Several of these heterocyclic amines have creatinine as a key precursor and the possibility that arginine might substitute has led to speculation that similar compounds could occur in roasted coffee. That this is not the case may reflect the ability of certain phenols in coffee to scavenge key carbon-centred radical intermediates, as has been shown for pyrolysis model systems [143].

Extracts of roasted coffee may be used commercially to produce a coffee flavour in other products. Careful control of the extraction procedure, for example choice of solvent, might lead to an extract with useful antioxidant properties, either accompanied by or free from coffee flavour and odour to suit the application. The application of such an extract to seafoods has been described [144].

CONCLUDING REMARKS

The author was asked to consider whether chlorogenic acids are advantageous components of coffee. This has been addressed from the biological (consumer-oriented) and technological (product-oriented) points of view.

Coffee is a unique beverage which owes its character to the unique physical and chemical properties of the coffee bean. One of the unique compositional features of the coffee bean is its high content of CGA. Without CGA, roasted coffee would surely be different, perhaps more like roasted nuts or cereals which are poor substitutes, and on that basis we can conclude in a philosophical vein that the coffee producers, processors and consumers owe a great deal to CGA. More concrete advantages are more difficult to prove, but it is possible that the CGA minimise the formation of potentially mutagenic pyrolysis products, and that CGA or their transformation

products minimise oxidative deterioration in the roasted coffee. Whether this ability defines a market for coffee extracts as technological or functional additives remains to be seen.

The author has found no evidence in the literature that suggests any undesirable biological effects associated with CGA consumption at realistic doses. At our present state of knowledge there is, however, insufficient evidence to suggest an unequivocal advantage associated with dietary CGA. Nevertheless, there are interesting pointers to the possibility that CGA metabolites, at concentrations that would be associated with modest coffee consumption, might offer a modicum of protection against oxidative challenges otherwise associated with vascular disorders such as CHD and atherosclerosis. Of course the opposing effects of coffee diterpenes must not be overlooked, but fortunately it is not difficult for people to drink CGA-rich beverage which is free, or virtually free, from the diterpenes. Similarly, there might be a modicum of protection against colon cancer. More work is required to clarify the position.

Therefore, the answer must be, 'Yes, chlorogenic acids are useful and might ultimately prove to be very useful components of coffee'.

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SUMMARY

This paper reviews the literature on chlorogenic acids and related compounds, in particular that published since the author's previous review in 1985. Aspects discussed are their occurrence and behavior during coffee roasting., their absorption and metabolism, possible effects on the consumer, and on the stability of coffee products.

RÉSUMÉ

Cet article passe en revue les ouvrages consacrés aux acides chlorogéniques, en particulier ceux qui ont été publiés depuis la revue précédente de l'auteur en 1985. Les aspects discutés sont leur présence et leur comportement au cours de la torréfaction du café, leur absorption et leur métabolisme, leurs effects possibles sur les consommateurs, et sur la stabilité des produits dérivés du café.

SOME PRELIMINARY INVESTIGATIONS OF OIL BIOSYNTHESIS IN THE COFFEE FRUIT AND ITS SUBSEQUENT RE-DISTRIBUTION WITHIN GREEN AND ROASTED BEANS

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1. Introduction

Plant seeds store lipids within intracellular oil bodies (syn. oleosomes or spherosomes) as food reserves. These are used during active periods of metabolism which in the case of the seed is the onset of germination. Oil bodies are an extremely prominent organelle in the storage parenchyma and other tissues of the coffee seed.

A variety of lipidic constituents are to be found in the oleosomes of coffee fruits (which are drupes) and dried roasted beans (*Folstar et al* 1975) and these include triacylglycerides (TAGs), free fatty acids and 16-O-methylcafestrol esters. The two most important coffee species, *Coffea arabica* and *Coffea canephora* var. *robusta* contain between 7 and 17% lipid. The mean lipid content of *C. arabica* coffee beans is 15% whilst that of *robusta* is less (10%).

The investigations reported here are some initial ultrastructural studies concerned with the biosynthesis and ultimate fate of oil in coffee. Our studies have attempted to gain a greater understanding of the processes of biosynthesis, emulsification, stabilisation, de-stabilisation, migration of oil bodies (oleosomes) and eventually re-distribution of free oil within fresh coffee fruits and processed beans, up to the final roasting stages.

Conventional preparation techniques for electron microscopy have limitations for this difficult type of specimen in terms of promoting dissolution and eventual loss of oil and difficulty in infiltration of the thick cell walls with embedding resins. Thus, recourse has been made to the efficacy of low temperature methods and special buffer formulations, in order to retain the oil,and to low viscosity resins to improve infiltration and embedding.

If consideration is paid to specimen preparation, for light and electron microscopy these structures persist and are visible as lightly electron opaque spheres.

2. Methods

2.1 Sampling

The coffee fruits of *Coffea arabica* at various ages used in this study were all sampled from the same plant which was grown in Cerrado, Brazil (21°latitude South). After harvesting, the fruits were processed according to traditional local methods. The youngest green cherries were sampled on 25-6-96 and the oldest (red cherries) 54 days later. After sampling, fruits were split into two halves and immediately immersed in primary fixative solution for transportation to the UK..

2.2 Fixation and Preparation of Coffee Berries

Fixation of fresh coffee fruits is difficult because of the very thick cellulose walls which surround storage parenchyma cells and also because of the high lipid content of the cells. Accordingly, prolonged fixation times were used and a buffer system (imidazole) was employed which has been previously demonstrated to improve fixation/retention of lipids (*Angermuller & Fahimi* 1982).

Tissue was fixed, stored and transported in half-strength Karnovsky's fixative (Karnovsky 1965). After excision of 1 mm cubes of parenchyma and other tissues, specimens were washed in buffer, then post-fixed in 1% osmium tetroxide/imidazole buffer for 1 h. (*Angermuller & Fahimi* 1982).

2.3 Initial Preparation of the Coffee Beans

Green and roasted beans were immersed in a porcelain mortar containing a small volume (30 ml) of liquid nitrogen (-196°C). After cooling, the beans were just gently cracked open (not crushed or ground) using a pestle. Cracked material was stored under liquid nitrogen until required.

2.4 De-oiling of the Dried Green and Roasted Bean Fragments

Fragments of bean excised from the storage parenchyma region of the perisperm were transferred to at least ten times their volume of chloroform for two weeks with at least five changes. After this de-oiling treatment, the fragments were transferred to 2 changes of 100% dry acetone prior to critical-point drying (see 2.4.1 below).

2.5 Scanning Electron Microscopy (SEM)

2.5.1 Critical-point Drying

De-oiled fragments were critical-point dried from liquid CO_2 according to the methods described in *Cohen* (1973) and *Robards & Wilson* (1993). Fragments in acetone were transferred to the pressure chamber of a *Polaron* E-3000 critical-point apparatus and dried from liquid carbon dioxide under pressure at 1300 psi and 32°C. Specimens were stored in a desiccator prior to mounting and observation in the SEM.

2.5.2 Conventional SEM

Small pieces of de-oiled, dried coffee bean were mounted on SEM stubs using epoxy cement. After polymerisation they were coated in a *Polaron* sputter-coater using gold. Coated specimens were subsequently observed in a *Hitachi* S-2400 SEM using an accelerating voltage of 8kV. Micrographs were prepared on 35 mm film.

2.5.3 Cryo-SEM

Low temperature SEM of roasted coffee bean was performed using a *VG Microtech* E-4500 cryo-preparation unit. Small pieces of liquid nitrogen fractured bean were mounted on the cryo-stub using *Tissue-Tec*.

2.6 Transmission Electron Microscopy (TEM)

2.6.1 Resin Embedding

After fixation, specimens were washed in distilled water, dehydrated in acetone series and embedded in a low toxicity, modification of *Spurr's* (*Spurr* 1969) low viscosity resin (see *Robards & Wilson* 1993).

Specimens were sectioned using a Reichert OMU-2 ultramicrotome and a diamond knife. Sections were stained using lead citrate and uranyl acetate (*Reynolds* 1963, *Watson* 1958, *Robards & Wilson* 1993) and observed using a JEOL 1200EX transmission electron microscope.

2.6.2 Freeze-Fracture

Freeze-fracture of untreated (ie no de-oiling) green and roast coffee bean was performed using a *Leybold Heraeus Bioetch* 2005. Specimens were fractured at -140°C then unidirectionally shadowed with platinum and carbon at an angle of 45°. Replicas were cleaned in 30% chromic acid for 1 h. prior to washing in distilled water (2 changes).

3. Results and Discussion

In the majority of seeds the nucellus of the ovule remains incipient and thus never develops into a storage tissue. However, in some taxa, eg. *Coffea* and *Yucca*, the structure develops and part of it is retained within the seed and accumulates storage materials as a perisperm (from the Greek 'about the seed').

The storage parenchyma cells of the perisperm of very young green coffee fruits (at the youngest stage of ripening) bear a mass of oil droplets (approx 0.5 - 1 μm in diameter) which form as a thin cytoplasmic layer of four or five droplets thick, close to the cell wall (**Mic 1**). These oil droplets or spherosomes are lightly osmiophilic (**Mic 1 large arrows**), but dispersed in an aqueous proteinaceous matrix which has a high electron scattering capacity due to binding of heavy metal stains (**Mic 1 small arrows**). The spherosome/protein layer thus constitutes a liquid oil-in-water emulsion.

Proteins, which are well-documented stabilisers of emulsion systems (*Dickinson* 1989) impart a high degree of stability to the lipid droplets, with little evidence of coalescence even in the 14 day post-harvest fruits examined. It is considered (*Yatsu & Jacks* (1972) and *Tzen et al* 1993) that the oleosome droplet is surrounded by a monolayer of phospholipid onto which are embedded the oleosins (the protein bodies which stabilise the lipid droplets and are distributed throughout the emulsion). *Tzen* believes the acidic lipids are assumed to interact with basic amino acids of the oleosins on the surface of the phospholipid layer. Because of the high density of staining of the proteins surrounding the lipids a discrete phospholipid layer was never resolved sandwiched between protein and lipid. Proteins are present in two distinct morphological forms within the cytoplasm: as discreet (0.5 μm diameter) protein bodies, scattered amongst the mass oil droplets, but also as an amorphous continuous matrix surrounding the oleosomes. Both forms of the protein had a high affinity for the heavy metals used to stain the ultrathin sections.

The remaining cytoplasm of the storage parenchyma cells,is composed of faintly-staining polysaccharide with some diffusely-scattered protein. Large, centrally-located vacuoles are present within the cell. These contain electron opaque material which is probably phenolic (possibly chlorogenic acid) in origin.

After 50 days or so, the emulsion layer has grown in thickness (four to twelve droplets thick) and phenolics such as chlorogenic acid, seen as dark flattened osmiophilic bodies (**Mic 2 small arrows**) are deposited both within the cell vacuoles, and also in locations between the plasmalemma and the cell wall (*Dentan* 1985). While the mean diameter of oleosins appear to be the same as in the very young fruits, the thickness of the amorphous protein layer around each of the lipid droplets appears to be thinner.

According to *Vance & Huang* (1987) oleosins have two principal functions: firstly, to form a stable amphipathic layer on the surface of the oleosome and arrest coalescence with neighbouring bodies but secondly, to serve in a recognition role on the surface of the oil body for the binding of newly synthesised lipase at the onset of germination (*Wang & Huang* 1987).

In more mature cherries there are, in places, significant modifications to the architecture of the cell walls. Secondary and tertiary cell wall layers have been deposited in an erratic manner along the length of the cell wall, throwing it into folds and giving it a 'nodular' or ridged appearance (**Mic 3**). At thin areas of the wall where secondary and tertiary cellulose layers have not been deposited there are large pit fields bearing numerous plasmodesmata. Presumably the origin of these nodular structures (ie thick and thin areas of wall) stems from secondary and tertiary wall layers being unable to be deposited in the immediate area around a pit field traversing a primary wall.

In the fourteen day post-harvest cherries, oleosomes still persist as stable, uncoalesced sub-micron droplets within the darkly-stained proteinaceous matrix.

After drying of the green beans, the lipid is present as 0.3μm droplets, in the form of large masses within the cell lumen and dispersed within a solid dry proteinaceous / polysaccharide continuous phase (**Mic 4**).

Higher magnification investigation of the dry cytoplasmic contents reveals a solid emulsion structure. Fragments of dry cell contents appear to be porous, each pore being about 0.2 - 0.3 μm in diameter. Presumably each pore on the surface of the solid fragments contained at one time an oil droplet which vapourised under the action of the vacuum of the SEM, leaving a cup-shaped void (**Mic 4.**)

There is no evidence from freeze-fracture images of pre-existing channels within the walls of green beans (**Mic 5**). Cellulose microfibrils are obvious and appear to have a polarised orientation (eg **Mic 5 arrows**). Freeze-fracture reveals the 0.2 - 0.3 μm diameter lipid droplets to be layered (ie. multi-lamellar) with an obvious liquid crystalline structure (**Mic 6 arrows**) possibly indicating alternate layers of lipid and water.

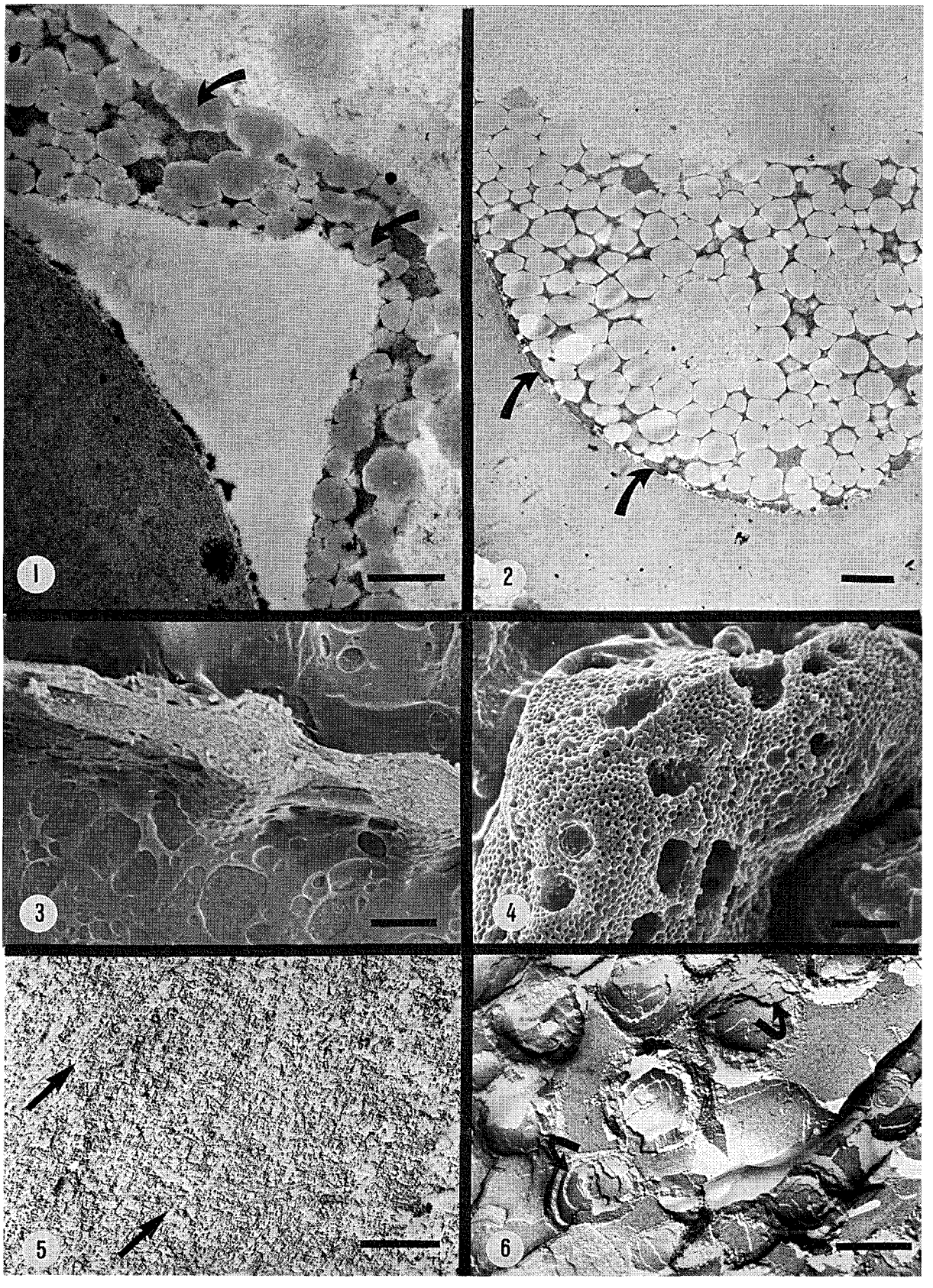
The oil droplet sizes recorded using SEM are similar to those observed in green coffee bean cell contents using freeze-fracture for TEM (**cf Mics 4 & 6**). Oleosomes are remarkably stable, after such severe desiccation, and do not coalesce even after being closely appressed to each other as a result of drying and shrinkage of the proteinaceous matrix.

Only after the the initial stages of roasting does the proteinaceous/polysaccharide matrix start to denature, allowing the sub-micron oil droplets to fuse and form much larger (>10μm) coalesced droplets (**Mic 7**). As roasting continues these droplets 'flow' around the inner surface of the cell wall (**Mic 8**).

Meanwhile, the elevated temperatures cause fundamental changes to the structure and chemistry of the thick cellulose walls of the parenchyma cells. Roasting of the beans also results in the dried hemicelluloses and pectic substances in the walls being thermally denatured and decomposed resulting in vapourisation (principally to CO_2 and other hydrocarbons). Such release of vapour might cause the cell walls to torrify, resulting in the formation of escape channels or 'blow holes' for the escaping gas (**Mics 9-11**). The effect of roasting temperatures on the denaturation of polysaccharides such as arabinogalactans and galactomannans in green beans has been reported by *Leloup & Liardon* (1993) who found that roasting considerably reduces the molecular weight range in these two polysaccharides

Hydrated components such as hemicelluloses, and to a lesser extent arabinogalactans and galactomannans, volatilise as a result of the extreme temperatures, causing a torrification of the cellulose wall with the subsequent formation of large 100 nm pores (**Mics 9 - 11 small arrows**). The cellulose matrix itself undergoes some major ultrastructural alterations with the formation of an extensive network of much smaller (10-20 nm) pores (**Mic 9 between small arrows**) and loss of polarisation of microfibrils (**Mic 9 cf with Mic 5**). This whole process of wall disruption with resultant increase in wall porosity has been termed by us 'microcanalisation'.

As a result of the increased porosity of the wall, the low viscosity lipids flood into the canals under the action of capillarity and the whole thick, secondary/tertiary cellulose wall acts as a reservoir for the coffee oil (**Mics 10 &11**).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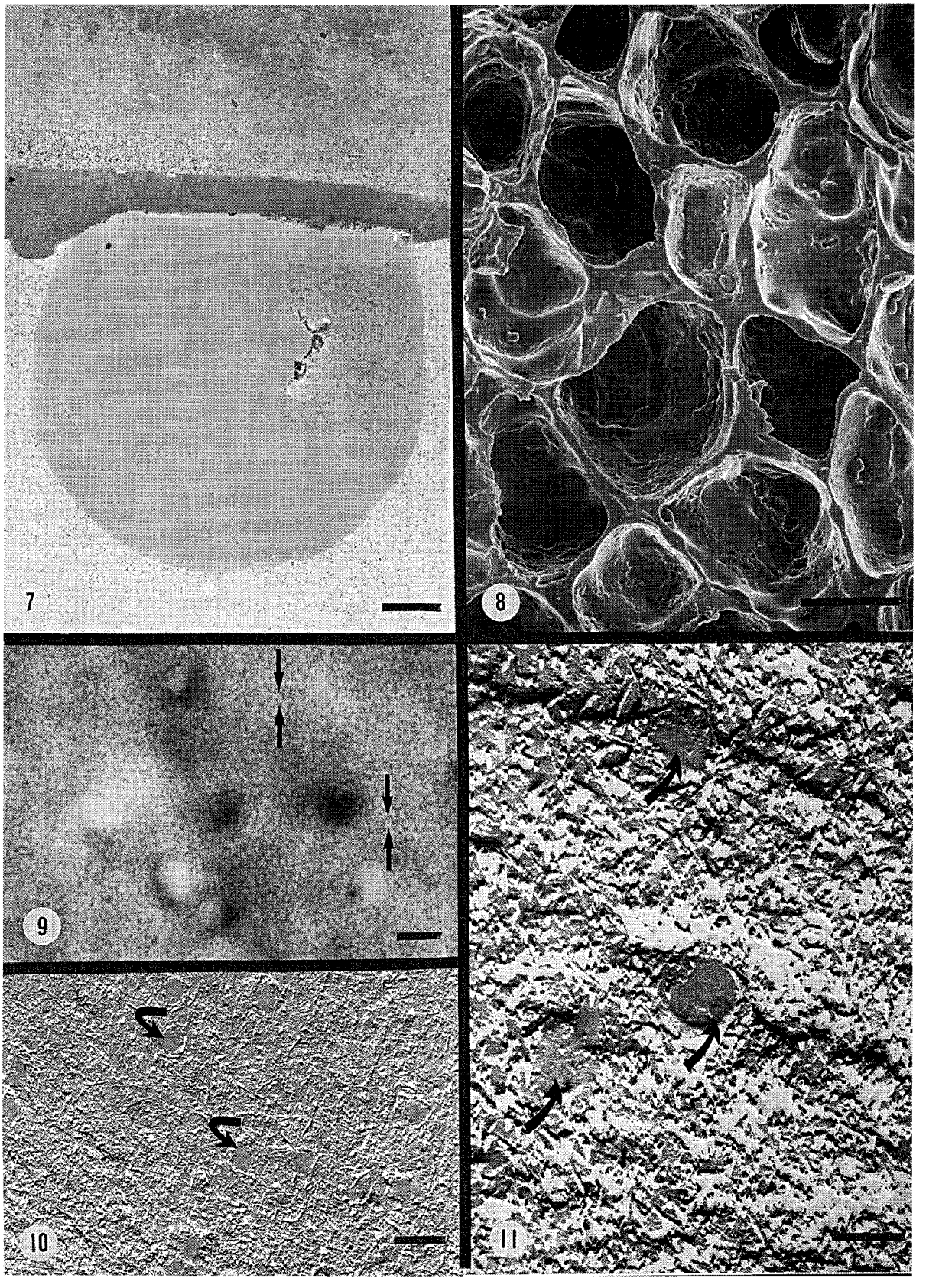
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CAPTIONS:

Mic 1. TEM demonstrating storage parenchyma of very young green coffee fruit Bar = 1.0μm

Mic 2. TEM demonstrating storage parenchyma of red coffee fruit (50 days). Bar = 1.0μm

Mic 3. SEM demonstrating nodular appearance of cell wall in older (50 days) red coffee fruit. Bar = 5.0μm

Mic 4. SEM of dry green coffee bean demonstrating solid emulsion structure. Bar = 5.0μm

Mic 5. Freeze/fracture TEM of green coffee bean, cell wall demonstrating polarised cellulose microfibrils (direction of arrows). Bar = 100nm

Mic 6. Freeze-fracture TEM of lipid droplets (oleosomes) in solid emulsion of green coffee bean, demonstrating lamellated (liquid crystal) structure. Bar = 100nm

Mic 7. TEM of roasted coffee bean demonstrating large coalesced oil droplet. Bar = 100nm

Mic 8. Cryo-SEM of roasted coffee bean demonstrating oil around internal surface of cell wall. Bar = 25μm

Mic 9. TEM of de-oiled roasted coffee bean demonstrating micro-porous nature of the wall (20 nm pores) and larger 100nm pores. Bar = 100nm

Mic 10. Freeze-fracture TEM of de-oiled roasted coffee bean demonstrating large (100 nm) pores within the 'torrified' cellulose wall. Bar = 100nm

Mic 11. As Mic 10. higher magnification image. Bar = 100nm

The oil in green coffee beans is locked up in an emulsion within the cell prior to roasting, just as a piece of butter on a slice of bread exists as a solid emulsion at room temperature. On roasting, the oil is released from the solid emulsion within the coffee bean just as on warming, the fat within the butter liquidifies and starts to flow.

The walls of green, unroasted beans are non-permeable to oil because of the presence of pectins and hemicelluloses just as the piece of fresh bread prior to being made into toast is relatively impermeable to liquid fat. However, on roasting, the pectins in the walls of green beans are denatured rendering the now-roasted bean walls permeable to oils, just as on toasting, the piece of bread becomes more permeable to the liquid fats in butter.

It appears that the 'toast and butter' analogy is indeed a very good one!

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5. Summary

Specialist fixation and cryo-preparation techniques for light and electron microscopy have been used to investigate the biosynthesis and re-distribution of oil, either as oleosomes or as free droplets, in coffee fruits and roasted beans.

In the young and maturing fruits the oil is present as 0.5 μm droplets in a protein-stabilised liquid bi-phase emulsion. On drying, and prior to roasting however, most of the oil is seen to be contained within the cell lumen in the form of a solid emulsion. During roasting the protein in the solid emulsion breaks down and much of the oil migrates or is drawn by capillarity into two sizes of micro-channel (hence 'microcanalisation') formed within the thermally denatured wall.

5. Sommaire

Des techniques spécialisées de fixation et de cytopréparation pour la microscopie optique et électronique ont été utilisées pour examiner la biosynthèses et la redistribution d'huile, soit comme olèosomes soit comme gouttelettes libres, dans les fruits de café et les grains de café.

En ce qui concerne les jeunes fruits et les fruits en voie de maturation, l'huile se présente sous forme de gouttelettes de 0.5 μm dans le emulsion biphasée liquid stabilisée par protéines. Pendant le séchage et avant la torréfaction, on constate pourtant que la plupart de l'huile se contient à l'interieur de la cellule sous forme d'émulsion solide.se décompose et la plupart de l'huile se dissémine ou est attirée, par l'action capillaire, dans les micro-canaux de deux tailles (d'où le terme "microcanalisation") formés dans la paroi thermodénaturée.

INFLUENCE OF COFFEE BOTANICAL VARIETY ON ESPRESSO CUPTESTING QUALITY

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1. INTRODUCTION

ESPRESSO is a way to enjoy a cup of coffee which is gaining large popularity world-wide [1]. Its roots are to be searched for in the Italian culture of foods and beverages, that developed a typical lifestyle linked to coffee drinking [2]. Its main marks are:

- **extemporaneous preparation**, on express order
- brewing by a specific method, using **high water pressure**
- **rapid extraction**, admitting into the cup just the best material.

The resulting beverage is very peculiar from a physical and chemical perspective too [3]. However, the ESPRESSO main characters are of sensory nature. All human senses, with exception of hearing, are involved in appreciation of an ESPRESSO cup:

- **vision** evaluates foam's aspect, examining its colour and its consistency and persistence
- **touch** assesses the beverage mouthfeel, or "body", a property linked with density and viscosity
- **taste** judges the bitter/acidic balance and the presence of a sweet caramelic after-taste
- **olfaction** appreciates both **fragrance**, by direct inhaling of the vapours arising from the cup, and **flavour**, or nasal perception of the volatile substances evolving in the mouth.

ESPRESSO extraction method produces in the cup a high concentration of sensorially active substances: special care must therefore be devoted to prevent and to eliminate defects from the raw material. Moreover, in order to offer the customers the finest sensory quality, attention should be devoted to several variables tending to affect the sensory balance appreciated by consumers.

Common knowledge claims that factors like growing region and altitude, as well as climatic situation and processing technique, can have an effect on overall coffee quality, mainly in the meaning of increased acidity and aroma. Unfortunately, little is known about their influence on ESPRESSO beverage, and genetic parameters are often neglected to the benefit of local traditional cultivars.

We consider in this paper the significance, on "ESPRESSO quality", of the genetic variability brought by diverse characteristics of coffee plants grown together in the same geographical conditions and under the same farm practice.

2. MATERIALS AND METHODS

About 20,000 (twenty thousand) individually identified *Coffea arabica* plants were sown and grown in a premium coffee producing region, located in Brazil at about 21° South, 1040 m altitude, where the average temperature varies between 20°C and 26.4°C [4].

A possible clustering criterion to describe this coffee population is to group them according to the shared botanical variety origin:

- CA: acronym for Caturra
- CR: " Catimor
- CTR: " Red Catuai
- CTY: " Yellow Catuai
- IC: " Icatu

The farming practices utilizedwere typical of the local tradition: full sunlight, semi-dense plot of 1666 plants/ha, with some quality-oriented operations like adequate fertilisation, regular pesticide spraying, weed control, handpicking harvesting, depulping, cherry processing by fermentation.

Besides agronomic and chemical evaluations, the individual adult plants were assessed for "ESPRESSO quality". Harvest plant by plant was accomplished by careful handpicking to avoid the presence of unripe, overripe or damaged cherries. The crop of each individual plant, of about 3 kg of cherries, was depulped and the resulting mucilaginous parchment beans were fermented in individual buckets, with the help of a pectolitic enzyme (Isozym) to standardise the fermentation time among all samples. After sundrying and dehulling, the samples were shipped to the illycaffe' corporate laboratory in Trieste (Italy), where the organoleptic analysis was performed.

The green coffee samples were prepared according to the illycaffe' lab procedures established for evaluating commercial samples [5]:

- visible defects (black, broken, unripe beans) were eliminated manually

- each sample was roasted to a typical Italian roast, using 80g per sample under controlled conditions in lab scale roasters (Probat), and ground to appropriate particle size in professional grinders (Faema)

- each roasted and ground sample was evaluated in three different cups, according to three key preparation techniques:

  - espresso: the classic espresso cup, prepared under standardised and thoroughly controlled conditions [6]. The resulting beverage is a solution of sugars, acids and caffeine, in which a distinct phase made of lipid micronic droplets is dispersed as an emulsion. Overall concentration can be as high as 60 g/l

  - infusion, a brewing method widely used in Northern Europe and in the U.S.A.: boiling water is poured on coarsely ground coffee powder and allowed to rest for a given period before filtering away the spent grounds from the remaining clear liquid coffee.
    The concentration of the beverage is low (below 20 g/l) and only the soluble substances pass into the cup, imparting it an aromatic pattern typical of a filtered coffee beverage

  - diluted espresso, where a little aliquot of espresso is taken and diluted with hot water up to the same total solids content of the infusion. This way the high concentration of regular espresso does not hinder any longer the evaluation of some weaker aroma nuances, and the difference between the solution aromatic pattern and the emulsion one can be determined.

Twelve samples per day were submitted to a cuptesting panel, formed by at least three trained professional experts, and were examined in blind absolute tests where some complex variables, e.g. overall merit, were determined by comparison with a mental paradigm present in each assessor's memory by previous experience. The scale used encompassed scores ranging from -3 to 0 in the negative rank and from 0 to +3 in the positive one, defining "good for espresso" only those samples which score from +1 to +3.

3. RESULTS AND DISCUSSION

Two data sets are available, pertaining to two consecutive crops 1990 and 1991. A total of 585 plants were evaluated in 1990 and 225 more in 1991, giving a total pool of more than 800 samples.

As can be seen in the overall merit distribution of these sensory analyses, (see fig.1), just a few samples (less than 10%), namely those scoring ≤ -2, were penalised for objectionable taste (stinking, harsh, immature). This result may be related to the "wet" processing method which allows to eliminate by floating some improper beans, so enhancing quality [7].
Unfortunately can be observed that only as little as 20% of samples were reported as "good for espresso" as defined above (merit $\geq +1$).

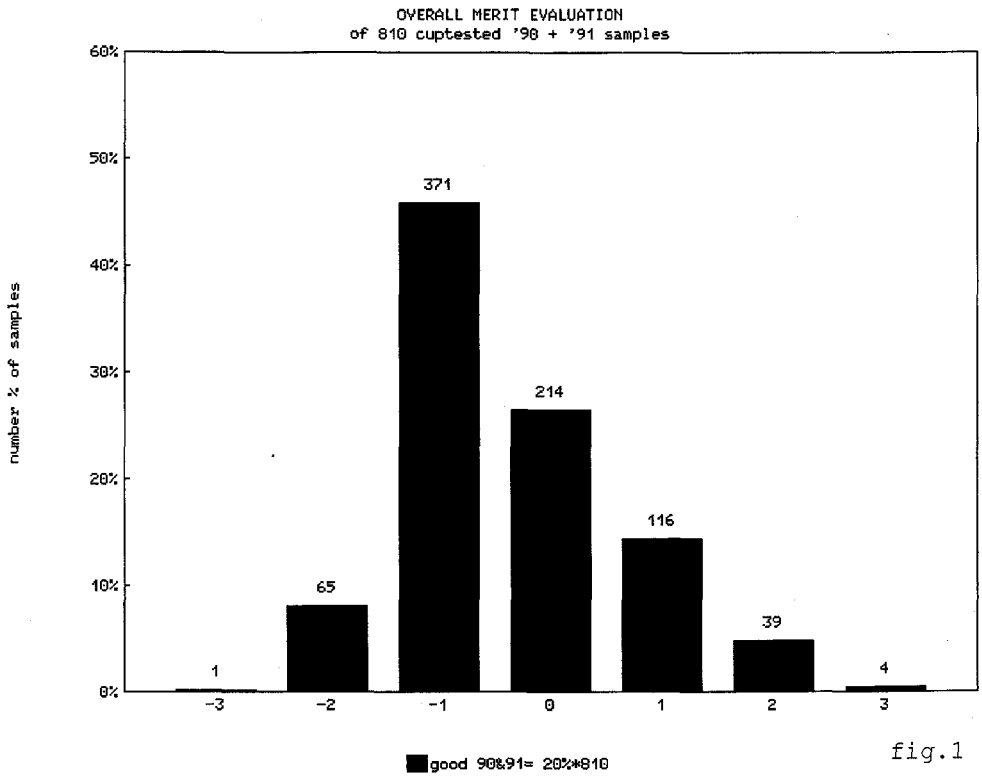

fig.1

With the objective of finding a relationship between coffee genotype and "ESPRESSO quality", we organised the 1990 data as displayed in fig.2:

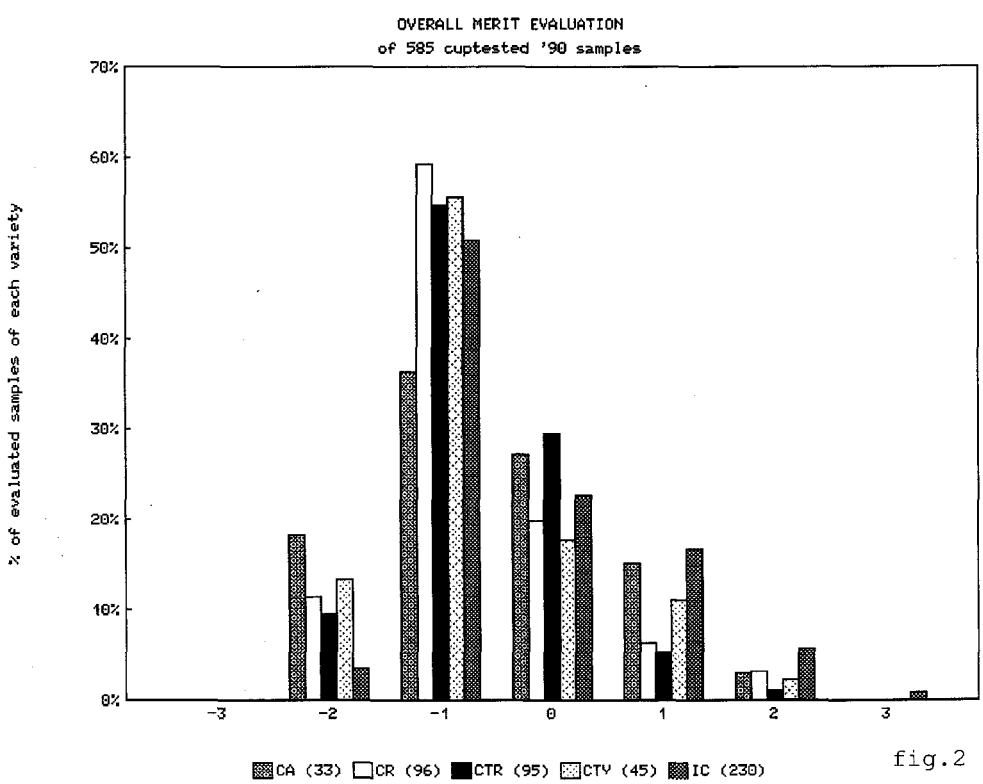

fig.2

All the examined coffee varieties exhibit irregular sensory characteristics, with presence of both good and bad samples.
However an evident "ESPRESSO quality" trend emerges when considering the percentage of "acceptable for espresso" samples (merit ≥ 0):

Caturra	CA	45 %
Catimor	CR	29 %
Red Catuai	CTR	35 %
Yellow Catuai	CTY	31 %
Icatu	IC	46 %

This tendency is confirmed when examining the 1991 data (where Caturra figures had to be removed due to the too little number of samples analysed):

Catimor	CR	41 %
Red Catuai	CTR	49 %
Yellow Catuai	CTY	64 %
Icatu	IC	63 %

All coffee varieties show fluctuation in year to year appraisal: this may be explained by a general quality improvement in 1991 samples.

When comparing directly the (1990 + 1991) combined data of the two varieties Catimor and Icatu, the quality gap is particularly striking, as appears evident from fig.3:

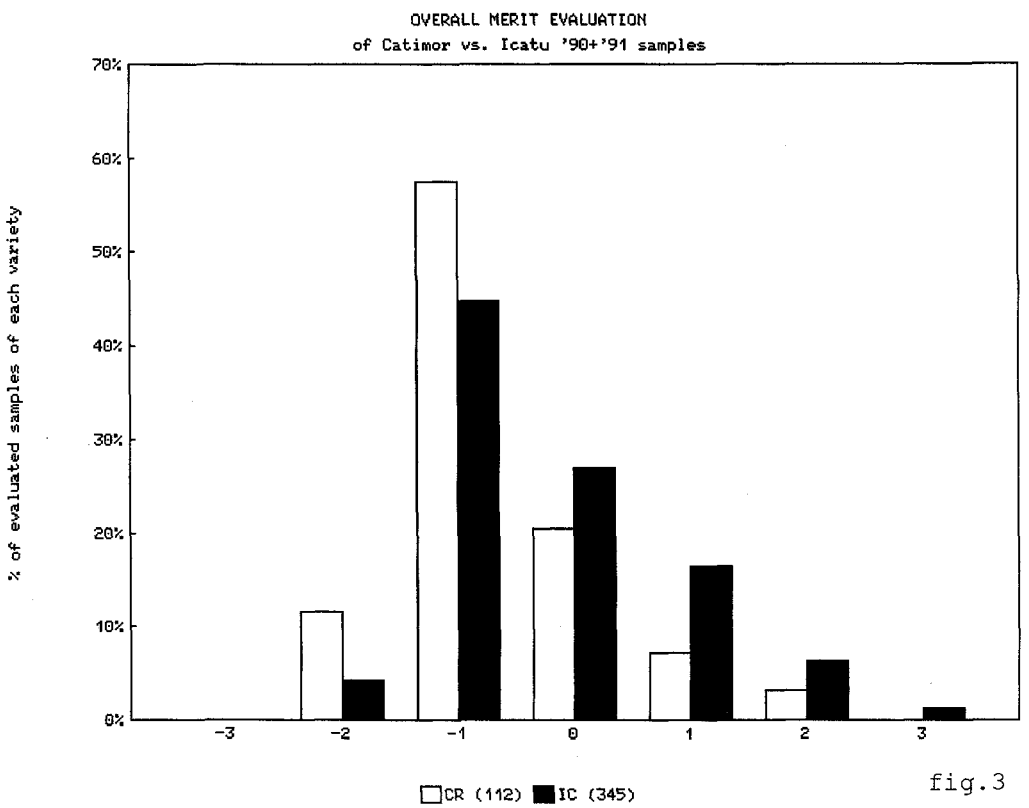

fig.3

In the range of "acceptable for ESPRESSO" (merit ≥ 0) Icatu scores an outstanding 51% compared to Catimor's 31%. In a more strict classification criterium, namely the quality class called "good for ESPRESSO" (merit ≥ +1), the performance of Icatu is more than double of Catimor samples: 24% vs. 10%. The presence of 12% of bad Catimor samples (merit ≤ -2), compared to the 4% of bad Icatu samples, appears not so important when considering that some defective or ill-fated samples may be present due to process accidents.

I call your attention, on the other hand, on the gap of samples declared unacceptable without being offensive (merit = -1): this score, as a rule, means an unbalanced bitter/acidic ratio, a lack of aroma and body, and a slight presence of astringency. All these traits can be seen as a peculiarity of a botanical origin, and induced by genetic traits of a particular variety.

According to our current data, we conclude that there is initial evidence that, at least under specific growing and processing conditions as described above, the botanical origin of *Coffea arabica* plants is important to provide the best industrial outcome in the ESPRESSO coffee production.

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SUMMARIES

Espresso, a way to enjoy coffee which is gaining large popularity worldwide, has in its cup a high concentration of sensorially active substances: special care must therefore be devoted to prevent and to eliminate defects from the raw material. Moreover, attention should be paid to the variables tending to affect the sensory balance appreciated by consumers. Little is known about their influence on Espresso beverage quality, and genetic parameters are often neglected (to the benefit of local traditional cultivars). This paper indicates that the botanical origin of *Coffea arabica* plants is important to get the best industrial outcome in the production of Espresso coffee.

L'Espresso, une façon de goûter son café qui gagne en popularité, contient dans sa tasse une concentration élevée de substances très actives du point de vue organoleptique: il faut donc soigneusement prévenir et rejeter les défauts du matériel brut. En plus, il faut surveiller les variables qui jouent sur le bilan sensoriel apprécié par les consommateurs. On connaît peu leur influence sur la qualité de la boisson Espresso, et les paramètres génétiques sont souvent négligés (favorisant comme ça les cultivars traditionnels de la région). Ce travail montre l'importance de l'origine botanique des plantes de *Coffea arabica* pour obtenir le meilleur résultat industriel dans la production de café pour Espresso.

PATHWAYS INVOLVED IN THE BIOSYNTHESIS AND CATABOLISM OF CAFFEINE IN *COFFEA* AND *CAMELLIA*

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Introduction

Over the years, the occurrence of purine alkaloids, such as caffeine (1,3,7-trimethylxanthine) and theobromine (3,7–dimethylxanthine), has been reported in a large number of taxa (see Suzuki et al. 1992). Re-examination of the literature suggests strongly that this phytochemical character is confined to six higher plant genera which include species used to produce beverages such as *Coffea arabica* L. (coffee), *Camellia sinensis* (L.) O. Kuntze (tea), *Theobroma cacao* L. (cocoa), *Ilex paraguariensis* St.Hil. (maté) and *Paullinia cupana* H.B.K. (guaraná) and *Cola acuminata* (P. Beauv.) Schott et Endl. (cola) (Baumann, unpublished). Recent studies on the metabolism of caffeine have focused on leaves of coffee and tea. The young expanding leaves of *C. arabica* comprise ca 3-4 % caffeine on a dry weight basis while young tea leaves contain up to 5-7 % caffeine. Mature and aged leaves of both species contain lower levels of caffeine. Feeding experiments with radiolabelled adenine and guanine have shown that young leaves of *C. arabica* have a much higher capacity for caffeine biosynthesis than older leaves (see Table I) and a comparable situation exists in *C. sinensis* (Ashihara et al. 1996a, 1997). In keeping with these observations, the activities of the methyltransferases involved in caffeine biosynthesis are highest in young, emerging coffee leaves (Mösli Waldhauser et al. 1997b).

Caffeine Biosynthesis

The routes by which caffeine originates from the products of purine biosynthesis de novo and salvage pathways, via a minor side branch of the purine catabolism pathway have been discussed in some detail in previous reviews

Table 1. Incorporation of [8-^{14}C]adenine and [8–^{14}C]guanine into caffeine by young, mature and aged leaves of *C. arabica.* Leaf sections incubated with 10^6 dpm of substrate for 18 h at 27°C. Radiolabel incorporated into caffeine expressed as a percentage of total radioactivity taken up by the leaves ± standard error. (n = 3) (after Ashihara et al. 1996a)

	Leaf Type		
Substrate	Young	Mature	Aged
[8-^{14}C]adenine	20.5 ± 0.5	7.1 ± 0.7	0.8 ± 0.2
[8-^{14}C]guanine	9.1 ± 0.5	0.6 ± 0.1	0.3 ± 0.1

(Suzuki et al. 1992; Waller et al. 1993). It has been accepted for some time that caffeine biosynthesis involves a xanthosine ➔ 7–methylxanthosine ➔ 7-methylxanthine ➔ theobromine ➔ caffeine pathway. A combination of in vitro and in vivo studies with [8-^{14}C]theobromine have confirmed that the dimethylxanthine undergoes *N*-1 methylation and is converted almost exclusively into caffeine by young leaves of tea and coffee (Ashihara et al. 1996a, 1997; Kato et al. 1996). While there is general agreement about the metabolism of 7-methylxanthine ➔ theobromine ➔ caffeine, and the *N*-7 > *N*-3 > *N*-1 sequence of methylations, there is now an interesting debate about the first committed step in the caffeine biosynthesis pathway as Baumann and co-workers in Zürich have questioned the role of xanthosine as the substrate for the initial *N*-7 methylation step.

The arguments of the Swiss investigators are based on a possibly 'weak' specificity of the caffeine methyltransferases and on the fact, that the in vivo formation of 7-methylxanthosine, identified by paper chromatography (Negishi et al. 1985a,b, 1992), has been confirmed by HPLC, albeit in trace quantities, in only one subsequent study (Ashihara et al. 1996a). In their first study with suspension cultures of *Coffea arabica* cells, the Swiss investigators initiated enhanced rates of caffeine production with adenine, ethephon and light. Despite up to 20-fold increases in caffeine levels, 7-methylxanthosine did not accumulate in detectable quantities (Schulthess and Baumann 1995a). In further experiments, the 'stimulated' test system was fed with the well-established caffeine precursors [^{14}C]adenine and [methyl-^{14}C]methionine. Traces of [^{14}C]7-methylxanthosine were detected only in cultures preincubated with 'cold' adenine which contained 'artificially' high concentrations of endogenous xanthosine either strongly labelled, after [^{14}C]adenine-feeding, or unlabelled, after feeding [methyl-^{14}C]methionine. Kinetic studies with [^{14}C]adenine showed that the specific activity of xanthosine was lower than that of 7-methylxanthine. This precludes xanthosine as a precursor of caffeine unless there are two separate xanthosine pools, one undergoing

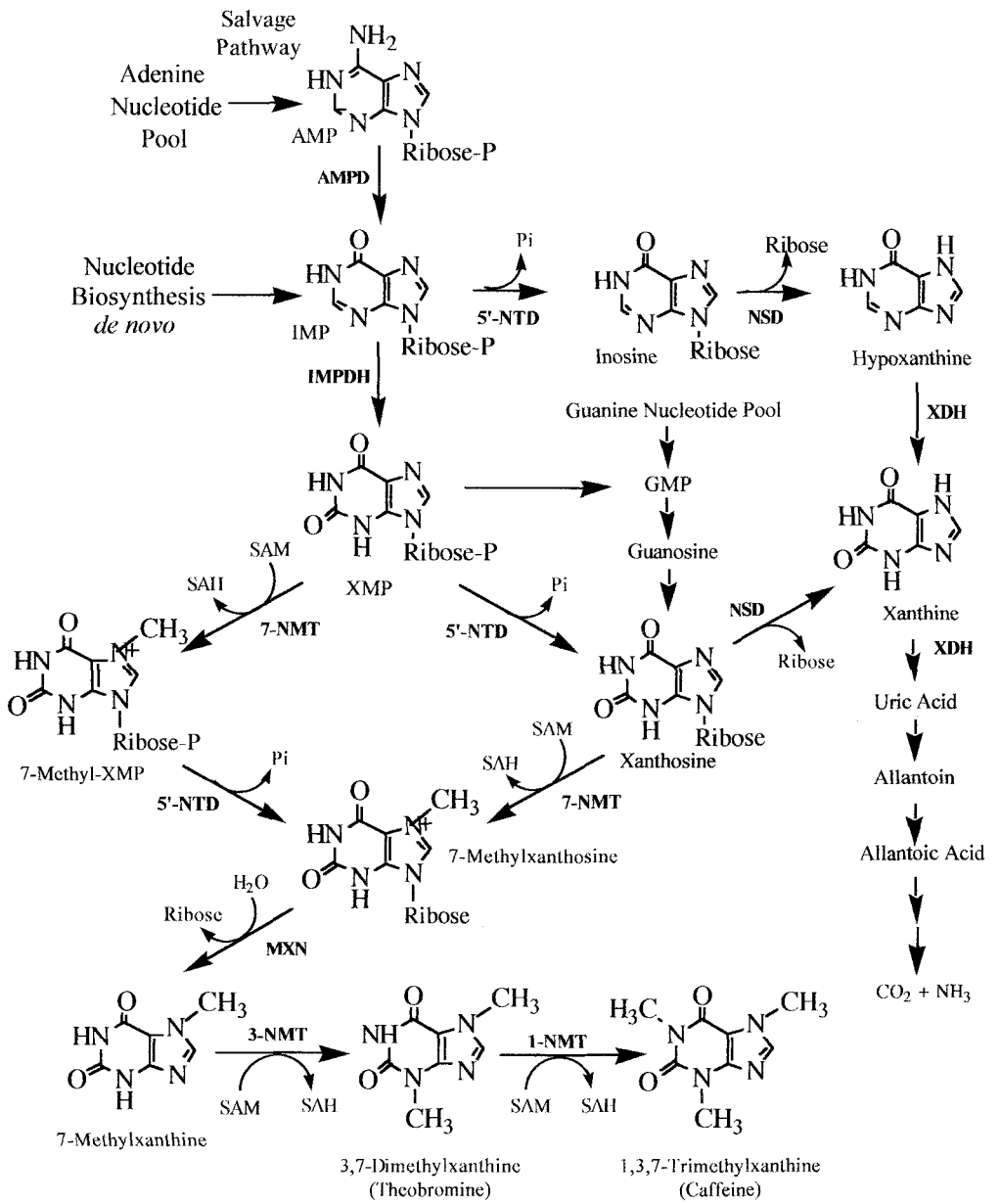

Figure 1. Key steps in the purine catabolism pathway and proposed pathways for the biosynthesis of caffeine from purine nucleotides via xanthosine and XMP in young leaves of *Coffea arabica*. AMP deaminase (AMPD), 5'-nucleotidase (5'-NTD), nucleosidase (NSD) 7-methylxanthosine nucleosidase (MXN) IMP dehydrogenase (IMPDH), 1-*N*-methyltransferase (1-NMT), 3-*N*-methyltransferase (3-NMT), 7-*N*-methyltransferase (7-NMT) xanthine dehydrogenase (XDH).

conversion to xanthine and acting as an entry point into the purine catabolism pathway, and the other acting as a substrate for purine alkaloid biosynthesis (Schulthess and Baumann 1995b). Subsequent in vitro studies demonstrated that cell-free preparations from young coffee leaves rapidly convert xanthosine monophosphate

(XMP) to 7-methyl-XMP which was further converted to 7-methylxanthine. On the basis of these observations, it was suggested that XMP, rather than xanthosine, is the in situ acceptor of the first methyl group in the caffeine biosynthesis pathway in young expanding coffee leaves (Schulthess et al. 1996). The alternative routes to caffeine along with the purine catabolism pathway are illustrated in Figure 1. At present, it remains to be determined whether *N*-7 methylation of XMP also represents the first committed step in the caffeine biosynthesis pathways operating in *Camellia sinensis* and other purine alkaloid containing species.

More controversial ideas about the caffeine biosynthesis pathway have been published by Nazario and Lovatt (1993a,b). In these studies, with *Coffea arabica* leaves, use was made of a range of radiolabelled precursors including formate, glycine and bicarbonate. The identification of products and the level of incorporation of radiolabel are questionable as they were based on co-crystallisation rather than more rigorous analytical methodology such as HPLC or GC-MS. The data obtained were interpreted as indicating that caffeine is synthesized from xanthine and that theobromine, produced by separate de novo and salvage pathways, is not converted to caffeine. The authors have produced no further experimental evidence to support their cavalier hypothesis and recent in vitro and in vivo studies with coffee leaves have established that theobromine is the immediate precursor of caffeine and that xanthine is a key purine alkaloid catabolite (Ashihara et al. 1996b; Kato et al. 1996) (see Fig. 1).

The three methylation steps in the caffeine biosynthesis pathway are catalysed by *S*-adenosylmethionine (SAM)-dependent *N*-methyltransferases. Suzuki and Takahashi (1975) first detected *N*-3-methyltransferase (3–NMT) and *N*-1-methyltransferase (1-NMT) activities in extracts from tea leaves which are also a source of *N*–7-methyltransferase (7-NMT) activity (Negishi et al. 1985a,b). The results of mixed substrate experiments with cell suspension cultures of *Coffea arabica* have indicated that separate enzymes catalyse the *N*-3 and *N*-1 methylations (Baumann et al. 1983). This is in line with reports that theobromine but not caffeine is synthesized in leaves and flower buds of *Camellia irrawadiensis* (Ashihara and Kubota 1987; Fujimori and Ashihara 1990) and that leaves of *Theobroma cacao* clone "Pound 12" contain only theobromine rather than the more typical mixture of theobromine and caffeine (Baumann and Fritz, unpublished). These and other observations suggesting the existence of discrete NMTs can, however, also be interpreted as the actions of a single enzyme that carries out all three methylation steps. This view is supported by the parallelled 3- and 1-NMT activities observed during the culture cycle of coffee cell suspensions (Baumann et al. 1983) and during coffee leaf development (Mösli Waldhauser et al. 1997b) and by the fact that all three NMT activities from young tea leaves co-chromatograph when subjected to gel-filtration and anion-exchange chromatography (Kato et al. 1996). Attempts to clarify the situation, and to isolate the NMTs, have proved less than straightforward because the enzymes are extremely unstable and, as a consequence, rigorous precautions must be taken if activity is not to be lost during even the simplest of purifications (Gillies et al. 1995).

Recently, chromatofocusing of anion-exchange-purified enzyme extracts from young coffee leaves has facilitated the clear separation of 7-NMT activity from the 3-NMT and 1-NMT activities (Mösli Waldhauser et al. 1997a). All three NMTs co-eluted when analysed by gel-filtration chromatography and their native molecular mass

was ca. 67 kDa. Photoaffinity labelling with [methyl-^{3}H]SAM, using procedures devised by Yu (1983) and Hurst et al. (1984), followed by SDS-PAGE of a chromatofocusing-purified preparation containing only 7-NMT activity, demonstrated the presence of a single labelled band of 40 kDa which could be a subunit of the 7-NMT since gel-filtration indicated molecular mass of 67 kDa. Mösli Waldhauser et al. (1997a) speculated that the native NMT in coffee leaves may a multimer, with at least one subunit containing the binding site for SAM. Based upon the native molecular mass of ca. 60 kDa found in coffee endosperm (Mazzafera et al. 1995) and coffee (Mösli Waldhauser et al. 1997a) and tea leaves (Kato et al. 1996), the existence of a diameric NMT is most likely. Mösli Waldhauser et al. (1997a) also carried out photoaffinity labelling and SDS-PAGE analysis of a gel-filtration purified preparation containing all three NMT activities and this revealed the presence of three labelled bands at 49, 43 and 40 kDa. It remains to be determined whether the 49 kDA and 43 kDa bands are associated with the 3-NMT and 1-NMT activities or whether they are unrelated SAM-dependent methyltransferases or other SAM-binding proteins.

Caffeine Catabolism

The degradation of caffeine to xanthine, which is further degraded via uric acid to CO_2 and NH_3, by the purine catabolism pathway, was first demonstrated in *Coffea arabica* leaves by Kalberer (1964,1965). Recent reports on the metabolism of [8-^{14}C]caffeine, [2-^{14}C]theobromine, [8-^{14}C]theophylline and [2-^{14}C]xanthine by leaves of coffee and tea, in the presence and absence of allopurinol, which inhibits xanthine dehydrogenase activity, have provided detailed insights into the catabolic pathways that operate in the two species (Ashihara et al. 1996b, 1997; Ito et al. 1997). In both species [2-^{14}C]theobromine is converted almost exclusively to caffeine with little or no release of $^{14}CO_2$ demonstrating that theobromine is a precursor and not a catabolite of caffeine. [8-^{14}C]Caffeine is degraded very slowly with only trace levels of $^{14}CO_2$ being detected. In contrast, [8-^{14}C]theophylline is metabolised rapidly by both tea and coffee leaves, indicating that the accumulation of endogenous caffeine in these tissues is a consequence of a lack of adequate 7-demethylase activity to convert caffeine to theophylline.

[8-^{14}C]theophylline absorbed by young, aged and mature leaves of *Coffea arabica* is metabolised rapidly via 3-methylxanthine to xanthine which enters the purine catabolism pathway and is released as $^{14}CO_2$. Likewise, more than 80% of [2–^{14}C]xanthine taken up by coffee leaves is released as $^{14}CO_2$ over a 42 h incubation period. The inclusion of 5 mM allopurinol in the incubation medium has a major effect on [8-^{14}C]theophylline metabolism. The production of $^{14}CO_2$ declines dramatically, as a consequence of xanthine degradation being blocked, and there are concomitant increases in the incorporation of label into, not only xanthine and 3-methylxanthine, but also 7–methylxanthine. It is especially noteworthy that the metabolism of both [8-^{14}C]theophylline and [2–^{14}C]xanthine by aged leaves in the presence of allopurinol results in >70 % of the recovered radioactivity being incorporated into 7–methylxanthine. The conversion of xanthine to 7-methylxanthine in such large amounts is unexpected as the established role of 7–methylxanthine is that of a precursor of theobromine in the caffeine biosynthesis pathway. Some form of strict compartmentation would appear be operating as the allopurinol-

induced accumulation of 7-methylxanthine in coffee leaves is not associated with incorporation of label into either theobromine or caffeine (Figure 2, Ashihara et al. 1996a).

The main fate of [8-^{14}C]theophylline incubated with mature and aged tea leaves, and to a lesser extent young leaves, is conversion to xanthine via 3-methylxanthine, and entry into the purine catabolism pathway (Ashihara et al. 1997; Ito et al. 1997). However, in [8-^{14}C]theophylline feeds to young leaves, a significant amount of the label is salvaged for the synthesis of caffeine via a 3-methylxanthine → theobromine → caffeine pathway. Trace amounts of [2-^{14}C]xanthine are also salvaged for caffeine biosynthesis, and this is increased when purine catabolism is blocked by allopurinol. Salvage of xanthine occurs as a consequence of its conversion to 3–methylxanthine which is metabolised to caffeine via theobromine. Interestingly, the [2-^{14}C]xanthine feeds to young leaves show that 3–methylxanthine, as well as yielding theobromine, is also converted via 1-methylation to theophylline. Supporting evidence for the existence of these pathways, which are illustrated in Figure 2, comes

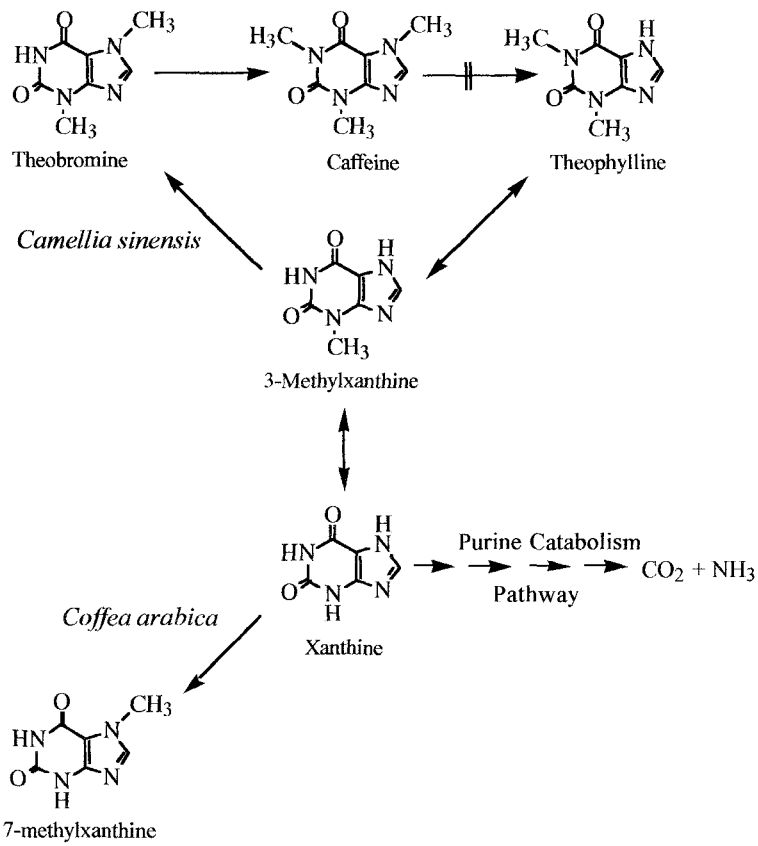

Figure 2. Purine alkaloid catabolism pathways operating in leaves of *Camellia sinensis* and *Coffea arabica*. Arrow with two vertical bars represents a blocked conversion. Double headed arrows indicate reversible conversions.

from in vitro studies with *N*-methyltransferase activity from young tea leaves which have shown that (i) xanthine is metabolised to 3–methylxanthine (Negishi et al. 1985a,b), (ii) 3-methylxanthine is converted to theophylline, theobromine and caffeine (Kato et al. 1996) and (iii) theophylline does not act as a methyl acceptor and therefore

does not undergo direct conversion to caffeine (Kato et al. 1996).

There are, therefore, distinct differences in the purine alkaloid catabolism pathways operating in leaves of *Coffea arabica* and *Camellia sinensis*. In both species caffeine accumulates, because its catabolism to theophylline is blocked, and conversion of [8-^{14}C]theophylline → 3-methylxanthine → xanthine links theophylline to the purine catabolism pathway which results in breakdown to CO_2 and NH_3. In young tea leaves there is detectable salvage of 3-methylxanthine and xanthine and resynthesis of (i) theophylline and (ii) caffeine via theobromine. There is no evidence for the operation of such pathways in coffee leaves. Instead, young, mature and aged *Coffea arabica* leaves, treated with allopurinol, convert xanthine to 7-methylxanthine which does not appear to be metabolised to any extent (Figure 2, Ashihara et al. 1996a, 1997).

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Summary

Recent investigations on caffeine biosynthesis pathways in leaves of *Coffea arabica* and *Camellia sinensis,* using both in vitro and in vivo test systems, are reviewed with special interest being focused on the proposal that the *N*–7 methyl acceptor is xanthosine monophosphate rather than xanthosine. In addition, studies on the isolation on the *N*-methyltransferases involved in caffeine biosynthesis which have succeeded in separating *N*-7 methyltransferase activity from the *N*-1- and *N*-3 methyltransferase activities are discussed. Purine alkaloid catabolism pathways are also discussed in the light of recent evidence demonstrating salvage of 3-methylxanthine and xanthine for the resynthesis of not only theophylline but also caffeine via theobromine. These pathways operate in leaves of tea but not those of coffee.

CHEMICAL COMPOSITION AND CHARACTERISTICS OF A ZINC(II)-CHELATING FRACTION IN INSTANT COFFEE

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One of the food-chemical characteristics of a coffee brew is browning of the phenolic type. The brown-colored materials formed by roasting coffee beans contribute to both the taste and nutritional factors to reduce the availability of trace elements. The authors have investigated the chemical characteristics of zinc(II)-chelating compounds with a dissociation constant of the 10^{-8} order. In this study, we report the chemical composition of degradation products from a zinc(II)-chelating compound with an alkaline or oxidative reaction, and a coffee-model study on the compounds involved in the formation of metal-chelating compounds.

Preparation of the zinc(II)-chelating compound from instant coffee.

The zinc(II)-chelating compound used in this study was prepared from instant coffee of the lyophilized type. A hexamine buffer (10mM) solution with $ZnCl_2$ (20 mM) was added to instant coffee, and swirling formed an insoluble zinc(II) complex. This complex was dissolved in an aqueous ammonia (1%) solution, and precipitated by acidification with HCl. The precipitate (Sample Ap) formed was further purified by ion-exchange chromatography in Amberlite IRA-410(OH-) and IR-120(NH4+) columns. Monitoring the zinc content by atomic absorption spectrophotometry indicated the non-adsorbed fraction to contain a zinc(II)-chelating compound, and further chromatography resulted in 6 fractions (I-VI) from a cellulose column developed with a propanol-1% ammonia aqueous solution.

The Ap-V fraction was found to have the highest zinc(II)-chelating activity with a dissociation constant of the 10^{-8} order. The yield was 0.3-0.4 % of the instant coffee used.

General properties of Ap-V.

Ap-V was an amorphous brown powder and was soluble in water. Its molecular weight was estimated to be about 48,000 by HPLC, using proteins as standard markers. The content of polyphenol was 30.4% by the Folin Denis method, and the contents of sugar and amino acid were about 3% and 4%, respectively. The chemical formula was experimentally found to be $C_{16}H_{21}O_9N_3$. The nitrogen content of more than 10% is indicative of the involvement of the Maillard reaction in the formation of the zinc(II)-chelating polymer.

Chemical structure of Ap-V.

The partial structure of Ap-V was investigated by characterizing the products formed through such degradative reactions on the Ap-V sample as alkaline fusion (350℃), alkaline decomposition (250℃) in glycerol, oxidative degradation of methylated samples with $KMnO_4$-$NaIO_4$, and degradation with $NaClO_2$. The products formed were extracted with ether and fractionated into a neutral and basic group, and an acidic group. These fractionated samples were subjected to 3D-HPLC, GC-MS and LC-MS analyses.

Alkaline fusion of the Ap-V fraction gave about 11% of ether-soluble compounds, of which the major compounds were low-molecular-weight polyphenols such as pyrogallol (2.16%), protocatechuic acid (3.56%), catechol (2.16%), and p-hydroxybenzoic acid (0.76%). Fig. 1 shows 3D-HPLC and LC-MS data for the fraction produced by these degradative reactions.

The alkaline degradation in glycerol gave similar chromatographic patterns by LC-MS to those for alkaline fusion. Some peaks by LC-MS showed the presence of more than two benzene rings in the molecule.

Oxidative degradation of Ap-V with $KMnO_4$-$NaIO_4$ gave different HPLC patterns by methylation. This shows that Ap-V contained a hydroxy group and carboxyl group which could be methylated.

Degradation with $NaClO_2$ gave pyrogallol and caffeic acid, which is indicative of the presence of 3 benzene-rings connecting with an ether bond.

The degradative reactions other than alkaline fusion gave fewer ether-soluble compounds than those by alkaline fusion, and similar phenolics were determined in the degradation products. This shows that

the benzene-rings involved in Ap-V were connected with strong bonds which alkaline fusion could release to the greatest degree to produce phenolics.

Fig. 2 shows GC-MS data for the acidic and basic fractions prepared by the alkaline fusion of Ap-V. These data show the presence of polyphenolics, benzoic acid and its derivatives, and carboxylic acids with 4-5 carbon chains. The basic fraction shows the presence of amides, which is indicative of the involvement of sugar and protein in the formation of Ap-V.

Model-coffee system.

The formation mechanism for brown compounds with Zn(II)-chelating activity in a coffee brew was investigated by a model coffee system. Models were prepared by one or mixtures of two to four combinations of chlorogenic acid, sucrose, bovine serum albumin (BSA) and cellulose, and roasted at 200℃ for 30 min. The aqueous soluble fraction from each of these roasted models was lyophilized, the yield being the largest in the model consisting of chlorogenic acid, sucrose and BSA.

The Zn(II)-chelating ability of the aqueous soluble fraction was determined by colorimetry with tetramethyl murexide the for Zn(II) complex. The metal-chelating ability per gram of a sample was found to be largest in the model prepared by using only chlorogenic, and the smallest in the model with all four compounds. The partial structure of chlorogenic acid in the brown pigment contributed to the chelating ability.

Antioxidative activity and enzyme-like activity.

Since Ap-V had metal-chelating activity, it was subjected to an antioxidative assay against linoleic acid. This assay involved incubating in a 0.02M phosphate buffer (pH 7.0) with ethanol at ambient temperature in the dark. The peroxide formed was measured by colorimetry at 500 nm with ammonium thiocyanate-$FeCl_2$. The effect of the amount of chelating Zn(II) and hydrogenation with Pd/C catalysis on the antioxidative activity of Ap-V was investigated.

Ap-V was found to be antioxidative. The less chelating metal Ap had, the more it was antioxidative. Hydrogenation of Ap-V reduced the antioxidative activity and Zn(II)-chelating activity by half. Olefinic moieties such as enol and enaminol in the Ap-V structure seem to have been involved in both these activities.

Ap-V was also found to show catalase and superoxide dismutase activities, the specific activities being much less than those of the respective enzymes.

Summary

A metal-chelating fraction was prepared from instant coffee, and its chemical composition and properties were investigated.

A hexamine buffer (10 mM, pH 5.0) with 20 mM $ZnCl_2$ was added to instant coffee to form an insoluble zinc(II) complex. This Zn(II) complex was dissolved in an aqueous ammonia (1%) solution, and precipitated by acidification with HCl. The resulting precipitate (sample Ap) was fractionated by chromatography in anion-exchange and cellulose columns. The zinc content determined by atomic absorption spectrometry was used as a purification marker for the Zn complex.

Fraction Ap V (0.3-0.4% yield) was found to have the strongest chelating activity, with a Zn(II)-dissociation constant of -log Kd $\fallingdotseq$ 8, among the six fractions prepared by cellulose column chromatography.

Ap V was a brown amorphous compound, was soluble in water, and was antioxidative against linoleic acid. Its molecular weight was estimated to be 48,000 by HPLC. The contents of polyphenol by the Folin Denis method, of sugar, and of amino acid were found to be 30.4% as chlorogenic acid, 3%, and 4%, respectively. The chemical formula was experimentally found to be $C_{16}H_{21}O_9N_3$, and the nitrogen content of more than 10% is indicative of the involvement of the Maillard reaction.

Alkaline fusion of the Ap V fraction gave about 10% of low-molecular-weight polyphenols for the ether-soluble fraction.

Various mixtures were prepared from chlorogenic acid, sugar and protein as coffee models which were roasted and fractionated into Zn(II)-chelating fractions as instant coffee. These three compounds were essential for the formation of a brown polymer with Zn(II)-chelating activity. Increasing the proportion of chlorogenic acid in the mixture of these three compounds resulted in greater Zn(II)-chelating activity of aqueous substances in the roasted model.

Ap-V was found to be antioxidative against linoleic acid, and to show enzymatic activities of catalase and superoxide dismutase.

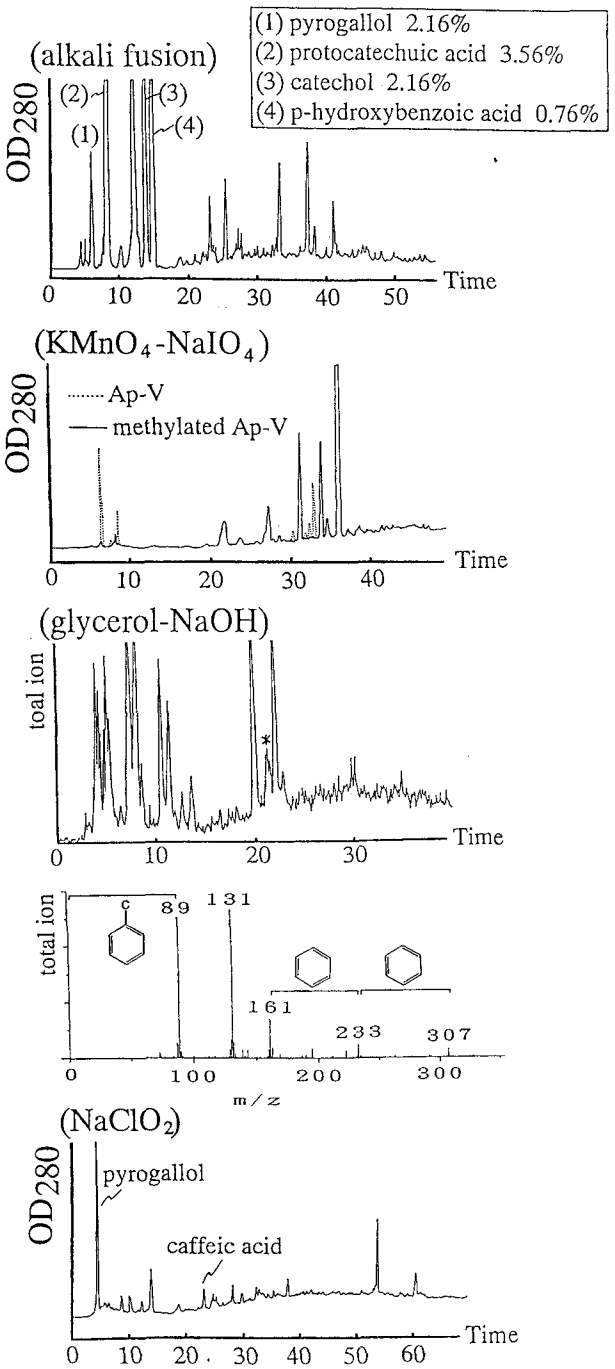

Fig.1. HPLC and LC-MS of Ap-V

Column: ODS

Eluent: Acetonitrile/Acetic acid/water

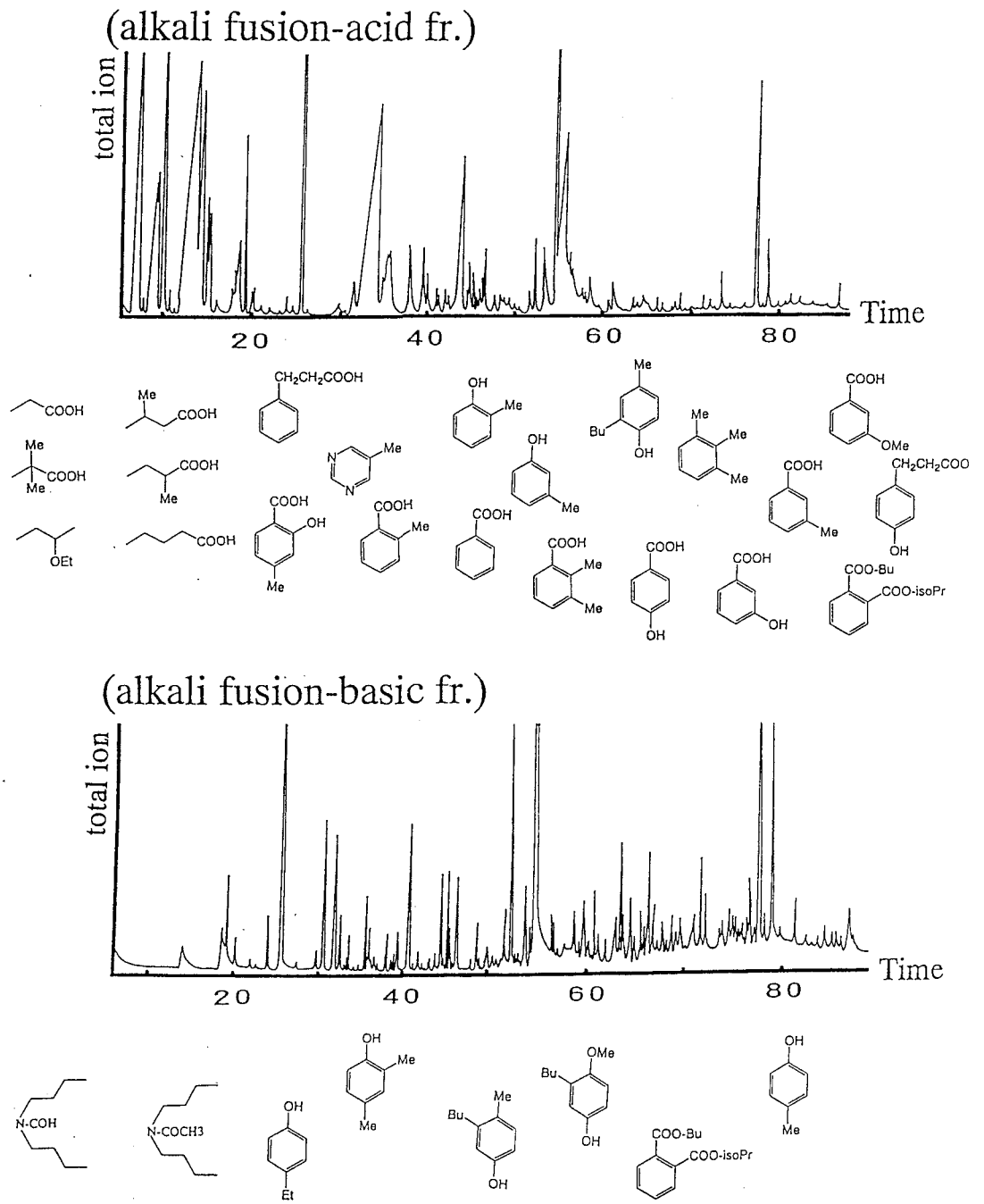

Fig.2. GC-MS of Ap-V

CHARACTERISATION OF OLIGOSACCHARIDES IN COFFEE EXTRACTS

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Green coffee bean is a carbohydrate-rich structure containing almost 50% of polysaccharides, among which arabinogalactans and mannans have been the object of detailed studies[1-4]. Arabinogalactan is a cell wall polysaccharide ranging between 14 and 17% of coffee bean dry matter, composed of a β-(1-3)-galactan backbone substituted with mixed arabinose/galactose branchings. Mannan is the storage carbohydrate typically accounting for 22%, the primary structure of which is essentially a straight chain of β-(1-4)-mannan[3-4].

Industrial coffee processing includes two successive thermal treatments : roasting responsible for pyrolysis reactions and high temperature extraction accompanied by hydrolysis[5]. Different studies have considered the fate of monosaccharides and polysaccharides upon roasting[6-7] and extraction[5,8]. Enhancement of mannan solubility, polysaccharide depolymerisation and debranching, free monosaccharides generation and degradation have been already suggested[5,8]. However, the effect of these thermal treatments on carbohydrate characteristics and properties have been little explored, partly because of the lack in analytical method for the oligosaccharide characterisation.

The complexity of oligosaccharide characterisation in coffee products results mainly from the diversity of oligosaccharide species (e.g. oligomannans, oligogalactans) and the absence of commercial standards. Different methods have been developed for characterising oligomannans produced from enzymatic hydrolysis of galactomannans using size-exclusion chromatography, n.m.r and chemical techniques[9-10].

The purpose of this work was to develop an analytical approach for characterising coffee carbohydrate with a particular attention given to the oligosaccharide fraction. Two techniques for oligosaccharide characterisation were tested and compared. These techniques were first applied to coffee extracts prepared from different roasts of a Colombian coffee.

MATERIALS and METHODS

Roasted coffees

Green *Arabica* coffee originated from Colombia. Roasting was performed in a Neuhaus Neotec RFB-S roaster using batches of 200g green coffee. Roasting temperature was set at

245°C. Light, medium and dark roast levels were obtained by stopping roasting after 160, 190 and 330 seconds respectively.

Coffee extracts
Roasted coffees were ground using a Ditting K-1400 mill. The average size of coffee particles was ca. 600µm. 10 g of coffee were suspended in 80mL of distilled water and extracted in small metallic vessels at 180°C for 20 minutes. The suspension was centrifuged at 16000g for 15 minutes. The supernatant was further filtered on a sintered glass funnel (G4). The total solid content was determined after oven drying for calculating the extraction yield. The remaining extract was kept for carbohydrate characterisation.

Carbohydrate fraction preparation
Free monosaccharides were purified from coffee extracts by filtration through a C18 cartridge. Oligosaccharides were isolated by cold precipitation of 10mL of coffee extract in 40mL of ethanol. The suspensions were centrifuged at 10000g for 10 minutes and the supernatants evaporated to dryness at 60°C. The oligosaccharides were recovered in 5mL.

Standards
Mannose, galactose, arabinose, glucose, xylose and fructose (HPLC grade) were supplied by MERCK (D-Darmstadt). Oligomannans were prepared by enzymatic hydrolysis of ivory nut β-(1-4)-mannans (Megazyme, AUS-Sydney). Mannan solutions (20mg/ml) were hydrolysed with 0.1%(V/V) gamanase (Novo-Nordisk, DK-Bagsvaerd) at 60°C for 5 to 30 minutes. The solution was filtered through sintered glass funnel (G4) and freeze-dried.

Acid hydrolysis of carbohydrates
Coffee extracts (1mL) or oligosaccharide preparations (2.5mL) were hydrolysed at 100°C for 2 hours in the presence of 200µL sulfuric acid 72%. The hydrolysates were cooled down, neutralised and completed to 50mL. The solutions was then purified on a C18 cartridge.

Characterisation of coffee carbohydrate by anion-exchange chromatography
Free monosaccharides, oligosaccharides and total carbohydrates in coffee extracts were analysed by anion-exchange chromatography using a Dionex DX300 chromatograph. 20µL were injected on a Carbopac PA 100 column (25x0.4cm, Dionex, USA-Salt Lake City) and eluted with water at 1mL/min. Detection was achieved with a PED detector after post column addition of 0.25mL/min sodium hydroxide 0.2N to the eluat.

Molecular weight distribution of coffee carbohydrates
The carbohydrate molecular weight distribution in coffee extract was analysed by size-exclusion chromatography on two superose columns 6 and 12 in series (30x1cm, Pharmacia, S-Uppsala) eluted with potassium hydroxide 0.1N at 0.5mL/min. A continuous detection was achieved with orcinol-sulfuric acid reactant[5].

Oligosaccharide separation by anion-exchange chromatography
20µL of coffee extract or standard solutions were injected on a pre-column (5x0.4cm) and two columns Carbopac PA 100 columns (25x0.4cm) in series. The oligosaccharide separation was performed at 1mL/min with a linear gradient of eluents 1 (NaOH 50mM) and 2 (NaOH 50mM/$NaCH_3CO_2$ 20mM), starting with 100% eluent 1 to reach 100% eluent 2 after 80 minutes. The columns were then washed with eluent 3 (NaOH 500mM) for 15 minutes and allowed to equilibrate with eluent 1 for another 15 minutes. Continuous post column reaction using orcinol-sulfuric acid was performed. Identification of oligosaccharides was achieved with

oligosaccharides prepared from ivory nut mannans. Quantification was performed using arabinose, galactose and mannose standards applying the correction factors (Table 1) to convert monosaccharide into oligosaccharide.

Table 1 Correction factor of monosaccharide into oligosaccharide

	DP1	DP2	DP3	DP4	DP5	DP6
Correction factor	1.00	0.95	0.93	0.93	0.92	0.92

(DP : degree of polymerisation)

Purification and identification of oligomannans

Enzymatically hydrolysed mannans were separated on a size exclusion chromatography using Sephadex G10 column (50x1.6cm, Pharmacia) and eluted with water at 0.1mL/min. Fractions were recovered every 15 minutes and checked for purity by the method developed for oligosaccharide separation. Pure fractions of each oligomannans were pooled. The degree of polymerisation was determined from the ratio reducing sugar to total mannose content.

RESULTS and DISCUSSION

Carbohydrate characterisation in coffee extract

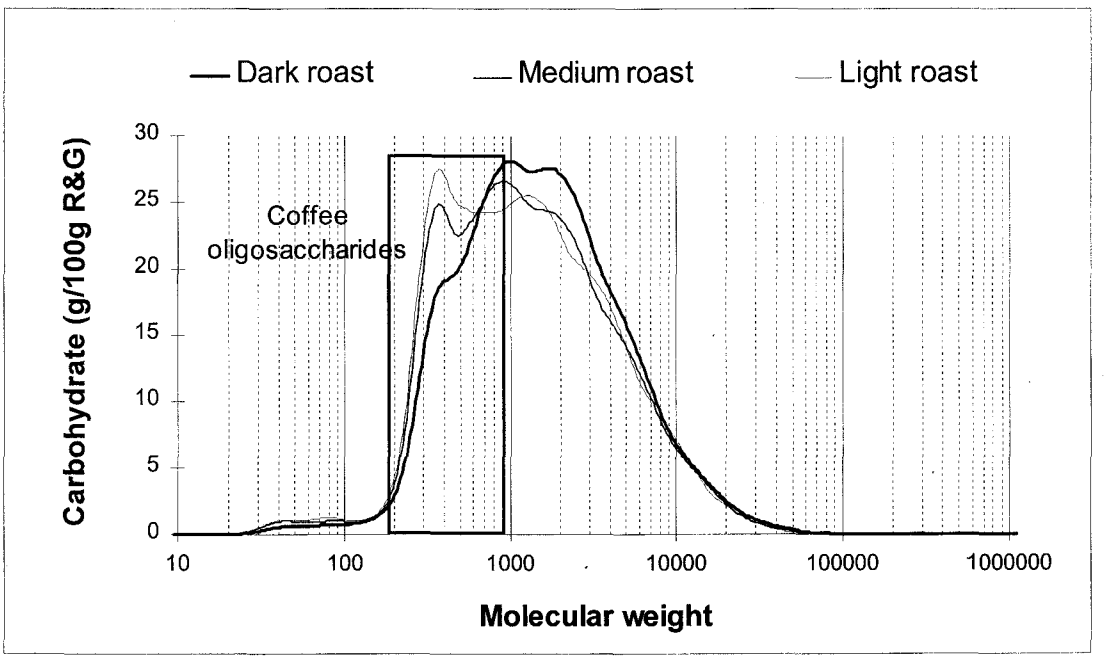
The extraction levels of the three different roasts of Colombian *Arabica* coffee were almost identical (38.4-39.2%) as well as the total carbohydrate contents in their extract (31.2-32.7%). However, the detailed carbohydrate compositions (i.e. mono-, oligo-, poly-saccharides) exhibited significant differences according to roasting intensity (Table 2). Arabinogalactan level was nearly halved with increased roasting (from 22.4 to 14.4%), the reduction affecting all carbohydrate families : mono- (2.6 to 1.5%), oligo- (4.0 to 3.1%), and polysaccharides (15.7 to 10.8%). On the other hand, mannan content was almost doubled in extract at darker roast (8.7 up to 16.0%). This increase is essentially due to high molecular weight species (4.9 up to 11.8%).

Table 2 Extraction yields and carbohydrate composition of coffee extracts

	Ara	Gal	Glu	Xyl	Man	Fru	Total + other
	(g/100g of dry extract)						
Extract 1 - Light roast - Extraction yield 38.4%							
DP 1	1.70	0.92	0.21	0.02	0.31	0.18	3.64
DP 2 to 6	0.00	4.02	0.49	0.07	3.45	0.00	8.03
DP>6	0.67	15.04	0.21	0.01	4.92	0.00	20.92
Total	2.38	19.99	0.91	0.10	8.68	0.18	32.59
Extract 2 - Medium roast - Extraction yield 39.2%							
DP 1	1.45	0.83	0.14	0.01	0.32	0.14	3.16
DP 2 to 6	0.00	4.41	0.60	0.07	4.49	0.00	9.57
DP>6	0.20	12.53	0.04	0.00	5.60	0.01	18.44
Total	1.65	17.77	0.79	0.08	10.41	0.15	31.17
Extract 3 - Dark Roast - Extraction yield 39.1%							
DP 1	0.90	0.60	0.08	0.01	0.28	0.14	2.29
DP 2 to 6	0.00	3.08	0.40	0.06	3.84	0.00	7.37
DP>6	0.57	10.24	0.22	0.02	11.84	0.00	23.05
Total	1.47	13.92	0.70	0.08	15.96	0.14	32.71

An other aspect of coffee carbohydrate characterisation are the molecular weight distributions obtained by size-exclusion chromatography (Figure 1). These results are in perfect agreement with the data in Table 2, confirming that polymers are more abundant in extract obtained from dark roast whereas mono- and oligosaccharides are in greater amount in light and medium roast extracts.

Figure 1 Carbohydrate molecular weight distribution of coffee extracts obtained for different roasting levels.

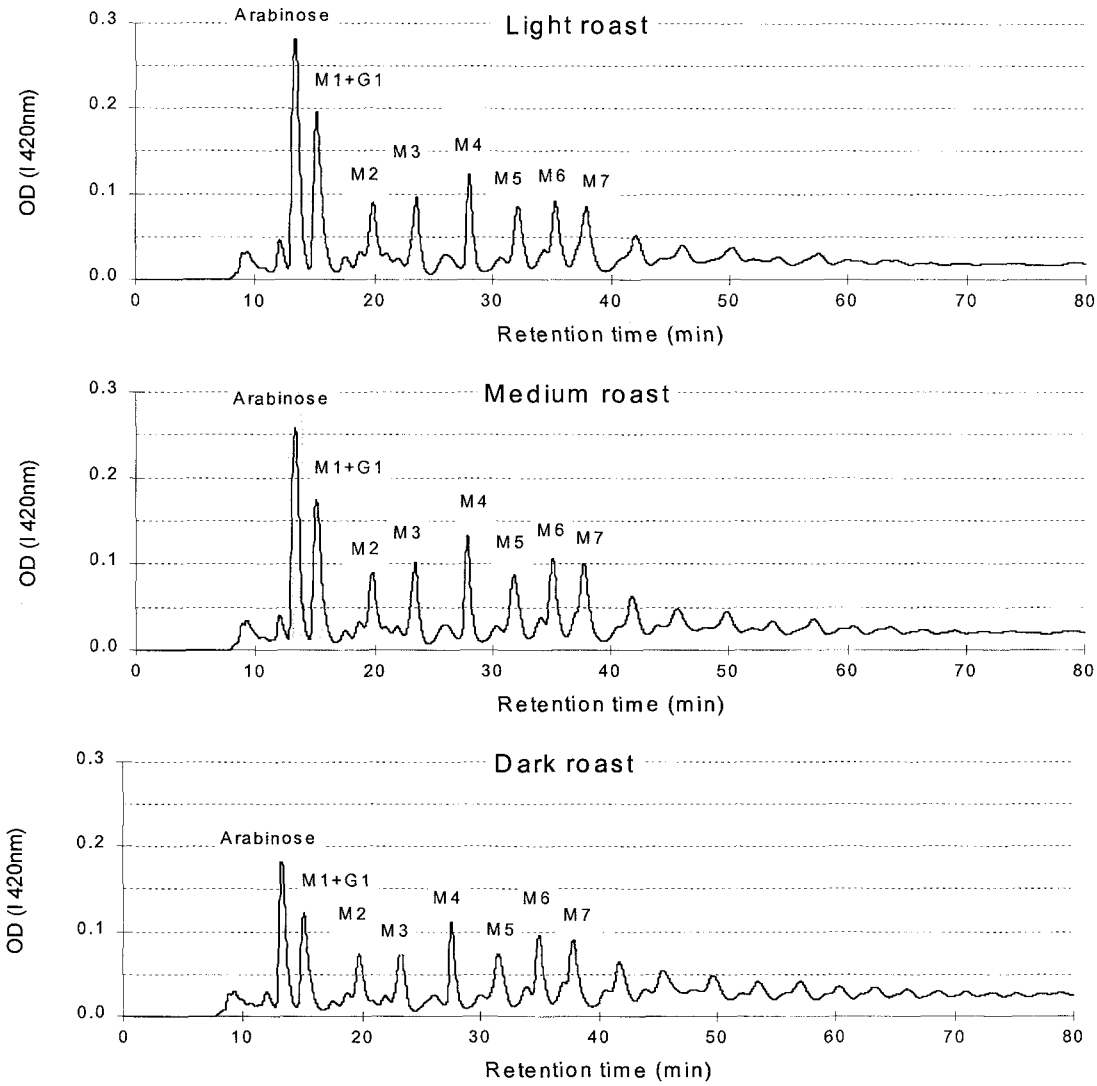


Oligosaccharide separation

Figure 2 presents the separation of oligosaccharides by anion exchange chromatography for the three different extracts. The chromatographic profiles are fairly comparable for the different roasts. In order to ensure the identification and quantification of coffee oligosaccharides, oligomannans have been prepared through enzymatic hydrolysis from ivory nut mannans. The purification of mannobiose and mannotriose are illustrated in Figure 3. Oligomannans have been named on the chromatograms of coffee extracts. Intermediary peaks are oligogalactans the structure of which has not yet been totally elucidated. The quantification of oligosaccharides could be easily performed owing to the orcinol-sulfuric detection by using the correction factors of mannose and galactose reported in Table 1.

The oligomannan and oligogalactan contents calculated from the chromatograms are presented in Table 3. The profiles of oligogalactans from light and medium roast extracts are almost identical, whereas that obtained from darker roast shows lower content in species of low degree of polymerisation (DP≤3). On the opposite, similarities can be observed for oligomannans between medium and dark roast extracts, the extract from light roast presenting a deficit in large oligomannans (DP≥4).

Figure 2 Separation by anion-exchange chromatography of oligosaccharides in coffee extracts prepared from coffee of different roasting levels.



The quantification of oligomannans and oligogalactans by chromatography is in fairly good agreement with the contents obtained after precipitation in ethanol, when taking into account oligomers up to 5 units (Table 3).

This approach allows an accurate characterisation of the coffee carbohydrates according to size and composition. An excellent agreement has been obtained between the different analytical procedures (e.g. chromatography of extract and carbohydrate hydrolysates, size exclusion chromatography).

The detailed knowledge of the structures of the oligosaccharides contained in these coffee extracts provides information on the effect of roasting on coffee carbohydrate structure. Roasting has an ambivalent action on coffee carbohydrates. On the one hand roasting improves mannan extractability and on the other hand it favours polysaccharide depolymerisation, and oligo- and mono-saccharide formation and degradation. Maximal levels

of both oligomannans and oligogalactans are thus found in extracts prepared from medium roast.

Table 3 Oligomannan, oligogalactan and oligosaccharide quantification. Comparison of the chromatographic and the ethanol precipitation methods.

	DP1	DP2	DP3	DP4	DP5	DP6	ΣDP_{1-5}	Total EtOH
				(g/100g of dry extract)				
Extract 1 - Light roast								
Oligomannans	0.31	1.05	1.08	1.08	1.13	1.08	4.65	3.76
Oligogalactans	2.62	0.82	0.73	0.76	1.66	1.08	6.59	6.64
Oligosaccharides	2.93	1.87	1.81	1.84	2.80	2.16	11.25	10.40
Extract 2 - Medium roast								
Oligomannans	0.32	1.08	1.14	1.18	1.26	1.19	4.98	4.81
Oligogalactans	2.28	0.76	0.70	0.77	1.79	1.19	6.30	6.69
Oligosaccharides	2.60	1.84	1.84	1.95	3.05	2.38	11.28	11.50
Extract 3 - Dark Roast								
Oligomannans	0.28	0.99	1.06	1.16	1.28	1.23	4.77	4.12
Oligogalactans	1.50	0.72	0.56	0.72	1.80	1.23	5.30	4.58
Oligosaccharides	1.78	1.71	1.62	1.88	3.08	1.46	10.07	8.70

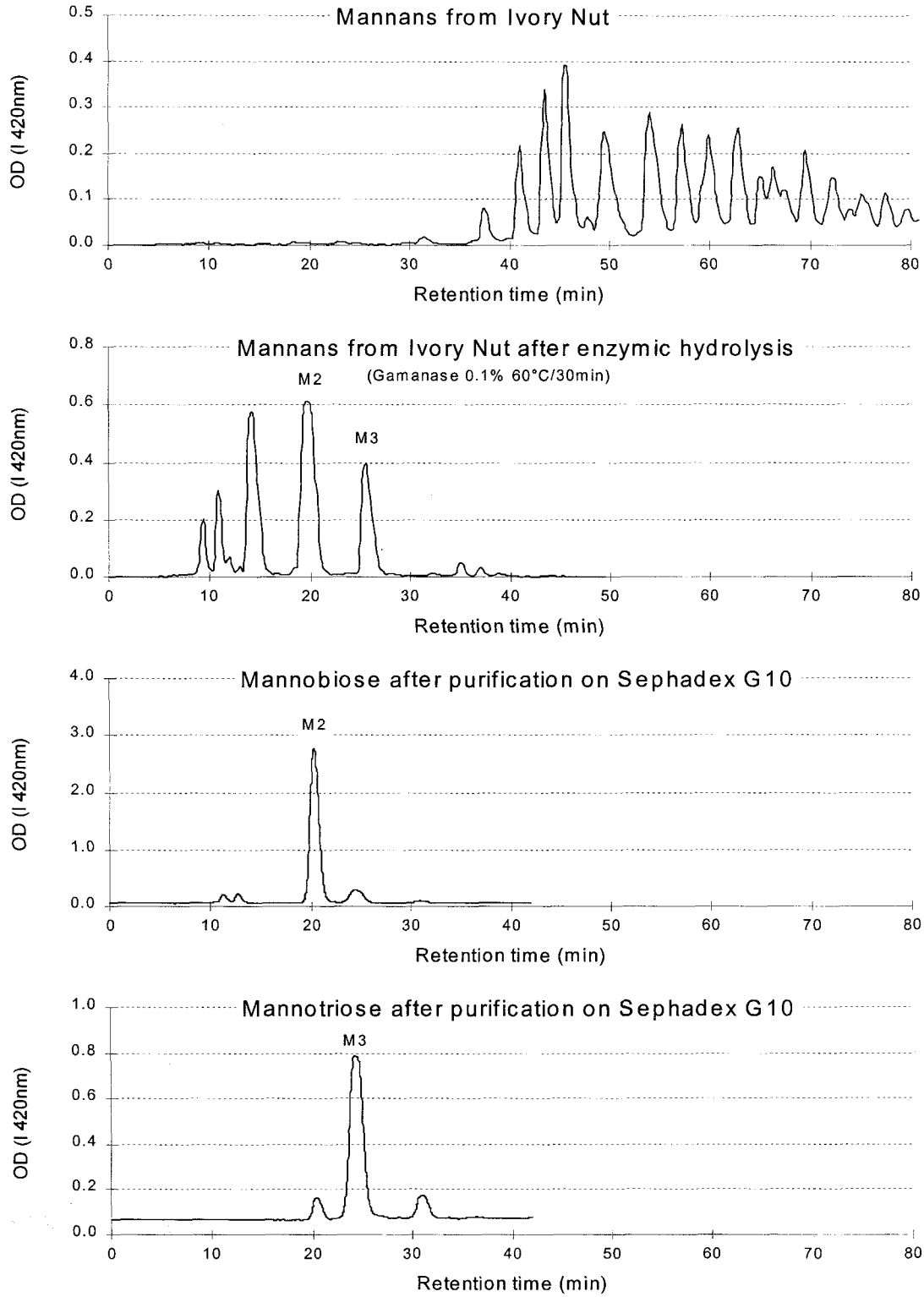
SUMMARY

With a view to extending the knowledge on the composition of coffee extracts, an anion-exchange chromatography method has been developed for the characterisation of oligosaccharides. A good separation has been obtained for coffee extracts prepared at 180°C and from three different roasts of an *arabica* coffee. Identification and quantification have been performed with oligostandards prepared by enzymic hydrolysis of ivory nut mannans. The results agreed very well with complementary analyses such as molecular weight profile and chemical characterisation of the carbohydrates, thus filling a gap in the already existing methods for the characterisation of carbohydrates in coffee products.

RESUME

Une méthode de séparation des oligosaccharides par chromatographie d'échange d'ions est présentée dans le but d'affiner la caractérisation de la fraction glucidique des extraits de café. Une bonne séparation des oligosaccharides a été obtenue sur des extraits préparés à 180°C à partir de trois différentes torréfaction d'un café *arabica*. L'identification et la quantification ont été réalisées à l'aide d'oligomannanes préparés par hydrolyse enzymatique de mannanes de noix d'ivoire. Les résultats sont en bon accord avec des analyses complémentaires telles que la distribution de masse moléculaire et la caractérisation chimique des glucides, indiquant que cette méthode pourra efficacement combler un vide analytique dans la caractérisation des extraits de café.

Figure 3 Preparation of mannobiose and mannotriose from ivory nut mannans through enzymatic hydrolyis.



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FACTORS AFFECTING MANNAN SOLUBILITY IN ROAST COFFEE EXTRACTS

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Introduction

A common feature of a beverage prepared from soluble coffee powders is the formation of precipitate or sediment on standing. Sediment formation during the soluble coffee process is also a known problem and has been the subject of some inventions, e.g. (1). Little attention has been given to the chemical nature of these sediments, although it has been claimed that protein derived compounds are present (2). Also potential components of the sediments are the extracted polysaccharides, in particular the linear mannan molecules, which have are known to have low solubility (3).

Coffee mannan is a linear polymer of β(1-4) linked anhydromannopyranose units. At approximately 22% dry weight basis, it has the highest content of the many constituents of roasted coffee (4). At extraction temperatures of up to 100°C (e.g. as in percolated coffee), solubilized mannan yields are low. However, at higher extraction temperatures, e.g. as used in commercial instant coffee production, mannan yields increase so that, in a typical process, about one third of the linear mannan fraction is extracted. In this publication the important role of the mannan fraction in the sedimentation from solutions of soluble coffee is demonstrated.

Experimental

Total carbohydrate analysis

Samples (40 mg) were heated in sealed vials with 2N triflouroacetic acid (120°C, 1 h). After dilution and passage through a C-18 cartridge, the hydrolysed residue was analysed for its component monosaccharides using Dionex ion exchange chromatography (5).

Preparation of sediments

Solutions of commercial soluble coffees (10 g/200 ml in 95°C water, stirred while cooling to room temperature) were centrifuged. The remainder of the supernatent was decanted off, the sediment stirred with water (200 ml) and the mixture then centrifuged and the supernatent decanted off as before. This was repeated twice, at which stage the supernatent was almost clear. The sediment pellet was dried in a convection oven overnight at 40°C and weighed.

X-ray diffraction analysis

X-ray diffraction analyses were made on finely ground material (pestle and mortar) using a Debye-Scherrer powder camera. Experimental details will be published elsewhere (6).

Preparation of mannans

Coffee mannan was isolated from green Arabica coffee beans as described elsewhere (3). Ivory nut mannan was prepared by alkali extraction of Ivory Nut meal (6).

Results and Discussion

Sediment composition

The total carbohydrate profiles of a series of commercial soluble coffees and their corresponding sediments are given in Tables 1 and 2, respectively.

Table 1. Total carbohydrate content of soluble coffees.*

	Carbohydrate Content % wt.			
Sample	Ara	Gal	Glc	Man
1	3.2	17.7	0.8	14.2
2	2.7	13.6	0.5	13.8
3	3.2	16.2	0.6	14.3
4	2.8	15.0	0.9	19.1
5	3.5	18.2	0.6	13.2

* Expressed as monosaccharide (calculated as anhydro-monosaccharide) produced by acid hydrolysis.

The mannan content is represented by the values for the anhydromannose yields; the arabinogalactan is given by (Ara+Gal).

Table 2. Yields and carbohydrate composition of coffee sediments.

	Yield, % wt.	Carbohydrate Content % wt.			
Sample		Ara	Gal	Glc	Man
1	2.28	0.4	2.5	0.0	60.1
2	2.48	0.4	2.7	0.3	66.8
3	2.41	0.5	2.8	0.0	63.4
4	2.19	0.4	2.2	0.0	55.8
5	2.16	0.5	2.8	0.0	59.0

It was apparent that enrichment of the mannan fraction occurred in the sediment; i.e. the mannan was less water soluble than the other components. The other principal polysaccharide in soluble coffee, the arabinogalactan, was more soluble and was not found to any significant degree in the sediments. Other analyses indicated that the sediments also contained a proteinaceous component (ca. 5 to 10% total amino acids produced by acid hydrolysis). The remainder of the sediments was not readily characterizable and was attributed to roasting products of the melanoidin type.

An explanation was sought for the marked difference in polysaccharide solubility. With respect to the chemical structures (3), the β(1-3) galactan backbone with the frequent irregular short side chains, would be expected to give the arabinogalactan polymer high water solubility. In contrast, the linear β(1-4) linked mannan polymer, which has a minimum of one-unit galactose side chains (3), should have a rather stiff two-fold helical conformation in solution. Such molecules show a high propensity to hydrogen bond and to form crystalline regions (7) leading to a significant reduction in solubility. In order to see whether this was occurring in the coffee extracts, X-ray diffraction was used to monitor the crystallinity of the mannan containing fractions.

Crystallinity of mannan

The X-ray powder diffraction pattern of a soluble coffee sediment revealed several sharp rings (Figure 1A). Comparison of this pattern with that of Ivory Nut mannan showed that they were identical (Figure 1B). This crystalline pattern was attributed to the Type I mannan structure (7) which is associated with lower molecular weight mannan and relatively rapid crystallization (8). Isolated coffee mannan also gave the Type I pattern. The other well characterized form of mannan is that attributable to the Type II structure, which occurs in high molecular weight mannan (9). End-group determinations have indicated that the average molecular weight of the mannan fraction in green coffee is about 6,000 (3).

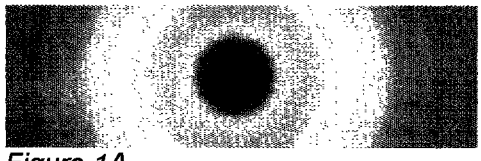


Figure 1A

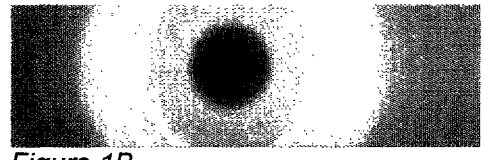


Figure 1B

X-ray diffraction measurements of the freeze-dried soluble coffee fraction, after removal of the sediment, only gave a faint mannan pattern (Figure 1C). No sign of crystallinity was evident in a roasted coffee sample (Figure 1D). Consequently, the mannan in the R&G coffee and in the solubilized extract was essentially non-crystalline or amorphous. Thus it can be concluded that, on standing of the solubilized coffee solids, a gradual conversion of the soluble amorphous mannan to an insoluble form occurred, which resulted in the formation of precipitated polymer. This would explain the faint pattern in Figure 1C, where some mannan crystallization presumably occurred during the freeze-drying process.
The formation of crystalline mannan in the coffee solution can be attributed to the ability of the molecules to interact, and thus align to form hydrogen bonds which leads to areas of crystallinity and eventually insolubility. This process is favored by the molecular linearity and relatively low molecular weight of the coffee mannan.

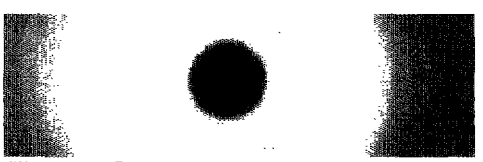


Figure 1C

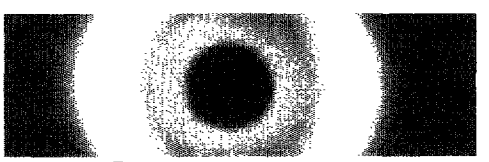


Figure 1D

Effect of Storage Temperature and Time

Holding of solubilized soluble coffee at elevated temperatures led to an increase in sediment yield (Figure 2), suggesting that low temperature storage favored lower sediment yields. Mannan contents initially increased but the yield at 80°C was less than that at 60°C. The meaning for this was not clear although it was possible that crystal stability was enhanced at the higher temperatures leading to incomplete acid hydrolysis in the carbohydrate analysis procedure. Thermally induced crystallization is not unusual in polysaccharides (10). The crystalline mannan was shown to be stable in boiling water which indicated the stability of the coffee sediment.
Sediment yields increased steadily with time of storage (Figure 3). The yield of precipitated mannan also increased thereby emphasizing the importance of the mannan crystallization step as a driving force in sediment formation in soluble coffee beverages and extracts.

Figure 2: Effect of Storage Temperature on Sediment and Precipitated Mannan Yields

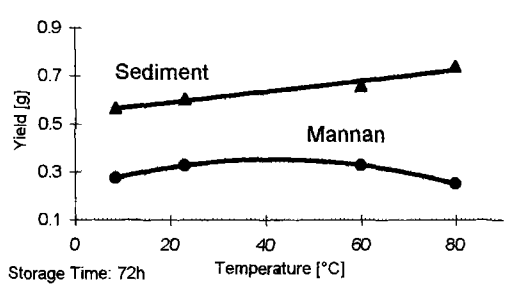
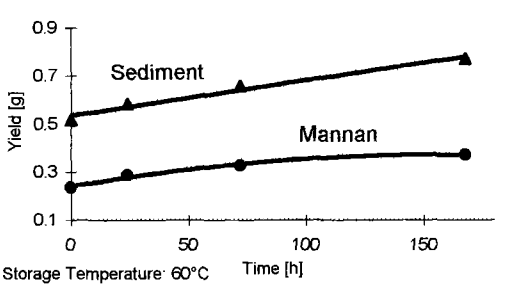


Figure 3: Effect of Storage Time on Sediment and Precipitated Mannan Yields



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Summary

β(1-4) mannan was shown to be a major component (ca. 60%) of insoluble sediment formed from soluble coffee. In aqueous solution of soluble coffee, the mannan, which was amorphous in roast coffee, gradually converted to an insoluble crystalline form, resulting in precipitation. Most of the precipitate remained insoluble on prolonged boiling, indicating the stability of the mannan Type I crystal formed. Yields of crystalline mannan and consequently sediment, were increased by higher temperatures and longer storage times. The other components of coffee sediment were proteinaceous (ca. 5-10%) and uncharacterized, probably melanoidin type, fractions.

Résumé

Le β(1-4) mannane apparaît comme un composant majeur (environ 60%) du sédiment insoluble formé à partir de café soluble. Dans une solution acqueuse de café soluble, le mannane, qui était amorphe dans le café torréfié, se convertit graduellement en une forme cristalline insoluble aboutissant à la précipitation. La plupart du précipité reste insoluble durant une ébullition prolongée, ce qui indique la stabilité du cristal de mannane type I formé. Les rendements en mannane cristallin, et par conséquent en sédiment, sont augmentés par des températures plus élevées et des temps de stockage plus longs. Les autres composants du sédiment de café sont d'une part des dérivés de protéines (environ 5-10%) et d'autre part des composants non identifiés, probablement des fractions de type mélanoidine.

FATTY ACID ESTERS OF CAFESTOL

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Introduction

In different scientific investigations, authors have reported that the consumption of coffee can lead to an increase of the serum cholesterol level [1-5]. In the case of filter coffee, this effect is very low and can be disregarded, but after drinking boiled coffee (Scandinavian-type), the effect was very high. In order to prepare Scandinavian coffee, the coffee powder is directly boiled with water so that more substances pass from the coffee powder into the brew [6-8].

Investigations have shown, that some components of the coffee oil are responsible for the increase of the serum cholesterol level. Recently published investigations have revealed that the diterpene cafestol in coffee oil induces this effect [9-12].

Besides cafestol, further diterpenes such as kahweol and 16-O-methylcafestol are present in the coffee oil. The total diterpene content of the lipid fraction in coffee beans is about 20 %.

There is only a small amount of cafestol in free form, whereas the greatest part is esterified with fatty acids. Up to present, only a few cafestol fatty acid esters have definitely been identified, but as twelve different fatty acid esters have already been detected in the diterpene 16-O-methylcafestol [13, 14], a similar number of esters were therefore expected for cafestol.

In the case of the cholesterol problem, it is important to know, whether the content or the distribution of the cafestol esters is different in various coffees, which influences the roasting has and, of course, whether or how many esters are passed into the prepared coffee brew.

Identification of the Cafestol Fatty Acid Esters of a Robusta Coffee

In 1962, Kaufmann and Hamsagar [15] identified six cafestol fatty acid esters by using paper chromatography. Folstar et al. [16] analysed three further fatty acids by gas chromatography after saponification of a diterpene mixture from an Arabica coffee containing cafestol esters and kahweol esters. They were not, however, able to definitely attribute these acids to the diterpene cafestol.

The cafestol fatty acid esters are components of the coffee oil.
In order to identify the individual esters it is necessary to separate them from the lipid matter. This can be accomplished in three chromatographic steps (Fig. 1). To begin with, the mixture of diterpene esters is separated from the higher molecular and quantitative predominant triglycerides using gel permeation chromatography (Bio Beads S-X3) and ethyl acetate/cyclohexane (1/1). Following this, further components such as sterol esters and the 16-O-methylcafestol esters can be extracted using solid phase extraction on silica cartridges, so that a solution is finally obtained, which solely contains the different cafestol fatty acid esters.

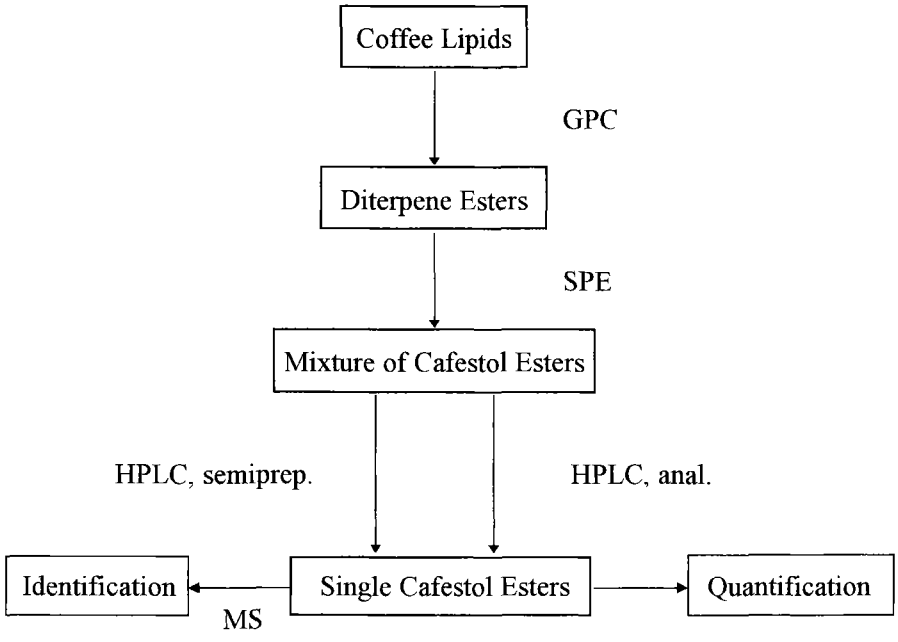

Fig. 1. Scheme of identification and quantification of cafestol esters

In the third stage, the mixture of cafestol fatty acid esters is chromatographed on a semipreparative RP-18 column based on a separation method for 16-O-methylcafestol esters, developed by our work group [14]. The elution areas of the individual peaks were collected, concentrated and analysed using solid-probe mass spectrometry.
The cafestol fatty acid esters, which have already been described by Kaufmann and Hamsagar and by Lam et al. [17] as well as the acids identified by Folstar et al. after the saponification of a diterpene fatty acid ester mixture, could now definitely be proved to be esters of the cafestol. In addition, the cafestol esters with the unsaturated fatty acid $C_{20:1}$ and some odd-numbered fatty acids such as C_{17}, C_{19}, C_{21} und C_{23} were identified (Fig. 2). The occurrence of these minor components is not surprising because various odd-numbered fatty acids were already analysed in the triglycerides and in the 16-O-methylcafestol esters of the coffee oil [14, 18].

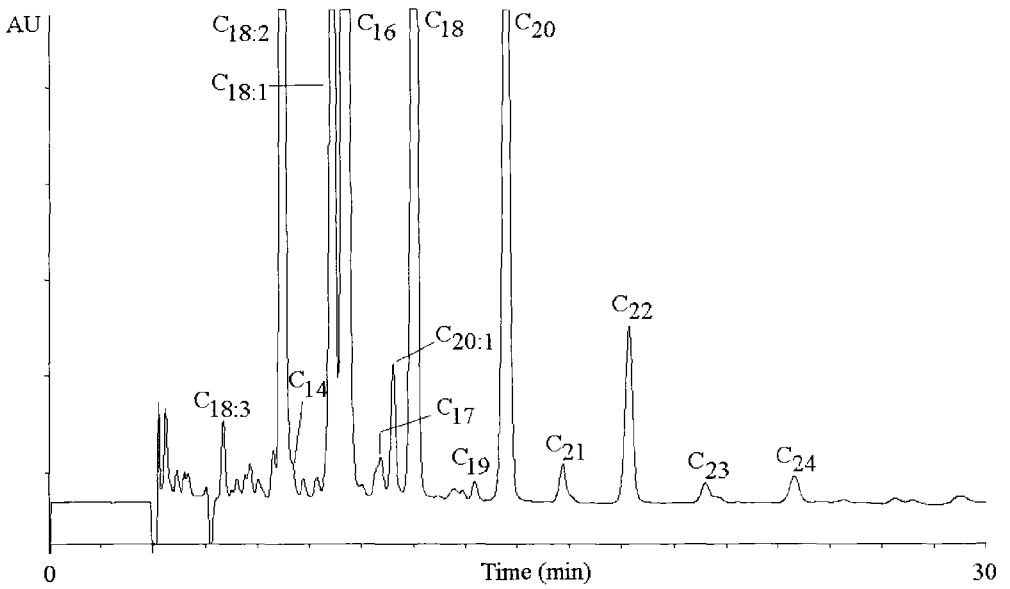

Fig. 2. HPLC chromatogram of cafestol fatty acid esters from a green Robusta coffee (Zaire) Nucleosil 120-3 C_{18}, 250x4 mm; acetonitrile/iso-propanol (60:40), 0,6 ml/min; UV 220 nm

Synthesis of the Cafestol Fatty Acid Esters

In order to be able to investigate the cafestol fatty acid esters, it was necessary for them to be synthesised. The principle of the synthesis is described below in short (Fig. 3): Free cafestol reacts with the fatty acid chloride at room temperature. After this reaction, the formed diterpene fatty acid ester as well as the free fatty acid and the cafestol, which is not converted, are present in the reaction vessel. Having added sodium carbonate solution to precipitate the free fatty acid and applying chromatogaphic methods, the pure synthesised ester is finally obtained. This can be tested with respect to purity by means of HPLC and diode array detection.

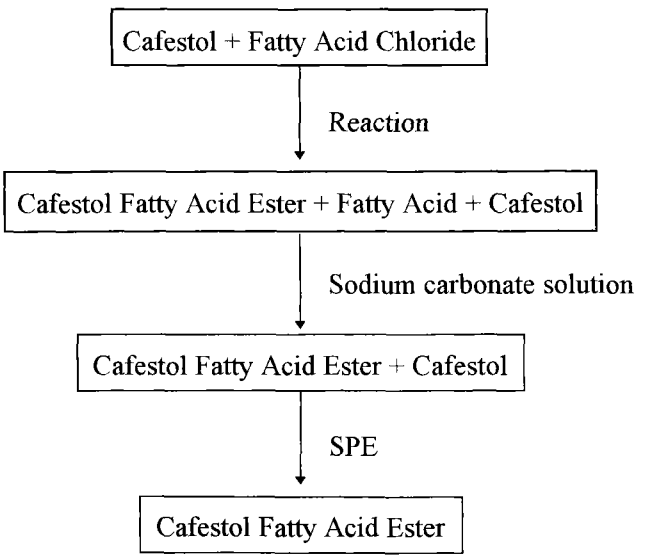

Fig. 3. Scheme of synthesis

Distribution of Cafestol Fatty Acid Esters in Green Robusta coffees

Green Robusta coffees from different Asian and African countries were analysed in order to find out whether there are differences in the distribution of the cafestol esters.

It was observed that the individual cafestol esters were irregularly present in the coffee oil. The odd-numbered fatty acid esters were minor components, whereas cafestol palmitate, cafestol linoleate, cafestol oleate, cafestol stearate, cafestol arachidate and cafestol behenate existed in large amounts. During the following analyses the focus was therefore placed on these six cafestol fatty acid esters.

In Figure 4, the proportional distribution of the six cafestol esters from some green coffees is shown. Hence, it is obvious that the distribution of the six esters is not significantly different.

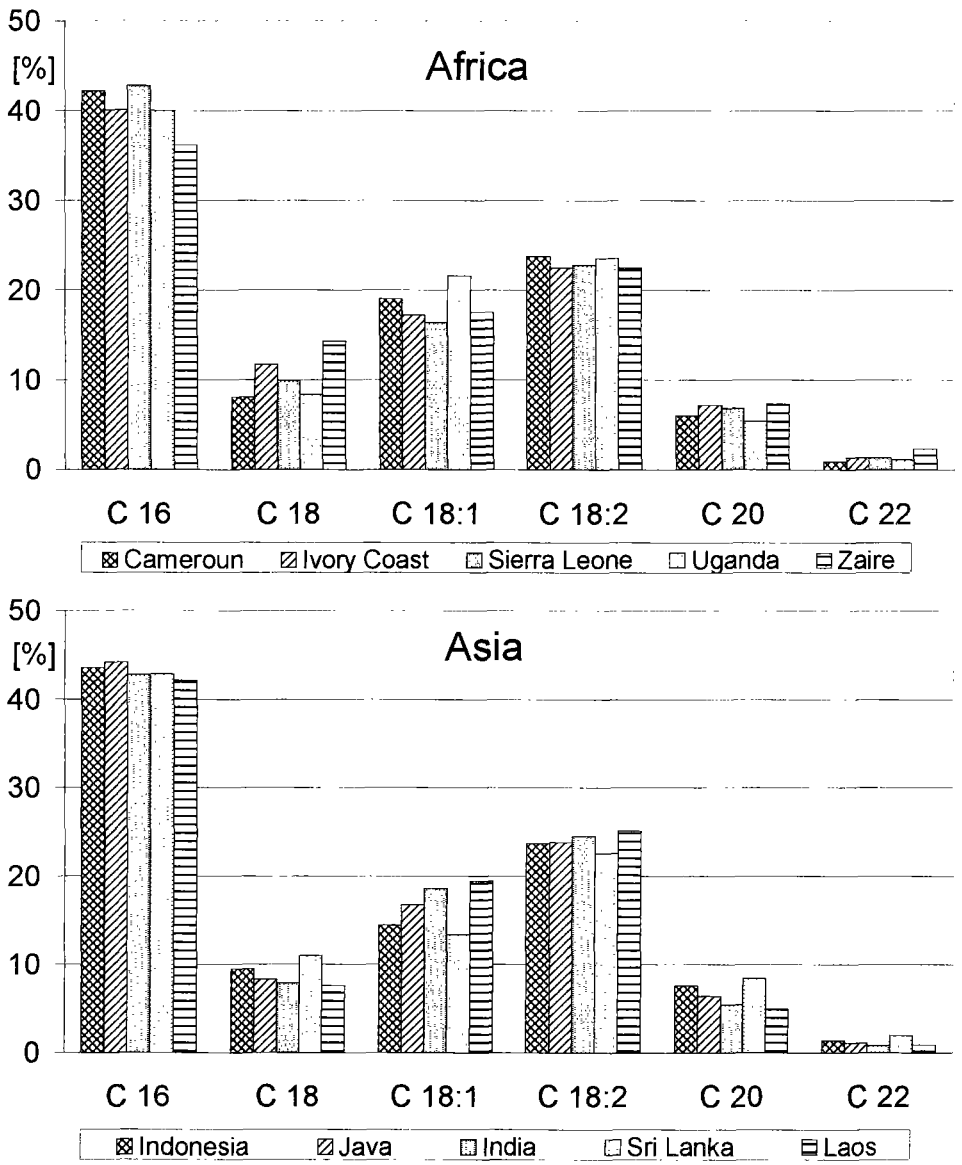

Fig. 4. Distribution of the main cafestol esters in some green Robusta coffees

Total Content of Cafestol Esters in Green Robusta Coffees

The ester content of the analysed green Robusta coffees is represented by sums in Figure 5. In comparison with the proportional distribution, differences were analysed between the various green coffees. Quantities from 2,2 to 7,6 g/kg dry weight were found. Coffees from Asia showed a slight variation in their quantity from 2,3 to 5,5 g/kg dry weight, whereas the range for coffees from Africa was much higher. Here, quantities from 2,2 to 7,6 g/kg dry weight were found.

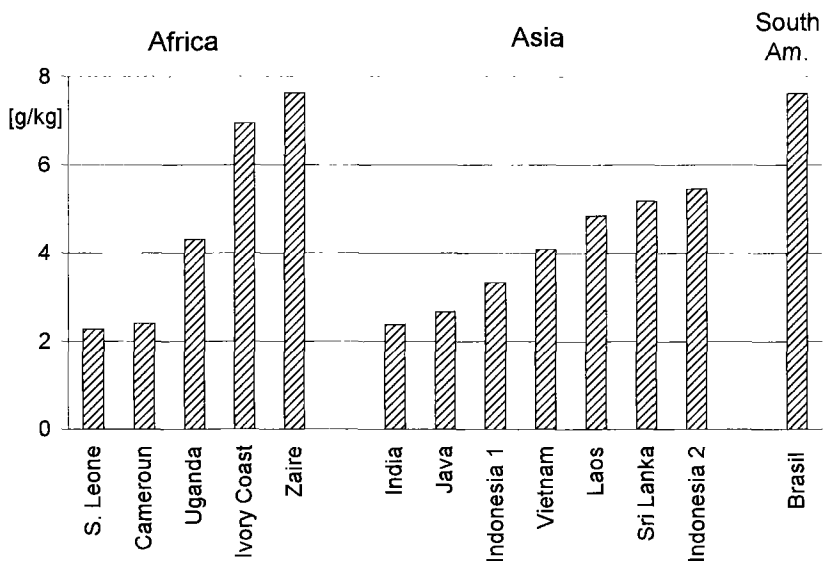

Fig. 5. Total contents of the main cafestol esters in green Robusta coffees in g/kg dry weight

Cafestol Fatty Acid Esters in Green Arabica Coffees

These six cafestol fatty acid esters were likewise analysed in green Arabica coffees. Whereas there is only a small difference in the proportional distribution of the coffees, the total content is clearly higher in Arabica coffees than in Robusta coffees. These results will be published shortly.

Stability Behaviour of some of the Cafestol Esters during Roasting

In order to be able to analyse the influence of roasting on cafestol fatty acid esters, a green coffee was roasted at two different temperatures. The outcome was that the total cafestol ester content, related to the fat, of the first roasting stage increased slightly compared to the green coffee. The rise in roasting temperature, however, let to a slight decrease of the total cafestol ester content.

The proportional distribution of the cafestol esters can be seen in Figure 6. It is therefore obvious that the esters have homogenous roasting properties.

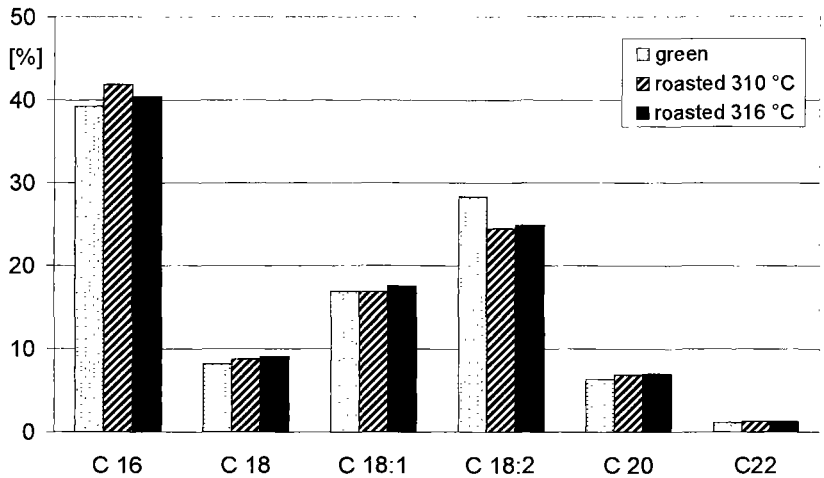

Fig. 6. Distribution of the main cafestol esters in green and roasted coffees (roasting time: 180 sec)

Cafestol Fatty Acid Esters in Beverages

The increase of the serum cholesterol level due to filter coffee is small, whereas after drinking Skandinavian coffee, this level is high. For our exemplary investigations, an espresso brew was therefore analysed, which represents a position in the middle.

An espresso brew was prepared by using a household Krups espresso machine with coffee roasted at 316 °C. The brew was freeze-dried and the cafestol fatty acid esters analysed as described in Figure 1.

From the total content of the six esters of the roasted coffee only around 0,5 % passed into the beverage. There was no significant difference in the proportional distribution of the cafestol esters in the coffee powder and the prepared brew, so that all esters were extracted during the preparation of the espresso in the same way (Fig. 7). This finding is in accordance with the results of the diterpene 16-O-methylcafestol [6].

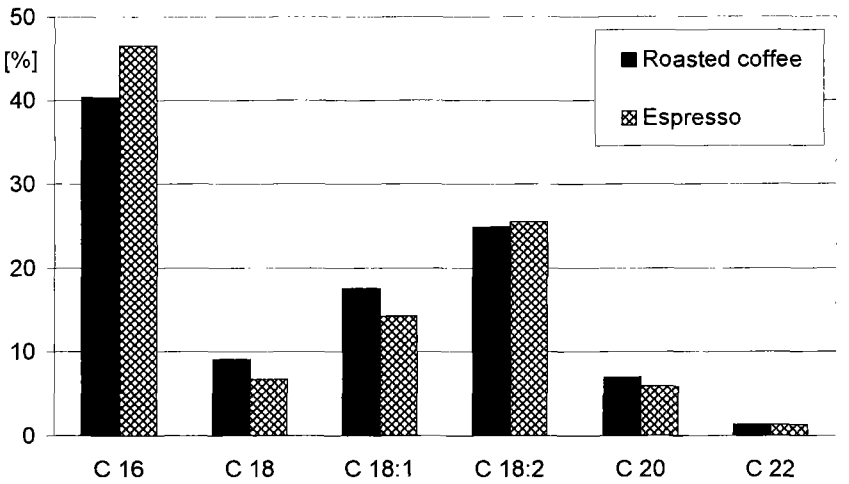

Fig. 7. Distribution of the main cafestol esters in the roasted coffee powder and the espresso

Acknowledgement
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Summary
The diterpene cafestol occurs in Robusta and in Arabica coffees. There is only a small amount of cafestol in free form, whereas the greatest part is esterified with fatty acids. Using gel permeation chromatography equipped with a Bio Beads S-X3 column and subsequent chromatography on silica cartridges, it is possible to separate various cafestol fatty acid esters. Fourteen fatty acid esters were isolated by semipreparative RP-HPLC and identified by solid-probe mass spectrometry. The cafestol esters detected in coffee oil can be obtained as standard matter by synthesis. The behavior of individual esters could therefore be investigated directly for the first time. The content of some cafestol esters was analysed in green coffees from different countries. By roasting coffee at various temperatures it was proved that the proportional ester distribution is not changed. The distributions of the esters in coffee powder and in the prepared espresso show no significant differences as well.

Zusammenfassung
Das Diterpen Cafestol kommt sowohl in Arabica- als auch Robusta-Kaffees vor. Nur geringe Anteile liegen in freier Form vor, der größte Teil hingegen ist mit Fettsäuren verestert. Durch Gelpermeationschromatographie an Bio Beads S-X3 und anschließender Säulenchromatographie an Kieselgel-Einmaltrennsäulen gelingt es, die Cafestolfettsäureester aus Kaffeeöl abzutrennen. Vierzehn Fettsäureester des Cafestols wurden durch HPLC an einer semipräparativen RP-18-Säule isoliert und anschließend mit Schubstangen-Massenspektrometrie identifiziert. Die im Kaffeeöl nachgewiesenen Ester konnten als Standardsubstanzen durch Synthese gewonnen werden, so daß das Verhalten einzelner Ester untersucht werden konnte. Gehalte und Verteilungen ausgewählter Ester wurden in Robusta-Rohkaffees verschiedener Länder analysiert. In einem bei unterschiedlichen Temperaturen gerösteten Kaffee konnte nachgewiesen werden, daß sich die prozentuale Esterverteilung kaum verändert. Auch zwischen der Verteilung der Ester im Kaffeepulver und in einem daraus hergestellten Espresso ergaben sich kaum Unterschiede.

MALDI-MS, A NEW ANALYTICAL TECHNIQUE AND ITS POTENTIAL FOR COFFEE ANALYSIS

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1. Introduction

Since its introduction in 1987 by Karas and Hillenkamp[1] and Tanaka [2], **M**atrix-**A**ssisted **L**aser **D**esorption **I**onization **T**ime-**o**f-**F**light **M**ass **S**pectrometry (MALDI-TOF MS) has received a great deal of interest. Commercial instruments began appearing in 1991. The increasing publicity and expansion of this technique went together with the rapid development of biotechnology and its growing need for accurate and sensitive molecular mass determination of bio-polymers. The potential of MALDI-MS to satisfy these needs has been demonstrated in a wide field of applications. The capability of MALDI-MS for characterization of peptides, proteins, carbohydrates and oligonucleotides has been demonstrated [3, 4, 5]. To date, only a few applications of MALDI-TOF MS in food analysis are published [6].
It is aim of this work to review the principles of MALDI-TOF MS and to assess its potential for coffee analysis. Examples are shown on how MALDI can be used for mass determination of coffee carbohydrates and coffee proteins.

Direct use of MALDI as a **quantitative** technique is very limited by principle experimental parameters for instance by local concentration variations in the crystal structure [7]. The second part of this paper describes fundamental examinations to overcome these problems. The applicability of using internal standards for quantitative oligosaccharide analysis in coffee is demonstrated and the repeatability of quantitative MALDI is discussed.

2. Principle

The principle of MALDI-TOF MS is shown in Fig. 1 and 2. Sample compounds are embedded in crystals of a matrix substance and irradiated with a laser beam. The most common matrix substances are aromatic compounds such as 2,5-dihydroxybenzoic acid and sinapinic acid. The laser energy is

absorbed by the matrix and transferred to the sample molecules, which are desorbed and ionized without major fragmentation.

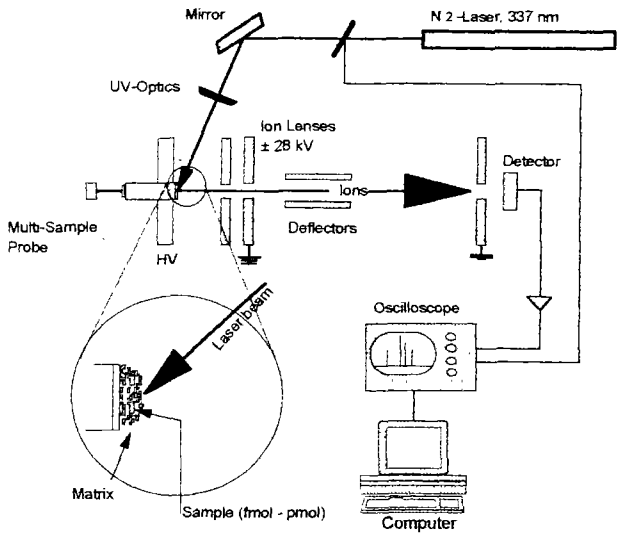

Fig. 1: Principle of MALDI-TOF Mass Spectrometry

Usually, MALDI is coupled with a *time-of-flight* (TOF) mass spectrometer (see Fig. 2). Due to the unlimited mass detection range of these instruments, (bio-) polymers up to 500,000 Da can be measured by this kind of ionization.

After desorption and ionization of the sample ions, these ions are first accelerated by an applied electrical field and subsequently enter the flight tube. The whole system is operated at a vacuum of approx. 10^{-7} torr. Ions at different masses have different energies, thus arrive at the detector at different times (µsec range). After entering the flight tube, the time of flight from the entrance to the detector, which is a function of the molecular mass, is measured.

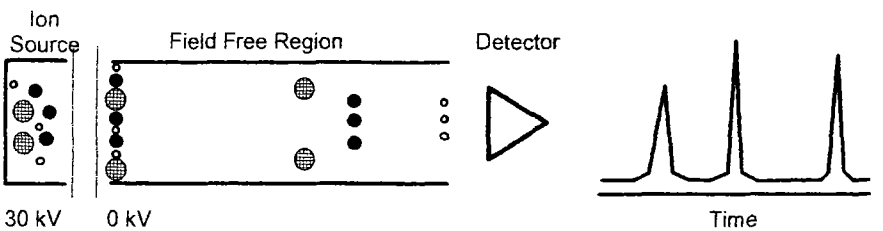

Fig. 2: Fundamentals of Time-of-Flight (TOF) Mass Spectrometry

As most other analytical techniques MALDI-TOF MS needs to be calibrated by compounds with known molecular masses. Because only very little fragmentation by the soft ionization procedure takes place, the measured mass spectrum mostly shows only single charged ions. Occasionally double charged ions or dimers are detected. Saccharides are known to possess high affinity to alkaline or alkaline earth metal ions. Therefore, sugars are generally measured as adducts of these ions, in particular as sodium adducts, even though only traces of these ions are present.#

3. Experimental

3.1. Instrumentation

All experiments were carried out on LASER Desorption Mass Spectrometer type G2025A, Hewlett Packard, Waldbronn, Germany.

Anion Exchange Chromatography for quantification of free and bound carbohydrates was performed using a metal-free liquid chromatograph equipped with pulsed amperometric detection (Dionex, Idstein Germany). Details of this method are described somewhere else [8,9].

3.2. Chemicals

Internal mass calibration of the instrument has been done based on commercially available mixtures of peptides purchased from Hewlett Packard.

All chemicals used were of highest purity available. Aminochinolin (AC), α-cyano-hydroxycinnamic acid (CHC), hydroxyisochinolin (HIC), 2,5-dihydroxybenzoic acid (DHB), α-, β- and γ-cyclodextrin, 2,6-di-o-methyl-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, N-acetylgalactosamine and dextrin-20 was purchased by Fluka, Buchs. Calibration of oligosaccharides was performed by using dextrin-20.

3.3. Sample Preparation

Coffee extracts were made by dissolving 10 mg coffee powder in water followed by filtration of the solution with Sartorius membrane filter (0.45 mm) and desalting the filtrate with Dowex mixed bed ion exchange resin. 10 ml of this solution were mixed with 10 ml of a 0.1 M 2,5-dihydroxybenzoic acid in acetonitrile. 1 ml of this mixture was transferred to the probe of the instrument. Finally, the solvent was evaporated in vacuum. The mass spectrometer was calibrated using dextrin 20 as reference prior to analysis of the unknown sample.

3.4. Instrumental Conditions

Instrumental conditions for all oligosaccharide determinations were:

- Polarity: positive modus
- Ion optics: 10 kV
- Laser energy: ca. 7 µJ
- Mass range: 5,000 Da
- Mass filter: 0 Da
- Detector-sensitivity: 1000 mV FS
- Detector voltage: - 4.75 V
- sampling rate: 5.0 nsec
- Vacuum: $< 9 \times 10^{-7}$ torr
- Calibration: 3-point-calibration

4. MALDI Analysis of Coffee Oligosaccharides

4.1. General considerations

Oligomannans as all other saccharides are detected as sodium and/or potassium adducts in MALDI-TOF MS. Owing to the higher concentration of sodium in natural samples intensive signals of sodium adducts and minor signals of potassium adducts are measured.

4.2. Optimization of the matrix

On the basis of own investigations of saccharides and of literature data the following substances were examined for their suitability as matrix :

i 2,5-dihydroxybenzoic acid (DHB),
ii aminochinolin (AC),
iii hydroxyisochinolin (HIC)
iv α-cyano-hydroxycinnamic acid (CHC) and
v mixtures thereof.

Criteria for optimization were linearity of baseline, high resolution, low noise, high yield of spectra and laser energy needed. All chemicals were tested using the same concentration but different solvents because of differences in the solubility of the matrices.

DHB has proven to be best suited for the analysis of oligomannans. Compared to commercially available standard solutions of DHB in methanol as solvent a further improvement was obtained by

preparing the matrix in acetonitrile. Fig. 3 shows a typical MALDI mass spectrum of a coffee extract obtained with DHB and CHB.

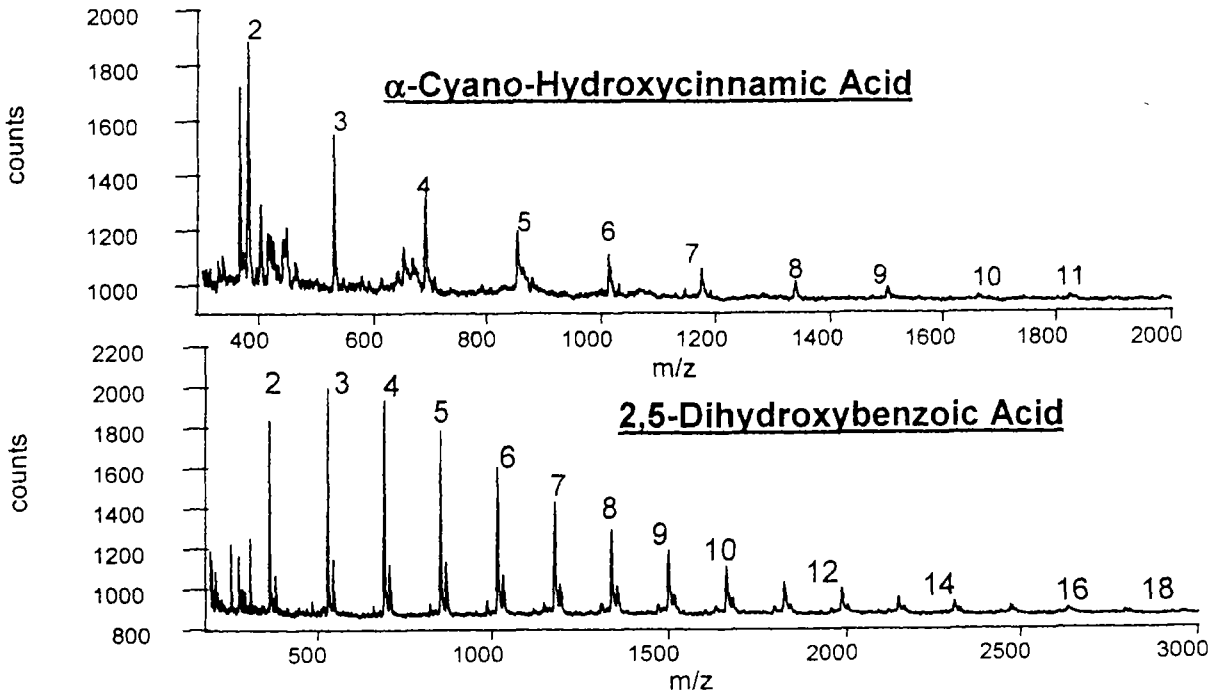

Fig. 3: MALDI Spectrum of Instant Coffee: Comparison of DHB (below) with CHC (above) as matrices

In theory all ions are detectable by TOF MS, even low masses such as sodium and potassium ions. In practice interferences caused by impurities and / or the matrix reduce the applicability of MALDI to higher masses. As a consequence carbohydrate monomers aren't clearly detectable.
Using DHB as matrix oligosaccharides of ca. 20 carbohydrate units (mol weight 3100 DA) were found in the coffee sample as shown in Fig. 3. MALDI cannot differentiate between single monomers (all same MW). Using High Performance Anion Exchange Chromatography (HPAEC) after total hydrolyzation of the sample the carbohydrate profile has been determined (see table).

Table: Total carbohydrate composition of the investigated Instant Coffee sample:

Arabinose	Rhamnose	Galactose	Glucose	Xylose	Mannose	**TOTAL**
3.65 %	0.45 %	17.11 %	1.00 %	0.00 %	16.09 %	**37.30 %**

Based on this carbohydrate composition, the theoretical structure of an oligosaccharide of a DP 20 found in soluble coffee has been calculated. The following formula was received:

$$(Ara)_{3.7} - (Rha)_{0.5} - (Gal)_{9.2} - (Glucose)_{0.5} - (Xylose)_0 - (Mannose)_{8.6}$$

4.3. Selection of the internal standard

Preliminary experiments revealed the external standard method not to be suited for quantitative analysis by MALDI-TOF MS. Thus a method was developed based on an internal standard, which was added to the sample in a known concentration. For the choice of an internal standard substance a number of prerequisites had to be fulfilled:

1. the substance must be pure and well defined,
2. the substance must not interfere with signals of sample components
3. the substance must possess similar properties like the analytes
4. the substance should be commercially available
5. the substance must be soluble in a suitable solvent.

For this purpose α-, β- and γ-cyclodextrin, 2,6-di-o-methyl-β-cyclodextrin, 2-hydroxypropyl- β-cyclodextrin and N-acetylgalactosamine were examined. All cyclodextrin derivatives were not suited because of heterogeneity of the chemical composition and interference with sample signals. For instance, potassium adduct of β-cyclodextrin gives the same signal as the sodium adduct of DP-6 and DP-7. However, N-acetylgalactosamine showed to be suited as an internal standard for the analysis of oligosaccharides (see Fig. 4).

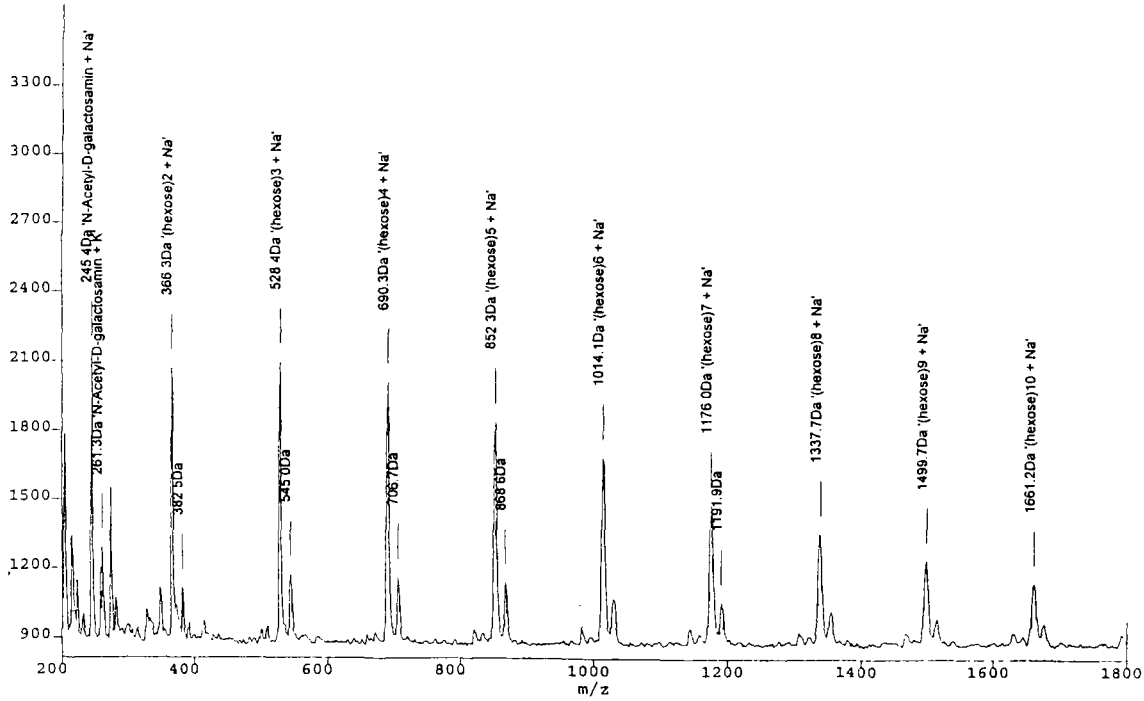

Fig. 4: MALDI mass spectrum of Instant Coffee with N-acetylgalactosamine as internal Standard

4.4. Determination of the useful concentration range and linearity

The optimal concentration range of internal standard and sample was investigated by using mixtures of defined oligosaccharides (maltose, maltohexaose and maltoheptaose) as model compounds.

Linearity of peak area and peak height were measured over a range of approx. one order of magnitude at two different concentrations of the internal standard.
Mass spectra were evaluated with respect to the ratio of peak area and peak height, respectively, of the internal standard to the sample. The following concentration ranges were studied:

Concentration of internal standard	125 - 1250 [mM]
Concentration of DP-2, DP-6 and DP-7	25 - 417 [mM]

It became obvious that better linearities are obtained, when peak areas are used. Correlation coefficients of better than 0.99 demonstrate the potential of MALDI as a quantitative method.
It was observed that the slopes of the linear correlations which represent the individual response factors of the oligosaccharides depend on the degree of polymerization (DP). The higher the DP of the oligosaccharide is, the bigger the corresponding signal response is.

4.5. Investigation of the reproducibility

The reproducibility of the analytical method was investigated by 4 replicate analyses of an oligosaccharide model (dextrin-20) using *N-acetylgalactosamine* as internal standard . Evaluating the peak areas resulted in reproducibilities between 5,5 % to 16,7 % (relative standard deviations, RSD).

4.6. Quantitative determination of the concentration of a maltooligosaccharide model system by means of the Standard Addition procedure

The principle of the standard addition procedure is based on the addition of known concentrations of the sample components to a sample with unknown concentration. This technique is well suited in cases where matrix effects influence the detection signal. For this purpose three different concentrations of maltodextrin DP-2, -3, -6 and -7 were added to a given concentration of dextrin-20 (see fig.5).

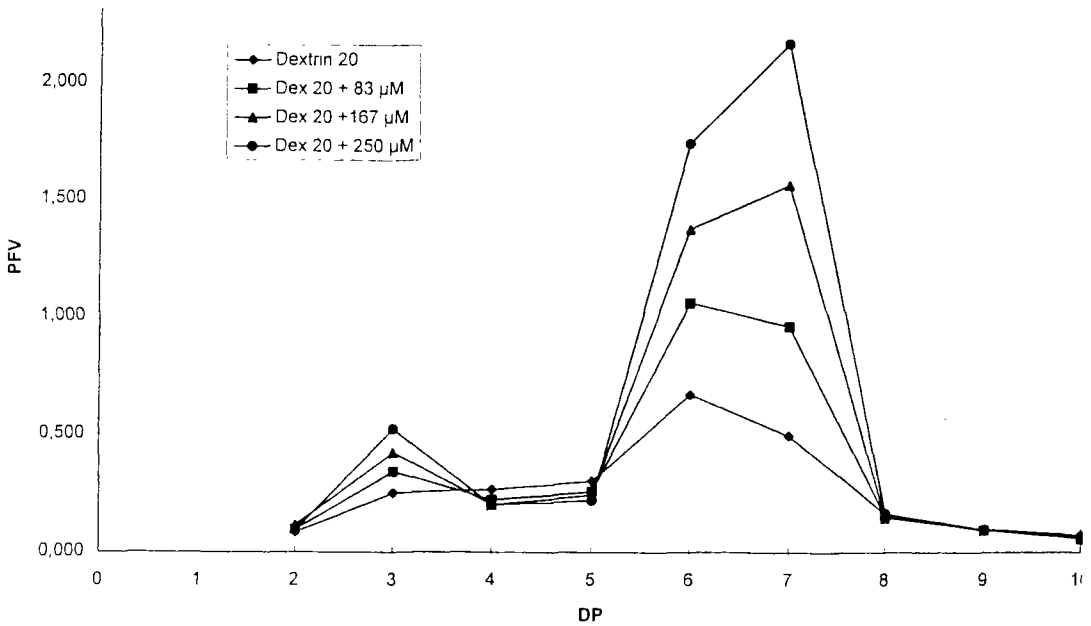

Fig. 5: MALDI MS signal intensities of dextrin oligosaccharides with different amounts of DP-2, -3, -6 and -7 added

Evaluation of mass signals shows, that only the ratios of the peak area of those compounds added to the sample are increased. The varying increases in peak areas after addition of defined concentrations of the four oligosaccharides illustrate the different responses of the

corresponding maltodextrins. While maltose (DP-2) exhibits only a small response to a concentration change, the influence of the concentration on the ratio of peak areas becomes bigger with increasing molecular weight.
By considering these results, it can be concluded, that MALDI-TOF mass spectrometry can be used for the quantitative determination of oligosaccharides with DPs higher than 6.

4.7. Quantitative determination of the concentration of oligomannans of a coffee extract by means of the standard addition procedure

The standard addition technique was applied to the determination of the concentration of oligomannans in coffee extracts. Known concentrations of malto-oligosaccharides with DP-2, DP-3, DP-6 and DP-7 (standards) were added to the coffee sample. Fig.6 shows the obtained mass spectrum of same coffee sample previously discussed (see Fig. 4).

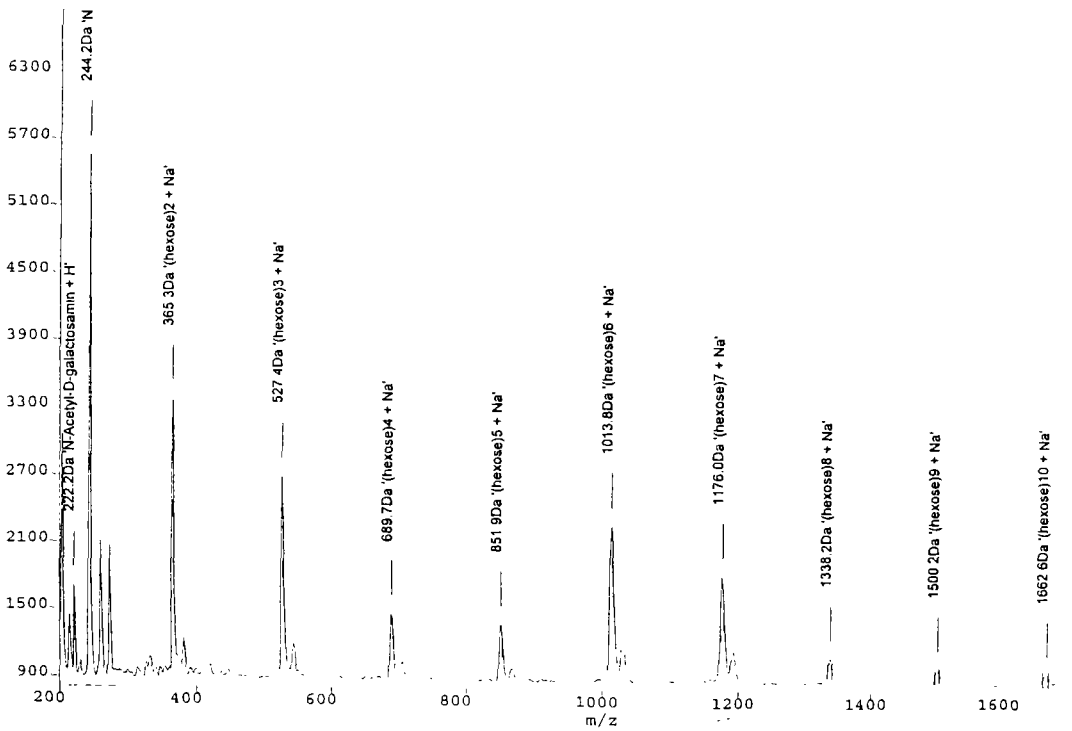

Fig. 6: MALDI mass spectrum of a coffee sample to which known standards (DP-2, -3, -6 and -7) are added (1 mg/mL coffee sample was mixed with 1250 mM of internal standard and 20 mM of each DP-2, DP-3, DP-6 and DP-7)

To examine the linearity of the method, different amounts of standards were applied. In all cases excellent linearities were found (correlation coefficients 0.96 - 0.99). Again different response factors of the coffee oligosaccharides were measured. In addition the response factors were different compared

To examine the linearity of the method, different amounts of standards were applied. In all cases excellent linearities were found (correlation coefficients 0.96 - 0.99). Again different response factors of the coffee oligosaccharides were measured. In addition the response factors were different compared to the dextrin oligosaccharides. These results imply, that the composition of the sample (nature of the carbohydrate units, non-carbohydrate constituents) has an impact on the response of individual signals.

Concentrations of oligomannans in a coffee extract have been calculated from the Y-intercepts obtained by standard addition method and were found as follows:

DP	2	3	4	5	6	7	8	9	10	11	12	13	14
concentration [µM]	707	173	139	74	81	21	16	10	6.5	4.9	4.3	1.8	1.5

5. MALDI Analysis of Arabica Green Coffee Protein

Further aim of this work was to explore the applicability of MALDI for characterization of proteins in coffee. For this purpose preliminary experiments were carried out where MALDI was applied for characterization of fractions obtained from green Arabica coffee beans. Aqueous extracts of ground Arabica beans were separated by gel permeation chromatography using Sephadex G-20 stationary phase material and freeze-dried. Using amino acid analysis protein containing fractions were identified and submitted to MALDI analysis. No further sample preparation was carried out. Sinapinic acid has been used as matrix substance.

The suitability of MALDI for protein characterization is exemplary demonstrated in Fig 7. Two main constituents with MW 7324 and 7445 Da are detected.

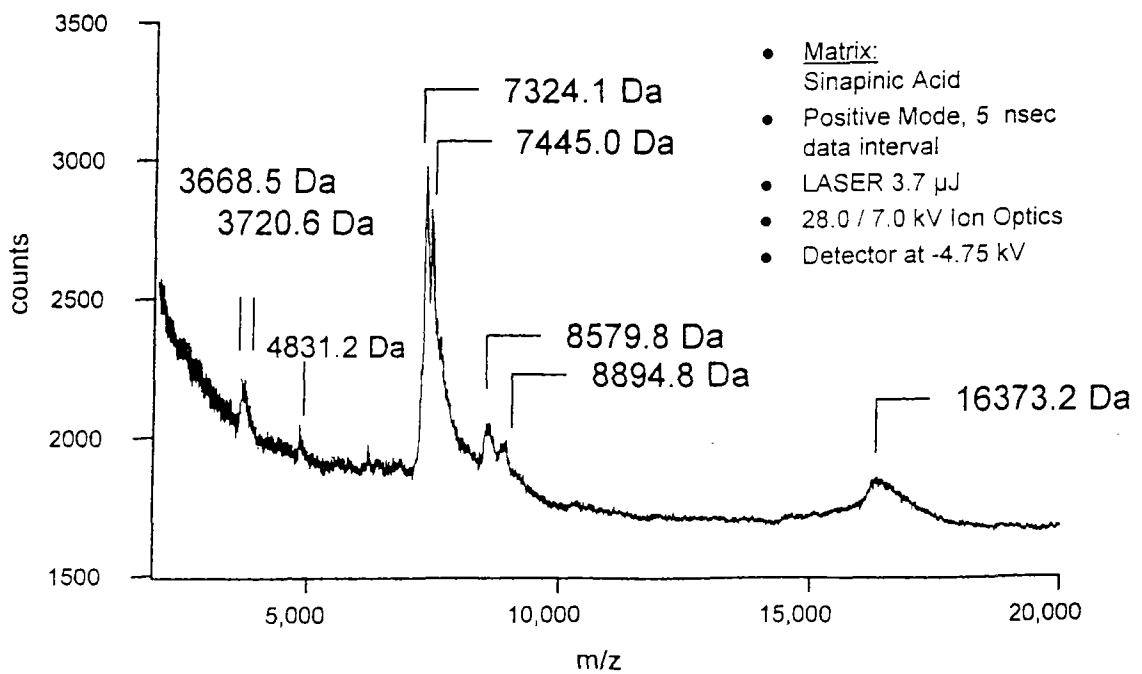

Fig. 7. Characterization of coffee proteins by MALDI-TOF mass spectrometry.

Summary

For the first time MALDI-TOF mass spectrometry was used in coffee analysis. Successfully exact molecular weight determination of oligosaccharides in coffee extracts was performed. Without any comprehensive sample clean-up step, large oligosaccharide up to a degree of polymerization of 20 could be identified. Procedures for quantification were developed based on using internal standards and standard addition. The developed methods proved to be linear and reproducible.
In addition the suitability of MALDI for molecular mass determination of coffee proteins has been demonstrated.

Résumé

Pour la première fois la spectrométrie de masse MALDI-TOF a été utilisée dans l'analyse du café. La détermination exacte du poids molaire d'oligosaccharides du café a été accomplie avec succès. Sans étape complète de nettoyage de l'échantillon, les oligosaccharides allant jusqu' á 20 de degré de polymérisation ont pu être identifiés. Des procédés de quantification basés sur l'utilisation de standards internes ainsi que sur l'addition de standard furent développés. Il a été prouvé que les méthodes dévelopées sont linéaires et reproductibles.
De plus il a été démonté que MALDI était approprié à la détermination de la masse moléculaire des próteines du café.

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DITERPENES IN COFFEE LEAVES

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Introduction

Coffee diterpenes have always been of great scientific interest. Due to this it is obvious that many questions have already been answered. It is known that cafestol and kahweol are present in Arabica coffee beans, whereas Robusta coffee beans contain cafestol, small amounts of kahweol and additionally 16-O-methylcafestol.

Nothing, however, is known about the presence of the three diterpenes in other parts of the coffee tree. For example, are there diterpenes in the coffee leaves or in the pulps?

Diterpenes in Green Leaves

In order to answer this the leaves from a Coffea Arabica and some from a Coffea Canephora were analysed first. The leaves were therefore freeze-dried, ground and saponified. The unsaponifiable matter was then extracted and the diterpenes were investigated as described below (Fig. 1). The identification was performed on HPLC with diode array detection and by GC-MS.

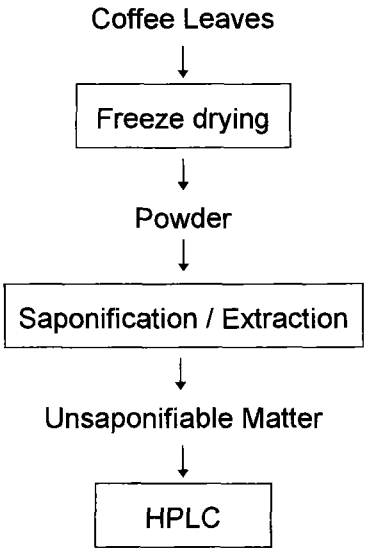

Fig. 1. Scheme of analysis

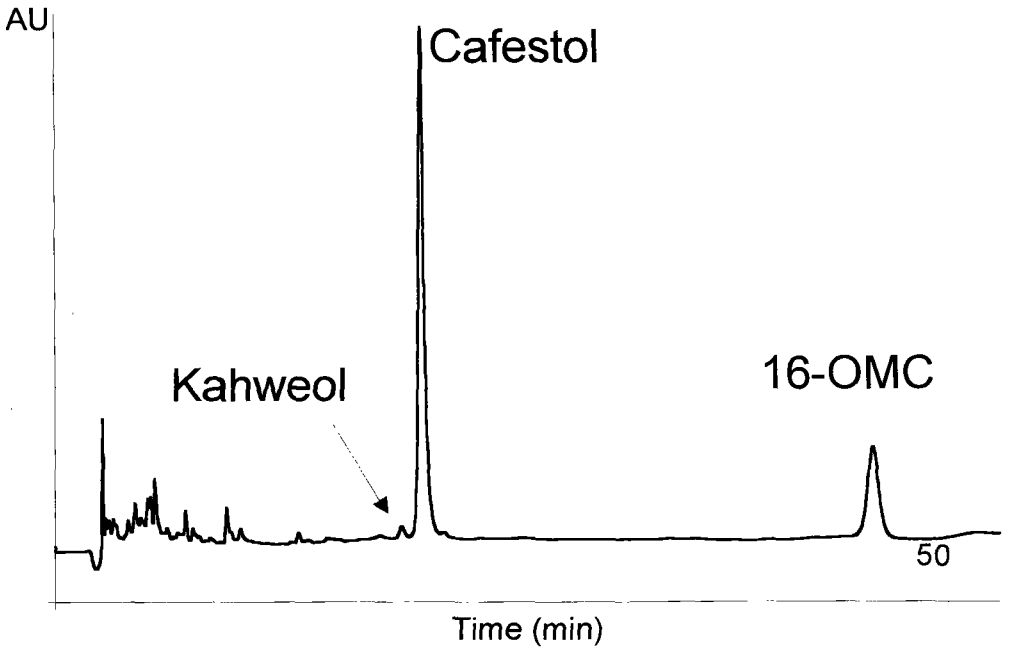

Fig. 2. HPLC chromatogram of Coffea Arabica San Ramon leaves
Nucleosil 120-3 C_{18}, acetonitrile/water (50:50); 0,6 ml/min; UV 220 nm

Figure 2 shows the chromatograms of the analysed Arabica and Canephora leaves. In both cases, diterpenes could be detected: in the Arabica leaves cafestol and 16-O-methylcafestol, in the Canephora leaves cafestol and kahweol. These findings were surprising, because they were in absolute contrast to the results of the corresponding coffee beans.

Hence, the leaves of other Arabica varieties were analysed: Catui, Murta and Caturra yellow. The results were the same as before for Arabica San Ramon. In each Arabica sample cafestol and 16-O-methylcafestol were present but either only small amounts of kahweol or even none at all, were found (Fig. 3).

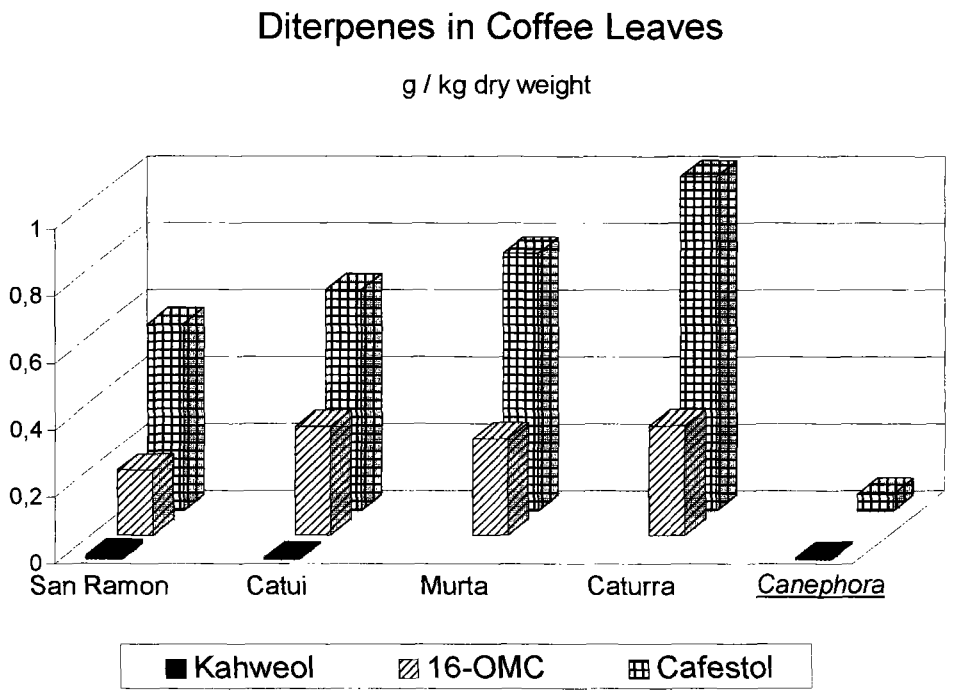

Fig. 3. Contents of the diterpenes in leaf samples of four Arabicas and one Canephora

The cafestol contents ranged from 0,56 to 1,04 g/kg solid matter of coffee leaves, the 16-O-methylcafestol from 0,19 to 0,33 g/kg, whereas the kahweol contents were very slight with less than 0,012 g/kg (The solid matter was between 30 and 36 % of the fresh weight.).
In comparison to this, the canephora leaves, which were examined, contained 0,049 g of cafestol as well as 0,004 g kahweol/kg; 16-O-methylcafestol was not detectable (detection limit is 0,002 g/kg dry weight).

Diterpenes in Fresh and Faded Leaves of Arabica San Ramon
Both fresh green and faded yellow-brown leaves of San Ramon were examined. The analysis revealed that the diterpene contents vary during the fading process, especially the cafestol content (see Fig. 4).

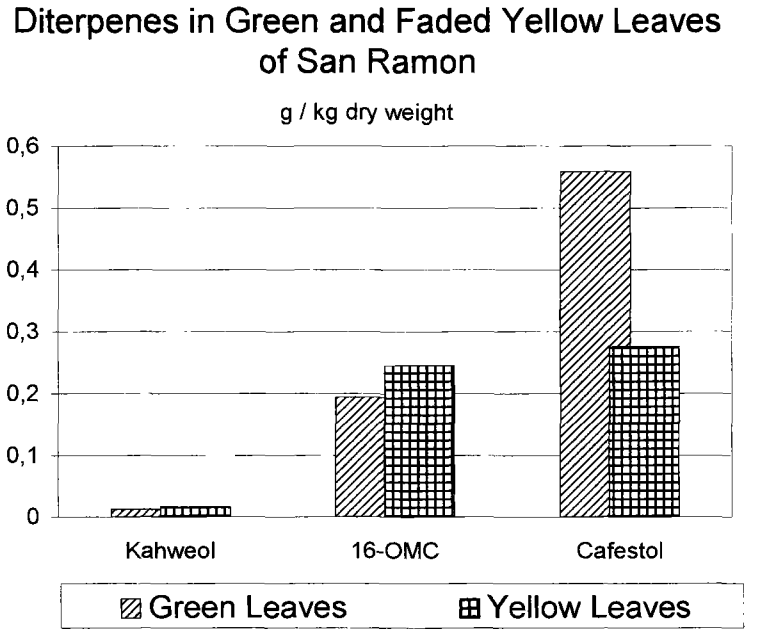

Fig. 4. Contents of the diterpenes in fresh green and faded yellow-brown leaves of Coffea Arabica San Ramon

Diterpenes in Pulps and Beans of Arabica San Ramon
The coffee tree Arabica San Ramon bears fruit so it was possible to analyse fresh coffee cherries as well as the leaves of the same tree.
The pulps were manually separated from the beans with a knife and processed as described in Figure 1. Differing from that, the beans covered here with the endocarp were ground first and subsequently soxhlet-extracted with tert-butyl methyl ether. After saponification, the diterpenes were analysed by HPLC in the unsaponifiable matter.

In Figure 5, the findings of the pulps and beans are compared to those of the leaves. The kahweol and cafestol contents of the pulps were substantially higher than those of the leaves. In contrast to this, the amount of 16-O-methylcafestol was analysed with 0,012 g/kg dry weight only.
In the San Ramon beans, as expected, 16-O-methylcafestol was not detectable, and the contents of cafestol and especially of kahweol were higher than in the pulps and the leaves.

These are the first results obtained from only a few samples. We are continuing our investigations.

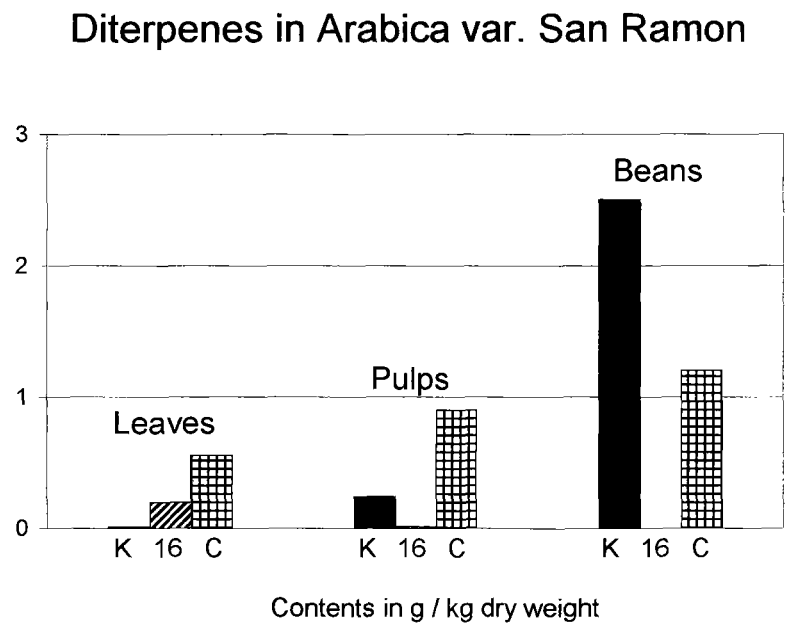

Fig. 5. Contents of diterpenes in green leaves, pulps, and beans of Coffea Arabica San Ramon

Acknowledgements
We would like to thank Prof. Jutzie and his work group from the Deutsches Institut für Tropische und Subtropische Landwirtschaft Witzenhausen der Gesamthochschule Kassel for placing the coffee material at our disposal, and Mrs Schirmer for her assistance in performing the analyses.

Summary
The three diterpenes cafestol, kahweol and 16-O-methylcafestol, known as ingredients of the coffee beans, were also identified in the leaves of Coffea trees.
Whereas cafestol and kahweol are present in Arabica beans, cafestol, only traces of kahweol and additionally 16-O-methylcafestol were found in the leaves of four Arabica varieties.
Although 16-O-methylcafestol is an indicator for Robusta in coffee mixtures, it was not detected in the leaves of the analysed Canephora. On the other hand, only small amounts of cafestol and kahweol were present.

Zusammenfassung
Die drei Diterpene Cafestol, Kahweol und 16-O-Methylcafestol, die aus den Kaffeebohnen bekannt sind, konnten auch in den Blättern von Kaffeepflanzen nachgewiesen werden.
Während die Arabica-Bohnen bekanntlich nur Cafestol und Kahweol enthalten, wurden in den Blättern von vier Arabica-Varietäten Cafestol, nur z. T. Spuren an Kahweol, aber zusätzlich 16-O-Methylcafestol analysiert.
In den Blätter eines Canephora waren nur geringe Mengen an Cafestol und Kahweol, dagegen kein 16-O-Methylcafestol nachweisbar, obwohl es als Indikator für Robusta in Kaffeemischungen gilt.

AN ANALYTICAL DISTINCTION BETWEEN UNTREATED AND STEAM-TREATED ROASTED COFFEE

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INTRODUCTION

Since Lendrich (LENDRICH et al., 1933) developed the steam treatment of coffee beans prior to the roasting process as a means to make the coffee beverage acceptable even to persons with stomach problems, analytical chemists have sought for a method to distinguish between these coffees and their untreated counterparts after the roasting took place. In the raw coffee beans, quite a lot of different procedures are available, for instance the detection of 3-methoxy-4-hydroxystyrole proposed by WINDEMANN (1974) in treated coffee beans or the differences in the contents of diterpens (WURZIGER, 1971). Nevertheless, all these possibilities vanish after the roasting took place. MOHR and WICHMANN (1977) stated that pyridine and furfurol contents of the treated roasted coffees are significantly lower than those of the untreated. But this method seems not to be applicable to every coffee.

Up to now, only Switzerland has brought forward an act dealing with this problem. In Switzerland, coffee that is advertised as being „reizarm" (less irritable), has to qualify with a low content in carbon acid-5-hydroxytryptamides (less than 400mg/kg). These contents vary in normal roasted coffee beans between 600 and 1000mg/kg, making it necessary to analyze the raw coffee as well. Since this type of coffee gains more and more market shares every year in Europe and the United States, legal action is expected quite soon in these countries as well, especially because the steam-treated coffees are sold for a higher prize than the untreated coffees.

Since substances of the coffee bean with a low molecular weight provide no significant difference for the distinction between these two types of coffee, STEINHART et al. (1990) began investigation of the melanoidins, a group of brown pigments of high molecular mass formed out of amino acids, carbohydrates and chlorogenic acids during the roasting process by means of a Maillard reaction. The melanoidins were separated on a gel column into five fractions, the first one with a molecular mass over 100.000 Dalton being significantly reduced in pretreated coffees. STEINHART and PACKERT (1993) confirmed this and reported that this fraction had 50% less mannose in its molecular structure. Therefore, a significant difference in the quantity as well as in the structure of those high molecular melanoidins seems likely. This observation might lead to a successful analytical procedure.

In order to look closely into the carbohydrate contents of this coffee melanoidin fraction with the highest molecular mass, we chose to analyze eight roasted coffees from different brands, bought in several shops in Hamburg. Three of them were steam treated, three were so-called mild coffees, two were normal roasted coffee beans and one was an untreated Maragogype.

MATERIALS AND METHODS

Samples. Samples were bought as whole coffee beans in different shops in Hamburg during one day. It was chosen to analyse the three main kinds of coffees: untreated coffees, mild coffees and the steam-treated coffees. Trademark (and manufacturer) of the selected coffee samples were as follows:

Steam-treated coffees:	Sana (Tchibo, Germany), Idee Kaffee Classic (Darboven, Germany), Kaffee Hag klassisch mild (Kaffee Hag, Germany)
Mild coffees:	Feine Milde (Tchibo, Germany), Milde Natur (Eduscho, Germany)
Untreated coffees:	Beste Bohne (Tchibo, Germany), Gala Nr.1 (Eduscho, Germany), Maragogype (Arko, Germany)

Sample Preparation. 50g of the coffee beans were ground in a water-cooled centrifugal mill (A10S, Janke&Kunkel, Germany). 20g of the coffee powder were weighed into a beaker and poured over with 125ml of boiling water. After closing the beaker with a watch glass, the brew was prepared on a magnetic stirrer for 5min at 70°C. The brew was filtered (Schleicher & Schuell, Germany) and membrane filtered (0,6µm, with glass fibre first filter, both Schleicher&Schuell, Germany).

Gelfiltration. 20ml of the coffee brew were applied to a sephadex G-25 fine column (gel bed: 54cm height, 5.0cm diameter, Pharmacia XK-50, Pharmacia, Sweden). Bidistilled water was used as eluent with 5.5ml/min. Peaks were recognized by a UV-photometer (Eppendorf 1101M, 405nm, Eppendorf, Germany) and collected with a fraction collector (Serva Linear II, Serva, Germany). The first fraction (see fig.1) was freeze dried (Beta 1-16, Christ, Germany).

Hydrolyzation and Derivatization. 20mg of the freeze dried powder were weighed into a 20ml-pyrex glass. After addition of 4ml 2N trifluoro acetic acid, hydrolysis was performed for 2h at 110°C in a drying cabinet. When the solution had cooled down to nearly room temperature, it was diluted with distilled water and filtrated into a 50ml graduated flask, thereby rinsing out the pyrex glass four times with distilled water, then filled ad vol with distilled water. 500µl of the solution was pipetted into a 2ml vial and brought to dryness in a vacuum centrifuge (Univapo 150H, Vacuubrand, Germany). 50µl of a propanthiole-trifluoro acetic acid-mixture (4:1, v:v) were added, the first reaction took place in 30min at 22°C ± 2°C and was ended by adding 50µl of pyridine. After drying again in the vacuum centrifuge, 50µl of pyridine and 50µl of 5% trimethyl chloro silane in BSTFA were added. Silylation took place for 20min at 75°C in a metallic heating appliance placed in a drying cabinet. When the mixture had reached room temperature, it was transferred to a glass insert (Hewlett-Packard, Germany).

Gas Chromatography. Gas Chromatography was performed on a Hewlett Packard 5890 Series II Gas Chromatograph, equipped with a Hewlett Packard 7673 autosampler and a flame ionization detector (all Hewlett-Packard, Germany). An HP-5 phase (Hewlett Packard) on fused silica (30m l., 0.32 i.d., 0.25µm film thickness) was used for the separation. Injection took place with a 1:10 split, temperature program was as follows: 180°C, 0min hold, with 10°C/min to 220°C, with 2°C/min to 225°C, with 7°C/min to 270°C, 10min hold. Carrier gas was helium with 1ml/min, make-up gas was also helium, with 30ml/min.

RESULTS AND DISCUSSION

To obtain the coffee melanoidins with the highest molecular mass, a gel filtration according to PACKERT(1993) had to be carried out. The exact procedure is described above, the achieved chromatogram is shown in figure 1. Table 1 shows the molecular masses of the obtained fractions.

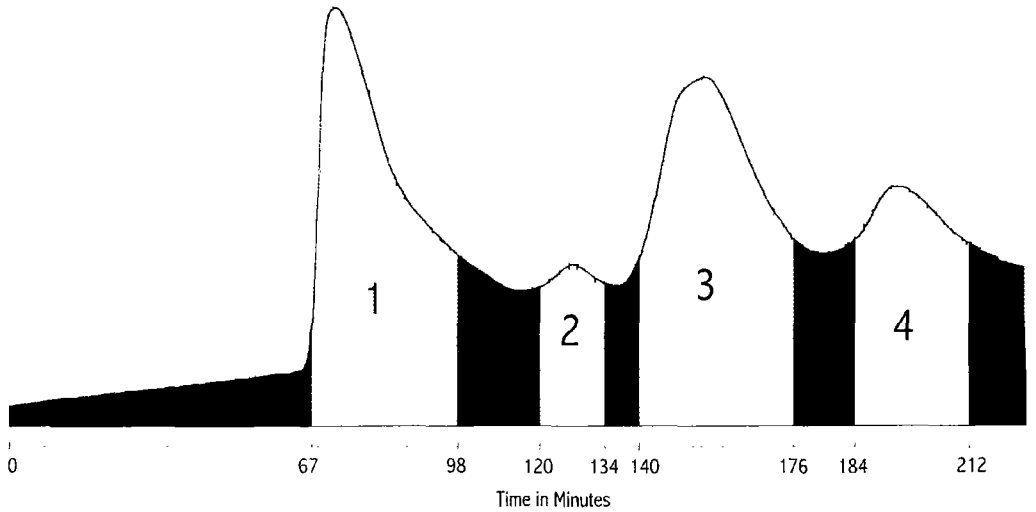

Figure 1: *Gel chromatogram with marked melanoidin fractions (nos. 1-4)*

Table 1: *Molecular masses of the coffee melanoidin fractions*

Coffee Melanoidin Fraction	Molecular Mass
1	63 100 - 12 600
2	5 750
3	5 750 - 1 510
4	3 630

The fraction containing the melanoidins with the highest molecular mass, marked "1" in fig.1, was lyophilised and afterwards analysed for its carbohydrate contents by means of an acid hydrolysis, thiolysation and silylation to volatile derivatives of the monosaccharides and gas chromatography. The exact procedure is described above. Table 2 depicts the obtained data.

Table 2: *Yields and total carbohydrate contents of the first coffee melanoidin fractions of the analysed coffees (data in g/100g lyophilisate, others mentioned)*

	Steam-Treated Coffees			Mild Coffees		Untreated Coffees		
	San	Ide Kaffe	Kaffe Ha	Fein Mild	Mild Natu	Best Bohn	Gal Nr.	Marago gyp
Yield (%)	14,0	8,5	16,4	13,9	15,0	17,1	14,8	11,3
Arabinose	6,2	6,3	8,0	4,4	4,8	6,4	5,2	4,9
Rhamnose	1,1	1,4	1,7			0,7		1,1
Fructose	3,4	1,6	2,5	1,6	1,5	2,1	1,7	0,6
Glucose		0,5		0,7	1,3	0,5	1,2	1,4
Mannose	8,0	12,3	9,1	11,9	13,6	15,3	17,1	19,7
Galactose	21,6	20,5	22,9	13,7	16,1	20,3	18,1	14,8
Sum	40,4	42,8	44,4	32,4	37,5	45,5	43,3	42,7

The arabinose content of pretreated coffees is slightly higher than that of the untreated or mild coffees, as can be seen in table 1. Only the "Beste Bohne" has a higher content of arabinose than the pretreated coffee with the lowest content, "Sana". Rhamnose is easily detectable in pretreated coffees, with an amount of 1.2 to 1.7 g/100g lyophilisate, but it contributes hardly to the polysaccharides of the untreated coffees, except the "Maragogype". With fructose, the picture is more mixed: the pretreated coffees show quite a high amount of fructose, but there are three coffees between the other six that display a higher amount of fructose than the pretreated coffee with the lowest content. Glucose seems to be very promising for a distinction: it is hardly detectable in pretreated coffees, but it amounts to 0.6 to 1.4g/100g lyophilisate in the untreated coffees. As has already been reported by PACKERT

(1993), the content of mannose in pretreated coffees is significantly lower than in untreated coffees; whereas the galactose contents are quite higher in the pretreated coffees than in the untreated coffees, except for the "Beste Bohne" again.

These data show that a distinction between pretreated and untreated coffees on the basis of a single carbohydrate is unthinkable of. But on a basis of a few carbohydrates, it seems to be achievable. From the carbohydrates listed above, fructose and arabinose do not qualify for such a task, since the differences in these carbohydrate contents between our two types of coffee are too small. So there are four carbohydrates left to form an equation: rhamnose, glucose, mannose and galactose. Since the contents of rhamnose and galactose are higher in pretreated coffees and those of glucose and mannose are lower in these coffees, they have to go by different signs in the equation. This leads to the following equation:

$$[\text{rhamnose}] - [\text{glucose}] + [\text{galactose}] - [\text{mannose}] = k_1 \qquad (1)$$

with [ ] = content of the monomer in % of the lyophilisate

Fig.2 shows the data of k_1 obtained by this equation.

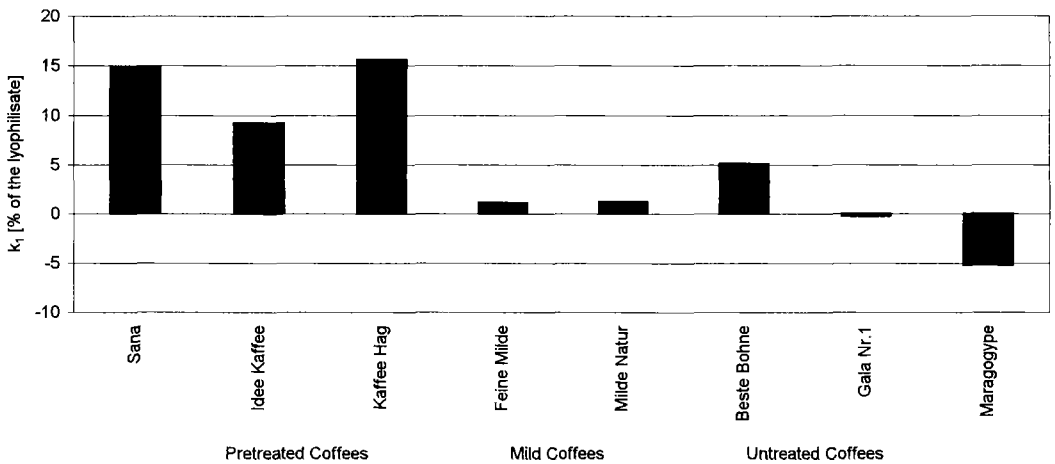

Figure 2: *Data of k_1 for the analysed coffees, obtained by equation 1*

As can be seen in fig.2, no overlapping between pretreated and untreated coffees occurs. The result of the equation lies between -5.1 and 5.1g/100g lyophilisate for the untreated coffees and between 9.2 and 15.6g/100g lyophilisate for the pretreated coffees.

Another possibility is to single out the two carbohydrates with the highest differences in their amount, galactose and mannose, and to divide one of them by the other. This seems to be promising because, as can be seen from the data in table 1, the galactose content of pretreated coffees is about the two- to threefold amount of the mannose content, whereas it is only slightly higher in the untreated coffees. To strenghten the expected distinction, rhamnose and glucose should join the equation as before. This leads to two possible equations:

$$[\text{rhamnose}] - [\text{glucose}] + [\text{galactose}] / [\text{mannose}] = k_2 \qquad (2) \text{ and}$$
$$[\text{rhamnose}] - [\text{glucose}] - [\text{mannose}] / [\text{galactose}] = k_3 \qquad (3)$$

with [ ] = content of the monomer in % of the lyophilisate

The data obtained for k_2 by the first equation are shown in fig.3, the second equation's results for k_3 are displayed in fig.4.

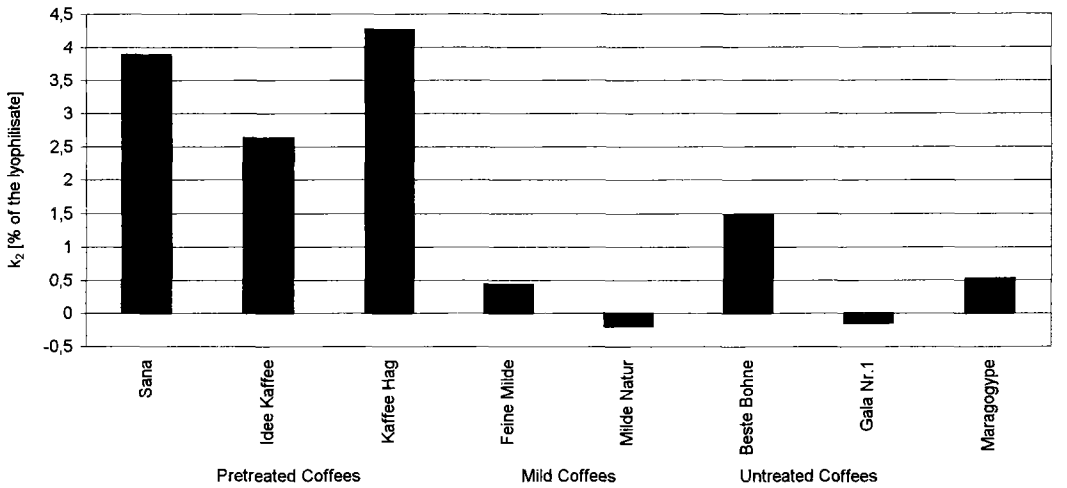

Figure 3: *Data of k_2 for the analysed coffees, obtained by equation 2*

As for the last equation, there is no overlapping between the results of the pretreated and those of the untreated coffees. The obtained data ranged from -0.2 to 1.5g/100g lyophilisate for the pretreated coffees and from 2.6 to 4.2g/100g lyophilisate for the untreated coffees.

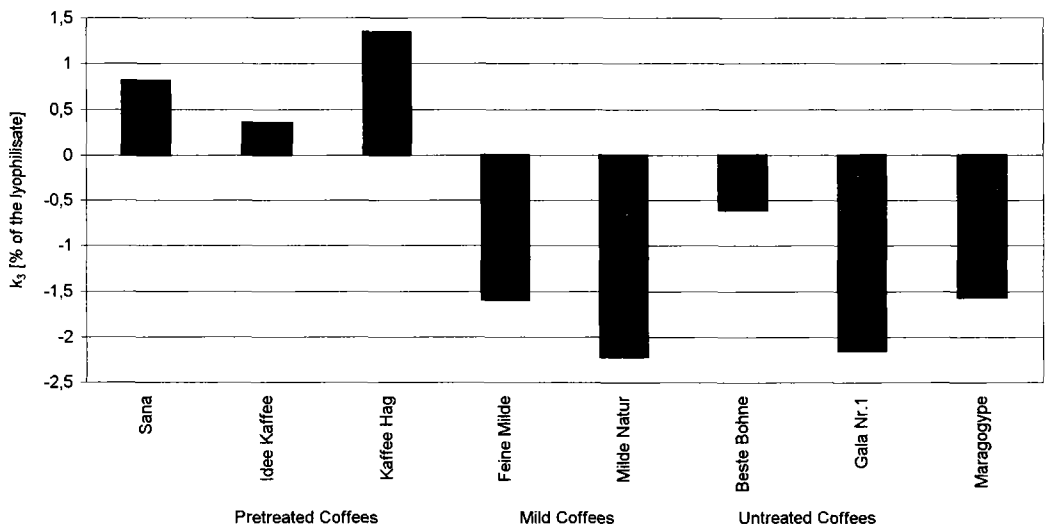

Figure 4: *Data of k_3 for the analysed coffees, obtained by equation 3*

The results of the formula rhamnose-glucose-mannose/galactose, shown in fig.4, seem to have a significant advantage over the previous data: the results for the pretreated coffees and those for the untreated coffees go by different signs which would make a distinction very easy for every laboratory. The results for the pretreated coffees were between 0.4 and 1.3g/100g lyophilisate whereas those for the untreated coffees were between -2.2 and -0.6g/100g lyophilisate.

Each of these three proposals for possible equations for a distinction between pretreated and untreated coffees after the roasting process certainly has its advantages. To distinguish between the three equations and in order to find out the most suitable one for this task, the average and standard deviation of the results of the pretreated and the untreated coffees in each equation were calculated. To obtain a probability level of 95%, the twofold standard

deviation was then added to and subtracted from the average. A suitable equation should develop no overlapping in these data. The results are shown in table 3.

Table 3: *Median and range of k_{1-3} for the different coffee types, data in % of the lyophilisate*

Equation	Type of Coffee	Median	Range
k_1	pretreated	14,81	6,37
	untreated	1,08	10,23
k_2	pretreated	3,88	1,63
	untreated	0,43	1,66
k_3	pretreated	0,81	0,98
	untreated	-1,59	1,61

As can be seen from the above data, all three equations qualify for the distinction between pretreated and untreated coffee after the roasting process. None of these show any overlapping in the data. Which equation is the most suitable in practice has to be proven by more data about more different coffees from different brands. Nevertheless, these equations seem very promising to fulfill the task.

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SUMMARY

Up to now, no possibility exists to distinguish analytically between untreated and steam-treated coffees after the roasting process. In this paper, a method is proposed for this using the contents of carbohydrates in the gel chromatographic fraction of the coffee melanoidins with the highest molecular weight. This method was developed by the analysis of eight regular coffees, bought in different shops in Hamburg. Three equations are proposed, each with the carbohydrates galactose, rhamnose, glucose and mannose, that allow the aforementioned distinction equally well. Which of these three equations is the most suitable has to be proven by analysis of more coffees.

AN 11S-TYPE STORAGE PROTEIN FROM *COFFEA ARABICA* L. ENDOSPERM : BIOCHEMICAL CHARACTERIZATION, PROMOTER FUNCTION AND EXPRESSION DURING GRAIN MATURATION

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INTRODUCTION

We describe the biochemical and molecular characterization of the major storage protein in *Coffea arabica* grain endosperm together with the functioning of four DNA regions located upstream from the genetic coding region of this protein as grain-specific genetic promoters in transformed tobacco. While the search for a grain-specific genetic promoter has been the primary objective in this work, the storage protein which has been characterized is also the most abundant source of protein and amino acids in the mature grain and is one of its principal components. The protein bears strong sequence homology to the 11S (legumin) family of plant storage proteins and is thought to be coded for by a multigene family within the Coffea genome. The expression of specific 11S mRNA and accumulation of the protein during grain maturation are reported. The function of several promoter regions from the 11S protein gene, linked to the GUS reporter gene, have been tested in transformed tobacco in order to assess rapidly their specificity and strength.

The full length cDNA sequence encoding for the Coffea 11S-type protein, together with the promoter region sequences, will be reported in forthcoming articles. To the authors' knowledge, this sequence is only the third complete native sequence from the genus Coffea to be described in the literature, the others being those of an α galactosidase (Zhu and Goldstein, 1994) and a metallothionin I-like protein (Moisyadi and Stiles, 1995).

MATERIALS AND METHODS

Plant Material

For samples of Coffea, all protein and nucleic acid extractions were performed on material obtained from greenhouse-cultivated trees of *C. arabica* var. Caturra. Fruit were harvested at specific weeks after flowering (WAF). Grains were separated from fruit pericarp and seed coat tissue before analysis. Fruit were frozen whole in liquid nitrogen and stored at -85°C until required. For genetic transformation, tobacco (*Nicotiana tabacum* var. XHFD8) was grown *in vitro* on solid MS medium (Murashige and Skoog, 1962) and transformed plants were cultivated in a greenhouse.

Preparation of Samples for Immobilized pH Gradient Two Dimensional Electrophoresis (IPG-2DE)

Grains were finely ground in liquid nitrogen. A reduced acetone powder was produced according to Damerval et al. (1986). Proteins were extracted in solubilization solution (100 µL per 10 mg powder) containing 3 % (w/v) CHAPS, 8.5 M urea, 0.15 % (w/v) DTT and 3 % (v/v) carrier ampholytes pH 3-10. Following incubation at room temperature for at least 30 min, and centrifugation (13,000*g* for 5 min) the supernatant was used for electrophoresis.

First Dimension IPG-2DE

IPG-2DE was performed using the Pharmacia Multiphore system with prefabricated pH-gradient gel strips (pH 3-10 NL), according to the manufacturers' instructions and following the recommendations of Bjellqvist et al. (1993a). Samples (50 µL per gel strip) were focalized at 150 V for 30 min, 300 V for 60 min and 3500 V to a total of 75,000 Vh at 15°C.

Second Dimension Electrophoresis

The second dimension electrophoresis was carried out using Protean II equipment (BioRad) on 11-18 % gradient gels (190 x 160 x 1 mm) using standard SDS-PAGE conditions (Laemmli, 1970), with additives as recommended by Hochstrasser et al. (1988). Gel strips were washed and mounted on the second dimension gels according to Gorg et al. (1987). Migration was performed at 15°C, 40 mA per gel.

Staining and Image Analysis of Two Dimensional Gels

Silver staining of gels was carried out according to Bjellqvist et al. (1993b). Image analysis was performed with an XRS 12CX gel scanner, BioImage 2-D Analyser software, and a SUN Microsystems Sparc Classic ISC microprocesser. Image intensities were calibrated with a series of spots previously demonstrated to maintain a constant abundance during maturation.

Protein Microsequencing

Proteins on IPG-2DE gels were transferred to PVDF membranes (ProBlott membranes. Applied Biosystems) using a Transblot Cell (BioRad) at 4°C, 420 mA constant for 1.5 hours, and spot detection with light Coomassie blue staining followed Problott instructions. Microsequencing of N-terminals of excised protein spots was performed on a Beckman LF 3000 sequencer together with a Beckman Gold Sytem HPLC and SPHEROGEL MicroPTH column. Internal sequences were obtained following trypsin digestion of protein released from membrane spots and separation of peptides by HPLC on a C18 column using an H_2O / acetonitrile gradient system. Peptide peaks were collected, concentrated, rediluted in 30 % (v/v) acetonitrile, 0.01 % TFA and sequenced as before.

Isolation of RNA and mRNA

Extraction of RNA was performed following an adaptation of Mahé et al. (1992). The extraction of 10 grains normally yielded between 0.5 and 1 mg total RNA. Poly-A^+ mRNA was purified from total RNA on an oligo-dT column (Oligotex-dT Kit, Qiagen).

Construction and Screening of cDNA libraries

cDNA was prepared from 1 µg of mRNA using the Riboclone cDNA synthesis system M-MLV (H^-) and the Riboclone *EcoRI* adaptator ligation system (Promega) according to the instructions supplied. Approximately 100 ng of cDNA were ligated into pBluescript II SK(+) (Stratagene) and the ligation was used to transform competent *E.coli* XL1-Blue MRF' (Stratagene).

Transformants were transferred directly from petri dishes onto nylon membranes (Boehringer Mannheim) according to the manufacturers' instructions. Preincubation and hybridization were similarly carried out according to instructions supplied with the DIG Oligonucleotide 3'-end labelling Kit (Boehringer Mannheim). The membranes were probed with a 17-mer oligonucleotide 5'GCNGAYGTNTTYAAYCC (Y = C+T and N = A+G+C+T), labelled at the 5' end with digoxigenin (Genosys). This probe was derived from the amino acid sequence ADVFNP obtained from a predominant polypeptide belonging to the 11S protein β arm group revealed by IPG-2DE profiles. Membranes were then washed in a solution containing tetramethylammonium chloride (TMAC) according to Wood et al. (1985). A DIG luminescent detection kit (Boehringer Mannheim) with CSPD (Tropix) was used for chemiluminescence detection of bound probe following the manufacturer's instructions. This screening led to the identification of a full length 11S cDNA (1706 bp).

Northern Blotting

Total denatured RNA from grains of between 4 and 35 WAF was subject to Northern blotting according to Sambrook et al. (1989). Filters were prehybridized and hybridized with the 11S [^{32}P]-labelled cDNA probe. Gel loading of RNA samples was controlled by measuring the abundance of 16S and 23S rRNA.

DNA Sequence Analysis

DNA sequencing was performed with a T7 sequencing kit (Pharmacia) together with [^{35}S]dATP (Amersham). Sequence analysis was performed using the GCG software package (Madison, WI). Amino acid and nucleotide sequences were screened against SwissProt (release 31.0) and Genbank (release 91.0) databases respectively employing the FASTA search programme (Pearson and Lipman, 1988).

Isolation of the 11S Storage Protein Gene Promoter

The 11S 5' flanking region was isolated by two inverse PCR (IPCR) following the method of Ochman et al., 1988. Nuclear DNA was isolated from *C.arabica* leaves according Rogers and Bendich, 1993, subject to separate digestions with restriction enzymes *HincII* and *NdeI* and self-ligated in the presence of T4 DNA ligase. IPCR was performed in the presence of the ligated genomic DNA, *Taq* DNA polymerase and two primers, localized in the 5' 11S coding region, which were designed so that their 3' ends faced away from each other. Amplified fragments were screened with a further oligonucleotide representing a region overlapping one of the initial primers. This led to the identification of a 1.7 kb fragment originating from a *HincII* digestion, which, following cloning and sequencing, was found to contain a 748 bp

sequence upstream from the 11S ATG codon. In order to obtain sequences upstream from the *HincII* site, a second IPCR was carried out using another set of primers localized in the 5' region of the 1.7 kb fragment. A similar strategy of screening and sequencing, using a second oligonucleotide probe based on the new sequence information, led to the identification of a another *NdeI* 1 kb fragment which provided 250 bp upstream from the 748 bp section already described.

Finally, this (approximately) 1 kb sequence was amplified by PCR from the genomic DNA using the *Pfu* polymerase, which is known to have a lower error rate compared to the *Taq* enzyme (Lundberg et al., 1991).

Construction of Vectors Incorporating 11S Promoter Regions

The pBI101 vector (Clontech Laboratories Inc.) was used as host to clone four fragments of the 1 kb sequence described above. This vector incorporates in its T-DNA the gene *uidA*, which encodes the β-glucuronidase (GUS) reporter enzyme and the gene *nptII*, which confers resistance to kanamycine (Bevan, 1984). The original (promoterless) pBI101 was used as the negative control, while a positive control was furnished by the vector pBI121, in which the expression of the *uidA* gene is under the control of the strong, constitutive CaMV35S promoter.

For PCR reactions accomplished in the presence of the vector housing the 1 kb fragment described in the preceding section, primer pairs were designed to include *BamHI* sites (to assist ligation into pBI101), and either one site corresponding to a region within the 1 kb fragment, or one site corresponding to the first five amino acids of the 11S gene. Fragments isolated from the PCR reactions were then ligated into the pBI101 vector and used to transform *E.coli* strain XL1-Blue MRF. Four pBI101 plasmid derivatives (**p1 to p4**) were isolated, incorporating promoter regions of approximately 1000, 750, 500 and 250 bp respectively.

Transformation and Regeration of *Nicotiana tabacum* and Analysis of Genomic DNA of Transformed Plants

The vectors pBI101, pBI121, and p1 - p4 were introduced into disarmed *A. tumefaciens* (C58pMP910) (Koncz and Schell, 1986) as described by An et al. (1993). Tobacco plants were transformed with these vectors according to Horsch et al. (1993). For each transformation, 30 plants were selected and self pollinated to obtain seeds. DNA from the transformed plants was analysed beforehand in order to select correctly orientated, single integrations of T-DNA in the plant genome (data not shown). The unique insertion event was confirmed by analysing the segregation of resistance to kanamycin in germinating grains.

GUS Activities in Transformed Tobacco

Leaves and grains were analysed for GUS activity according to Jefferson et al. (1987). Protein was quantified by the method of Bradford (1976).

RESULTS / DISCUSSION

Characteristics of the Full Length cDNA and Amino Acid Sequence of the *C.arabica* 11S-Type Storage Protein

The 1706 bp fragment isolated from the 30 WAF cDNA library corresponds to a full length cDNA for the coffee grain 11S storage protein. The sequence includes an untranslated leader sequence of 32 bp, an open reading frame of 1476 bp and an untranslated 3' sequence of 195 bp. The coding region is able to encode for a protein of 492 amino acids with a theoretical molecular weight of 55 kD (Figure 1). Screening of the translation product against the SwissProt database revealed a close sequence homology to the 11S family of plant storage proteins. The most closely related sequences include glycinin from *Glycine max* (Nielsen et al., 1989), 12S from *Arabidopsis thaliana* (Pang et al., 1988), cruciferin from *Brassica napus* (Ryan et al., 1989), glutelin from *Oryza sativa* (Takaiwa et al., 1987), the legumins of *Pisum sativum* and *Vicia faba* (Heim et al., 1989; Rerie et al., 1990), and 11S from *Cucurbita maxima* (Hayashi et al., 1988). Absence of the amino acid motif Asn-X-Ser/Thr (where X may be any amino acid) would suggest that the protein is not glycosylated, in agreement with other 11S proteins (Shewry, 1995).

N-terminal amino acid sequencing of polypeptides separated by IPG-2DE allowed the identification of the two principal parts of the sequence, the 'α and β arms' (a terminology adopted from descriptions of other 11S globulins). The α arm N-terminal amino acid sequence begins at a distance of 26 codons downstream from the theoretical start codon of the open reading frame while the start of the ß arm N-terminal amino acid sequence occurs at amino acid 305 (GLEET), after the cleavage site N/GLEET. This cleavage site has been described for all 11S-type protein precursors. Analysis of the initial 26 amino acid section of the α arm suggests that this is markedly hydrophobic (data not shown) as is generally observed for plant peptide signal sequences of secretary proteins (Bennett and Osteryoung, 1991). The polypeptide α and β arms are also likely to possess different hydrophobicity characteristics (data not shown).

The molecular weights deduced from the coding sequence are 31.2 kDa and 20.9 kDa for the α and ß arms respectively. These molecular weights are in agreement with those determined for the α and β arms by electrophoretic analysis. Sequence alignment also indicates that the two cysteines, at amino acid positions 36 and 311 probably correspond to those involved in the formation of a disulfide bridge between the α and ß arms (Borroto and Dure, 1987; Wright, 1988).

Analysis of amino acid content (Figure 2) shows that this 11S precusor is enriched in glycine and glutamine and is similar in composition to the 11S storage protein of soybean seeds (Bewley and Black, 1994).

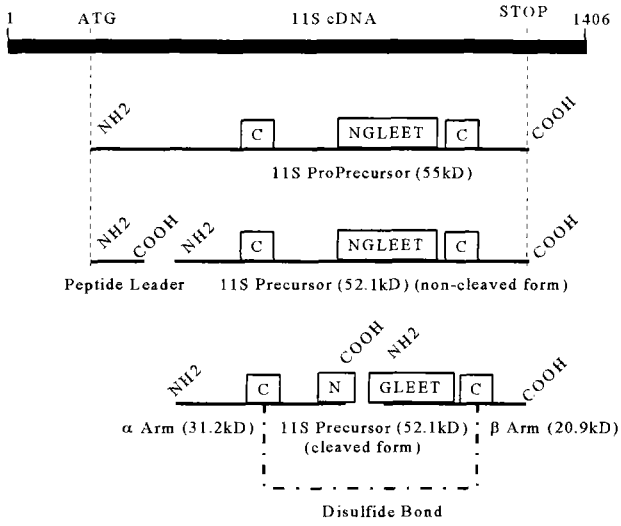


Figure 1. Diagramatic representation of coffee 11S cDNA and derived seed storage proteins. C . cysteines involved in formation of the disulfide bridge following cleavage. The cleaved and non-cleaved forms of the precursor exist *in vivo*.

Figure 2. Analysis of amino acid content of the *C.arabica* 11S grain storage protein. Amino acid content calculated from the full length cDNA coding for an 11S storage protein from mature *C.arabica* grains.

Amino Acid	Number of Units	% Total
A alanine	24	4.9
C cysteine	6	1.2
D aspartic acid	19	3.9
E glutamic acid	35	7.1
F phenylalanine	26	5.3
G glycine	**50**	**10.2**
H histidine	10	2.0
I isoleucine	21	4.3
K lysine	26	5.3
L leucine	**47**	**9.6**
M methionine	3	0.6
N asparagine	26	5.3
P proline	24	4.9
Q glutamine	**53**	**10.8**
R arginine	28	5.7
S serine	31	6.3
T threonine	16	3.3
V valine	32	6.5
W tryptophan	5	1.0
Y tyrosine	10	2.0

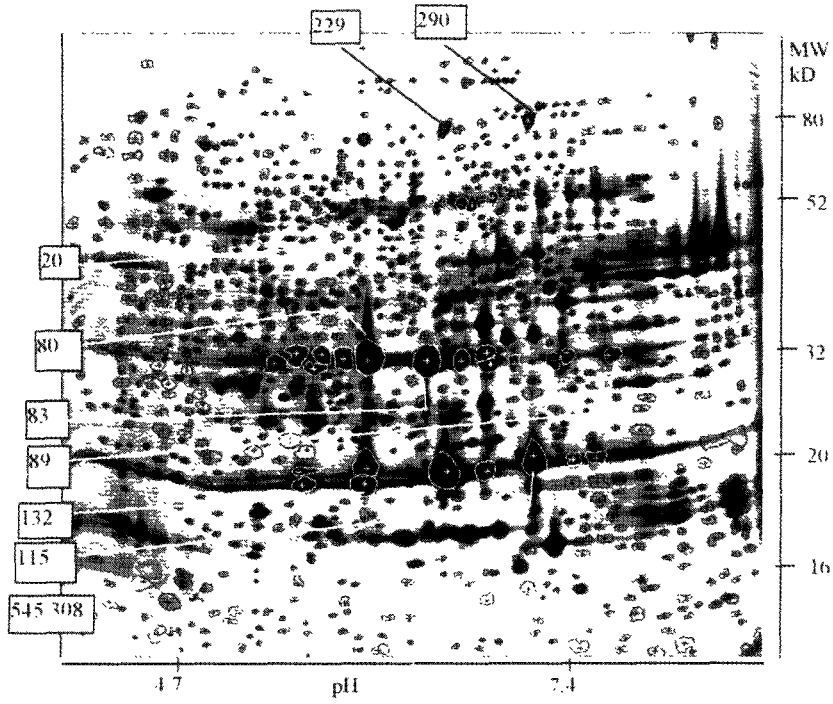


Figure 3. Silver stained IPG-2DE profile of mature whole grains of *C.arabica* var Caturra. Polypeptide components of the 11S storage protein family for which amino acid sequencing information is presented are numbered and contoured in white. Other polypeptides considered to belong to the same group, due to their abundance, molecular weight and accumulation profile during maturation, are also contoured in white: 229 + 290 : β arm complexes (β arm N-terminal) ; 20 : mature precursor (α arm N-terminal) ; 80 + 83 + 89 : α arm (α arm N-terminal) ; 132 + 115 : β arm (β arm N-terminal) ; 545.308 : α arm fragment (α arm N-terminal).

Figure 4. Polypeptides identified as belonging to the 11S storage protein group in C.arabica endosperm IPG-2DE profiles by N-terminal and internal amino acid sequencing following 2-dimensional electrophoresis Internal sequences were obtained following trypsin digestion and separation by *HPLC*. For trypsin digestion, cutting sites are normally preceded by either arginine (R) or lysine (K).

Spot	Amino Acid Sequence	Identity, MW, pI
229	Identical to spot 115 N-terminal	ß N-terminal, 76kD, pI 6.4
290	Identical to spot 115 N-terminal	ß N-terminal, 76kD, pI 6.9
20	Identical to spot 83 N-terminal	α N-terminal, 55kD, pI 6.5
80	Identical to spot 83 N-terminal	α N-terminal, 32kD, pI 6.0
83	E P R L G G K T Q E N I Q K L N A Q E P S F R F P	α N-terminal, 33kD, pI 6.3
	2:EGHQG	Internal peptides spot 83
	3: NTVQPK	"
	4: LPHXS	"
	5: LNAQE (also in the N-terminal)	"
	8: FVYVVEGTGVQGTVI	"
	9: FPSEAGLTEFWDSNNPEFGX	"
	10: NIFSGFDDQLLADAFNV	"
	12:GDVLILLPGFTQWTYNDGDV	"
89	Identical to spot 83 N-terminal	α N-terminal, 33kD, pI 7.4
132	Identical to spot 115 N-terminal	ß N-terminal, 21kD, pI 6.0
115	GLEETLETVKLSENIGLPQEADV FNPYAGYITTVN	ß N-terminal, 21kD, pI 7.0
545-308	Identical to spot 83 N-terminal	α N-terminal, 16kD, pI 4.7

aa: amino acids; X: uncertain amino acid determination

Figure 5. Expression of the 11s storage protein and specific mRNA in C.arabica endosperm during maturation

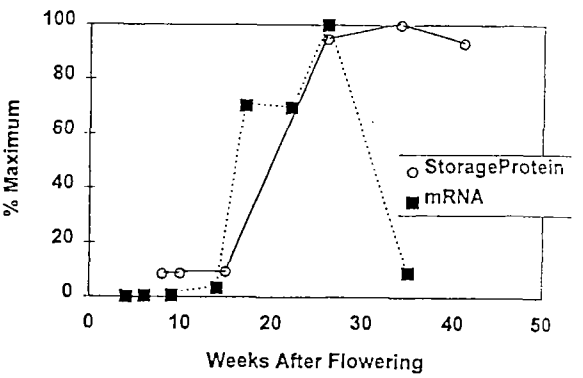


Figure 6: Schematic representation of constructions used in transgenic tobacco plants

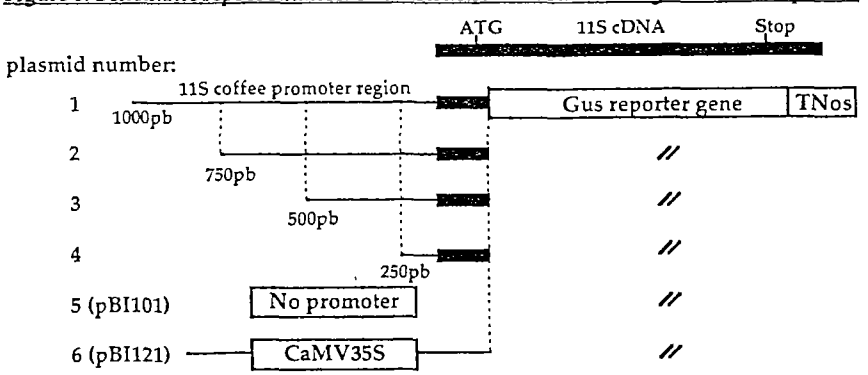
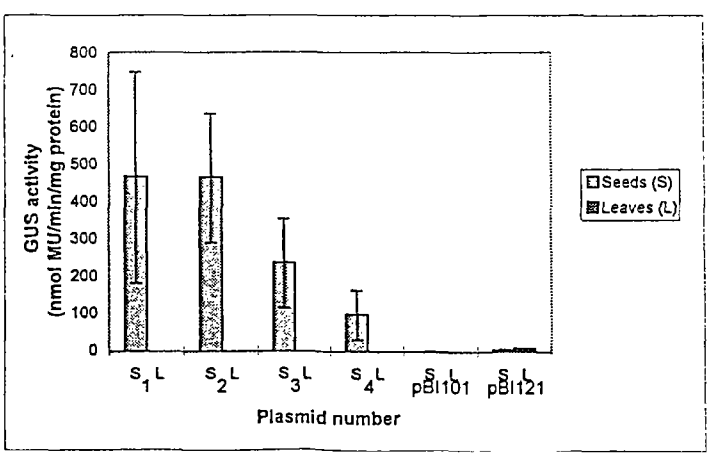


Figure 7: GUS activity in transgenic tobacco plants



Polypeptide Profile of the 11S Storage Proteins

The IPG-2DE profile of the mature *C.arabica* grain (Fig. 3) is dominated, under denaturing and reducing conditions, by several groups of abundant polypeptides. These are observed to accumulate during the maturation process (data not shown). Those polypeptides for which amino acid sequencing evidence is presented are numbered, while the totality of polypeptides considered to belong to the 11S group are contoured in white. All forms of the protein - the precursor and the α and β arms - exist as isoforms possessing different isolelectric points but approximately the same molecular weights, indicating slight differences in amino acid content. The precursor occurs at 52 kDa (represented by spot 20, possessing the α arm N-terminal). Reduced α and β arm polypeptides occur at 32 kDa (represented by spots 80, 83 and 89, possessing the α arm N-terminal) and 20 kDa (represented by spots 132 and 115, possessing the β arm N-terminal) respectively. β Arm complexes occur at approximately 80 kDa (represented by spots 229 and 290, possessing the β arm N-terminal).

The amino acid sequences obtained for these polypeptides corresponded to the cDNA sequence obtained subsequently, as did a series of internal peptide sequences obtained for spot 83 (Figure 4). Thus the common N-terminal sequence of polypeptides 229/290/132/115 (GLEETLETVKLSENIGLPQEADVFNPYAGYITTVN) corresponds to the N-terminal of the β arm located within the cDNA. The common N-terminal sequence of polypeptides 20/80/83/89/545-308 (EPRLGGKTQENIQKLNAQEPSFRFP), together with the internal peptide sequences of 83 (EGHQG; NTVQPK; LPHYS; LNAQE; FVYVVEGTGVQGTVI; FPSEAGLTEFWDSNNPEFG(G); NIFSGFDDQLLADAFNV; GDVLILLPGFTQWTYNDGDV) are located within the α portion of the same cDNA sequence. The α arm N-terminal commences at amino acid 27 of the coding region.

The *in vivo* condition of the 11S protein is probably the precursor in either its non-cleaved or hydrogen bridge form, as suggested by SDS-PAGE profiles under non-reducing conditions in which the 52 kD form is clearly dominant (data not shown).

Six minor codon differences were detected between the cDNA and peptide sequences. These differences may indicate the base of the multiple pI isoforms of the protein. The pI diversity of the polypeptides possessing identical N-terminal amino acid sequences observed in the IPG-2DE profile (which ranges from pH 5.5 to 7.4 for the α arm polypeptides and from pH 5.5 to 7.0 for the β arm polypeptides) points to the presence of a gene family coding for the 11S storage protein in the coffee genome.

The signal peptide described by the first 26 codons of the cDNA sequence has a theoretical molecular weight of 2.869 kDa and a pI of 8.45. This is too small to be detected on IPG-2DE profiles. However, an acidic polypeptide (pI = 4.7) possessing the α arm N terminal sequence is located on the IPG-2DE profile at approximately 16 kD. The origin of this component is uncertain, but it may be an example of cleavage at a site interior to the α arm as previously described for some of the glycinins from soybean (Wright, 1988).

mRNA and protein expression during grain maturation

11S storage protein mRNAs are easily detectable by Northern analysis using total RNA indicating that they are very abundant in the grain. 11S mRNAs are barely detectable at 14 WAF, begin to accumulate in the cell at 18 WAF and reach a maximum at 27 WAF. After this period they decline dramatically, being undectectable in mature grains. This pattern of expression is similar to that observed in other seeds expressing 11S-type proteins, such as *Pisum sativum* (Boulter et al., 1987). The summed abundance profile of all polypeptides thought to belong to the 11S protein follows the mRNA profile between 17 and 27 WAF. This protein level is then maintained relatively constant during maturation (Figure 5).

Specificity of expression

Analysis of IPG-2DE profiles of perisperm separated from young grains and also pericarp gave no evidence for the existence of the 11S protein in these tissues. However, the major polypeptides representing the α and ß arms were present at very reduced quantity in separated embryo tissue (data not shown). The pronounced peak of expression of specific 11S protein mRNA occurs at the beginning of the period of endosperm expansion and concommitant synthesis of storage compounds in the grains, from which time onwards the perisperm is engulfed and reduced. These observations indicate that the expression of nuclear genes encoding for 11S coffee storage proteins is localized in the endosperm tissue of the grain. It can be postulated that the expression of the 11S coffee gene is controlled mainly at the transcriptional level by an endosperm-specific promoter which is activated during coffee endosperm expansion between the 18 and 27 WAF, as has been described in the literature for pea legumin (Shirsat, 1991).

The Function of 11S Promoter Regions in Transgenic Tobacco

A representation of the plasmids constructed with putative 11S promoter regions is shown in Figure 6. Results from measurements of GUS activity (Figure 7) show that no activity was observed from leaves or grains of plants incorporating the T-DNA of plasmid pBI101 (negative control). For all other experiments, differences in GUS activity observed between plants transformed with identical plasmids can be explained by position effects resulting from the random integration of T-DNA into the genome (Peach and Velten, 1991).

In the case of plants transformed with the plasmid pBI121, GUS activity did not vary significantly between plant and leaf extracts, being between 1.5 and 20 nmol MU min-1 mg protein-1, confirming the constitutive function of the CaMV35S promoter in higher plants (Odell et al., 1985). No GUS activity was observed in leaves of plants transformed with plasmids incorporating the coffee grain 11S protein promoter constructs, p1 to p4. However, activities detected in the grains of these plants were, for the constructs p1 to p4, 60, 60, 30 and 12 times greater than average activities detected in grains transformed with the CaMV35S contruct pBI121. Maximum GUS activity was measured in transformants host to the p1 and p2 constructs, which attained an average of 465 nmol MU min-1 mg protein-1. Thus, while the magnitude of the GUS activity was inversely related to the size of the deletion, all 11S promoter constructs demonstrated spatial specificity, expression in all cases being confined to the grains.

CONCLUSION

The protein family described in this report represents the most abundant protein accumulating in mature *Coffea arabica* endosperm. It accounts for approximately 50 % of total proteins in this tissue, representing between 5 and 7 % of coffee bean dry weight. The close sequence homology of the cDNA translation product with the 11S-type plant storage proteins (for reviews see Wright, 1988, and Shewry, 1995) supports the assumption of a storage function for this protein. The division of the protein into α and ß sections, the occurrence of the precursor, and the molecular weights of the major components is representative of the 11S-type storage proteins. The protein is probably predominant *in vivo* in its precursor form with the two arms remaining linked by a disulfide bridge following post-translational enzymic cleavage, or as the uncleaved form. Storage is probably within protein bodies in the mature coffee endosperm (Dentan, 1987).

The range of pIs observed for the protein probably represents the existence of multigene copies within the genome. At least 5 (and possibly as many as 14) proteins can be identified at the position of the precursor group, suggesting that the Arabica storage gene family may contain this number of copies.

In order to test the promoter function of regions upstream of the 11S protein coding region, transformed tobacco was selected to contain only one insertion of the respective genetic promoter-GUS gene (*uidA*) construct. Assuming that the GUS activity represents the strength and specificity of the promoter regions controlling the *uidA* gene expression, it is concluded that the 1000 bp region upstream from the 11S coding region translation initiation codon, together with the regions resulting from deletions within this that were tested, inclose the genetic information capable of provoking a stronger expression than the CaMV35S reference promoter which is spatially specific to the grain tissue of transformed tobacco.

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ABSTRACT

Coffea arabica L. endosperm proteins have been analysed by 2-dimensional electrophoresis (IPG-2DE) and amino acid micro-sequencing. The principal grain storage protein has been characterized and a full length 1706 bp cDNA coding for this protein has been isolated. The sequence is closely homologous to the 11S (legumin) family of plant storage proteins. The protein accounts for at least 50 % of total grain protein and exists *in vivo* as a mature precursor of approximately 52 kDa. It is also detected on 2DE profiles as reduced cleavage products with MW's of 20 (the β arm), 32 (the α arm) and 80 kDa (β arm complexes). The expression of the 11S gene and protein during grain maturation is reported. Upstream promoter regions of the 11S protein gene have been isolated and sequenced. Transformation of tobacco with constructs of 11S promoter regions linked to the GUS reporter gene has shown that these promoter regions are capable of inducing high level expression which is specific to the grain tissue in this species.

RESUME

Les protéines de l'endosperme de *Coffea arabica* L. ont été analysées par électrophorèse bidimensionnel (IPG-2DE) et micro-séquençage des acides aminés. La protéine de réserve principale a été caractérisée et un ADNc de pleine longueur (1706 pb) codant pour cette protéine a été isolé. La séquence codante possède une forte homologie avec la famille 11S (légumine) des protéines de réserve végétales. Cette protéine représente environ 50 % des protéines totales du grain mûr et se trouve *in vivo* sous la forme d'un précurseur ayant un poids moléculaire approximatif de 52 kDa. Elle est aussi détectée dans les profils IPG-2DE sous sa forme réduite, composée des bras α (32 kDa), des bras β (20 kDa) et des complexes des bras β (80 kDa). L'expression du gène et de la protéine pendant la maturation du grain est décrite. La région génétique en amont de la partie codante du gène 11S a été isolée et séquencée. La transformation des plantes de tabac avec des constructions génétiques liant ces régions au gène rapporteur GUS a permis de montrer leur capacité d'induire une forte expression de ce gène uniquement dans les graines des plantes transformées.

EFFET INHIBITEUR DE L'ACIDE CAFÉYL 5-QUINIQUE SUR LA FORMATION DE PYRAZINES DANS LES RÉACTIONS DE MAILLARD

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INTRODUCTION

Les réactions de Maillard sont impliquées dans la formation de l'arôme de produits alimentaires ayant subi un traitement thermique ; elles ont fait l'objet, ces dernières années, de nombreuses publications concernant l'identification et le mécanisme de formation de ces composés aromatiques (Mottram, 1994 ; Tressl et al., 1994). L'arôme du café torréfié est constitué environ de 900 composés identifiés, formés en grande partie au cours de ces réactions à partir de sucres réducteurs et d'acides aminés ou de peptides (Flament, 1989 ; Shibamoto, 1991 ; Tressl et al., 1993 ; Silwar et Lullman, 1993). Sur l'ensemble de ces composés, 8 % seulement joueraient un rôle réel pour l'arôme café (Holscher et Steinhart, 1994) en particulier certains dérivés de la furanone et de la pyrazine (Semmelroch et al.1995), composés formés par les réactions de Maillard au cours de la torréfaction. Le mécanisme de formation de l'arôme café (Tressl et al., 1993; Tressl et al., 1994; Rewicki et al., 1994) a été largement étudié en systèmes modèles en fonction des précurseurs (Ho, 1993; Bohnenstengel, 1992) , des conditions de torréfaction (Shaw et Ho, 1990) et du milieu réactionnel en particulier en fonction du pH, de l'activité de l'eau (Leahy et Reineccius, 1990) et de la présence de lipides (Farmer et Mottram, 1990 ; Whitfield, 1992). Les composés phénoliques du café vert (les acides chlorogéniques), de 7 à 14 % suivant l'espèce *Coffea arabica ou Coffea robusta* constituent une famille aussi importante en quantité que les lipides. Ces composés, outre le fait qu'ils se dégradent sous l'action de la chaleur et donnent certains composés phénoliques préjudiciables à la qualité (Leloup, 1995), peuvent comme les lipides intervenir dans les réactions de Maillard ; leur interaction peut donc être soit directe, soit indirecte par l'intermédiaire de composés phénoliques provenant de leur propre dégradation thermique.

Le but de notre étude a donc été de mettre en évidence le rôle de l'acide caféyl 5-quinique, composé majoritaire des acides chlorogéniques, sur la formation de pyrazines dans les réactions de Maillard. Les réactions en système modèle ont été suivies par la formation des pyrazines qui représentent la deuxième famille de composés trouvés dans l'arôme café et qui contribuent directement à la note "grillé ou torréfié"de l'arôme (Shibamoto, 1991). Elles ont été effectuées dans les conditions de température et de durée de la torréfaction, en faisant varier d'une part la nature des précurseurs constituant les systèmes modèles (saccharose et acides aminés) et d'autre part la concentration en acide caféyl 5-quinique.

MATERIELS ET METHODES

Conditions expérimentales :

Les réactifs, de qualité analytique, sont fournis par les sociétés Merck et Fluka. Les réactions entre saccharose (100 mg), acides aminés (100 mg de valine ou leucine) et acide caféyl 5-quinique (de 0 à 15 mg) sont effectuées dans des fioles scellées chauffées dans un four. Ces différents composés, additionnés d'eau ou de tampon pH 5 (50 µl), sont mélangés intimement à un support inerte, de la Célite 545 (silice diatomée pour filtration). La température de grillage est de 220°C pendant 6 min. Après réaction, la fiole est refroidie rapidement puis le mélange est extrait aux ultrasons 4fois par 1 ml d'éther contenant de la chloropyrazine comme étalon interne (25 mg/100 ml).

Conditions analytiques :

Les extraits sont analysés par chramatographie phase gazeuse dans les conditions suivantes :

- colonne capillaire J&W 30 m, phase DB-WAX, diamètre interne 0,32 mm, film 0,25 µm
- programmation de température de 60 à 200 °C, 2 °C/min

L'identification des différentes pyrazines est effectuée par couplage chromatograhie phase gazeuse-spectrométrie de masse . Une confirmation peut être faite par rapport à des témoins.

RESULTATS ET DISCUSSION

Afin d'étudier l'effet de l'acide caféyl 5-quinique sur la formation des pyrazines présentes dans l'arôme café, nous avons choisi les systèmes modèles à base de saccharose et d'acides aminés neutres (valine et leucine). Le saccharose a été choisi car sa teneur est importante, de 3 à 8 % dans le café vert, et dans les conditions expérimentales de température, celui-ci peut s'hydrolyser en glucose et fructose, sucres réactifs dans les réactions de Maillard. Les acides aminés choisis sont présents sous forme libre dans le café vert et leur teneur varie suivant l'espèce *Coffea arabica ou Coffea robusta* et le traitement post récolte (Tressl 1982).

Un support inerte, Celite 545, est mélangé aux réactifs afin de favoriser la réaction sans carbonisation des produits et ce support facilite également l'extraction des composés aromatiques formés. L'ajout d'eau ou de tampon pH 5 permet d'obtenir un mélange réactionnel plus homogène avant grillage.

Caractérisation des pyrazines formées à partir du système modèle valine-saccharose.

Le mélange réactionnel est constitué de 100 mg de valine (0,85 mmole), de 100 mg de saccharose (0,29 mmole) et de 50 µl d'eau ou tampon pH 5. Les principales pyrazines formées sont identifiées Tableau I.

Tableau I : Pyrazines formées à partir du système modèle valine-saccharose.

PYRAZINES	REF	Quantité formée (mg)	
		Eau	pH 5
Méthyl 2-pyrazine	1	0,46	0,48
Diméthyl 2,5-pyrazine	2	1,97	1,77
Diméthyl 2,6-pyrazine	3	0,49	0,52
Ethyl 2,méthyl 5-pyrazine	4	0,27	0,27
Triméthylpyrazine	5	0,40	0,41
Méthyl 2, (méthyl 2-propyl) 3-pyrazine	6	0,75	0,69
Méthyl 2, propyl 3-pyrazine	7	0,33	0,34
Diméthyl 2,5 (méthyl 2-propyl) 3-pyrazine	8	2,63	2,36

L'influence du tampon pH 5 par rapport à l'eau n'est pas significative sur la formation des pyrazines sauf pour la

diméthyl 2,5-pyrazine et la diméthyl 2,5 (méthyl 2-propyl) 3-pyrazine où une diminution de l'ordre de 10 % est observée.

Influence de la concentration en acide caféyl 5-quinique sur la formation des pyrazines à partir du système modèle valine-saccharose.

Les mélanges réactionnels, identiques au précédent, sont effectués en présence d'acide caféyl 5-quinique à des concentrations croissantes de 5, 10 et 15 mg soit 0,014, 0,028 et 0,042 mmole. L'évolution quantitative des pyrazines formées est résumée Tableau II en présence d'eau ou de tampon pH 5.

Tableau II : Teneur en pyrazines en fonction de la quantité d'acide caféyl 5-quinique.

PYRAZINES	Teneur en acide caféyl 5-quinique							
	eau				pH 5			
	sans	5mg	10mg	15mg	sans	5mg	10mg	15 mg
Méthyl 2-pyrazine	0,46	0,33	0,15	0,13	0,48	0,35	0,20	0,12
Diméthyl 2,5-pyrazine	1,97	1,17	0,77	0,49	1,77	1,20	0,97	0,51
Diméthyl 2,6-pyrazine	0,49	0,42	0,21	0,17	0,52	0,41	0,27	0,17
Ethyl 2,méthyl 5-pyrazine	0,27	0,21	0,11	0,09	0,27	0,20	0,14	0,09
Triméthylpyrazine	0,40	0,28	0,13	0,10	0,41	0,27	0,16	0,09
Méthyl 2, (méthyl 2-propyl) 3-pyrazine	0,75	0,43	0,16	0,12	0,69	0,43	0,21	0,11
Méthyl 2, propyl 3-pyrazine	0,33	0,25	0,14	0,16	0,34	0,25	0,21	0,19
Diméthyl 2,5 (méthyl 2-propyl) 3-pyrazine	2,63	1,58	0,55	0,40	2,36	1,57	0,77	0,57

Quelle que soit la nature du milieu, eau ou tampon pH 5, on observe une décroissance dans la formation des différentes pyrazines en fonction de la quantité d'acide caféyl 5-quinique ajouté. Pour une teneur de 15 mg en acide caféyl 5-quinique du melange réactionnel, le taux d'inhibition relatif à la formation des différentes pyrazines (Tableau III) est compris entre 70 et 85 % sauf pour la méthyl 2, propyl 3-pyrazine où il est plus faible (44 % à pH 5 et 52 % avec eau).

Tableau III : Taux d'inhibition de formation des pyrazines avec 15 mg d'acide caféyl 5-quinique.

PYRAZINES	REF	Taux d'inhibition %	
(15 mg d'acide caféyl 5-quinique)		Eau	pH 5
Méthyl 2-pyrazine	1	72	75
Diméthyl 2,5-pyrazine	2	75	71
Diméthyl 2,6-pyrazine	3	65	67
Ethyl 2,méthyl 5-pyrazine	4	66	66
Triméthylpyrazine	5	75	78
Méthyl 2, (méthyl 2-propyl) 3-pyrazine	6	84	84
Méthyl 2, propyl 3-pyrazine	7	**52**	**44**
Diméthyl 2,5 (méthyl 2-propyl) 3-pyrazine	8	85	76

Influence de la concentration en acide caféyl 5-quinique sur la formation des pyrazines à partir du système modèle leucine-saccharose.

Les résultats obtenus à partir des mélanges réactionnels leucine-saccharose en présence d'acide caféyl 5-quinique à différentes concentrations (de 0,014 à 0,056 mmole) sont résumés tableau IV.

Tableau IV : Teneur en pyrazines obtenue à partir du système modèle leucine- saccharose en présence d'acide caféyl 5-quinique (milieu tampon pH 5).

PYRAZINES	Teneur en acide caféyl 5-quinique				
pH 5	sans	5 mg	10 mg	15 mg	20 mg
Méthyl 2-pyrazine	0,43	0,33	0,22	0,18	0,08
Diméthyl 2,5-pyrazine	0,93	0,77	0,57	0,42	0,28
Diméthyl 2,6-pyrazine	0,44	0,42	0,31	0,25	0,13
Ethyl 2,méthyl 5-pyrazine	0,20	0,15	0,11	0,08	0,05
Triméthylpyrazine	0,39	0,23	0,16	0,12	0,07
Ethyl, diméthylpyrazine	0,20	0,12	0,08	0,06	0,05
Méthyl, pentyl pyrazine (isomères)	1,81	1,33	1,00	0,79	0,52
Diméthyl 2,5 (méthyl 3-butyl) 3-pyrazine	2,98	2,15	1,56	1,17	0,70
Triméthyl, hexylpyrazine	0,42	0,29	0,21	0,15	0,13

Comme pour le système valine-saccharose on observe une diminution de la formation des différentes pyrazines, cette diminution étant fonction de la quantité d'acide caféyl 5-quinique présent dans le mélange réactionnel. Le taux d'inhibition pour l'ensemble des pyrazines formées (Tableau V) est cependant légèrement inférieur, à concentration égale en acide caféyl 5-quinique, à celui du système modèle valine-saccharose.

Tableau V : Taux d'inhibition de formation des différentes pyrazines avec 15 mg et 20 mg d'acide caféyl 5-quinique.

PYRAZINES	REF	Taux d'inhibition %	
Acide caféyl 5-quinique		15 mg	20 mg
Méthyl 2-pyrazine	1	58	81
Diméthyl 2,5-pyrazine	2	55	70
Diméthyl 2,6-pyrazine	3	43	70
Ethyl 2,méthyl 5-pyrazine	4	60	75
Triméthylpyrazine	5	69	82
Ethyl, diméthylpyrazine	6	70	75
Méthyl, pentylpyrazine (isomères)	7	56	71
Diméthyl 2,5 (méthyl 3-butyl) 3-pyrazine	8	61	77
Triméthyl, hexylpyrazine	9	64	69

La présence dans le milieu réactionnel d'acide caféyl 5-quinique, même à faible concentration, diminue donc la formation des pyrazines, et ce quel que soit l'acide aminé. Le milieu, neutre (eau) ou légèrement acide (tampon pH5), a peu d'influence sur la formation des pyrazines.

Caractérisation physique et olfactive des systèmes modèles après réaction.

Parallèlement à la détermination qualitative et quantitative des pyrazines formées, la couleur et l'odeur de chaque milieu réactionnel ont été caractérisées après grillage. Les résultats sont résumés Tableau VI.

Tableau VI : Caractérisation de l'odeur et de la couleur du milieu réactionnel après grillage.

Ac. Aminés	Ac. Caféyl 5-quinique (mg)	Odeur	Couleur
Valine	0	noisette grillée (++)	brun clair
	5	noisette grillée (+)	brun
	10	noisette grillée (-)	brun foncé
	15	acre, desagréable	brun noir
Leucine	0	amande grillée (++)	brun clair
	5	amande grillée (+)	brun
	10	amande grillée (-)	brun foncé
	15	acre, désagréable	brun noir

L'odeur et la couleur du milieu réactionnel varie peu en fonction de l'acide aminé mais surtout en fonction de la quantité d'acide caféyl 5-quinique présent dans le milieu. L'odeur agréable de noisette ou d'amande grillée diminue fortement avec la teneur en acide caféyl 5-quinique, tandis que l'intensité de la couleur augmente. A partir d'une teneur de 15 mg en acide caféyl 5-quinique, une odeur acre de type phénolique apparaît et masque la flaveur agréable à note "grillé"due aux différentes pyrazines.

Applications aux cafés verts ROBUSTA : influence de la teneur en acides chlorogéniques sur la qualité aromatique.

Ces résultats obtenus à partir de systèmes modèles simples expliquent les corrélations entre la composition chimique (teneur en acides chlorogéniques et saccharose) et les tests organoleptiques (qualité aromatique) observéeslors d'études sur la qualité de différents clones de cafés Robusta (Tableau VII).

Tableau VII : Composition chimique et qualité aromatique de différents clones cafés Robusta.

Cl. ROBUSTA	M. préparation	Saccharose %	Ac. Chloro. %	Qual. Arome
461	VS	2,5	13,7	très faible
126 (1)	VS	5,8	11,8	bonne
126 (2)	VS	3,2	14,5	très faible
503	VS	4,0	13,2	faible
628	VS	4,8	11,9	bonne
J21	VS	5,1	11,0	bonne
J21	VH	5,8	11,1	très bonne

(1) : café mature (2) : café immature

Les teneurs en acides chlorogéniques et en saccharose sont en définitive des paramètres très importants pour les caractéristiques organoleptiques. La qualité aromatique des cafés augmente quand la teneur en acides chlorogéniques diminue et quand la teneur en saccharose augmente. Ces résultats ont été déjà observés lors d'études

sur la qualité des cafés :

- qualité aromatique des cafés Robusta en fonction de la maturité : coefficient de corrélation de -0,50 entre la teneur en acides chlorogéniques et de 0,89 entre la teneur en saccharose respectivement avec la qualité au niveau organoleptique (Guyot, 1987).

- qualité des cafés Arabica (Catuai) en fonction de l'altitude et de l'ombrage : coefficient de corrélation de -0,17 entre la teneur en acides chlorogéniques et de 0,55 entre la teneur en saccharose respectivement avec la qualité au niveau organoleptique (Guyot, 1996).

CONCLUSION

Les résultats de cette étude montrent donc que la teneur en acide caféyl 5-quinique, et donc en acides chlorogéniques pour le café vert, joue un rôle important dans les réactions de Maillard en inhibant la formation de composés aromatiques comme les pyrazines. Cette inhibition pourrait être due aux propriétés antioxydantes de l'acide caféyl 5-quinique qui bloqueraient certaines réactions de condensation. Ce résultat est intéressant pour la sélection génétique car à partir de la composition chimique du café vert, en particulier de sa teneur en acides chlorogéniques et en saccharose, la qualité organoleptique d'un café Robusta pourra être sinon prédite du moins estimée : les cafés à forte teneur en acides chlorogéniques (>13%) devant être éliminés de la sélection pour la qualité. Une étude complémentaire devra cependant être entreprise pour définir l'origine de ce mécanisme d'inhibition dû à l'acide caféyl 5-quinique et à ses propriétés chimiques.

RESUME

L'arôme café est constitué de composés formés en grande partie par les réactions de Maillard, réactions de condensation entre sucres et acides aminés. L'arôme obtenu est fonction de ces composés mais aussi de l'environnement biochimique et de paramètres extérieurs comme la température et la durée de torréfaction.

L'étude de ces réactions en systèmes modèles ont montré que la qualité de l'arôme obtenu, caractérisée par la teneur en pyrazines, varie en fonction de la concentration en acide caféyl 5-quinique, constituant majoritaire des acides chlorogéniques dans le café vert. Les résultats montrent que la teneur en pyrazines formées diminue lorsque la concentration en acide caféyl 5-quinique augmente. Cette diminution est particulièrement nette dans le cas de réactions mettant en oeuvre des acides aminés neutres apolaires : la valine et la leucine.

Les conclusions de cette étude permettent de mettre en évidence le rôle inhibiteur des acides chlorogéniques du café vert sur la formation de l'arôme café par les réactions de Maillard. Ce critére devra donc être pris en compte pour la sélection de nouveaux clones afin d'obtenir un arôme optimal.

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VOLATILE COMPOUNDS ASSOCIATED WITH THE OVER-FERMENTED FLAVOUR DEFECT

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INTRODUCTION

One of the most objectionable organoleptic defects is introduced into coffee products by over-fermented green coffee or by so called *'stinker'* beans. The occurrence of *'stinkers'* is associated with an intolerable *fruity, silage-like* or *rotten* flavour and will usually lead to rejection of spoiled batches by the coffee trade since over-fermented lots may contaminate large quantities of coffee even at low levels. Research into this special field of coffee technology has been undertaken since decades. Early investigations aimed at empirical elucidations of the major risks in green coffee processing that may generate *stinker* beans (1-3). It was found that various factors may yield uncontrolled fermentation such as increased bean moisture, elevated temperatures, the presence of certain microbes and extensive fermentation times (4). A few researchers looked into the chemistry of volatiles that could be linked to *'stinker'* beans (5,6). However, odour quality and intensity of the suggested compounds reveal that the relationship with the over-fermented flavour was more indicative rather than causative. The objective of the present work was to identify the key aroma compounds using the current toolbox of aroma chemistry and to establish a method for quantitative assessments for tracking *'stinker'* beans by objective analytical means.

EXPERIMENTAL

Materials and Sample Preparation. Two washed Arabicas of different origins and one Robusta with a distinctive over-fermented flavour defect were supplied by green coffee experts. One bag (60 kg) of green Arabica coffee with a moderate over-fermented flavour was available for most of the investigations (AOF I). A second Arabica sample (AOF II) and another Robusta (ROF) were available in small quantities. Two Robustas and eight Arabicas with a neutral flavour were selected for reference purposes.

2-Methyl butanoic acid ethylester (2-MBEE) and 3-Methyl butanoic acid ethylester (3-MBEE) were purchased from Aldrich, Steinheim, Germany. d_3-3-MBEE, cyclohexanoic acid ethylester (CHEE) and its deuterium labelled derivative were synthesised according to reference (7) using ethyl-2,2,2-d_3 -alcohol for preparation of the labelled reference compounds. Roasting was performed on a benchtop scale fluidised bed roaster for 3 min at 270°C. Coffee beverages were prepared at a recipe of 50 gms per liter using a drip filter machine.

Shelf life tests were executed with Colombian green coffee. Moisture contents were adjusted to 14, 15, 16, 18 and 20% with distilled water at 80°C. Samples were kept at 30°C and 90% rel. humidity. The control was kept at room temperature. For comparison one sample was steam treated at 100°C for 5 min using a simple laboratory autoclave. The final moisture level was adjusted to 20%.

Threshold values were determined by spiking coffee beverages (commercial Arabica blend) by adequate amounts of ester stock solutions and presenting the samples as triangular testing versus control to 6 experienced cup testers. The threshold data given in Table 3 represent the lowest and highest threshold value as observed by the panellists.

Isolation of Volatiles. Green coffee samples were ground with a Condux® mill (average particle size 0.5-1 mm) and 100 gms were placed in a 2 l round bottom flask together with 1 l of distilled water. For quantitative measurements the internal standards d_3-MBEE and d_3-CHEE (ca. 2 µg each in 1 ml tert. butylmethyl ether) were added. Simultaneous distillation/extraction was carried out for 2 h (8). 50 ml of a mixture of n-pentane/diethylether (1+1, v/v) was used as solvent. The raw extract was dried over anhydrous sodium sulphate, filtered off into a 50 ml finger flask and concentrated to about 1.5 ml by means of a Vigreux column (250 x 10 mm) at a water bath temperature of 45°C, subsequently. Reproducibility was checked by analysing 10 samples of the same fermented lot that was ground and carefully mixed previously .

Gas Chromatography (GC), GC-Olfactometry (GC-O) and Mass Spectroscopy (MS). GC-separations were performed on Hewlett Packard® gas chromatographs type 5890 series II equipped with a DB-1701 capillary column (60 m x 0.25 mm, film thickness 0.25 mm; J&W Scientific®). Injection volumes were 1 µl applied by a programmable temperature variable injector system (Gerstel®, Mülheim, Germany). The temperature profile ranged from 60°C to 200°C at a heating rate of 12°C/min. The oven temperature profile was 35°C held for 2 min then raised 5°C/min to 220°C and held for 10 min.

GC-O was conducted on a Carlo Erba® gas chromatograph MEGA type 5300 using the same capillary columns mentioned above but with larger inner diameter (0.32 mm). The GC-effluent was split 1:1 by means of a glass-cap-cross splitter (9) for flame ionisation detection as well as for simultaneous olfactometric evaluation by the human nose. Sniffing was performed by 3 trained testers.

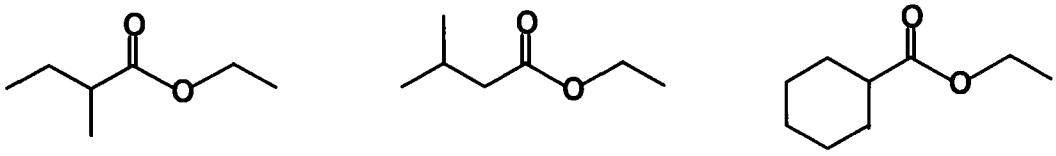
GC-MS was performed on a Hewlett Packard® MSD 5972 (EI mode, ionisation energy 70 eV). The components of interest were quantified by SIM, using the internal standard method (see also Table I).

RESULTS AND DISCUSSION

Identification of the Off-Flavour Causing Compounds. One of the key issues in aroma chemistry is the availability of authentic sample material that actually represents the positive or negative flavour attribute to be investigated. Green coffees with an over-fermented flavour defect usually do not arrive in the consumer countries. The present sample set was selected from a large number of healthy shipments and showed a distinctive *fruity, silage-like* flavour defect at different intensities.

GC-O is the method of choice to characterise single or few key aroma compounds in complex matrices such as coffee. The non-fractionated volatiles concentrate isolated from the spoiled coffees was evaluated by means of the human nose of experienced testers. Besides numerous odour notes typically present in green coffee aroma extracts (10) basically three *fruity* odour notes were perceived at the sniffing port at different intensities. The corresponding chemical compounds were identified as 2- and 3-MBEE and CHEE (Figure 1) by comparison of GC retention and mass spectral data with authentic reference chemicals (Table I). The present work suggests that these three esters are by far the most important contributors to the over-fermented flavour defect despite the fact that the ethyl esters of almost all food relevant fatty acids occur in coffee. GC-effluent sniffing was conducted over the whole chromatogram and performed on two capillary columns of different polarity but no further *fruity* off-notes were perceived that might also contribute to the over-fermented flavour. These three compounds have not been reported yet as coffee aroma compounds (11).

Figure 1: Odourants associated with the over-fermented flavour defect.



2-MBEE **3-MBEE** **CHEE**

The presence of 2- and 3-MBEE is not surprising since the corresponding precursor acids are well known coffee volatiles (12) whereas, that of CHEE actually is. In food CHEE has been detected in rum aroma earlier (13). It is interesting to note that these compounds were found amongst other ethyl esters in spoiled milk that was contaminated by certain bacteria (14). Since the cyclohexane carboxylic structural frame is not very common in higher plants it is likely that specific microbes may play an important role for the generation of CHEE.

Table 1: GC retention data and MS target ions relevant for the present work, calculated according to (15), column parameter: 60m x 0.32 mm, 0.25 mm film thickness.

Compound	DB-1701	FFAP	m/z	Odour Description
2-MBEE	904	1062	115	*fruity*
3-MBEE	911	1077	115	*fruity*
d_3-3-MBEE	909	1077	118	
CHEE	1209	1441	101	*fruity, silage-like*
d_3-CHEE	1207	1441	104	

Analytical Procedure. The method of choice to quantify trace odourants in complex matrices is the use of stable isotopes as internal standard to minimise the risk of losses or other inaccuracies during the isolation and separation of volatiles. In the present case deuterium labelled 3-MBEE and CHEE were easily synthesised and added for internal standardisation. The relative standard deviation of this method was between 3.8-5%. The detection limits were 0.2 µg/kg for 2- and 3-MBEE and 0.05 µg/kg for CHEE.

Quantitative Results. As Table 2 and Figures 2 and 3 show, 2- and 3-MBEE and CHEE are obviously detectable in the healthy Arabica and Robusta green coffees at low levels. A flavour defect is therefore likely only above certain quantitative levels. The quantitative data of the spoiled green coffees demonstrate that at least one compound occurs in significantly elevated amounts. CHEE was significantly elevated in the washed Arabicas only whereas, the spoiled unwashed Robustas showed higher levels of MBEE and no increase in CHEE. The reason therefore is not yet clear. 2- and 3-MBEE show a quite constant quantitative ratio of 1:10 which reflects the ratio of the corresponding acids in roasted coffee fairly well (16).

Table 2: Contents of key aroma compounds related with the over-fermented flavour defect in green coffee; all data given as µg/kg (RSD = Relative Standard Deviation; *: lowest and highest value detected in 10 healthy controls).

	RSD	Controls*	AOF I	AOF II	ROF
2-MBEE	5 %	0.15 - 2.5	1.8	37.4	8.7
3-MBEE	3.8 %	2.3 - 13.3	25.7	345	86.4
CHEE	3.8 %	0.1 - 0.7	10.4	19.8	0.2

Quantitative Distribution of Ethyl Esters Across a Green Coffee Lot. It is well accepted in the world of coffee that the compounds associated with the over-fermented flavour defect are not distributed homogeneously across a given coffee lot. Since the off-flavour intensity can vary from cup to cup it is likely that the off-notes may be concentrated in certain parts or even single beans (*'stinkers'*) across the batch.

Figure 2: Distribution of 3-MBEE in green and roasted coffee.

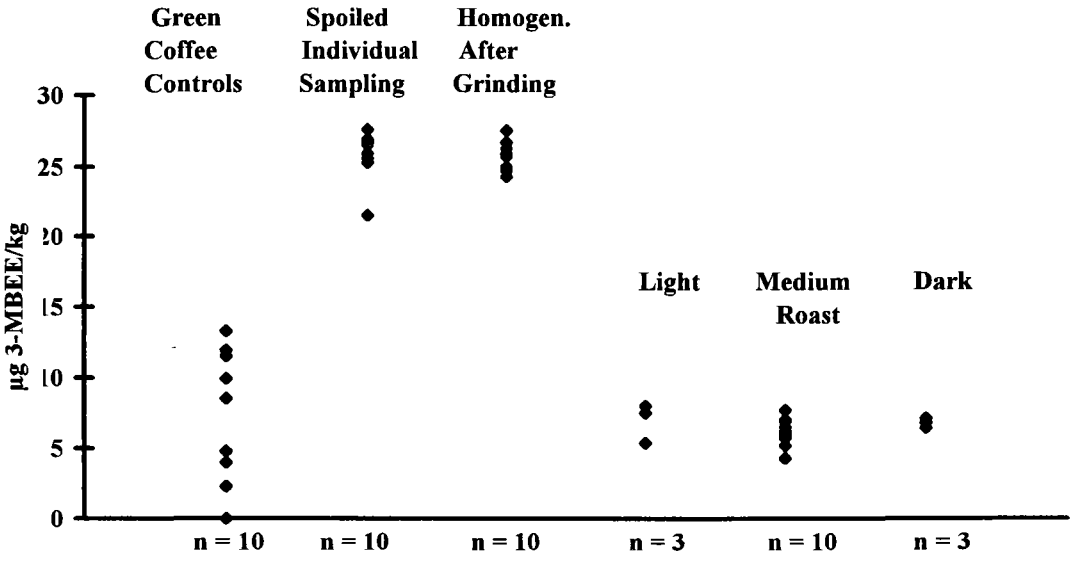
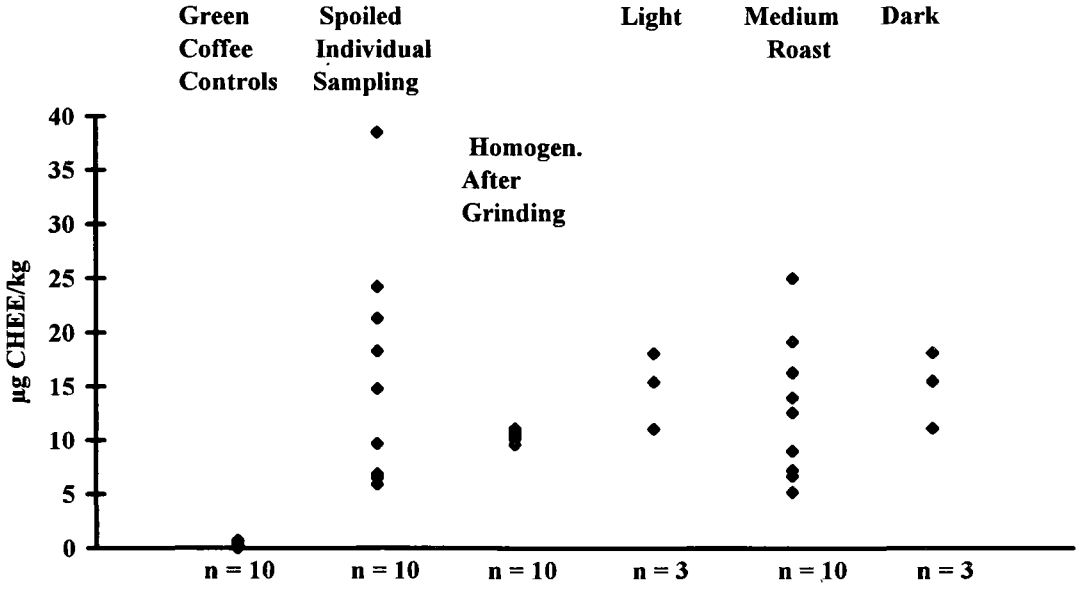


Figure 3: Distribution of CHEE in green and roasted coffees.



As mentioned above one bag of over-fermented green coffee was available for a more detailed and broader experimental approach to find evidence that may support the empirical findings. In one experiment ten spot checks were sampled from the bag and analysed after individual grinding of each sample. Another ten spot checks were sampled individually from the bag but were then combined again and ground and carefully mixed as one single batch. Ten samples of this ground material were taken for analyses. The readings of 3-MBEE and CHEE are depicted in Figure 2 and 3 and compared to those of healthy green coffees. Figure 2 shows that small quantities of 3-MBEE generally occur in coffee with maximum levels around 13 µg/kg. The spoiled beans showed slightly elevated levels and fairly good repeatability across the bag no matter whether the sample had been homogenised or not. This finding indicates that 3-MBEE is homogeneously distributed across the lot.

Traces of CHEE were detected in the control samples at levels less than 1 µg/kg (non washed coffees) or much less than 1 µg/kg (washed coffees). As opposed to 3-MBEE, the content of CHEE varied very broadly across the spoiled bag and ranged from 3 to 40 µg/kg (Figure 3). Ten replicates of the homogenised material gave little analytical variation and an average value of 10 µg/kg. It may be speculated that in particular CHEE is related with the *'stinker'* bean phenomenon.

Influence of Roasting. Figures 2 and 3 also show that roasting of the spoiled beans will reduce but not eliminate the light volatile 3-MBEE but will not affect the levels of CHEE significantly. In roasted beans CHEE varied across the lot similar to the green matter. This kind of variation was more dominant than the effect of roasting.

Table 3: Calculation of aroma values via sensory threshold values and quantitative data.

	Roast Coffee (µg/kg)	Beverage (µg/l)	Threshold Value Coffee (µg/l)	Aroma Value
2-MBEE	1.8	< 0.05	n.d.	n.d.
3-MBEE	13.9	0.14	0.2 - 0.5	0.3 - 0.7
CHEE	14	0.1	0.005 - 0.01	10 - 20

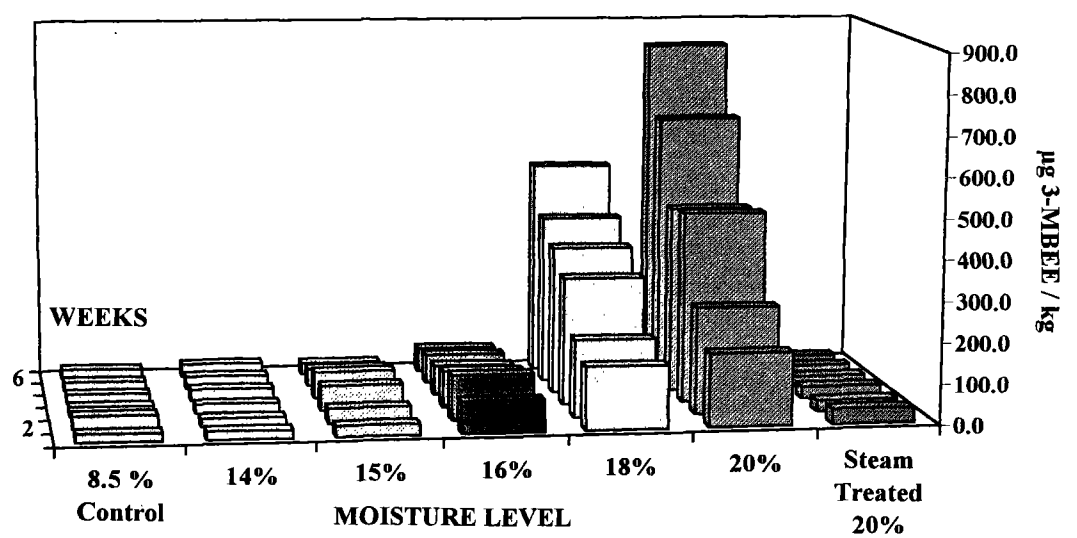
(n.d.: not determined)

Sensory Relevance. The individual contribution of a particular odourant X in a given food matrix is roughly estimated by the term aroma value$_{(X)}$ which is the concentration$_{(X)}$ divided by the sensory threshold value$_{(X)}$ in the matrix. An aroma impact will be likely if the aroma value$_{(X)}$ is larger than 1. To estimate the individual impact of the three target esters to the over-fermented flavour one spot check from lot AOF I was roasted and ground. 2(3)-MBEE and CHEE were measured in the roasted and ground matrix as well as in a coffee beverage prepared from the same coffee (Table 3). From these readings and the corresponding sensory threshold values in coffee the aroma value can easily be calculated. For sample AOF I it came out that the aroma value of 3-MBEE was somewhat below 1. A sensory impact at this level remains questionable. It can furthermore be assumed that 2-MBEE does not yet contribute to the off-flavour of that particular sample since the aroma value ought to be much below 1. The concentration of CHEE however, exceeds the threshold value by a factor of 10-20.

The quantitative levels detected in sample AOF II (Table 2) reveal that all three esters will contribute to the off-flavour to a different extend. 3-MBEE appears to be the major off-flavour causing compounds in sample ROF. 2- and 3-MBEE possess fairly low odour threshold values compared with many other ethyl esters (17). The threshold value of CHEE was experimentally determined to be even significantly lower by several orders of magnitudes (Table 3).

Green Coffee Shelf Life Test. Elevated moisture contents and temperatures are known to be critical factors for the formation of over-fermented flavour defects even during shipment or storage (1). Figure 4 shows that these influences are not only important for green coffee processing but can be simulated on the benchtop using a given green coffee. Over the shelf life period of six weeks CHEE did not change significantly. The contents of 2- and 3-MBEE however, went up dramatically and approached the parts per million level as a function of the moisture and storage time. A moisture content of 14 % may be considered as the critical limit to avoid flavour deterioration related with microbial growth but does not seem to leave much margin to stay on the safe side. The quantitative readings of the short time steam treated coffee remained fairly unaffected even at moisture levels of 20% and the over-fermented flavour was not perceived. This finding gives excellent evidence that microbes are involved in the generation of the over-fermented flavour defect.

Figure 4: Generation of 3-MBEE in green coffee during storage at 30°C and 90% relative humidity as a function of moisture level and time.



SUMMARY AND CONCLUSIONS

The results of the present work reveal that three ethyl esters of short chain fatty acids, 2- and 3-MBEE and CHEE, are actual or potential key aroma compounds responsible for the over-fermented flavour defect. The individual contribution is a function of the actual concentration in a given sample measured by an optimised methodology that delivers reliable readings even in the low ppb level. CHEE is not homogeneously distributed across a green bean lot but is concentrated in certain beans. Investigation of the corresponding coffee beverage and the sensory threshold values gave an idea of the relationship between quantitative levels and the corresponding sensory impact of individual esters. Elevated levels of these aroma potent ethyl esters may not only occur in wet processed Arabicas but were observed in unwashed Robustas as well. The occurrence of an over-fermented flavour defect is not limited to inappropriate green coffee processing. Critical factors such as elevated moisture levels and temperatures may support growth of microbes that generate the off-flavour causing key compounds during shipping or storage.

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SUMMARY. *The results of the present work reveal that specific ethyl esters of short chain fatty acids are actual or potential key aroma compounds responsible for the over-fermented flavour defect. The individial contribution is a function of the actual concentration in a given sample. For quantification an optimised methodology was developed that delivers reliable readings even in the low ppb level. Elevated levels of these off-flavour causing compounds may not only occur in wet processed Arabicas but were observed in un-washed Robustas as well. The occurrence of an over-fermented flavour defect is not limited to inappropriate green coffee processing. Critical factors such as elevated moisture levels and temperatures in green coffee may generate an over-fermented flavour defect during shipping or storage.*

RESUME. *Les résultats de cette étude scientifique révèlent que certains esters d'éthyle composés d'acides gras à chaines courtes sont les composés clés réels ou potentiels responsables de fèves trop fermentées ou de fèves puantes qui se développe dans quelques cafés verts trop fermentés. La contribution individuelle de chacun des composés est fonction de leur concentration dans l'échantillon. Afin de quantifier cela, une méthodologie optimisée a été élaborée. Celle-ci donne des résultats fiables même pour les concentrations de l'ordre de la ppb. Des teneurs élevées de ces composés puants ne se trouvent pas seulement dans les cafés verts Arabica mais aussi dans les cafés naturels de Robusta. La formation du goût puant peut ne pas être seulement dûe à un procédé inapproprié pour le café vert. Des facteurs tels qu'une forte humidité et des températures élevées dans le café vert peuvent être à l'origine d'un goût puant après la recolte.*

DETECTION OF AROMA ABOVE A COFFEE POWDER : LIMITS AND PERSPECTIVES OF ELECTRONIC SENSORS

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Introduction

Since a few years, electronic gas sensor arrays, so-called electronic noses, are being tested in various R&D laboratories for their ability to characterise the headspace composition of products of different nature, like foods or packaging. Their main advantage lies in the simplicity of use, combined with an integrated data treatment allowing the comparison and discrimination of samples. These features make these instruments particularly well suited for screening and quality control applications where they could be more easily introduced than traditional methods based on gas chromatography.

Various studies related to coffee have been reported, using either metal oxide sensors, or to a lesser extent, conducting polymers[1-8]. Most studies were concerned with the possibility of discriminating between coffees at different roast levels, or freeze dried versus spray dried instant powders. Other issues such as batch-to-batch variations in production, or correlation with sensory profiling were not investigated.

In our laboratory, we were interested in establishing if such electronic sensor arrays could be of real use for analysing coffee aroma, one of the most complex volatile mixture. This study has focused on the characterisation of the aroma fraction released from instant coffee at the first opening of the package.

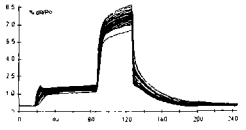
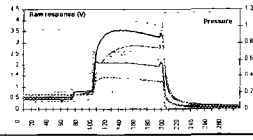
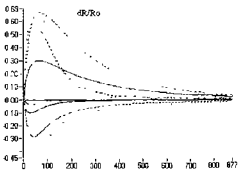
Experimental

Two types of sensors were investigated based on conducting polymers (CP) or metal oxides (MO). Three different instruments were used: one equipped with CP (Aromascan Ltd.) and two equipped with MO (in-house development; Fox 3000, Alpha M.O.S. Ltd.). The main features of the three systems are presented in Table 1 and 2.

Table 1: Instruments configuration: sensor arrays and aroma sampling procedures.

Instrument	Sensors type and number	Aroma sampling	Sample conditioning
A32/50S from Aromascan	Conducting polymers (CP) 32 sensors (array at 35°C)	• Automated static head-space sampler from Tekmar (vial pressurisation) with stop flow on the sensor array • Aspiration using regulated flow; with continuous flow on the sensor array • Air_{ref} at 10% rel. humidity	• HS vials 22 ml equilibrated at 40°C • Tedlar bags or refill pouches at 20°C
Taguchi head "in-house" development	Metal oxide (MO) 7 Taguchi (> 300°C)	• Sensor chamber put in contact with sample headspace • Static analysis	• Jars, refill pouches or Tedlar bags at 20°C
FOX 3000 from Alpha M.O.S	Metal oxide (MO) 2x 6 sensors Sy type T and P type (> 300°C)	• Automated static headspace sampler from CTC (gas syringe) • Manual injection with gas syringe • Purging air at 250 ml/min and 20% relative humidity	• HS vials 11 ml equilibrated at 45°C • Jars, refill pouches and Tedlar bags at 20°C

Table 2: Data acquisition and treatment.

Sensor type	Raw data signal (dR/R_o or R_{max})	analysis and cycle times*	data treatment
A32/50S (CP)		26" for reference air 60" for the sample 60" for washing **7' total cycle time**	sensor response averaged over 20" (40" after sample injection)
Taguchi (MO)		60" for reference air 140" for the sample **10' total cycle time**	sensor response averaged over 20" (1' after sample injection)
FOX 3000 (MO)		300" for the sample **20' total cycle time** automatic check for base line level before acquisition	maximum intensity for each sensor calibration for sensor drift available

** Cycle time corresponds to acquisition time plus time needed for sensors to return to base line*

Different samples were studied to understand to what components the sensors would respond. Neutral oil, pure chemicals dissolved in neutral oil, neutral oil aromatised with roast and ground coffee aroma, and instant coffee powders coated or not coated with the aromatised coffee oil were analysed on the different instruments.

The headspace composition of the samples was confirmed by gas chromatography. Standards and oils were analysed in 22 ml vials with an HS100/Sigma2000 (Perkin Elmer Ltd). Headspace from jars, pouches, or Tedlar bags were injected in a Hewlett-Packard 5890 gas chromatograph. Volatile compounds were separated on DBWAX columns at 8°/min (60 m, 0.25 mm i.d, 0.25 µm film thickness).

Oxygen and carbon dioxide concentrations were analysed with a Servomex 1400. Water activity of coffee powders was determined by a Rotronic analyser.

Results

The results obtained with the three sensor arrays are compared for the different categories of samples under study.

Superficially aromatised powders versus uncoated powder

Conducting polymers were not able to discriminate between an uncoated powder and a powder coated with aromatised oil, neither from a refill pouch, nor from samples conditioned in vials.

On the opposite, both sensor arrays equipped with metal oxides could distinguish aromatised samples from the reference powder. Due to the sampling method used with the Taguchi sensors (the headspace was displaced into the sensor chamber), the amount of oxygen in the jar headspace was also a factor of discrimination. The discrimination obtained with samples conditioned with or without gassing with inert gas is shown in Figure 1. Aromatisation and gassing are discriminated respectively on x and y axis.

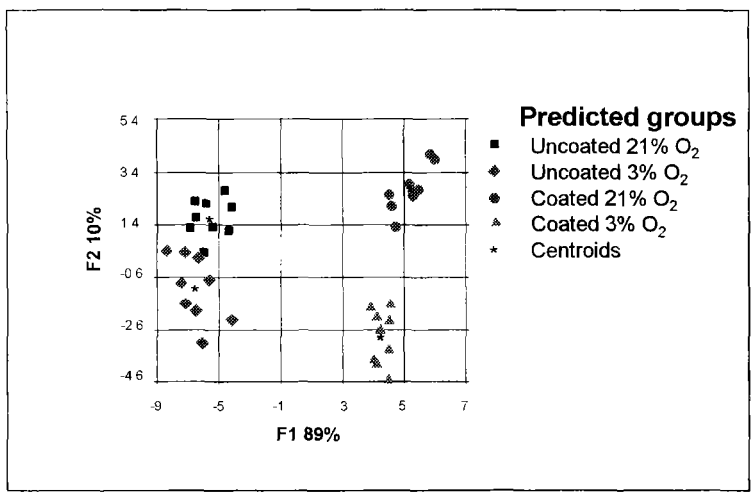


Figure 1: Plot of the discriminant functions from a linear discriminant analysis. Taguchi sensor response to different in-jar aroma. Results obtained from raw data.
The same coffee powder was coated or not with aroma; jars were gassed or not before sealing.

A good discrimination was also obtained with the Fox 3000. The sum of absolute intensities measured with the twelve sensors is reported in Figure 2. Due to the low amount of gas injected in the main flow of air passing through the sensor chambers (250 ml/min), oxygen variations between samples at different gassing levels was not detected.
The variation of intensity observed between aromatised samples was a good indicator of the jar-to-jar variability of global aroma concentration.

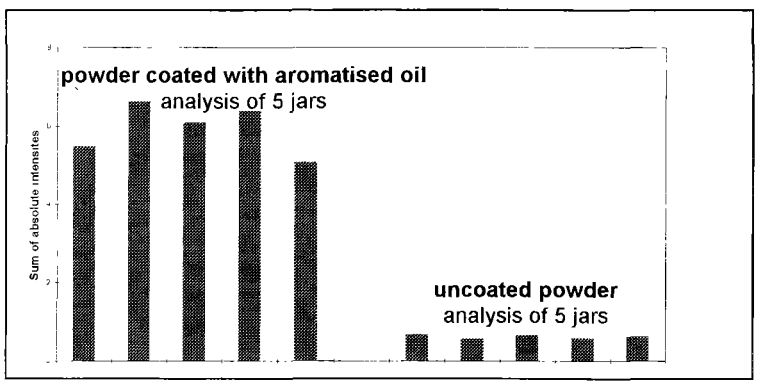


Figure 2: Headspace analysis of sealed jars with the FOX 3000. Injection of 50µl of gas.

When both types of powder were transferred into headspace vials for analysis (FOX 3000), the discrimination was still possible but the difference between uncoated powder and aromatised powder was reduced. This was explained by aroma losses occurring at jar opening.

Aromatised oil versus neutral oil

All three sensor arrays were found able of discriminating between aromatised and neutral oil. Figure 3 shows results obtained with conducting polymers. The small amount of oil put in the vials was representative of the amount of oil coated on a few grams of instant coffee. As expected, the discrimination increased with increasing amounts of aroma in the vial. These results, which contrast with those obtained for the powders, suggest that coffee powder itself has an important influence on the response of conducting polymers, overwhelming the differences due the presence or absence of aromatized oil. Furthermore, the reproducibility of analyses made in triplicate was not too good with CP, and significant sensor drifts were observed.

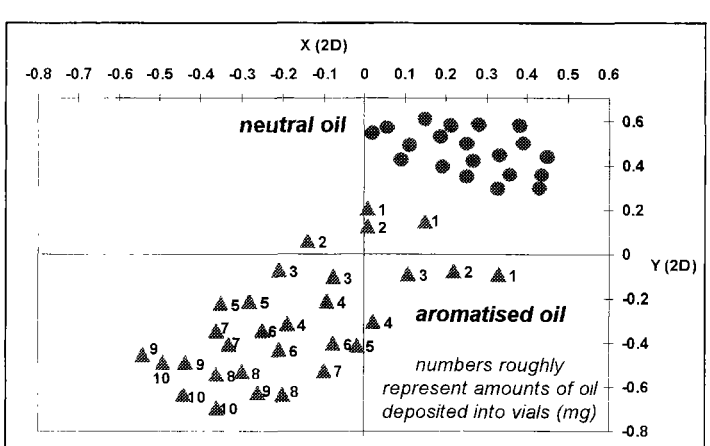


Figure 3: Non-linear mapping of analysis of neutral and aromatized oils with the A32/50S. Samples were conditioned in sealed vials with nitrogen in triplicate.

Sensitivity and selectivity of sensors towards chemical standards

The sensitivity of the sensors used was checked for a selection of coffee aroma components using standards dissolved in coffee oil or in water. The corresponding headspace concentrations were determined by means of air/oil, or air/water[9] partition coefficients. In these tests, metal oxides were found to be more sensitive than conducting polymers (see Figure 4 and 5).

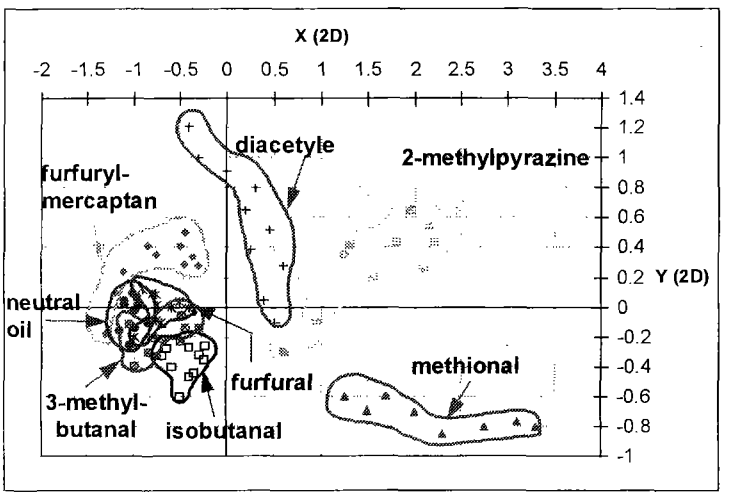


Figure 4: Test of discrimination between some standard components diluted in neutral coffee oil, using CP (A32/50S). Concentrations ranged from 20 to 2000 ppb_{vol} (ng/ml) in the headspace. Normalized data.

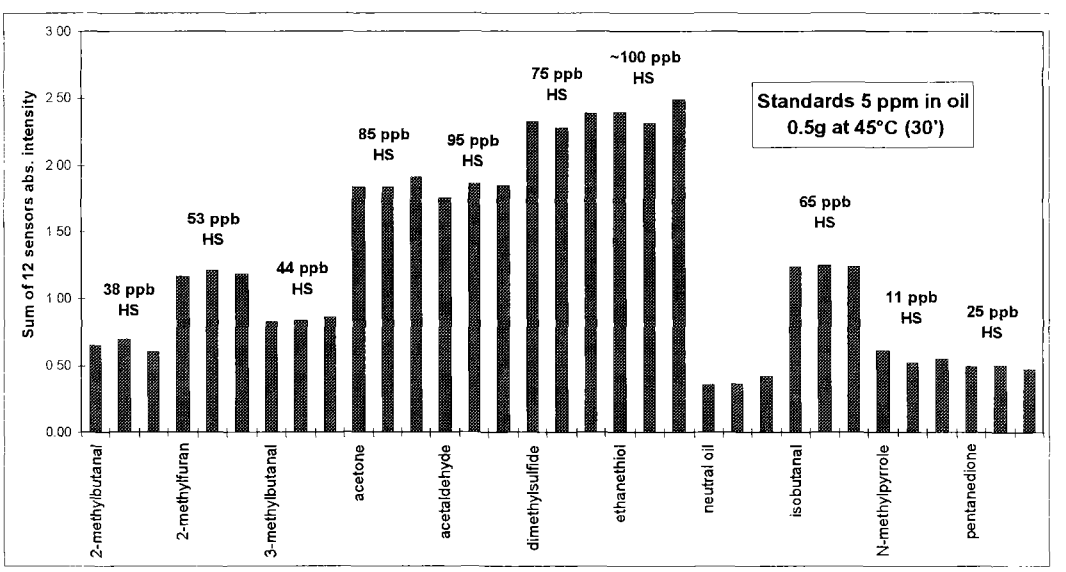
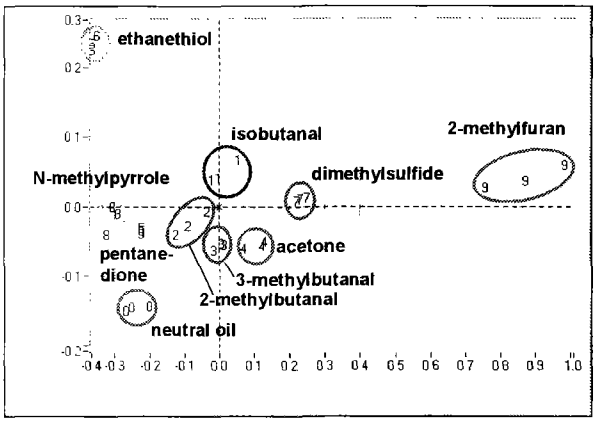


Figure 5: Reproducibility and sensitivity in ppb_{vol} (ng/ml) of MO sensors in the FOX 3000 in measuring headspace above standard solutions in neutral oil (50µl injected).

Conducting polymers achieved a good discrimination of diacetyl, 2-methylpyrazine, and of the two sulphur-containing compounds, but could hardly distinguish the various aldehydes from neutral oil. Metal oxides were indeed able to discriminate neutral oil from different chemicals, even for those



present at a concentration as low as 10 ppb_{vol}. The data reported in Figure 5 have been normalised and analysed by PCA using the 12 MO sensors as variable. The results are shown in Figure 6. Different aldehydes could be discriminated. A high discrimination power was observed for thiol and furan.

Figure 6 : Selectivity of MO sensors in the FOX 3000 in measuring headspace above solutions in standard oil. Relative data (PC1 91%, PC2 7%)

Sensitivity of sensors towards water vapour and carbon dioxide

The headspace above a coffee powder is not only composed of aroma components of high volatility, but also of water vapour (a_w of coffee powders: 0.1-0.3), and carbon dioxide[10]. The latter two components are present in significantly higher concentration than the aroma itself (> 10x).
The high sensitivity of conducting polymers towards water and carbon dioxide is evidenced in Figures 7 and 8. These results demonstrate why (CP) sensors do not discriminate aromatised coffee from non aromatised powder.

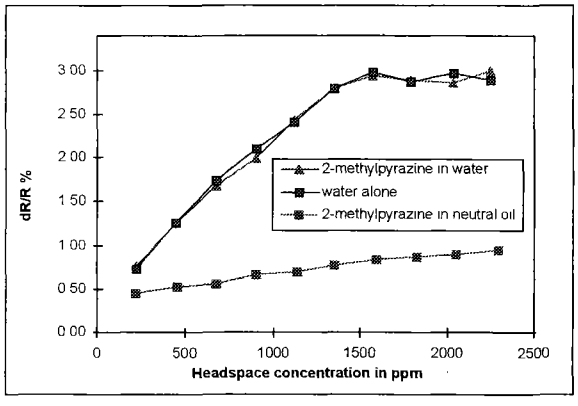


Figure 7: Response of CP sensor 18 (A32/50S) to 2-methyl-pyrazine diluted either in water or in neutral coffee oil. All CP sensors behaved similarly.

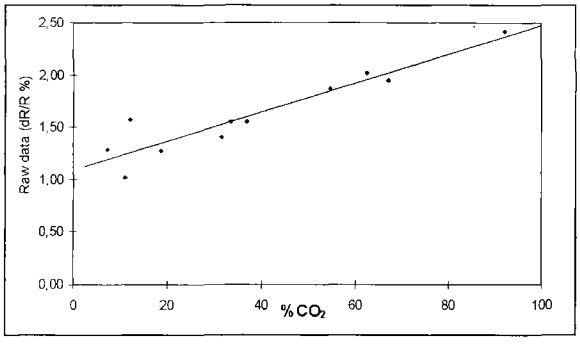


Figure 8: Response of CP sensor 1 of the A32/50S to increasing ratios of CO_2 in nitrogen. The behaviour of all sensors was similar. The gas was sampled in Tedlar bags. Its concentration in carbon dioxide was measured with a Servomex infra-red analyser.

Conclusion

A systematic approach was chosen to test the ability of conducting polymers and metal oxide sensors to respond to coffee aroma components present in the headspace of instant coffee powder.

Both types of sensors could discriminate small amount of aromatised oil from neutral oil in the absence of coffee powder. However for this application, conducting polymers did not succeed in discriminating aromatised powder from non aromatised powder. The higher proportion of water vapour and carbon dioxide compared to the small amount of aroma present in the headspace, was probably the reason of this failure. V.P.Shiers et al.[11] already reported the difficulty of using conducting polymers in the detection of spoilt beef. They supposed moisture to be the limiting factor. In another study however, beers adulterated with 200 ppb of diacetyle or dimethylsulfide were successfully differentiated from regular ones with CP sensors (T.P.Bailey[12]). Unfortunately in that case, no other analytical method was reported to confirm the results obtained with the sensors.

Metal oxide sensors were indeed able to distinguish aromatised coffee powders from uncoated powder. Because of the sensitivity of MO towards oxygen concentration, the small volume injected with the FOX system compared with the main air flow, was found adequate to avoid the influence of oxygen level in the samples. For the time being, total headspace intensity and thiols concentration seem to be the most relevant factors of discrimination between samples. The detection threshold was found in the range of 10 ppb_{vol} in the headspace. Knowing that many important fragrant components of coffee aroma are in very low concentration[12-13], the correlation with aroma quality will be difficult to achieve without still improving the sensitivity of the sensors.

Abstract

Electronic gas sensors, also known as "electronic noses", are new type of instrument allegedly capable of measuring the intensity and quality of food flavours in a rapid and easy way. In this study, we have investigated the application of such devices to the quality control of instant coffee in-jar aroma.

Two types of sensor were studied: conducting polymers (CP; Aromascan Ltd., Crewe, UK), and metal oxides (MO; in-house head equipped with Taguchi sensors and Fox 3000, Alpha M.O.S Ltd., Toulouse, F). Using a systematic approach, both type of sensors were tested for their ability of discriminating between instant coffee powders, coated or not coated with an aromatised oil. Samples analysed ranged from simple model solutions to more complex matrices. The response of the gas sensor array was compared with those obtained from more conventional determinations (headspace-GC, relative humidity etc.).

The results obtained for the conducting polymers were rather disappointing. These sensors were able of distinguishing aromatised oil from neutral oil only in the absence of coffee powder; they failed when coffee powder was present. These results were explained by the high sensitivity of conducting polymers towards moisture and carbon dioxide desorbing from coffee powder.

Better results were achieved with metal oxide sensors which succeeded in discriminating instant coffees coated with aromatised or non aromatised oil. The discrimination was mainly based on global intensity and the significant presence of some sulphur components. Limit of sensitivity was found for a concentration in the range of 20 ppb in the headspace (ng/ml).

Résumé

Les capteurs à gaz utilisés dans les nez électroniques sont décrits comme étant capables de mesurer simplement et rapidement la quantité et la composition d'un arôme. L'utilisation de tels capteurs a été testée pour caractériser l'arôme de flacon de cafés instantanés. Deux types de capteurs ont été étudiés: les polymères conducteurs (Aromascan Co., Crewe, GB), et les oxydes métalliques (tête développée par Nestlé et équipée avec des Taguchi et Fox 3000, Alpha M.O.S Co., Toulouse, F). Afin de déterminer plus précisément à quoi les capteurs répondent, une approche systématique a été suivie. En complément des poudres aromatisées ou non en surface, des huiles aromatisées, des huiles neutres, ainsi que des solutions de composés standards dans l'huile ou dans l'eau ont été étudiées sur les trois instruments. La composition de l'espace de tête des échantillons a été vérifiée systématiquement par des techniques de référence (GC, mesures de CO_2 et d'humidité relative).

Les capteurs à polymères conducteurs se sont révélés incapables de discriminer l'arôme de flacon d'une poudre aromatisée de celui d'une poudre de base, bien qu'étant sensibles à de petites quantités d'huile aromatisée en l'absence de poudre. Ces résultats peuvent s'expliquer par la grande sensibilité de capteurs polymères conducteurs utilisés envers l'humidité et le dioxyde de carbone désorbés de la poudre de café.

De meilleurs résultats ont été obtenus avec les capteurs à oxydes métalliques qui se sont avérés capables de discriminer des cafés aromatisés superficiellement ou non. Néanmoins la discrimination est principalement basée sur l'intensité globale de l'arôme et la présence de certains composés soufrés. La limite de sensibilité des capteurs a été estimée à 10 ppb d'arôme total dans la phase gazeuse (ng/ml).

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COMPARISON OF A BREW AND AN INSTANT COFFEE USING A NEW GC-OLFACTOMETRIC METHOD

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INTRODUCTION

More than 800 compounds[1] have been identified in coffee aroma. Instead of continuing a systematic investigation of volatiles that often have no significant impact on the overall aroma, the current trend in flavor research is to determine key odorants. For that purpose, GC-olfactometric methods have been developed in the last decade:

- dilution methods (CHARM[2] and AEDA[3]) for which a series of dilutions of a given sample is injected and smelt. The series is stopped when the last dilution does not allow the detection of any odor at the sniffing port.
- intensity methods: odor intensities of GC peaks are recorded as a function of time by moving a cursor.

Recent papers now focus on a more quantitative aspect[4,5]: are olfactometric techniques suitable for differentiating 2 products based on their aromagram differences? To answer this question, the aroma sample must be representative of the perceived odor, and the variability of the GC-sniffing profile must be known.

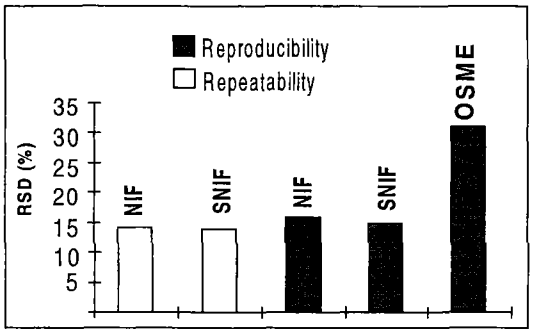
Sampling: as an alternative to injection of a sample extract, the vapor phase surrounding the product can be directly analyzed. This was firstly applied to coffee aroma by Holscher[6], and more recently by Semmelroch and Grosch[7]. As this alternative seems to better represent the aroma composition, this approach was preferred for this study. However, when sampling the gas phase with a gas-tight syringe[6,7], the injection volume is limited. A purge-and-trap technique would allow the collection of greater volumes of headspace, but their representativeness would be altered compared to equilibrium conditions. To overcome this difficulty, the use of a recently developed headspace cell was preferred[8]. It traps headspace volatiles that are in equilibrium with a food, onto an adsorbent. This better mimics the conditions of the air surrounding the coffee cup, and the sampling repeatability has been established.

Data treatment: dilution methods exhibit reproducibility problems[9] inherent to specific anosmia, lack of attention, or to discontinuous breathing. Comparing 2 aromagrams is a quantitative task which requires determining the method's variability. This has never been achieved for AEDA. Quantitative use of CHARM would require at least 3 repetitions at 10 different dilution levels[5]. Intensity methods (OSME) show "vast differences" of peak intensities between replications for a single panelist[10]. To overcome these difficulties, we recently proposed a new method[11].

Factors affecting the aromagram variability are due to the insufficient number of assessors involved in the sniffing process. Since sensorial analysis is able to generate reliable results using panels consisting of several judges, the same approach was chosen. Only one concentration level was used. The sampled volume was adapted for detection of 10-30 odorants. Each assessor pressed a button for the duration of an odor perceived at the sniffing port. The computerized averaging of these individual aromagrams led to a profile in which peak heights represent the detection frequency of odorants by the panel. Peak heights are called "NIF" (Nasal Impact Frequency) and peak areas "SNIF" (Surface of Nasal Impact Frequency).

The method is repeatable and reproducible with a panel of 6 members: two independent panels were able to generate the same aromagram without training. Mean relative standard deviations (RSD) are reported in Fig. 1.

Fig. 1. Mean relative standard deviation of NIFs and SNIFs, comparison with OSME[5,11].



The relationship between odor detection frequencies (NIF) and compound concentrations was established. As this calibration is tedious if applied to every peak of a complex mixture, and since looking for differences between aromagrams requires a quantitative comparison, a statistical approach was used[11]. Based on a panel of 21 assessors, the Least Significant Difference (LSD) has been computed, to determine which difference between 2 SNIF values was statistically significant.

MATERIALS AND METHODS

Coffee.

Roast coffee: the blend used was made of 80% Arabica and 20% Robusta. It was roasted for 5 min Roast beans were stored at -40°C until use. The beans were ground and brewed (17 g in 200 mL Vittel, "Grande Source") in a domestic coffee maker (Turmix,"Gold Filter 720") just before analysis.

Instant coffee: the same roast beans were used. The dried powder was prepared in our pilot plant according to usual procedure[12]. It was stored at -40°C before use. The drink was prepared by pouring 200 mL boiling water (Vittel "Grande Source") onto 5.7 g powder. This amount takes into account the extraction difference between the instant coffee and a home-made brew.

Headspace sampling. As soon as the hot drink was prepared, 1 mL of brew was introduced into the sample receptor of the previously described cell[8], which was adapted to an internal headspace volume of 60 mL and thermostated at 30°C. The liquid and gas phases were then equilibrated for 30 min before pressing the piston and collecting the volatiles on a Tenax trap. The cell was cleaned and dried at 50°C under vacuum between experiments.

Sniffing procedure. This was achieved as previously reported[11] with a panel of 8 assessors. Each person participated in one half of the sniffing run, and smelt the second half in a distinct session to avoid lassitude. Sensorial descriptors of the odorants were recorded by the assessor on a tape recorder during peak elution.

Headspace-GC/MS. Flavorings were identified using 2 systems:

SPTD/HP 5971: a 5890 gas chromatograph hyphenated to a 5971A mass spectrometer (Hewlett Packard, Palo Alto, CA) was equipped with a Short Path Thermal Desorption (SPTD), model TD2 (Scientific Instrument Services, Ringoes, NJ). This headspace injector desorbs Tenax traps.

Headspace was sampled either with our new cell, as for ATD injections or in the following way: 5 mL of freshly prepared brew poured into a stripping vessel connected to a Tenax trap. Helium was then bubbled through the

liquid at 30 mL/min for 2 h 20 min at room temperature. Volatiles trapped were thermally desorbed and eluted on a DBWax column (J&W): 30 m x 0.25 mm i.d. x 0.25 µm film thickness. The oven was held at 20°C for 5 min and programmed at 4°C/min to 220°C.

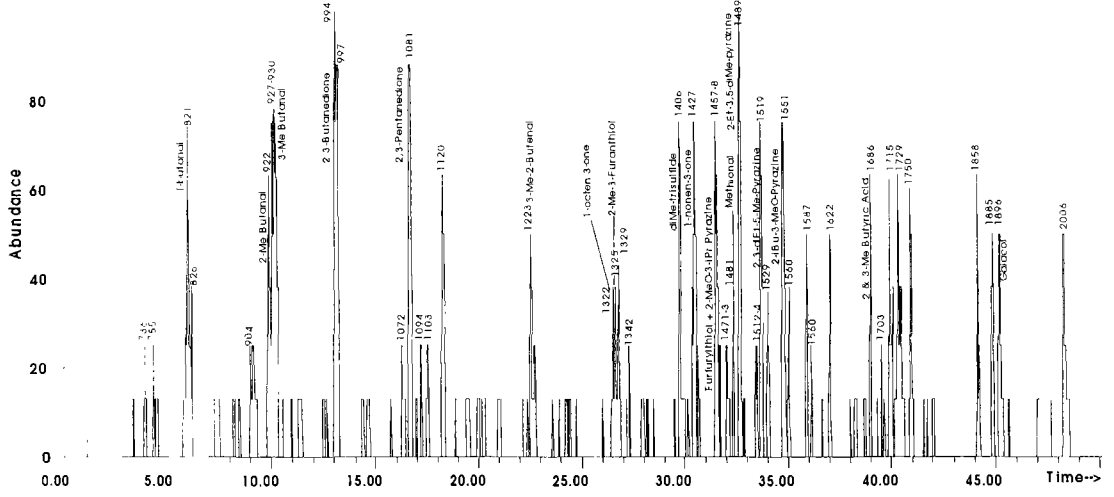
Tekmar/HP 5995: a 5995 mass spectrometer (Palo Alto, CA) was connected to a Tekmar LSC 2000 purge-and-trap system. Five mL of freshly prepared brew were introduced into the glass vessel and stripped with nitrogen for 20 min at 40 mL/min Flavorings were separated on a Nukol column (Supelco): 30 m x 0.25 mm i.d. x 0. 25µm film tickness.

DISCUSSION

"NIF" profile of the coffee brew aroma. (Fig. 2).

About 30 main peaks were observed in the aromagram. Individual perception differences of panelists were minimized as each judge only participated in 1/8 of the final olfactometric profile. Under such conditions, a 100% NIF means the peak was detected by all 8 panelists. In other terms, its concentration was above everybody's odor threshold. Smallest peaks (12.5% intensity) were only detected by 1 panelist. They represent either artefacts (e.g. interaction of the room odors with the panelist's perception) or low intense odors which exceeded the detection threshold of only one panelist.

Fig.2. "NIF" profile of the coffee brew aroma (DBWax column, retention indices on peak apex).



Headspace-GC-MS analyses were performed either under the same sampling conditions (combined static/dynamic headspace) as sniffing acquisitions, or using a purge-and-trap method. In spite of the enrichment provided by the latter, not all the peaks of the aromagram could be detected by mass-spectrometry (Table I). Impact pyrazines and sulfur-containing components occurred at concentrations below the MS detection limit, while their very low odor thresholds allowed their olfactometric perception.

Identification was quite consistent with published data[6,7,13] using only one, instead of 6 different headspace volumes, like with AEDA. In spite of this single volume level, the "SNIF" method provides us with differentiated peak intensities. Peak heights (NIF) or areas (SNIF) are not a direct measurement the odor intensity, but detection frequencies have been proved to increase with flavoring concentrations. Calibration curves could be drawn[11], but this should be an unrealistically tedious task.

Key components observed for the present blend are consistent with Semmelroch's results[7]: 2-methyl propanal, 2-methyl butanal, 3-methyl butanal, 2,3-butanedione, 2,3-pentanedione, 3-methyl-2-butenal, and gaiacol were positively identified by mass spectrometry and found to have high aroma impact. In addition, a peak containing a mixture of 2 and 3-methyl butyric acid was also identified, in agreement with Holscher [6] who could not clearly distinguish odors of both isomers .

2-Methyl-2-butenal, never previously mentioned as an impact flavoring in coffee, was positively identified in this brew, however its possible artefact origin must be further checked.

Because GC/MS did not give any signal for 1-octen-3-one, 2-methyl-3-furanthiol dimethyl-trisulfide, furfurylthiol, 2-methoxy-3-*iso*-propyl-pyrazine, methional, 2-ethyl-3,5-dimethyl-pyrazine, 2,3-diethyl,5-methyl pyrazine, and 2-*iso*-butyl-3-methoxy pyrazine, these components were only identified by their descriptors and by comparing their retention indices with those of authentic samples injected into the same headspace-GC system. As they have already been reported in roasted beans or in brew[6,7,13,14], their occurrence in the headspace seems reasonable. Although (E)-b-damascenone has been mentioned in roast coffee[13], its contribution appeared strongly reduced in the brew[7,14] and it could not be detected in this study. Furaneol® and substituted gaïacols eluted outside the limits of this aromagram, and will be considered in another investigation.

Special attention was given to a peak eluting 100 index units later than 1-octen-3-one, which also exhibited a mushroom odor. We recently positively identified this compound in yogurt aroma as 1-nonen-3-one[15]. Up to now its spectral confirmation in coffee was not possible because its very low concentration, whereas it possesses one of the lowest known odor thresholds (8 pg/kg in aqueous solution).

Table 1. *Comparison of SNIFs of Coffee brew & instant coffee beverage.*

INDICES *DBWax (measured)*		***COMPOUNDS***	***SNIF***		***SIGNI-FICANCE*** *(a)*
Brew	*Inst. Cof.*		*Brew*	*Instant coffee*	
821	*820*	*2-methyl propanal*	*2764*	*1681*	
826	*824*	*unknown*	*779*	*2324*	
923	*922*	*2-methyl butanal*	*2141*	*1869*	
927-30	*927*	*3-methyl butanal*	*2252*	*9253*	+++
994-7	*994*	*2,3-butanedione*	*11681*	*7277*	- - -
1081	*1081*	*2,3-pentanedione*	*7370*	*5291*	- - -
1120	*1119*	*unknown*	*5239*	*2667*	- - -
1223	*1223?*	*3-methyl-2-butenal*	*1924*	*0*	
-	*1267*	*unknown*		*1821*	
1322	*1322*	*1-octen-3-one*	*1216*	*2075*	
1325	-	*2-methyl-3-furanthiol*	*762*	*0*	
1329	*1330*	*unknown*	*1511*	*5050*	+++
1406	*1407*	*dimethyl-trisulfide*	*2970*	*3253*	
1427	*1429*	*unknown*	*5671*	*1873*	- - -
1457-8	*1457*	*furfuryl thiol + 2-MeO-3-i-Pr-pyrazine*	*6264*	*6454*	
1481	*1480*	*methional*	*1407*	*2801*	
1489	*1490*	*2-Et-3,5-dimethyl-pyrazine*	*7695*	*4884*	- - -
1512-4	*1514*	*unknown*	*1533*	*3640*	+++
1519	*1521*	*2,3-diethyl-5-methyl pyrazine*	*5339*	*2258*	- - -
1529	-	*unknown*	*2114*	*0*	+++
1551	*1552*	*2-iso butyl-3-methoxy pyrazine*	*6677*	*7284*	
1587	*1587*	*unknown*	*2704*	*2083*	
1622	*1621*	*unknown*	*2256*	*1306*	
1686	*1686*	*2- & 3-Me butyric acid*	*2282*	*2097*	
1703	*1704*	*unknown*	*1040*	*3573*	+++
1715	*1716*	*unknown*	*1251*	*1580*	
1729-34	*1733*	*unknown*	*1725*	*1522*	
1750	-	*unknown*	*3155*	*0*	- - -
-	*1846*	*unknown*	*0*	*1054*	
1858	*1859*	*unknown*	*2597*	*2097*	
1885	*1887*	*unknown*	*2370*	*1421*	
1896	*1899*	*gaïacol*	*2868*	*6406*	+++
2006	*2005*	*unknown*	*2584*	*682*	
-	*2013*	*unknown*	*0*	*2245*	+++
		SUM of SNIFs	***102 141***	***97 821***	

(a): +++ , - - -: SNIF increase or decrease of more than LSD value at 90% confidence level.

Comparison of NIF profiles of brewed and instant coffee (Table 1).
GC-sniffing analysis was performed on a soluble powder prepared with the roasted beans used for the above "SNIF" analysis. The qualitative NIF profile of the instant coffee drink was very similar to that of the brew as the majority of flavorings were common to both products. Results are summarized in Table I, in which significant quantitative variations are indicated according to the LSD previously established (2,000 SNIF units) at 90% confidence[11]. One observes that:

- small peaks in the background disappeared (12.5% height), in the first part of the chromatogram. They probably correspond to the most volatile flavorings, which are present at concentrations corresponding to their detection limit in the brew, and which are lost during instant coffee drying. *Iso*-butanal seems to decrease for the same reason.
- methyl furanthiol was not detected in the drink prepared with the instant powder, confirming Semmelroch's results[1].
- an unknown peak was enhanced at index 1330, while another appeared at index 1267.

Unlike Semmelroch's observations, dimethyltrisulfide, and 2-*iso*-butyl-3-methoxypyrazine still seem to participate in the aroma of the soluble powdered drink. Based on their SNIFs, they do not seem to be affected by processing.
At last, the sum of SNIF values for both beverages was found to be almost equal. This suggests that **flavor strengths were identical in both cases**. This observation is in agreement with preliminary results of classical sensorial analysis. Direct evaluation of the overall flavor by a panel did not find any differences between the perceived intensities of the brew and the instant coffee beverage.

CONCLUSIONS

With the frequency detection of the "SNIF" method, the most potent odorants above a coffee cup were determined using a single level of injected volume. The 8-member panel averaged individual differences of judges' perceptions and allowed quantitative profile comparisons. Using an LSD previously established, the "SNIF" method identified which of the coffee's impact odorants significantly increased or decreased during the manufacture of instant coffee.
About 30 and 20 main contributors were found in the brew and the instant coffee drink, respectively. The odor impact of most volatile compounds was lower in the instant coffee drink, meanwhile a few others were enhanced, but the qualitative composition of both drinks as well as their overall flavor strength remained very similar.

ACKNOWLEDGMENTS

We specially acknowledge Dr. E. Prior, and M. Baumgartner, for their kind help in reviewing this paper, and Dr. S. Bobillot for preliminary results of sensorial analysis.

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SUMMARY

A new GC-Olfactometry approach has been used to evaluate impact aroma compounds of coffee. It is based on headspace sampling mimicking a coffee cup in equilibrium with its surrounding air, and on detection by a panel of 6-10 assessors instead of 1 or 2 for other methods. Intensities of aromagram peaks are then measured by detection frequencies of odorants perceived at the sniffing port. Because of the use of several assessors, these frequencies appear to be repeatable, and reproducible by 2 independent panels without training prior to the analysis. This approach allows the calculation of standard deviations and last significant differences, therefore aromagrams can be compared on a more quantitative basis than with previous GC-Olfactometry methods.
The method was applied to the comparison of impact flavorings of a coffee brew and to the corresponding instant coffee, both prepared from the same beans. Both profiles exhibited little qualitative differences, but intensities varied. These results are consistent with literature data. In addition, a new key odorant was detected in the coffee aroma.

RESUME

Une nouvelle approche par CG-Olfactométrie a été utilisée pour évaluer les composés impact de l'arôme de café. Elle est basée sur un échantillonnage par headspace qui imite bien une tasse de café en équilibre avec l'air qui l'entoure, et sur une détection par un panel de 6-10 personnes au lieu de 1 ou 2 comme dans les autres méthodes. L'intensité des pics de l'aromagramme est alors mesurée par la fréquence de détection des substances odorantes perçues à l'olfactomètre. En raison de l'emploi de plusieurs juges, ces fréquences apparaissent répétables et reproductibles par 2 panels indépendants, sans entrainement préalable. Cette approche permet de calculer les déviations standards, et les différences significatives minimales. Par consequent, les aromagrammes peuvent être comparés sur une base plus quantitative qu' avec les méthodes antérieures de CG-olfactométrie.
La méthode a été appliquée à la comparaison des aromatisants impacts du café-filtre et du café instantané correspondant, tous deux préparés à partir des mêmes grains. Les profils sont apparus qualitativement peu différents, mais avec des variations quantitatives. Ces résultats sont en accord avec la littérature. De plus, un nouvel odorant-clef a été détecté dans l'arôme de café.

DETERMINATION OF THE GEOGRAPHIC ORIGIN OF GREEN COFFEE BY STABLE ISOTOPE TECHNIQUES

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1 INTRODUCTION

In-cup taste, aroma, and aspect of the green beans (size, shape, colour), are markedly influenced by the country of origin of the coffee. However, due to the large number of producing countries and intracountry variability, no taster can reliably identify them. Therefore, objective methods are needed to determine the geographic origin of coffee.
Thermo-gravimetric (1) and pyrolysis (2) mass spectrometry failed to discriminate green coffees from different origins. Infrared spectroscopy was able to identify coffees from 4 different countries, but the proposed sample preparation does not seem to be very reliable (3). Several authors looked at minerals as potential markers of the origin of coffee (4-6). Analysing 10 green coffees from 8 countries, Krivan *et al.* (7) found manganese to be the best indicator, followed by C, Co, Cs, Na and Rb. Chlorogenic acids were also considered as possible tracers (8). The most promising results were obtained by Bicchi *et al.* (9), who discriminated washed Arabica coffees from Colombia, Cameroon, Honduras, Costa Rica and Guatemala. Finally, Danho *et al.* (10) reported the discrimination of African and American green coffees using multivariate analysis of stable isotope ratios. However, as all American samples were Arabicas and most of the Africans were Robustas, it is not sure that the differentiation was not on the basis of their botanic origin. Therefore, the technique needed further investigation. This study describes the analysis of stable isotope ratios of 25 green coffees from various origins.

2 EXPERIMENTAL

2.1 Sampling

Twenty-five green coffee samples (16 Arabica, 9 Robusta), originating from 16 different countries and 3 continents (America, Africa, Asia) were analysed. A detailed description of the samples is given in Table 1.

2.2 Extraction and purification of caffeine

Caffeine was extracted from finely ground beans of green coffee (150 g) in 1500 ml boiling water for 16 h. The solution was then centrifuged and filtered through cotton wool. The filtered solution was adjusted to pH 12.0 with K_2CO_3 and extracted with 4 x 200 ml CH_2Cl_2. The organic phases were dried with Na_2SO_4. The solution was evaporated and the residue was loaded on a silica gel column. Caffeine was eluted with a mixture of CH_2Cl_2/acetone 90/10. After evaporation of the solvent, caffeine was purified by crystallisation from ethanol. Purity of the caffeine was checked by NMR (> 99 %).

2.3 Determination of $(D/H)_i$ ratios

Site-specific $(D/H)_i$ ratios, one for each of the three N-CH_3 groups present in caffeine, were determined by ^{2}H-NMR on an AM500 Bruker spectrometer. The spectra were recorded at 76.77 MHz using a specific 10 mm (o.d.) probe equipped with a ^{19}F locking device. Broad band ^{1}H decoupling was applied continuously. The following acquisition conditions were applied: T = 308 °K, PW = 90° pulse (13 µs), t = 6.8 s, NS = 2200. The NMR tube was filled with tetra methyl urea (TMU), CCl_4, formic acid, pyrrole and the purified caffeine. $(D/H)_i$ values were calculated with respect to the TMU reference (10).

2.4 Determination of ($^{13}C/^{12}C$) and ($^{15}N/^{14}N$) ratios

Overall carbon and nitrogen stable isotope ratios in caffeine were determined by on-line analysis using a Carlo-Erba NA 1500 II Elemental Analyser fitted with a Fisons Instruments Optima mass spectrometer. The results were calculated as described earlier (10), and expressed as $\delta(^{13}C)$ and $\delta(^{15}N)$, respectively.

3 RESULTS AND DISCUSSION

3.1 Isotope ratios

Overall isotope ratios $\delta(^{13}C)$ and $\delta(^{15}N)$, and site-specific isotope ratios $(D/H)_2$, $(D/H)_3$ and $(D/H)_4$ were measured in caffeine extracted from 25 green coffees from various geographic and botanic origins. The results are given in Table 1.
$\delta(^{13}C)$, $\delta(^{15}N)$, and $(D/H)_3$ values were in good agreement with those previously reported (10, 11). However, significantly lower average $(D/H)_2$ and, in particular, $(D/H)_4$ ratios were measured. Clearly, the methyl groups at sites 2 and 4 are depleted compared to that on position 3. It is difficult to discuss this difference in metabolic terms.
Univariate analysis of the data showed that neither of the 5 isotopic ratios can separately and clearly discriminate green coffees from different continents or botanic origins (Arabica vs Robusta). Therefore, the results were analysed using multivariate techniques.

Table 1. Stable isotope ratios of caffeine extracted from green coffees of different origin

COUNTRY OF ORIGIN	GROWING AREA	BOTANIC ORIGIN	CROP YEAR	$\delta(^{13}C)$ ‰	$\delta(^{15}N)$ ‰	$(D/H)_2$ ppm	$(D/H)_3$ ppm	$(D/H)_4$ ppm
Kenya	Ruiro, 1600 m	Arabica	1994	-25.2	0.50	137.0	142.5	129.2
Kenya	Kisii, 1700 m	Arabica	1994	-27.7	0.10	128.9	136.3	122.7
Burundi	Ngozi, 1500 m	Arabica	1995	-25.0	2.20	132.1	138.7	125.3
Costa Rica	Heredia, 1200 m	Arabica	1994	-26.6	-0.22	118.1	125.9	112.5
El Salvador	El Molino, 1280 m	Arabica	1995	-26.7	-0.50	130.1	135.1	123.6
Ecuador	Pinas, 1380 m	Arabica	1995	-29.0	1.40	131.0	137.0	126.3
San Domingo	Peralta Azua, 800 m	Arabica	1995	-29.7	-0.20	129.8	132.7	125.7
Trinidad	Monte Cafe, 850 m	Arabica	1995	-26.6	1.80	129.8	137.9	125.4
Brazil	Amparao, 960 m	Arabica	1995	-26.6	1.10	133.9	140.0	136.1
Colombia	Pensilvania, 1650 m	Arabica	1995	-27.6	4.10	127.0	131.1	128.9
Colombia	El Canõn, 1700 m	Arabica	1995	-27.7	1.10	126.8	130.4	127.7
Colombia	El Jordan, 1700 m	Arabica	1995	-27.3	0.10	127.6	135.0	126.3
Colombia	El Silencio, 1700 m	Arabica	1996	-26.6	2.40	125.5	132.3	122.4
Mexico	Tapachula, 1200 m	Arabica	1996	-26.3	0.50	121.9	127.4	116.2
Laos	Ban Itou, 960 m	Arabica	1994	-26.4	1.90	128.5	131.5	122.5
Indonesia	Takengon, 1500 m	Arabica	1995	-26.5	0.80	128.3	139.7	122.6
Cameroon	Abong Mbang, 600 m	Robusta	1995	-26.8	2.75	129.8	137.4	123.3
Central Africa	Kongbo, 400 m	Robusta	1995	-26.9	0.70	133.8	142.2	126.4
Ivory Coast	Belleville, 300 m	Robusta	1994	-28.2	1.20	132.5	137.5	122.5
Cameroon	unknown	Robusta	unknown	-26.6	2.30	133.8	137.1	130.9
Ecuador	Latacunga, 400 m	Robusta	1995	-27.8	0.80	133.3	135.9	128.5
Mexico	Alianza, 620 m	Robusta	1996	-27.1	-0.50	131.9	135.7	126.5
Laos	Bolovens, 600 m	Robusta	1995	-27.5	1.10	136.2	128.8	128.2
Vietnam	Viet Duc, 470 m	Robusta	1995	-26.9	0.44	130.1	134.9	124.5
Indonesia	Ngarip, 950 m	Robusta	1995	-25.3	1.60	128.4	136.9	119.9

3.2 Discrimination of geographic origin

Principal component analysis (PCA) is a powerful method to visualise high-dimensional data sets. With the objective of differentiating green coffee according to its country of origin, PCA was carried out on the 16 Arabica and 9 Robusta samples separately, without using information about the geographic origin. The results are shown in Figure 1.
No apparent grouping of the Arabica or Robusta samples according to their continent of origin was observed. Nevertheless, within the same continent, the green coffees from different countries were well separated. The intracountry variations seem

too high (e.g. Colombia, Kenya, Cameroon) and the number of samples analysed is too low to speculate on the use of this technique for an unambiguous identification of the geographic origin of green coffee. However, it certainly has the potential, in favourable cases where two countries are very well discriminated (e.g. Costa Rica/San Domingo, Mexico/Ecuador, Indonesia/Laos, Cameroon/Ivory Coast), to correctly classify a sample of known but questionable origin.
It is also noteworthy that for both sets of coffee, the samples could not be differentiated with respect to the altitude of their growing area.

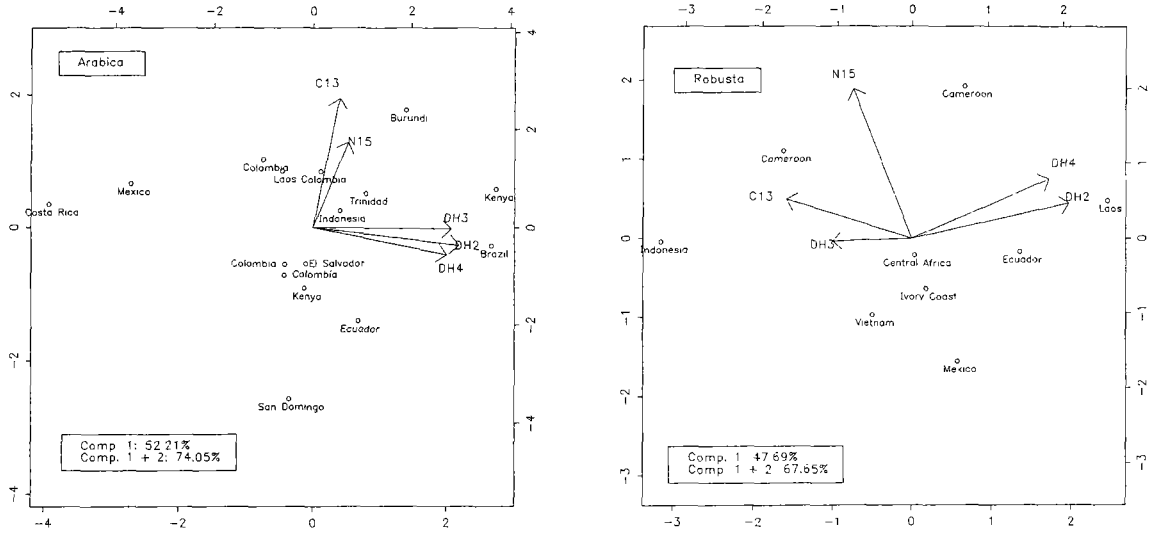

Figure 1. Discrimination of geographic origin of coffee (PCA).

3.3 Discrimination of botanic origin

With the objective of differentiating Arabica from Robusta coffees, PCA was carried out on the whole set of 25 samples. No apparent grouping of the samples according to their botanic origin was observed. However, when a predictive quadratic discrimination analysis was carried out on the $\delta(^{13}C)$ and $(D/H)_2$ only (Figure 2), the two relevant variables for the discrimination, all but 4 coffees were correctly classified (76 % 1-fold cross-validation classification rate). One Robusta sample (Indonesia) was classified as Arabica, and 3 Arabica samples (El Salvador, Trinidad, Brazil) were classified as Robusta. This is a rather promising result which should be further validated by analysing a larger number of samples.

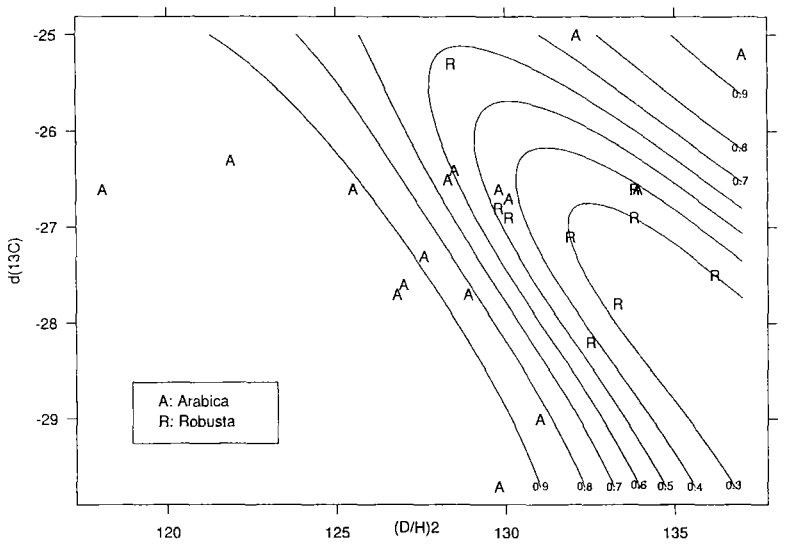

Figure 2. Discrimination of Arabica and Robusta coffee (Predictive quadratic discrimination). Contour plot of posterior probabilities for being classified as Arabica.

4 CONCLUSIONS

Despite the limited number of samples analysed, it can be concluded that the determination of stable isotope in caffeine does not allow an unambiguous identification of both the geographic and botanic origins of green coffee. Neither univariate nor multivariate analysis of the data resulted in an apparent grouping of the samples with respect to their country or continent of origin, nor to a differentiation between Arabica and Robusta species. In very specific cases, and given further validation, the technique may correctly classify a green coffee of known but questionable origin.
Work is currently in progress to investigate the analytical potential of the ^{18}O content of caffeine as an additional parameter for geographic discrimination.

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SUMMARY

No reliable analytical methods are currently available to determine the geographic origin of coffee. For this purpose, we evaluated a recently published technique based on the measurement of stable isotope ratios.
Caffeine was extracted from 25 green coffee samples (16 Arabica, 9 Robusta) of 16 different countries. The overall ($^{13}C/^{12}C$) and ($^{15}N/^{14}N$) isotope ratios in the purified caffeine were determined by mass spectrometry, and the site-specific $(D/H)_i$ isotope ratios by ^2H-NMR.
Neither univariate nor multivariate statistical analysis of the data resulted in an apparent grouping of the samples with respect to their country or continent of origin, nor to a differentiation between Arabica and Robusta species. Therefore, the technique does not allow an unambiguous identification of green coffee of unknown origin. In very specific cases, and given further validation, the technique has nevertheless the potential to correctly classify a green coffee of known but questionable origin.

RESUME

Il n'existe actuellement pas de méthode d'analyse fiable pour déterminer l'origine géographique du café. Dans cette optique, nous avons évalué une technique récemment publiée, basée sur la mesure de rapports d'isotopes stables.
La caféine a été extraite de 25 échantillons de café vert (16 Arabica, 9 Robusta) provenant de 16 pays différents. Les rapports isotopiques ($^{13}C/^{12}C$) and ($^{15}N/^{14}N$) de la caféine purifiée ont été mesurés par spectrométrie de masse, et les rapports isotopiques spécifiques $(D/H)_i$ par ^2H-NMR.
Une analyse statistique des valeurs obtenues, aussi bien univariée que multivariée, n'a pas mis en évidence un groupement des échantillons selon leur pays ou leur continent d'origine, ni selon leur origine botanique (Arabica, Robusta). Par conséquent, la technique ne permet pas l'identification non-équivoque d'un café vert d'origine inconnue. Dans des cas très particuliers et moyennant une validation plus poussée, elle pourrait néanmoins être utilisée pour classifier correctement un café vert dont l'origine est connue mais douteuse.

CAFESTOL AND DEHYDROCAFESTOL IN ROASTED COFFEE

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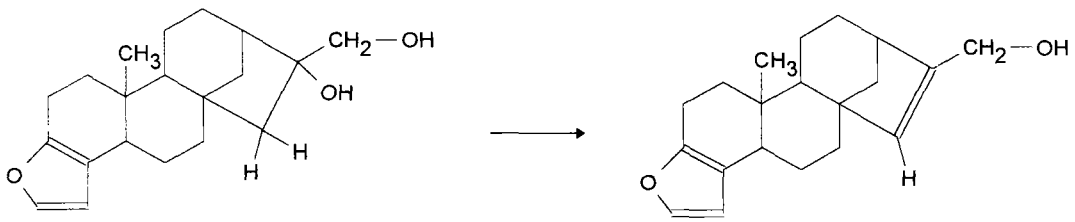
Many compounds of the coffee bean are changed by technological treatments, especially by the roasting process. The diterpenes are also effected.
Cafestol. for example, is degraded to dehydrocafestol [1,2]:



Cafestol Dehydrocafestol

Dehydrocafestol is formed during the roasting process and is not present in green coffee. The amount is dependent on the roasting temperature and the cafestol content of the green coffee. These results were achieved from investigations of two Arabica coffees and one Robusta coffee roasted for three minutes at different temperatures. Figure 1 shows the findings of an Arabica Tanzania coffee.

In order to be independent from the total amounts of cafestol and the formed dehydrocafestol, the ratios of cafestol/dehydrocafestol were calculated. The investigations revealed that there is a relationship between the roasting temperature and the cafestol/dehydrocafestol ratio both for the two Arabica coffees and the Robusta coffee in the same way (Fig. 2). The relationship is approximately linear, especially for each of the first three roasting temperatures. The slight deviation for the higher roasting temperatures can be attributed to the formation of further cafestol by-products [3].

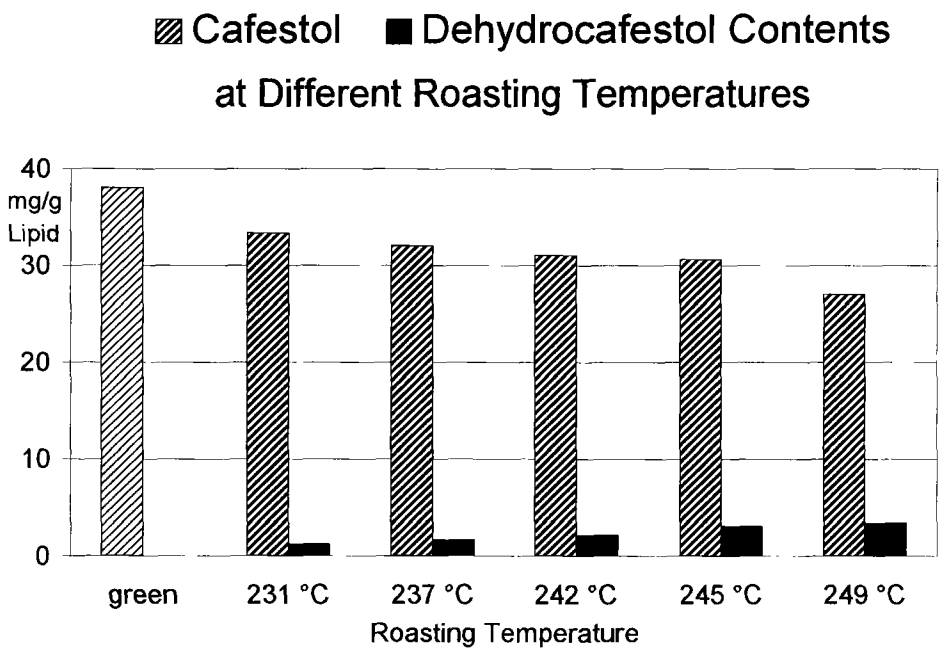


Fig. 1. Cafestol and dehydrocafestol contents of a Tanzania Arabica at different temperatures

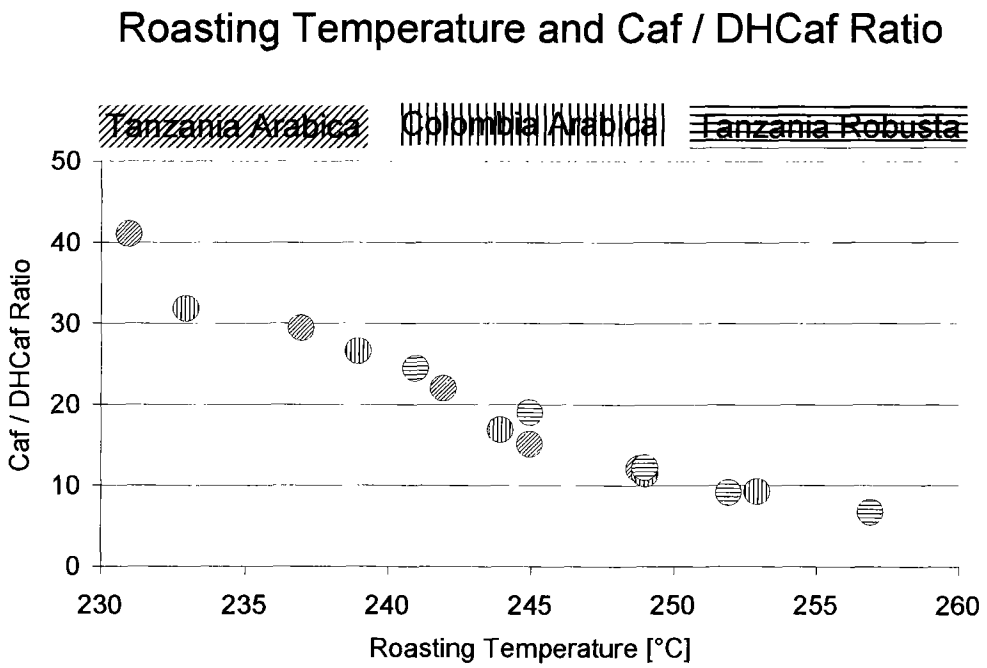


Fig. 2. Relationship of the cafestol/dehydrocafestol ratio and the roasting temperature

The individual roasted coffee samples were organoleptically judged by well-trained coffee tasters. Each third roasting grade was classified as being strongly roasted. The organoleptic results and the corresponding cafestol/dehydrocafestol ratios were now related to each other (Fig. 3).

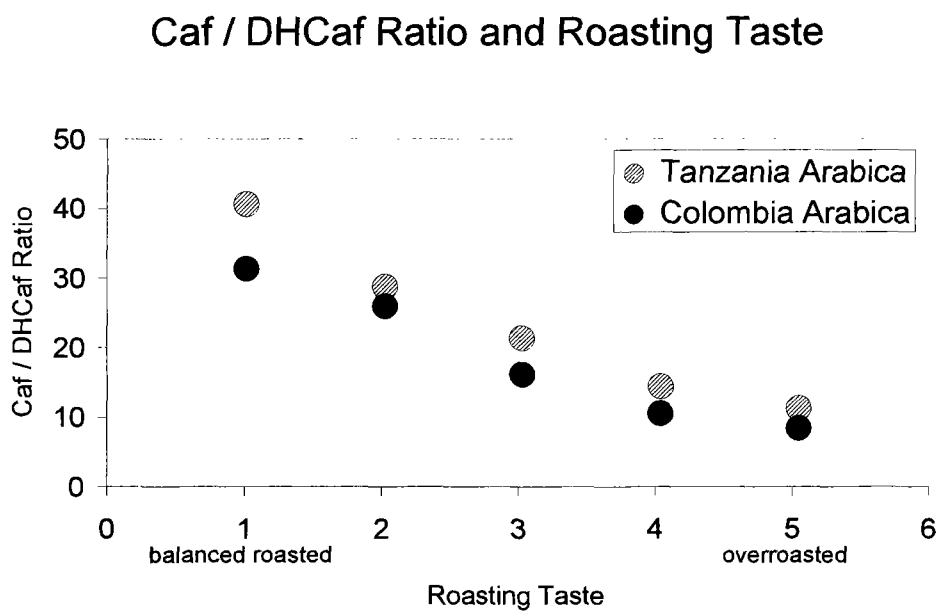


Fig. 3. Relationship of the cafestol/dehydrocafestol ratio and organoleptic roasting grade

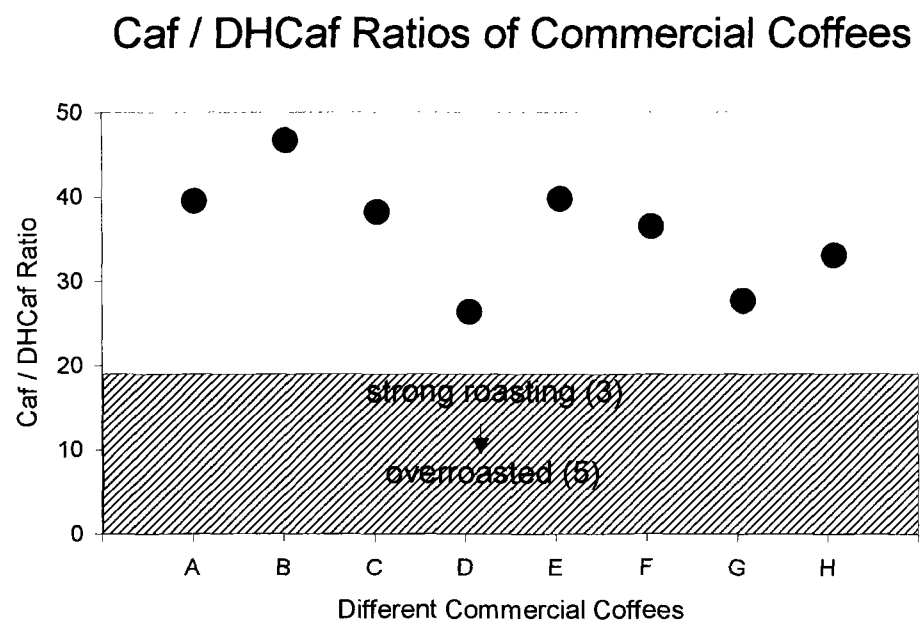


Fig. 4. Cafestol/dehydrocafestol ratios of commercial coffees

Several German coffees were analysed for their cafestol/dehydrocafestol ratios (Fig. 4). The ratios ranged from 26,6 to 46,7. None of the samples reached a value which could be classified as being strongly roasted (based on the results of the Arabica Tanzania coffee).
These findings also corresponded with the results of our sensorial investigation.

The cafestol/dehydrocafestol ratio can therefore be used for the organoleptic estimations of roasted coffees as an objective criterion.

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Summary

The diterpenes cafestol and dehydrocafestol, which is formed during the roasting process, were analysed in two Arabica coffees and one Robusta coffee roasted at five different temperatures. For each coffee sample the cafestol/dehydrocafestol ratio was calculated and related to the roasting temperature. The investigations revealed that there is an approximate linear relationship for all coffees in spite of containing different amounts of cafestol in the green coffee.
Furthermore the cafestol/dehydrocafestol ratio can be used for the organoleptic estimations of roasted coffees as an objective criterion.

Zusammenfassung

Die Diterpene Cafestol und das bei der Röstung gebildete Dehydrocafestol wurden in zwei Arabica-Kaffees und einem Robusta-Kaffee bestimmt, die bei fünf verschiedenen Temperaturen geröstet worden waren. Das jeweilige Cafestol/Dehydrocafestol-Verhältnis wurde berechnet und in Beziehung zur Rösttemperatur gesetzt. Dabei ergab sich für alle Kaffees - trotz unterschiedlicher Cafestolgehalte des Rohkaffees - ein nahezu linearer Zusammenhang.
Die vorgestellten Ergebnisse zeigen ferner, daß das Cafestol/Dehydrocafestol-Verhältnis auch als objektives Kriterium für die sensorische Beurteilung von Kaffees herangezogen werden kann.

CHANGES IN COMPONENTS OF CANNED COFFEE BEVERAGE STORED AT HIGH TEMPERATURE

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INTRODUCTION

Canned coffee beverage was introduced into the market for the first time in 1969 in Japan. The canned coffee market has expanded rapidly since the launch. Now, the total market volume exceeds ten billion cans/year, which is the biggest category occupying a quarter of the soft drink market in Japan. One of the reasons for this rapid growth is the fact that the canned coffee is sold either warmed (about 60℃) or chilled (about 5℃) all the year round.

General production flow of the canned coffee beverage is shown in Fig.1.

Through the production line and the distribution, exposure to high temperature of the canned coffee beverage is stronger than regular coffee, which results in unfavorable taste. Therefore, it becomes more important to clarify the mechanism of deterioration by heat in order for us to supply better products to consumers.

In this paper, our recent findings on the mechanism of the deterioration of canned coffee beverage is reported.

MATERIALS AND METHODS

Preparation of canned coffee beverage

Coffee beans : Colombia and Brazil arabica coffee

Soluble solid : 1.35° Brix

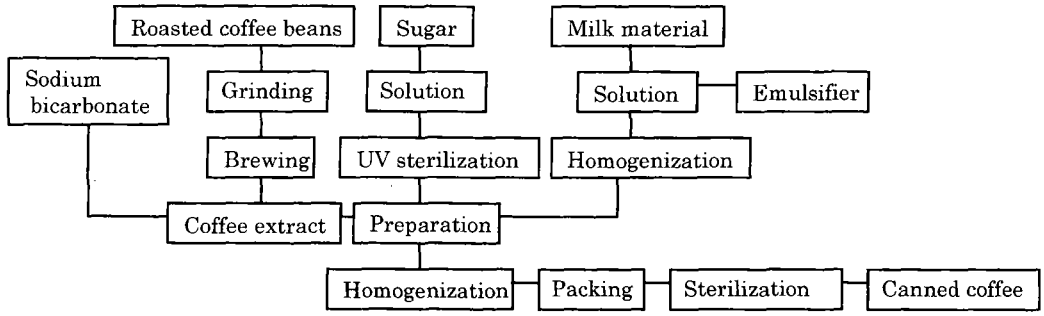

Fig. 1. General production flow of the canned coffee beverage

The above coffee extract was heated to 70℃ and filled in a can. After the head space was substituted with nitrogen gas, the can was seamed. This sample was quickly sterilized in a retort cooker at 124℃ for 18min.

Analysis of chlorogenic acids and caffeic acid[1)]

Sample preparation

Sample solution was diluted 10 times and filtrated through membrane filter (0.2 μ m).

HPLC analysis

HPLC (JASCO LC-900 HPLC system) analysis was done under the following conditions;

guard column, YMC-guardpack ODS-A; column, YMC-pack ODS-A; injection volume, 20 μ l; eluent, 5% acetic acid and CH_3CN (98 : 2,v/v) for 10 min, the linear gradient from (98 : 2) to (70 : 30) for 50 min, and (70 : 30) for 10 min; flow rate, 1.0ml/min; wavelength for detection, 320nm.

Analysis of hydroxycinnamic acid derivatives[2),3)]

Sample preparation

Sample preparation was done using RAPID TRACE (ISOLUTE C18 500mg). Solid-phase extraction cartridge was conditioned by washing with 2ml of CH_3CN followed by 2ml of 0.1M phosphate buffer. Two ml of sample was passed through the cartridge. This cartridge was then washed with 2ml of phosphate buffer. This portion of elution was discarded. The target compounds were eluted using 4ml of ethyl acetate. The fraction containing the target compounds was filled up to 25ml with isopropanol and was filtrated through membrane filter (0.2 μ m).

HPLC analysis

HPLC analysis was done under the following conditions;

guard column, Jasco crestpak C18T-5P; column, Jasco crestpak C18T-5; injection volume, 20 μ l; eluent, 2% acetic acid, tetrahydrofuran and CH_3CN (77.6 : 18.9 : 3.5,v/v)

for 50 min; flow rate, 1.0ml/min; wavelength for detection, excitation at 265nm and emission at 335nm.

Analysis of quinic acid, phosphoric acid and phytic acid

Sample preparation

Sample solution was used after filtrated with membrane filter (0.2 μ m).

HPLC analysis

HPLC analysis was done under the following conditions;

guard column, Shodex Ionpak KC-LG; column, Shodex Ionpak KC-811; injection volume, 20 μ l; eluent, 4mM perchloric acid for 50 min; flow rate, 1.0ml/min; reaction reagent, bromothymol blue solution; wavelength for detection, 445nm.

RESULT AND DISCUSSION

Chlorogenic acids and caffeic acid

Changes of chlorogenic acids by retort sterilization are shown in Table 1. Increase in 3-caffeoyl quinic acid (3-CQA), 4-CQA, decrease of 5-CQA were observed. 3-CQA and 4-CQA are thought to be isomerized from 5-CQA by heat[4]. Changes of chlorogenic acids during storage at 60℃ are shown in Fig.2. 5-CQA showed the biggest decrease among chlorogenic acids, all of which showed downward trend. This fact shows that 5-CQA is unstable to heat. On the other hand, increase in caffeic acid due to the decomposition of chlorogenic acids was observed.

Table 1. Changes of chlorogenic acids by retort sterilization

	3-CQA(ppm)	4-CQA(ppm)	5-CQA(ppm)
Before retort sterilization	405	298	499
After retort sterilization	594	423	374

Hydroxycinnamic acid derivatives

HPLC pattern of the coffee stored at 60℃ showed two peaks. One of the peaks is identified as 4-vinyl guaiacol (4-VG). The second peak has the same retention time as the degradation product of caffeic acid (DPCA) by heat, which was thought to be 4-vinyl catechol (4-VC) based on the information presented in the previous studies[5]. Therefore, the second peak, which is surmised to be DPCA, is thought to be 4-VC. Both 4-VG and DPCA showed linear increase during storage at 60℃ as shown in Fig.3. Due to the unavailability of the standard sample of 4-VC, DPCA was quantified as 4-VG. 4-VG is one of the influential flavor component in coffee with low threshold limit. However, sensory evaluation revealed that it influenced the flavor unfavorably at higher content as shown in Fig.4. The vertical axes indicate the overall evaluation of coffee aroma and the intensity of 4-VG smell. The horizontal axis indicates the period stored at 60℃. Sensory evaluation was scored in five point scale by trained panelists

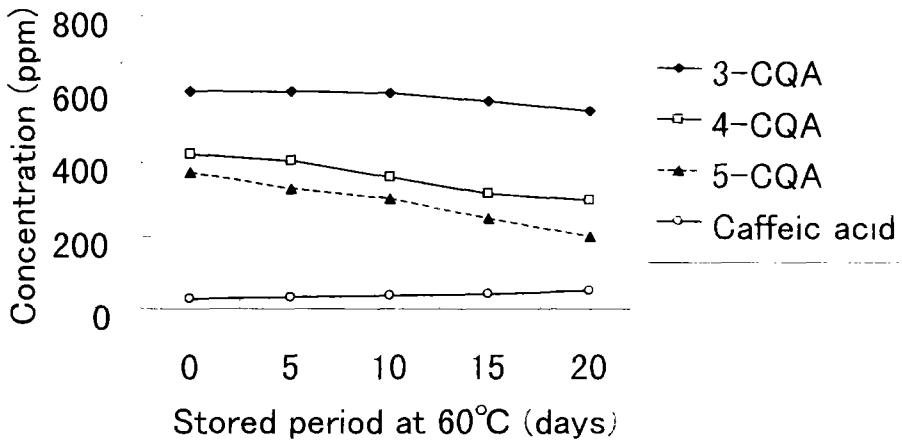

Fig.2. Changes of chlorogenic acids and caffeic acid

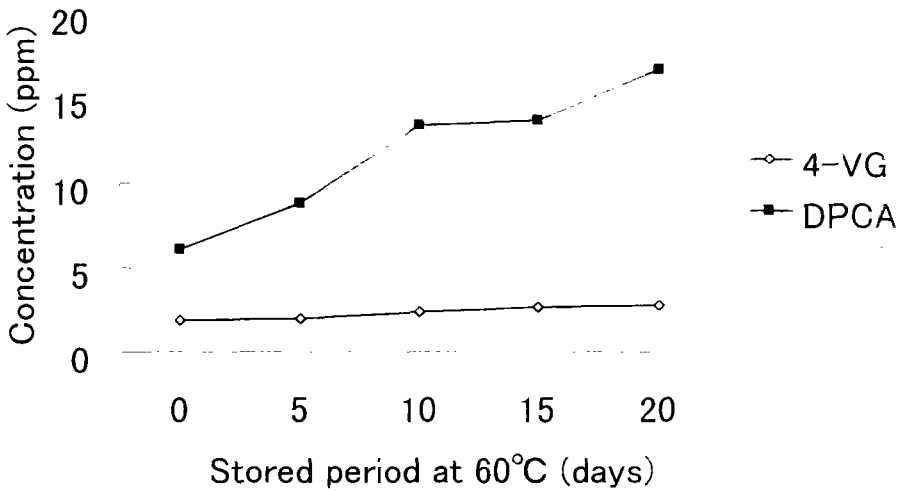

Fig.3. Changes of 4-VG and DPCA

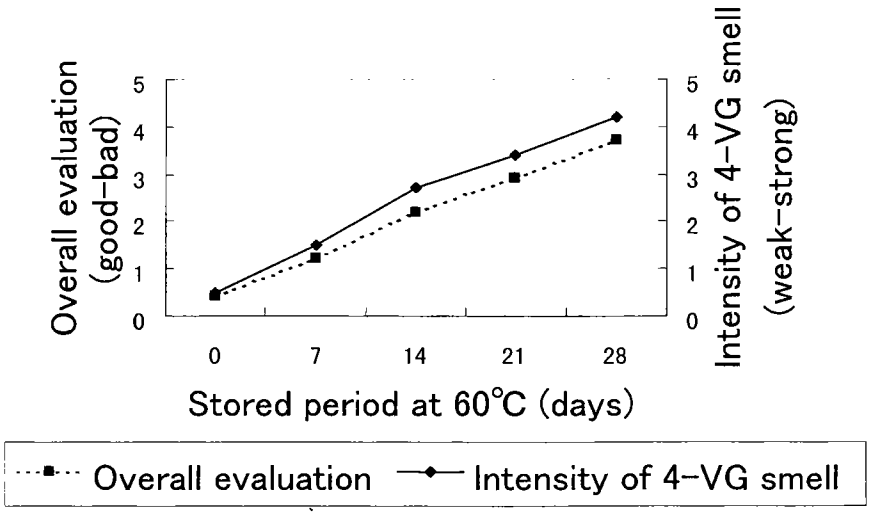

Fig.4. Relation between overall evaluation and intensity of 4–VG smell

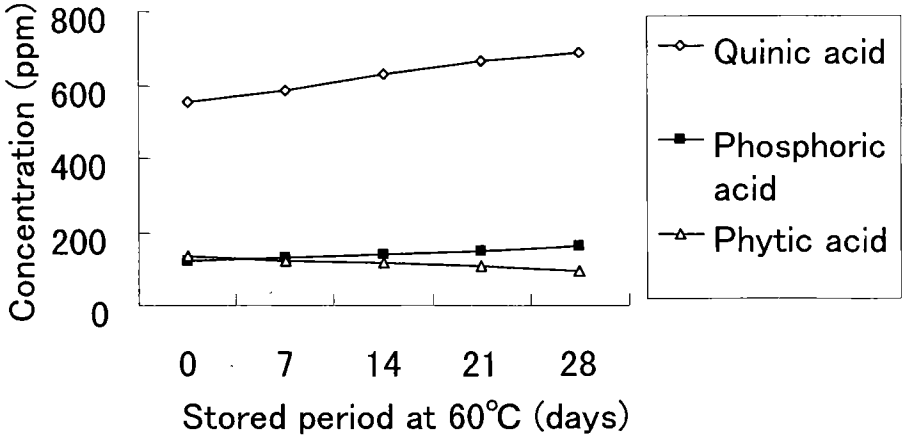

Fig.5. Changes of quinic acid, phosphoric acid and phytic acid

in our lab. In addition, it was confirmed that 4-VG was formed in the ferulic acid solution stored at 60℃. From these findings, hypothetical mechanism of component changes in a canned coffee beverage during storage at 60℃ is concluded as follows;

Chlorogenic acid (CQA) → Caffeic acid → 4-Vinyl catechol → Bitter taste

Chlorogenic acid (FQA) → Ferulic acid → 4-Vinyl guaiacol → Unfavorable flavor

Quinic acid, phosphoric acid and phytic acid

Changes of quinic acid, phosphoric acid and phytic acid during storage at 60℃ are shown in Fig.5. It was observed that phytic acid continuously decreased and that quinic acid and phosphoric acid continuously increased. Quinic acid is the degradation product of chlorogenic acids. In addition, it was confirmed that phosphoric acid was derived from decomposition of phytic acid. These components are thought to be involved in the reduction in pH value of the canned coffee beverage during retort sterilization and storage at high temperature.

REFERENCES

1) M. Murata, H. Okada, S. Homma, Biosci. Biotech. Biochem., 59, 1887-1890 (1995)
2) R. L. Rouseff, G. R. Dettweiler, R. M. Swaine, Journal of Chromatographic Science, 30, 383-387 (1992)
3) H. S. Lee, S. Nagy, Journal of Food Science, 55, 162-166 (1990)
4) L. C. Trugo, R. Macrae, Food Chem., 15, 219-227 (1984)
5) T. Nakabayashi et al., Kougakushuppan, KOHI BAISEN NO KAGAKU TO GIJUTU (Chemistry and Technology of Coffee Roasting), 81-83 (1995)

SUMMARY

In Japan, canned coffee beverages have been usually sold at around 60℃ by being kept in a vending machine with heating device. Deterioration caused by the prolonged duration in a vending machine is considered as a problem. In this study, continuous changes of a canned coffee were investigated, analyzing the chemical components of the coffee stored at 60℃ by HPLC. It was confirmed that 4-vinyl guaiacol, degradation product of caffeic acid, phosphoric acid and quinic acid increased; and that chlorogenic acids and phytic acid decreased. Phosphoric acid derived from decomposition of phytic acid and quinic acid are responsible for the pH decline of the content. It was indicated by sensory evaluation that 4-vinyl guaiacol and degradation product of caffeic acid took part in the unfavorable changes in flavor and taste (especially bitter taste), respectively.

MEASUREMENT OF FLAVOUR RELEASE IN COFFEE PRODUCTS

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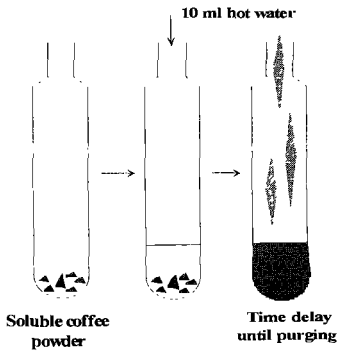
Objectives

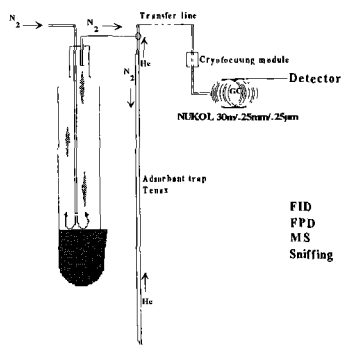
The objectives of this study were to develop a method which allows us to evaluate the amount of volatiles compounds above the liquid, just after reconstitution and to differentiate coffee products in terms of release.

Methods

The purge-and-trap system is used to measure aroma release after brewing or reconstitution[1,2] of coffee.
Hot water is poured in the finger glassware containing coffee. The whole mixture is left open without stirring (time delay) until the measurement (the first measurement is taken just after reconstitution).
Different trapping periods (20 s and 60 s) were investigated, corresponding to a volume of 7 and 50 ml head-space, respectively.
Four different signals were recorded in the same operating conditions: MS, FID, FPD and sniffing.



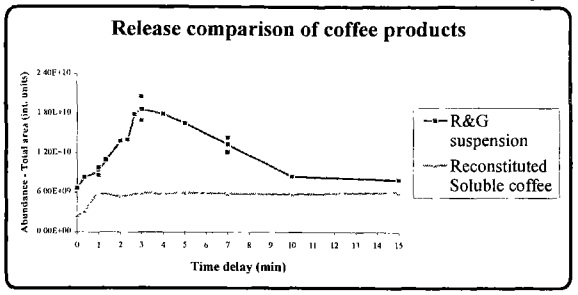
Sample preparation



Release measurement system

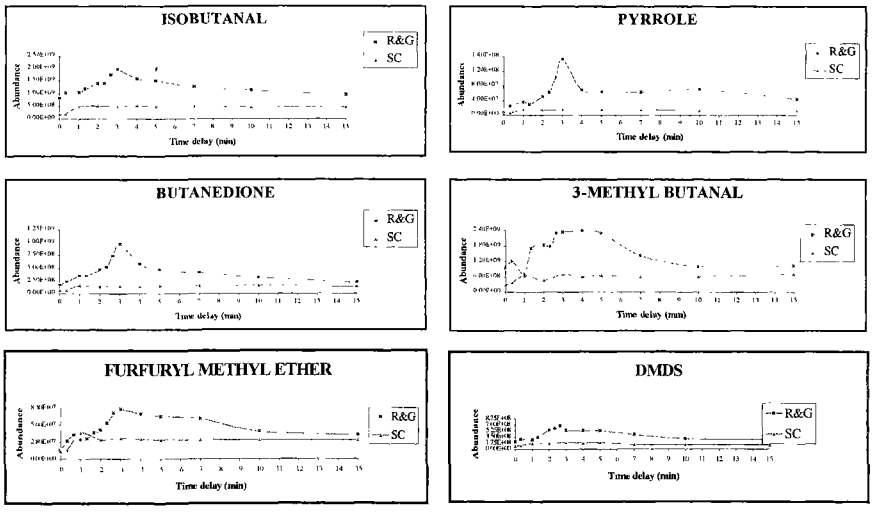
Results

Comparison of coffee brew versus soluble coffee highlights different release behavior. Strong quantitative differences can be pointed out.
R&G suspension shows a later release, but signals are at least three times higher. Aroma quantification of these products showed similar order of magnitude.



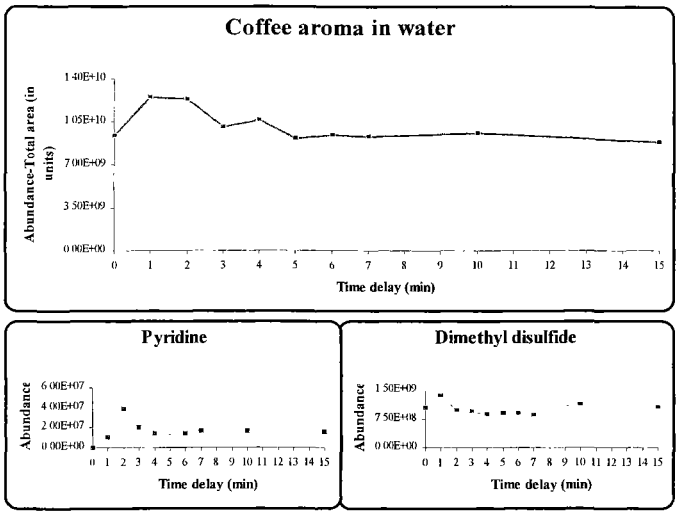
The effect of magnetic stirring was also investigated. Three minutes after reconstitution, signals are enhanced by a factor of 4.
Both results obtained with short or long purge time show large quantitative differences.

The individual release curves follow the same pattern as the global ones. The maximum individual aroma release is observed at the same time as for the global curve.
Different type of release curves can be observed. A sharp and high release for compounds as diones and pyrrole (eight times the starting level), a lingering effect for methyl butanals, DMDS and furfuryl methyl ether.



To explain these observations, we suggest that matrix effects play a role not yet clearly understood.

The influence of the matrix can be pointed out by comparing different release systems: coffee aroma in water versus R&G coffee suspension.



The maximum intensity of release is reached earlier in the first case. The behavior of individual compounds is more pronounced in this system characterized by the absence of any matrix interactions (except potential aroma-aroma interactions).
Later release (pyridine) corresponds to compounds with high solubility in water, as those with lower solubility are quickly found in the head-space (DMDS).

Conclusions

Release measurements starting from a complex system can be performed with the purge-and-trap method.
Chromatographic separation remains one of the most useful technique to follow the time evoglution of individual chemicals. The technique was tested with several coffee products. Differentiation in terms of aroma release could be clearly established.

Outlook

The main emphasis of the forthcoming work will be given to aroma-matrix interactions.

References

1- Elmore, J.S.; Langley, K.R. Novel vessel for the measurement of dynamic flavor release in real time from liquid foods J. Agric. Food Chem. 1996, 44, 3560-3563
2- van Ruth, S.M.; Roozen, J.P.; Cozijnsen, J.L. Comparison of dynamic headspace mouth model systems for flavour release from rehydrated bell pepper cuttings H.Maarse & D.G. van der Heij (Eds.) Trends in flavour research, 1994 Elsevier science

Summary

A purge-and-trap system was used to measure the flavour release of different coffee products. Differentiation in terms of release was observed.

GC-SNIFFING METHOD FOR IDENTIFICATION OF COFFEE AROMA PROFILES

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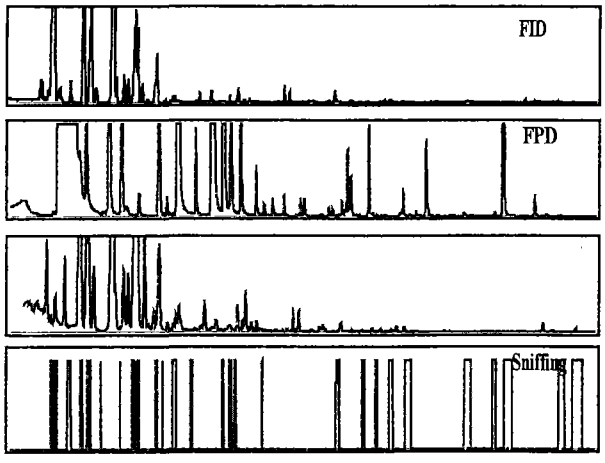
OBJECTIVE

To develop a direct method for analysing key aroma components in coffee-based products.

PRINCIPLE

Volatile components (KI < 1700) can be trapped by head-space concentration techniques and analysed by gas chromatography (GC) using specific detectors (FID, FPD, etc). For identification purposes, GC is combined with mass spectrometry (MS).
Instrumental detection of volatile components does not indicate their aroma relevance (odor threshold). Coupling these different techniques with detection by sniffing is used to determine key aroma components.

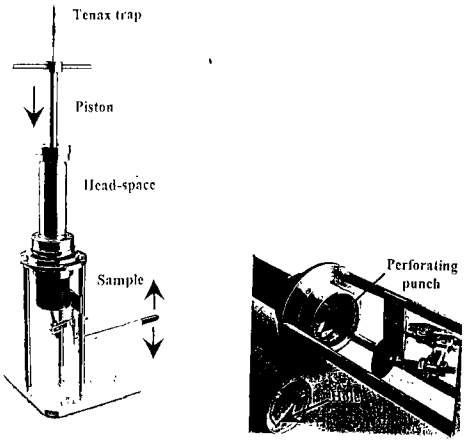


Gas chromatography and mass spectrometry analysis

METHODS

Direct trapping of the aroma

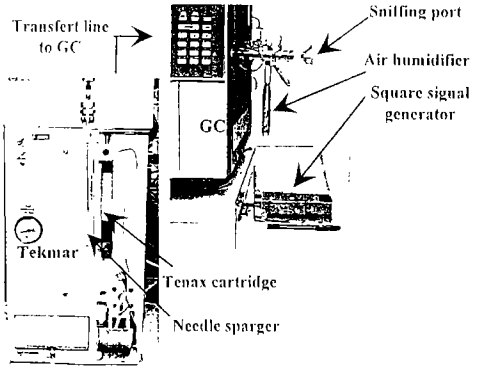
The product (can, jar or sachet) is opened in a device which allows direct sampling without contact with the external atmosphere. After equilibration (time is as a function of the product) the piston is pushed and the head-space aroma trapped on a Tenax cartridge.



Head-space cell

Analysis of the aroma

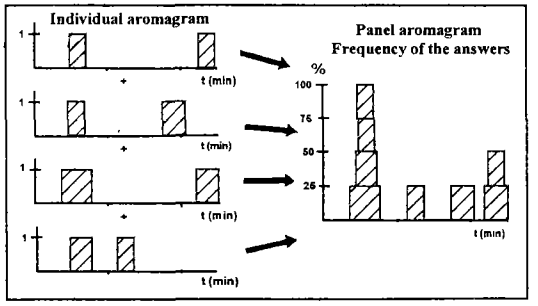
Volatiles are thermally desorbed from Tenax using a Tekmar apparatus into an HP 5890 GC equipped with a NukolTM column. The column outlet is either connected to an FID, an FPD and a sniffing port or to an MS detector.



Head-space and GC-sniffing technique

Head-space sniffing analysis

The improved sniffing method is based on combining individual responses (panel of 8 persons). Square signals are combined to build an aromagram indicating the key aroma components.

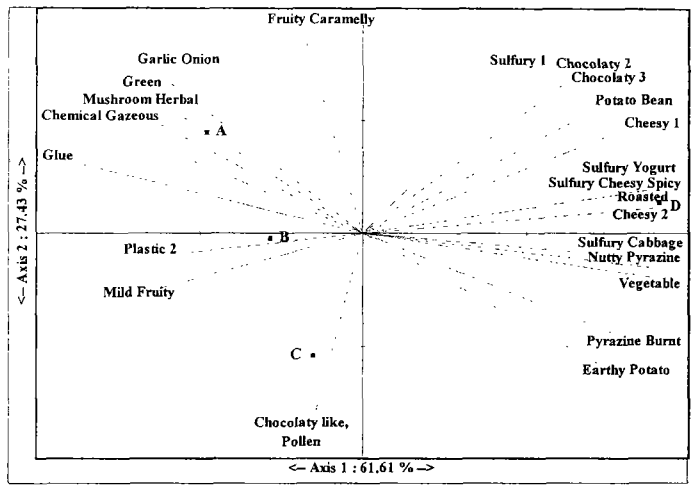


Data treatment procedure (4 sniffing additions)

RESULTS

Head-space analysis

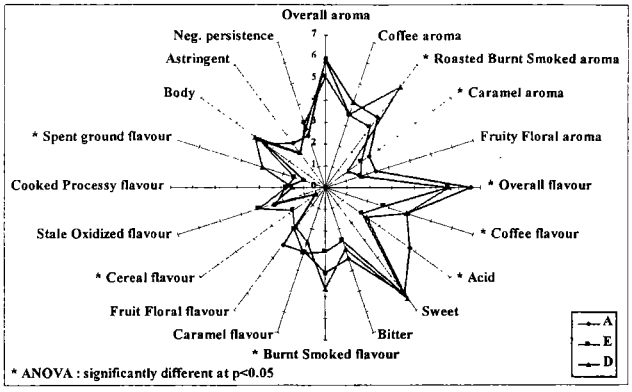
Results were analysed using Analysis of variance (Anova) to determine the most significant descriptors, and Principle Component Analysis (PCA) to characterize the products. Key aroma components can, if necessary, be identified by mass spectrometry.



PCA of selected samples

Example : Aromagram of sample D

Sensory analysis
Sensory evaluation is performed with a panel of 12 trained tasters. Each tasting is focused on profiling both aroma and flavour. Tasting is carried out blindly with three repetitions.
Preliminary sensory profiling of selected samples indicate good relationships between analytical and sensory data. A more complete study on a large range of products is on-going.



Sensory profiles of selected samples

CONCLUSIONS

The trapping method (head-space cell) is reproducible, clean, user-friendly and highly performant. However it is restricted to the detection of highly volatile components (head-space, KI < 1700) and, therefore, is not suitable for the characterization of low volatile components (KI > 1700).
The improved sniffing method is reproducible, fast, precise, is by-passing specific individual anosmia and allows key aroma components to be precisely characterized.
When necessary, the key aroma components can be identified by mass spectrometry.

OUTLOOK

To establish relationships between sensory and sniffing (analytical) descriptors.

SUMMARY

A system for direct trapping of aroma (highly volatile components) from coffee based-products (liquid or powder) combined with an improved sniffing method has been developed. The aroma can be characterized and key aroma components determined with this technique.

EXTRACTION IN COFFEE-PROCESSING AND BREWING

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INTRODUCTION

For household coffee brewing many procedures exist. Common to all these methods is the extraction of ground roasted coffee beans with water to obtain the coffee brew. Solid/liquid extraction is a common method in domestic food preparation. The brewing methods may be subdivided into continuous and discontinuous processes, they work with or without recirculation and are characterized by certain process parameters, e.g. pressure or temperature (below or above 100 °C). Procedures which operate at overpressure are used in cases where large amounts of coffee brew are made for commercial purposes or in special cases like the making of espresso. Pressureless procedures on the other hand are used for household purposes or in catering trade.
Generally, the extraction process depends on several parameters, like apparative design, transport kinetics and equilibrium between soluble substances, water and ground dependent on pressure and temperature.
Diffusion is the main mechanism responsible for the passage of the solid constituents from the ground roasted coffee into the coffee brew. This mass transport takes place in several steps:

1. penetration of water into the coffee bed, displacement of gases (air, roast gases), moisturing and whirling-up of particles
2. washing of material off the surface of the coffee particles, which were ruptured by grinding
3. penetration of water into the pores of the particles
4. swelling of the particles
5. solubilization of water-soluble substances, possibly hydrolysis of non-water-soluble substances
6. diffusion of dissolved substances to the particle surface (rate determining step)
7. convective mass transfer into the surrounding solution.

The amount of hydrolysis of non-soluble substances depends on temperature and pressure during the extraction process. Hydrolysis plays an important role in the making of soluble coffee, but in household coffee machines its role is questionable because of lower temperatures and shorter extraction times.
In the literature, some models exist to describe the extraction of coffee [1-4]. All experiments hitherto described were carried out in an ideal stirred vessel. While Charm [1] considers the resistance of the liquid film as rate determining, the other authors found the inner particle diffusion to be the slowest process. Zanoni [4] states an additional wash off process of solubles from the cut surface, which is responsible for nearly 90 % of the overall extraction yield.
From the view of process technology, coffee brewing is a highly instationary solid/liquid separation process. For an overview see [5, 6].
The aim of this work is not to compare different brewing techniques but to examine for a single technique, filtration under gravity, the influence of process and material parameters on kinetics of mass transfer, extraction yield and sensoric quality.

MATERIALS AND METHODS / EXPERIMENTAL METHODS

All extraction experiments were carried out in a self-constructed pressureless extraction apparatus (figure 1). It basically consists of a thermostated water tank with pneumatic flow controlling and -measuring (turbine) devices, a thermostated vessel which can hold different (geometry, volume) extractors, and a collecting device. The following data are recorded continuously: temperatures (tank, coffee bed, three heights within the extractor, outlet tube of extractor) and flow rate of hot water into the extractor. To determine temperature and concentration profiles within the extractor, three probe valves are integrated within the extractor at different heights. Here it is possible to measure temperatures and to take samples with the help of a special probing syringe. For the samples taken the solute concentration of the liquid phase (specific electrical conductivity, see below) and the residual solubles content of the coffee particles (by total extraction) were determined.

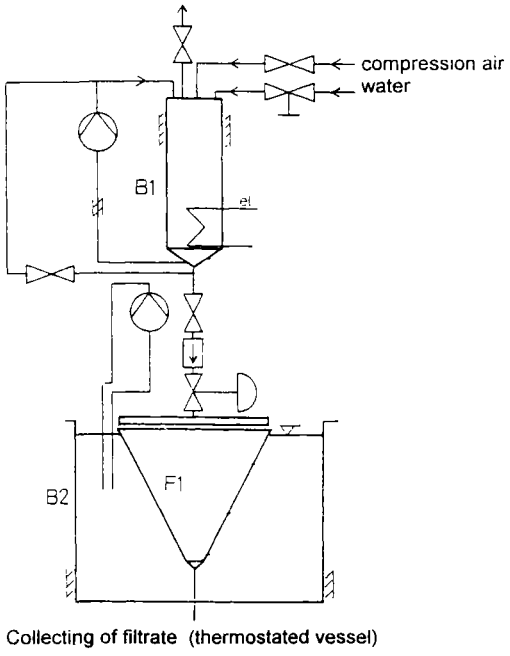

Figure 1: Extraction apparatus (B1: thermostated water tank; B2: thermostat; F1: interchangeable extractor)

The determination of solid content of the coffee brew according to DIN 44539(2) „Elektrische Haushalts-Kaffeebereiter: Gebrauchseigenschaften, Prüfungen" is by placing 10 ml of the filtered coffee brew for 3 h in an

oven at a temperature of 105 ... 110 °C. After cooling in a desiccator the amount of extracted soluble substance is determined gravimetrically.
For our experiments a faster method was necessary. Thus we examined the following physico-chemical parameters of the coffee brew and their correlation with the total content of solids: specific electrical conductivity, pH, acidity, density, viscosity, K^+-content. As known from the literature [5] the specific electrical conductance is directly correlated to total solubles concentration (up to about 10 % solutes) and both quick and easy to determine. Our results are summarized in figure 2.

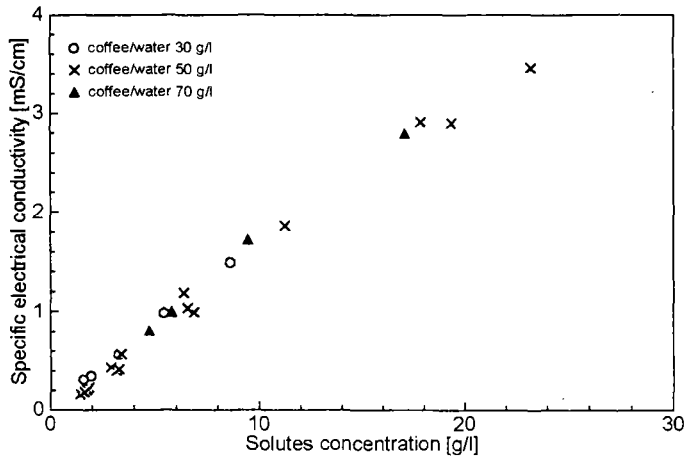

Figure 2: Specific electrical conductivity vs. solid content of the coffee brew

The extraction apparatus allows the variation of the process parameters within a wide range. The parameters under examination are listed in figure 3.

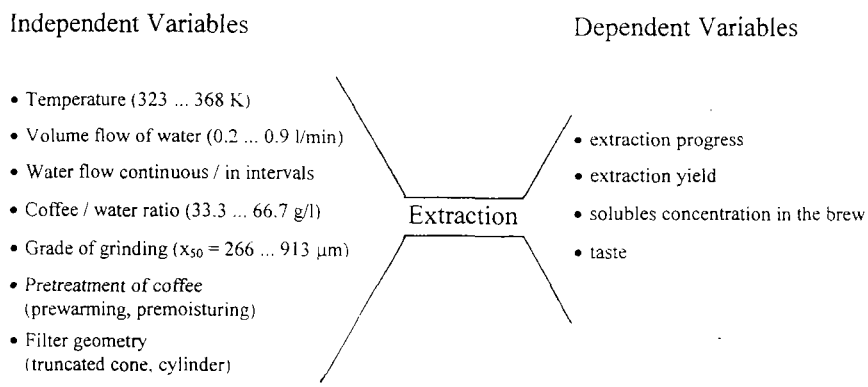

Figure 3: Independent and dependent parameters in coffee extraction

Reference parameters were:
- extraction temperature 368 K,
- coffee/water ratio 50 g Arabica/l drinking water,
- flow rate of water 0.5 l/min,
- middle grind (x_{50} = 391 µm),
- 1.5 l truncated cone extractor.

The pre-moisturing of the coffee particles was done by storage in a desiccator over different saturated salt solutions with known relative humidity until mass constancy was reached. By „wetting" via the gaseous phase a pre-extraction was prevented.
To examine the mass transfer within the extractor, during the extraction 6 samples were taken in different positions of the extractor (every minute). Each sample was separated into solution and solid. For the solution the total solubles content was determined via its specific electrical conductivity, the solid (coffee ground) was then totally extracted with water to determine the residual load.
Additional sensoric tests should show, how certain extraction parameters influence the taste of the coffee brew. The parameters examined where the grade of grinding (x_{50} = 913, 716, 508 µm), the extraction temperature (60, 75, 95 °C) and the flow rate of water (0.25 and 0.5 l/min). The tests were realized as triangle tests according to DIN 10951, the test panel consisted of 10 students.

Results and Discussion

All extraction curves show a non-linear rise, when the total extracted mass is plotted against the total amount of water. A typical example is represented in figure 4. Additionally, the equilibrium load (determined in a stirring vessel) of 0.11 g solubles /g filtrate and the initial gradient of the extraction curve are shown. Obviously, coffee extraction with filtration is not determined by the equilibrium solubility in water. In the first phase, where one observes a nearly linear rise, solubles are washed off the ruptured surface of the coffee particles (wash-off zone). The convective mass transfer is here limited by a surface layer. During the further progress of the extraction, the inner-particle-diffusion becomes the rate determining step, the extraction curve clearly deviates from a linear rise (diffusion zone).
Besides, filtration superimposes the extraction. This means that extract with equilibrium load mixes with fresh water from the upper region of the filter to give a load less than in equilibrium.

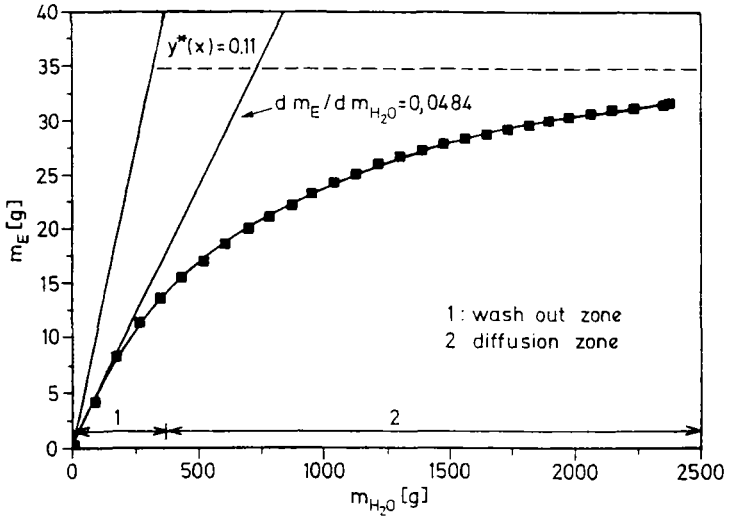

Figure 4: Extraction progress: mass of extracted solubles vs. the amount of water

Influence of Process Parameters on Extraction Kinetics and Solid Content

The temperature of the solvent (tap water) was varied in the range 323 ... 368 K. As shown in figure 5, the extraction yield generally rises with temperature, most destinctively between 343 and 363 K, due to increasing diffusion rate and saturation concentration of solubles in water. There is no effect of temperature on filtering time.
In the literature, the influence of temperature is discussed controversially. Heiss et al. [7] found in the temperature range 331 ... 373 K only a minor influence on extraction progress and yield for a coarser grind, for finer

grinds only during the first 30 s. Voilley et al. [2] detected an influence of temperature on extraction yield in the region 303 ... 364 K, especially for higher coffee/water ratios. While they state that there is no influence on organoleptic properties between 343 and 378 K, Rothfos [8] found no significant differences in extraction yield, but minor differences in taste in the region 353 ... 368 K.
Increasing the flow rate of solvent will lead to a shorter residence time and time of contact between water and coffee, consequently the yield decreases (figure 6). The effect is not so clear as one would expect, because high flow rates will result in good mixing within the upper central region of the filter bed and in improved convective transport of solids into the brew.
When the water is not added continuously, but in several (1 ... 4) consecutive pulses (constant total amount of water), the extraction progress differs, but there is no prominent effect on extraction yield.

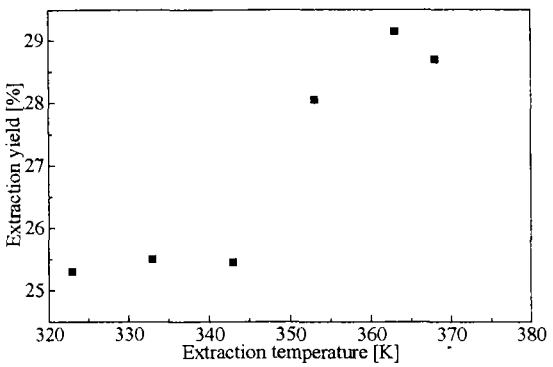

Figure 5: Influence of temperature on extraction yield

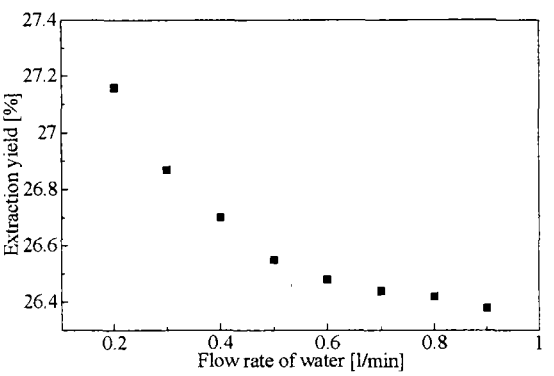

Figure 6: Influence of flow rate on extraction yield

Increasing the coffee/water ratio leads to higher solubles concentration in the coffee brew, but the extraction yield decreases, as shown in table 1. The reason is the higher water uptake capacity of the ground coffee (about double its weight), which lowers the amount of coffee brew. Voilley [2] found the coffee/water ratio to be the main influencing factor on solubles concentration. The filtration time increases by 15 % when doubling the coffee/water ratio.

Table 1: Influence of coffee/water ratio on extraction results

coffee/water [g/l]	solubles concentration [%]	extraction yield [%]	filtration time [s]
33.33	1.030	27.72	332
40	1.232	27.28	359
50	1.573	27.25	372
66.67	2.086	26.29	381

The geometry of the filter for pressureless household coffee makers is usually a truncated cone. We examined several cylindrical filters with different volume/surface ratios. Due to the unfavourable ratio of the filter surface and the extraction volume very long preparation times are necessary to obtain reasonable extraction yields (20 ... 30 min, 27.5 %). For normal filtering times (6 min) one gets a very low extraction yield of only 5.9 %, which is merely about one fifth of normal yields by using a truncated cone filter (table 2). The latter obviously represents an optimal balance between duration of filtration and extraction yield. Cylindrical filters are nevertheless used for filtration under pressure, e.g. in espresso making.

Table 2: Influence of filter geometry on extraction results

volume [l]	filtration area [cm^2]	filtration area/ volume [m^{-1}]	solubles concentration [%]	extraction yield [%]	preparation time [s]
cylindrical					
0.60	7.55	1.26	-	-	-
1.50	32.17	2.15	1.586	27.62	1708
2.75	67.93	2.47	1.616	27.57	1150
4.00	169.72	4.24	0.351	5.91	328
truncated cone					
		27.50	1.573	27.25	425

An important influencing factor is the grade of grinding of the coffee beans. Grinding means rupture of the coffee bean cells. Small particles have a large specific surface. They show a relatively constant extraction progress. Courser grinds on the other hand show an obvious fractionation effect. The first fractions leaving the filter have a high content of solids, the latter decreasing rapidly with continuing extraction time, as shown in figure 7.

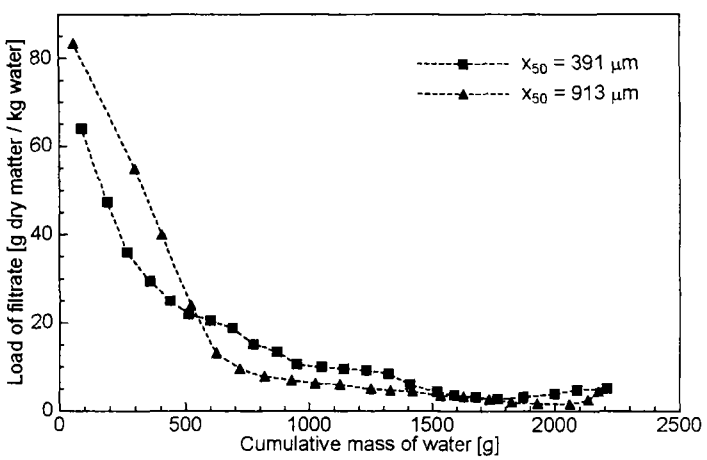

Figure 7: Extraction progress for different grades of grinding

Table 3: Influence of grinding on extraction yield, solubles concentration and filtration time

medial diameter x_{50} [µm]	solubles concentration [%]	extraction yield [%]	filtration time [s]
266	1.488	25.75	386
391	1.573	27.25	372
508	1.555	26.98	351
716	1.531	26.66	337
913	1.527	26.64	318

Thus the first amount of the filtrate mainly determines the extraction result. Considering the dependence of total extraction yield on size distribution of coffee particles, one gets a maximum for a middle grind (x_{50} = 391 µm). For finer grinds the extraction yield is smaller due to agglomeration and flotation effects and insufficient wetting

of the particles. For courser grinds on the other hand the yield decreases (see table 3) due to the smaller volume specific surface, which strongly influences the diffusive transport. The filtration time, i.e. the time between the first adding of water and transition of the continuous flow of filtrate into dripping, decreases with increasing particle size (decreasing flow resistance).

Pre-moisturing of the coffee particles solubilizes the dry matter within the solid matrix, a widening of the pores by swelling lowers the diffusion resistance. By pre-moisturing the extraction rate in the first stage of the extraction as well as the overall extraction yield rise (from 27.12 % for 2.2 % moisture content to 27.63 % for 10 % moisture content). In the further course of the extraction, after total wetting of all particles, no difference is obtainable, see figure 8.
Pre-heating the coffee particles to 70 °C has no major influence on the extraction progress, but rises the extraction yield from 26.15 % to 27.49 %, probably due to a widening of the pores by thermal expansion (figure 8). Nevertheless, in daily praxis this treatment would be too expensive.

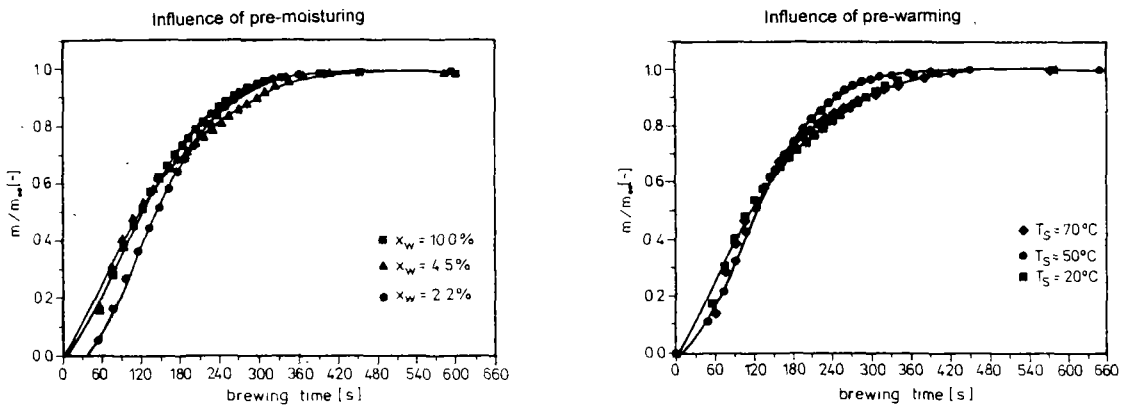

Figure 8: Influence of pre-moisturing (a) and pre-warming (b) of coffee particles

The taste of the coffee brew is not influenced by the grade of grinding. The flow rate of water on the other hand has a high significance, higher extraction times are preferred. The difference between an extraction temperature of 95 °C/ 75 °C is not distinctive, whereas the difference 95 °C/ 60 °C is significant. As one would expect, the higher temperature is slightly preferred.

Temperature and Concentration Profiles within the Extractor

Generally, the solubles concentration first rises and then decreases. In the centre of the extractor it is lowest in all steps. Along the axis of the extractor one finds a low content of solubles, because here is the main flow of the freshly added solvent. The residual load of the coffee particles along the axis of the extractor is higher in the upper region, in the lower region the extracted particles collect. Within the first minute, the residual load decreases sharply, just to rise again in the further progress. This can only happen by feeding fresh particles from the rim. This moving of the particles is supported by video recording. The flow of fresh water results in a strong stirring (recirculation) in the middle of the extractor. Two regions with different extraction processes are discernible within the filter: percolation (i.e. slow flowing of a solvent through a bed of ground solid) through the still fixed bed in the outer region and an ideal mixing vessel in the centre of the filter (immersion extraction). As long as water is added, the latter region grows at the expense of the percolation region. From the low solubles concentration within the suspension phase it follows that the extraction mainly happens in the fixed bed (high ratio coffee/water, low flow rate). When the flow of fresh water ends, the residual load decreases, because the stirring effect ends.
Supplementary measurements during the extraction with the help of a special conductivity cell gave similar results. As soon as the water reaches the cell, the conductivity increases rapidly to then remain at a constant level

for some time. When the cell comes into the area of the ideal stirred vessel, the conductivity rises again. When the water flow stops, the conductivity decreases to a constant level which is maintained until the cell runs dry.

Course of Extraction

After the flow of water starts, a channel of liquid forms which spreads from the axis outward. In the upper region of the extractor the impact of water leads to a stirring effect, simultaneously the extractor fills. When supply and outlet flow are constant, a constant filling rate is observed, until the water feed and thus the stirring effect stop. The last stage is the draining of the extractor.

CONCLUSIONS AND PERSPECTIVES

On the basis of the results presented a model was developed to describe the extraction of roast & ground coffee in pressureless household coffee machines [9]. Therein four phases are considered:

1. filling of the extractor
2. flow through the fixed bed and immersion extraction
3. immersion- and percolation extraction in combination
4. percolation extraction and draining of extractor.

ACKNOWLEDGEMENTS

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ABSTRACT

The extraction of coffee in pressureless household coffee machines depends on several parameters. In a pressureless extraction apparatus, the influence of some process parameters on kinetics of mass transfer and extraction yield is examined. The determination of concentration profiles within the extractor and measurements with a conductivity cell allow a more detailed description of the course of the extraction.

ZUSAMMENFASSUNG

Die Extraktion von Röstkaffee in drucklosen Haushalts-Kaffeemaschinen hängt von vielen Parametern ab. Mit Hilfe einer eigenkonstruierten Extraktionsapparatur wird der Einfluß verschiedener Prozeßparameter auf die Kinetik des Stoffübergangs und das Extraktionsergebnis untersucht. Die Bestimmung von Konzentrationsprofilen im Extraktor und Messungen mit einer Leitfähigkeitsmesszelle erlauben eine detailiertere Beschreibung des Extraktionsvorgangs.

A REACTION-DIFFUSION COMPUTATIONAL MODEL TO SIMULATE COFFEE PERCOLATION

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1. INTRODUCTION

The espresso coffee brewing process involves complex chemical and physical phenomena. Knowledge of these phenomena affects the quality of the final product in the cup [Petracco and Suggi 93]. In this work the Reaction Diffusion Machine (RDM) [Bandini *et al.*, 1995], [Bandini *et al.*, 1996] which combines features of two existing abstract machines: the CHAM [Berry *et al.*, 1992] and some classes of Cellular Automata [Chopard *et al.*, 1990], [Dab *et al.*, 1990], is proposed into the modeling of the diffusion process of soluble substances. The defined computational model allows us the qualitative analysis of the phenomenon and the study of the appropriate diffusion function that could be utilized to integrate the fluid dynamic model based on automata [Petracco *et al.*, 1993], [Bandini *et al.*, 1994] for better simulating the percolation process. The main aim of the RDM is to incorporate the reactions and all the diffusion-related features into a unique, formal, and computational distributed model.

2. THE EXTRACTION PROCESS

The percolation process consists of hot, pressurized water flowing through a layer of ground roasted coffee in a brewing chamber in which soluble and scattered substances are extracted by water. The extraction process develops following a sequence of phases: *washing* and *diffusion.*

Different authors have given different weight to each of the two phases. In Zanoni [Zanoni *et al.*, 1992] the extraction process is principally considered as a washing process which involves the dissolution of "free" solutes found on the surface of the particle. According to the authors, approximately 90% of the theoretical product is attained in this first phase, while the diffusion of the solutes from the internal porous parts is considered insignificant. In fact, the studies by Zanoni, Pagliarini and Peri point out how most of the soluble substances are

extracted during the first minute with the remains being extracted in relation to the external surface area. As a result, the washing phase is the principal method.Contrarily, Spiro [Spiro 1993] argues against the results obtained by Zanoni, Paglierini and Peri, affirming that in their phenomenological model, developed through the kinetic study of coffee, the actual diffusion D is characterized by ample variations, in function with the average diameter of the particles, and no defined trend. Better yet, Spiro claims that the data collected relate to the successive instances from the first minute and that the values of the remaining extraction supplied [Zanoni *et al.*, 1992] incorporate the concentration of solutes derived not only from a possible washing phase, but also from a diffusion phase.

Other authors have inspected Italian espressos from the characterization point of view [Petracco, 1989] or from limited to soluble solids and caffeine kinetics [Nicoli *et al.*, 1987]. In conclusion, the coffee preparation cannot be treated as a simple diffusion phenomenon, but as a complex process which includes two phases. However, since more than 90% of the theoretical product is obtained through the washing phase, it is evident that this latter phase is of greater importance in the beverage preparation.

3. SOLUBLE SUBSTANCES EXTRACTION MODEL

Percolation is a complex physical phenomenon studied from several theoretical [Stauffer *et al.*, 1992], applicative and computational standpoints [Sahimi, 1993, 1994]. In particular, *invasion percolation* models (liquids spreading throughout porous media) [Lenormand *et al.*, 1985], [Wilkinson *et al.*, 1983] find their best application in invasive pollution phenomena or in the extraction of physical-chemical substances (e.g., petroleum).

Percolation problems can be studied by numerical analysis [Stauffer *et al.*, 1992] or by fractal theory [Mandelbrot, 1983]. Cellular automata (CA) [Wolfram, 1983] has offered new approaches for modeling and simulating percolation problems [Sahimi, 1993]. In the specific case of percolation invasion using fluid-dynamics models, CA systems have been suitably created to avoid numerical approaches [Rothman, 1988], [Bandini *et al.*, 1994]. In these models, the uniformity of invasive substances (e.g., water) and the hypothesis that the porous medium is immovable allow good results to be obtained. However, real percolation processes are more complex as in the case of free particles moving into channels. In order to model this situation, CA have to be extended [Worsch, 1997], weakening the computational advantages of the approach. The adoption of a RDM model for the invasion percolation modeling is our proposal in challenging these limitations. In this manner, we consider coffee percolation, that is the passage of water throughout a compacted bed of both ground and roasted coffee particle aggregates and small pieces of coffee (singletons) not belonging to aggregates. Singletons and aggregates form the *percolation bed* which has a fixed dimension defined by a *percolation chamber* (the filter).

As mentioned previously, the extraction process of soluble substances (e.g., caffeine) from the percolation bed can be divided into two main phases: *washing* and *diffusion*. The *washing stage* corresponds to the reaction which occurs between water and the surface of both coffee aggregates and singletons. This reaction, as well as the reaction between water entities carrying some amount of solubles, is based on an *equilibrium search law*.

The *distribution stage* of solubles is due to the diffusion of water through the channels while the caffeine distribution in the medium is disregarded [Zanoni *et al.*, 1992]. Therefore, aggregates are represented as elements where no diffusion occurs and reaction involves only particles located on their surfaces.

The following few elements are sufficient to model a very complex system:

•the filter, composed of two lateral walls and a perforated floor; the top is free and accepts water coming from a source;

•the percolation bed;

•the water, flowing through the channels washing coffee particles and dragging singletons;

•the mechanism of the distribution of concentration solubles occurring during the washing stage of the extraction process.

The computational space presented in a visualizing interface is partitioned in the following sub-spaces:

•*Source*: where water type elements enter the percolation bed. In the initial configuration, the source is saturated with water type entities.

•*Aggregates*: containing just coffee type elements.

•*Room*: containing wall type elements located at the border of the solution to represent the filter. In the initial configuration there is a finite amount of coffee type elements. In any intermediate configuration, there are also water type elements.

•*Output*: in the initial configuration all of its sites are empty. In the intermediate configurations, there are water type elements.

The RDM modeling the above percolation system can be described as follows. The space of sites is a regular bidimensional grid and the adjacency relation among places is a von Neumann neighborhood (four adjacent cells).

The passive diffusion of elements is determined by a force field (e.g., gravity). The force field distribution function is a non-zero constant in all sites except for the sites contained in the aggregates, where its value is null.

The model contains the following types of individual elements.

•*Wall type* : elements of this type serve as physical obstacles for the moving entities, assume just a single state, and are not fully sensitive to any force field (e.g. gravity).

•*Water type* : each state is a 4-tuple whose elements are pairs $<state^x, C^x>$ where x is one of the adjacent places and the state can assume one of the values "quite, unbalanced"; C is the concentration soluble which ranges from 0 (pure water) to a maximum integer value representing a water saturation constant - wsc. Water elements in this state are quite sensitive to the gravity field which drives their movements from the top to the bottom of the grid.

•*Coffee type*: each state is a 5-tuple whose initial four elements are the same as the previous case (also considering a soluble substance contained in the coffee- e.g., caffeine - saturation constant - csc); the fifth component is an integer which counts the number of reactions involving the entity in a specific configuration (this will be explained later). Coffee entities are sensible to the force field in all states where the state is quite motionless and the counter is greater than a fixed number.

There are two *movement rules*, one involving water entities and one involving coffee singletons (if their representation is explicitly needed, for instance, simulating through singletons the dragging of microscopic particles contained in the percolation bed):

•A site-select rule representing the movement of water entities is defined for the movement of water elements (here informally) as follows: if a south place is empty then the element can occupy this place or else it will non-deterministically choose a place between its east place or west one if they are empty; otherwise no movement occurs.

•The second site-select rule for the movement of singletons accounts for the fact that singletons in the channels have to receive "some energy" from the reaction with the north "pushing" water entities before becoming sensitive to gravity. In the case of coffee type elements within aggregates, the non-null sensitivity to gravity is irrelevant.

There are two *reaction rules*, one describing the interaction among water entities, and one describing the interaction between water and coffee entities. These rules describe how the distribution of concentration solubles follows an equilibrium search law (corresponding to the mechanism of the physical diffusion equation considering the concentration diffusion) when combined with a *trigger rule* whose effect is to make the concentration solubles homogeneous within each element involved in the reactions.

The reaction rules suppose that reacting entities can be ideally divided into four portions with a balanced amount of caffeine and that each portion exchanges particles with the adjacent portion of the neighboring entity. Actually, this rule turns out to be equivalent to the finite difference formula for the diffusion equation. An eventual one unit remainder is assigned to one of the portions.

In a more formal way, we report here a partial description of such rules in terms of RDM.

Let OR(p,q) = <x,y>, where <x,y> is <north,south> or <east,west>, denote the relative position of adjacent sites p and q.

$$R^{R}_{w,w} = \frac{\text{water}_p, \text{water}_q, OR(p,q) =< z,y >, C^{z}_{p} < C^{y,}_{q}, C^{z}_{p} < \frac{1}{4}\text{wsc}, C^{y,}_{q} < \frac{1}{4}\text{wsc}}{x'_p =<\ldots,<\text{unbalanced}\ ^{z}, C'^{z}_{p} = \frac{C^{z}_{p} + C^{y}_{q}}{2} + \alpha >, \ldots > \text{ and } \alpha \in \{0,1\}}$$

$$x'_q =<\ldots,<\text{unbalanced}\ ^{y}, C'^{y}_{q} = \frac{C^{z}_{p} + C^{y}_{q}}{2} + (1-\alpha) >, \ldots >$$

$$R^{R}_{w,c} = \frac{\text{water}_p, \text{coffee}_q, OR(p,q) =< z,y >, C^{z}_{p} < \frac{1}{4}\text{csc}}{x'_p =<\ldots,<\text{unbalanced}\ ^{z}, C'^{z}_{p} >, \ldots >}$$

$$x'_q =<\ldots,<\text{unbalanced}\ ^{y}, C'^{y}_{q} >, \ldots, k' >$$

$$\text{where } C'^{z}_{p} = \frac{C^{z}_{p} + C^{y}_{q}}{2} \text{ if } \frac{C^{z}_{p} + C^{y}_{q}}{2} < \frac{1}{4}\text{wsc}, C'^{z}_{p} = \frac{1}{4}\text{wsc}, \text{ otherwise };$$

$$C'^{y}_{q} = C^{y}_{q} - (C'^{z}_{p} - C^{z}_{p})$$

$$\text{and } k' = k+1 \text{ if } < z,y >=< \text{north}, \text{south} >, k' = k \text{ otherwise}$$

The forementioned trigger rule balances the concentration again and changes the entity to a quite inert state so that further reactions and movements can occur.

$$R^{T}_{w/c,B} = \frac{\text{water / coffee}_p, \text{unbalanced}_p FSV(\text{water / coffee}_p) =< 0,1 >}{x'_p =<< \text{quite}^{z}, C'^{z}_{p} >_{z \in COORD}, [k] >}$$

$$\text{where } C'^{z}_{p} = \frac{1}{4}\sum_{h \in COORD}(C'^{h}_{p} + \alpha^{h}) \text{ and } \alpha^{h} \in \{0,1\} \text{ and } \sum_{h \in COORD}\alpha^{h} = \text{mod}\left(\frac{1}{4}\sum_{h \in COORD}(C'^{h}_{p} + \alpha^{h})\right)$$

The above set of rules preserves the fundamental principle of matter conservation and avoids anisotropic distribution as in the case of diffusion dynamics CA models.

4. SOME SIMULATION RESULTS

Three different sets of experiments were performed by variation of particle size parameter. Water injection was maintained at the same ratio during the experiments.

The first situation (figure 1) displays a slice of a coffee bed of coarse particles, the second one (figure 2) depicts a section of fine particles and the third situation (figure 3) represents a mixture of both, better resembling real percolations.

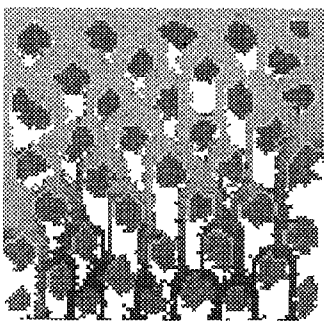


FIGURE 1. Coarse Particles

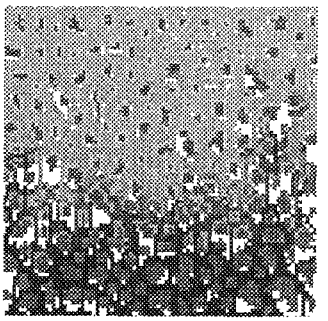


FIGURE 2. Fine Particles

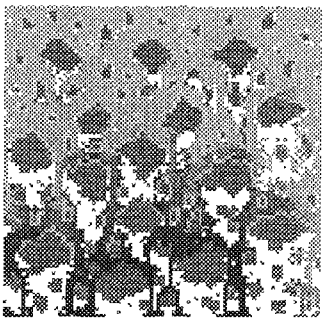
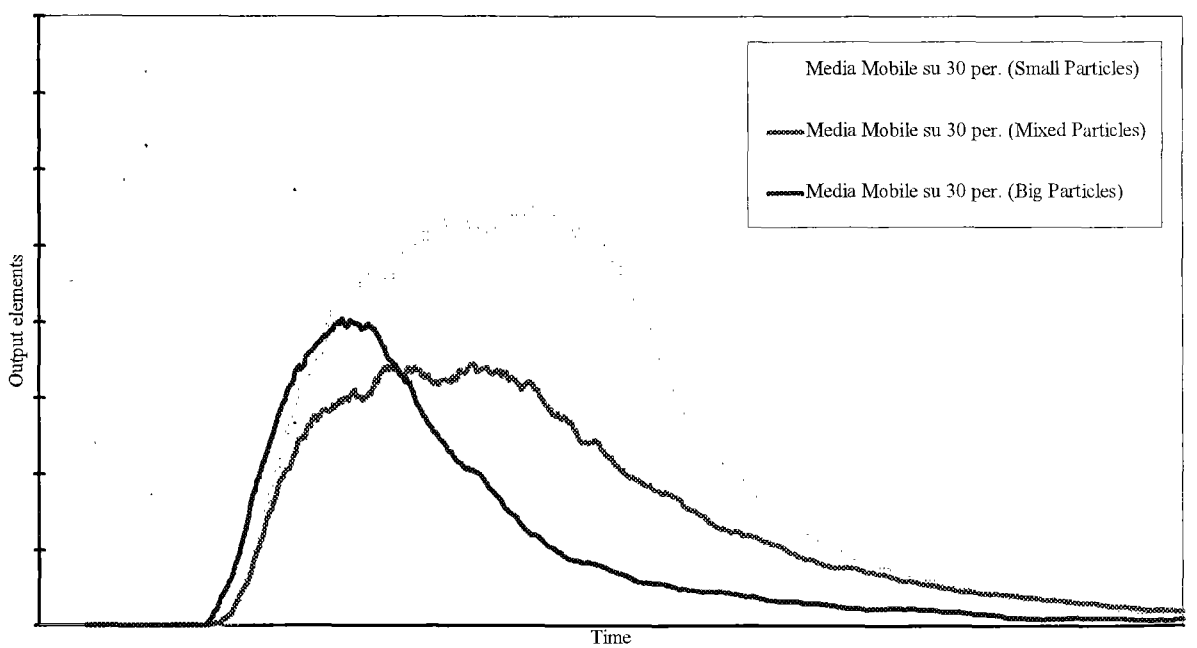


FIGURE 3. Mixed Particles

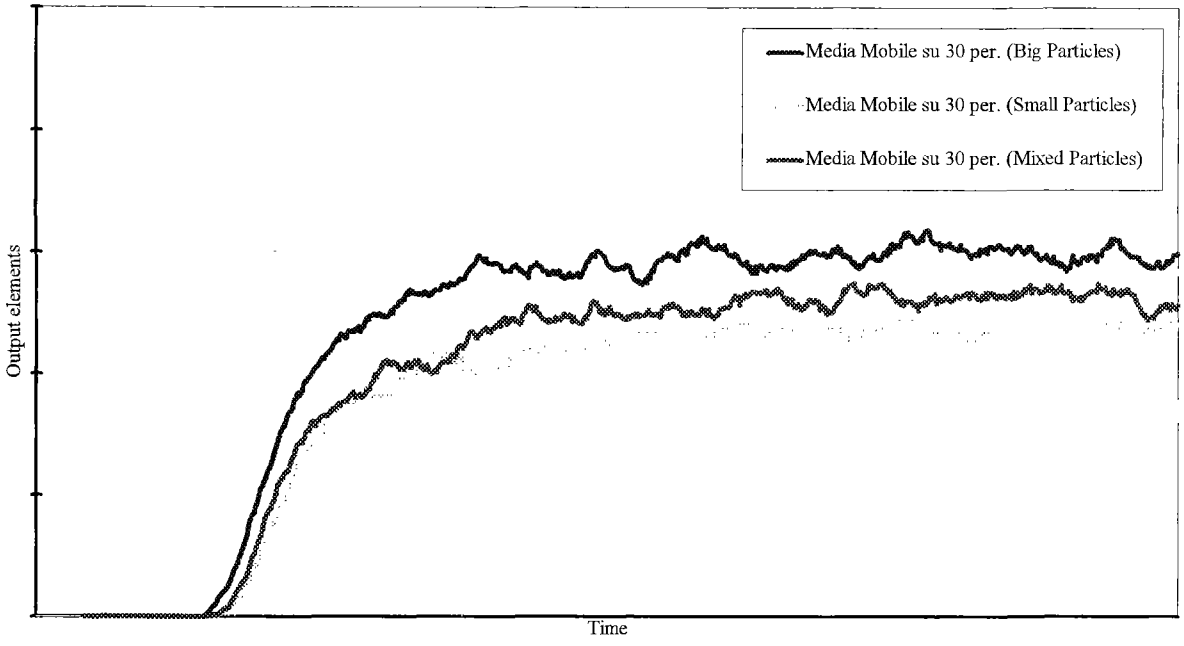
Figure 4 displays the number of water elements leaving the system. The number of elements refers to the temporal intervals corresponding to 20 computational instances. As evident from the graph, following the initial growth (the first phase), the number of elements exiting tends to stabilize near a constant value (second phase). The first phase corresponds to the first instances of the percolation processes where the liquid is placed in the percolation room. In the second phase, the liquid has formed preferential channels inside the porous media.

Figure 4. Solubles Extraction Simulation



The second graph illustrates the average substance concentration alpha (the maximum concentration equal to 16) verifiable with the water elements leaving the system after 20 computational ticks. In the simulation which produced the data for these graphs, all the soluble substances present in a coffee element were available during the extraction phase.

Figure 5. Water Flow Simulation



During the simulation it is possible to observe the concentration change of both the water and coffee entity. In fact, the coffee particle, yielding part of its hydrosoluble substances through a series of intermediate steps which visibly represents the concentration changes, changes its color from dark, indicating the presence of a high concentration of substances, to light, indicating the absence of hydrosoluble substances. Analogously, water changes from light, noted by the solute absence, to dark, noted by reaching the saturation threshold (wsc). At any computational instant it is possible, however, to observe a qualitative representation of the diffusion and extraction process.

Furthermore, at any instant, the number of coffee particles and water particles leaving the system is memorized, in addition to the total concentration of substances leaving the system according to the attribute values.

Even though the system only represents a small portion of a real coffee layer with bidimensional limited topology and apart from the simplicity of the behavior rules, it should be noted that the obtained results compared to the observed phenomenon are qualitatively similar to those obtained in laboratory experiments.

Acknowledgments

The implementation of the RDM computational platform in the Java object-oriented language is due to Luca Demarchi; we would like to thank him for his contribution. We are in debt with Marino Petracco and Paola Arosio for their fruitful suggestions and comments throughout the preliminary stage of this research.

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SUMMARY

A REACTION-DIFFUSION COMPUTATIONAL MODEL TO SIMULATE COFFEE PERCOLATION

The increase of computation power at low cost and the development of dynamical computation systems has offered new approaches for modeling and simulating percolation problems.
This paper presents a computational model based on the Reaction-Diffusion Machine (RDM) for simulating both the motion of water throughout a compacted bed of particles of ground and roasted coffee, and the extraction process of soluble substances (e.g. caffeine) from the percolation bed.
The RDM allows us to consider particles as individual entities endowed with solute concentration. Moreover, in the RDM it is possible to consider individual entities (e.g. water) not reacting with each other, preserving their concentration value during their movement in space and the actual behavior of concentration dynamics or brownian motion as given by the solution of the standard diffusion differential equation. It allows us to describe in two well-separated phases the diffusion as a movement of entities in a given space and the reaction as a distribution of concentration dynamics.

RÉSUMÉ

UN MODELE INFORMATISE A REACTION-DIFFUSION POUR SIMULER LA PERCOLATION DU CAFE

Une utilisation toujours croissante de l'informatique à bas prix et le développement de systèmes informatisés dynamiques ont apporté de nouvelles manières pour modeler et simuler les problèmes de la percolation.
Il s'agit ici d'un modèle informatisè qui se base sur la Machine à Réaction-Diffusion (R-DM) pour simuler soit le mouvement de l'eau à travers une couche compactée de particules de café moulu et torréfié soit le processus d'extraction de substances solubles (par exemple la caféine) venant de la couche de percolation.
La R-DM permet de considérer les particules comme des entités individuelles (par exemple l'eau) ne réagissant pas entre elles et capables de conserver leur valeur de concentration pendant le mouvement dans l'espace et le comportement réel de la dynamique de concentration ou du mouvement brownien comme il résulte de la solution de l'équation différentielle de la diffusion standard. Cela nous permet de décrire en deux phases bien distinctes la diffusion comme un mouvement d'entités dans un espace donné et la réaction comme une distribution de la dynamique de concentration.

KINETICS OF COFFEE INFUSION
Rates and diffusion coefficients of caffeine and mineral ion infusion from medium roasted coffees

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INTRODUCTION

The kinetics of extraction of soluble materials from coffee is important especially when some of these materials are either harmful or beneficial to the human health. The rate of extraction of caffeine for example is of relevance to the decaffeination processes. The amount extractable depends on several factors such as the size to which the coffee bean is ground, the temperature and type of the solvent, the length of the extraction period and the form in which the material exists in the bean (Spiro and Selwood 1994, Haslam et al 1987).

One aspect which has received very little attention is the rate of mineral extraction. It is expected that the total amount of different elements in the coffee bean will depend on the country and area of origin. The main subject of this paper is to investigate the rate of extraction of mineral ions and caffeine from coffee beans from different geographical regions.

EXPERIMENTAL

The coffee beans investigated were medium roasted Kenyan Special, Sumatra Blue Mountain Java, Brazilian Santos, Ethiopian Mocha and Zimbabwean coffee. All these coffees were Arabica except for Ethiopian Mocha which was a Robusta (Colombo Coffee and Tea Co., Durban). These were ground using a Glen Creston mill equipped with agate mortar and pestle and then sieved through a standard set of stainless-steel Endecotts sieves, using an Endecotts sieve shaker machine. The bean fraction between 1.70 and 2.00 mm sieve size was used for study. All containers used were made of plastic and the water was demineralised Milli-Q-water (Milli-Pore). Extran detergent (Merck) was used in the cleaning process and the apparatus was then rinsed with a 0.1M HCl (BDH Arista) solution.

All kinetic experiments were carried out using a conical flask made of plastic containing 200 ml of water. These were allowed to equilibrate to 80°C in a thermostat controlled waterbath. The lid of the flasks had a hole

through which a sampling tube made of a thin plastic tube passed. The sampling tube was attached on the outside of the flask to a 2 ml sabre syringe. On the inside, the end of the tube had a hollow plastic cone to which a Gilson filter (Anachem) was inserted so as to exclude coffee beans and particulate during sampling. It has been found (Spiro & Siddeque, 1981) that simply dropping tea leaves into the hot water tended to give irreproducible results. Because of this 4 g of coffee beans was quickly added to the 200 ml of water via a long wide-spout glass funnel. The stop-watch and the immersible magnetic stirrer attached to the waterbath were started as soon as the coffee was added.

Samples (1 ml) were taken at half minute intervals for the first five minutes and the last equilibrium sample after 90 minutes. The samples taken were transferred to plastic sample tubes containing 9 ml of water and were mixed thoroughly. After each sampling, the sample tube was cleaned of any solution by attaching a clean syringe and injecting air through it and the filter. Corrections were made to the concentrations of the soluble (Spiro & Jago, 1982; Jaganyi, 1992) for volume lost through sampling and evaporation by weighing the flask and the content before and after each run.

RESULTS AND DISCUSSION

The variation of caffeine and mineral ion concentration c, with time t, followed first-order behaviour according to the equation

$$\ln\left(\frac{C_\infty}{C_\infty - C}\right) = k_{obs}\, t + a \qquad (1)$$

which is predicted by the steady state theory of extraction (Spiro and Elswood 1994). The concentration of the soluble components at equilibrium is represented by C_∞, the first -order rate constant by k_{obs} and the intercept by a. Plots of the ln function against time were indeed linear with small intercepts. A typical graph first order rate plot is shown in figure 1.

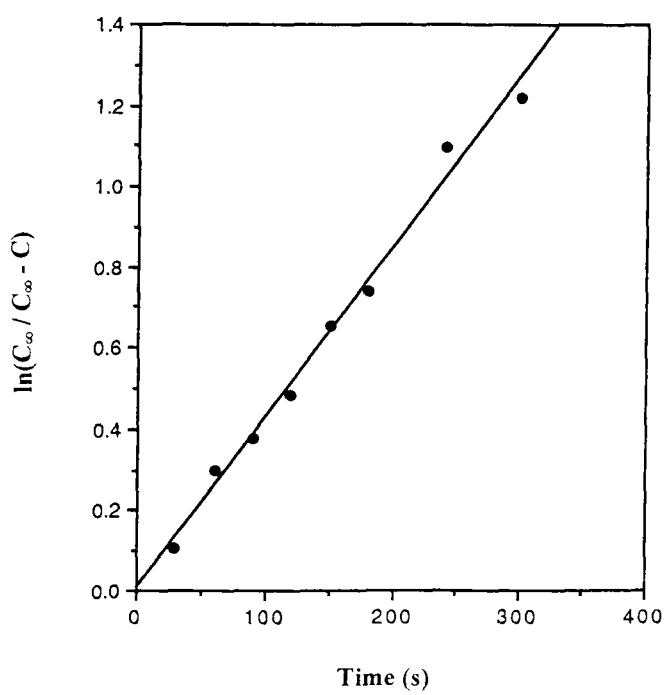

Figure 1 First order rate plot for infusion of potassium from medium roasted Mocha coffee (Ethiopia)

The mean rate constants obtained from the slope of the graphs of equation (1) and the half-life values $t_{1/2}$ of infusion determined from equation (2)

$$t_{1/2} = (\ln 2 - a) / k_{obs} \qquad (2)$$

are listed in Table 1. Each result is based on at least three independent experiments whose results agreed satisfactorily.

Table 1 Mean kinetic data for the infusion of caffeine and mineral ions from different types of coffees (1.70-2.00 mm) into Milli-Q-Water at 80°C

Type of Coffee (Country)	Species	C_∞ (ppm)	C_∞ (mM)	k_{obs} ($10^{-3}s^{-1}$)	Mean intercept a	$t_{1/2}$
Special Kenya (Arabica)	Caffeine	262.6	1.35	2.71	0.11	215
	K^+	353.9	9.69	3.50	0.14	176
	P as ($H_2PO_4^-$)	26.6	0.86	1.89	0.14	293
	Mg^{2+}	27.5	1.13	1.16	0.14	477
	Mn^{2+}	0.37	0.06	0.98	0.09	615
Santos (Brazil)	Caffeine	247.0	1.27	3.59	0.08	171
	K^+	319.5	8.40	3.95	0.11	135
	P as ($H_2PO_4^-$)	25.2	0.81	2.42	0.13	233
	Mg^{2+}	23.6	0.97	1.55	0.12	370
	Mn^{2+}	0.33	0.06	0.98	0.16	544
Blue Mountain Java (Sumatra)	Caffeine	284.0	1.46	3.37	0.11	173
	K^+	374.9	10.3	4.23	0.06	150
	P as ($H_2PO_4^-$)	24.3	0.78	2.13	0.13	264
	Mg^{2+}	26.4	1.08	1.25	0.16	427
	Mn^{2+}	0.36	0.06	0.93	0.20	530
Zimbabwe (Zimbabwe)	Caffeine	282.0	1.45	3.42	0.14	162
	K^+	182.8	5.01	5.66	0.25	78
	P as ($H_2PO_4^-$)	21.1	0.68	2.23	0.17	235
	Mg^{2+}	25.6	1.05	1.24	0.91	406
	Mn^{2+}	0.40	0.07	0.95	0.13	593
Mocha (Ethiopia)	Caffeine	297.6	1.53	2.72	0.12	211
	K^+	334.5	9.16	3.08	0.13	183
	P as ($H_2PO_4^-$)	26.5	0.86	1.96	0.13	287
	Mg^{2+}	26.4	1.08	1.34	0.09	450
	Mn^{2+}	0.26	0.05	1.04	0.14	532

Kinetic and equilibrium data

The equilibrium concentrations were found to be independent of the origin of the coffee beans. The equilibrium amount of potassium leached from the beans was the highest, followed by caffeine. The values for Phosphorus and magnesium were next with similar magnitude while manganese had the least. This trend was the same in all the coffees investigated. The difference in equilibrium concentration of all the individual species in various coffees was 10% between the highest and the lowest. The only inconsistencies were with potassium and phosphorous concentrations from the Zimbabwean coffee.

Since kinetic study on Kenyan coffee has been carried out, the rate constant obtained with the Special-Kenyan beans will be examined first. The k_{obs} obtained was 2.74×10^{-3} s^{-1}

The literature value by Spiro and Selwood (1984) is 9.0×10^{-3} s^{-1} for particle size 0.85-1.18 mm at 84.1°C. This is equivalent to a rate constant of 2.68×10^{-3} s^{-1} taking into account the present experimental conditions of particle size and temperature. This is because the rate of infusion varies inversely with the square of the particle radius (Spiro and Selwood, 1984: Spiro 1988). To account for temperature difference, Arrhenius equation is used since ln k_{obs} is inversely related to temperature. The agreement with the results tabulated in Table 1 is very good. Inspection of the results in Table 1 shows that the order of infusion is K^+ > caffeine > P as $(H_2PO_4^-)$ > Mg^{2+} > Mn^{2+}. This order is true for all the coffees.

Diffusion coefficients and hindrance factors:

The diffusion coefficient was calculated in two different ways. The first calculation made use of Steady-state theory equation (3)

$$k_{obs} = 12\, D_{bean}/r^2 \qquad (3)$$

where r and D_{bean} are the radius of the bean particle and diffusion coefficient of the species respectively. The second value was determined by use of half-life values shown in table 1 and applying equation (4) (Spiro et al, 1989), which is a solution of Fick's second law of diffusion (Crank, 1975).

$$1/t_{1/2} = 32.7\, D_{bean}/r^2 \qquad (4)$$

The values due to Fick's Law were lower than those obtained using Steady-state. An average value Mean D_{bean} was used in calculating the hindrance factor HF from equation (5).

$$HF = D_{aq} / D_{bean} \qquad (5)$$

where D_{aq} is the diffusion coefficients of the species in aqueous media. Price and coworkers (1989) have determined the aqueous data for caffeine at 80°C. Spiro and Lam (1995) have tabulated in Table 4, the values for K^+, Mg^{2+} and P as $(H_2PO_4^-)$. Assuming that the major orthophosphate ion analysed as phosphorus was $H_2PO_4^-$ since the average pH of the coffee liquor was 4.5, Nernst equation.

$$D_{aq} = RT\lambda^\circ / |Z| F^2 \qquad (6)$$

was used to determine the limiting tracer diffusion coefficient for Mn^{2+} at 80°C. The symbols R, T, λ. Z and F in equation (6) represents gas constant, temperature in Kelvin, limiting equivalent conductance, ionic charge number and Faraday constant respectively. Since the limiting equivalent conductance for Mn^{2+} is only available at 25°C (CRC 1991-1992), the Walden rule

$$\lambda^\circ \eta = \text{constant} \qquad (7)$$

was used to determine the value of λ° for Mn^{2+}. The values for viscosity (η) of water were taken from Robinson and Stokes (1959). The diffusion coefficient and the resulting hindrance factors HF are listed in Table 2.

Table 2 Effective diffusion coefficients and Hindrance factors of caffeine and mineral ions for various types of coffees (1.70-2.00 mm) at 80°C

Type of Coffee	Species	D_{bean} (from k_{obs}) ($10^{-11}m^2s^{-1}$)	D_{bean} (from $t_{1/2}$) ($10^{-11}m^2s^{-1}$)	Mean D_{bean} ($10^{-11}m^2s^{-1}$)	D_{aq} ($10^{-9}m^2s^{-1}$)	HF
Special Kenya (Arabica)	Caffeine	19.3	12.2	15.8	2.2	14
	K^+	22.5	14.9	18.7	5.0	27
	P as ($H_2PO_4^-$)	13.5	8.9	11.2	2.6	23
	Mg^{2+}	8.3	5.5	6.9	2.0	29
	Mn^{2+}	7.0	4.3	5.7	2.1	37
Santos (Arabica)	Caffeine	25.6	15.3	20.5	2.2	11
	K^+	30.6	19.2	24.9	5.0	20
	P as ($H_2PO_4^-$)	17.3	11.2	14.3	2.6	18
	Mg^{2+}	11.1	7.1	9.1	2.0	22
	Mn^{2+}	7.0	4.8	5.9	2.1	36
Blue Mountain Java (Arabica)	Caffeine	24.0	15.1	19.6	2.2	11
	K^+	30.2	17.4	23.8	5.0	21
	P as ($H_2PO_4^-$)	15.2	9.9	12.6	2.6	21
	Mg^{2+}	8.9	6.1	7.5	2.0	27
	Mn^{2+}	6.6	4.9	5.8	2.1	36
Zimbabwe (Med-Arabica)	Caffeine	24.4	16.2	20.3	2.2	11
	K^+	40.4	33.5	37.0	5.0	20
	P as ($H_2PO_4^-$)	15.9	11.1	13.5	2.6	19
	Mg^{2+}	8.8	6.4	7.6	2.0	26
	Mn^{2+}	6.8	4.4	5.6	2.1	38
Mocha (Robusta)	Caffeine	19.4	12.4	15.9	2.2	14
	K^+	22.0	14.3	18.2	5.0	27
	P as ($H_2PO_4^-$)	14.0	9.1	11.6	2.6	22
	Mg^{2+}	9.6	5.8	7.7	2.0	26
	Mn^{2+}	7.4	4.9	6.2	2.1	34

Analysing the HF values, Mn^{2+} is the most hindered species in all the coffees. Its diffusion being approximately 3.5 to 4 times slower than caffeine, which is the least hindered of the species investigated. The hydrodynamic radii in water for Mn^{2+}, Mg^{2+} and caffeine are very similar as indicated by their D_{aq} values, but their HF values are very different. The value of Mg^{2+} being approximately half-way between the other two. Even though some of the HF values for a particular species differ for different coffees, their magnitude remain very similar.

Combining the outcome of Table 1 and Table 2, it is clear that the diffusion of caffeine and mineral ions within the coffee bean particle is a hindered process. The comparison of the HF values for caffeine, Mg^{2+} and Mn^{2+} clearly indicates that one of the causes of slower diffusion is the association between the species and insoluble components of the bean.

Another explanation put forward to explain this process is the complex formation between the dissolved species and other solubles to create a more bulky and slower-moving entity. One of the compounds suspected to fall into

this group are caffeine-potassium chlorogenate molecular complex (Haslam, et al, 1987, Horman and Viani, 1972, Spiro, et al, 1984, 1989) and caffeine self-association. Spiro (1997) has demonstrated that the slow infusion of caffeine is not due to its self-association. The potassium cholorogenate complex has been isolated as a 1:1 complex with caffeine. The equilibrium concentration in Table 1 show that the ratio of caffeine to potassium is 1:7. This indicates that potassium in the coffee bean possibly, exists in more than one form. The other possible explanation is that the complex ionises and free potassium diffuses out of the bean as a single specie. These ideas are clearly reflected in the large rate constant and hindrance factor for potassium in comparison to caffeine. In addition to the above explanation one factor which is believed to be the main cause of slow diffusion is the physical restraints within the bean matrix. This forces the solubles to follow a diffusion path which is tortuous in nature.

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SUMMARY

The rates of infusion of caffeine, $H_2PO_4^-$, K^+, Mg^{2+} and Mn^{2+} into Milli-Q-water were measured at 80°C. Five different types of medium roasted coffees; Special Kenya (Kenya), Santos (Brazil), Blue mountain Java (Sumatra-Dutch East Indies), Mocha (Ethiopia) and Zimbabwe (Zimbabwe) in the particle size range 1.70-2.00 mm were used in the investigation. The analytical techniques employed were HPLC and ICP-AES. Diffusion coefficients of caffeine and mineral ions were calculated in two separate ways using rate constants and half lives of infusion. These were then compared with known diffusion coefficients of the same species in water for determination of hindrance factors. The hindrance factor for caffeine was found to be much smaller than the corresponding factors recorded at 25.5°C (Spiro and Chong 1977). In general the hindrance factors in the bean were all in the order of 10. Indicating that the infusion of the various species through the coffee bean is a hindered process. This is because of the association of caffeine and mineral ions with other coffee soluble, and the physical restraints within the bean matrix. The equilibrium concentration of the different species was found to be independent of the various coffees.

ELECTRONIC NOSES : STATE OF THE ART AND IMPLICATIONS FOR THE COFFEE INDUSTRIES

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ABSTRACT

There is an increasing interest in gas sensors to analyze complex gas mixtures and odors. The required performance in most applications can only be achieved by increasing the amount of chemically "'orthogonal" information deduced from a sensor system. The evident need to increase the information requires to increase the number of independently determined features in such sensor systems. This can be accomplished by building a large sensor feature space by three different concepts, i.e.,

- several sensor material/one transducer approach,
- several transducers/one sensors materials approach , and
- modulated parameter approach.

Subsequently, the pattern recognition has to be optimized to characterize the feature space.
These concepts are described briefly. To make use of these concepts in a commercial instrument suitable for a broad spectrum of applications, modularity is of key importance. In this context, a new most flexible design of a modular sensor system ("MOSES") is introduced which includes all these options by extending the feature space as large as needed for specific practical applications of gas or odor analyses. The choice of a sufficiently large number of modular components with their individual sensor elements and modes of operation leads to a sufficiently large number of features which in the final feature extraction and pattern recognition yields orthogonal chemical- (or odor-) relevant parameters.

Results of an analysis with MOSES may either be referenced to parameters obtained from classical tools of analytical chemistry (such as GC/MS couplings) or from human odor panels. Examples from the food industry and quality control (and, in particular from first applications in the coffee industries) demonstrate, that even complex gas mixtures and complex odors may be analyzed with this modular system.

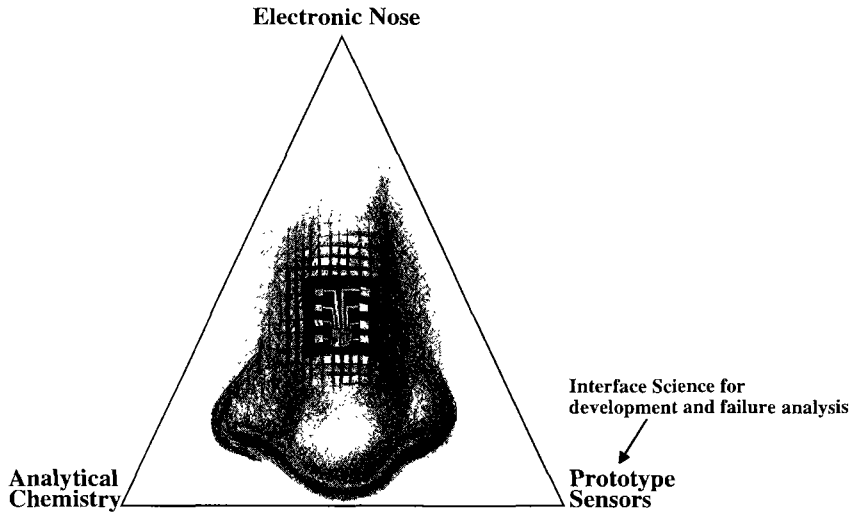

Fig.1: The concept of electronic noses in the context of analytical chemistry and sensor research and development.

1. Introduction

Chemical and biochemical sensors with their broad spectrum of applications aim in particular at environmental monitoring, process control, and quality analysis [1-3]. Several commercial instruments are already available which typically make use of one of the various possible signal transduction principles[4]. In this context even the well-established gas sensors to be discussed in this paper are known to have insufficient performances for most applications if compared with the established instruments of analytical chemistry (like GC/MS) because such sensors are usually not totally selective, exhibit certain sensitivities only towards classes of molecules, show drifts and in many cases are not sensitive enough. Therefore electronic noses are developed now by combining several sensors and transducers (Fig. 1) [5]. In the future these systems may also complement the kind of information traditionally obtained in Analytical Chemistry, e.g., by characterizing odor sensation or monitoring toxicity.

Considering the broad spectrum of transducers and of chemically sensitive materials which may be used in principle in such "electronic noses", two classes of materials appear to be most promising for future chemical sensor elements:

- Metal oxides with electron, ion or mixed conduction[6, 7], and
- polymers or supramolecular structures with systematically varied recognition sites[8, 9].

Many research groups develop new materials and transducers with particular emphasis on optimizing interface properties between the gas phase, the sensitive material and the transducer. By comparing the performance of their prototype devices with existing sensors or transducers on the market, they try to find out principal advantages of new materials and transducer designs for new future applications. The necessary quantitative comparison with components of existing complete sensor systems requires standardized experimental setups and test procedures. This includes the sample uptake, feature extraction, and pattern recognition procedures. Standardization, illustrated schematically in Fig. 2, was the main criterion in the design of a new modular sensor (MOSES) system to be discussed in chapter 3.

Basic concepts for systematically extending the feature space in chemical sensor systems are outlined in chapter 2 before the modularity concept of MOSES is introduced.

2. Extending the Feature Space in Chemical Sensing: Concepts and Typical Examples

The traditional use of chemical sensors focuses at the output of one individual chemical sensor with only one sensor signal ("feature"). The sensor with its chemically sensitive layer and transducer is exposed to the analyte molecule. Often a filter is used to prevent contamination and to reduce cross sensitivity. The filter may either be integrated or added as a separate component. The chemical information concerning the analyte concentration is

converted by the transducer via the chemically sensitive layer into an electrical signal and yields a "feature". The desired chemical information is then obtained in a comparison of this feature with calibration data. The latter are obtained by monitoring independently calibration points of gases with known composition and implementing the calibration function, e.g., in analog electronic circuitry.
Obviously this setup delivers only one single value, i.e. one concentration. Whenever more information is desired, a more complex setup including several sensors or different modes of sensor operation is required. This increases the number of independent features for a more complex gas analysis. In this context, the term "feature" is defined as one quasi-independent variable determined from the measuring setup. The most important reason for increasing the dimensions of the feature space is the fact that no gas sensor is totally selective. If species other than the analyte to be detected are present, cross sensitivities influence the reading of the instrument. Determining a feature space with sufficient dimension and evaluating the data by means of pattern recognition allows in principle to eliminate the influence of cross sensitivities.

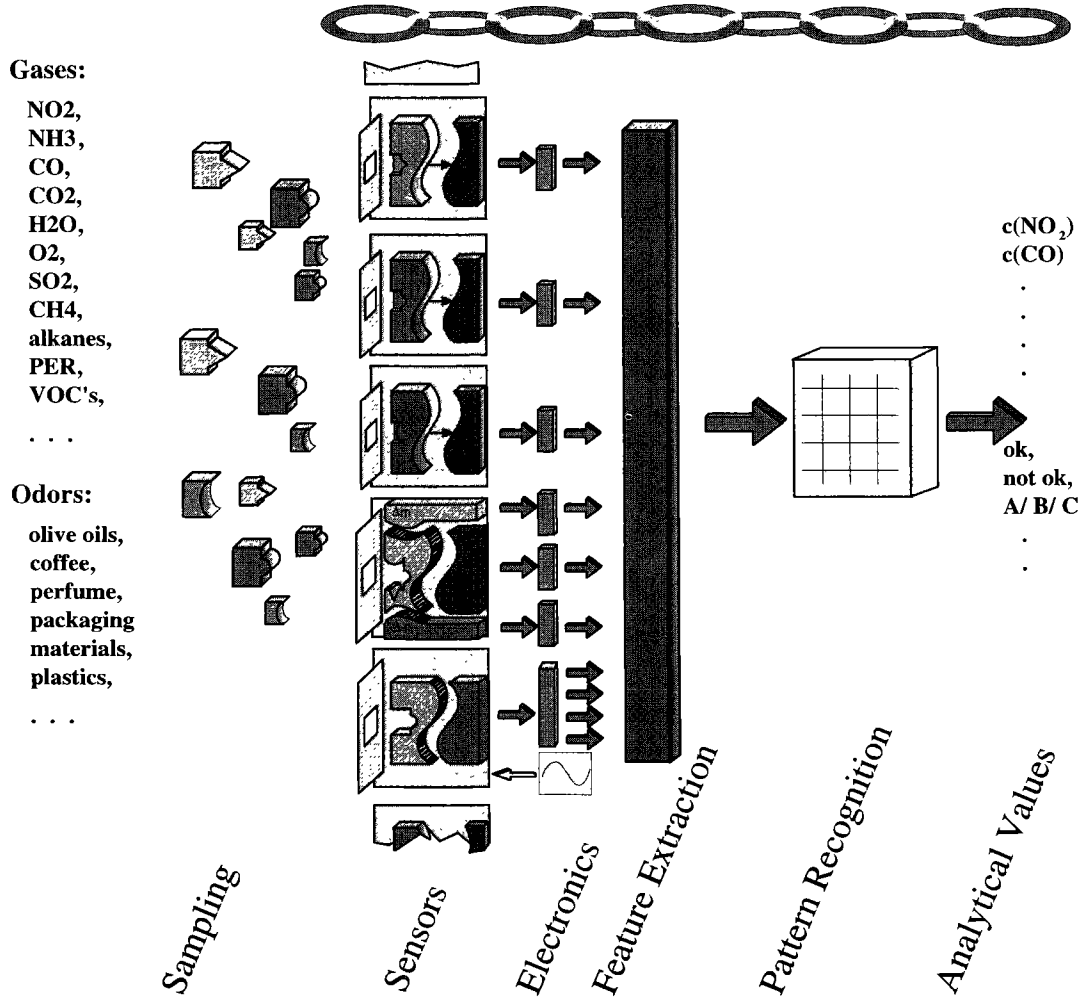

Fig. 2: Schematic set-up of the electronic nose introduced here, i.e., the modular sensor system MOSES for gas and odor analysis. Sensors consists of chemically sensitive materials (with their recognition sites) and of transducers (to allow the transfer from chemical to electronic information).

2.1 Several sensor materials / one transducer approach

To achieve a discrimination between different analytes, several gas sensors are often combined to form an array. By modifying the properties of each sensitive layer, these sensors with their partly overlapping sensitivities

generate independent features with the same type of transducer. The signals of these sensors are recorded simultaneously. Instead of a simple calibration function a multicomponent analysis or pattern recognition is used to obtain the desired analytical information [4, 10, 11]. In most cases this data evaluation is performed by a computer or microcontroller.

In this context, different polymers and supramolecular compounds have been used as sensitive materials to, e.g., detect organic volatiles (VOC`s). These materials offer many options for chemical modifications and hence a huge flexibility in tailoring molecular recognition sites by controlled organic synthesis [8, 12-14]. By attaching, for example, specific functional groups with different chemical properties to the backbone of a polymer, the sensitivity for the detection of certain analyte molecules is enhanced.

The cyano-group of poly(cyanopropyl)methylsiloxane as a specific example introduces a higher polarity to the polymer, polarizabilty is introduced by adding phenyl-groups (e.g. in poly(phenyl)methylsiloxane). This enhances specific selectivities as to be seen in Fig.3. The 3 different polymers shown here span a three-dimensional vector space. The quartz crystal microbalance (QCM) results of three sensors determine a vector which increases in length proportional to the gas concentration and which after normalization to a unit length determines a substance-specific direction in Fig.3.

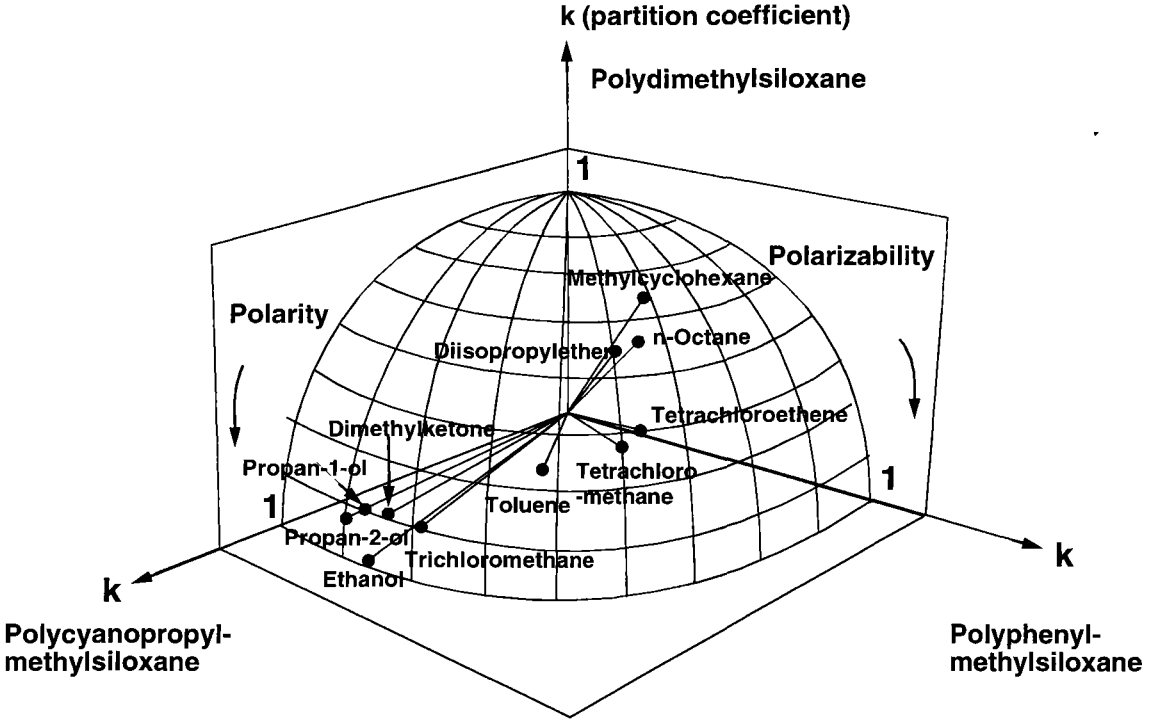

Fig. 3: Different analyte modules monitored with three different polymer-coated QCM`s. For details, see text.

To illustrate the selectivity and long-term stability of this type of materials combined with this type of transducer, QCM results of an array with 6 different polymer coatings, i.e.,the quantitative analysis of organic volatiles in air was investigated systematically over long times. At the first day, the calibration data set was determined. Even after more than 200 days the concentrations of the test mixtures could be predicted with errors below 5% by using the same calibration data set without any recalibration [15].

The high selectivity of specific interaction sites in modified polymers becomes of increasing interest in current sensor work. In this context it was for the first time possible to perform a quantitative chiral discrimination with polymer gas sensors. Chiral groups in the side-chain of this polymer provide enantioselective coatings. By using an array of R-, S- and nonchiral polymer phase coatings, it was possible to discriminate between the R- and the S-analyte and to even determine the composition of racemic mixtures to within a few percent of error[16]. This result is of particular interest since our human nose is known to differentiate between certain enantiomers (like spearmint and caraway attributed to R- and S- carvon, respectively).

2.2 Several transducers/one sensor material approach

An alternative approach is the recording of several independent parameters of the same sensor coating. The simplest option is to use different separate transducers for recording several independent physical properties of one sensitive layer.

As an example Haug et al. [17] used 4 different transducers to measure changes in the optical thickness Δd, mass Δm, temperature ΔT and capacity ΔC of a polymer layer upon the incorporation of organic volatiles.

In the next degree of sophistication, the different transducer principles may be integrated in one design. As an example, mass changes and changes in the conductance of a conducting polymer have been monitored with a special electrode arrangement on a quartz resonator [18].

2.3 Modulated-parameter approach

An often applied method to increase the feature space of chemical information is the use of a specific modulation either in the operation conditions of the sensor [19-25], or in the gas composition [26, 27].

- Modulation of the gas composition can be achieved by, e.g., switching between a reference gas and the gas to be analyzed by using filters or catalysts to change the gas composition. The dynamic response of the sensor due to these changes can then be evaluated.
- Modulation of the operation conditions of the sensor itself can be achieved in many ways and usually depend on the chosen transducer. To enhance the feature space, metal oxide gas sensors, for example, may be used in a broad range of operating conditions by varying the operating temperature and the potential of the measuring electrodes (besides using the options for systematic modifications of their transducers, e.g. the contact arrangements, and of their electron conducting materials like SnO_2 including their nanocrystallinity, dopants, etc.[26]).
- Fig.4 shows a schematic of typical reactions taking place at the surface of the metal oxide and an equivalent circuit which formally describes the contributions from the surface, volume, contact, and grain boundaries to the overall electrical characteristics of the sensor. By using an AC-modulated working potentials of the sensor it is possible to enhance the performance of the sensor, if the operating frequency is chosen to enhance a specific contribution to the equivalent circuit. Measuring at different frequencies leads to detailed features for the subsequent pattern recognition[19].

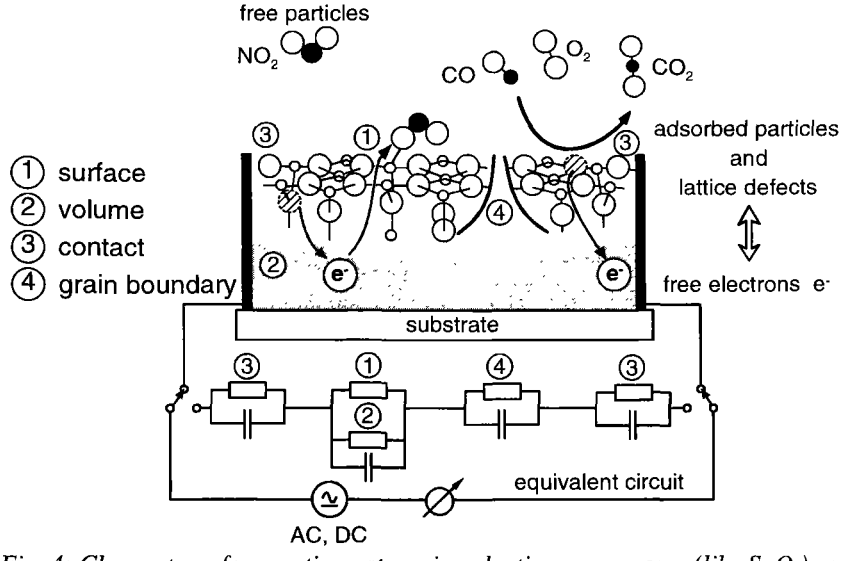

Fig. 4: Charge transfer reactions at semiconducting gas sensors (like SnO_2) and corresponding equivalent circuit describing frequency dependent features[16].

It is well known, that the sensitivity of a metal oxide sensor also depends critically on its working temperature. By modulating the temperature it is possible to discriminate between different analytes. To allow for fast changes in the temperature, microstructured transducers have to be chosen with their low thermal inertia [22].

Using Fast Fourier Transformation (FFT) and subsequent neural network analysis of the resulting changes in the resistance of the metal oxide it was possible to determine concentrations of gas mixtures of NO_2 and CO in air with only one sensor [22].

2.4 Modular system approach (MOSES)

The examples described above indicate, that the feature space may easily be extended to a huge number of independent features. The latter are generated by varying the sensor materials, the transducer principles and the mode of operation for each sensor/transducer combination. Because of the large number of possible variations in a complete sensor system, all of which determine the overall system's performance, a modular experimental setup offers the best flexibility for optimizing the choice of these features by optimizing each individual component of the system (the "links" in the chain in Fig. 2) for specific applications. This is the basic design principle of the multiparameter modular sensor system MOSES to be described in the next chapter.
Besides its practical applications, the system also provides an ideal tool to perform basic research in the fields of new improved materials for chemical sensing, molecular recognition and odor perception. This aspect is essential also for introducing improved electronic noses in the real world for the following reason: It should be emphasized clearly, that the weakest link in the total chain of a sensor system in all practical applications is the chemically sensitive coating. It must allow for completely reversible chemical reactions to the outer world (the gas phase) and completely irreversible coupling to the inner electronics world (the transducer). The optimization of properties of these two different types of interfaces can only be achieved by systematically varying materials and preparation procedures ("empirical approach") and/or by analyzing interfaces down to the atomic scale with the well-established tools of interface analysis ("systematic approach" see also Fig. 1). To achieve quick R&D times, both approaches for optimizing sensors must be chosen in parallel: A variety of recent examples illustrate, that only the combined empirical approach and systematic research make it possible to significantly reduce the traditionally large development times for chemical sensors and transducers. It should be kept in mind, that in the past the development times for new reliable chemical sensors were in the order of 30 years: A classical example is the ZrO_2-based oxygen λ-probe. Such times are unrealistic for present-day improvements of electronic noses.

3. Instrumentation of Modular Sensor Systems (MOSES)

A general sensor system for gas and odor analysis consists of four main parts with corresponding interfaces:
1. analyte sampling,
2. sensor modules and electronics,
3. signal recording, and
4. external data processing.

In the MOSES system all parts of the system are exchangeable without interfering with the other parts. This is achieved by standardizing all interfaces (see Fig.5). The standardization concerns the interface 1 between the different sampling units and the different sensor chambers (gas connections), the electrical interface 2 between the different sensor modules and the data evaluation unit ("MOSES module bus"), and the interface 3 to link the data evaluation and visualization units (inside the software).

3.1 Gas connections (interface 1):

The gas to be analyzed is guided from the sampling unit (from the headspace sampler, purge and trap unit, ...) through the system from one module to the next in a serial or parallel flow arrangement.

3.2 MOSES-Module-Bus and system controller (interface 2):

The system comprises different transducer principles, which determine different types of modules in the "hybrid" modular system. Each sensor module consists of a measurement chamber with a sensor/transducer unit, a printed circuit board for the sensor electronics, and the microcontroller or digital signal processor (DSP). Several sensor modules are already available (see section 3.4.), others like surface acoustic wave (SAW), electrochemical, optical and high temperature solid electrolyte modules are under development.

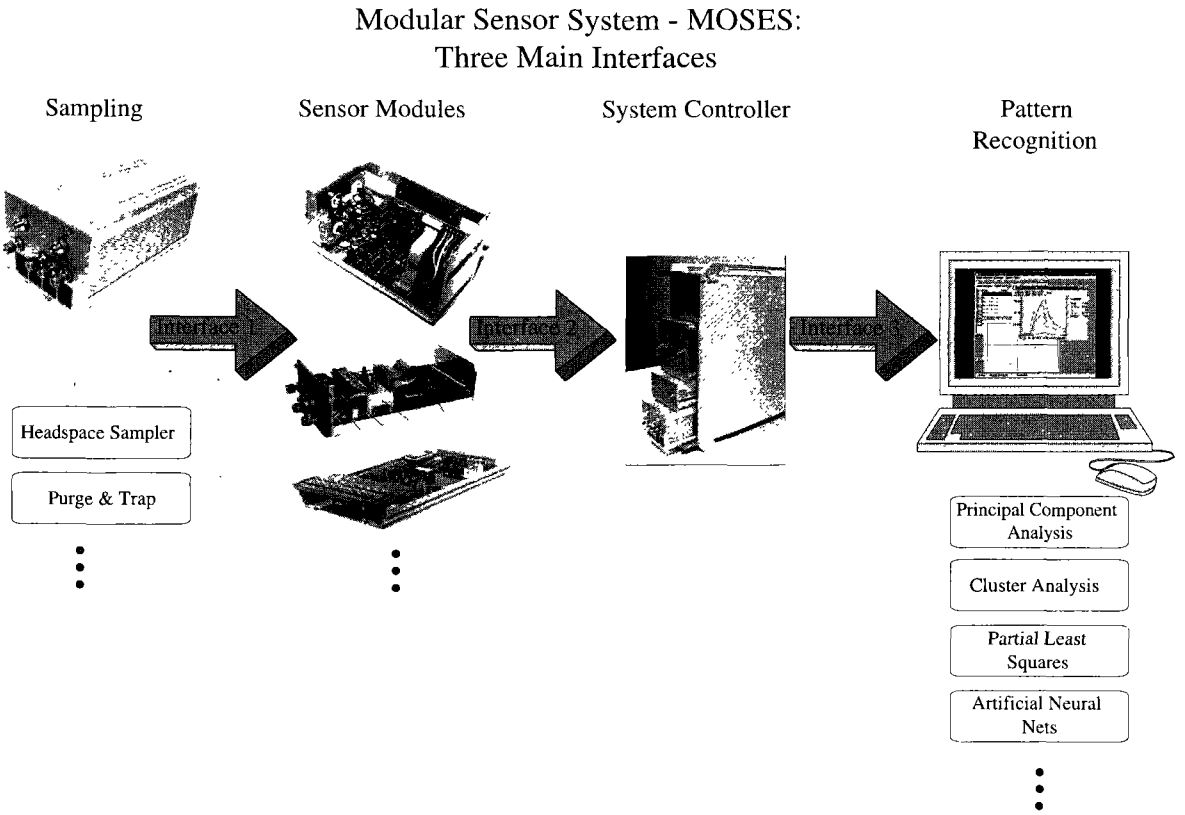

Fig.5: Basic modules of the MOSES system.

The total system may easily be extended by adding new modules with their new transducers, since the interface between the individual modules and the data evaluation part allows to transmit specific control and measurement signals in both directions from and to the individual modules in standardized form.
This was achieved by using a bi-directional, addressable, digital bus system and intelligent modules. Each module is equipped with a dedicated microprocessor and/or digital signal processor (DSP) to perform the necessary control of the module and to perform the measurements by using a specially designed circuitry for each sensor. On the digital bus system all modules are controlled using standardized parameters and signal transmission. The microprocessors onboard the modules receive commands via the digital bus. This makes it possible to also address special modules with their individual pumps, valves, filters, catalysts or general I/O to enhance the feature space analysis. All modules reside in standard racks with the digital bus connector on the rear side. They can be easily installed and removed from the MOSES system.
The bus is controlled by a system controller(i386), which performs all the time-critical commands. Additionally a dedicated sync signal line is used on the bus to assure that measurements are taken at exact time intervals.

3.3. External data processing (interface 3):

The controller converts all messages on the bus and sends the filtered data to an external personal computer via standard RS232. For stand-alone applications the controller can be programmed with a c-similar script language. As the controller is based on a standard PC 104 computer running with MS-DOS it can be extended by hard disks, floppy disks, a display, CAN, Ethernet or other interfaces.

3.4 Modules

3.4.1 Input Module:

Since sampling is a critical step in any gas and odor analysis, a variety of sampling devices optimized for the use in gas chromatography may easily be integrated into the MOSES system.

Headspace autosamplers are often used for comparative studies, e.g., of samples from the food and packaging industry. Headspace samplers like the Hewlett Packard HP 7694 are supported by the MOSES software.
For samples with very low concentrations, purge and trap units like the Hewlett Packard HP 7695 are utilized.
For on-line applications a dedicated Input Module has been developed.
This input module is equipped with a gas pump, mass flow controller, temperature and humidity sensor and an input valve array for the selection of the gas samples (with three inputs, e.g. for reference-, test- and purge-gas). For on-line control the system may sample air by using the pump and the flow controller. The gas flow can be controlled from 0 to 50 ml/min. In applications where the sample air is delivered to the system from outside (e.g. when connected to a headspace sampler) the pump is bypassed and the flow controller is used to measure the gas flow.

3.4.1 Quartz Crystal Microbalance (QCM) Module:

In the QCM measurement chamber eight quartz crystals are used as mass sensitive transducers. The quartzes are operated as thickness shear mode resonators "TSMR" at a fundamental frequency of 30 MHz.
The sensor array is mounted inside a 3 ml stainless steel chamber and thermostated by a Peltier element. The chamber can be bypassed using two valves.
In the standard configuration of MOSES, a set of 8 selected polymers was chosen which shows optimum stability and "chemical orthogonality" for a broad spectrum of common applications. In our lab, new materials are continuously synthesized and tested for their use as QCM coatings. This includes in particular the use of polymers known to form stable coatings in gas chromatography, of polymers with incorporated specific adsorption sites of supramolecular monolayers, cyclopeptides prepared by combinatorical chemistry, etc.

Provided that a careful selection of polymer coatings is made (see, e.g. Fig. 3) the advantages of these QCM sensors are their good reproducibility, long term stability and long lifetime. Another additional advantage especially in applications where the sample could be influenced by oxidation, is the possibility to use inert carrier gas like nitrogen without significant changes in the base line. This is in contrast to SnO_2-based sensors. In contrast to the use of conducting polymers in chemical sensing, drift effects and water cross sensitivity can be neglected or easily corrected for.
Adjustable parameters of the quartz module are chamber temperature and gate time of the frequency counters. By changing the gate time the frequency resolution of the measurement can be adjusted. Three gate times are possible: 0.1 sec (resolution 10Hz), 1 sec (resolution 1Hz) and 10 sec (resolution 0.1Hz).

3.4.2 Metal Oxide Module:

This module is equipped with eight different metal oxide sensors. However any other sensor-active material or transducer type with changing resistance from 100Ω to 50MΩ may also be measured in this module. In the simplest case, the DC resistance is measured either at constant potential or at constant current. For each sensor in this module the heater voltage and the measuring potential as well as it`s polarity may be adjusted individually. All these parameters may be changed dynamically during the measurement using the script language of the system controller. This leads to a very broad range of modes of operation which can be exploited for either systematic research of the sensor behavior or for optimizing the performance in a particular applications.
In the standard configuration 8 metal oxide sensors were selected from several commercial suppliers to optimize chemical orthogonality in their responses. Alternatively, our own microstructured oxide sensors have been used, which at least in the present test stage show better sensitivities and selectivities than commercial ones[29]. Their long-term stabilities are checked now under conditions of permanent use. All sensors can easily be exchanged. Thereby almost every commercially available or self-made conductivity sensor can be used within this module.
Provided that a careful selection of metal oxides, their doping, contact geometry, working temperature and mode of operation is made, the advantages of metal oxide sensors are their high sensitivities and long lifetime. This has led to a broad variety of already available commercial or to new laboratory type products.

3.4.3 Calorimetric Module:

The calorimetric consists of an array of 8 microstructured calorimetric sensors and a complex sampling arrangement. An onboard digital signal processor (DSP) evaluates the sensor responses during gas changes between analyte and inert gas [30, 31].

Calorimetric transducers measure the temperature change in the sensitive layer due to the ad- and absorption or due to the desorption of analyte molecules. The change of the temperature is therefore related to changes in the concentration of the analyte in the gas phase. Several operating conditions are possible with this module. To measure the absolute concentration it is necessary to switch between a reference gas and the analyte gas. If catalytically active coatings are chosen like in the "classical pellistor" setup, a continuous flow operation is possible.
A variety of feature extraction algorithms can be performed by the digital signal processor onboard this module. The sensors may be coated with the same sensitive layers as the quartz module. This makes it possible to examine different properties of the same sensing layer in one array, since masses and energies usually change differently during the molecular recognition of different molecules.

3.5 Software

Processing of the acquired information can be performed at three different stages:

- The microcontrollers or digital signal processors onboard the modules are normally used for data preprocessing (e.g. for noise reduction) and evaluation of very fast signals(e.g. fast fourier transform of modulated signals).
- The controller is used to control the timing and to collect the data from all modules.
- The external computer extracts the features from the incoming data stream for the subsequent pattern recognition, performs the pattern recognition itself and displays and stores the results.

For the subsequent pattern recognition the principal component analysis (PCA) is the preferably applied algorithm and hence fully integrated into the software. Due to it's modular, object oriented design, the software can easily be extended by new pattern recognition techniques. Linear discriminant analysis (LDA) and neural nets may also be integrated. As the software is running under Windows 95 a broad variety of pattern recognition, statistical methods, data visualization tools can be applied by using standard software interfaces to external programs.

4. Typical Application Areas and Selected Examples for Electronic Nose Analysis

The main goal in the development of the MOSES system was to create a flexible system that can be adapted easily to the different basic research tasks and to the different application areas of chemical gas sensors. Applications areas in which the MOSES systems has already been tested are shown in Fig. 6:

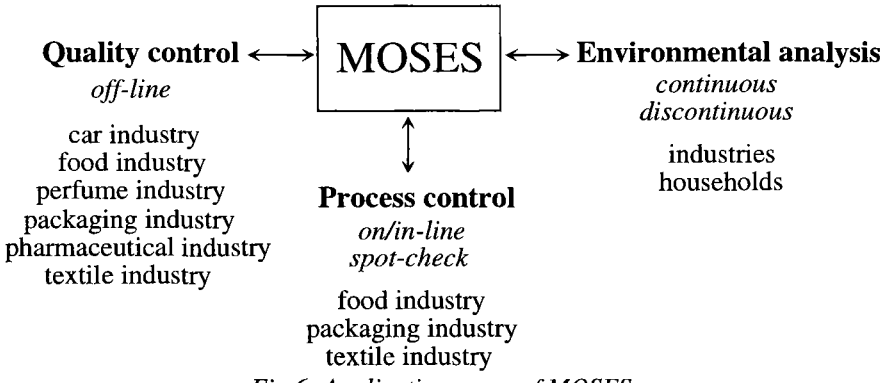

Fig 6: Application areas of MOSES.

The MOSES system is particularly suitable for quality control with the need of quantitative gas mixture analysis or odor characterization. Excellent reproducibility of results obtained with the quartz and a metal oxide module over a period of more than several months could already be demonstrated for a variety of case studies. For details, see earlier papers [32,33] and http://www.ipc.uni-tuebingen.de/moses.
Particular details on the quality control of packaging materials in the food industry and the identification of olive oils have been published in [32].

Typical results on analyzing different coffee brands are shown in Fig.7. A cluster analysis of Robusta, Columbia and Santo`s beans is stated in Fig.8. The discrimination of coffee mixtures with different levels of

coffee brands is illustrated in Fig.9. It should be pointed out, that the qualitiy of an identification is termined by the reproducibility of measurements within one cluster on the one hand and the distance between the different clusters and the principal component plots. An excellent reproducibility over several months could be achieved.

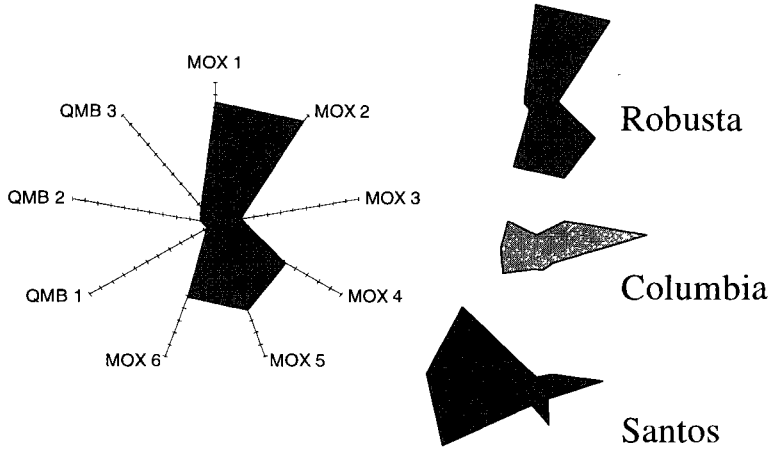

Fig 7: Characteristic patterns for Robusta, Columbia and Santos beans. The polar plots are obtained with a selection of 6 different metal oxide and 3 QMB sensors.

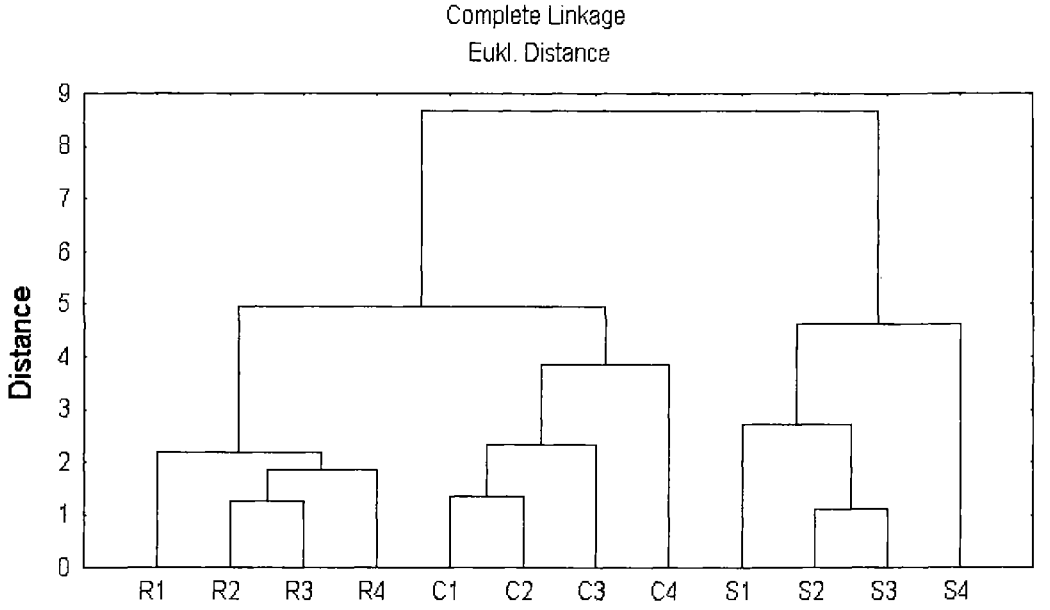

Fig.8: Cluster analysis of Robusta, Columbia and Santos beans. For each brand 4 measurements are performed. The different brands are well discriminated. A larger distance occurs between the Santos and the other two samples. This implies a larger difference in the headspace composition of the Santos beans as compared to Robusta and Columbia.

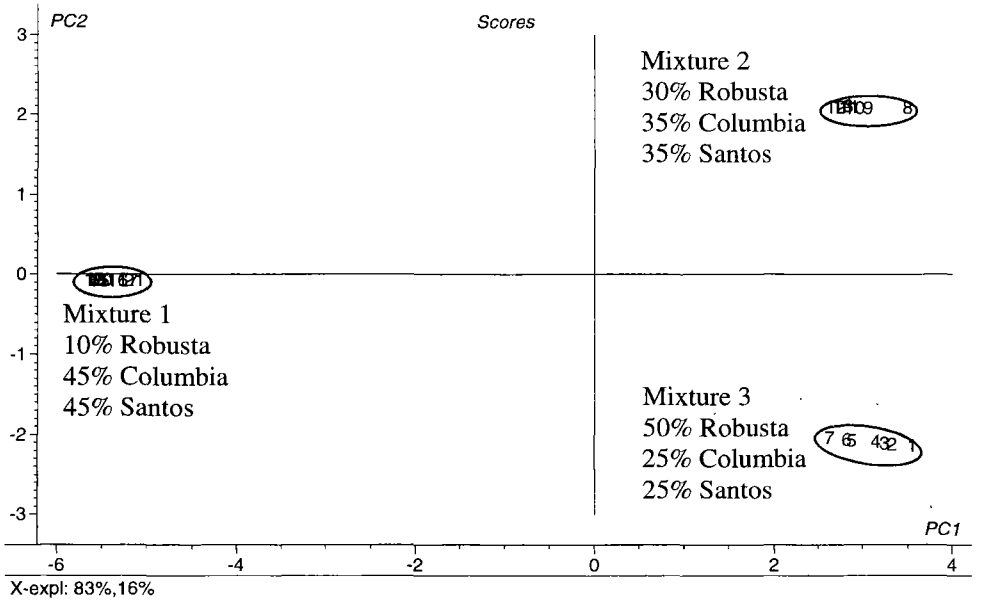

Fig.9: Discrimination of coffee mixtures with different levels of coffee brands. The mixtures consist of Robusta, Columbia and Santos coffee (grinded beans). The amount of Robusta increases from 10% to 50%. For each mixture seven measurements are performed. A clear separation of the different mixtures occurs for the different headspace compositions. Here, 8 QMB as well as 8 metal oxide sensors were used.

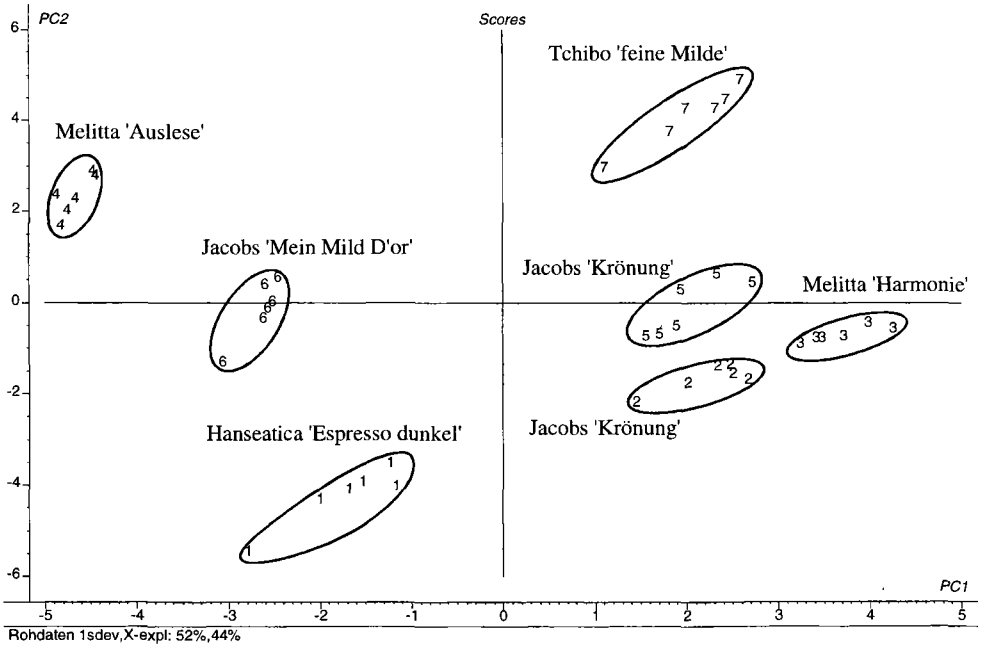

Fig. 10: Discrimination of 7 different commercial coffee brands. There is a clear separationand only a small scattering within the brands. For one type of brand (Jacobs 'Krönung') we measured two packages from different production dates. Although the same brands are separated they are in the same area of feature space.

5. Conclusion

The general application of electronic noses or, more precisely, of chemical sensor systems, requires the individual optimization of each of the different components in the total system. Evidently, a modular design concept is most suitable for this optimization. The use of modules with different transducer principles, in

particular, is extremely important to provide a feature space of chemical information which is sufficiently large to cope with increasingly more complex environments to be analyzed by future electronic noses. This will complement the information obtained by classical analytical chemistry or by individual sensors (Fig. 1).
This general modular design concept was realized in the MOSES instrument. Its open architecture makes it possible to adapt the total system to many special applications and to extend the system by incorporating new modules with new sample uptake units, filters, transducers, multicomponent analysis concepts etc. without changing the overall architecture.
Several research groups collaborate already by using the same MOSES system or at least the same interface architecture in order to standardize and to objectively characterize the performance of the different components of their sensor systems. The latter is done in view of the specific contribution of each component to the overall performance ("link in the chain"). It is specific for each application. Different researchers, developers and application-oriented engineers now have available a common tool for quantitatively comparing new designs with existing well established ones. This makes it possible to characterize specific needs or deficits for further development that aims at *new applications* for electronic noses.
On the other hand, the modular sensor system may also be used as a benchmark tool for *basic research* to understand the thermodynamics, kinetics and molecular-scale aspects of molecular recognition or of odor perception for well-defined key-lock systems. Conditions for comparative studies with systematically varied properties of sensor-active materials lead to a systematically varied feature space and hence pattern recognition results. In these studies, just those parameters which have to be optimized are varied for constant parameters of the rest of the total sensor system (such as the sample uptake or the pattern recognition). The atomistic understanding of sensing mechanisms and of failure analysis is subsequently obtained from interface analysis[34] (see Fig.1) and leads to new design strategies for better sensors.

6. Acknowledgments

We gratefully acknowledge Lennartz electronics (Tübingen, Germany) for the cooperation in developing the now commercially available MOSES and Hewlett-Packard (Little Falls, USA) for providing us with the HP 7694 headspace-sampler and HP 7695 Purge and Trap.
The Calorimetric Sensor Module was developed by J. Lerchner, D. Caspary and G.Wolf from TU Bergakademie Freiberg and M. Krügel and M. Nitzsch from Eurotronics, Leipzig and we gratefully acknowledge the cooperation with these groups.
For details on the MOSES system see http://www.ipc.uni-tuebingen.de/moses.

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CAFFEINE RECOVERY FROM ACTIVATED CARBON

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Reasonable consumption of caffeine is not at all dangerous for human beings, nevertheless there are many consumers preferring decaffeinated coffee, mainly in the evening. Therefore an entire industry developed for the decaffeination of coffee beans.

Parallel there involved a large beverage industry applying the natural caffeine from coffee for their alcohol-free refreshment drinks. This paper shall inform about a new process, developed to recover caffeine from activated carbon.

As introduction there is a summary of the existing decaffeination procedures and the available processes to recover a maximum of caffeine.

Decaffeination with Organic Solvents

The first process for a caffeine extraction has been developed by Ludwig Roselius in Bremen, the first commercial production started in 1906 with an organic solvent, the product was called "Kaffee HAG". Since that time many patents have been granted for producing decaffeinated coffee with organic solvents. The basic process is a moisturisation step with steam to approximately 40 % H_2O, followed by the caffeine extraction at 80° C with Methylenchloride, Ethylacetate, Di- or Trichlorethylene. After decaffeination the residual solvent is removed by steam and the beans are finally dried before roasting.

In the "indirect solvent method" there is at first an extraction of water soluble substances including caffeine from green coffee with hot water of 80° C. Out of the thereby generated green coffee extract the caffeine is extracted with organic solvents which do not mix with water in a liquid-liquid extraction process. The caffeine-free extract is then used for the decaffeination of the next batch of caffeine containing beans.

The caffeine, separated from the solvent by distillation, can be further cleaned and sold.

In order to avoid chemical solvents for the decaffeination process, three "chemical free" methods have been developed:

Decaffeination with Water (SECOFFEX)

Fresh hot water (~ 80° C) is first used to extract caffeine out of prewetted green beans. Simultaneously soluble substances are extracted from the beans until an equilibrium between soluble concentration in the coffee and extract is achieved. This caffeine containing "equilibrium extract" is passed through a bed of activated carbon to selectively adsorb the caffeine. In order to avoid the adsorption of other green coffee substances the carbon is preloaded with a sugar solution. Having passed the activated carbon, the extract is free of caffeine and is used for the decaffeination of the next batch.

The caffeine loaded carbon is reacativated at about 800° C, the caffeine is lost. A recovery of caffeine is possible in a "quasi chemical free" process where the caffeine containing equilibrium extract is decaffeinated by means of an organic solvent in a liquid-liquid extraction. Out of the solvent caffeine is achieved by distillation.

Decaffeination by means of Supercritical CO_2

The green beans, prewetted with water to approximately 35 %, undergo a highly selective caffeine extraction with supercritical Carbondioxide at 80° - 90° C and 250 - 300 bar pressure. The caffeine containing CO_2 stream passes an adsorption vessel, filled with activated carbon before it is - caffeine free - recycled to the extraction vessel. When the decaffeination is finished, the system has to be depressurised, the beans dried (eventually under vacuum), and the carbon reactivated - normally with loss of the caffeine.

In a variant of this process the caffeine can be removed from the supercritical CO_2 stream with water in a pressure scrubber; then the recovery of caffeine from water is possible.

Decaffeination with Oil

The premoisturised green beans are brought into contact with a liquid, water-immiscible fatty material (e. g. glycerol esters) for several hours at 50° C to 120° C. In a liquid-liquid extraction process the caffeine is transferred into the waterphase out of which it can be recovered. The fatty material can then be recycled to the decaffeination.

The Caffeine Market

The world wide caffeine demand is about 10,000 t/a of which approximately 75 % are used for cola type beverages (s. Fig. 1)

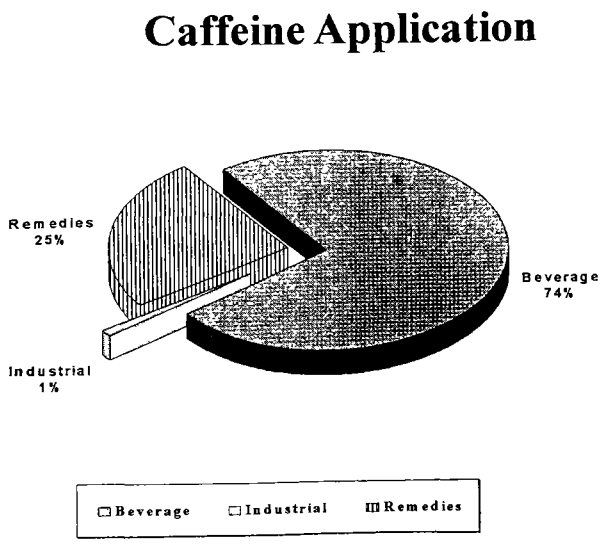

Fig 1

About 60 % of the total volume is produced synthetically, the remaining 40 % or 4,000 t are natural caffeine from coffee beans. Assuming an average caffeine content of 1.5 % over all Robusta and Arabica coffees and an estimated world wide decaffeination capacity of 510,000 t/a then there are 7,650 t caffeine per anno theoretically available (s. Fig.2). That means: Only slightly more than 50 % of the caffeine demand is recovered in decaffeination plants, mainly from MECI, EA and Oil processes (if all capacities are fully utilised).

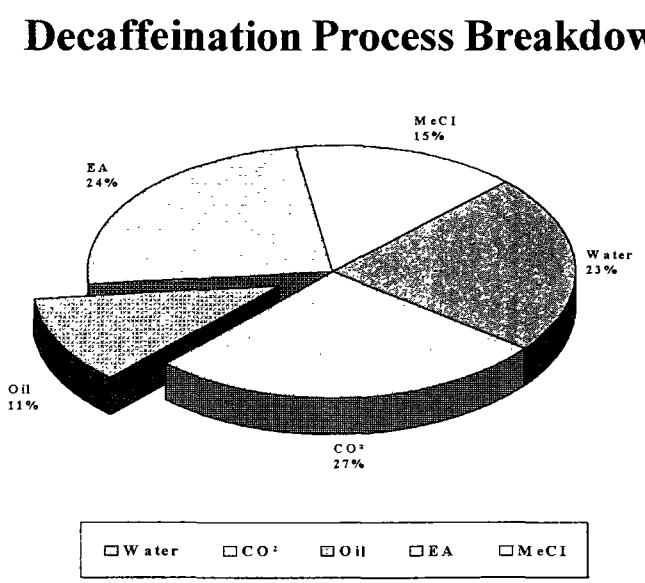

Fig 2

As there is an increasing demand for natural caffeine we developed a method to recover caffeine in processes where supercritical CO_2 and activated carbon are applied.

Caffeine Recovery from Activated Carbon

In principle it is possible to recover caffeine from loaded activated carbon with special solvents. The following table covers most of the recovery systems - described in various patents.

Patent	**Process**		
82305 113.1 General Foods 09/1982	Solvent:	Acetic acid of 70 % concentration (to avoid flammable glacial acetic acid) Temp: < 100 ° C Yield: 80 %	
EP 0251 364 Douwe Egberts 05/1987	Solvent:	Benzoic acid Temp: 140° - 150° C Yield: 89 %	Dichloroacetic acid 120° C 80.8 %
87201 491.5 Douwe Egberts 08/1987	Solvent:	70 - 98 % acetic acid 2 - 30 % citric acid 0 - 25 % water Temp: 100° - 150 ° C Yield: > 90 %	

Recovery Systems with Solvents

Patent	Process	
4.298.736 General Foods 06/1980	Solvent:	Glacial acetic acid or acedic acid azetrop (e.g. with butyl alcohol) Temp: > 100° C Yield: ≤ 73 %
4.548.827 Genberal Foods 03/1982	Preceding step:	Removal of non caffeine solids by aqueous basic solutions (e.g. potassium hydroxide)
	Solvent:	Acidic acid (70 %) Temp: 93° C 4 hours Yield: 70 %
DE 3213 G36 A1 HAG GF 04/1982	Solvent:	Formic acid Temp: 100° C for 1 - 2 hours Yield: 78 %
4.443.60 General Foods 09/1982	Solvent:	Aqueous solution of ethylene carbonate or propylene carbonat Temp: 70° - 80° C for approx. 1 hour Yield: Approx. 70 %

After an evaluation of the different solvent recovery systems the conclusion is that obviously a number of disadvantages avoid their broad commercial application:

- The claim of a "chemical free" decaffeination process is hardly to defend when chemical solvents for the caffeine recovery are used.
- There is a two step process necessary to get via a solvent the crude caffeine.
- There are solvent losses in cleaning the activated carbon and separating caffeine from the solvent.
- Applied solvents are volatile and often inflammable.
- The carbon has to be cleaned and regenerated before further application.

The recently developed

Direct Caffeine Desorption Process

is based on tow HAG patens:

1. *The "Wilkens Patent" (DE 351119C2) s. Fig. 3.*

The desorption of caffeine from the loaded carbon takes place in a so called "cross-flow" reactor, designed in a way to keep the carbon in a continuous thin down-stream (~ 40 mm) in order to avoid read-sorption of desorbed caffeine. The recycling inert gas stream (CO_2, N_2, combustion gas) is heated by means of a natural gas burner to 420° C and passed through an activated carbon prefilter to reduce the oxygen content below 100 ppm. The carbon moisture shall be less than 15 %, preferable below 1 %, the residence time of the carbon in the desorption zone is 20 - 30 minutes. The caffeine loaded gas is cooled with water in water scrubbers, the caffeine absorption into the caffeine-water solution is supported by fibrous micro filter. The aqueous caffeine water solution with approximately 100 g caffeine/litre is then passed to a crystallisation plant to finally get crystalline, wet, crude caffeine.

The caffeine free gas stream is recycled through a blower to the burner, dimimished by about 10 - 20 % exhaust gas.

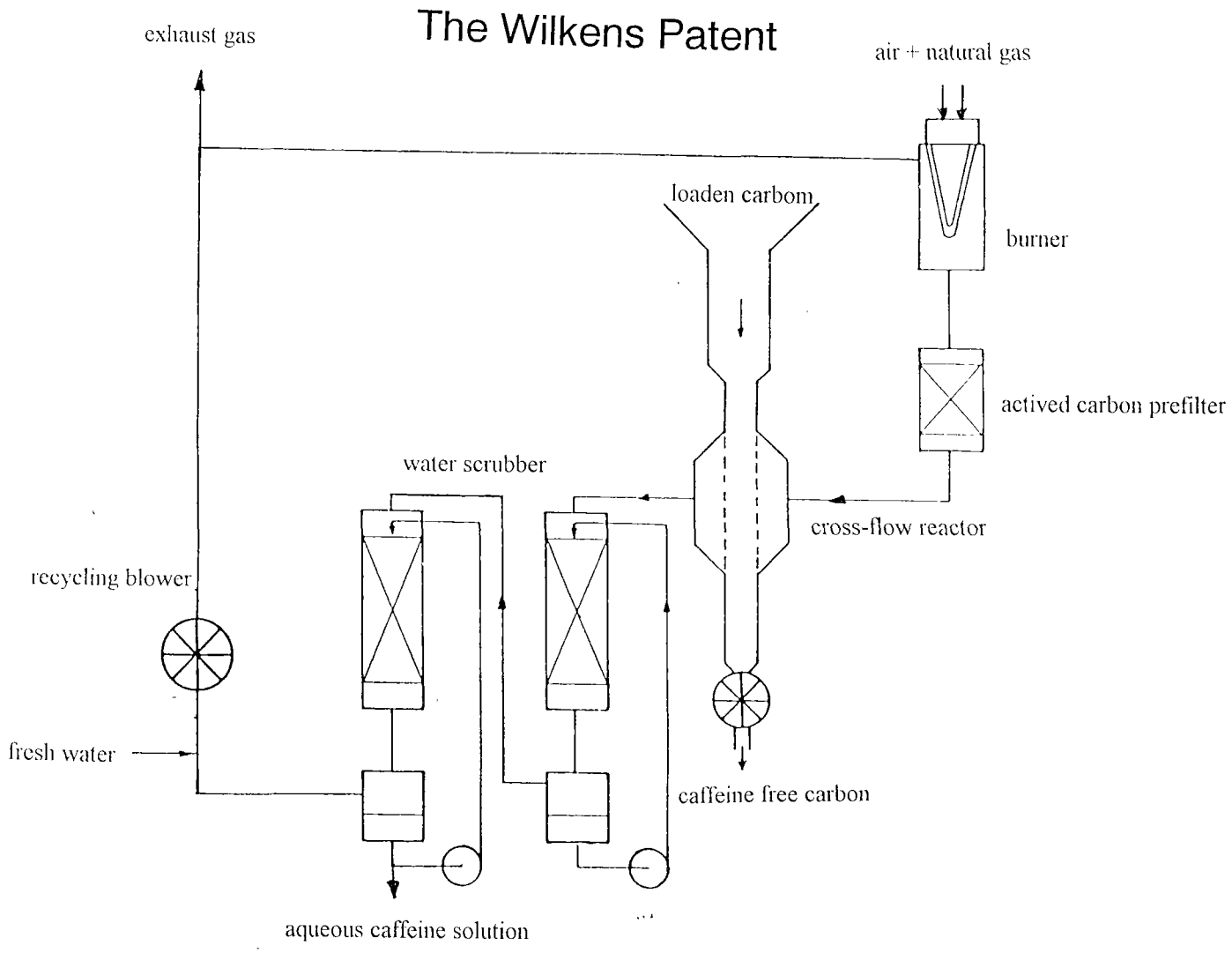
The Wilkens Patent
exhaust gas
air + natural gas
loaden carbom
burner
actived carbon prefilter
water scrubber
cross-flow reactor
recycling blower
fresh water
caffeine free carbon
aqueous caffeine solution

Fig. 3

2. *The "Sipos-Jones" Patent (92113069.6) s. Fig. 4*

The process described in this patent is based on the Wilkens patent but with two major improvements. First it is not restricted to the extreme thin bed layer and second the activated carbon is treated in a fixed or fluidised bed and it is preheated prior to desorption to 200 ° C to 320° C. The corresponding heat source ("heat carrier") can be other than the sweeping gas or the sweeping gas itself.

The clean sweeping gas, an inert gas from combustion, almost free of oxygen, is passed through a heat exchanger to achieve the desorption temperature of 360° C. In case of fixed bed operation the gas stream is top-down (Fig.4), in case of a fluidised bed the gas stream is from bottom to top. The carbon residence time in the reaction vessel is between 30 and 180 minutes. A major advantage of the system is the possibility to reactivate the carbon in the third layer immediately after the caffeine desorption - by applying an additional heat source at approximately 800 ° C. After the reaction the carbon is water quenched to room temperature and recycled to the decaffeination plant.

The caffeine loaded sweeping gas is transported to an absorption vessel to be brought in contact with water of 60° C for cooling.

The dispersed condensed caffeine particles are kept back by sintered micro filter and washed down into the waterphase. The aqueous caffeine solution is recycled via a cooler, the excess solution with 10 % caffeine is further cooled and fed to a crystallisation system. The achieved crystalline crude caffeine for sale has less than 5 % impurities and contains approximately 35 % water.

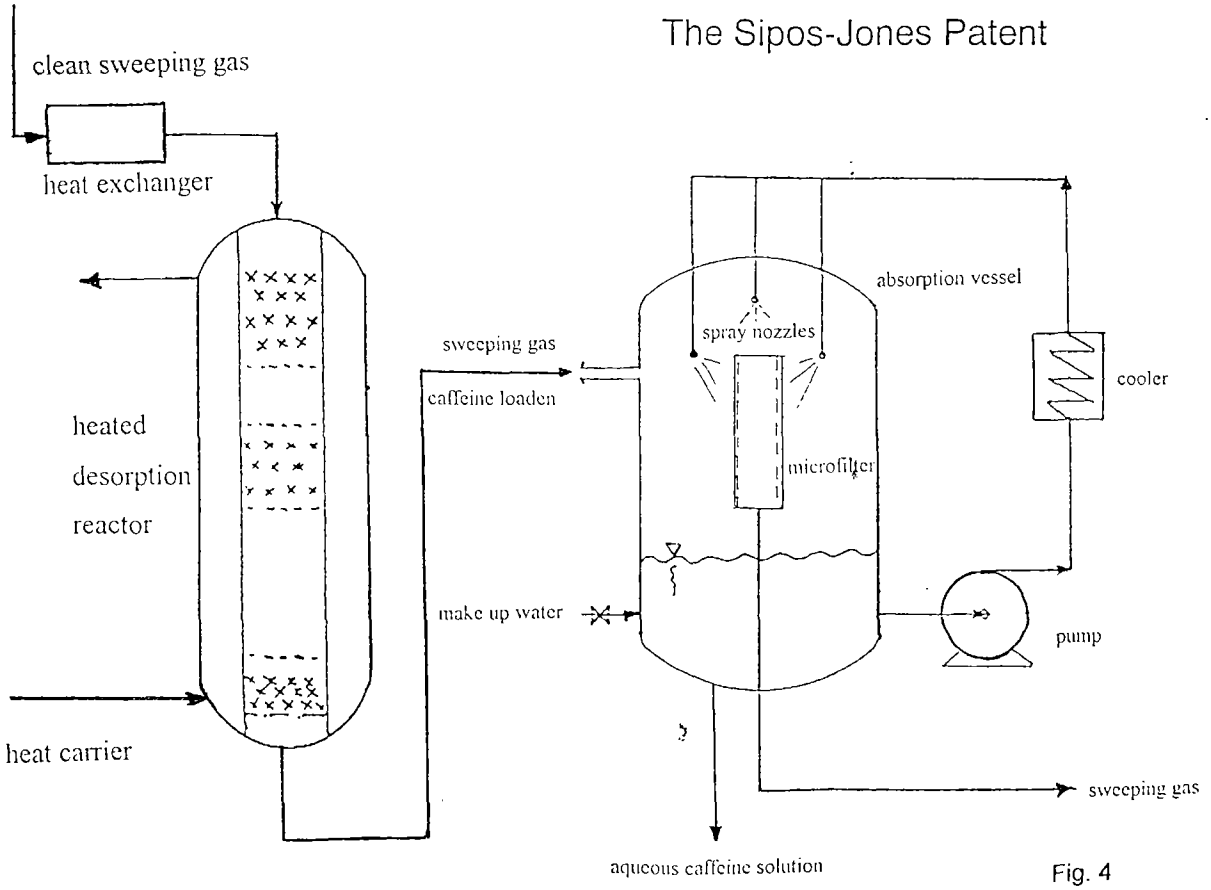

Fig. 4

Summary:

Caffeine Recovery from Activated Carbon:

In the supercritical CO_2-Decafeination process the caffeine, extracted from prewetted coffee beans, is absorbed on activated carbon.

To reactivate the carbon it undergoes a high temperature process in which all caffeine is cracked, resp. burnt.

Several patents are issued describing processes to recover caffeine from the carbon before reactivation with different kind of acids or alcohols - requiring complicated separation and cleaning processes.

We developed an atmosphere and temperature controlled 3-stage process, where caffeine is desorbed into the gasphase. By means of water scrubbers it can be dissolved in water, cooled and crystallised. After filtration it can be sold as wet crude caffeine or refined to USP grade. A corresponding patent has been applied.

CAFFEINE-CAFFEIC ACID COMPLEX FORMATION IN COFFEE EXTRACTS

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INTRODUCTION

Based on caffeine solubility in water, highly concentrated hot coffee solutions can be prepared which should exceed caffeine saturation limits when cooled. It is widely accepted that caffeine can not be crystallized from coffee using this simple approach. The ability of caffeine to form physical complexes with many aromatic compounds is well known [1-6]. These hydrophobically bound complexes derive their stability from overlapped π-electron orbitals between the stacked ring systems [7-10]. Complex strength and solubility vary widely depending on the structure of the caffeine adjunct [1-6]. Association strength is described by an equilibrium constant, K, which has units of inverse molarity. K is equal to the molar concentration of the complex divided by the product of the molar concentrations of caffeine and its adjunct. The K-values which have been reported for numerous caffeine complexes span many orders of magnitude [1-10].

Caffeine occurs naturally in coffee as a 1:1 molar complex with chlorogenic acid (CGA) [11]. CGA is the most abundant phenolic acid in coffee, with molar concentration in excess of caffeine. CGA forms caffeic acid (CA) and quinic acid upon hydrolysis. Caffeine can also complex with CA, a phenolic acid found at very low levels in coffee [8,9]. Caffeine-CGA and caffeine-CA K-values are 17 and 12 L/mole, respectively, in 40°C water [8,9]. Caffeine-CGA K-values decrease with increasing solution pH, attributed to greater solvation of the salt than the acid [11]. Caffeine-CGA K-values also decrease with lower solvent polarity [9]. The authors have discovered that addition of CA to coffee extracts can displace CGA from caffeine-CGA to precipitate caffeine-CA crystals [12-14] despite reported stronger association of caffeine with CGA. The near insolubility of caffeine-CA can easily produce supersaturated solutions even at low caffeine levels to provide effective decaffeination. This paper summarizes the conditions which favor caffeine-CA formation and crystallization from caffeine solutions and coffee extracts.

MATERIALS

Reagent grade CA (3,4-dihdroxycinnamic acid; 97% purity; predominantly *trans*; Aldrich), CGA (Sigma), and caffeine (Sigma) were used. CA was further purified by recrystallization from water. Spray-dried Instant Maxwell House® (IMH), Instant Sanka® (IS), and atmospheric solids (AS) coffees contained 4.1, 0.08, and 8.7% wt/wt caffeine, respectively. Green extracts were produced by contacting water with unroasted beans to attain 0.4-0.5% caffeine in 25% wt/wt total solids. Solvent extracts were produced by contacting roasted coffee extracts with methylene chloride and vacuum distilling to attain 7-8% caffeine in 10-12% wt/wt total solids.

EXPERIMENTAL

Decaffeination

Decaffeination of aqueous caffeine solutions and coffee extracts was conducted by adjusting to desired pH with HCl or KOH, heating to 95°C on a stir-plate, adding CA while stirring to completely dissolve, and turning off heat with continued stirring while cooling to 25°C over 3-4 hours. Caffeine-CA crystals usually became visible after cooling to 50-70°C. Solutions were left undisturbed to allow crystals to precipitate overnight, after which supernatants were sampled, diluted, and analyzed by HPLC. Decaffeination of methylene chloride extracts was conducted at 25°C by contacting with a slurry of excess CA in water. Caffeine partitioned into the aqueous phase causing rapid precipitation of caffeine-CA crystals. Crystals were removed from all systems by paper filtering through a Buchner funnel under vacuum.

HPLC Analysis

Solubilities of compounds and complexes were determined by analysis of pure saturated aqueous solutions equilibrated at 25°C and native pH unless otherwise indicated. Caffeine, CGA, and CA concentrations in water and coffee extracts were measured by HPLC (Waters Corporation, Model 721, 280 nm UV detector) using a method developed to provide mutual resolution. A Resolve Radial-Pak Z-module column (5μm spherical C-18, 3.9 mm diameter x 15 cm length) was used with 1.5 mL/min isocratic flow rate of mobile phase (3.3 mM KH_2PO_4, methanol, acetic acid at ratio of 80:20:4). Solutions were diluted 10-100X with water to ensure retention of solubility and then diluted to 0.1% wt/wt with mobile phase prior to analysis.

RESULTS

Caffeine Solubility

The caffeine content of coffee powders ranges from less than 1% in extended autoclave fractions to nearly 10% in mildly extracted atmospheric fractions. Commercial products usually have 4-5% caffeine. The solubility of caffeine in water is about 2% wt/wt at 25°C and increases to 40% wt/wt at 100°C. Highly concentrated coffee extracts can be prepared which greatly exceed caffeine solubility limits in pure water without resultant caffeine crystallization. For example, a hot 40% wt/wt AS coffee solution containing 3.5% wt/wt caffeine in solution did not precipitate caffeine upon cooling. This prompted measurement of the actual solubility of caffeine in coffee. The solubility of caffeine was measured in 20% wt/wt IMH solutions at 25°C over a range of pH by adding excess caffeine and analyzing supernatants after several days of equilibration. The initial 0.8% wt/wt solution caffeine level increased to over 6% (Figure 1). This means that IMH powder would have to contain over 30% caffeine to saturate a 20% solution.

Complex Solubility

In coffee at pH 4.9, caffeine-CGA is 95% dissociated (pKa 3.6) as a potassium salt. The apparent solubility of this caffeine-CGA salt was measured versus temperature by adding equimolar amounts of caffeine and CGA to water and increasing pH to 6.0 with KOH (Figure 2). Caffeine solubility was increased by association with CGA and likely contributes to the very high solubility of caffeine in coffee extracts. The solubilities of CA (pKa 4.8; native pH 3.5) and caffeine-CA in water were measured as a function of temperature and pH (Figures 3 and 4). Caffeine-CA demonstrated much lower solubility than caffeine-CGA. HPLC analysis and acid titration of caffeine-CA crystallized from water verified a stoichiometric 1:1 molar complex.

Water Decaffeination

Having established the low solubility of caffeine-CA and its ease of crystallization from caffeine solution, experiments were conducted to determine whether CA could be added to aqueous solutions of caffeine-CGA to precipitate caffeine-CA. It was found that caffeine-CA could be crystallized from caffeine solutions containing CGA without a reduction in the extent of expected decaffeination based on caffeine-CA solubility. However, CGA inhibited caffeine-CA crystallization as evident from slow nucleation and growth of smaller crystals. Precipitation of caffeine-CA from solution drove its continued formation at the expense of caffeine-CGA. The conditions which favored decaffeination efficiency of aqueous caffeine solutions were addition of a molar excess of CA with respect to caffeine, low solution pH, and high caffeine concentration (Figures 5-7).

Coffee Decaffeination

Initial attempts to decaffeinate aqueous brown coffee extracts by addition of CA yielded low levels of decaffeination. Elucidation of key variables led to attainment of up to 94% decaffeination in both IMH and AS coffee extracts. Coffee pH reduction was limited to 4.2 since lower pH resulted in protein precipitation. Addition of a molar excess of CA with respect to caffeine, low solution pH, and high initial solution temperature all increased the extent of coffee decaffeination (Figures 8-10). CA addition to green coffee extracts and solvent extracts of roasted coffee produced up to 93% and 99% decaffeination, respectively, and will be detailed in a subsequent publication.

Crystallization Properties

Caffeine-CA was easily crystallized from water, but this could not be achieved for caffeine-CGA. The visual appearance and crystal habit of caffeine-CA differed from both caffeine and CA. Caffeine crystallized from water as thin needles and CA as rhomboidal plates. Caffeine-CA crystals grown in the presence of CGA often formed large bundles of closely packed rods or spherical clusters of rods emanating from few nucleation centers, in contrast to rapid growth of free crystals in CGA-free solutions. Caffeine-CA crystallized from water as long square rods and from coffee extracts as bundles of closely packed rods or elliptical clusters comprised of stacked plates. Caffeine-CA crystals grown from water were highly regular, while those grown from coffee were marred by extensive face and edge defects which gave them a more rounded appearance.

Coffee extracts decaffeinated using this method often contained high levels of residual CA. Coffee inhibited nucleation of CA crystals, which precipitated as small yellow-orange spheres having an amorphous appearance. In an effort to better understand this phenomenon, the solubilities of CA and caffeine-CA were measured at two different pH levels and temperatures in 10% wt/wt IS coffee solutions (Tables 1 and 2). It is evident that the solubility of CA is much higher in coffee than in water, perhaps due to the presence of CGA, and that the solubility of caffeine-CA is not affected as much as pure CA by higher pH and temperature. Caffeine and CA retained their stoichiometric molar proportions at 25°C, but deviated somewhat at 95°C.

Figure 1: Solubility of Caffeine in a 20% wt/wt IMH Coffee Solution

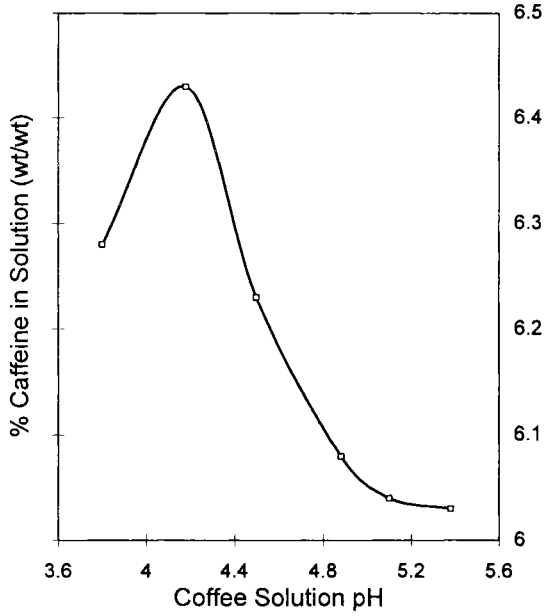

Figure 2: Solubility of Caffeine and Caffeine-CGA in Water at pH 6

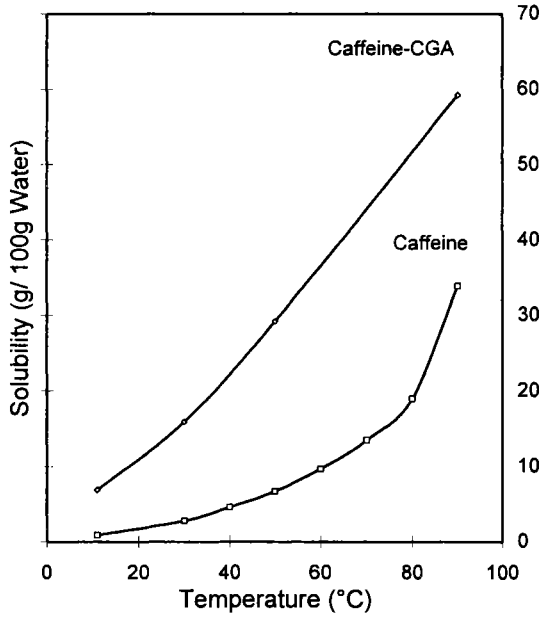

Figure 3: Solubility of Caffeine-CA and CA in Water vs Temperature

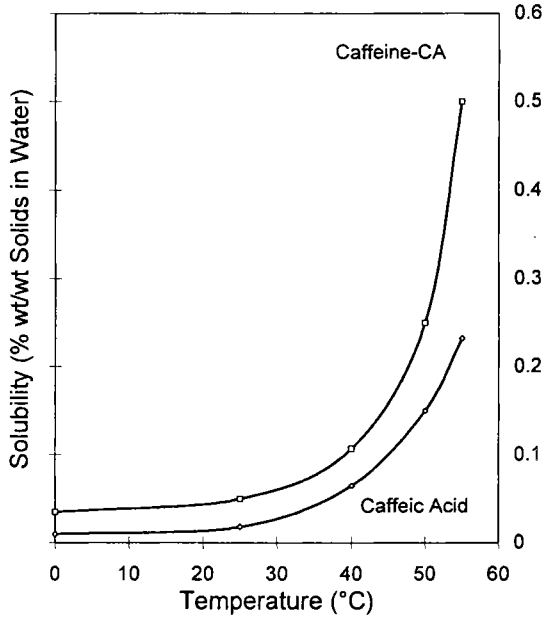

Figure 4: Solubility of Caffeine-CA and CA in Water vs pH at 25°C

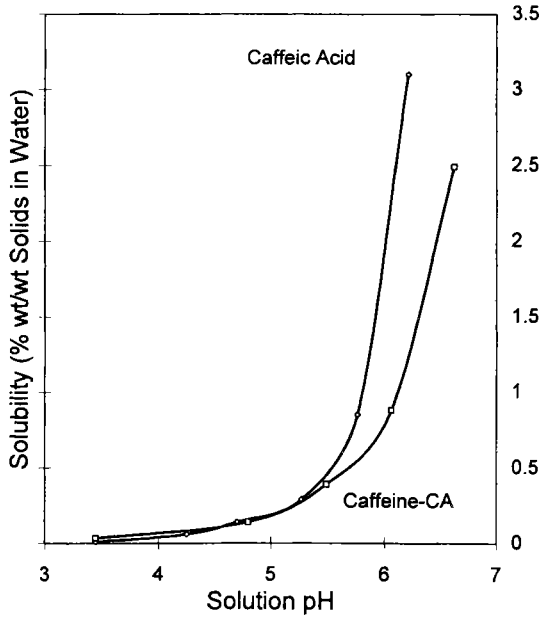

Figure 5: Extent of Decaffeination of Water vs Amount of CA Added

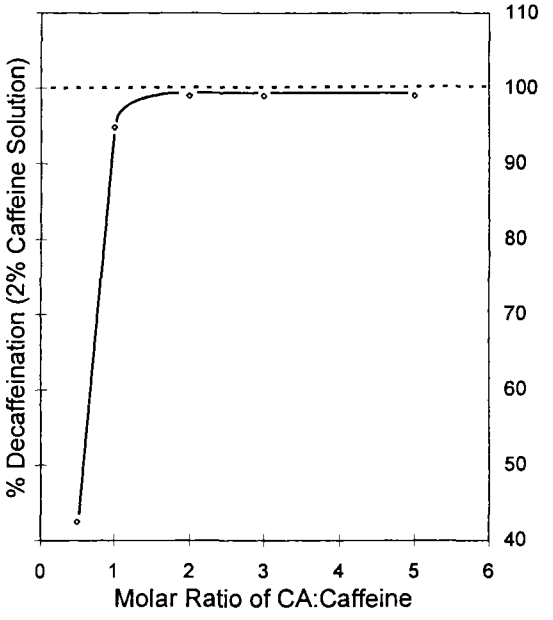

Figure 6: Extent of Decaffeination of Water vs pH at 3:1 Molar CA:Caffeine

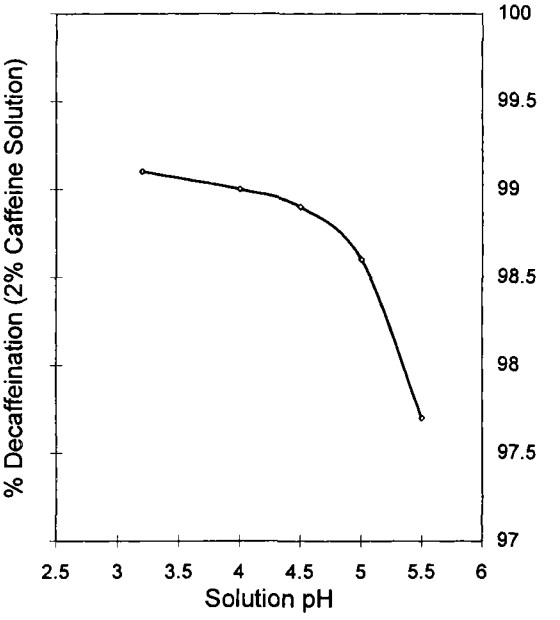

Figure 7: Extent of Decaffeination of Water vs Caffeine Concentration

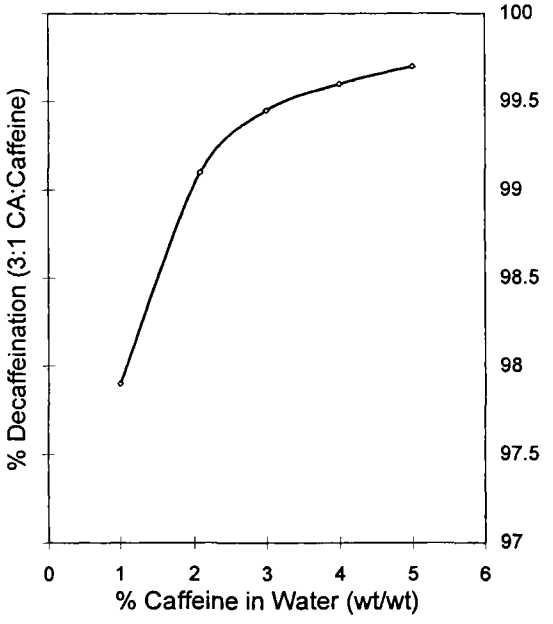

Figure 8: Extent of Decaffeination of 30% AS vs Amount of CA Added

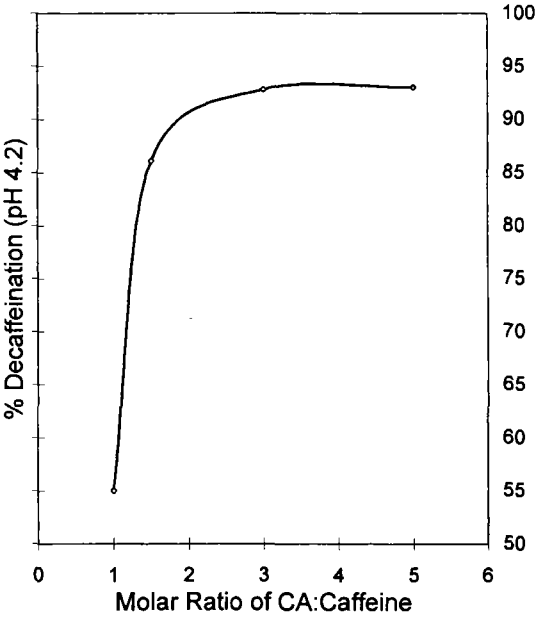

Figure 9: Extent of AS Decaffeination vs Concentration at 3:1 CA:Caffeine

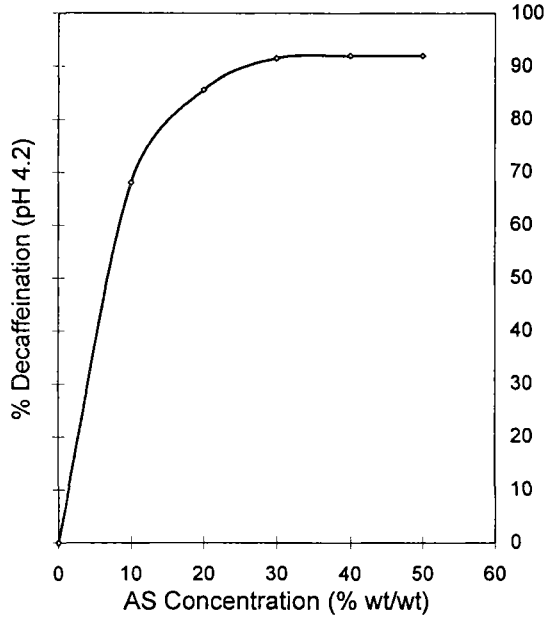

Figure 10: Extent of Decaffeination of 30% AS vs Temperature at pH 4.2

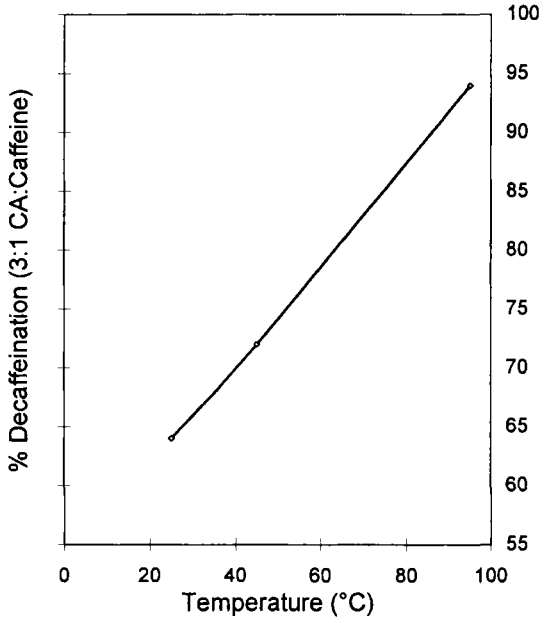

Table 1: Solubility of CA in 10% IS vs Temperature and pH.

Temp. (°C)	Coffee pH	CA (%/wt)
25	4.2	0.079
25	4.7	0.103
95	4.2	0.903
95	4.7	1.184

Table 2: Solubility of CA and Caffeine from Caffeine-CA in 10% IS vs Temperature and pH.

Temp. (°C)	Coffee pH	CA (%/wt)	Caffeine (%/wt)	Molar Ratio
25	4.2	0.103	0.118	1.0:1
25	4.7	0.127	0.142	1.0:1
95	4.2	0.216	0.269	1.2:1
95	4.7	0.365	0.327	0.8:1

DISCUSSION

The extent of decaffeination obtained from caffeine solutions closely matched theoretical limits calculated from equilibrium solubilities of caffeine-CA measured as a function of temperature and pH. The slightly higher solubility of caffeine-CA in coffee extracts only marginally decreased the extent of decaffeination attained from pure water. Coffee decaffeination closely paralleled similarly calculated limits but was typically a few percent lower than predicted. Like CGA, coffee inhibited nucleation of caffeine-CA as evident from slow crystallization and numerous crystal defects. Because of the effect of excess CA on coffee flavor and the desire to recycle CA for economy, it is advantageous to recover as much as possible. CA was recovered from precipitated caffeine-CA crystals by Soxhlet refluxing with methylene chloride. The caffeine preferentially dissolved in the solvent while the extraction thimble retained crystalline CA to yield 99% pure caffeine and CA in a single separation step.

CONCLUSIONS

Caffeine is much more soluble in instant coffee extract than in water and it appears that this phenomenon is at least partially attributable to physical association of caffeine with CGA. Like caffeine-CGA, caffeine-CA occurs as a stoichiometric 1:1 molar complex. Addition of CA to coffee extract to precipitate caffeine-CA crystals is an effective method to selectively effect decaffeination. Use of an excess molar amount of CA to caffeine, low solution pH, high caffeine concentration, and high temperature all increase the extent of coffee decaffeination which can be attained. Despite higher caffeine-CA solubility in coffee and slower crystallization, the extent of decaffeination attained is only marginally lower than from pure caffeine solution. Caffeine and CA can be efficiently recovered from caffeine-CA crystals, but further research is needed to improve recovery of excess CA from decaffeinated coffee extracts.

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SUMMARY

Addition of caffeic acid to caffeine solution or coffee extracts causes formation of a 1:1 molar physical complex with caffeine which can be crystallized from solution. This process can be used to selectively decaffeinate brown, green, and solvent extracts of coffee. Caffeine is normally present in the form of a 1:1 molar complex with chlorogenic acid which can not be crystallized from coffee extracts due to its very high solubility. The caffeine-caffeic acid complex has very low solubility and can be precipitated from solution in the form of pure crystals by complexing at high temperature and cooling to supersaturate.

QUICK ROASTING OF COFFEE BEANS, AN OVERTURE TO THE FLUID BED TECHNIQUE

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ABSTRACT

From a couple of decades, Drying and Roasting of coffee beans in fluid beds has offered an interesting alternative, since thorough mixing and effective gas-particle contact during fluidisation ensures a good homogeneity of thermal treatment of the product and more stringent control over the bean temperature. In the existing method of fluidisation , material fluidisation perfomed in a multi jet hot air plenum and the roasted product will be either discharged by the help of vibratory attachment or by increasing the fluidisation air velocity

The present work explores a new method of fluid bed technique for quick roasting of coffee beans. The main difference between the proposed method fluid bed and an existing one is related to the manner in which the material fluidisation takes place and the manner in which the roasted product are discharged are discussed in brief. Experimental trials were conducted on a lab scale roaster and process parameters were standardised as roasting temperature of 260°C and roasting time of 5 min.

Each trials have received an interesting results and that are analysed by referring to the bio-chemical and organoleptic properties of the roasted samples and the results are tabulated .

Finally, the roasted product from the proposed method of roasting and commercial roasting operations were compared in terms of energy consumption and final product quality.

COFFEE QUALITY : THE IMPORTING COUNTRIES PERSPECTIVE

J. A. J. R. VAESSEN

Secretary General of the European Coffee Federation
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Mr. Chairman,
Ladies and Gentlemen,

It is a great privilege and an equally great pleasure to address this Conference, especially since the kind invitation of the organisers has allowed me to visit one of the worlds most distinguished coffee producing countries. At the same time I must confess to a slight feeling of unease: here I am, a non-scientist addressing an audience of scientific experts on the issue of coffee quality.

First of all, let me briefly introduce the European Coffee Federation, or ECF in short. The ECF is a private sector organization. It represents the European green coffee trade and coffee roasting industry. The ECF has as its members national coffee associations in 13 European countries. Its combined membership accounts for the annual imports of about 36 million bags of coffee, or close to half of world trade. The ECF is active in areas such as import duties and import documents, maritime transport, standard green coffee contracts, International Coffee Organization matters and European Union legislation affecting our industry (environment, food safety, labelling and such).

Now to the actual subject of my speech: coffee quality.

It may perhaps be sacrilegious to say this in a scientific conference, but in my view coffee quality is not a subject that lends itself to the natural tendency of the human species to measure and classify. The notion of 'quality' is by itself imprecise. It involves subjective judgements. It is also a multidimensional concept. This afternoon, I would like to highlight four of the many dimensions of the concept of coffee quality, seen from the perspective of coffee importing and consuming countries.
These are:
1. Quality in international trade
2. Quality in international and national legislation
3. Marketing and consumer perceptions
4. Quality requirements of the 'emotional' consumer

1. QUALITY IN INTERNATIONAL TRADE

Obviously, the buyer of green coffee wants to be assured that he receives the quality he has ordered. A shipment of coffee is not like a faulty piece of household equipment that you return to the store. For buyer and seller any dispute over the quality of the product creates at least irritation.

A number of tools have been developed to assist buyers and sellers of green coffee in the determination of the quality of coffee bought and sold.

I call these 'tools for objectivation'.

The first applies to ***terminology***. If you want to describe something, especially something as elusive as 'quality', you must start with using terms that have the same meaning for all parties. The International Organization for Standardization has developed a coffee vocabulary (ISO 3509). The third edition was published in 1989. It contains many purely factual descriptions, such as:

> **Arabica coffee:** Coffee of the botanical species *Coffea arabica* Linnaeus

Other descriptions touch on the quality aspects. To quote just one example:

> **Large stone:** Stone retained by a screen having round holes of 8,00 mm diameter

As you probably know, ISO standards are not mandatory in themselves. However, if an ISO member country decides to put into effect a national standard, that must be based on existing ISO standards. But even on a voluntary level the ISO coffee vocabulary may be of assistance, for example to any institution setting up a grading system.

This brings me automatically to the second 'tool for objectivation': the various ***grading systems of exporting countries***.
The simple descriptions 'Kenya AB', 'Colombia Excelso' or 'Indonesia Robusta Grade 4' - to mention but a few - hide elaborate systems of grading, often based on defect counts, but in many instances also including sensory analysis and cupping. When implemented consistently, such grading systems are indispensable for buyers and sellers to avoid to the maximum extent possible differences in appreciation concerning the quality of the traded coffee.

Further assistance in 'objectivation' is provided by the ***quality assessment of arbitration panels*** in consuming countries. Apart from resolving the immediate dispute between trading partners, arbitration also provides feedback to the countries of origin on the functioning of the grading system and the interpretation of quality descriptions. Some producing countries have themselves provided support to the arbitration institutes to arrive at consistent verdicts. When Indonesia changed to the new defect-based grading system a number of years ago, it produced elaborate information on the new system to the arbitration institute in The Netherlands, including physical examples of what constituted a 'small stick', 'large stick', a 'broken bean' etcetera. Thus samples submitted for arbitration could be compared directly with the standard applied in Indonesia.

The fourth and final 'tool for objectivation' that I would like to mention is the ***grading applied by the terminal markets***. Even though much of the trading is paper-based, delivery of coffee against a terminal market contract is still possible and the buyer must be assured of the quality of the coffee. That terminal market grading and the criteria applied must not be taken lightly is illustrated by the recent discussions around the changes applied by LIFFE in London.

Despite these instruments to provide a certain measure of objectivity, consistency and predictability in the quality assessment of internationally traded coffee, still

virtually all coffee is traded on samples. This illustrates the fact that even the most sophisticated vocabulary or even the most refined and strict grading system can not provide sufficient information to the buyer on the quality of the coffee. He will still rely on his own judgement.

2. QUALITY IN INTERNATIONAL AND NATIONAL LEGISLATION

As any traveller can confirm, coffee served around the world can differ from bad to indifferent to excellent. 'Quality' in this sense is not the subject of any legislation that I am aware of.
On the level of the European Union, food legislation is predominantly 'horizontal', which means that a subject is regulated for all foodstuffs together. This is the case for a great variety of subjects, ranging from pesticide residues to flavourings and from hygienic production to labelling. The EU has deliberately avoided as far as possible product-specific legislation. This would simply be unworkable given the enormous and ever-changing number of food products on the market. Less than ten products are the subject of specific EU legislation, and one of them is instant (soluble) coffee. Many of the provisions relate to sales names and labelling, but some involve quality aspects. For example, minimum levels of dry matter and maximum levels of anti-caking agents for instant coffee used in vending machines.
There is no EU legislation for green or roasted coffee.
National rules and regulations exist in individual countries. The level of detail differs. Again, their purpose is to ensure the integrity of the product and a very basic quality level. A few examples:

- on green coffee, the food law of several EU countries contains provisions regulating the maximum percentage of broken beans or foreign matter;
- on roasted coffee, the legislation frequently regulates such aspects as adulteration with skins and husks or maximum moisture level.

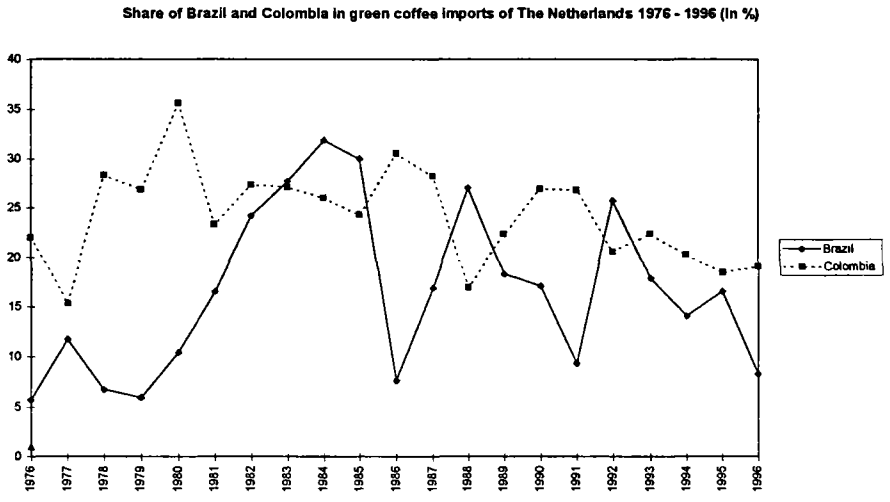
There is no direct relationship between the existence of national legislation and the quality of coffee offered for sale in a country. Denmark, for instance, has no specific national rules for green or roasted coffee at all, but there is no doubt that the quality offered to the consumer is excellent, witness the fact that Denmark has a very high per capita consumption. Tradition and quality conscious consumers play at least as important a role as legislation.

3. MARKETING AND CONSUMER PERCEPTIONS

Which brings me to the next quality aspect: quality as perceived by the consumer and the efforts of the coffee roasters to satisfy consumer demand.
For the average European consumer, coffee quality is linked to the brand that he or she buys. The roasters obviously tailor their production and marketing to consumer requirements. Blending and roasting are not only economic operations; they also give each coffee its identity. In Europe virtually all of the coffee is a blend of a number of origins, types and qualities.
Because of the single market of the European Union, some concerns can be heard that coffee will become a standardised 'Euroblend'. Apart from the question if this is a negative development (McDonalds thrives on the sameness of its hamburgers all over the world), I feel that this concern is unwarranted for two reasons: national traditions and the increasing consumer penchant for an ever-growing variety of food products.
Therefore blending and roasting are - and will for the foreseeable future continue to be - :

- based on traditional national preferences
- tailored to prevailing brewing methods
- specific for sub-sectors in the market (home consumption, institutional market, cafés and restaurants)

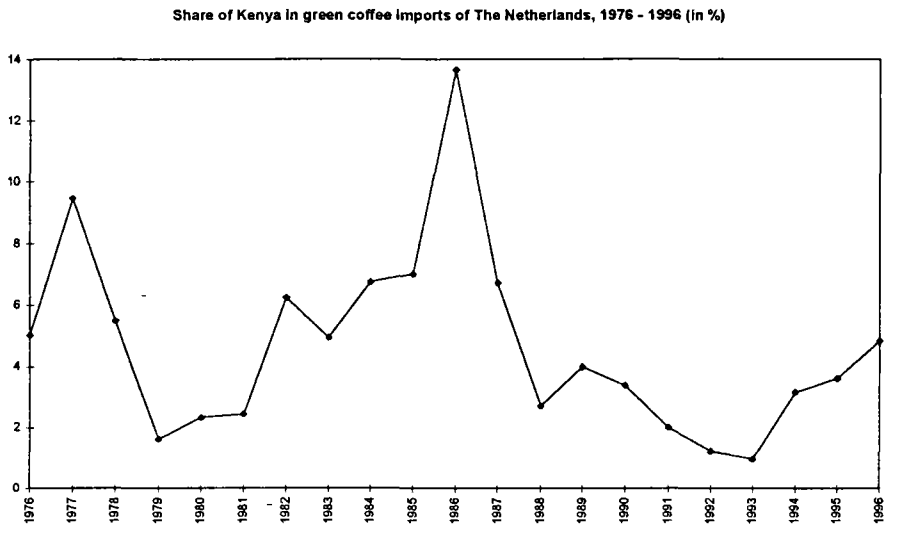
It is very important to note that blends are not fixed recipes. Roasters have a certain amount of flexibility to switch between origins and qualities and still obtain the same taste pattern in the end product. This flexibility can be traced back to the 1975 'killer frost' in Brazil. Before the frost, the European roasting industry never contemplated blends with such a low Brazil content, but the temporary scarcity of this major origin forced them into developing alternatives.
To illustrate the switches between origins I have taken the imports into the Netherlands in the last 20 years from Brazil and Colombia (expressed as a percentage of total Dutch imports):



As you can see, there is a marked increase in Colombia's share whenever there was a reduced availability of coffee from Brazil trough frost or drought. Please be careful how you interpret this graph. For the sake of readability I included only the two major suppliers to the Netherlands. I do not intend to suggest that a direct replacement of Brazilian coffee by Colombian coffee took place. It is more likely that a lack of Brazil was compensated for by a higher share of a number of origins, including Colombia.
The graph does illustrate the wild swings in import shares: for Brazil ranging from about 5% to above 30% and for Colombia from 15% to 35%.

It is perhaps a bit risky, but since I am in Kenya I will also show you the import share from Kenya in imports of green coffee in The Netherlands over the same period.

Do not be overly alarmed by the apparently large swings: I used a different scale for this graph. And let me assure you: the same swings can be observed with any origin and any destination. The most important is the basic message: import patterns can vary enormously. No exporting country is automatically guaranteed a share of the market and constant attention to the availability of sufficient coffee with an attractive price/quality relationship is required.



4. THE 'EMOTIONAL' CONSUMER

For a small but growing number of consumers, coffee quality is no longer restricted to the taste of the product. Quality also involves other factors:

- wholesomeness (food safety, free from contaminants)
- respect for the environment (during primary production, during manufacturing as well as in respect of packaging waste)
- social aspects (adequate remuneration for farmer and farm labourers)

I would like to expand a little more on the first item, because recent EU policy changes will have an important impact on primary production and manufacturing of all foodstuffs.

Several food safety scares in the EU have contributed to a political climate where consumer protection and food safety, always priority items, are receiving even more attention.

One of the instruments in ensuring food safety is an approach that is variously known as 'from stable to table', 'from farm to fork' or 'from plough to plate'. This means that the whole chain of food production, from the raw material to the finished product, is taken into consideration. In the case of coffee and other raw materials produced outside the EU, this involves the countries of primary production.

At the moment I can only speculate about the practical implementation of this new approach. Obviously, the EU legislation itself cannot be applied beyond its borders, but we can expect proposals for more stringent import controls on raw materials and/or requirements for buyers to apply stricter norms in their contractual relations.

A concrete example involving coffee is mould formation in green coffee. As you may know, the prevention of mould formation is the subject of an ICO project, but it is also a subject under debate in the EU and many member states are advocating import checks.

At the moment, I cannot be more precise because the discussion is still going on, but in the context of the new food safety policy of the EU it may become of considerable importance for the importing as well as the exporting countries.

All this serves to illustrate that 'quality' also means that the consumer has to have a 'good feeling' about the product he buys. And this is perhaps the most intangible and difficult to measure, but at the same time the most important of all quality aspects.

Thank you for your attention.

TRICHROMATIC COLOUR SORTING OF ARABICA AND ROBUSTA COFFEE BEANS

J. JUSTUS

ELEXSO Sortiertechnik GmbH, Hamburg

The first photoelectric sorting machines were developed 50 years ago to separate white beans or peas according to their light / dark value, at 50 kg/h throughput capacity. This basic technology is still used today in **monochromatic** machines which range in throughput from 200 to 4 000 kg/h. Monochromatic sorting of green coffee beans is often sufficient when only black beans are to be rejected, mostly for robusta applications.

Arabica coffee or washed robustas have to be sorted not only in a light / dark model, but require also sorting machines capable to remove discoloured beans of similar light / dark values. In order to distinguish between grey and green, yellow and light-green, brown and dark-green, etc., at least a **bichromatic** two-dimensional mode is required, measuring the reflected light through two different colour filters. Bichromatic machines have been employed throughout West and East Africa since 40 years. The product is first singulated and then viewed in free fall from one or more sides under diffuse light of fluorescent tubes or incandescent lamps.

It can also be scanned by one or more lasers of one or more colours. However, bichromatic sorting machines have only limited capability to simultaneously remove mould or foreign matter.

Sorting under ultra-violet light is also known in East Africa. In this mode only fluorescence is measured, but no further colours.

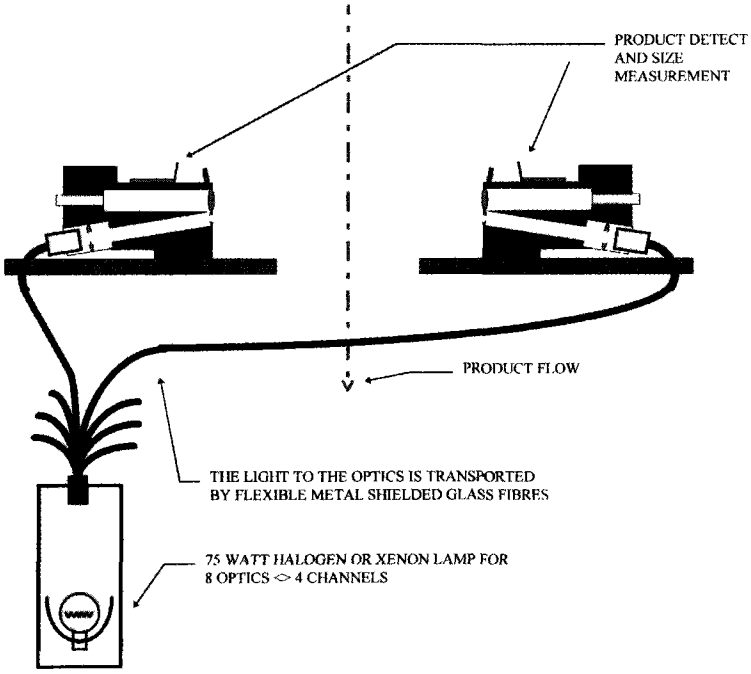
The purpose of this presendation is to inform about the latest technology of **trichromatic** colour sorting pioneered by the ELEXSO Sorting Technology Company of Hamburg, Germany. The advantage of trichromatic sorting is that it covers the full range of the spectrum from ultra-violet (mould) to the near infra-red (foreign matter). 262 000 possible colour combinations are evaluated in a three-demensional mode. By analysing colours beyond the light visible to human eyes this trichromatic machine has even proven its efficiency to lower the aflatoxin level when sorting peanuts. Research is presently going on to determine which colour combination visible to this machine might be an indicator also for ochratoxin.

The product is singulated in 4 parallel slides achieving a conveying speed of 5-6 m/sec. Behind the lower end of each slide the product is illuminated from each side via 2 sharply focused light beams to create a viewing plane of 1,5 x 25 mm. 8 such light beams emanate from glass fiber bundles which converge below just one 75 W halogen lamp located in a dust-proof enclosure outside the viewing box of the machine. The light reflected from each side of each coffe bean is collected by optical lenses mounted in front of further glass fiber bundles. These are split up to carry the light to 3 filters ranging from 400 nm ultra-violet to 1 650 nm infra-red. On the other side of the filter opposite each strand of glass fibers are photocells which measure the reflected light. After the analog to digital converter the signals are processed at very high speed to permit 12 scans per bean. Real time signal processing and 1 000 Hz air ejectors enable the machine to remove defective beans or foreign matter with pin-point accuracy at up to 500 beans per second below each of the 4 slides, for a total sorting capacity o 800 to 1 200 kg/h on just 1 m^2 floor space.

PRINCIPLE OF *ELEXSO*'S PATENTED TR4 TRICHROMATIC OPTICS

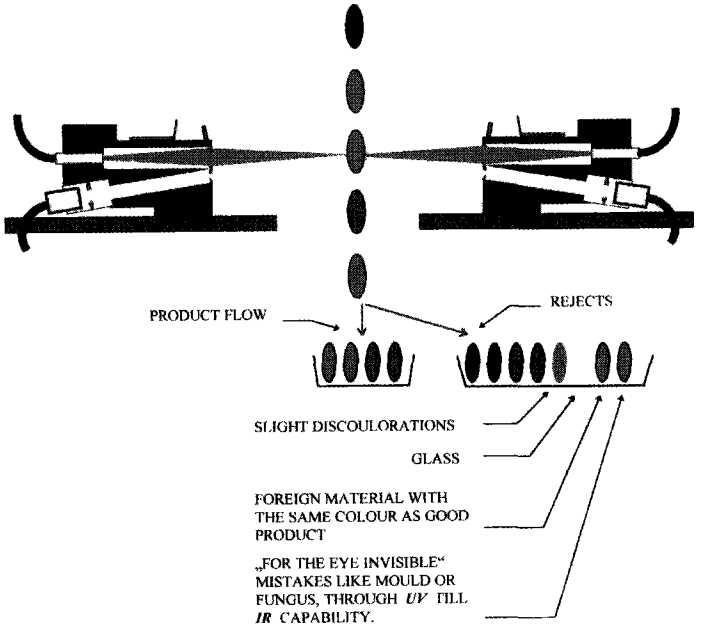
1. ILLUMINATION

Direct illumination with sharp bundled light for a very high light output with only *one* lamp for four channels. The whole light output is used to illuminate the product only.

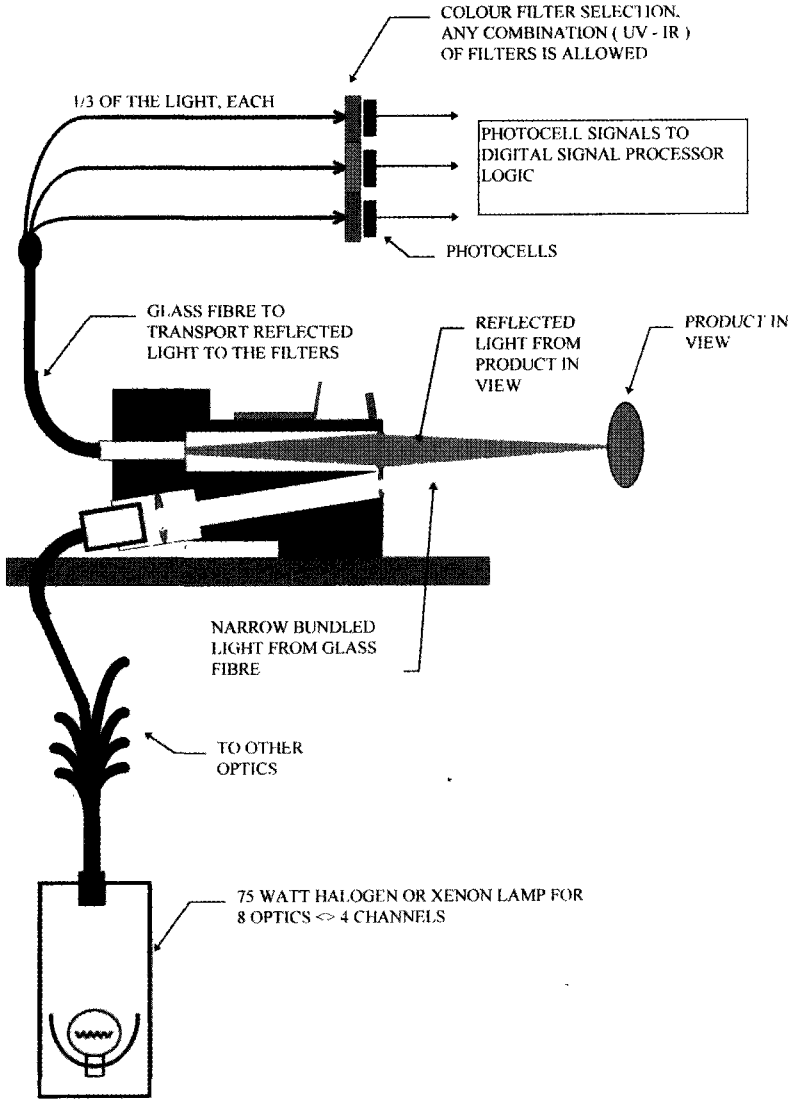


2. INSPECTING

No backgrounds or any other colour references are used. All light received by the receiving fibre bundles is coming directly from the product in view.



3. LIGHT RECEIVING (shown only one side of a two side view)



A NEW WAY TO SPREAD COFFEE KNOWLEDGE : THE MULTIMEDIA APPROACH

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Throughout the world millions of espressos are served daily. However only recently has the espresso coffee itself become so well-known while its history, characteristics and qualities are still widely unknown. Solely the knowledge of its existence is due to legends passed on among generations and its eventual diffusion from Ethiopia throughout the Middle East and onwards to Europe. Now drunk worldwide, many still do not know much about the coffee culture including its history, characteristics and various ways of preparation. Coffee culture can be considered diffuse as well as global as it includes the history of the Brazilian coffee plants to the drinking styles in European cafès. Advancements in worldwide and local communication, however, have made it easier to spread news, information and facts about coffee or any other interest in a faster and more direct means. Information is now quickly and easily spread through multimedia technology.

Multimedia is the combination of communication means primarily used to spread, market or advertise information locally and worldwide. Radios, televisions and newspapers have been among the most popular measures for years, though technological growth has now incorporated the use of computers with cd-roms and websites to allow the quick transfer of data, information, and media. The multimedia approach is an ideal style for the advancement of the broad and global culture of coffee. Various media tools can incorporate the long path from the coffee plant to the coffee beverage in one unified, but diverse source. The once used text book with photos of the coffee plant and coffee drink can now become vivid by integrating the text book media source with the video source leading to the newer tools such as the CD-ROM and internet sites.

The CD-ROM

No longer a novelty, the CD-ROM has become one of the leading education sources and a standard in the software distribution media. Presently there are more than 6,000 titles available throughout the United States alone. Its overwhelming popularity throughout the world is due to its numerous advantages. Today the CD-ROM is easily attainable and available on both PC and Apple platforms. Not only is it easy to acquire and operate, but it also is able to hold a vast amount of information, up to 650 megabytes or the equivalent of a twenty volume encyclopedia set, on one small disc at a considerably low wholesale price. The CD-ROM printing costs average around $1.00 per copy. However, the growing popularity is not solely due to its marketing and financial advantages. Multimedia combines text, sound, picture and motion into one unique and portable source. The combination of these features offers an optimal learning environment whether in the classroom, at home, or in the office. This tool can be used by many ages as well as in all academic and business areas. As previously stated, the CD-ROM is ideal for the widespread coffee habitat and culture. It offers a new way of explaining coffee, its history and other aspects of from the plant to brewing. By using short simple texts accompanied with images and videos, the coffee concepts can be easily learned. In addition to the text, photos and videos, interaction is required by the user, thereby offering a simulated "hands-on" experience which would otherwise generally be unavailable due to the great costs incurred from traveling to coffee regions, plantations and roasting companies.

The Planet Espresso is the first CD-ROM available on the market dedicated to the instruction of coffee. It is divided into two sections: the ***cup*** and the ***nut*** each of which are divided into subsections covering the various aspects of espresso coffee. The ***nut*** is a tool primarily for bartenders who have problems making a perfect espresso. Answering a series of simple questions leads to a diagnosis regarding their problems with hints in correcting them and thus leading to their perfect cup (figure 1).

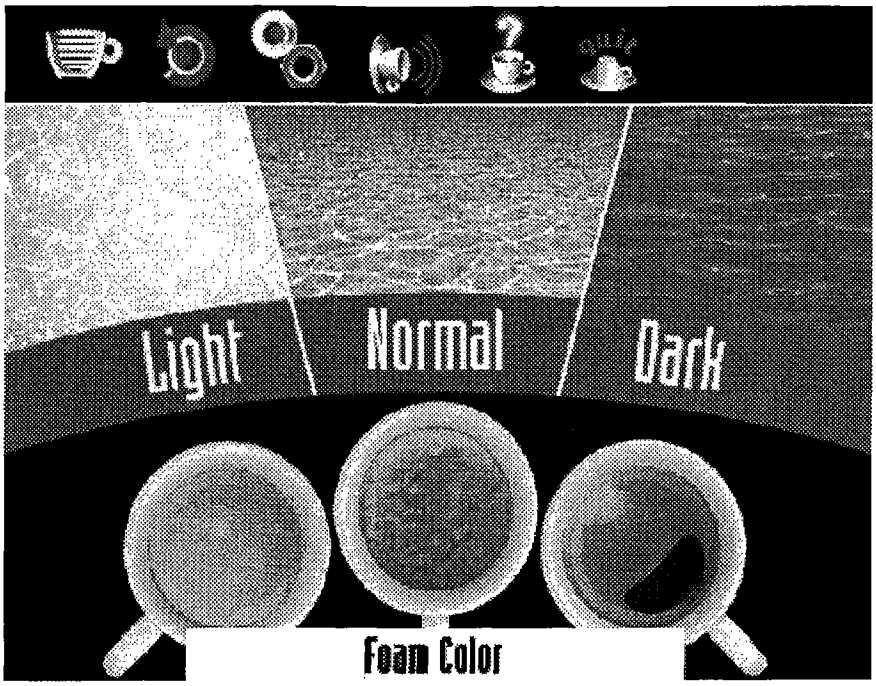

FIGURE 1. The Barman's tool. The Nut is designed to diagnose problems with espresso preparations. By clicking on the area applicable, methods to resolve espresso preparation problems are found.

The Planet is subdivided into four sectors (figure 2) dedicated to the knowledge of espresso coffee. "What is It about an Espresso?" includes the true espresso definition as well as the description and characterization through the smell, taste, touch and sight senses. "From Planet to Pack" offers the step-by-step coffee processes: how coffee is grown and how it gets to the coffee shop, supermarket or store while "At the Bar" demonstrates the different stages in preparing an espresso based on the type of machine being used: Conventional, Fully Automatic, and E.S.E. And for the espresso market, "The Market" gives a market overview, the current styles of drinking an espresso as well as the best way to drink it. The interaction required by the user makes the CD-ROM an ideal media tool. The user decides which path to click on rather than having to leaf through the 200 pages of information that he/she may not be interested in at the moment.

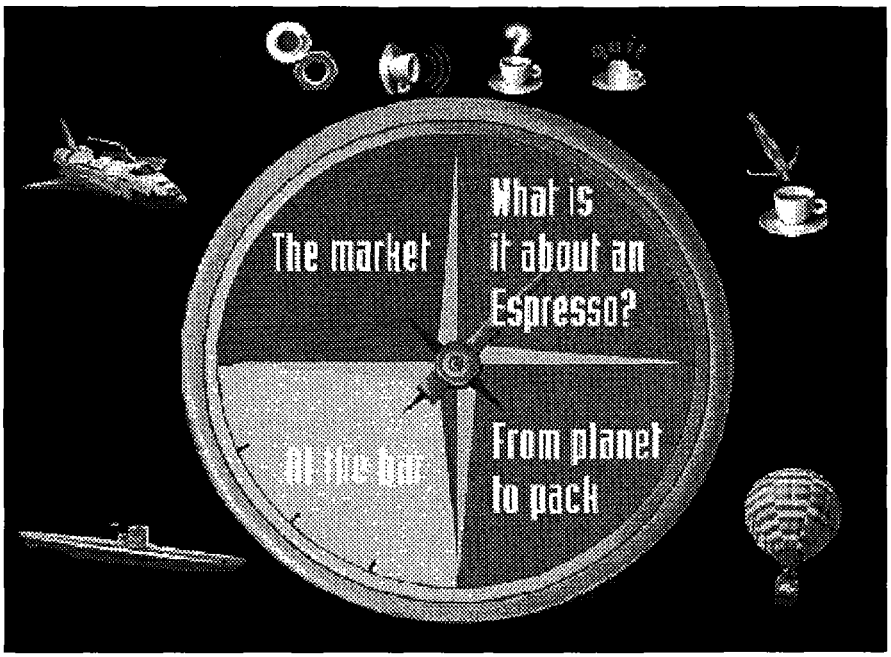

FIGURE 2. The Planet Espresso. Each area of the compass relates to a different part of the espresso culture.

From the CD to the Internet

Although the Planet Espresso is able to provide an efficient means of learning and understanding of the coffee culture, today the CD-ROM has become a transition technology for reaching a larger audience via the internet. The internet holds an even greater influence on Information Technology with its capacity to modify mass communications. Whereas the CD-ROM can target a large audience, it still remains on an individual or group level such as in the classroom or office; the internet, also in the classroom or office, from one location is able to reach another location and simultaneously another and another. Continually growing there are currently more than 50 million private and professional users with daily access to the various sites and newservers. Further, the internet is updateable while the CD-ROM, once produced, is not. Unlike the CD-ROM, the millions of users on the internet are able to have their information updated by a click of a button. To a greater extent the internet offers the same advantages as the CD-ROM. Through a series of clicks the user can choose which path he wants to take.

The teaching principles of both the CD-ROM and the internet are similar through the combination of texts, sounds and videos, yet through the internet the information goes one step farther. The internet is currently the largest databank of information open and free worldwide. The coffee culture itself can be divided into three branches of the internet system: World Wide Web servers, Newsgroups and Databases. Among the servers there are presently 250 coffee sites, 700 coffee retailers and 200 cybercafès in addition to numerous green and roasted

coffee producers and established coffee organizations such as ICO, FAO and CA&CE. Due to the mass of sources and material offered by so many distinct groups, it is necessary to keep the information current. Falling behind signifies losing to the competitors. As a result, the internet can be safely considered the latest aside from the quickest news source for the coffee world.

Like the CD-ROM, the illyweb provides institutional material offering coffee history, data, characteristics of both coffee and espresso coffee plus other features such as commercial, technical product information, and service information including retail, coffee culture, production, preparation and the scientific aspects of coffee. It covers the entire espresso-making process from growing the coffee to enjoying a properly prepared espresso. The countless photographs, economic data, and information on different types of coffee compiled together narrow the large geographical boundaries of the coffee world into the limitless, but accessible, confines of cyberspace. However, unlike the CD-ROM, the site offers the choice of communication in all these areas. It is an interactive website, where anyone who wants to know more about espresso can communicate directly with the company. At any point during the user's navigation, he/she may ask questions or comments through an email sent directly to the company. Learning and understanding are not only offered through the visuals, texts, and videos, but through the possibility to inquire about any matter presented. Neither the CD-ROM, nor any other media tool can offer this quick source of information and, when necessary, clarification of a topic. Learning is achieved worldwide through one source while questions or doubts which arise during the learning process are not left unattended as might happen when studying from a reference which does not have a means of communication such as studying through films, books or even a CD-ROM..

The main topics on the website, science, art, news, espresso and the company itself, are generally organized through deductive reasoning. Through the various links and hotwords, navigation is simple. The user only needs an idea of what he/she wants to discover, confirm, or answer. For instance, if one wants to learn about the chemical aspects of coffee, he/she logically starts by clicking into the general science category, the "Science of Espresso" (figure 4), which leads to a series of choices including how the coffee is characterized. Choosing the characterization offers another set of choices among which is the chemical aspect of coffee. Starting from the general and moving towards the specific keeps the site organized and allows the individual to easily find the information he/she is looking for in addition to receiving an overall picture of espresso and coffee.

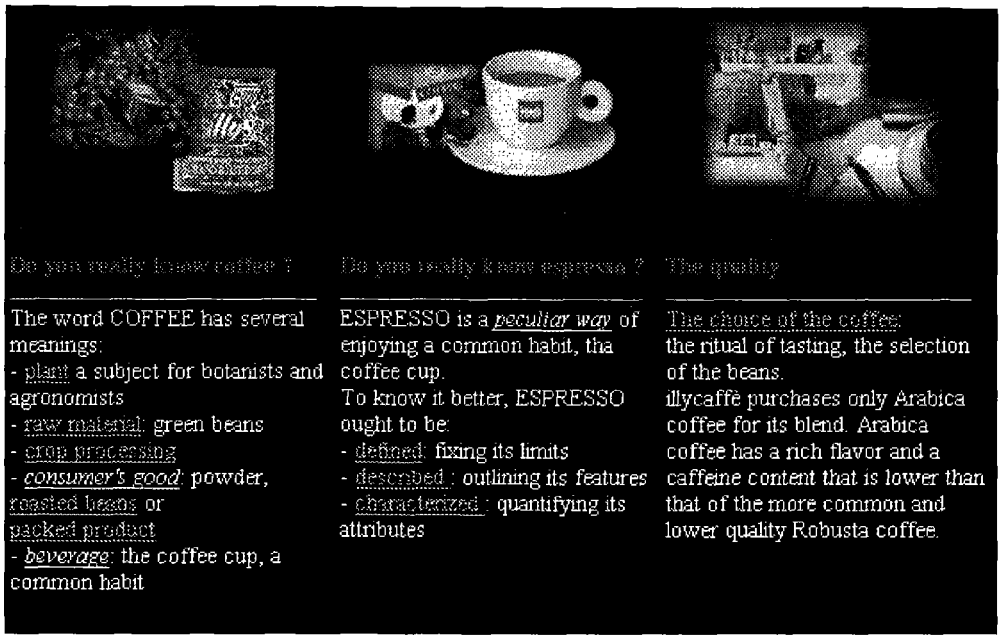

FIGURE 4. The Science of Espresso. Clicking on the underlined words leads to more detailed and specific information of espresso coffee

CD-ROMS, the internet and other media tools have rapidly grown in popularity due to their multi-purpose uses. Not only do they serve as a resource of information, but they can be used as commercial and marketing tools in addition to teaching aids in both the business and academic world. The multimedia approach has become the latest and most effective means of universal communication and learning in many business and educational fields. In the coffee sector it is not only an ideal approach, but a functional way to spread espresso coffee information worldwide. The combination of the teaching approaches is one of the most effective ways of transferring concepts to large widespread groups of individuals. The fusion of texts, sounds and videos coupled with the required interaction on the part of the learner allows the individual to deeply involve himself in the area of interest not always tangible in the real world. Through the multimedia approach the vast territory of the coffee culture cannot only be found in one location, but can be updated universally, directly, and instantaneously. Multimedia offers companies, individuals or entities the opportunity to provide, spread, and acquire decades of espresso and coffee information at a small cost in an almost immediate fashion thereby gaining extra time to be dedicated to the research of espresso and coffee.

SUMMARY

A NEW WAY TO SPREAD COFFEE KNOWLEDGE: THE MULTIMEDIA APPROACH

Coffee culture and coffee knowledge has spread by word of mouth, papers, articles and conferences. As technology advanced, information has continued to spread through these means as well as through the use of films, slides and videos. Today, it is possible to expand these learning approaches with the use of multimedia technology, integrates in a non linear context pictures, text, sound and video.

The multimedia presented, in the form of a CDROM, offers a new proposal of learning: knowledge and facts related to various aspects of coffee, from the plant to brewing, are described with plain text supported by images and short videos. By combining different media, one can better transfer the concepts at hand.

Furthermore, this method offers interaction from the user thereby allowing an individual to deeply involve himself in all coffee areas, that are not readily at hand due to distance, or other unavailable resources.

RÉSUMÉ

UNE NOUVELLE FAÇON DE REPANDRE LA CONNAISSANCE DU CAFE: UNE APPROCHE MULTIMEDIA

La culture et la connaissance du café se sont diffusées de bouche à oreille, à travers les journaux, les articles et les conférences.

Au fur et à mesure que la technologie avançait, l'information a continué à se répandre à travers ces moyens et également par l'utilisation de films, diapositives, vidéos. Aujourd'hui, on peut développer les méthodes de connaissances en utilisant la technologie multimédia qui, dans un contexte non linéaire, comprend: photos, texte, audio et vidéo. Le support multimédia présenté sous forme de CD-ROM offre une nouvelle proposition d'apprentissage: la connaissance et les informations concernant les différents aspects du café, de la plante jusqu'à la préparation, sont décrites par un texte clair et simple accompagné d'images et de brefs vidéos. A travers l'emploi des différents médias, il est possible de mieux matérialiser les concepts.

En outre, cette méthode offre à l'usager la possibilité d'interagir et de pouvoir s'intéresser à tous les secteurs du café qui, à cause de la distance, ne sont pas à la portée, ou à d'autres ressources non disponibles.

KINETICS AND DEVELOPMENT OF BOILER SCALE FORMATION IN COMMERCIAL COFFEE BREWING MACHINES

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1 Introduction

Water is the second raw material, which is needed for coffee brewing (see figure 1). Water content of the ready final coffee brew is about 98 %, thus the influences of water quality are very important.

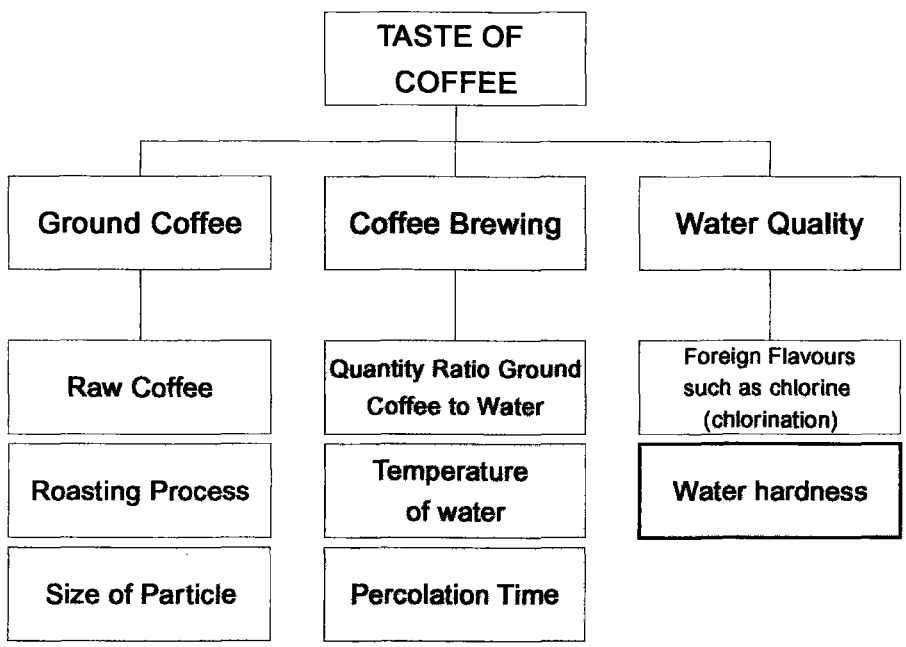


Figure 1: Taste of coffee brew depends on several variables

Water with a high hardness downgrades the coffee beverage taste. The ideal hardness is 8 - 10 °dH [1]. 1 °dH [German hardness degree] corresponds to 10 mg/l CaO. Boiling of water which is hard due to bicarbonate salts of calcium and magnesium (temporary hardness) will drive off CO_2 and precipitate insoluble carbonates. Simple boiling, however, will soften temporarily hard water, but not permanently hard water.

2 Material and Methods

2.1 Preparation of water

For the experiments water with a high concentration of HCO_3^- was needed. Therefore solid calcite was dispersed as a fine powder in drinking water of the city of Braunschweig ($c\ Ca^{2+}$ = 16.3 mg/l, $c\ CO_3^{2-}$ = 2.4 mg/l, $c\ HCO_3^-$ = 25.6 mg/l). The solid calcite was dissolved by bubbling gaseous carbon dioxide into the stirred suspension. But now the pH-value of the water was too low, thus compressed air was bubbled through the water until the lime / carbon dioxide equilibrium was reached. The pH-value was measured with a pH-meter.

2.2 Assays

Ca^{2+} concentration was determined as well by complexometric titration with ethylenediaminetetraacetate as by atomic absorption spectrophotometry.
The concentration of CO_2 and hydrogencarbonate ion was estimated from measured pH-value and determined capacity of acidity ($K_{a4,3}$ and $K_{a8,2}$) and of basicity ($K_{b8,2}$) [2].

3 Results and Discussion

3.1 Lime / Carbon Dioxide Equation

The reaction kinetics of the precipitation effect of temporarily hard water when such hard water is heated has been examined by STUMPER [3].

$$Ca^{2+} + 2\ HCO_3^- \rightleftharpoons CaCO_3 + H_2O + CO_2\uparrow \qquad (1)$$

The steps involved in this reaction are summarised in the following reaction equations:

$$Ca(HCO_3)_2 \rightleftharpoons Ca^{2+} + 2\ HCO_3^- \qquad (1a)$$

$$2\ HCO_3^- \rightleftharpoons CO_3^{2-} + H_2CO_3 \qquad (1b)$$

$$H_2CO_3 \rightleftharpoons H_2O + CO_2\ (\text{aqueous}) \qquad (1c)$$

$$CO_2\ (\text{aqueous}) \rightleftharpoons CO_2\ (\text{gaseous}) \qquad (1d)$$

$$Ca^{2+} + CO_3^{2-} \rightleftharpoons CaCO_3\ (\text{aq}) \rightleftharpoons CaCO_3\ (\text{solid}) \qquad (1e)$$

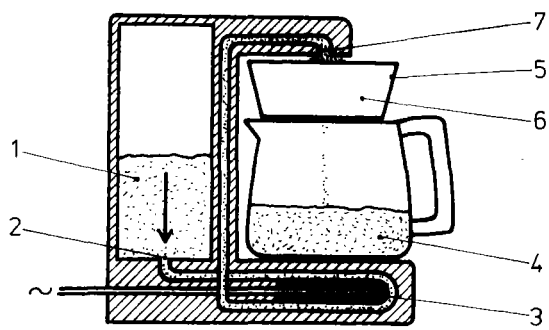
Equations (1c) and (1d) show that the solubility of carbonic acid depends on:

- the partial pressure of CO_2 in the atmosphere in contact with the water surface,
- the saturation concentration of CO_2 (gaseous) in liquid water (HENRY-DALTON-law), which decreases with increasing temperature.

3.2 Brewing Principles

Commercial coffee machines use many types of coffee brewing. The "water softening" by heating of water with a high temporary hardness depends on the kind of heating. This is the reason why different heating principles were examined by us. The following common type of household machine uses the principle of a continuous water flow heater and the restaurant machine works according to the boiler principle (water is stored at brewing temperature).

3.3 Home Percolation of Coffee



1 Water storage tank 2 Valve 3 U-shaped heater
4 Coffee beverage 5 Funnel 6 Ground coffee in paper filter
7 Transfer pipe

Figure 2: Household coffee maker

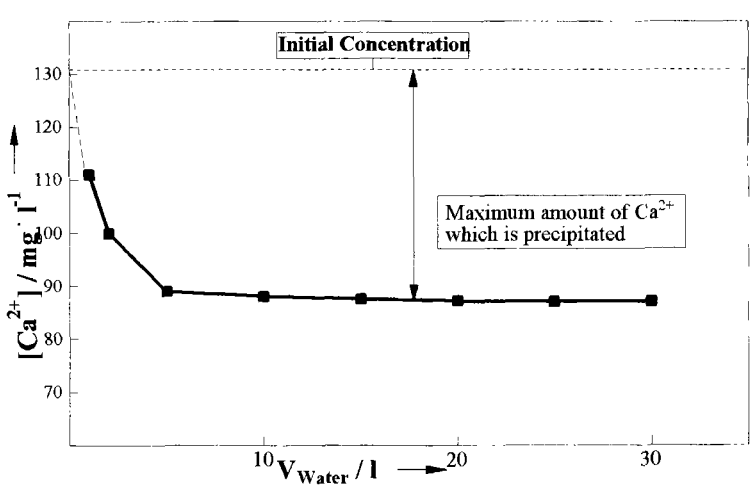
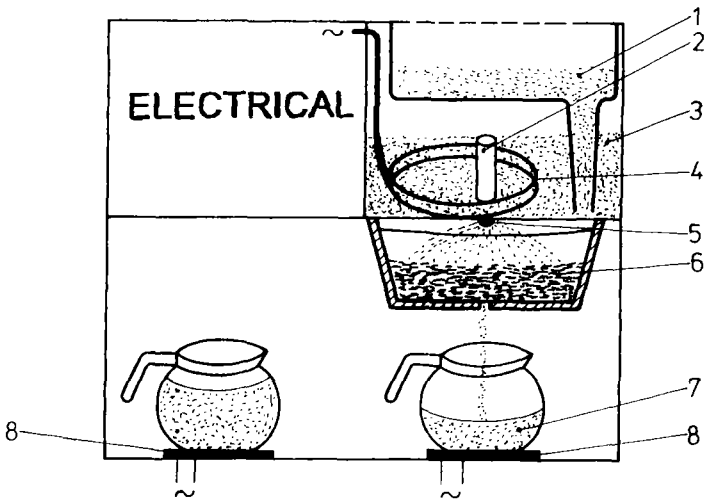


Figure 3: Change in hardness of decoction water as a function of volume prepared. Initial concentration c Ca^{2+} of decoction water = 130,6 mg/l

The principle of coffee brewing, as demonstrated in figure 2, is for household machines very popular in central Europe.
The ground coffee is placed in a funnel with a suitable paper filter (5,6). The cold water flows from the storage tank by gravity into the heater (3). The machines are constructed such that the U-shaped heater (3) is used both for boiling the water and warming the plate. The boiling water is pushed by the vapour produced through the transfer pipe into the funnel with ground coffee in the paper filter. Following percolation of the ground coffee bed and filtration the coffee beverage is collected in a vessel.
After the deposited boiler scale in the household machines has been removed by washing with weak acid solutions the water softening effect is not very efficient because no $CaCO_3$ nuclei are left (see figure 3). Before crystals can grow nuclei must be present. The nucleation step in crystallisation (the rate of crystal growth is very low → concentration of Ca^{2+} in the hot water is high) is then followed by crystal growth. When the U-shaped heater has accumulated a uniform precipitation layer of boiler scale a maximum deposition of boiler scale is reached .

3.4 Restaurant Coffee Brewing

Many restaurant coffee makers use the boiler principle for water heating, which is demonstrated in figure 4.



1 Cold water
2 Transfer pipe
3 Hot water
4 Heating coil
5 Nozzle sprayer
6 Funnel with paper filter and ground coffee
7 Coffee beverage
8 Plate warmer

Figure 4: Restaurant Coffee brewer, which works as a boiler (storage of hot water)

This type of coffee machines does not boil water, but is designed to hold water at 98 °C at atmospheric pressure. Thus coffee can be brewed immediately, if necessary. The boiler vessel contains 3.3 litres of hot water. Maximal 1.5 l of cold water are added from the top onto the false bottom (1). The cold water does not mix with the hot water, but forms a colder layer underneath. While the cold water is heating up the hot water from the water surface flows through the nozzle sprayer (2,5) into the funnel with paper filter and ground coffee (6).

Following the new addition of water the water temperature in the boiler decreases only at the bottom. At the water surface (this part of water is used for the extraction) the temperature is changed only by a few degrees (figure 5).
The extraction process only relies on gravity, and it is similar to the coffee brewing with the household machine.

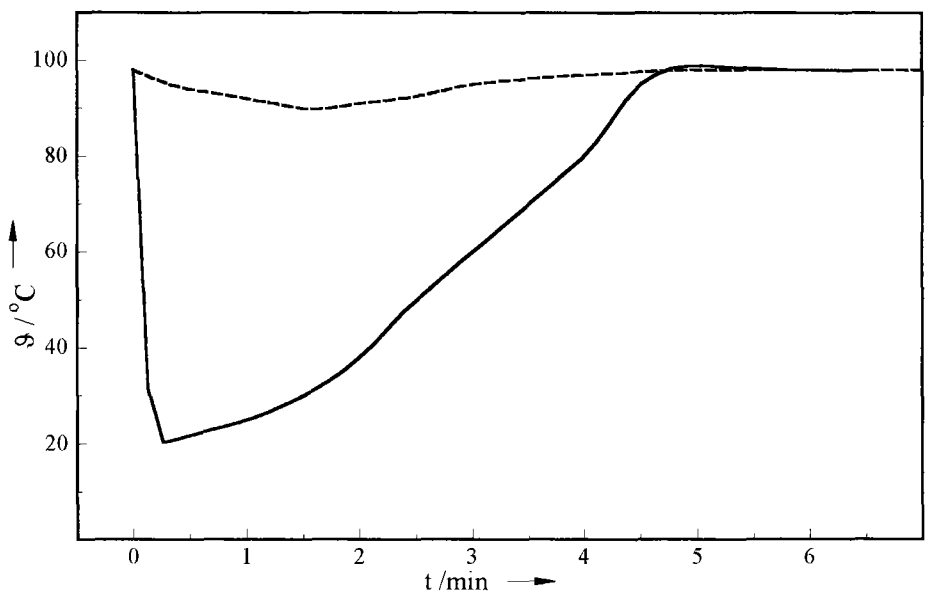
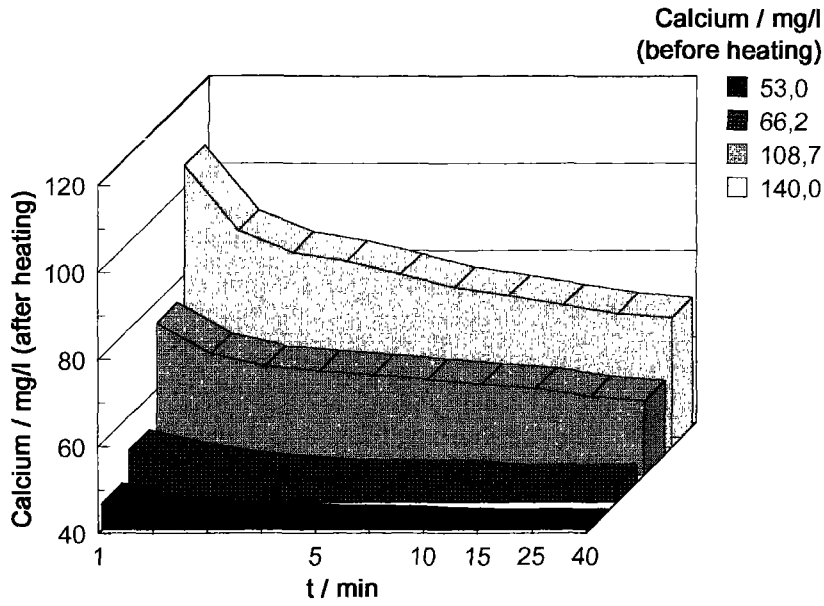


Figure 5: Temperature-time-diagram within the boiler at the water surface (- - -) and at the bottom (—)

CO_2 (g) escapes from the water into the atmosphere during the storage of water at the decoction temperature; the longer the storage time the higher is the amount of boiler scale which is deposited (see figure 6). It is advisable to provide a suitable opening for partial release of vapour carrying away the CO_2 evolved ("venting"). Nucleation sites (e.g. marble pieces) may facilitate precipitation at positions other than the heating coils.



Initial c Ca^{2+} in decoction water mg/l	Decrease in c Ca^{2+} %
140.0	53
108.7	49
66.2	35
53.0	25

Figure 6: Remaining concentration of Calcium depends on the time of heating of make-up water and initial concentration

4 References

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3 STUMPER, R. (1931): Zeitschrift für Anorganische und Allgemeine Chemie, **202,** 227-276.

Acknowledgements

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MESSAGE D'UN CHERCHEUR POUR L'AMÉLIORATION DE LA PRODUCTION CAFÉIÈRE PAR UNE MEILLEURE UTILISATION DES RÉSULTATS DE LA RECHERCHE

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Il m'a été donné, lors des XVème et XVIème Colloques de l'ASIC, à Montpellier puis à Kyoto, de montrer, en m'appuyant sur les productions moyennes à l'unité de surface obtenues dans les différents pays producteurs de café, que cette production moyenne était extrêmement faible par rapport à celles qui sont obtenues non seulement dans les stations de recherche, mais aussi par certains caféiculteurs. On constate en effet que si, dans ces cas, les rendements peuvent être de 3 tonnes/hectare de café marchand pour le robusta, et de 4 tonnes/hectare pour l'arabica, une production de 1500 kg pour l'une et l'autre de ces espèces ne devant pas être considérée comme un exploit, la moyenne mondiale n'est que d'un peu plus de 500 kg à l'hectare, beaucoup de pays se situant à des niveaux bien inférieurs.

Il apparaît ainsi, à l'évidence, qu'une énorme distance sépare le possible du réel, et que l'immense majorité des caféiculteurs ne mettent pas en pratique les recommandations de la recherche. Pourtant de nombreuses et coûteuses opérations ont été conduites depuis longtemps dans la plupart des pays producteurs de café dans le but d'améliorer la caféiculture par le transfert en milieu rural des connaissances accumulées par les chercheurs.

Dans mes précédentes interventions citées au début de cet exposé, j'avais tenté de mettre en évidence quelques-unes des raisons qui ont conduit à ce qu'il faut donc bien appeler un échec. Je ne reviendrai pas ici sur ces éléments et me permettrai de vous renvoyer à la lecture des Actes des deux Colloques précédents. Mais dans la suite logique de ces textes, je voudrais centrer mon propos d'aujourd'hui, plus en détail, sur le point précis de la saisie du message scientifique et de sa transmission. C'est un élément fondamental du problème posé; c'est aussi un sujet de controverse entre chercheurs et développeurs: combien de fois en effet n'ai-je pas entendu les chercheurs regretter que l'on ne suive pas leurs recommandations, persuadés qu'ils sont de la pertinence et du réalisme de leurs résultats, combien de fois n'ai-je pas entendu dire en contrepoint que les propositions de la recherche sont trop compliquées, ou trop coûteuses dans leur application, ou trop ignorantes des contraintes des paysans, pour tout dire, inadéquates ! Si, dans la suite de cet exposé, je serai amené à faire état d'erreurs commises dans le cadre de certaines opérations de développement, ce ne sera pas pour attiser ce genre de controverses, mais au contraire pour en tirer les conséquences raisonnables et tenter

de trouver les formules d'entente entre les chercheurs et les développeurs, entente sans laquelle les uns et les autres agiront indépendamment, se feront d'inutiles reproches, en pure perte et au détriment des paysans, qui, en quelque sorte, et d'une manière ou d'une autre, sont cependant les financeurs des activités des uns et des autres.

Je traiterai donc successivement les points suivants:

1.- Ne pas dissocier les différentes propositions de la recherche, mais les transmettre dans leur globalité car elles constituent un paquet technique cohérent indissociable.

2 - Respecter scrupuleusement les données de la recherche, sans les modifier pour des raisons de commodité et/ou de coût.

3 - Considérer que la recherche ne peut faire de miracles, et qu'on ne peut transformer la caféiculture sans effort: l'adoption d'une technique, nouvelle par rapport à ses habitudes, représente pour le paysan un investissement plus ou moins coûteux en travail et/ou en intrants achetés, investissement qu'il ne peut faire à bon escient que s'il en comprend bien les raisons et les enjeux, d'où l'obligation d'un important effort de formation.

4 - En conséquence, agir sur l'environnement socio-économique des paysans pour qu'ils soient en mesure de se saisir des propositions qui leur sont faites (formation technique et à la gestion, accès au crédit, groupements en coopératives ou autres types d'organisations professionnelles, etc.).

5 - Intervenir autant que faire se peut sur la politique des prix, afin que les paysans soient raisonnablement rémunérés.

1 - La nécessité de considérer le paquet technique proposé comme un tout indissociable doit être en permanence présente à l'esprit. On peut illustrer cette nécessité par quelques grands traits :

- s'il est par exemple recommandé d'utiliser telles ou telles variétés d'arabica ou tels ou tels mélanges de clones de robusta, ces variétés ou ces clones devront être plantés selon les normes définies par les chercheurs après de longues années d'expérimentation au champ, en particulier en ce qui concerne les densités de plantation, variables selon la structure de ces végétaux, selon leur vigueur et selon les caractéristiques des terroirs qui ont une énorme influence sur le développement de la plante: on ne plantera pas de la même façon un Caturra à port nain occupant peu de place et un Mundo Novo à port élevé, on ne les plantera pas au même écartement à 800 mètres ou à 1500 mètres d'altitude; à une variété donnée, à un clone donné, sont liés des modes de mise en place qui leur sont adaptés ;
- ceci étant admis, on devra appliquer une taille rigoureuse, sachant que le caféier fleurit sur le bois d'un an, et que le bois qui a fleuri ne fleurira plus; la taille a pour objet de provoquer la formation du bois florifère, et par conséquent de préparer la récolte future: elle est l'élément clé de la production caféière, et il ne sert à rien de planter une variété ou un groupe de clones à haut potentiel productif, si on ne leur donne pas, par la taille, la structure source de bois neuf leur permettant d'exprimer au mieux leurs potentialités ;
- mais il ne sert à rien de provoquer, par la taille, la formation d'un bois florifère abondant, si l'alimentation minérale est insuffisante pour permettre la croissance de ce nouveau bois, et le développement des fruits qu'il est destiné à porter en abondance : une fumure minérale adaptée au niveau de production escompté, dans les conditions de sol et de climat où l'on se trouve, doit être impérativement apportée, sous peine de rendre inopérants les efforts de taille consentis et d'aboutir rapidement à l'épuisement de la plante, avec mort des rameaux; inversement, apporter une fumure minérale sans tailler ne répondra pas à l'attente car les rameaux anciens sont de moins en moins nombreux du fait de l'élagage naturel du à la sénescence et n'ont qu'un segment florifère court, de plus en plus court avec l'âge ;
- s'il est maintenant universellement reconnu que l'expression du potentiel productif est meilleure en plein ensoleillement que sous ombrage, cela suppose que la fumure minérale soit apportée en quantité suffisante, sinon on va vite à l'épuisement de la plante ; inversement, apporter une fumure minérale à des caféiers sous ombrage n'a que peu d'intérêt car il est bien connu maintenant que l'absorption des éléments minéraux ne se fait pas correctement dans ces conditions: on ne ferait que provoquer le filage de rameaux peu fructifères, et la poussée d'inutiles gourmands ;
- les conditions pour une forte production ne seront réunies que si, parallèlement à la taille et à la fumure, un entretien soigné des plantations est assuré, évitant la concurrence des adventices, des graminées en particulier, grosses consommatrices des engrais destinés aux caféiers ;
- mais il ne sert à rien d'avoir une production abondante si les mesures de lutte phytosanitaire nécessitées pour

la protection des feuilles et des fruits ne sont pas appliquées contre les grands aléas parasitaires qui guettent ici et là, les caféiers et leur production, et qui sont capables de destructions massives, ruinant définitivement tous les efforts consentis par ailleurs ;

- la production arrivée à son terme devra être traitée dans les conditions les meilleures pour une valorisation maximum, donnant tout son sens aux travaux effectués jusque là: la cueillette sera faite au stade convenable de maturité des fruits sous peine de perte de poids et de qualité, les cerises devront être traitées selon des méthodes maintenant bien au point pour donner aux grains leurs qualités marchandes optimums.

On voit bien, à la lecture de cette séquence d'actions, qu'elles sont indissolublement liées pour constituer un paquet technique cohérent qu'il faut appliquer sans faille de la pépinière à la mise en sac du produit marchand: chacune de ces actions représente en effet une étape ayant des répercussions sur les autres, positives si elle est mise en œuvre, négatives dans le cas contraire.

Or on lit très souvent dans la littérature spécialisée, que les paysans sont réticents à appliquer dans son ensemble le paquet proposé: on veut bien apporter des engrais, mais on répugne à tailler; on veut bien planter sans ombrage, mais on n'apporte pas la fumure qui s'impose alors; il n'est pas rare de rencontrer des plantations constituées de caféiers de port nain et de caféiers de port élevé, plantés aux mêmes écartements, dans une grande confusion, etc.

Une des causes de la stagnation de la productivité à un niveau bas, tient au fait que l'on n'a pas su faire comprendre suffisamment qu'une opération isolée, choisie parmi celles qui figurent dans le paquet technique proposé par la recherche, ne peut avoir d'effet positif, ou en tout état de cause, qu'un effet très limité.

La première recommandation que je pourrai donc faire ici, est de toujours veiller à ce que ce paquet technique soit appliqué dans son ensemble sans en dissocier les éléments, même si l'un ou l'autre de ces éléments apparaît plus attractif, plus facile à mettre en œuvre, moins coûteux que les autres: isolé, il ne sert à rien. Il appartient aux services d'encadrement des paysans, sous quelque forme qu'ils se présentent, de leur faire comprendre cet impératif.

2 - Le respect scrupuleux des données de la recherche doit être une règle intangible.

Or on peut lire très souvent que les paysans, lorsqu'ils se saisissent d'une des recommandations de la recherche, ne l'appliquent pas à la lettre, mais l'interprètent à leur manière, la modifient dans une mesure plus ou moins grande pouvant aller jusqu'à un véritable travestissement, le plus souvent pour minimiser la contrainte financière ou en travail qu'elle représente, ou par suite d'une mauvaise compréhension des conséquences de sa non application rigoureuse. C'est vrai pour le recépage cyclique des caféiers multicaules, qui devrait être total pour répondre à ce qu'on en attend, mais, que l'on essaie de transformer en une sorte de taille tournante conservant un ou plusieurs axes, avec pour résultat, sous le couvert des frondaisons que l'on a ainsi malencontreusement conservées pour garder un minimum de récolte, le filage des rejets qui n'auront jamais la structure leur permettant de produire convenablement. Dans le domaine phytosanitaire les exemples sont nombreux où le paysan ne fait pas tous les traitements chimiques recommandés pour une lutte efficace contre tel ou tel parasite ou ravageur; une telle pratique conduit à un résultat nul, ou très modeste comparé aux efforts qui sont malgré tout consentis, surtout lorsqu'il s'agit de combattre une maladie cryptogamique: dans ce cas en effet on est confronté à un problème de tout ou rien, et il faut que les développeurs sachent persuader l'agriculteur que le succès n'est pas proportionnel au nombre des interventions; trois traitements au lieu de six ne conduisent pas à un demi-succès, mais sont effectués en pure perte, et il vaut mieux s'abstenir.

Lorsque c'est l'agriculteur lui-même, dans l'ignorance où il se trouve, qui décide ainsi de tronquer les recommandations qui lui sont faites, on peut à la rigueur l'excuser. Mais lorsque le message scientifique est modifié par ceux qui ont pour mission de le transmettre, la chose est grave. Et pourtant cela existe. J'ai personnellement été témoin d'un cas particulièrement éloquent: bien qu'il ne touche pas la caféiculture, mais la cacaoculture, il peut être cité ici car les problèmes de transfert de technologie sont identiques pour les deux cultures. S'agissant de la lutte chimique contre le Phytophthora megakarya, agent de la pourriture brune des cabosses du cacaoyer au Cameroun où cette maladie détruit au moins 50% de la production, et beaucoup plus en maints endroits, des travaux expérimentaux rigoureux effectués pendant de longues années, avaient conduit, à l'époque où l'on ne disposait que des fongicides cupriques devant être fréquemment renouvelés, à recommander dix à douze traitements annuels appliqués à deux semaines d'intervalle, et consommant au total par hectare et par an, de 1500 à 2000 litres d' une bouillie à 1% minimum d'oxychlorure tétracuivrique titrant 50% de cuivre métal. Ces traitements testés en vraie grandeur pendant trois ans,

avaient fait passer la production de 350 kg à 800 kg à l'hectare sur les 5000 hectares que comptait cette expérience de transfert, et ce dans des conditions parfaitement rentables. Malgré cela, les responsables de l'organisme d'encadrement chargé de la modernisation de la cacaoyère camerounaise, décidèrent ex abrupto que la formule était trop lourde, et donnèrent pour consigne, dans le cadre de la généralisation de la protection à l'ensemble de la cacaoyère, de faire seulement six traitements annuels pour un total de 800 litres/ha/an d'une bouillie allégée dosée à seulement 0,8% du produit cuprique recommandé. Pendant vingt ans d'une telle dérive, le problème de la pourriture brune des cabosses est resté identique à lui-même, avec 50% de pertes en moyenne, malgré des dépenses considérables.

Heureusement, une erreur aussi grave est exceptionnelle, du moins on peut l'espérer. Elle est citée ici non pour condamner en bloc les responsables du développement, mais seulement pour en tirer des leçons pour le futur. Sans doute peut-on penser que les responsables de cette opération n'ont pas voulu sciemment nuire aux paysans, ni au pays et qu'ils ont seulement agi par manque total de compréhension du problème qui leur était posé. Car ils ont été guidés par des raisons financières ou de facilité de transmission (il doit en effet être plus facile d'obtenir l'adhésion des paysans pour six traitements annuels ne consommant qu'une faible quantité de fongicide, que pour douze nécessitant plus d'efforts et plus du double de fongicide), alors qu'ils auraient du se ranger à l'avis souvent réitéré des chercheurs qui savaient bien que ces demi-mesures ne pouvaient conduire qu'à l'échec, compte tenu du phénomène épidémiologique auquel ils étaient confrontés, compte tenu aussi des caractéristiques des fongicides disponibles à l'époque.

D'une manière plus générale, il faut comprendre que, lorsque le paquet technique semble difficile à appliquer, ce n'est pas ce paquet, ni les opérations qui le constituent, qu'il faut modifier ou réduire, mais les surfaces auxquelles ils doivent être appliqués. Prenons l'exemple d'un agriculteur possédant quatre hectares de caféiers ayant, dans les conditions de l'extensif, une productivité de 300 kg de café marchand à l'hectare. Il récolte donc 1200 kg de café marchand au total, certes sans intrants achetés ou presque, et avec un faible apport en travail, consacré à un entretien minimum et surtout à la cueillette. S'il décide d'adopter les recommandations de la recherche, mais avec des moyens financiers et en force de travail lui permettant de les appliquer à la lettre seulement sur deux hectares, et s'il a la sagesse de se limiter effectivement à ces deux hectares, il pourra récolter 1500 kg par hectare soit 3000 kg. Si au contraire il n'a pas cette sagesse, et s'il disperse ses efforts financiers et en travail sur les quatre hectares plantés, n'apportant ainsi que la moitié des engrais nécessaires, une taille insuffisante, un entretien peu soigné, sans doute augmentera-t-il quelque peu sa production globale qui atteindra peut-être 500 ou 600 kg à l'hectare, soit un total de 2000 à 2400 kg. Pour la même dépense en intrants achetés, il récoltera donc moins, et il travaillera davantage, car il est plus coûteux de répandre de l'engrais sur quatre hectares que sur deux, il est plus coûteux d'entretenir, même mal, quatre hectares que deux, il est peut-être dix fois plus coûteux de récolter 2000 à 2400 kg de café sur quatre hectares que 3000 kg sur deux hectares.

Ces chiffres ont pour but d'indiquer une ligne de conduite: "travailler mieux sur des surfaces réduites" devrait être le mot d'ordre dans toute opération de développement, en proposant le paquet technique de la recherche dans sa globalité, en appliquant à la lettre les consignes données pour chacune des opérations qui le constituent, et ce, sur les surfaces compatibles avec les possibilités de chacun, plutôt que de "saupoudrer" des quantités modestes d'intrants et de travail sur des surfaces trop grandes pour en tirer un réel bénéfice. A la limite, il faut comprendre qu'un petit agriculteur qui ne dispose que d'un hectare sur lequel il produit en mélange ses vivriers et 200 ou 300 kg de café, devrait s'occuper convenablement de 200 caféiers groupés dans une petite partie de son terrain et occupant pour la même production, moins d'un cinquième d'hectare, libérant ainsi les quatre cinquièmes de sa terre où ses vivriers, conduits en culture pure, pourront recevoir les soins qui correspondent à leurs exigences, sans la gêne que constituent pour elles les caféiers, et, réciproquement, sans gêner les caféiers. Il est temps de faire comprendre que polyculture n'est pas association; cette dernière n'étant compatible avec le caféier qu'à des périodes bien particulières de la vie de ce dernier, où il laisse de la place libre pour d'autres cultures, c'est-à-dire les deux années qui suivent la mise en place au champ des jeunes plants, les deux années qui suivent chaque recépage dans le cas de l'adoption du recépage cyclique.

Ce principe de l'application intégrale du paquet technique et de chacun de ses éléments, n'est d'ailleurs pas incompatible avec le souci de rentabilité; au contraire, s'agissant par exemple de traitements phytosanitaires, ils peuvent paraître économiquement lourds lorsqu'on doit les appliquer à des plantations à faibles rendements, conduites sans beaucoup d'autres soins, mais il est clair que leur poids économique, rapporté au kilo de produit récolté, sera réduit s'ils sont appliqués à des plantations ayant une haute productivité obtenue par la mise en pratique de l'ensemble du paquet technique proposé: en effet, si l'on pose qu'il faut deux fois plus de produit et de travail pour traiter un caféier portant deux kilos de café, que pour traiter un caféier ne portant que 300 grammes — et ce parce que les caféiers les

plus productifs ont un plus grand développement ---- il faudra cependant au moins trois fois moins de produit et trois fois moins de travail pour traiter 300 kg de production portée par 200 arbres que pour traiter les mêmes 300 kg portés par 1000 arbres. Ceci confirme bien l'intérêt de la formule "travailler mieux sur des surfaces réduites". On devrait aller plus loin dans la formulation des objectifs visés par une opération de développement et proposés aux paysans: au lieu de parler de moderniser une surface exprimée en hectares, ce qui effraie les agriculteurs qui se croient alors dans l'obligation de moderniser la totalité de leur surface plantée, il faudrait présenter le problème sous une forme très différente, et proposer de moderniser, dans un premier temps au moins, la production obtenue par le paysan, exprimée en poids de produit récolté; de cette manière le paysan comprendrait plus facilement que l'effort demandé est davantage à sa portée puisque ne devant alors s'appliquer qu'à une surface limitée.

Cette exigence de respect des résultats de la recherche implique bien évidemment que ces résultats soient pertinents, et qu'ils aient été obtenus en prenant en compte les contraintes qu'entraîne, pour le paysan, toute adoption d'une technique nouvelle pour lui. Les chercheurs doivent donc tenter d'obtenir des résultats dont la mise en pratique soit économiquement acceptable. C'est bien le sens des orientations de la recherche depuis un certain nombre d'années: après s'être, d'une façon générale, consacrée au problème de l'augmentation des rendements par la sélection de variétés à haute productivité, la mise au point des moyens de la lutte directe efficace contre les aléas parasitaires majeurs, et la définition des modalités optimums de conduite culturale y compris l'usage des engrais pour la majorité des cas, elle s'oriente maintenant vers des solutions plus qualitatives que quantitatives susceptibles d'alléger la contrainte paysanne. Parmi les objectifs prioritaires actuels de l'amélioration génétique est en effet inscrite la résistance à l'égard des parasites et une moindre attractivité pour les ravageurs, afin de réduire les coûts de la protection phytosanitaire; on essaie aussi de trouver des formules raisonnées pour la production endogène de l'indispensable élément azoté, par l'exploitation de Légumineuses diverses associées aux caféiers; on s'oriente vers l'exploitation des propriétés des mycorhizes pour que les plantes utilisent au mieux les ressources minérales des sols; on cherche des formules diverses de lutte biologique pour au moins réduire les dépenses nécessitées par la lutte chimique directe contre les ravageurs, etc. En bref, même si quelquefois encore les solutions proposées par la recherche peuvent paraître coûteuses, en particulier dans la lutte contre parasites et ravageurs du fait de réalités bioécologiques incontournables dans l'état actuel des choses, les chercheurs sont bien conscients de la nécessité d'être "pratiques", et tentent de progresser dans ce sens, au pas à pas, au fur et à mesure de l'acquisition des connaissances. Cela implique qu'ils restent au plus près des réalités du monde paysan pour élaborer les propositions qui lui seront faites, et, s'il ne peuvent évidemment pas être à la fois chercheurs et développeurs, c'est en dialoguant avec ces derniers qu'ils pourront répondre à cette exigence, sachant de plus que ce contact est de nature à rétroalimenter la recherche en lui demandant soit de mieux préciser ses propositions, soit de les adapter à des situations diverses.

3 - Mais il convient d'être honnête, et de ne pas laisser croire que l'on trouvera la formule magique permettant une haute productivité sans travail, et sans intrants.

La voie génétique permettra sans doute, grâce aux progrès en cours et à prévoir, de résoudre au mieux certains problèmes parasitaires, mais elle ne changera sûrement pas de si tôt la nature profonde des caféiers qui continueront à produire sur le bois jeune, et demanderont donc toujours un investissement en travail pour la taille, sous une forme ou sous une autre, qui est à l'origine de ce bois. Peut-on croire aussi que la production d'une partie de l'azote utile au caféier par l'association des Légumineuses sera gratuite, alors qu'il faudra réguler la croissance de ces plantes pour qu'elles répondent à ce que l'on attend d'elles sans qu'elles deviennent gênantes ou carrément nuisibles ? Est-il sain de laisser croire que la lutte biologique se fera sans contrainte et sans dépenses, alors que l'élevage des parasitoïdes est une affaire délicate, nécessitant un minimum d'installations et un personnel éclairé pour les amener à maturité en nombre suffisant au moment le plus opportun pour assurer leur efficacité ? On pourrait ainsi énumérer nombre d'autres éléments qui, dans le futur, apporteront peut-être un plus et un certain soulagement à la tâche de l'agriculteur, mais ils ne seront jamais une substitution gratuite à ses efforts actuels.

Je crois qu'il faut être clair: trouver les formules les plus économiques en travail et en intrants achetés doit être le souci constant des chercheurs, tout le monde peut s'accorder sur ce point.

Mais tout ce qui a été dit précédemment montre bien que, si l'on veut que les données de la recherche soient utilisées et bien utilisées, non seulement elles exigeront un effort, mais elles devront être saisies par des esprits éclairés. On aimerait pouvoir dire que les techniciens en charge du transfert des connaissances soient tous suffisamment avertis et formés pour que, d'eux au moins, ne surviennent pas les dérives signalées précédemment. On ne peut donc trop

recommander que ces agents au rôle fondamental pour l'évolution du monde rural, avant d'être lancés sur le terrain, soient éduqués directement par les chercheurs qui ont mis au point les techniques à divulguer, afin qu'ils en comprennent bien les mécanismes biologiques qui en sont les fondements, les liens qui les rendent indissociables, et la nécessité impérative qu'il y a à les appliquer à la lettre compte tenu des réalités bioécologiques concernées: ils ne devraient plus ainsi être tentés de les simplifier, de les tronquer, de faire dans le paquet technique proposé par la recherche, des choix plus ou moins malheureux.

Les développeurs étant eux-mêmes bien formés aux techniques qu'ils ont à divulguer, ils doivent avoir à leur tour pour tâche de former les paysans pour que ces derniers soient à même de les comprendre, et par conséquent de les appliquer intelligemment et sans dérives. Cet effort de formation technique des paysans est fondamental: certes il ne serait guère raisonnable ni réaliste, de vouloir en faire des savants, mais il faut trouver les mots simples, et les méthodes adaptées à chacun pour que le message soit saisi et accepté, ce qui suppose bien sûr que nombre de routines, d'idées préconçues, de pesanteurs ancestrales soient bousculées. Dans les campagnes, et ceci s'adresse aux politiques, est-ce que cette formation ne devrait pas commencer à l'école, parallèlement à l'éducation de type classique ? Des "leçons de choses", b-a-ba des connaissances nécessaires à la compréhension des techniques à mettre en œuvre pour telle ou telle culture, bien orientées dans le sens de ce qui sera le futur des enfants lorsqu'ils seront adultes et qu'ils reprendront les plantations et les champs de leurs parents, devraient être inscrits aux programmes des classes primaires et du premier cycle du secondaire: des enfants ainsi formés verraient sans doute d'un œil plus favorable leur devenir d'agriculteurs, et seraient peut-être moins enclins à quitter la terre pour la ville qui ne leur offre généralement aucun avenir.

4 - Voilà donc qui m'amène à un quatrième volet du message que je voudrais faire passer aujourd'hui: la nécessité d'organiser le milieu socio-économique dans lequel évolue le paysan.

Supposons donc que nous ayons à notre disposition les résultats de bonnes recherches, applicables pour une amélioration de la productivité caféière; supposons aussi que nous ayons des agents du développement bien formés à leur tâche, connaissant parfaitement le message scientifique qu'ils ont à transmettre et par conséquent capables de le transmettre dans son intégralité; supposons enfin que nous ayons des agriculteurs suffisamment éduqués pour bien comprendre ce qui leur est proposé pour une amélioration substantielle de leurs plantations.

Est-ce suffisant ?

D'évidence, c'est non. Car, pour que les paysans puissent se saisir des techniques qui leur sont recommandées, il faut qu'ils soient placés dans un environnement socio-économique leur permettant de s'en saisir.

Toute innovation, nous l'avons vu, va se traduire, pour le paysan, par un coût, en travail et/ou en intrants achetés, et l'on sait bien qu'il ne dispose ni d'une force de travail extensible, ni des fonds nécessaires pour faire appel à une main d'œuvre extérieure rémunérée, ou pour se procurer les intrants prescrits dans les propositions qui lui sont faites.

Toute opération de développement doit donc impérativement prendre en compte cette dimension socio-économique, indispensable à son succès. A la transmission du message technique, à l'effort de formation technique qui doit accompagner ce message, doivent être associées des actions susceptibles de modeler le milieu socio-économique dans lequel évolue le paysan: on devra ainsi s'attacher à organiser les paysans en coopératives ou autres formes d'associations, de telle sorte, par exemple, qu'ils aient accès au crédit lorsqu'il est plus facilement attribué à des groupes solidaires, ce qui leur permettra de disposer des moyens financiers qui leur manquent pour créer des stocks d'intrants disponibles en quantité et en qualité au moment voulu (ce qui est rarement le cas sans cette organisation), ou pour constituer des ateliers communs de traitement du produit, garants d'une meilleure qualité, et points de départ d'une commercialisation directe poussée aussi loin que possible en aval, leur assurant une certaine indépendance vis-à-vis de la chaîne traditionnelle d'écoulement de leur production, et donc une meilleure rémunération.

Si ces efforts d'organisation ne sont pas inclus dans les programmes de développement, le paysan restera étranger à ces programmes, car il n'aura pas les moyens de substituer à ses activités et à ses dépenses traditionnelles de fonctionnement les tâches nouvelles qui lui seront demandées. Mais cette intégration du paysan dans ce nouvel univers suppose qu'il ait reçu, parallèlement à la formation technique évoquée plus haut, une formation à la gestion certes élémentaire: c'est donc vers une véritable professionnalisation du paysan que doivent tendre tous les efforts de ceux qui sont à son contact pour l'amener à un niveau de compréhension de son métier tel qu'il saura profiter des innovations qui lui sont proposées.

Tout étant en place pour que passe la bonne parole, cette bonne parole ne passera cependant que si l'on a une méthode de transfert efficace: si l'on se contente de diffuser des textes ou des films, si bien faits qu'ils puissent être, si

l'on se limite à établir des champs de démonstration, si parfaits qu'ils soient, on ne convaincra pas: tous ces supports ne vaincront pas la méfiance des paysans qui y verront, à juste titre dans la plupart des cas, un certain côté artificiel, en ce sens que, s'agissant par exemple des champs de démonstration, ils sont conçus, établis et conduits par des acteurs rémunérés par ailleurs et non pas intégrés dans l'économie de l'activité qu'ils prétendent représenter. Si l'on veut que le message passe comme on le souhaite, ce sont les paysans eux-mêmes, une fois éduqués, qui doivent le mettre en application, chez eux, avec toute la progressivité nécessaire pour que cet effort reste compatible avec la vie de son exploitation.

Cette notion de progressivité de la mise en application des connaissances est fondamentale : on ne peut imaginer qu'un caféiculteur applique d'un seul coup, sur l'ensemble de sa surface les innovations proposées qui peuvent être lourdes en travail et en intrants, et s'accompagner d'un délai important pour les rentrées monétaires attendues. C'est donc en insérant de façon bien dosée les efforts demandés pour la modernisation de la caféiculture dans l'ensemble de leurs exploitations et de leurs contraintes, que l'on obtiendra le maximum d'adhésion des paysans: les transformations exigées pour la modernisation de la caféière devront être proposées en tenant compte des autres activités courantes et indispensables à la conduite de la ferme.

Ceci rejoint ce qui est dit plus haut: "travailler mieux sur des surfaces réduites". On obtiendra la même production sur quelques centaines de caféiers bien conduits que sur quelques milliers d'arbustes laissés en extensif. Cette notion est fondamentale car elle relativise l'effort demandé: une opération de développement n'implique pas nécessairement qu'elle doit s'appliquer à l'ensemble des surfaces plantées. Ce serait d'ailleurs une aberration en cas de succès car on aboutirait alors à une surproduction désastreuse. Il faut donc que les programmes de développement soient conçus de telle sorte qu'ils visent, dans un premier temps, l'obtention de la production de départ sur une surface aussi réduite que possible, quitte à les étendre progressivement, en fonction des possibilités offertes par le marché.

Sans doute sera-t-il nécessaire aussi, au départ, de faire un effort de sélection des paysans les plus aptes à faire l'expérience proposée, sachant qu'ensuite, par effet "boule de neige", le nombre des adhérents à l'opération augmentera: une erreur à ne pas commettre est de considérer dans une région donnée, tous les paysans comme également réceptifs, et, en conséquence, de diluer les efforts de transfert sur des populations trop nombreuses et trop disparates pour en saisir le bien-fondé.

5 - Mais il ne faut pas se cacher que le succès de toute tentative d'amélioration de la productivité caféière, et par conséquent le succès de toute opération de développement ayant cet objectif, est pour beaucoup conditionnée par la rémunération des efforts supplémentaires qu'elle entraîne, c'est-à-dire, en définitive, par le prix du produit payé au paysan.

C'est sans doute le point le plus délicat, car cette rémunération dépend des fluctuations du marché, international de surcroît, dans le cas d'une production vouée pour sa plus grande part à l'exportation. On ne peut que souhaiter voir s'instaurer un juste équilibre entre l'offre et la demande, afin que les producteurs reçoivent un prix décent leur permettant de s'engager dans la voie de la modernisation. Cette question dépasse bien sûr le cadre de ce Colloque, mais ne serait-ce pas, d'une certaine façon, l'intérêt des pays consommateurs plus riches en général, de faire un effort en ce sens? Et n'appartient-il pas aussi aux Etats producteurs de moduler leurs prélèvements, en fonction de la conjoncture? A ce propos, j'avais signalé, dans mon intervention de Kyoto, que le Guatemala avait renoncé à ses prélèvements au moment de la crise de 1989, ce qui avait pu être considéré comme l'un des éléments ayant permis à ce pays de se maintenir très favorablement sur le marché, au contraire d'autres pays qui n'avaient pas eu cette sagesse et qui ont vu alors leurs paysans prendre la caféiculture en désaffection.

S'il n'est sans doute pas aisé d'agir sur les prix du marché, il est sûrement plus facile de convaincre les paysans en leur expliquant, ce qui est maintenant bien admis, que, pour un prix donné à l'unité de poids de café marchand, la rémunération de la journée de travail est beaucoup meilleure lorsque les rendements à l'unité de surface sont élevés que quand ils sont faibles, et ce dans une proportion qui peut aller du simple au triple, ou même plus; ceci devrait être un argument fort à faire passer dans l'esprit des paysans dont la tendance naturelle est au contraire de croire aux vertus apparentes de l'extensif, en particulier en période de crise: croyant avoir une bonne stratégie ils se pénalisent au contraire. Il n'est pas exagéré de dire que les pays qui ont le plus souffert de la crise de 1989, au point de voir réduire leur production, sont ceux qui avaient une caféiculture très extensive, alors que ceux qui se sont bien maintenus sont ceux où la caféiculture est la plus intensifiée.

Quelques suggestions

De tout ce qui a été dit précédemment, il ressort d'évidence qu'une parfaite cohésion entre le chercheur et le développeur devrait être recherchée et adoptée: seul le dialogue permanent entre ces deux protagonistes permettra d'éviter la controverse stérile qui consiste à dire que les résultats de la recherche sont inappropriés, et, inversement que ces résultats ne sont pas, ou mal, exploités.

Un premier pas serait que les agents chargés du développement soient, comme je l'ai suggéré tout à l'heure, directement formés par les chercheurs. Mais ne peut-on pas penser aller plus loin, en imaginant que les organismes de recherche s'impliquent eux-mêmes plus directement dans le transfert de technologie, en créant leurs propres services d'intervention à ce niveau ? Je me permettrai de citer ici à ce propos, un exemple de réussite particulièrement remarquable, celui de la CEPLAC, dont on peut dire qu'elle a été l'outil efficace du développement de la cacaoculture brésilienne dans les années 60 et 70: cet organisme avait en effet su intégrer sous la même autorité la recherche (CEPEC), la formation avec plusieurs centres spécialisés, le crédit; et la vulgarisation des techniques par l'encadrement des agriculteurs. Cette centralisation de tous les échelons d'activité assurait le passage des recommandations avec le minimum de dérives, sans hiatus majeur entre les concepteurs et les utilisateurs. Sans doute pourrait-on trouver d'autres cas analogues, en Colombie par exemple, avec la Fédération des Cafeteros. Il conviendrait, me semble-t-il, de s'inspirer le plus souvent possible de ces formules d'intégration, pour une transmission plus directe du message scientifique, le dialogue entre chercheurs et vulgarisateurs n'étant pas rendu difficile par des barrières administratives ou autres, et, si le besoin se fait sentir, l'arbitrage apparaissant plus facile au sein d'une même maison.

Comme "on n'est jamais si bien servi que par soi-même", cette intégration serait sans doute la meilleure formule pour la recherche pour faire passer son message; confrontée directement au monde paysan dans sa diversité, la recherche serait de plus mieux placée pour mesurer la pertinence de ses résultats, leurs failles ou imperfections éventuelles, afin d'être mieux à même de les amender. Ce serait aussi la meilleure façon de valoriser ses travaux, et de se créer des ressources.

Je voudrais pour terminer ajouter que, bien utiliser les résultats de la recherche est évidemment une chose souhaitable à laquelle des efforts importants doivent être consacrés. Mais essayer d'utiliser tous les résultats de la recherche serait encore mieux, ce qui permettrait sans doute de toucher un nombre accru de caféiculteurs. Il est en effet des cas où, faute des aménagements nécessaires, de bonnes recherches susceptibles d'améliorer considérablement la productivité, restent éternellement inutilisées: c'est ainsi qu'une méthode de lutte contre l'anthracnose des baies de l'arabica (Coffee berry disease ou CBD, due au Colletotrichum kahawae), inventée au Cameroun il y a maintenant une trentaine d'années, n'a jamais été mise en application autrement que par le planteur chez lequel la recherche l'avait conçue, vérifiée et mise au point; cette méthode très "écologique" consiste à provoquer, par l'irrigation de saison sèche, la floraison précoce des caféiers; par voie de conséquence, les stades jeunes des fruits, seuls vulnérables, se situent en période sèche, hors de la période d'activité du pathogène, et échappent ainsi à la maladie, économisant au total quatre traitements chimiques (deux ou trois traitements restant cependant encore nécessaires pour combattre les rouilles foliaires). Il a été montré en même temps que ces apports d'eau de saison sèche assuraient la floraison totale des arbustes, ce qui est rarement le cas dans les conditions naturelles où cette floraison est souvent imparfaite, évitaient la coulure survenant souvent lorsqu'une période de sécheresse suit la floraison, et garantissaient la tenue maximum des fruits en réduisant considérablement la chute physiologique naturelle; il a été montré surtout que ces apports d'eau provoquaient une croissance des rameaux deux à trois fois supérieure à ce qu'elle est dans les conditions naturelles, préparant ainsi une floraison et donc une production abondante pour la saison suivante. Cette méthode de lutte efficace, non polluante, et que l'on peut considérer par ailleurs comme une assurance pour une production régulière de haut niveau, n'est jamais sortie des revues où elle a été publiée, personne n'ayant fait l'effort de la transférer dans des programmes concrets de développement, intégrant les aménagements nécessaires au stockage de l'eau, alors qu'il y aurait sans aucun doute de nombreux sites, touchés ou non par le CBD, où elle aurait pu être mise à la disposition des paysans pour une considérable amélioration de la productivité de leurs caféière, et, profitant de l'eau mise en réserve, pour un développement d'autres cultures.

N'y a-t-il pas d'autres exemples où, faute des nécessaires aménagement des terroirs, on prive les caféiculteurs d'importants outils de progrès ?

Mais les organismes de recherche sont-ils suffisamment agressifs pour assurer à certains de leurs résultats un destin autre que celui qui consiste à rester dans des tiroirs ou sous forme d'articles dans les revues spécialisées ? Ils ont, en tout état de cause, fait preuve d'une faiblesse coupable en maintes occasions, lorsqu'ils sont restés muets devant les

dérives auxquelles étaient soumis leurs résultats au moment de leur mise en application: je n'en veux pour preuve que l'exemple particulièrement significatif et caricatural du mépris dans lequel ont été tenues les recommandations des chercheurs dans le cas de la lutte contre la pourriture brune des cabosses du cacaoyer cité plus haut.

Conclusion

Pour conclure, je dirai en résumé que l'amélioration de la caféiculture et le mieux-être des caféiculteurs passent par une utilisation des résultats de la recherche dans leur globalité et sans "aménagements" particuliers, ce qui suppose une parfaite compréhension des problèmes à résoudre, et donc un gros effort de formation et d'organisation socio-économique, à charge pour les chercheurs d'améliorer progressivement leurs propositions dans le sens de la valorisation maximum des efforts des producteurs: le dialogue entre chercheurs et développeurs, ou leur intégration dans les mêmes structures, pourrait garantir le succès des opérations de transfert des connaissances en milieu rural, au bénéfice des paysans, ceux-ci pouvant devenir, si on sait favoriser leurs organisations professionnelles, les meilleurs acteurs de leur propre modernisation.

RESUME

L'auteur a fait ressortir (Colloques ASIC Montpellier 1993, Kyoto 1995) la distance séparant les possibilités offertes par l'application des connaissances (3 T/ha de café marchand pour le robusta, et 4 T/ha pour l'arabica), et la réalité (moyenne mondiale de 500kg/ha).

Il est donc bien clair que le plus grand nombre des producteurs n'appliquent pas les techniques proposées par la recherche, et ce malgré l'existence des organismes chargés d'effectuer leur transfert en milieu rural.

Il faut s'interroger sur les raisons de cet état de fait et en tirer les conséquences. L'analyse de quelques situations concernant la caféiculture ou d'autres cultures importantes, car le phénomène ne concerne pas que la production caféière, conduit à faire quelques recommandations:

-1.- Ne pas dissocier les données de la recherche, mais proposer le paquet technique cohérent établi par les chercheurs.

-2.- Respecter les données de la recherche sans les modifier pour des raisons de commodité ou de coût.

-3.- Considérer que l'adoption d'une technique, nouvelle par rapport à ses habitudes, est, pour le paysan, coûteuse en travail et/ou en intrants.

-4.- Agir en conséquence sur l'environnement socio-économique des paysans pour leur permettre d'adhérer aux propositions qui leur sont faites (accès au crédit, groupement en coopératives, aménagement des terroirs, etc.).

-5.- Intervenir sur la politique des prix afin que les paysans soient raisonnablement rémunérés.

Chercheurs et développeurs doivent travailler étroitement ensemble pour que les techniques disponibles soient bien utilisées et toutes utilisées: c'est en s'impliquant directement dans le transfert des connaissances en milieu rural, que les organismes de recherche valoriseront au mieux tous leurs résultats passés, donnant ainsi tout leur prix à leurs résultats futurs.

Ce message s'adresse donc à la fois aux organismes de développement et aux organismes de recherche.

CONSIDÉRATIONS GÉNÉRALES SUR LES PROBLÈMES POSÉS PAR LE TRANSFERT DE TECHNOLOGIE EN MILIEU RURAL

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LE TRANSFERT DE TECHNOLOGIE, SON CONTENU

Introduction

Le transfert de technologie peut être défini comme les opérations par lesquelles les outils, les procédés et les méthodes préconisés en caféiculture sont mis à disposition, puis adoptés par les agriculteurs. Cet exposé se limite aux producteurs de café Robusta de la zone francophone (Côte d'Ivoire, Cameroun, Centrafrique, Togo, Madagascar) et à certains aspects techniques.

Les travaux de recherche conduits dans ces pays (par l'IFCC) à partir de 1958, ont permis d'obtenir un matériel végétal (clones ou hybrides) aux performances considérablement améliorées, comparées à celui des caféières en place.

En effet, ce matériel sélectionné, cultivé selon certaines normes agronomiques (densité, taille, fertilisation, ensoleillement...) :

- permet d'obtenir, en moyenne plus de 1 500 kg/ha de café vert,
- produit des fèves de bonne granulométrie,
- s'agissant de Robusta, présente une tasse au goût franc, neutre et corsé,
- est adapté à son milieu et se révèle résistant (ou tolérant) aux divers parasites et maladies cryptogamiques.

Ces aptitudes se sont confirmées en grandes plantations industrielles (Cameroun, Madagascar) avec des rendements ayant atteint parfois en grandes parcelles 6 tonnes de café vert/ha.

L'IMPACT DU TRANSFERT DE TECHNOLOGIE EN MILIEU RURAL

Le transfert

Le transfert a débuté au cours des années 60 et l'impact sur la production n'est apparu qu'au début des années 70.

Que constate-t-on ?

Rendement en café vert kg/ha

Années	1948-52	1961-65	1970	1980	1989-91	1993-95	1993-95 / 1948-52
Cameroun	396	389	368	300	373	210	0,5
Côte d'Ivoire	264	375	369	239	203	120	0,5
Centrafrique	444	302	369	358	628	568	1,3
Togo	575	515	480	385	324	600	1,0
Madagascar	320	345	300	411	374	370	1,1
Sources FAO - Annuaire de la Production							

A l'exception de la RCA, il s'avère donc que, pour ces cinq pays, les rendements actuels sont inférieurs ou, au mieux, équivalents à ceux du début des années 50. Par contre, en Amérique Latine, le Costa Rica et la Colombie, semblent avoir réussi le transfert de technologie.

Rendement en café vert kg/ha

Années	1948-52	1961-65	1970	1980	1989-91	1992-92	1992-93 / 1948-52
Costa Rica	455	744	789	1 331	1 433	1 468	3,2
Colombie	453	534	534	668	813	905	2,0

Il y a donc bien un problème spécifique au transfert de la technologie caféière.

En effet :

- en Côte d'Ivoire, la caféière ne serait constituée que de 10 à 15 % de matériel sélectionné (environ 150 000 ha),
- au Cameroun, la situation serait comparable,
- au Togo, par contre, une grande partie de la caféière traditionnelle (coffea Nialoui) a été remplacée et reconstituée de matériel sélectionné (clones) sur environ 25 000 ha,
- à Madagascar, seulement 10 % de la caféière serait composée de matériel clonal,
- en Centrafique, aucune évaluation précise n'existe mais il semble que le matériel sélectionné représente moins de 10 % des surfaces.

Le constat est donc qu'après trente ans de diffusion, le matériel végétal sélectionné n'a pas été largement adopté par les planteurs.

Là où il l'a été, l'agrotechnie proposée y a-t-elle été appliquée ? Sur ce point, peu d'enquêtes et d'évaluations ont été réalisées.

On peut toutefois affirmer que :

- les densités de plantation sont respectées,
- les tailles de formation (multicaulie) sont largement pratiquées,
- beaucoup de ces plantations sont en plein soleil ou sous ombrage partiel,
- l'apport d'engrais est irrégulièrement appliqué,
- le contrôle des adventices est très variable,
- la récolte et les traitements post-récoltes demeurent traditionnels.

S'il existe des plantations dont les rendements sont supérieurs à 1 000 kg de café vert par hectare, la plupart ont des productions beaucoup plus faibles et beaucoup de celles qui sont en plein soleil et sans engrais, dépérissent. Dans certaines régions, on a pu même constater que les planteurs avaient abandonné des plantations sélectionnées.

ESSAI D'ANALYSE DU TRANSFERT DE TECHNOLOGIE

Généralités

Les développeurs ont pensé que la diffusion des technologies nouvelles serait réalisée par des structures, relais de l'Administration en milieu rural, accompagnées des moyens financiers nécessaires.

Ces structures (SATMACI en Côte d'Ivoire, SRCC au Togo, CEAMP à Madagascar, ADECAF en Centrafrique, SODECAO au Cameroun) ont été à l'orée des années 60 mises en oeuvre avec l'aide française (FAC et CCCE) relayée et complétée par les aides multilatérales de l'Union Européenne (Fonds Européen de développement - FED), du Fonds Africain de Développement (FAD), de la Banque Africaine de Développement (BAD), de la Banque Mondiale, du PNUD dans certains cas. De plus, les Etats concernés ont toujours apporté leur contrepartie financière à ces programmes.

Ce n'est donc pas l'absence de financements qui aurait bloqué le transfert de technologie. Il y a donc d'autres facteurs qui sont intervenus, de nature économique, structurelle, agrotechnique et sociaux.

Les facteurs économiques

Le prix est une des composantes majeure des décisions du planteur relatives à son intervention sur la caféière. La crise du marché international du café, survenue au cours des années 80, caractérisée par une baisse drastique des cours mondiaux, a montré l'importance de l'interaction prix/production. Deux éléments du prix sont à considérer :

- la stabilité du prix qui sécurise le planteur et favorise les investissements à long terme,
- le niveau du prix, composante des revenus et de la rentabilité.

La stabilité des prix du café qui a prévalu en Afrique francophone, a été favorisée par :

- le maintien d'une parité fixe au sein de la zone franc jusqu'en 1994,
- par les mécanismes de stabilisation mis en oeuvre ; cette politique a très probablement largement incité à la création de caféières extensives.

Elle s'est, par ailleurs, accompagnée de subventions au matériel végétal, aux intrants, à la plantation en Côte d'Ivoire, au Gabon, à Madagascar... qui ont favorisé l'extension des caféières améliorées ; mais le niveau du prix payé aux producteurs a freiné l'adoption des nouvelles technologies intensives fortement consommatrices de capital (main d'oeuvre plus abondante, acquisition d'intrants). Sur ce point la comparaison des prix payés aux producteurs en Afrique et en Amérique est révélatrice des écarts.

Les prix payés au Brésil, au Costa Rica et en Colombie ont été plus fluctuants mais plus élevés que ceux pratiqués en Afrique. Les hauts niveaux de prix ont été plus rémunérateurs et par conséquent plus incitateurs à l'adoption de nouvelles technologies.

A cet égard le tableau suivant est révélateur.

Productivité comparée

	Côte d'Ivoire			Cameroun		Costa Rica		
Niveau technique kg/ha	400	900	1 200	250	900	600	1 000	1 200
Kg de café par J. de travail	5,5	7	8	6	7	6	9	14
Indice	100	127	145	100	117	100	150	233
$ par jour de travail	7,4	9,5	9,9	8,3	8,4	11,5	17,4	25,7
Indice	100	128	134	100	101	100	151	223
Source : B. LOSCH et PETIT HUGUENIN 1991 - P. SFEZ (91-92) dans compétitivité des cafés africains								

En Afrique, comme en Amérique, l'adoption des nouvelles technologies a amélioré la productivité du travail, mais en Afrique, l'insuffisance des prix en a réduit la valorisation, décourageant les caféiculteurs à s'engager plus dans l'intensification et la modernisation des caféières.

Les structures, moyens et techniques

Les prix ne sont pas les seuls facteurs qui ont inhibé le transfert de technologie. Il convient aussi de considérer le rôle joué par :

- les structures de développement,
- les méthodes et moyens de formation,
- les moyens d'accompagnement de la production,
- enfin, la nature même des techniques.

Les structures de développement devaient avoir un rôle déterminant dans la diffusion de la technologie. La nature du cycle du caféier (délais d'entrée en production, montée en croisière...) et les délais de réponse du monde rural à toute innovation impliquaient que l'intervention de ces structures s'inscrive dans la durée et la continuité.

Or ces structures, mises en place au cours des années 1960 ont toutes disparu aujourd'hui et certaines d'entre elles n'ont pas dépassé dix années d'existence (CEAMP à Madagascar, ADECAF en RCA...)

De plus le financement des programmes dont elles bénéficiaient fut renouvelé tous les trois ou cinq ans. Chaque nouveau programme était l'occasion d'inflexions ou de changements plus ou moins profonds.

En outre, les équipes nationales et/ou expatriées ont été fréquemment changées.

Cette discontinuité a été préjudiciable à la perception des messages, à leur acceptation par les planteurs.

Méthodes et moyens de formation.

Des années 60 à la fin des années 1980, les méthodes de formation et de vulgarisation s'appuyaient sur une organisation pyramidale et en cascade, de cadres et de formateurs, souvent fonctionnaires.

Aujourd'hui il est admis qu'un tel système, hérité de la période coloniale, procédant de haut en bas, imposant l'application parfois autoritaire de techniques, sans concertation ni prise en compte des avis du planteur ni des contraintes réelles de l'exploitation, était voué à l'échec ou, tout au moins, peu adapté, à l'éveil du monde rural.

Moyens d'accompagnement techniques

⇒ Matériel végétal

De gros investissements ont été mis en place pour la production du matériel végétal sélectionné, qu'il s'agisse de champs semenciers, de parcs à bois, de propagateurs.

Ces infrastructures ont bien fonctionné au début, mais depuis une dizaine d'années, elles ont périclité dans un certain nombre de pays, de sorte qu'aujourd'hui, beaucoup de planteurs n'ont plus accès à ce matériel sélectionné, ralentissant ainsi son implantation.

⇒ Intrants

La disponibilité en intrants, et particulièrement d'engrais, a été liée essentiellement aux financements extérieurs ; subventionnés ils sont aujourd'hui vendus au prix du marché.

Leur disponibilité a souvent été aléatoire, et leur usage est resté limité en raison :

- de l'éloignement des centres distributeurs,
- du manque de moyens de transport pour accéder aux zones rurales excentrées,
- des prix jugés trop élevés lorsque les subventions se sont taries,
- du montant de l'avance financière à consentir en cours de campagne, jugé à la fois onéreuse et risquée.

La crise caféière a provoqué une stratégie de repli à l'égard des intrants qui semble s'être installé durablement.

⇒ Crédit

Le financement de la création de petites surfaces (0,25 à 0,50 ha) peut être supporté par le planteur lui-même. Au delà, le financement devient plus lourd. En l'absence d'aides et de subventions le planteur est contraint d'avoir recours au crédit bancaire ; malgré l'existence d'institutions de crédit (FONADER au Cameroun, BNM à Madagascar, BNDA en Côte d'Ivoire), les planteurs ont eu beaucoup de difficultés à accéder au crédit en raison :

- de l'absence d'agences de proximité,
- de procédure administratives jugées compliquées,
- de taux et de durées de prêts inadaptés à la production caféière,
- de garantie, demandées que les planteurs ne pouvaient satisfaire.

Depuis, la disparition de la plupart de ces institutions, a créé un vide rendant l'accès au crédit de faisance valoir ou à long terme encore plus problématique.

Les thèmes techniques

Certains thèmes techniques (densité, taille de formation) se sont bien diffusés dans le cadre des interventions des structures de développement ; il est vrai, que dans bien des cas, leur application conditionnait l'attribution d'aides et de subventions. Par contre les thèmes relatifs à la fertilisation, à l'entretien des parcelles, à la taille (quinquennale), à l'ombrage sont très irrégulièrement adoptés.

⇒ La fertilisation demeure insuffisamment pratiquée. L'absence ou l'insuffisance d'engrais ne permet pas d'atteindre les niveaux de production spécifiques des clones ou des hybrides, de sorte qu'en plein soleil, les caféiers, dès la deuxième récolte, présentent des signes de dépérissement décourageant les planteurs.

La fertilisation paraît donc bien être un thème qui a contribué, en Afrique, à freiner la diffusion de la technologie.

⇒ L'entretien des parcelles, en plein soleil, implique :

- d'effectuer des désherbages manuels (4 à 6 passages par an), consommateurs d'une main d'oeuvre abondante souvent non disponible sur l'exploitation. Sa mobilisation extérieure est parfois difficile et toujours onéreuse et souvent incompatible avec la trésorerie des planteurs,
- ou, d'utiliser des herbicides, dont la disponibilité, comme celle des engrais est aléatoire et dont le coût est considéré comme onéreux et difficile à financer.

  En l'absence d'un désherbage suffisant, la concurrence des adventices, en particulier graminéennes devient rapidement, comme le manque d'engrais, préjudiciable à la production de la caféière.

⇒ La récolte des caféières à haut rendement (1 tonne ou plus/ha, soit environ 5 tonnes de cerises) pose problème aux planteurs :

- quant à la disponibilité de main d'oeuvre, analogue en période de pointe à celle posée par le désherbage,
- quant aux moyens de transport pour acheminer les cerises de la caféière au lieu de séchage,
- quant aux équipements de traitement de post-récolte (aires de séchage, décortiqueuses, ...).

⇒ La taille quinquennale se révèle mal acceptée ; elle est, soit différée dans le temps ou même non réalisée, soit remplacée par des tailles traditionnelles dites tournantes, ou par l'écimage.

Cette attitude se justifie :

- par le travail jugé considérable du recépage et des entretiens complémentaires (désherbage accru, égourmandage) et de son coût récurrent,
- par la baisse de production et du revenu.

⇒ L'ombrage des caféières a été, semble-t-il, un thème mal vulgarisé et par conséquent mal adopté.

Deux situations prévalent :

- celle de l'absence d'ombrage, instituant une conduite en plein soleil, qui implique une forte intensification (apports conséquents d'engrais, désherbage plus nombreux) peu compatible, on l'a vu, avec l'environnement technique des planteurs.

- A de rares exceptions, cette situation conduit à une faible productivité, voire des échecs.
- celle de l'excès d'ombrage qui réduit le niveau d'entretien et de fertilisation et par conséquent celui des intrants mais ne procure que des productions très faibles.

Les cas de plantations à ombrage équilibré et contrôlé restent l'exception.

Les motivations socio-économiques

L'essor de la production caféière africaine a débuté dès l'après guerre, à l'époque coloniale, porté par des cours rémunérateurs et Poussé les contraintes de l'Administration. De plus la culture du café était la principale source de revenu monétaire, destinée à payer l'impôt per capita et à accéder aux biens de consommation. Mais, rapidement, l'opportunité de produire cacao, hévéa, palmier, fruitiers ou vivriers (riz, manioc, tubercules...) s'est présentée aux planteurs.

Ces nouvelles cultures pratiquées au sein des exploitations ont conféré un intérêt tout relatif au café.

Les stratégies

Les planteurs ont intégré dans leur stratégie les contraintes liées à la production, les éléments de rentabilité, les risques encourus...

En terme de contraintes :

- physiques, ils ont su mieux adapter les spéculations aux contraintes naturelles,
- de travail, ils l'ont mieux intégré dans leurs choix stratégiques et analysé les notions de disponibilité de main d'oeuvre, de coût, de productivité, de pénibilité et de valorisation et l'ont mieux reparti à l'égard des spéculations.

La caféiculture intensive ne s'est pas trouvée mieux placée face au cacao, moins consommateur de travail et plus rémunérateur en terme de valorisation du travail.

La caféiculture (comme la cacoculture) a été l'opportunité d'appropriation des terres dans la conquête des fronts pionniers. Les aides de toute nature semblent avoir été mises à profit pour faciliter les nouvelles occupations. En respectant les normes de création des caféières les agriculteurs bénéficiaient des subventions; au-delà, dans la mesure où aucune aide n'accompagne la maintenance, les planteurs ont travaillé en extensif.

C'est la stratégie d'accumulation extensive pour laquelle on plante l'arbre dans une vision d'extension du patrimoine.

En conclusion, malgré leur intérêt, les nouvelles technologies caféières n'ont été, en Afrique francophone que partiellement adoptées et appliquées ces trente dernières années en milieu rural.

Cette situation a des causes multiples et complexes. Elle relève des politiques de prix, de l'environnement structurel et économique, des stratégies des agriculteurs (trop occultées des développeurs) et de la nature même de l'agrotechnie proposée par la recherche.

Ces échecs conduisent à s'interroger sur la nécessité d'une approche différente du transfert de technologie.

QUELLES APPROCHES POUR L'AVENIR DU TRANSFERT DE TECHNOLOGIE ?

Les conditionalités

L'acceptation des techniques passe par l'adhésion des producteurs de café.

Cette adhésion est acquise par la sécurité des revenus, par l'amélioration et l'adaptabilité des techniques à leurs contraintes et à leur environnement.

Ces conditions sont des préalables incontournables à l'adhésion des producteurs.

En effet les planteurs ne s'engagent que lorsque les débouchés existent et lorsque la politique des prix leur offre :

- une stabilité des cours
- des prix rémunérateurs et incitatifs qui permettent la rentabilité de l'activité caféière et une valorisation du travail au moins égale à celles des autres spéculations qu'ils peuvent pratiquer. Mais aujourd'hui les producteurs agissent dans le cadre du marché mondial qui, en période de prix déprimés, laisse une faible marge de manoeuvre.

L'accès à l'information et à la formation relatives aux nouvelles technologies est indispensable. De la même manière

- les intrants (engrais, pesticides, petits équipements) devraient être en permanence accessibles à des prix compatibles avec l'économie caféière,
- l'organisation de la profession caféière doit devenir un appui au transfert de technologie.

  L'Etat a longtemps maintenu sous sa tutelle l'organisation de la production et de la commercialisation du café.

  Son désengagement conduit progressivement les producteurs à s'organiser pour mieux défendre leurs intérêts économiques face aux négociants mais aussi pour s'informer et se former, accéder aux intrants et au crédit.

  Dans un proche avenir, on peut espérer l'émergence d'une profession interlocutrice des intervenants de la filière et en particulier de la recherche.

Le rôle de la recherche

Afin d'être en prise avec les vrais problèmes des exploitations caféières la recherche se devra d'établir des liens plus étroits avec la profession et les producteurs.

Ces liens plus étroits devraient :

- permettre d'analyser les stratégies, les motivations et les attentes des producteurs,
- faciliter un dialogue permanent dans l'élaboration des programmes, leurs mise en place et leur ajustement,
- faciliter la décentralisation de la recherche et l'insérer davantage dans le milieu rural, avec l'opportunité de mieux intégrer les caféiculteurs dans le processus de définition des programmes. Cette approche n'est pas sans poser des problèmes institutionnels et financiers ; mais cette évolution paraît indispensable,

- permettre de proposer des thèmes techniques acceptables par les producteurs (matériel végétal adapté aux conditions locales, niveau de fertilisation réduit, techniques intégrant l'amélioration de la productivité du travail, ...).

Tous les aspects du transfert de technologie caféière n'ont pu être abordés dans ce propos. On a pu juger de la complexité du sujet où interfèrent techniques, économie, sociologie, évolution des sociétés et des Etats. D'autres aspects : problèmes fonciers ou technologiques et en particulier celui concernant la qualité, dont on sait l'importance pour l'avenir du Robusta, auraient pu être abordés.

Ces considérations montrent la nécessité d'une meilleure écoute et par conséquent d'une meilleure approche du monde rural.

RESUME

L'analyse de l'évolution des rendements/ha du café dans les cinq principaux pays d'Afrique (Cameroun, Côte d'Ivoire, Centrafrique, Togo, Madagascar) producteurs de café Robusta, montre que la production/ha a, selon les pays, régressé ou est demeuré au même niveau que celui atteint au début des années 60. Cette situation à montré que le transfert des technologies amélioratrices de la productivité n'a été que partiel. En effet le matériel sélectionné n'occupe qu'un faible pourcentage des surfaces caféières et les techniques recommandées ne sont que partiellement appliquées.

Les raisons de ce transfert technologique limité sont nombreuses et complexes : politique des prix inadaptée, insuffisance de l'accompagnement technique et économique (approvisionnement en instrants, crédit, matériel végétal) et, plus encore, technologies passe par l'adhésion des producteurs de café ; celle-ci est conditionnée par des politiques des prix appropriés, par la formation et l'information adaptée et par une nouvelle approche de la recherche caféière. A ce titre, elle devrait se rapprocher de la profession et établir des programmes qui correspondent à la demande des planteurs.

DIX ANS D'EXPÉRIENCE DANS LE BASSIN CAFÉIER DE COATEPEC AU MEXIQUE

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Le bassin caféier de Coatepec, situé dans l'Etat de Veracruz au Mexique, a servi de cadre à de nombreuses actions franco-mexicaines de recherche et de développement. L'objet de la communication est d'exposer, dans leurs grandes lignes, les travaux réalisés en insistant non pas sur leur contenu (pour cela, on se référera aux publications existantes) mais plutôt sur leur enchaînement.

I. Le contexte

Au Mexique, la surface en café est de l'ordre de 600 000 ha répartis entre 200 000 producteurs. La production annuelle s'élève à environ 300 000 tonnes de café vert dont 70 à 80% sont exportées.
Plusieurs caractéristiques confèrent une certaine spécificité au bassin de Coatepec par rapport à d'autres régions caféières du Mexique. En premier lieu, le développement de la propriété dite moyenne aux marges des *haciendas* et l'importance prise régionalement par le procès de réforme agraire ont contribué à limiter le nombre et la taille des grands domaines. Ensuite, dans les plus petites structures, la juxtaposition cultures vivrières-café est rare : depuis les années 70, la plupart des agriculteurs minifundiaires consacrent presque exclusivement leur assise foncière au café. Enfin, les modes de conduite des plantations sont relativement homogènes : il s'agit de systèmes "traditionnels" avec cultivars Typica dominants et ombrage diversifié.

La figure I indique la distribution de la surface plantée (environ 24 000 ha répartis entre 6 700 producteurs) dans la région de Coatepec. On remarque que l'essentiel des producteurs (85% de l'effectif) gèrent des plantations de taille inférieure à 5 ha. Les limites des classes de surface de la figure 1 correspondent souvent à des "seuils de différenciation fonctionnelle" entre planteurs :

- un hectare de plantation est une dimension souvent présentée comme seuil de viabilité d'une exploitation spécialisée. Un rapide calcul montre qu'un planteur doit disposer de cette surface pour, en conditions moyennes de prix et de rendement, dégager un revenu monétaire lui permettant de satisfaire les besoins élémentaires familiaux,
- la taille de 5 ha plantés constitue une limite à partir de laquelle on observe des changements dans l'organisation du travail (emploi d'ouvriers agricoles hors récolte, moindre participation de la main d'oeuvre familiale aux activités productives). C'est également à partir de cette limite dimensionnelle

que les planteurs investissent dans des *beneficios* humides (unités de transformation par voie humide). En réalisant de tels investissements, les planteurs prétendent s'affranchir des intermédiaires locaux, acheteurs de café cerise, et ainsi négocier leur production dans de meilleures conditions,

- les exploitations avec plus de 20 ha plantés sortent du cadre de l'agriculture de type familial. Elles sont souvent détenues par des producteurs ayant un profil de chefs d'entreprise gestionnaires de *beneficios* humides et secs ; ceci apparaît sur la figure II qui présente les types de café mis en marché par les différentes catégories de planteurs.

Figure I . Distribution de la surface plantée dans le bassin de Coatepec

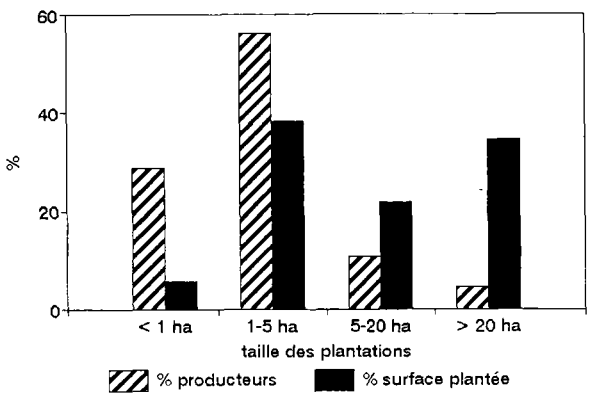
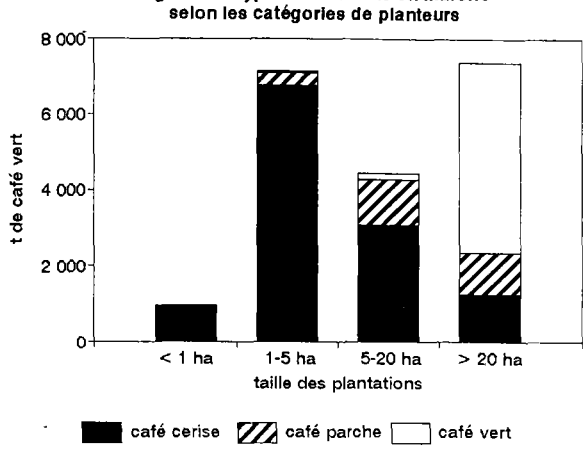


Figure II . Types de café mis en marché selon les catégories de planteurs



II. 1984-1988 : un observatoire des dynamiques agricoles régionales

Les activités de coopération franco-mexicaine commencèrent dans le cadre d'une convention, signée mi-1983, entre l'ORSTOM (Institut français de recherche scientifique pour le développement en coopération) et l'INIREB (Instituto nacional de investigaciones sobre recursos bióticos). L'objet de cette convention était la réalisation d'une étude multidisciplinaire du bassin caféier de Coatepec. Il s'agissait, en particulier, d'analyser le comportement des agriculteurs face à l'instabilité de leur environnement. Celui-ci, défini de manière très large, incluait les aspects physiques du milieu et les facteurs d'ordre socio-économique (Gondard et *al*, 1988).

La problématique était posée de la manière suivante :

- la production caféière est en crise et cette crise va s'accentuer du fait, notamment, de la diminution tendancielle des prix du café et de la progression des dégâts dus à la maladie de la rouille orangée (*Hemileia vastatrix*),
- la propre reproduction des exploitations familiales était compromise et des solutions alternatives devaient être trouvées.

Un observatoire fut donc créé et pris le nom de LIDER (*Laboratorio de investigación y desarrollo regional*). Les travaux du LIDER démarrèrent avec une double approche, géographique et historique. Il s'agissait principalement de rendre compte de la place du café dans l'espace agraire, que constituait le bassin de Coatepec, et de rechercher les facteurs historiques qui expliquaient la formation de cet espace.

Les premiers résultats furent assez surprenants. On peut les résumer de la manière suivante :

- depuis l'apparition du café, vers 1870, le rythme de plantation n'avait pas été uniforme ; aux accélérations, souvent consécutives aux hausses des cours mondiaux, avaient succédé des pauses mais force était de constater que la "tache" caféière n'avait presque jamais cessé de s'étendre,
- la surface en café, loin de régresser, s'étendait notamment chez les petits paysans du Sud du bassin,
- l'effondrement - très relatif - des cours et l'incidence de la rouille - surestimée et limitée aux plantations particulièrement mal conduites - apparaissaient comme des composantes secondaires

de la crise du café. En revanche, celle-ci était fortement alimentée par les dysfonctionnements de la filière étatique chargée de contrôler la production et la commercialisation du grain.

Ces résultats invalidaient largement les hypothèses de départ qui demandaient donc à être reformulées. Le LIDER n'y parvint pas ; il éprouva des difficultés pour redéfinir une ligne directrice susceptible de donner une cohérence à ses activités. Jusqu'à son arrêt, en 1988, un grand nombre de travaux furent néanmoins conduits. Pris individuellement, beaucoup d'entre eux ont une indéniable valeur scientifique. Mais il est difficile de les replacer dans une problématique d'ensemble.

Sans prétendre à l'exhaustivité, on peut prendre quelques exemples de travaux illustrant les principales thématiques abordées par le LIDER :

- pédologie. De nombreuses études se rapportant aux caractéristiques des sols de la région de Coatepec ont été entreprises. On citera en particulier la réalisation d'une carte morphoédaphologique du bassin (Rossignol, 1987) et la description des grandes catégories de sols (andosols, sols ferralitiques, brunizems).
- socio-économie. Parmi les travaux relevant de ce domaine de connaissance, deux méritent d'être soulignés. Le premier a porté sur le fonctionnement des unités de production d'un *municipe* du Sud du bassin caféier (Bernard, 1988) ; le deuxième est une analyse rétrospective d'un groupe social dénommé "bourgeoisie caféière" (Beaumont, 1988) qui a dominé l'aval de la filière régionale jusqu'aux années 50 et a joué un rôle déterminant dans la consolidation du bassin de Coatepec.
- histoire et géographie humaine. La question qui sous-tend les travaux menés dans ce domaine est : comment les hommes ont, à travers le temps, construit l'espace qui les entoure ? Il s'agit donc d'études, conduites à différentes échelles, visant à intégrer les facteurs (écologiques, économiques, sociaux, etc.) qui ont interagi pour générer le système agraire actuel. Elles ont notamment débouché sur la réalisation d'un atlas graphique (Marchal et Palma, 1985) et sur des analyses détaillées de micro-régions du bassin caféier, en particulier le *municipe* de Xico (Hoffmann, 1992).

III. 1988-1991 : un dispositif de recherche-développement centré sur l'exploitation familiale

Les travaux du LIDER, de nature essentiellement cognitive, ont servi de base pour une opération concrète de recherche-développement, menée dans le cadre du projet DIMAC (*Desarrollo integral de los márgenes del área Xalapa-Coatepec*) associant le CIRAD, la SARH (Ministère mexicain de l'agriculture) et de nombreux autres partenaires institutionnels mexicains (recherche, formation, développement). Les objectifs centraux de ce projet étaient ainsi définis (Lefort et Muller, 1986) :

- l'intensification. Il s'agissait de maintenir la caféiculture, en termes de tonnage, à son niveau actuel et par conséquent de réduire les surfaces sous caféiers,
- la diversification. Il s'agissait de rendre les exploitations économiquement moins fragiles (ou moins dépendantes du café), en dégageant des terres et du temps pour d'autres cultures.

Dès son démarrage, le projet DIMAC fut confronté au problème de la coordination des activités entre les différents partenaires ; les structures mexicaines étaient en effet fragilisées du fait du désengagement de l'Etat et donc de la forte réduction des moyens alloués aux structures publiques. Par ailleurs, les objectifs assignés, discutés avec les producteurs et leurs organisations, se révélèrent par trop éloignés des réalités. L'intensification se heurtait à l'effondrement des cours (à partir de 1989) qui décourageait tout investissement lourd dans les caféières ; les producteurs préféraient maintenir leurs systèmes "traditionnels", peu performants mais jugés moins risqués car adaptés aux variations d'intensité des soins culturaux (par exemple, arrêt de la fertilisation et diminution des entretiens en cas de mévente).
La diversification n'entrait pas dans les stratégies de la plupart des exploitants, notamment les plus petits d'entre eux.

Face à ce constat, le projet DIMAC resserra ses liens avec la société civile (en particulier, les organisations de petits producteurs) et bâtit ses activités autour de deux thèmes centrés non plus sur l'innovation structurelle mais sur l'amélioration de l'existant. Ces thèmes, complémentaires, étaient

agronomiques et économiques.

la conduite de la caféière

Le projet DIMAC définit un référentiel technique qui visait à améliorer le système traditionnel sans alourdir les coûts de production (Sallée et Pasquis, 1992). Ce référentiel reposait sur trois points :

- l'adoption d'une politique de taille cyclique, alternant écimage et recépage,
- l'aménagement de l'ombrage,
- l'amélioration de la fertilisation en qualité, quantité, fréquence et mode d'application.

Ces points étaient considérés comme indissociables. Néanmoins, l'intérêt des producteurs se porta principalement sur la fertilisation ; ce thème se convertit en véritable "porte d'entrée" ou instrument de dialogue entre chercheurs et producteurs.

La fertilisation fit l'objet d'une démarche de recherche-développement combinant le diagnostic et l'expérimentation. Les résultats de cette démarche ont été présentés dans plusieurs communications dont une au colloque ASIC de San Francisco (1991). On se contentera de rappeler les principaux acquis :

- le diagnostic fut réalisé à l'échelle du bassin caféier. Il se basa sur des prélèvements en horizons de surface d'environ 350 caféières. Les résultats soulignèrent, d'une part, l'acidification et la désaturation de la zone amendée autour du caféier ainsi que la déficience généralisée en potasse et, d'autre part, l'inadéquation de la formule NPK 18-12-6 communément utilisée par une grande majorité de producteurs,
- l'expérimentation visa à valider les traitements proposés par la méthode du diagnostic-sol. Elle fut conduite en milieu réel avec la participation active des producteurs à toutes les étapes. D'une manière résumée, on nota, d'une part, les incidences positives, sur les rendements et la qualité de la récolte, de la fertilisation déduite avec, toutefois, un effet dépressif du chaulage et, d'autre part, l'intérêt économique de cette même fertilisation.

Parallèlement, un programme de fertilisation basée sur la méthode du diagnostic-sol fut proposé à des coopératives de petits producteurs ; il porta sur 320 ha de caféières. L'évaluation indiqua, entre autres, une diminution du coût moyen de la fertilisation et une amélioration de la qualité de la récolte. Elle mit également en évidence les principaux facteurs limitants de diffusion de l'innovation :

- les difficultés d'obtention, par les petits planteurs, d'un crédit de campagne pour l'achat d'intrants,
- la faible disponibilité en engrais simples sur le marché local.

la gestion de l'unité de production

La méthode définie par le projet DIMAC pour le conseil de gestion aux exploitants comprenait :

- l'enregistrement et le traitement de données techniques et économiques de l'exploitation,
- le conseil de gestion proprement dit à savoir la restitution, aux producteurs, des informations issues du traitement précédent.

Cette méthode a été développée durant la période 1988-91 et a concerné une cinquantaine de producteurs répartis dans six groupes "pilotes". Force est de reconnaître qu'elle n'a pas atteint l'objectif fixé, à savoir aider les producteurs dans leurs prises de décisions. Une place trop importante a été accordée à la mise au point du logiciel gérant la base de données ; la qualité des informations qui l'alimentaient n'a jamais été vraiment contrôlée.

IV. 1991-1994 : des actions en partenariat avec les producteurs organisés

A partir de 1991, le projet DIMAC dut tirer les conséquences d'une approche trop centrée sur l'exploitation. La conjoncture était loin d'être favorable : la crise du marché du café se prolongeait et le désengagement de l'Etat s'accentuait. Il apparut nécessaire de renforcer les actions à l'aval de la filière, au niveau des structures de type coopératif. L'hypothèse était que seule une meilleure valorisation de la récolte pouvait inciter les petits producteurs à adopter des innovations améliorant leurs systèmes de production. Les

actions furent principalement conduites avec la ROCA (*Red de organizaciones cafetaleras autogestivas*).

la ROCA : une instance fédératrice de coopératives de petits planteurs

La ROCA fédérait sept coopératives de petits planteurs qui avaient acquis une expérience organisationnelle et avaient pour objectif la prise en charge de la première transformation (gestion d'un *beneficio* humide). Le degré de consolidation ainsi que le niveau d'activités de ces coopératives variaient fortement.

L'analyse justifiant la création de la ROCA était qu'aucune coopérative ne présentait une surface économique suffisante pour, d'une part, valoriser correctement sa production et, d'autre part, intervenir efficacement auprès des institutions publiques. Il convenait donc de s'unir pour, notamment, rechercher en commun des appuis extérieurs (crédits, assistance technique) et des débouchés commerciaux.

La ROCA fut conçue comme une structure souple au sein de laquelle chaque coopérative conservait son autonomie. Certaines de ses activités étaient menées en partenariat avec le projet DIMAC :

- approche de type recherche-développement pour l'amélioration de la conduite des caféières,
- conseil aux opérateurs des usines sur des thèmes visant globalement à améliorer la qualité du café parche produit par les coopératives,
- publication périodique du bulletin *La voz del cafeto* présentant des conseils techniques et rapportant des interviews de producteurs.

le diagnostic de la campagne 1992/93

Suite aux mauvais résultats commerciaux de la campagne 1991/92, la ROCA demanda aux chercheurs du projet DIMAC de suivre de manière rapprochée le déroulement de la récolte 1992/93. Il s'agissait, en évaluant le fonctionnement des coopératives, d'effectuer un diagnostic de situation pour proposer des mesures correctives (Goud et Sallée, 1994). Ce diagnostic porta principalement sur trois points :

- l'évaluation du degré de consolidation des coopératives. Les indicateurs choisis montrèrent que seules deux coopératives présentaient un certain niveau de consolidation. Le fonctionnement des autres usines paraissait largement affecté par des conflits internes,

- le niveau de maîtrise de l'usinage. Les volumes traités durant la campagne 1992/93 par les *beneficios* de la ROCA furent particulièrement faibles en raison, notamment, des rendements au champ relativement bas et des difficultés d'entrer en concurrence avec les intermédiaires locaux pour l'achat ferme de café cerise. De plus, la maîtrise de la transformation fut imparfaite ce qui se traduisit, entre autres, dans la qualité irrégulière du café parche produit.
  On observa sur le plan physique une fréquence élevée de lots mal séchés et un taux toujours important de défauts. En dégustation, la qualité moyenne resta médiocre avec un niveau d'acidité bas. Une fraction significative de la production ne put être classée, selon les normes en vigueur dans le bassin de Coatepec, en café exportable et fut donc écoulée sur des marchés peu rémunérateurs. Plusieurs explications purent être avancées :
  - le contrôle insuffisant de la qualité des livraisons de cerises,
  - pour certains *beneficios*, une mauvaise fermentation des fèves due à un équipement incomplet, une eau de lavage en quantité insuffisante et de qualité médiocre et une mauvaise gestion de l'approvisionnement,
  - une qualité irrégulière du séchage.

- les résultats économiques des coopératives à partir des indicateurs suivants :
  - les marges brutes. Pour cinq coopératives, les marges brutes furent négatives ; pour les deux autres, elles furent très faibles. Ceci s'expliqua par des prix de vente peu rémunérateurs, reflets de la nature des débouchés de l'essentiel de la production des coopératives, ainsi que par des charges d'opération élevées.
  - l'endettement. En fin de récolte, seule une coopérative remboursa intégralement le crédit

de campagne ; les autres, en choisissant de rémunérer les livraisons des membres à un prix voisin du cours moyen du café cerise durant la récolte ne purent apurer la totalité de leurs dettes ce qui alourdit leur endettement déjà important.
- l'investissement. Du fait de leurs médiocres résultats financiers, la capacité d'investissement des coopératives a été pratiquement nulle.

les propositions

Les résultats du diagnostic de la campagne 1992/93 furent présentés dans chaque coopérative. Ceci permit d'instaurer - ou de restaurer - un débat interne, souvent constructif, sur la base d'indicateurs techniques et financiers précis. Des activités de conseil et de formation furent entamées dans les domaines de :

- la planification de l'approvisionnement des *beneficios*,
- la professionnalisation des opérateurs de ces mêmes *beneficios*,
- la réduction des coûts d'énergie et de main d'oeuvre.

Les mesures relevant de la gestion purent être rapidement mises en oeuvre ; en revanche, celles faisant appel à l'investissement furent plus difficiles à appliquer, notamment du fait de la précarité de la situation financière des coopératives et de la disparition de l'aide publique.

C'est en matière d'épuration des eaux résiduaires, domaine sans relation directe avec le processus de production, que les avancées les plus significatives ont été obtenues. Ce thème ne faisait pas partie des demandes prioritaires des coopératives. Néanmoins le projet DIMAC le proposa à l'une d'entre elles ; l'argument était que, à terme, les *beneficios* allaient devoir maîtriser la pollution qu'ils généraient sous peine d'être fortement pénalisés. Une unité pilote d'épuration fut donc installée. Son fonctionnement a déjà fait l'objet de nombreuses publications dont une au XV^{e} colloque ASIC (Castillo et *al*, 1993).

V. Eléments pour une analyse rétrospective

Les principaux enseignements de dix ans d'expérience dans le bassin caféier de Coatepec sont probablement d'ordre méthodologique. Ils résident dans :

- l'**approche interdisciplinaire** d'une problématique rurale complexe

Tout milieu rural est un ensemble complexe qui peut être étudié différemment suivant le point de vue que l'on adopte (par exemple : agronomique, géographique, sociologique). Néanmoins, force est de constater que, dans le contexte du bassin de Coatepec, les meilleurs résultats - en termes de qualité des travaux et d'impact sur le développement - ont été atteints lorsqu'une question donnée était traitée de manière interdisciplinaire.

- l'utilisation d'une **démarche participative** pour l'identification des contraintes et la recherche de solutions

Cette démarche essaie de :

- prendre en compte la diversité du milieu humain et physique,
- s'appuyer sur les demandes formulées par les producteurs,
- instaurer un dialogue entre producteurs et chercheurs afin d'échanger leurs savoir-faire respectifs.

Dans le contexte du bassin caféier de Coatepec, la démarche participative fut principalement utilisée pour le programme de fertilisation en milieu réel et pour celui d'appui à la gestion technique et économique des coopératives. Dans les deux cas, elle a compris une phase de diagnostic réalisé conjointement entre chercheurs et acteurs (producteurs, responsables des coopératives, etc.), une phase d'expérimentations et de mise en oeuvre des propositions et une phase d'évaluation. Les résultats peuvent être considérés comme satisfaisants : des innovations techniques et organisationnelles ont pu être identifiées et certaines, malgré une conjoncture peu favorable (effets de la crise du marché du café entre 1989 et 1994), ont été

adoptées par les producteurs et transformateurs.

- l'**approche globalisante**, *de type filière, pour traiter les aspects de production primaire*

Les différents acteurs économiques qui interviennent au sein d'une même filière ont souvent des objectifs contradictoires ce qui induit les classiques conflits entre producteurs, transformateurs et exportateurs. Au-delà de ces conflits, il existe pourtant un intérêt réciproque - celui du développement de la filière - dont tous les acteurs sont les bénéficiaires potentiels et qui peut entraîner l'émergence de formes de concertation.

Dans le cas du bassin de Coatepec, cette concertation s'est - timidement - instaurée au début des années 90 autour de la question de la qualité. L'idée était de restaurer la réputation du café produit dans le bassin, réputation passablement érodée par la généralisation de pratiques critiquables (par exemple, celle du "gommage" des défauts par mélange de café de qualité inégale). Une association a été créée pour, entre autres, uniformiser les critères qualitatifs en définissant une classification spécifique au bassin de Coatepec et ainsi s'insérer sur le marché international des appellations d'origine.

Cette initiative, à laquelle participait la ROCA, a été appuyée par les chercheurs du projet DIMAC. Il est rapidement apparu qu'elle pouvait se convertir en facteur déterminant d'amélioration des conditions techniques de production et de première transformation : les producteurs et leurs coopératives étaient susceptibles d'intégrer facilement la notion de qualité dans leurs pratiques si celles-ci étaient valorisées - même faiblement - lors de la mise en marché du produit.

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RÉSUMÉ

Le bassin caféier de Coatepec a servi de cadre à de nombreuses actions franco-mexicaines de recherche et de développement. Ces actions, principalement menées durant la période 1984-1994, peuvent être regroupées en trois grandes phases :

- un **observatoire** des dynamiques agricoles régionales (1984-1988),
- un **dispositif de recherche-développement** centré sur l'exploitation familiale (1988-1991),
- des **actions en partenariat** avec les producteurs organisés (1991-1994).

Cette succession de phases n'a rien de linéaire mais elle répond cependant à :

- **une évolution des modalités d'intervention** : une démarche de recherche suivie d'actions concrètes de développement.
- **la prise en compte des nouveaux enjeux** : l'amélioration de la qualité du café et la maîtrise de la pollution générée par les usines de traitement.
- **une adaptation aux changements institutionnels** : le désengagement de l'État et l'émergence d'organisations de producteurs.

Des innovations techniques et organisationnelles ont été proposées aux producteurs et transformateurs mais la crise du marché du café, intervenue entre 1989 et 1994, a fortement limité leur degré d'adoption. Dix ans d'expérience ont également permis de tirer des enseignements d'ordre méthodologique :

- l'intérêt du **travail interdisciplinaire** face à une problématique rurale complexe,
- la pertinence d'une **démarche participative** pour l'identification des contraintes et la recherche de solutions,
- la nécessité d'un **raisonnement globalisant**, de type filière, pour traiter les aspects de production primaire.

PROFESSIONNALISER LES PLANTEURS : RÊVE OU RÉALITÉ ?

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L'intensification de l'agriculture introduit la notion de rendement optimum par unité de surface.

Si l'intensification est raisonnable, elle contribue à la valorisation des efforts, mais aussi à l'économie des sols et au respect des ressources naturelles tout en assurant la sécurité matérielle de l'agriculteur.

Mais l'intensification implique avant tout l'adhésion de l'agriculteur à sa "professionnalisation". Cette professionnalisation de l'acteur principal de l'environnement et du développement qu'est l'agriculteur, est une option qu'il faut, selon moi, défendre aujourd'hui comme une priorité stratégique. Cette option impose l'encadrement du milieu rural.

L'encadrement en vue de la professionnalisation de l'agriculteur exige des moyens qu'il faut produire et qu'il faut fournir aux responsables.

Ces moyens doivent être d'autant plus convaincants que les pré-acquis de ceux à qui ils s'adressent sont limités et que l'autorité dont ils dépendent ne peut trouver son efficacité que dans la persuasion.

Parmi les moyens, certains outils, comme les outils audio-visuels ont aujourd'hui des qualités essentielles que le développeur ne peut dédaigner.

Ces qualités ont été testées et chiffrées dans un grand nombre de secteurs d'activités :

Apprentissage	11% par l'ouïe. 83% par la vue.	
Rétention	20% de ce qu'on entend. 50% de ce qu'on voit et entend.	
Méthodes de communication	On retient après 3 heures	On retient après 3 jours
Par parole uniquement	70%	10%
Seulement visuel	72%	20%
Images et paroles simultanées	85%	65%

Un proverbe africain résume ce tableau : "mieux vaut voir une fois que d'entendre mille fois" (Yiugol ndé wootéré no bhuri manugol no haalé).

A ces qualités chiffrées de l'audio-visuel, il faut ajouter celle qui relève de l'intégration du spectateur-auditeur dans son propre rôle qu'il suit à l'écran dans un décor qui lui est familier, ce qui l'incite à compléter son savoir.

L'outil didactique sur support audio-visuel à l'usage des formateurs et des formateurs de formateurs, pour être convaincant et persuasif, doit cependant être conçu et produit par des spécialistes du transfert du savoir. Il faut intégrer les efforts des chercheurs, des enseignants, des pédagogues, des spécialistes de l'image scientifique et de son traitement en fonction de la réalité du terrain

C'est à ce prix qu'un tel outil peut prendre aujourd'hui une place de choix dans la panoplie des moyens à utiliser dans le cadre de la professionnalisation des agriculteurs.

*

* *

Mais qu'est-ce que l'outil didactique sur support audio-visuel et comment, malgré les diversités culturelles, permettrait-il mieux qu'un autre ou en tout cas complémentairement à tous les autres, d'imposer une base solide à la formation professionnelle ?

En ce qui concerne les loisirs, le documentaire, la "leçon filmée", l'auditeur-spectateur et le producteur doivent s'admettre au seuil de différentes démarches de pensée, au seuil de cultures différentes. Aujourd'hui, l'outil didactique audio-visuel profite de ce phénomène. Mais il peut aller plus loin, grâce au traitement de l'image et à l'infographie qui permet une analyse rigoureusement universelle de la réalité commune à toutes les cultures. On peut créer ainsi des outils dont chaque formateur, à quelque niveau que ce soit, usera quelque soit la démarche culturelle de son auditoire.

Comme vous le verrez, dans un outil didactique sur support audio-visuel, l'image de terrain alterne avec l'image traitée et avec l'infographie. Cet aller-retour constant provoque une curiosité technique, un désir de mimer les gestes proposés.

Un tel assemblage d'images soutenu par un texte adéquat suscite la discussion et l'interrogation en évitant les dérapages de l'imagination au cours des débats. En cela l'outil didactique complète l'éventail des outils de tous les formateurs : exercices pratiques, manipulations, travaux personnels et autres supports pédagogiques.

La cassette magnétique que je vais présenter dans un instant a trait à un problème caféicole : la taille de régénération. Cet outil a été construit avec la collaboration scientique du CIRAD et du Président d'honneur de l'ASIC, Monsieur René COSTE. Il doit être utilisé par les formateurs de vulgarisateurs mais certains de ses extraits peuvent servir à l'édification de supports audio-visuels en vulgarisation proprement dite.

Cette cassette comme d'autres est accompagnée d'un livret reprenant le commentaire intégral et des éléments de base pour une discussion thématique. Figurent également dans ce livret des conseils d'utilisation.

Il est conseillé en effet :

- dans un premier temps, de voir le film, sans interruption et sans prendre de note. Cette première approche doit être réalisée en exigeant uniquement une attention soutenue ;

- dans un deuxième temps, de revoir le film, en se ménageant des arrêts sur image consacrés aux questions-réponses;

- dans un troisième et dernier temps, de revoir enfin le film dans son entièreté.

De tels outils didactiques sur support audio-visuel destinés à professionnaliser l'agriculteur sont déjà utilisés avec succès dans le secteur privé, dans l'enseignement, dans les Instituts de recherche ayant la vulgarisation dans leurs attributions et même par les médias.

Pour autant qu'il soit bien conçu et bien utilisé, l'outil didactique sur support audio-visuel intervient et interviendra de plus en plus pour intensifier le transfert du savoir.

Il permet un discours univoque, focalisé sans ambiguïté sur les sujets les plus divers en offrant un véritable ordre du jour à la discussion et à l'élaboration du "mieux savoir "et du "mieux faire".

De tels outils ne devraient-ils pas faire partie du bagage utile au développement professionnel des caféiculteurs ? C'est la question que je suis venu vous poser en me disant que la professionnalisation des agriculteurs ne doit pas rester dans le domaine du rêve surtout dans les régions du globe où l'avenir de 80 % de la population dépend directement de la gestion des équilibres agro-sylvo-pastoraux.

Suite à cette intervention, il est présenté une cassette magnétique audio-visuelle ayant pour sujet :

"Le caféier : taille de régénération "

SUMMARY

PROFESSIONALISING FARMING PRACTICES : PIPE DREAM OR REALITY ?

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Knowledge transfer is too limited to promote the constant advance of know-how. This extraordinary situation calls for a huge effort of imagination, particularly regarding appropriate training for farmers, who are of particular importance as those with the largest share of responsibility for improving of deterioring the agro-sylvo-pastoral balance. The extensive farming systems in use in countries with high population growth rates are a scourge which must be addressed. The rational and reasonable intensification of farming pratices, incorporating the concept of yield per surface unit and thus the principle of conserving land and sustainable natural resources, can make a substantial contribution to environmental protection whilst also ensuring farmers' material security. The strategic priority for development must therefore be to make farming practices more professional by providing training in rural areas. Extension agents responsible for rural training programmes and those who train them must be given appropriate resources to complement those already available. Audiovisual resources possess fundamental advantages which have been tested and statistically proven the world over. Conventional teaching tools in developing countries must therefore make way for audiovisual teaching aids, designed and produced by experts in both knowledge transfer and the subject areas covered.

RESUME

PROFESSIONNALISER LES PLANTEURS : REVE OU REALITE ?

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Le transfert des savoirs acquis est si minime qu'il n'encourage pas une perpétuelle progression de ce savoir. Cette situation extraordinaire mérite un immense effort d'imagination notamment en matière d'encadrement rationnel de l'agriculteur, interlocuteur privilégié en tant qu'acteur principal de l'amélioration ou de la dégradation des équilibres agro-sylvo-pastoraux. Les systèmes extensifs d'exploitation en usage dans les pays à forte croissance démographique constituent un mal auquel il faut remédier. L'intensification rationnelle et raisonnable de l'Agriculture qui introduit la notion de rendement par unité de surface et donc celle de l'économie des sols et des ressources naturelles durables, contribue largement à la préservation de l'environnement tout en assurant la sécurité matérielle de l'agriculteur. Elle implique la "professionnalisation" de l'Agriculteur. La professionnalisation de cet acteur du développement est une option qu'il faut aujourd'hui défendre comme une priorité stratégique. Elle passe par l'encadrement du milieu rural. Il faut donner au personnel chargé de l'encadrement en milieu rural et à ceux qui sont chargés de la formation de ce personnel, des moyens appropriés complémentaires à ceux qui existent déjà. Les moyens audio-visuels ont des qualités essentielles testées, chiffrées, prouvées dans le monde entier. Dans cette perspective, l'outillage didactique traditionnel doit, dans les pays en voie de développement, faire une place à l'outil didactique sur support audio-visuel conçu et produit par des spécialistes du transfert du savoir au coeur même des savoir-faire.

ECONOMIC LIBERALIZATION AND PRIVATIZATION EFFECTS ON TECHNOLOGY TRANSFER IN EASTERN AFRICA AND KENYA IN PARTICULAR

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INTRODUCTION

Since coffee production in Eastern Africa by Missionaries in the early 19th century - the crop has remained an important cash crop. Arabica coffee centre of origin (diversity), Ethiopia, is one of the East African States neighbouring both Kenya and Uganda. The three major East African states, Kenya, Uganda, Tanzania and Ethiopia produce both Arabica and Robustas. Uganda is the second largest Robusta producer in Africa while Kenya is well known for its high quality Arabica coffee.

In terms of production, most of the East African coffees are produced by smallholder farms. In Kenya over 600,000 households produce over 70% and own also 70% of the national coffee acreage. Most of Ugandan coffee is also produced by smallholder farmers numbering over 500,000. The same case can be stated for the other East African states. This dispersion of producers over space makes technology transfer quite a challenge.

Economic liberalization and privatization started in the 1990. The main thrust of economic liberalization has been geared towards creating market driven economies rather than state controlled economies. This has entailed reduced role of governments through divestitures, from state corporations, deregulation of output and input markets, the floating of exchange rates and interest rates. These reforms are mainly towards enhancement of the private sector to enable the sector to be the engine of economic growth. These reforms have

transgressed through all sectors of economy, Agriculture sector and coffee sub-sector have not been spared either. The monopoly enjoyed by marketing boards has been reduced at various degrees. There is an increased involvement of private sectors in coffee marketing.

These policy changes have offered opportunities and created some challenges in the area of coffee production, processing and marketing. Research and technology transfer in the coffee sub-sector is also in a dynamic flux. The effects of these economic policy changes on Technology Transfer is the subject of this paper.

2.0 COFFEE INDUSTRY CONTRIBUTION TO THE ECONOMY

Coffee plays a crucial role in economies of the East African states by being one of the major foreign exchange earner contributing over US$ 1 billion in 1995/96. Coffee is the major foreign exchange earner in Uganda, contributing over 65% of the total foreign exchange. In Kenya, coffee ranks third after Tourism and Tea; contributing about 20% of the total foreign exchange earnings.

As at 1987, about 70% of the rural labour force in Kenya was employed in Agriculture of which one third was absorbed by the coffee industry. Coffee contributes about 25% of the gross farm revenues in Kenya as it happens to be the highest valued crop in Kenyan agriculture. It is estimated that coffee provides full or part-time employment to one-eighth (2.5 Million people) of the Uganda's population. Further more, the sector establishes important demand and supply linkages with the rest of the economy (ICO,1997). Thus, coffee industry in East Africa play a vital role in the general economy, contributes significantly on the distribution of income and rural development in general.

3.0 COFFEE PRODUCTION TECHNOLOGIES

As alluded to earlier, most of the coffee in Eastern Africa is grown by smallholder farmers under rain-fed conditions. Kenya and Tanzania have a significant plantation sector which contributes 15-30% of the total production.

The smallholder are resource poor farmers with small land holdings, labour intensive production systems, limited operating capital and access to credit. In some cases there is no security in land tenure. These underlying conditions are manifestations of a low-input/ low-output production technology which severely constrained coffee productivity and returns to labour.

For instance over the last thirty years the average yields in small scale farms in Kenya has been 534 kg of clean coffee/ha as compared to 1064 kg/ha in the plantation sector. Yields of up to 3,500 kg/ha are achieved in the irrigated plantations. In Uganda, Robusta yields are reported to be around the average African Robusta yield of 360 kg/ha (Kaberuka,1996)). These yields are nowhere comparable to the average yield of Asian Robusta producers which average 1000 kg/ha.

Over the last decade, a new high yielding and disease resistant Arabica variety has been developed in Kenya. Uganda has also developed high yielding clonal Robusta varieties. These varieties have the capacity to triple the current average yields of smallholder farmers equalling them with that achieved in Asia. The adoption of these promising technology has however remained low due to shortage of planting materials, lack of adequate farm credit and awareness. This indicates, in a way, the yield gap resulting from low adoption of modern coffee farming technologies (new varieties comparison to Asia and Latin America).

As it is well documented, technology adoption depends on its technical feasibility, its reliability, economic profitability and social acceptance (Clayton, 1983).

Technical feasibility involves identification, development and adoption of appropriate technologies. The main players are Researchers, Farmers and Extensionists. In Kenya, unlike other East African states, Coffee Research capacity is well developed and farmers contribute about 2.5% of the gross earnings for Research. Being the main financiers the farmers are involved in identification, prioritization, monitoring and evaluation. This might not be the case in other East African states.

Reliable technologies in a crucial ingredient of technology transfer and adoption (Wharton, 1969). Technologies which have

a higher change of adoption should have less variability in yield and hence profitability. Thus, breeding for yield stable technologies such as high yield coffee varieties with appropriate complementary field management practices and input packages stand a higher chance of adoption. This is the main feature of the emerging coffee production tigers of the Asia.

Economic profitability and social acceptance of production technologies highly depend on appropriate and reliable technologies. Farmers have been shown to respond very positively to economic incentives which do not dispute their social life.

Meeting these crucial ingredients of technology development and transfer in a new economic environment poses the major challenge of all the participants involved in this process in Eastern Africa coffee industry.

4.0 ENHANCED ROLE OF PRIVATE SECTOR AND TECHNOLOGY TRANSFER

Coffee production and marketing has undergone fundamental changes in Eastern Africa from 1990. The governments have divested or sold part of their shareholdings in the coffee marketing parastatals. A good example is the Uganda Coffee Marketing Board Limited (CMBL) where the government intends to sell 51% of its shares to private companies while the remaining 49% are to be sold to the public (Annon, 1997).

Private traders have been allowed to operate in competition with Coffee Marketing Boards in Uganda, Tanzania and Ethiopia. The private traders have offered stiff competition to marketing Boards and this has been translated into higher competitive prices. This has hard mixed results - in terms of production. In Uganda and Ethiopia, production has increased, while in Tanzania the situation has not been encouraging (Table 1).

This transfer of price determination and marketing roles from the state to the private sector and given the weak-bargaining position of most smallholder farmers calls into question the issue of funding and indeed safeguards towards preserving past investments in technology development and transfer. In some cases the smallholder co-operative societies

which used to operate as investment centres, credit source and vehicles of technology transfer are somehow threatened. The thesis is that privatization of the coffee industry might exerbate the current low level of technology adoption and may hinder its transfer. farmers find it difficult to amass savings necessary for replanting, adoption of new varieties and field management practices. The increases in coffee returns will change very little towards this situation.

4.2 AVAILABILITY OF FARM CREDIT

Credit and financial resources are the oil towards an efficient and effective technology transfer and adoption system. Economic liberalization has also met de-regulation of interest rates and input prices as well as removal of subsidies. Coffee production is an expensive undertaking, requiring the use of various purchased farm inputs such as fertilizers, pesticides and herbicides. These are required in order to improve to sustain or improve yields. For instance in Kenya, it was estimated that in 1995/96 production year it costed around US$ 1,000 (91 US cents/lb) to produce one tonne of clean coffee of which US$ 300 (27 US cents/lb) were in form of purchased farm inputs (Karanja, 1997). The cost of production was estimated at US cents 26/lb for Uganda smallholder farmers in 1996. Only 10% of this cost was spent on chemical inputs and fertilizers (ICO, 1997)

The de-regulation of interest rates and input prices resulted into high interest rates and high prices of farm inputs. This, in turn, has severely constrained the availability of Agricultural credit and use of off-farm inputs.

For instance, since the start of economic liberalization, in 1991, the percentage of Bank deposits loaned to agriculture in Kenya declined from 16% to around 12% in 1995 (Table 2). This is despite the fact that Agriculture contributes around 25% of the Gross Domestic Production (GDP).

This credit crunch need to be taken into consideration when technologies are being developed. It is one criteria which will significantly determine the appropriateness of coffee production technologies and their consequent transfer and adoption.

4.3 TECHNOLOGY TRANSFER AGENTS

Alongside research and credit, the role played by extension and producer organisations in technology transfer can not be over-emphasized. Apart from general economic liberalizations, governments are in the process of reforming the structure of the civil service. The Civil Service Reform Programmes (CSRP) are aimed at reducing the number of Civil Servants including extension workers. This is done through retrenchment programmes. It is hoped CSRP will result into smaller, efficient and highly productive civil service. The envisaged small civil service will exerbate further the already high extension worker to farmer ratio. For instance, in 1996, it was found that the overall access by farmers to public extension services in some districts in Kenya was low as 14% with an extension worker to farmer ratio of 1:1000 (Gatheru, 1996).

Farmers organisations are being challenged to take a greater role in technology transfer by engaging professionals to serve farmers under their jurisdiction. The need for mechanisms and structures to facilitate producers greater autonomy in a decentralized technology transfer system calls upon re-definition of the historical linkage between research and the new technology transfer system.

4.4 ECONOMIC AND FINANCIAL MANAGEMENT OF COFFEE SECTORS

Technology transfer is not only confined to coffee production. It extends to realm of coffee marketing. Macro-economic liberalization and privatization of coffee marketing has exposed producers especially to both price and foreign exchange fluctuation risks. The safety mechanisms such as guaranteed minimum prices and coffee pools are no longer visible within the new market driven economies. This calls into question the issue of reliability of coffee income which not only can reduce the preferability of coffee enterprise but also can hinder technology transfer.

There is need therefore for technology transfer in terms of new risk management instruments used in the developed countries, financial engineering mechanism used in developed coffee markets

can also become hardy in long term financing of the coffee industries in Eastern Africa.

The risk management and financing mechanisms are rarely used in Eastern Africa. There is need therefore to explore possibility of introducing such mechanisms in East African Coffee Trade.

5.0 CONCLUSION

Liberalization and Privatization of East African states economies has some encouraging results in terms of increased coffee production. However, coffee farming remains poorly served in terms of modern and appropriate production technologies. This being mainly due to low investment in research and technology transfer.

The strong emergence of private sector in coffee industry, which is mainly motivated by profits, will in a way lessen these investments as they are mainly viewed as public goods. Discretionally investment policies in Coffee Research and Technology transfer have to be made without which liberalization will not move.

Liberalization policies have constrained availability of credit and technology transfer man power. Modern financing mechanisms have to be adopted to feel in the credit gap while farmers organisations are strengthen to provide man power. This will avoid a vacuum being left out.

Re-orientation of Research towards more farmer participatory and financially supported structure will also go along way towards development of more appropriate technologies. The technologies developed should take to cognisance the changing economic landscape and try to address to resource poor smallholder farmers.

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Table 1: Total coffee exports from major East Africa Countries

Country	1992/93	1993/94	1994/95	1995/96
		('000' 60kg bags		
Kenya				
[CMA]*	1,412	1,460	1,325	1,874
Tanzania				
[CMA & BOA]	812	529	468	766
[R]*	197	149	174	150
Total	1,009	678	642	916
Ethiopia				
[BOA]*	1,160	1,332	1,321	1,777
Uganda				
[R]*	1,756	2,735	2,273	3,846
[OMA]*	254	332	420	358
Total	2,010	3,067	2,793	4,204

Source: ICO coffee statistics

CMA - Colombian Mild Arabicas
BOA - Brazilian & other Arabicas
OMA - Other Mild Arabicas
R - Robustas

Table 2: Agricultural Share of GDP and percentage (%) of total Bank deposits advanced to Agriculture in Kenya (1989 to 1995)

	1989	1990	1991	1992	1993	1994	1995
Agric. Share of GDP (%)	28.8	28.2	27.3	26.3	25.1	25.0	25.0
% Share of deposits advanced to Agric	16	15	16	13	14	13	12

Source: Central Bank of Kenya

ABSTRACT

Macro-economic and coffee sector liberalization and privatization policies implemented and their effects on coffee production, processing, marketing and technology transfer have been evaluated. Pricing and foreign exchange policies have increased production incentives while equally increasing venerability of coffee industry participants to price and exchange risks.

Coffee yields have remained low, at 350 kg of clean coffee/ha as compared to average yields of 1000 kg/ha in Asia and Latin America. Clonal Robusta coffee and Arabica hybrid varieties developed in Uganda and Kenya respectively have the potential to increase six-fold the current yields. The current varieties are also capable of producing four times the current yields.

Liberalization, Privatization and restructuring policies adopted have severely constrained use of farm inputs, availability of agricultural credit and exerbated the already low extension to farmer ratios. These factors have become major constraints in technology transfer and adoption.

The need to develop appropriate production technologies in line with the new economic environment, the participation of farmer's organisation in technology transfer process and more agricultural friendly credit policies have been identified as main future challenges.

LA DIFFUSION DE SEMENCES DE CAFÉIERS CANÉPHOROÏDES, UN MAL REDEVENU NÉCESSAIRE À MADAGASCAR

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INTRODUCTION

A travers cette communication, nous nous proposons de rapporter quelques résultats de recherches agronomiques, susceptibles à court et moyen termes d'améliorer la productivité et la qualité intrinsèque de *Coffea canephora* qui, jusqu'à ce jour, produit la presque totalité des exportations-café à Madagascar.

Nous parlerons des atouts et contraintes de la diffusion des boutures, de la diffusion des semences et de l'utilisation des banquettes de *Flemingia congesta* comme fertilisant biologique des caféiers cultivés en basse altitude à Madagascar.

DIFFUSION DES BOUTURES

Pour *Coffea canephora* et les autres canephoroïdes de type congusta, les sélectionneurs admettent en général que des plants issus de clones judicieusement triés sont toujours potentiellement meilleurs par rapport à ceux issus de semences, tant pour leurs valeurs agronomiques que pour leurs qualités commerciales. Néanmoins, les rendements obtenus par les paysans malgaches ainsi que la qualité du produit sont fréquemment décevants par rapport aux conclusions tirées des essais en milieu contrôlé.

A Madagascar, la sélection végétative a permis d'isoler en stations, 78 clones à la fois :

- -haut producteurs avec 2 à 3 tonnes de café marchand à l'hectare
- -à gros grains faisant plus de 18 grammes aux 100 graines
- -un goût neutre à la tasse
- -et/ou un taux de caféine faible

De cet ensemble, 14 clones sont proposés à la vulgarisation.

Le constat actuel fait ressortir, en milieu paysan, un rendement dépassant rarement 500 kilogrammes de café marchand à l'hectare pour une moyenne nationale d'environ 350 kilogrammes. Ceci malgré la mise en place d'un réseau national d'encadrement et de diffusion de boutures racinées de clones sélectionnés. Par ailleurs, si le pourcentage de café grade I des parcelles de démonstration atteint des valeurs supérieures à 90 % en stations, celui de la production nationale oscille autour de 50 %.

La cause principale de ces écarts peut être attribuée au non respect par les producteurs des normes culturales exigées par les clones. Cette attitude étant dictée par plusieurs facteurs, notamment la concurrence d'autres spéculations (vanille, cacao, poivre, girofle) et cultures vivrières, le coût élevé des intrants, et la fluctuation des prix. L'abandon et la reprise des plantations caféières se succèdent parallèlement aux grandes variations des cours internationaux.
Toutefois, de nouvelles caféraies sont souvent établies à partir de semences tout venant.

DIFFUSION DES SEMENCES

Parallèlement au triage des clones, des croisements contrôlés ont permis de sélectionner des plants hybrides, reproductibles à partir de champs semenciers biclonaux. Ces résultats découlent des essais de comportement d'hybrides issus de croisements contrôlés aux stations d'Ilaka-Est (Vatomandry) et de Kianjavato (Mananjary). Trois combinaisons pouvant produire 1,5 à 1,8 tonnes de café marchand à l'hectare ont été ainsi retenues :

-H 865 x 25-11-58 (*Congusta x Coffea canephora*).

-23-1-57 x SI 1900 et réciproque (*Coffea canephora x Coffea canephora)*

A noter que ces géniteurs font partie des meilleurs clones sélectionnés par voie végétative. Le dispositif génétique adopté, modèle diallèle, permet d'étudier l'héritabilité des caractères, le mode de transmission des caractéristiques gustatives n'a pas été abordé. Pour l'instant, les teneurs en caféine et les valeurs organoleptiques ne sont pas étudiées.

Si l'objectif de départ de ce volet de recherche a été la fourniture de semences «améliorées» aux planteurs des zones enclavées, il s'est avéré que les plants issus de semences sont également plus rustiques et répondent donc mieux aux techniques culturales pratiquées en milieu paysan.

De par la dissolution de l'organisme de vulgarisation opérant sur la Côte centre Est de Madagascar, les centres de bouturages des clones sélectionnés ne sont plus opérationnels. Face à cette situation et pour éviter l'utilisation de semences tout venant, le Projet PNUD/FAO en partenariat avec FOFIFA (Centre National de la Recherche Appliquée au Développement Rural) a mis en oeuvre depuis 1995 un volet de diffusion de semences biclonales.

BANQUETTES DE FLEMINGIA CONGESTA

Cette technique permet de pallier aux contraintes liées à l'emploi d' engrais chimiques sur la Côte Est mais aussi de limiter les dépenses d'entretien des plantations. L'utilisation du *Flemingia congesta* sous forme de banquettes assurant la couverture du sol dans les interlignes des caféiers a été expérimentée avec succès.

De plus, en stations, nous avons noté que les parcelles, avec banquettes de flemingia, doublent leur production et gardent intacte la granulométrie des clones par rapport à des parcelles nues ou ombragées, en l'absence de fertilisation minérale.

Le *Flemingia congesta*, qui s'adapte aisément sur toute la côte-est malgache du fait surtout de l'abondance et de la bonne répartition des pluies dans l'année, permet ainsi d'améliorer et de stabiliser la productivité.
En station, l'utilisation du *Flemingia congesta* pour la réhabilitation des vieilles plantations a fait ses preuves.

Depuis 1996 nous avons mis en place des parcelles de démonstration avec *Flemingia congesta* en milieu paysan. Le but étant de leur montrer les avantages de *Flemingia congesta* en tant que plantes de couverture, haies antiérosives et fertilisant biologique.

CONCLUSION

Le café, deuxième produit agricole d'exportation malgache, après la vanille, subit les contre-coups des fluctuations des cours internationaux entraînant une désaffection des planteurs.

Les techniques mises au point en stations :
- diffusion de semences « améliorées »
- utilisation de *Flemingia congesta*

seraient une solution adéquate pour la relance de la production par de nouvelles plantations à partir de semences biclonales d'une part, et d'autre part la réhabilitation de vieilles caféraies par l'installation de banquettes de *Flemingia congesta*. L'étape ultérieure serait la densification sur la ligne des anciennes plantations.

RESUME

Coffea canephora assure la presque totalité de la production de café à Madagascar. L'inexistence d'organismes de production et de diffusion de boutures avec comme corollaire l'utilisation de semences tout venant dans l'établissement de nouvelles caféraies, constitue un facteur prépondérant dans la baisse de la quantité et de la qualité des cafés exportés, deuxième source de revenus agricoles malgaches. D'où l'effort de recherches pour les semences biclonales, de diffusion rapide et directe auprès des planteurs.

Parallèlement à ceci, du fait des contraintes liées à l'emploi de fertilisants chimiques et de par les résultats probants obtenus en stations sur l'utilisation de banquettes de *Flemingia congesta*, le FOFIFA (Centre National de la Recherche Appliquée au Développement Rural) s'efforce de systématiser cette technique dans la réhabilitation de vieilles plantations et pour les nouvelles.

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ANNEXE

SELECTION VEGETATIVE ET DIFFUSION DES BOUTURES

A partir des 14 clones déjà vulgarisés, le FOFIFA a redéfini un mélange polyclonal composé de 3 clones de *Coffea canephora* (K43, 23-1-57, 278-59) et 2 clones du groupe des hybrides congusta (H865, H725). Ces clones présentent les mêmes caractéristiques de floraison (période, intensité, étalement) pour maximiser l'interfécondation. Sans fertilisation, ils sont très productifs en association avec des banquettes de *Flemingia congesta*. En parcelles de démonstration, les rendements moyens sont de 1,8 Tonnes de café marchand à l'hectare par an. Ils produisent plus de 95 % de café grade I et de taux moyen en caféine voisin de 2,2% MS.

Le tableau ci-après fait état des caractéristiques du mélange polyclonal ainsi recommandé.

TABLEAU DES CARACTERISTIQUES DU NOUVEAU MELANGE POLYCLONAL

Clones	Bouturage (1)	Production (2)	Granulométrie	Taux de caféine % MS	Période de grosses floraisons	Période de pointe de récolte (3)
H 865	Bon	8,850	21,4	2,2	Juil-Août-Sept-Oct.	Juin-Juil-Août
H 725	Moyen	8,720	17,8	2,4	Juil-Août-Sept-Oct.	Juin-Juil-Août
K 43	Moyen	7,170	18,3	2,2	Août-Sept-Oct.	Juin-Juil-Août
278-59	Normal	7,950	18,7	2,3	Juil-Août-Sept.	Juin-Juil-Août
23-1-57	Moyen	8,650	19,9	1,5	Juil-Août-Sept.	Juil-Août-Sept.

N.B. : (1) : Taux de réussite au bouturage :
. Bon : Supérieur à 60 %
. Moyen : 40 à 50 %
. Normal : 50 à 60 %

(2) : Production moyenne par pied par an sur 5 récoltes en kilogrammes de cerises

(3) : Période de pointe de récolte : réalisation d'au moins 80%

FARMER PARTICIPATORY IPM RESEARCH AND EXTENSION : EXPERIENCES IN KENYA

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BACKGROUND

In the central highlands of East Africa, cash crops such as coffee, are grown in mixed cropping systems with a variety of food crops, fodder and fruit trees (Njoroge and Kimemia 1993; Nyambo, personal observations). Recent studies have shown a dramatic increase in the use of pesticides by small-scale farmers (Dickinson *et al*, 1984; Maroko, 1987, 1989, and 1991; Mwanthi and Kimathi, 1990; Michalik, 1994; Ngatia *et al*, 1994; Nyambo *et al*, 1996a), this leading to increased concern about the escalating costs of production, reduced farm incomes as well as an increase in health and environmental risks. The costs of agrochemicals (fertilisers and pesticides) absorbs a large proportion of farmers' income (Nyambo, *et al*, 1996a) but the farmer is faced with little choice due to the lack of readily available alternative crop management options to improve production on sustainable levels. Cost effective and environmentally friendly pest (insects, diseases, weeds, nematodes) and soil management options are needed to reduce over dependency on synthetic chemical inputs, improve the sustainability of crop production and farm incomes as well as reduce potential health and environmental risks.

Sustainable methods of soil and other resource management, including crop plants and their pest problems as well as their potential natural enemies are the focus of farmer participatory integrated pest management (IPM) as an approach to improve agricultural productivity. This holistic approach can lead

to lower costs of production in terms of synthetic pesticides and inorganic fertilisers and so improve farm income.

To this end, a pilot farmer participatory IPM training project in coffee and vegetable small scale farming systems was initiated in Kenya in 1995. Its aim was to introduce farmer participatory IPM implementation in cropping systems, which have been shown to be using excessive amounts of chemical pesticides. The project focused on developing farmer participatory training methods for farmers in small scale mixed cropping systems, and drawing upon the methods developed in the successful farmer field schools (FFS) approach to IPM pioneered on rice in Asia (Kenmore, 1996). Farmer field schools (FFS) are an informal farmer driven 'bottom-up' education approach, which emphasise farmer empowerment through participatory technology development and transfer as well as the acknowledgement of the indigenous knowledge of farmers and their experiences.

Developing farmer participatory IPM through FFS requires several steps in order to build the necessary scientific information, cadre of trainers and an enabling national policy. In the East African region, there already exists an array of scientific information which is not used by farmers because it is not appropriately packaged (Nyambo, *et al*, 1996a). Such information can be tested and packaged by FFS participants for wider adoption.

A cadre of trainers/facilitators is a prerequisite for the implementation of FFS. The trainers/facilitators need training in IPM, facilitation and moderation skills so that they become resource persons in farmer field schools. To prepare them for this, they should have a special course that includes facilitation, leadership, management, and curriculum development. This may take several seasons before they become fully qualified.

A national policy is essential in order to make farmer participatory IPM research and extension approach a permanent aspect of national policies and farmer practices.

OBJECTIVE

The main objective of the project was to investigate the feasibility of adapting the successful participatory farmer field school (FFS) experiences developed by FAO, IIBC and others in SE Asia to the African situation. In particular, it was intended to find out whether the discovery-learning and farmer participatory experimentation methodologies used in Asian rice systems could serve as a useful model for farmer participatory IPM development and extension in small holder mixed cropping systems in East Africa.

This paper reports on the work done in coffee based agrosystems in Kenya during the pilot project.

METHODOLOGY

Systems approach

One of the major criticisms of the traditional approaches to the development and extension of pest control recommendations for farmers is lack of an interdisciplinary and systems approach to pest management (Rueda & Bentley 1993; Bentley & Andrews 1996). Traditionally, IPM components are developed for individual crops and pests. Some of the recommendations resulting from this approach contradict each other at farm level, which in turn leads to poor adoption by farmers (Nyambo, *et al* 1996a). To address this problem, it was deemed important for the project to target production systems as a whole as well as emphasising an inter-disciplinary approach so that techniques developed during this pilot project can be used in similar farming systems in Kenya and other East African countries.

Farmer participatory research and extension: an attitude issue

The traditional extension system in East Africa is based on a 'top-down' approach, with emphasis on delivering pre-designed messages/packages. The packages are usually formulated by a researcher and

delivered to the subject matter specialist at the regional/district extension level for packaging and delivery to farmers. In this system, the extension agent is an instructor and the farmer is expected to follow the instructions. This 'top-down' and decentralised decision making approach ignores existing farmer knowledge and skills in crop production, resulting frequently in limited adoption of research recommendations. Although lack of and/or weak research-farmer-extension linkage is often mentioned as the greatest constraint to adoption of technical information, the most serious and easily overlooked barriers in the implementation of farmer participatory IPM are the attitudes and perceptions of the key players in research and extension. For farmers to adapt IPM approaches, they need to understand the basis from which the technology was developed. To improve farmer-research-extension relationship and to equip the project implementing personnel with acceptable facilitation and moderation skills, the project included a substantial element of training in participatory community approaches for extension workers as part of the training of trainers (TOT) course, introduced local agricultural research staff to these approaches and made use of valuable local NGO and other expertise in farmer participatory methods.

Problem identification and selection of target farmer groups

Farmer participatory IPM is not limited to insects and pesticides, but to the practicality of field problems at hand. Plant nutrition and water management has as much to do with good yields as with plant protection. Therefore, the curriculum for FFS is based on the problems in the production system, which is a move towards the development of locale specific technologies. Although to develop robust technologies that are suitable to many agro-ecosystems or many site-specific ones (Bentley & Andrews, 1996) is challenging, this can be made simpler by more farmer involvement in the development, formulation and extension of such technologies. This is achievable by involving farmers in the identification and prioritisation of crop production problems in their respective areas. The logical starting point for the project was therefore to discuss and identify major pest problems with farmers in the context of local climate and agro-ecology using participatory rural appraisal (PRA) tools.

A semi-structured questionnaire was used to identify immediate pest problems and needs for potential IPM intervention for four different mixed farming areas in Central and Coast Provinces.

For sustainability and enhancement of continued learning, the FFS was introduced through existing self-help farmer groups. This was because such groups have already established mutual understanding and cause to work together. The target group sizes were 15-25 participants for each FFS. The groups were selected with assistance from KIOF and MoA, extension division. Two of the groups (Murang'a and Nyeri districts) were KIOF contact groups whereas the other two (Kiambu and Taita-Taveta districts) had no prior experience in participatory learning. This offered an opportunity for the organisers to compare the progress of the FFS between these groups. The farmers' groups that were selected and participated in the pilot project are:

- Othaya, Nyeri district, Central Province
- Karigu-ini, Murang'a district, Central Province
- Githunguri, Kiambu district, Central Province
- Wundanyi, Taita-Taveta district, Coast Province

All four areas have a bimodal rainfall pattern. All grow maize, banana, field beans and sweet potato as staple subsistence as well as for cash. Vegetables are grown as subsistence as well as for cash. Wundanyi farmers grows a wide range of vegetables solely for cash. For vegetable production, all groups rely on supplementary furrow irrigation during the dry season.

Coffee and vegetables are grown in all the selected project areas but there are differences in pest problems and the degree of reliance on synthetic agrochemical inputs. With regard to coffee, the Othaya farmers apply fungicides and inorganic fertilisers regularly, while the Githunguri farmers generally use fresh farmyard manure and no pesticides have been applied since 1989. The Karigu-ini

and Wundanyi farmers have completely neglected their coffee plantations in recent years due to low coffee prices and high costs of agrochemical inputs.

Selection of TOT candidates and course duration

For sustainability as well as to facilitate follow-up after the training, it was deemed necessary to link into the national extension and research system. The current 'top-down' extension system has a limited mechanism for farmer participation in the formulation/packaging of appropriate technologies related to agricultural production. To enhance effective and sustainable farmer participatory IPM, the training of trainers (TOT) course targeted the front-line extension workers (FEW). The front-line extension workers are in direct contact with farmers, and therefore should be well-equipped and conversant with participatory IPM approaches. The TOT course targeted the FEW for the pilot training. However, this was not well received as it was in violation of the existing top-down extension system, which gives training priority to senior staff.

The TOT candidates were selected to include both Ministry of Agriculture extension division and KIOF staff employed as extension agents. The choice of the MoA trainees was left to the District Agricultural Officers (DAO) in the target pilot project areas, with the following guidelines.

- Each DAO nominates only two candidates
- Both candidates should be FEW working with the farmer groups already identified in the target areas
- The nominees should go back to their duty stations to initiate and run the pilot FFS with the target farmer groups
- Nominated candidates should not be transferred during the course of the project.

The DAOs for the targeted districts nominated and allowed the FEW to attend the TOT course.

The TOT course consisted of a total of 8 weeks' residential training conducted in two separate blocks of 5 and 3 weeks between February and July 1996. This was sandwiched with two blocks of 10 weeks of practical work, April-mid June and Mid-July to end of September 1996, when the trainees conducted FFS sessions with regular technical support from the master trainers.

The residential course consisted of two main elements:

- *Participatory community approaches to agricultural extension*

This was designed to give the extension workers appropriate participatory community approaches to improve on their extension methods, with emphasis on changing attitudes towards farmers and examining which methods best foster a creative and equal relationship between farmers and extension workers.

- *Participatory approaches to IPM in coffee/vegetable small-scale production systems*

The majority of the extension workers lack technical information in IPM practices. A course curriculum was developed which focused on the principles of good crop husbandry with emphasis on coffee/vegetable systems and IPM. Additional topics covered during the course included group dynamics, improving facilitation skills and designing farmer experiments. Learning by discovery (hands-on) through simple experimentation and group discussion was emphasised throughout the course.

Establishment and running of FFS

After the intensive TOT training, the trainees went back to their duty stations to initiate and conduct FFS with the selected farmer groups in Othaya, Wundanyi, Githunguri and Karigu-ini, with regular technical support from the master trainers. The Karigu-ini group received the most attention as the farmers also served as the practice group during the residential TOT course.

As with the TOT training, FFS training focused on discovery learning, with emphasis on insect pest and disease life cycles, understanding natural enemies as well as plant nutrition and improved crop

husbandry. To be able to make informed crop management decisions, the farmers learned how to carry out Agro-Ecosystems Analysis (AESA) on a weekly basis in their experimental plots. The AESA involves regular crop inspection and analysis of plant growth and vigour as well as all the factors (insect and their natural enemies, disease, nutritional, water requirement) that may affect yield. This information together with records of previous crop management actions is used in decision making.

To accommodate both the illiterate and literate farmers and to make the training truly participatory, local languages (rather than Kiswahili or English) and live specimens of insects and plant materials were used during the FFS sessions to enhance the learning process.

Institutional collaboration

To optimise the benefits of the farmer participatory IPM research and extension, institutional and interdisciplinary approach is vital at all stages of technology development and implementation, particularly in the mixed cropping systems prevalent in East Africa. To harmonise technical information for use by farmers as well as to minimise antagonism at field level and so give farmers the best services, institutional and interdisciplinary collaboration in the pilot project was a pre-requisite.

The project was co-ordinated by the International Institute of Biological Control (IIBC) Kenya Station, in collaboration with the Coffee Research Foundation (CRF) of Kenya, the Kenya Institute of Organic Farming (KIOF), Kenya Agricultural Research Institute (KARI), Ministry of Agriculture, Livestock development and Marketing (extension division) and the Permanent Presidential Commission on Soil Conservation and Afforestation (PPCSCA).

IIBC's input focused on farmer participatory training methods, the identification and use of indigenous natural enemies and IPM components for pests. CRF provided inputs on coffee insect pests, diseases, weeds, nutrition and overall coffee production husbandry. KIOF contributed technical input on the use of botanical extracts for insect pest control and organic methods of soil fertility management, in addition to their experience in participatory training methods with small-scale vegetable growers. KARI provided input on aspects of vegetable production with a focus on disease management for small-scale producers. Staff from the PPCSCA ran the 2-week course on participatory community approaches to agricultural extension. The Ministry of agriculture extension division provided staff to become FFS trainers who facilitated the FFS sessions as well as the staff who participated in the evaluation of the project impact on farmers' practices.

Staff from all the collaborating institutions worked together as a team from planning and problem identification to implementation and evaluation of the project.

ACHIEVEMENTS AND LEARNING POINTS

The PRA revealed that:

- The pest problems and priorities differed in the targeted project areas and often, did not match the national priorities and groupings (Nyambo, *et al,* 1996b).
- At the farm level, farmers' crop production problems included pests, a decline in soil fertility and a lack of general information on some aspects of good crop husbandry.

To address these problems, a holistic approach to integrated crop management was adopted in an attempt to develop locale specific solutions as well as to improve crop production on sustainable level.

Curriculum development and farmer empowerment

The project successfully developed and tested curricula for training trainers and farmers, and conducted FFS on coffee/vegetable production systems at weekly intervals for six months. The FFS's empowered farmers, in particular women, to be able to make informed crop management decisions notably in crop protection, soil management and overall crop husbandry.

Training of trainers and FFS groups

Eleven FEW (4 women and 7 men) were trained as FFS facilitators to provide a core of master trainers for future expansion of farmer participatory IPM activities. Women were particularly encouraged to participate in the FFS sessions because they are rarely the primary beneficiaries of training in crop management through the traditional extension and research systems even though they do most of the farm work (Nyambo, *et al,* 1996a). Of the FFS participants, 42 were women and 23 were men, and both men and women held leadership positions in their respective groups and had equal say.

Participatory development and formulation of appropriate IPM components

A range of environmentally friendly and sustainable crop management options suitable for use by small-scale coffee/vegetable farmers were developed through farmer experimentation and discovery learning. These include the following:

- **Non-chemical soil treatment against soil-borne pests in vegetable nurseries.** The farmer groups had identified root knot nematodes, as one of the major problems in vegetable production. Chemical soil treatment has become increasingly prohibitive because of high costs and poor efficacy of the chemicals commonly used. As a result, many of the farmers were buying seedlings from the local market. Such seedlings were often already infected with a wide range of pests, this forcing farmer to spend a lot of money on chemical pesticides to raise the crop. To reduce potential pest problems and so enable many of the small scale farmers produce vegetables profitably with less chemical pesticide input, it was necessary to assess some of the recommended non-chemical soil treatment methods. Although root knot nematodes was a common problem at all project sites, the choice of treatment for adoption and subsequent use varied. Based on efficiency in pest control, plant establishment, growth and yield, the Wundanyi group ranked use of a mixture of subsoil and compost as the best treatment. The adoption rate at farm level in this group was 76.8%. In contrast, the Othaya, Karigu-ini and Githunguri groups selected the use of a mixture of burnt trash and compost for their respective areas. The adoption rate at farm level was 70.4% in Karigu-ini, 59. 4% in Othaya and 48.1 % in Githunguri.
- **Preparation and use of organic fertilisers to reduce dependency on inorganic fertilisers.** Crop production in recent years has been largely dependent on the use of inorganic fertilisers with limited use of fresh manure where available. This has resulted in soil degradation due to lack of organic matter content and in some areas, an accumulation of high nitrates in ground water and catchment areas e.g. high nitrate levels have been detected in Kishienyi dam and Mzazala river catchment areas in Wundanyi (Paul Sawo, 1993, unpublished). Many of the FFS participants practice zero grazing and so all try to apply fresh manure in their fields. Although this is a good practice, fresh manure could be a source of pest infection on the farm. In addition, it takes much longer for the benefits of raw manure to be realised. To reduce the potentials of pest infection and to optimise the benefits of organic fertilisers as well as to provide farmers with alternatives to inorganic fertilisers, farmers were taught how to make and use compost, liquid manure and plant teas. Within a very short time of its introduction, many of the farmers started making and applying organic fertilisers on their farms as well as teaching other farmers in their areas. The adoption rate in all the groups is very high so far i.e. 100% in Karigu-ini, 78.5% in Wundanyi, 87.5% in Othaya and 94.4% in Githunguri. The spill over in Wundanyi is 64.2%, 55% in Githunguri and 50% in Othaya areas. The greatest benefit cited by all groups was reduced over dependency on synthetic fertilisers, which has resulted in reduced production costs.
- **Use of natural products (botanical extracts) for pest control.** Natural products, if effective, are less costly and some e.g. neem extracts, are environmentally friendly and compatible with IPM practices. While some products were introduced to the farmer groups as alternatives to chemical pesticides by KIOF, farmers made contributions based on their knowledge and experiences. Before some of these were adopted for wider use, the participants conducted group experiments to ascertain their efficacy. Only a few of the suggested herbs and plants gave effective control of target pests in the different areas. Some e.g. the extract of Mexican marigold did not give effective control of aphids, webworms and diamondback moth. In Wundanyi, incorporation of fresh Mexican

marigold leaves in the soil as a non-chemical means of controlling soil borne pests resulted to very low germination (45% on tomato and 1% on cabbage compared to 75% and 99% respectively on farmer practice). The Githunguri group reported that a mixture of chillies and onions caused skin and eye irritation, and that when a solution of chillies is applied on vegetables, it kills some of the potential natural enemies. Inadequate information on preparation and dosage rates, residues on produce as well as effects on non-target organisms were identified by the farmers as some of the limiting factors for their wider adoption and use. Despite all these limitations, the adoption and spill over among the groups is still high. The adoption level is 88.8% in Karigu-ini, 87.5% in Othaya, 57.1% in Wundanyi and 77.7% in Githunguri, with a spill-over of 28.6% in Wundanyi, 70% in Othaya and 66.6% in Karigu-ini areas.

- **Improved crop management skills**. To know how to make informed crop management decisions, the farmers learned how to carry out Agro-Ecosystems Analysis (AESA) on a weekly basis in the experimental plots. Regular crop monitoring and analysis of crop development as well as constraints to production helps farmers improve pest management, (timing and selection of appropriate control options) and proper timing of other crop management practices e.g. fertilisation, harvesting, watering, mulching etc. The adoption rate among the FFS participants to date is 100% in Wundanyi, 88.8% in Karigu-ini, 62.5% in Othaya and 66.6% in Githunguri. However, the spill over is still very low, being 28.6% in Wundanyi and 55.5% in Githunguri and none for Othaya and Karigu-ini groups. This is because for a farmer to do a good AESA, basic knowledge on how to differentiate insect pests and natural enemies as well as the differences between damage due to insects, diseases and nutritional effects is necessary, and this needs training. Through regular field AESA, and using a combination of locally generated IPM components, Wundanyi FFS group were able to achieve an impressive 70% reduction in chemical pesticide use on their tomato IPM plot and obtained a yield increase of 16% over current farmer practices (Nyambo, *et al* 1996b). On coffee, through regular monitoring of pests and crop development, use of cultural practices, and a combination of a good choice of a curative fungicide, it was possible to reduce fungicide application for coffee rust control by 50% compared with the current research recommendation (Nyambo, *et al* 1996b).
- **Enhanced farmers' knowledge and skills in coffee management.** Coffee is an important cash and export crop for Kenya. However, production has declined in recent years due to escalating costs of agro-chemical inputs (Nyambo, *et al,* 1996a), which has forced many of the small scale producers to neglect their coffee plantations. To increase production (yield and quality) and to reduce health and environmental hazards associated with over dependency on agrochemical inputs, there is a need to provide farmers with effective but less costly coffee management options. The coffee sessions emphasised cultural practice (pruning, mulching, mbuni stripping), conservation of natural enemies, use of organic fertilisers and regular AESA. In Karigu-ini, a coffee IPM plot has as much yield potential as a plot that received the CRF recommended routine spray package despite the fact that the IPM plot had 50% fewer fungicide sprays to control leaf rust. Adoption has been slow in all the project areas (Karigu-ini 30%, Wundanyi 57%, Othaya 37.5% and Githunguri 38.8%) mainly because the yield results are not immediate. In addition, the training duration allocated for coffee FFS sessions was insufficient to expose farmers to the whole crop cycle. Ideally, one-year's training will be required for farmers to appreciate the benefits of IPM practices in coffee.

A number of supporting activities were invaluable to the success of the project, and these are:

- **Use of local language in FFS sessions**. It has been suggested that most local African languages have small vocabularies for soils, plants, insects, diseases and plant disorders and therefore, farmers have difficulties exchanging technical information with crop specialists and extension workers. Local languages (Kikuyu in Central and Kidavida in Taita-Taveta) were used throughout the FFS sessions in this pilot project, and contrary to expectations, farmers came forward with a wide range of names for plants, insects, diseases and plant disorder. However, some names changed within localities, even within the same ethnic group, and hence the need for documentation. Use of local languages made the training easy and truly participatory as it enabled even the illiterate farmers to

participate fully. However, farmers appreciated some of the limitations associated with use of local languages in training and suggested that both Kiswahili and English could be used where appropriate to enhance learning.

- **Institutional collaboration.** Collaboration (International, National research & extension, inter-governmental organisation and an NGO) enhanced harmonisation of information and approaches, removed animosity as well as antagonism, and ensured tapping of the best from each collaborator for the benefit of farmers. This also improved linkage and information flow between farmers-researchers-extensionists. However, lack of full commitment from some of the collaborating institutions, as well as poor infrastructure hindered smooth implementation of the project.
- **Exchange visits.** Farmers' exchange visits between FFS groups were arranged to foster farmer-farmer learning by exposing them to different agro-ecosystems. The visits provided an opportunity for farmer-farmer networking as well as for self-evaluation. However, the visits were brief and farmers were requesting for longer visits.
- **Training duration.** The training covered six months but this was not adequate to assess the impact of the training on farmers' practices and its socio-economic implications. Ideally, the training should cover complete crop season(s) to give both the facilitators and the farmers a chance to appreciate seasonality and changes in pest pressure and their natural enemies, as well as the benefits associated with IPM practices and improved crop husbandry. This is particularly important for tree crops such as coffee.
- **Cost sharing.** Current research approaches to technology development depends largely on 'inputs for data' (free handout) strategy. Researchers exchange free inputs for data and so in farmers' eyes they are not a source of advice, ideas or knowledge but of free inputs. No inputs, no data. This tendency for farmer-researcher interaction based on handouts, encourages researchers to seek out product (***targetitis***) rather than knowledge based solutions and therefore lack vision for sustainability. To encourage an environment conducive to discussion and exchange of knowledge and ideas on a sustainable level, cost sharing was encouraged throughout the project. Farmer groups paid for all the experimental inputs, snacks, travel costs and their time to enhance ownership and value of the information. This approach was not well received by all stakeholders. Once they realised that no free handouts were involved some farmers did not participate in the FFS sessions. However, those who participated in the FFS sessions eventually appreciated and accepted the long-term benefits of learning through cost sharing.
- **Recognition of farmers as partners in technology development**. Change in attitude, notably the ability to recognise farmers as equal partners in technology development and transfer, is essential to foster participatory research and extension of IPM. In particular, appreciation of farmers' knowledge and experiences in farming, is a milestone in IPM/FFS. This is an area that needs appropriate facilitation and moderation skills in order to develop an enabling learning environment. The learning process in farmer participatory research and extension demands that the different partners contribute knowledge and experiences derived from different ways by discussion and experimenting together to pick the best outputs for application. This requires a change in attitude. Many researchers and institutions are still very conservative, have an individualism approach culture to work planning, and are lacking in appropriate moderation and facilitation skills. To achieve truly farmer participatory IPM research and extension, training in facilitation and group dynamics management is a prerequisite before setting up FFS groups.

CONCLUSIONS

During the pilot project, trainers (facilitators) and farmers were trained in IPM, participatory experimentation was initiated and institutional collaboration was established. Satisfactory results were obtained in terms of reduced agrochemical use, improved crop health and quality in coffee as well as in vegetables. Increased knowledge of IPM for the trained farmers and FEW was another achievement. The role model effect of the trained farmer groups has created a demand for FFS input in other self-formed groups. In Wundanyi, IPM/FFS training groups have been formed, and the FFS graduates have been approached to assist in training. In Githunguri, several groups (women, men and youth groups)

have been formed and members are now requesting training through the FFS approach. In Karigu-ini, the FFS graduates have helped the formation of a new group for IPM training and already they are assisting in their training. In Othaya, the members organised a field day and invited their neighbours, school children and division administrative personnel to their experimental plots to create awareness of IPM.

In order to strengthen and build on the results of the pilot project and lay the foundation for scaling up farmer participatory IPM research and extension in Kenya and East Africa, the preliminary findings need to be tested rigorously so as to be able to make informed decisions. In particular, further work is needed on curriculum development, assessing the socio-economic impact of the training on farmers' practices, assessing the constraints to its adoption and gathering quantitative data on crop yields. Evidence from FFS programmes based on annual crops in Asia (Kenmore, 1996) points strongly to the need for continued support to the FFS groups and their facilitators over at least two cropping seasons for the changes in farmer practices to become sustainable and/or expanded. Tree crops, such as coffee, may require more than just two seasons for the results of IPM practices to be realised (Nyambo, *et al,* 1996b; de Waal, 1997).

Additional collaboration is also required from the research community to support the development of appropriate IPM technologies for a number of potential interventions, which have been identified by the FFS participants. Equally important is a thorough, independent evaluation of the impact of the training on farmers' practices in the medium term and an analysis of the constraints to adoption of farmer participatory research and extension of IPM through FFS as a model for national programmes in East Africa.

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ABSTRACT

In the central highlands of East Africa, cash crops such as coffee are grown in mixed cropping systems with a wide range of food crops. Recent studies have shown a dramatic rise in the use of pesticides by the small-scale farmers in such cropping systems, this leading to widespread concern about increases in crop production costs as well as health and environmental risks. The cost of agrochemical inputs (fertilisers and pesticides) absorbs a large proportion of farmers' income but the farmer is faced with little choice due to the lack of readily available alternative crop management options to improve production on sustainable levels. A holistic approach to sustainable pest (insects, diseases, nematodes, weeds) and soil management is needed to reduce agrochemical inputs, improve crop production on sustainable levels and so boost farm income.

To this end, a pilot farmer participatory integrated pest management (IPM) training project in coffee and vegetable systems was initiated in Kenya in 1995. The aim of the project was to introduce farmer participatory IPM implementation in cropping systems which have previously been shown to be using excessive amounts of agrochemicals, and drawing upon the methods developed in the successful farmer field schools (FFS) approach to IPM pioneered on rice in Asia.

The pilot project completed its initial phase in September 1996. It has developed and tested curricula for training of trainers and farmers, held farmer field schools on coffee and vegetable small scale farming systems which have captured the imagination and attention of the participants. It has demonstrated that coffee and vegetables can be grown with substantially reduced synthetic agrochemical inputs while maintaining or increasing yields. It has empowered farmers to be able to make informed decisions on crop management and it has clearly generated considerable enthusiasm amongst the participating farmers. This holistic approach to crop management is the way towards sustainable improved crop production and farm incomes as well as reduce both environmental and health risks.

RESOURCE ALLOCATION AND ENTERPRISE INTERACTION IN THE SMALLHOLDER COFFEE SECTOR IN KENYA

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Introduction

The first smallholder coffee production in Kenya was allowed in the 1930s in two far-flung districts of Kisii and Meru. However, it was not until after the implementation of the Swynerton Plan[1] in 1954 that production in this sector gained tempo, surpassing the estate sector in 1966 (Table 1). Presently the sector contributes about 54% of the total national production from an estimated area of 120,000 hectares. Although the sector is located in areas with some of the best soils and rainfall regimes, the yields have not been commensurate with the cropped area. At an average of less than half a tonne per hectare, the yields are far below those realised in the estates sector (Table 1).

The production scenario however, is a manifestation of a complex interaction of both physical and socio-economic factors within the smallholder farming system. The evolution of the farming system has been fashioned by the need to balance farmer goals and resource limitations, within a production environment whose enterprise possibilities are defined primarily by soil and climatic factors. The change of land tenure from communal to individual ownership in the 1960s further encouraged farmers to invest in cash crops.

This paper discusses the various factors that influence resource allocation within the main coffee zones in the smallholder highland areas in Kenya. Also discussed are the nature of interactions that exist among the various crops and livestock enterprises and their influence on adoption of research recommendations.

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[1] The Swynerton Plan was a colonial government initiative to allow small scale African farmers to grow cash crops. Hitherto, this was a preserve of the settler community.

Table 1: Kenya coffee production statistics (selected years)

Year	Smallholder		Estates	
	Production (Tonnes[t])	Yield (t/ha)	Production (Tonnes[t])	Yield (t/ha)
1963/64	15373	1.18	28405	0.87
1966/67	27558	0.64	25231	0.78
1969/70	26275	0.48	26521	0.88
1989/90	69483	0.59	34356	0.90
1991/92	41977	0.43	37520	0.98
1994/95	62561	0.51	32699	0.85

Source: Coffee Board of Kenya Annual Reports

Agro-ecological zones and enterprise-mix

Areas suitable for rainfed coffee production in the Kenya highlands are divided into three zones, the coffee-tea (UM 1), the main-coffee (UM 2) and the marginal-coffee zones (UM3). The zones are defined by their relevant agro-climatic factors, differentiated by soil pattern and their ability to support main crops and livestock enterprises, (MoA, 1983). The implication of this zoning is that the primary enterprise options within each zone are limited and common to all the farmers (Table 2). However, unique enterprises and practices may be observed among innovators.

Table 2: Distribution of major agricultural enterprises in the coffee zones

Agroecozone	Main crops	Main livestock
UM 1 (Coffee-Tea)	Coffee, tea, vegetables	Dairy cattle,
UM 2 (Main Coffee)	Coffee, maize, beans, vegetables	Dairy cattle
UM 3 (Marginal Coffee)	Coffee, Maize, Beans, Tobacco	Cattle (Unimproved breeds)

In the UM 1 zone, the twin cash crops (tea and coffee) and dairy farming are the major features of the agricultural landscape. Food crops production in this zone is carried out at a scale that cannot adequately cater for local consumption.

In the UM 2 zone, coffee is the main cash crop while food crops production (mainly for domestic consumption) assume a bigger priority compared to the Coffee-tea zone. Dairy farming however maintains its importance among livestock enterprises.

In the marginal coffee zone an entirely different enterprise mix emerges. The enterprise mix reflects the risk factor of unfavourable weather conditions. The types of livestock reared and crops grown are those that can cope with unreliable weather patterns. Coffee assumes a lesser position of importance as a cash crop, while surplus cereals and pulses are traded for cash. In some areas tobacco becomes an additional cash crop (MoA, 1983).

Land and labour allocation

The two prime resources in agricultural production in the smallholder sector are land and labour.

i) Land

Farm holdings within the smallholder sector in the Kenya uplands are estimated on average at 0.4 ha (Crandall, 1993). The holdings tend to be larger in the UM 1 and UM 3 zones compared to the UM 2. This could be explained by the nature of settlement before the advent of the cash crop agriculture. The coffee-tea (UM 1) areas were primarily forest lands while the marginal coffee areas (UM 3) were considered areas of low agricultural potential and could not therefore support meaningful settlement. Most of the population was therefore concentrated within the main coffee zone (UM 2).

Due to population pressure, there is currently heavy human settlement and the attendant agricultural activities in all the three zones. Being perennial crops, coffee and tea are almost permanent enterprises in the farms. Zero grazing, the most common system of dairy farming in all the zones, restricts competition for land resource to mainly fodder growing. The land area allocated to annual crops vary from one season to the other. Intercropping coffee with other crops has also been noted to be an emerging practice (Onsongo, in press).

ii) Labour

The primary source of labour in the smallholder sector is the household. Hired labour is only a consideration during times of simultaneity of labour intensive activities among enterprises. In the coffee-tea zone, for example, during the wet seasons tea picking competes for labour with other activities such as coffee harvesting and weeding. Communal mobilisation of labour from within the smallholder communities used to be a common practice in the past but has over time declined in importance.

Despite this dependence on family labour, only less than 40% of the household labour is available for farm activities (CRF, In press). Migration of the potential labour force from the farm sector and competition from non-farm activities could be the main factors behind this low labour supply. This may have been further aggravated by perceived low returns to labour in the farm sector.

In terms of labour allocation, specialised tasks, such as coffee pruning and spraying are undertaken by skilled individuals in the households. However, all members of the household participate in general tasks such as weeding, planting and livestock feeding.

Other considerations in allocating land and labour

There are numerous other factors that farmers consider in allocating their prime resources among various enterprises. These include food security and the nature of the enterprise.

i) Food Security

The smallholder coffee areas in the Kenya highlands have some of the highest human population densities outside the urban centres. Although large populations could be useful sources of labour they invariably constrains food supply.

Household food security is therefore a major consideration in farm resource allocation. Food security has been defined broadly as the ability to access adequate food always (Mellor, 1983). This implies that the households may either plant own food or access the market for supply. In the Kenya highlands, due to dietary habits, food security has been synonymous with the availability of cereals (mainly maize). Consequently, the level of adequacy of maize is usually used as the index to determine food security situations. Various household food surveys by the Ministry of planning indicate some interdependence between agro-ecological zones. Households in the coffee-tea zone access cereals from the markets. Farmers in this zone therefore concentrate on production of cash crops. In the main and marginal coffee zones farmers allocate their land and labour resource with an objective of meeting household consumption needs and generating surpluses for sale.

ii) The nature of the enterprises

The other consideration in resource allocation among farmers is the length of time a particular enterprise takes for a farmer to start reaping cash benefits. In an enterprise-mix involving only annual crops a farmer can alter crop combinations without losing the ability to revert back. However, the same may not be possible for tree crops. For instance, in spite of fluctuating incomes from a cash crop like coffee, farmers are hesitant to uproot their plantations. The worst they most have done is to reduce attention to the enterprise. The land resource remains unavailable for other uses.

Other enterprise interactions

The resource perspective of a smallholder farming system is essentially a competitive one. For example, an expansion of one farm enterprise without a reduction in another or compensatory benefits of superior production technology is bound to strain the land resource. If the existing labour force is fully employed then the system will demand supply of additional labour.

However, there are other interactions among enterprises that are not necessarily competitive. The interaction of coffee and livestock in the smallholder sector in Kenya is such a relationship. In view of expensive inorganic fertilizers, zero grazing systems have come in handy as major sources of manure. At the same time, in some areas most farm inputs (including livestock feeds) are purchased against coffee deliveries. Another example is a situation where farmers have intercropped coffee with food crops in times of market slump. Crandall (1993), in study of the smallholder production systems in Kenya noted that labour and intermediate cost were fully paid for by the maize and beans intercrop in coffee. Although farmers with such an intercrop would have wished to withdraw their efforts from coffee, the intercrop provided a motivation for continued tending.

Implication on adoption of research recommendations

Available land and labour resources, and the range of enterprises on a farm, influence the technologies a farmer is likely to adopt. Nyoro (1986), observed that smallholder farmers in Kenya are not likely to adopt research recommendations that do not suit their resource and enterprise situations. For instance, a farmer may modify a research recommended coffee spray programme due to inadequate supply of labour. Similarly, the two to three years time lag between planting and bearing in coffee and the attendant cash-flow disruption is a major disincentive to farmers wishing to convert to superior varieties.

Conclusion

Agricultural production in the smallholder sector in the Kenya uplands is diversified within an enterprise range that is limited by natural potential, land and labour resources. Food security and the nature of enterprises have also been considerations in resource allocation decisions.

The farmer circumstances have also an implication on adoption of research recommendations. It is also pertinent to note that most research systems in Kenya are organised along commodity and discipline lines, with weak linkages between each other. Consequently, most of the research recommendations are made without taking into consideration the resource endowment and the enterprise diversity on the farms.

In order to come up with research recommendations that farmers

would be willing to adopt there is need to clearly understand farmer circumstances in terms of their primary resources, production options and their interactions.

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Abstract

Smallholder coffee production in the Kenya highlands started earnestly after 1930s. About the same time there was introduction of other adaptable technologies in other crops and livestock enterprises. In addition, changes in land tenure systems coupled with population factors have shaped the smallholder farming system.

Resource allocation among various enterprises and the enterprise mix are influenced by numerous factors, namely, natural potential within the agro-ecozones, food security considerations and the nature of the enterprises.

These factors have also had implications on the adoption of research recommendations by the farmers.

SUSTAINABLE ENVIRONMENT AND PRODUCTION RESOURCES FOR HIGH QUALITY ARABICA COFFEE

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Introduction

Agriculture is the mainstay of Kenya's economy. It is a source of economic growth, food security and off-farm employment. About 80% of the population is rural based and depends on agriculture for their livelihood. Coffee a lone occupies about 160,000 ha of most fertile land between 1400 to 2100m above the sea level - with an average of 900mm of annual rainfall (Njoroge, 1993).

In Kenya, Arabica Coffee is grown in soils derived from volcanic deposits, basement complex rocks or an intermediate of the two (Michori, 1981). These soils are referred to as humic and eutric nitosols and are generally very deep, friable and free draining. They are very leached at higher altitude zones leading to low contents of plant nutrients (Michori, 1981). This demands external sources of nutrients in order to obtain high yields and quality coffee.

Coffee is cultivated as mono-culture which gives room to inputs such as the mobile nitrogen and potassium to find their way into the underground and surface waters. The situation is aggravated by poor cultural practices and rugged terrain which accelerate water and soil erosion. During rainy seasons agricultural chemicals and fertilizers are washed into the domestic water down stream thus adversely affecting human development index and production resources.

In an effort to sustain high coffee yield, quality and environment, soil nutrient maintenance and protection schemes must be adhered to; such schemes includes: judicious use of fertilizers, nurse and cover crops, grass mulch, green and farm yard manures, shade trees, irrigation, intercropping etc. Where used, these agricultural production practices have been found feasible.

Studies by NorthMore (1965) showed that the qualities of raw coffee bean, roast, acid liquor and flavour were

significantly lowered if certain maximum and minimum levels of potassium and calcium were exceeded in the raw bean. Therefore, application of potassium and calcium containing nutrient sources have to be carried out with care.

Inorganic fertilizer sources are expensive especially to the small scale farmer and organic sources are left to be the only alternative. Organically produced coffee is gaining popularity among coffee consuming countries and efforts should be directed to utilization of grass mulch, leguminous cover crops such as Desmodium spp, green manure from cowpeas, beans and trees such as Leucaena leucocephala, Sesbania sesban, etc. (Njoroge, 1993). Other materials include Farm Yard Manure and Coffee pulp. There should be longterm monitoring of the effects of green manure on soil structure, soil moisture conservation, rate of litter mineralization and soil organic matter and found dynamics.

To sustain the environment and resource base on which coffee thrives, there are several factors to consider:

(i) **Sustainability of Cultivars:**

The cultivar to be grown should be of proven quality, disease and pest resistance and high yielding. In addition, the cultivar should be efficient in utilizing inputs such as solar radiator, water and fertilizers. Should be morphologically adapted to trap solar radiation. The cultivar should be compatible with various forms of cropping systems such as intercropping, alley cropping, nurse and cover cropping systems. It is envisaged that with such traits, environment and resource conservation goals will be achieved.

(ii) **Ecological Conditions:**

It is important that the selected cultivar be grown in a suitable environment for it to express its maximum genetic potential. Arabica Coffee requires temperature in the range of 32^0C during the day and 7^0C during the night. The range between daily maximum and minimum should be around 19^0C. Arabica coffee requires 1000 to 1150mm of rainfall and maximum dry period should not exceed 4 months. Altitude influences rainfall and temperature. Arabica coffee grows well at altitude between 1400 to 2100mm above the sea level. To obtain high quality coffee shade trees are required to modify extreme temperatures and mulch is needed to conserve moisture where coffee is grown under minimal rainfall conditions. Irrigation after coffee has obtained good root systems is a suitable practice and is crucial in low rainfall areas.

(ii) **Soils:**

The best coffee soils are the humic nitosols which are red clay loams of volcanic origin. They are deep, friable and free draining. Arabica Coffee requires soils with a pH range of 5.4 to 6.5. These humic soils are highly leached at higher altitude zones leading to low contents of plant nutrients (Michori, 1981). To obtain high yields and quality coffee, these nutrients must be supplied from organic or inorganic sources.

The above three factors are considered to be the minimum ecological requirements which are required to obtain sustainable yield and high quality coffee. Most of other consideration are

easily manipulated by man and can adversely affect the yield and quality of coffee if certain requirements are not met.

Resource Sustainable Coffee Production Practices

(a) **cropping systems:**

Small scale coffee farmers are low external input users but they can effectively raise their productivity through a more effective use of natural (eg. light) and added (eg, fertilizer) resources through intercropping. This is possible provided that component crop demands for resources are well understood. Management of intercrops to maximize their complimentality and synergistic effects and to minimize competition follows simple natural principles and its practice is limited by the imagination of resource managers (Midmore, 1993). Intercropping is a way of life for subsistence farmers Malacela (1980) and a very common practice Vandermeer (1959).

Suitable intercropping systems in coffee enhances farmers income, food security at the same time improving soil fertility through better utilization of nutrient resources, soil erosion control and introduction of better shading and economic trees in terms of animal feed, mulching, woodfuel, nutrient recycling and nitrogen fixation. It has been shown that the most suitable time for Arabica Coffee intercrop with food crops is during the first two and half years of coffee establishment and during tree conversion (Njoroge, 1993).

Agro-horticulture is a cropping system where coffee is grown in mixtures with perennial fruit trees such as citrus, bananas, mangoes, pawpaw, passion fruit etc. This cropping systems give extra income to the farmers when coffee prices are low and during drought. The system promotes the health of coffee in that fields are kept weed free, disease and pests are controlled thus saving on their control while protecting the environment.

Alley-cropping and hedge row agroforestry system are sustainable production methods where coffee do benefit from N-fixed by selected leguminous trees and shrubs such as Sesbania sesban, Leucaena leucocephala etc. In addition, the hedge-row trees provide shade and windbreak to coffee plants, not withstanding the beneficial effect of having to provide fodder, woodfuel and timber. These systems require thorough study to establish their suitability as possible alternatives to conventional systems.

Mulching, cover cropping and nurse cropping are also farming practices whose impact on erosion control, moisture conservation, pest control and nutrient supply cannot be over emphasized. Their positive additive effects on various coffee intercrops have a major impact in raising especially organic coffee.

(b) **Plant Health:**

Pest and diseases of coffee are many and this is one area that takes most of the producers' profits. It is therefore necessary to limit their spread using environmentally benign means.

(i) **Weeds:**

Weeds compete for water and essential nutrients with coffee and at the same time interfere with coffee

management practices. Severe attack by weeds result to fewer and smaller beans which adversely affect both yield and quality of coffee. Yield losses due to the tune of 50% have been reported (Coffee Board of Kenya, 1993). It is therefore imperative to control weeds to obtain high yields and quality coffee.

Integrated weed control method is the most appropriate as there is no single method which is efficient enough. Weed control should start during land preparation and in mature coffee a combination of mulch, cover crop in inter-row cultivation are effective means.

(ii) **Diseases:**

There are several diseases of Arabica coffee and if not controlled, do result to reduced yield, total crop loss and poor quality of coffee beans. There are many methods used in disease control but resistant varieties like Ruiru 11 provide the best means. Timely and appropriate cultural practices enhance the level of disease protection and control. This integrated disease control method ensures environmental protection and high yield and quality of coffee. The method is also cheap for resource poor farmers.

(ii) **Pests:**

Arabica Coffee has a host of pest that range from root, stem, branch, leaf and fruit feeders. Among environmentally sound methods of control is the deployment of cultural and biological methods of control. Cultural methods involve pruning, mulching, clean weeding etc. Pruning helps to control Antestia-bug and scales while thrips are controlled by proper mulching. Yellow headed borer and Berry months can be controlled by physical means.

Another environmentally benign method involves the use of naturally occurring organisms which are either predators, parasites or pathogens. Ladybirds, leafminer parasitoids and macrorhaphis are some of the biological agents that help in the control of pests. For biological methods of control to succeed, farmers require to distinguish and protect them in their fields.

Integrated pest management (IPM) is yet another method which is employed in the control of coffee pests. The method has a great future in coffee industry but require farmers to know when and how to use the various components of the method.

Coffee Processing:

High quality coffee is produced from high quality coffee varieties such as SL series and it is impossible to produce class 1 or 11 coffee from poorly cultivated coffee trees. However, we can make the best of coffee produced through the best processing methods. A good batch of cherry should be delivered to the factory if high quality coffee beans are to be obtained. Here,

the method of processing determine the final quality of coffee. Dry or "Mbuni" processing produces brown colour beans of low quality.

Wet processing is laborious but produces beans of blue to grey colours of high quality. Production of such beans require proper fermentation to remove the sticky mucilage which attracts moulds and insects. Protopectin is a constituent of mucilage which is insoluble and must be removed in a process called" fermentation". Mucilage breakdown is enzymatic and requires pH. of 5.5 - 6.0.

Thorough washing of the parchment proper drying, grading and subsequent storage are partinent steps in high quality coffee production. These steps determine the final quality of coffee and subsequent prices. If properly utilized, by-products of coffee processing such as pulp and recycled water can save on inputs as compost in farms, charcoal and mulch; while pulping water can be recycled for pulping. This would help to save on pumping water cost. However, disposal of such effluents on pumping should be carried out in a way that they don't pollute the environment down stream.

Policy Issues governing Coffee growing:

Regulations governing coffee production at both farm and factory level must be reinforced. The water Act cap. 372 emphasizes the importance of protecting the environment through adherence to "Water Right Apportionment" hence safeguarding the public from organic pollutants found in rivers. These effluents are said to lower the quality of coffee if used for pulping. The polluted water is unfit for irrigation, livestock and fish culture.

Owners of coffee factories may they be private or cooperative must be held responsible to clean the environment and provide safety precautions against pollution of waters down stream. Taxation from such firms must strictly be utilized to clean and protect the environment in coffee areas. Agricultural ACT Cap.318 emphasizes among other things the need to limit cultivation to certain gradient and distances from rivers and streams. If these regulations are reinforced and adhered to will help to curb incidences of soil erosion, siltation of river banks hence conserving biodiversity a long the rivers and marshy areas.

Article '35' of the international coffee agreement 1994 states that "members shall give due consideration to the sustainable management of coffee resources and processing, bearing, in mind the principles and objectives on sustainable development agreed at the Eigth Session of the United Nations Conference on Trade and Development and united Nations Conference on Environment and Development".

This article emphasized the importance that producers and cunsumers should place on environmental issues that affect the environment and final coffee quality. Some consumer organizations such as the coffee Roasters Association of Sweden have expressed their support for the method of integrated farming and pest control. The emphasis as to preserve the land for future use and the products chosen and used in farming should not cause health problems.

Environmental concerns:

With environmental health issues dominating coffee future path, organically grown and fairly-traded coffee has a bright future. However, more studies on the relationship between cholesterol and cancer, cholesterol levels and coffee consumption needs attention. (UNCTAD, 1994). Other issues concern pesticide residues on green coffee and use of methylene chloride in the decaffeination process.

Environmentalists claim that costa Rican coffee production generates more pollution than any other sector in the economy. (Loria, 1992). Contaminants from coffee pulp affects marine life near river mouths because coffee requires extensive use of pesticides. Coocafe, a Costa Rica coffee manufacturer is focusing its marketing efforts on the new eco-conscious consumers with its new coffee product Foresta. The manufacturer plans to donate 25 cents from each container sold to preserve the costa Rican environment (Boxman1993).

Indeed, this is a move in the right direction.

Kenyan coffee manufactures should produce various coffee brands for local and international markets. These products will encourage more participation by both producers and consumers. Greater participation implies more incentives to the producers hence motivating them to produce high quality coffee and protect the environment at the same time.

Technology Transfer:

To sustain the environment and resource base on which coffee thrives farmers, change agents and coffee scientists should work as collaborators. This collaboration should start at the point of problem identification, planning, execution, analysis and evaluation of the new innovations. This approach will provide the continuity and hence sustainability of the innovations because the farmers will have a stake in the outcome. Indigenous knowledge systems if wisely incorporated into the conventional scientific information will promote and stimulate innovation acceptance for environmental protection and high quality coffee production.

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Abstract

The paper outlines those factors necessary in the production of high quality coffee. Agriculture is a major non-point environmental pollutant through the use of pesticides, fertilizers, sediment discharge, by-products from industries, poor and irresponsible cultivation practices (Njoka, 1994). In conventional agriculture, many agricologenic plant diseases are due to simplification of the ecosystem and introduction of foreign factors (Hodges and Scofied, 1983). Therefore, the importance of employing environmentally sound and resource sustaining production principles in reference to coffee industry cannot be over emphasized. Environmentally benign coffee production practices are discussed and cross referenced with the conventional production practices. This highlights those practices that promote environmentally and resource sustainability in coffee industry. Special emphasis is placed on the control of soil and nutrient erosion, through sustainable production cultural practices; exploitation of biological systems to combat coffee pests and diseases with ultimate goal to enhance soil fertility, coffee yield and quality. Suggested production practices are selected on the basis of practical ecological and economic principles deemed necessary to make coffee industry more environmentally sound, economically viable and socially acceptable. To sustain the environment and resource base on which coffee thrives, farmers and change agent should be treated as collaborators in the generation of innovations.

THE AFRICAN COFFEE RESEARCH NETWORK : PROSPECTS AND CHALLENGES INTO THE NEXT MILLENNIUM

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1.0 Background

The idea to create the African Coffee Research Network (ACRN) also known as RECA, the french version meaning, "Reseau de Recherche Cafeiere en Afrique", wasconceived in June 1992 in Portugal in a meeting facilitated by the Technical Centre for Agriculture and Rural Cooperation (CTA) and attended by 17 African Coffee Growing countries: Angola, Burundi, Cameroon, Central African Republic, Congo, Tanzania, Togo, Uganda, Democratic Republic of Congo, Zimbabwe, Cote d'Ivoire, Ethiopia, Guinea, Equatorial Guinea, Kenya, Madagascar and Rwanda. The formation of this regional network was necessitated by two major reasons: the need to save further decline in quantity and quality of African coffee, and the need to draw up a plan of action that would enable the Inter-African Coffee Organisation (IACO) and CTA to have sufficient information from all centres and ministries concerned with coffee research in Africa.

The Portugal meeting made recommendations towards a common strategy of doing research in Africa embracing: Creation of a Coffee Research Network; Increase of productivity per unit area; Improvement in quality; Creation of liquoring laboratories; Extension of research results; and identification of possible sources of funding. Priority research themes were recommended and it was resolved that more opportunities should be provided to African scientists for meetings and training. The ACRN was mandated to support regional research on key constraints of coffee production, processing, marketing and consumption; and also facilitate sharing of information, exchange of germ plasm and other research materials.

The Network was launched in March 1993 in London and its headquarters is in Abidjan, Cote'd'Ivoire under the umbrella of IACO. Membership is open to all African coffee producing countries who are IACO members and Associate membership is allowed for other international institutes. CIRAD, ORSTOM, France and CIFC, Portugal are associate members. Members make a symbolic financial contribution to the running of the network.

2.0 ACRN Activities

Following its inauguration in March 1993, ACRN has held several workshops and meetings in conjunction with IACO and is fully participating in the current Coffee Rehabilitation Programme in Africa. Regional research proposals have been prepared and submitted mainly to ICO for funding by the Common Fund for Commodities (CFC).

2.1 Workshops/Meetings

The first workshop of the network on the "Improvement of Productivity and Quality of Coffee through Genetics" was held in Montpelier, France from 14-15 June 1993. At that workshop, a strategy was laid and various groups from amongst members were assigned to write various collaborative projects for donor funding embracing:

i) Establishment, expansion and maintenance of germplasm centres in Ethiopia (Arabica), Cote d' Ivoire (Robusta) and Madagascar (Mascaro).
ii) Creation of coffee varieties resistant to Coffee Berry Disease (CBD).
iii) Improvement of Robusta coffee quality by establishing liquoring units as well as conditions of production.

Subsequent workshops and meetings held by ACRN included the workshop on "Improvement of the Cultivation Techniques on Coffee Plants" held in Nairobi, Kenya from 6-10 December 1993. The major purpose was to examine national programmes of member countries on agronomic practices, extension systems, their results and constraints. The workshop brought together Agronomists and extension experts from member and associate member institutions and enabled them familiarise themselves with the Kenya Coffee Industry. Members observed that coffee research results are numerous but most of them not disseminated and that the ineffectiveness of extension is partly due to a lack of appropriate teaching materials.

Two meetings were held in Nairobi, Kenya from 23-26 May 1994 and 20-24 March 1995 respectively. The purpose of the first meeting was to discuss and formulate a draft project on Coffee Germplasm in Africa while the second meeting was the "First meeting on the STD III CBD Project (creation of coffee varieties resistant to CBD)".

In line with the activities of ACRN, Uganda Coffee Development Authority (UCDA) organised a Regional Coffee Research and Development Conference in 1995 in which members participated and presented papers on the progress of research in various countries.

The ACRN organised a Workshop on "Entomology and Plant Pathology" which as held in Doula, Cameroon from 3-7 October 1995. The objective of this workshop was to draw up a programme for ACRN on entomological and phytopathological aspects in coffee production and intensified control of the coffee borer. Participants expressed the need to facilitate the exchange of experiences between scientists in order to harmonise differences in training, consolidate scientific knowledge and standardise working methods.

Some ACRN members attended the last ASIC Conference in Japan in 1995 and two General Assemblies have been held by ACRN in November 1995 in Libreville and November 1996 in Abidjan respectively. The latter Assembly invited ACRN members to participate actively in the 17th ASIC Conference in Nairobi in July 1997. It was also observed that the development of Tracheomycosis in Democratic Republic of Congo and Uganda required

urgent steps. Thus, it was recommended that a regional workshop be held to address the disease. In a meeting held on 21 February 1997, and attended by ICO, IMI and CIRAD, it was agreed that the workshop be held on 28-30 July 1997 in Kampala hosted by the Coffee Research Centre (COREC) of the National Agricultural Research Organisation (NARO).

The biennial work programme of 6th November 1995 focused on 3 elements for 1996/97: Follow up of on-going projects; preparation of new projects according to recommendations of previous workshops; and preparation for the 17th ASIC Conference.

2.2 Regional Research Projects.

ACRN is currently coordinating four regional research projects whose proposals have been submitted for funding mostly to CFC.

1. The Quality Improvement by Characterisation and Optimal Use of Coffee Soils for the Production of Quality Robusta Coffee. The project aims at varietal selection based on coffee quality; Identification of optimal coffee soils and optimal soil use; Identification of cultural practices favouring quality; and preparation of a catalogue of Robusta coffees according to their characteristics. The project is located in robusta producing countries i.e Cameroon, Cote d'Ivoire, Madagascar, Togo and Uganda. The project proposal is being submitted to the Common Fund for Commodities for funding through the ICO and CIRAD is the Executive Agency.

2. Integrated Pest Management (IPM) for Small Scale Farmers in Eastern Africa. This project aims at developing IPM packages which are appropriate for managing pest problems. Participating countries include Kenya, Tanzania, Rwanda, Uganda and Burundi.

3. The Genetic Resources Component. The objective is the conservation and use of coffee genetic resources for the benefit of African coffee farmers. This is a pre-proposal submitted to European Development Fund by ACRN and the International Plant Genetic Resources Institute (IPGRI).

4. The Control of Tracheomycosis. The project proposal is to be written following the Tracheomycosis Workshop in Uganda.

All of these research projects are not yet financed. Several other projects are being prepared for funding by willing donors.

2.3 Collaborative Linkages

ACRN has found strength in collaboration among countries to promote mutual understanding and interdependence in solving common problems. To further the Network's objectives, collaborative linkages have been established with a number of institutions:

1. GENAGRO, a Management-Environment-Agronomy Association which is a Belgian based non-profit organisation. The objective of this collaboration is for GENAGRO to provide and disseminate teaching materials based on audio-visual aids designed for information and training in rural areas in ACRN member countries. In the long-run,

GENAGRO would train ACRN correspondents (directors, assistant directors, camera men, officers) in techniques of producing teaching materials based an audio-visual aids. This will ensure that quality teaching materials will be available for all research projects thus facilitating dissemination of results.

2. The Coffee Anthracnose Research Network in Africa (CARNA) which is an Inter-African Research Network on the Coffee Anthracnose. The Inter-African Phytosanitory Council (IAPSC) plays a liaison role between the CARNA and ACRN to ensure a harmonious collaboration.

3. In preparing and implementing projects, ACRN collaborates with many other institutions in a number of aspects. Some are financing banks like African Development Bank (ADB) and PTA Bank, donors like European Union, ICO/CFC or scientific collaborators like CIRAD, ORSTOM a French Scientific Institute with similar objectives on coffee like ACRN, IPGRI, IMI and the Coffee Research Institute of Portugal. The Network is in touch with IACO concerning the Coffee Rehabilitation Programme in Africa which is aimed at developing Programmes for rehabilitation of existing coffee.

2.4. Leadership

The current Deputy Secretary General of IACO is also the Co-ordinator of ACRN, Mr. Mpungi Buyungu. Chairmanship is held by scientists from member countries in rotation for a two year term and is basically a voluntary non-paid service. The first Chairperson for ACRN was Dr. W. Opile from Kenya while the second and current Chairperson is Mr. Coulibaly. The Vice Chairperson is Dr. P.K. Ngategize from Uganda.

3.0 Prospects and challenges.

Despite the notable achievements registered by ACRN since its inauguration, much still remains to be done to consolidate and enhance the gains already made. Some of the challenging regional research issues facing ACRN member countries are discussed below.

1. The Fusarium Coffee Wilt disease (Tracheomycosis) which is devastating coffee in the Democratic Republic of Congo and Uganda. For example in Uganda, 13 out of 27 coffee growing districts are affected but with varying intensity. In Bundibugyo, the worst hit district, about 40% of coffee shambas are affected with an average disease incidence of 5-10%. Nationwide, it is projected that in a period of 2-5 years assuming current infection rates, the disease would have destroyed 2% of the total coffee acreage which represents a financial loss of about US$ 3.5 million. Therefore, there is urgent need for sustained research for short-term and long-term control of the disease in East and Central Africa.

2. Coffee production, productivity and quality has been declining in Africa. For example, the continents share of total production went from 21% in 1960 to 30% in 1974 then to 20% in 1994. Although Africa has one third of the world coffee acreage, it accounts for only one fifth of production. There is need for promoting cost effective coffee production methods, breeding for productivity and quality to meet new market niches and facilitating exchange of planting materials.

3. Small scale coffee farmers lack sustainable methods of pest (insects, diseased , weeds, nematodes) control. In recent decades, increasing reliance on the use of chemical pesticides has led to greater production costs, pest resistance , outbreaks of new pests as well as human health and environmental problems. Development of Integrated Pest Management (IPM) packages should be a priority goal.

As ACRN forges ahead, one of its goals will continue to be to pool the scientific skills and other resources of member countries which will improve productivity, increase output and farm income. In order to make the impact of ACRN felt, there is need to strengthen the coordination preferably with full-time staff for the network to ensure the following:

1) Quick preparation of projects for funding and follow up
2) Facilitation of lobbying for funds for coffee research and the network
3) Innovations in collection of subscriptions from member countries
4) Aggressively seeking for funds worldwide to support regional projects
5) Dissemination of research results among members and exchange of germplasm.

The possibility of having 2 arms of the research network i.e. East and Central, and Western should be explored. This would be advantageous in terms of ease of coordination, distances and language barriers, and there already exists other regional bodies within the proposed regions which are funded by EU.

4.0 Conclusion

ACRN has made tremendous progress in the last 5 years in tackling the challenges facing member countries in the coffee sector. However, coffee production, productivity and quality in Africa is still low and pests are on the increase. With the approach of the millennium, ACRN should be more focused and design strategies to effectively combat challenging regional research issues. Member countries of ACRN and other stakeholders in African coffee should strengthen ACRN through prompt payment of subscriptions, active participation in ACRN programmes and sharing research information and materials. ACRN is further challenged to strengthen the co-ordinating office to efficiently prepare regional research projects acceptable to donors. The future success of ACRN is dependent on each member country recognising the challenges facing the Network and working hand in hand with other member countries to solve the problems for a common cause and better prospects. We are interested in helping to set up an International Coffee Research Network, with a view of improving the performance of the coffee industry worldwide. This super structure should not overshadow the small research institutes. On the contrary, it should assist and reinforce them by facilitating the funding of research programmes.

ABSTRACT

The African Coffee Research Network (ACRN) was founded in June 1992 in Portugal out of the need to save further decline in quality and quantity of African coffee. The meeting was facilitated by CTA and attended by seventeen African Coffee growing countries. ACRN was mandated to support regional research on key constraints of coffee production, processing, marketing and consumption; and also facilitate sharing of information, exchange of germplasm and other research materials. Following its inauguration in March 1993, ACRN held several workshops in conjunction with the Inter African Coffee Organisation (IACO), and is fully participating in the current coffee rehabilitation programme in Africa. ACRN has found strength in collaboration among countries to promote mutual understanding and interdependance in solving common problems. Notable achievements have been registered. However, much still remains to be done, to consolidate and enhance the gains already made. As ACRN forges ahead, one of its goals will continue to be pooling the scientific skills and other resources of member countries which will improve productivity and increase output and farm income. With the approach of the millenium, ACRN should be more focused and design strategies to effectively combat challenging regional research issues. These include Fusarium wilt(Tracheomycosis) in East and Central Africa, development of Intergrated Pest Management (IPM) packages, facilitating exchange of planting materials, promoting cost effective coffee production methods and breeding for productivity and quality to meet new market niches. ACRN is further challenged to strengthen the coordinating office to efficiently prepare regional research projects acceptable to donors. Member countries of ACRN and other stakeholders in African coffee should strengthen ACRN through prompt payment of subscriptions, active participation in ACRN through prompt payment of subscriptions, active participation in ACRN programmes and sharing research information and materials.

SPECIAL WORKSHOP ON THE ENHANCEMENT OF COFFEE QUALITY BY REDUCTION OF MOULD GROWTH

Current overview of ochratoxin A – further suggested action

Mould formation on coffee beans creates product, which can be unacceptable to purchasers and processors. Some moulds produce toxins, which can be dangerous for human consumption. One of these is ochratoxin A [OTA] which is nephrotoxic and possibly carcinogenic to humans.

In the last year regulatory authorities of the European Union [EU] have been discussing the possible imposition of OTA limit values for green beans.

Owing to little factual knowledge existing regarding the mycology in the green beans and how moulds infect, develop and form toxins, the European coffee industry acted promptly by commissioning a study to create understanding and knowledge. This investigation will produce results permitting the development of actions to assist in the control of critical control points [CCP] during the production of coffee from the tree to the processor. These actions will further develop and form the basis of Good Agricultural Practices [GAP] and Good Manufacturing Practices [GMP].

As reported by India, the immediate implementation of practices based on current understanding of GAP and GMP have already resulted in improved quality of the product, resulting in greater export potential and therefore revenue earnings. These have been accomplished at low costs by internal training and education.

The development of CCP's, GAP and GMP procedures will form the basis of the program "Enhancement of coffee quality by the prevention of mould growth", where the International Coffee Organisation [ICO] will act as the supervisory board and the Food and Agricultural Organisation [FAO] will act as the Project Executing Agency [PEA]. This program aims to reduce mould formation leading to toxin development in coffee to the absolute minimum and is the internationally agreed and preferred action to reduce human exposure.

The establishment of limit values at the suggested level of 5 ppb by the EU would currently create rejection of substantial quantities of imported greens. Further suggested action by the London International Financial Futures Exchange [LIFFE] would cause even higher rejection levels of coffee. Coffee is produced in many countries some of whom are designed Least Developed Countries [LDC], and many rely on coffee export earnings as a major factor of their external revenues. Such levels of rejection would be of serious economical and social consequence to such countries.

A limit value imposed by the EU would inevitably be followed immediately by all major importer countries.

Eventually Codex Alimentarius will have to set an appropriate standard.

We seek the commitment by all the participants at this meeting to pursue immediate action on return to your country to develop and implement basic GAP and GMP action through training and education as a first step. The first set of draft guidelines is attached.

As soon as more detailed results become available through the Prevention program, with understanding of CCP's and other actions they will be made available through the Prevention program activities for inclusion into your programs.

Draft guidelines for good agricultural practices and good manufacturing practices

Below are listed some practical measures, which can be taken to minimise or prevent mould growth in coffee. Just like the coffee plant, fungi require water and nutrients in order to develop. The suggested measures are designed to minimise the time during which the fungi experience favourable conditions in coffee production. Healthy plants produce healthy fruits and the tissue layers of sound fruits can protect the moist, nutritious interior of the cherry from contact with mould. Nevertheless, contact with any obvious sources of fungal contamination (soil, poor water, and mouldy fruits) should be minimised to help the cherries' natural defences. Once harvested, drying should be done as expeditiously as possible to minimise the period during which there is sufficient water for growth. Coffee is produced using many different systems world-wide. The suggested observance of these practical rules developed from past experience can help you to regularly produce a high quality product and to maximise the return on y
our crop.

Orchard management

GAP is predisposition for healthy plants and fruits.

Harvesting

Avoid physical damage to the cherries.
Avoid soil contact by use of mats.
Sort out foreign material and defects.
Keep separate overripe, split and fallen to the ground cherries.
Keep mats clean.

Wet processing

Pulp on same day as harvesting.
Separate floaters.
Control quality of water.
Sanitize equipment.
Separate completely parch and pulp.
Complete fermentation within local standards.
Control quality of water and sanitation of equipment during washing.

Drying -	If possible, rapidly remove excess water with forced drying.
	Dry slowly to avoid cracking by excessive heat, control layer thickness and turn regularly.
	Avoid re-wetting.
	Cover with ventilation especially at night.
	Avoid re-wetting.
	Use mats or drying tables when possible.
	Maintain mats/tables in clean and sanitised condition.
	Achieve water content for parchment of maximally 12%.

Dry processing

Start drying on day of harvesting.
Spread immediately – never heap – control layer thickness – turn regularly.
Avoid soil contact – dry on mats or preferably drying tables.
Avoid re-wetting – cover and ventilate.
Achieve water content of cherries maximally 12%.
Clean and sanitise mats and tables or other drying surfaces.

Storage

Never store different types together, i.e., parchment with dry cherries or beans.
Store only below critical moisture levels.
Store only under adequate air circulation and ventilation.
Ensure waterproof floors, walls, roofs.
Design roof to insulate to minimise heat transfer.
Avoid wall contact.
Prevent exterior winds entering.
Minimise storage at humid/warm conditions – no storage in harbour unless in controlled warehouses.
Clean and sanitise bins and silos.

Hulling

Keep beans, parchment and husks completely separate.

Transport of beans, parchment, and dried cherries

Avoid re-wetting.
Clean and sanitise all transport means.
Keep period of uncontrolled temperature and humidity to minimum duration.

Training

Train farmers and processors adequately.
Involve local research.

Processing

Clean the beans well.
Clean and sanitise equipment.
Control dust adequately.
Do not re-incorporate chaff into product.
Remove residual husk.
Do not use cherry waste in soluble manufacture.

A GLOBAL NETWORK FOR COFFEE RESEARCH

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INTRODUCTION

The proposal to initiate a feasibility study into a possible global research network for coffee was put forward by the Executive Director of the International Coffee Organization (ICO) in January 1996 and approved by the International Coffee Council (ICC) in May 1996. This paper, acting as an interim report on the initial findings of the project and placing it in the wider context of the environment in which any implemented network will have to operate, addresses the benefits of forming a global information network covering scientific and technical research on coffee growing and processing. The scope of the study is to identify the potential for increased international cooperation, harmonization, linkages and communications between organisations implementing substantial coffee research programmes. The aim of any implemented network would be to provide relevant information, either direct or through gateways and hyper text links, and to assist the worldwide coordination of research on coffee, while maximising the use of information sources and other resources already available and providing a discussion forum for the international coffee information user community. Particular attention has to be paid to the users in developing countries in such a cooperative network, and the need for specific training. With regard to existing resources and facilities, the ICC, at its meeting in May 1997, noted that the ICO's Executive Board had encouraged the Executive Director to develop services, using the options available through the use of the Internet, both to facilitate ICO communications and to disseminate information (International Coffee Organization, 1997).

It is appreciated that there are already information sources on coffee research available, although at present these are fragmented and many are at a low level of awareness to the international coffee information user community as a whole. At present there is no specific and systematic global network for the exchange of information for this subject area and for cooperation on research. Such a network, in addition to providing access to new information identified and made available, could potentially provide cost effective and efficient access to the already known fragmented sources through links and hence avoid duplication of effort and waste of resources. In addition this may well facilitate access to peripheral information sources on the Internet, for example those at more generalised agencies, that are often required to provide information as background for research projects. All of this can result in increased justification for unconnected organisations to acquire the necessary facilities for access to the Internet as well as increasing the efficiency of cooperation which at present has to rely heavily on informal and personal links between researchers.

All of this can be placed in the general framework of the future of information work, although with the speed and breadth of information and communications technologies (ICT) development at present, it is not possible to predict accurately what will be the end result. However the trends are undeniable. The number of users of electronic communications is growing rapidly worldwide, although there are worrying geographical differentials in the speed of this growth. The technological constraints to this growth are decreasing while the

facilities are becoming more user friendly and the capacity for storage, processing and retrieval of information are increasing with a reduction in associated costs. Such trends are likely to result in an increased emphasis on intelligent and organised access to, and the availability of, information and the associated services, irrespective of where the information is held. In addition, this means that such developments are likely to be at the expense of more traditional information provision services. This raises the question as to whether any information user community can afford not to pursue such a development as opposed to investigating the desirability of it in isolation.

SURVEY OF POTENTIAL USER COMMUNITY

In order to provide evidence of attitudes to a proposed network a survey of potential users was conducted by the ICO in May 1997. The intention of this survey was to obtain information from potential user organisations conducting significant research into coffee, concerning the facilities available to make use of such a network particularly with regard to ICT, the activities of these organisations in relation to coffee research, the information and services that should be supplied and the contributions the users can make to a commonly owned network. For comparison, the introduction of services, now well established, related to development and the environment and based on the use of the Internet are described elsewhere (Ferguson, 1996), where a consortium of organisations is working to harness new ICT to tackle the problems of information related to Third World development. This is but one network involved in some aspects concerning the Third World.

The survey form attached as Appendix A and designed to obtain the above information, has been distributed in English, French, Spanish and Portuguese. It has been sent to all ICO members, 44 exporting members and 18 importing members, and to a supplementary list of 32 organisations known to be interested in the project. Wherever possible this data collection is being facilitated when staff from potential user organisations visit the ICO, or when ICO staff visit the organisations concerned, both primarily on other duties, in addition to taking advantage of similar occasions presented by conferences, seminars and other meetings. These last mentioned are also giving the opportunity for multipartite discussions and further contacts. Where necessary this information is being supplemented by directed correspondence initiated by the ICO.

At this stage 21 replies have been supplied from 19 countries. The encouraging factor here is that they are mainly from the major players and are particularly positive in their support for such a venture. This is demonstrated in the responses concerning the willingness to be considered for a national/regional coordinating role and the commitment to make available local resources, although these are unlikely to be financial. This includes the provision of endogenous information to be mounted on any such network to the benefit of a wider audience. The respondents give three closely scored indications of the importance they attach to such a development. These are, in importance ranking given, the testing of techniques in different environments, the avoidance of duplication and the harmonization of research. These are closely followed by the wish to share information and to exchange materials. All these factors received a score of between 60 and 75% on desirability ranking as tabulated below. There are also other isolated individual reasons given. With regard to duplication of effort and any associated waste of resources, many non electronic information provision services and related networks contain necessary duplication. Electronic networks have the potential to reduce this as well as eliminating additional unnecessary duplication.

Testing techniques in different environments	74
Avoidance of duplication	71
Harmonization of research	71
Sharing of information	68
Exchange of materials	61
Other aspects	20

To receive a greater volume of response at this early stage in the project could well prove an embarrassment and disappointment to some potential users as it would be unwise to initially implement any final network design with an excessive number of user organisations.

LEVELS OF CONNECTIVITY

This project is being developed in the recognition that research networks are not necessarily, and often not advisedly, concentrated primarily on electronic communications as opposed to more traditional forms. Networks are normally a mixture of such facilities based on the existing facilities and the resources available as related to the needs of the particular network and its community. Indeed networks are still initiated without any reference to electronic networking. An example of this is the European Union (EU) funded programme of Tropical Forestry Information Consolidation, Networking and Dissemination managed by the United Kingdom (UK) Overseas Development Institute (ODI), an extension to the Rural Development Forestry Network that has been operational since 1992 (Brown, 1997). However many products of this programme seem to be appropriate for an electronic network if the facilities were in place to accommodate such exploitation.

In considering levels of connectivity, it is important to bear in mind that although a particular country may have connectivity, even at a high level, it does not follow that the relevant user organisation that could make best use of any coffee electronic network, will also have access. Even if such connectivity is available in the same geographical area of the country, the facilities may not be available to make that final connection to the network. However this is unlikely to be based on the inadequacy of the technology potentially available and more likely to be related to economic and political barriers to supplying the resources required, including training. In many of these problem locations, multinational and international organisations such as banks and airlines, are already using these facilities through such means as dedicated lines and satellites. Often it is the cost and skills to operate such systems that are prohibitive to smaller and less well resourced users rather than the technological developments. However these factors do not preclude the use of the potential of the Internet and networking for the international coffee information community, but reinforce the need to provide an extensive range of connectivity levels. Such problems have already been highlighted (Panos Institute, 1995) while implemented networks, such as Devline (http://devline.ids.ac.uk), have been designed to offset such disadvantages, particularly for users in developing countries (Beer, 1996). In the latter, facilities were made available for the full range of connectivity levels from the World Wide Web (WWW) to mail and hard copy provision. Such a design will be important for any proposed global coffee network, although, with the high priority being given to, and the amount of work being put into, electronic networking, in Africa for example by the United States Agency for International Development (USAID) through the Leland Initiative covering, initially,

twenty countries (Butsch, 1996), hopefully this will only be a temporary necessity. Where necessary, email information provision can provide a practical alternative for Internet access in the absence of WWW access facilities as can Compact Disc-Read Only Memory (CD-ROM) and floppy discs as well as information broking through a third party and hard copy provision.

Initial results from the user survey mentioned above show an expected pattern. Returns from Central/South America and Europe show that high levels of connectivity, including the use of the Internet and WWW, are available to potential users of any global coffee research network, although there is a need to cope with a small number with very low connectivity levels. However the returns from Africa show a very different situation with low level of connectivity, if any. Returns from Asia also show a high level of connectivity, while those from Oceania suggest low levels. Such results emphasise the need for the provision of the full range of connectivity levels including third party information and document provision until the time when these developments make any of less ICT intense connectivity levels excess to requirements. The economics of an equitable provision of information to members of the coffee research information user community may well hasten such developments.

Connectivity can also cover languages and this is an area that will require debate within the international coffee information community. However it seems desirable, because so much of the global science and technology information activities uses English as its carrier language, that any worldwide coffee information network should initially do likewise. Future developments can certainly include a cost benefit analysis of introducing other carrier languages.

POTENTIAL SERVICES

Based on the study of other information networks, both electronic and otherwise, a considerable range of potential services can be established. The common occurrence of some of these can be interpreted as their having gained a standard status.

One of the most often used uses of electronic networking is to make available to a wide range of user communities, a series of databases which would otherwise be accessible to a limited audience only. These databases could cover researchers, research organisations and research projects. Such databases can be either bibliographic or statistical. A primary example of such a bibliographic database could be any one of the ICO series of national coffee profiles such as the one on Papua New Guinea (Muir, 1996) in full text form which in itself, could be a model on which to build, over time, a virtual library of full text coffee documents. A secondary form would be Coffeeline facilitating the identification of a subset of document surrogates to answer specific enquiries. A statistical database is exemplified by the ICO periodic publication Coffee Statistics (ICO, 1996). A non ICO database of interest to users would be the African Coffee Commodity database that was being investigated by the EU Technical Centre for Agriculture and Rural Cooperation (CTA) a few years ago. Subject to publishers' requirements and copyright regulations, hard copy directories such as the Coffee International Directory (International Trade Publications Ltd, 1996) could be made available in electronic form over the network under this heading.

The dissemination of news uses such services as bulletin boards and newsletters, while discussion lists provide the facility for interaction. The ICO already has a newsletter, Coffee

Newsletter, that could be made available over any implemented network in machine readable form in the four languages available, although this could complicate the provision of this publication in hard copy form on a subscription basis. Similar complications could occur with regard to databases mentioned above, but such difficulties could be circumvented by the use of password systems where the password to the network is provided either free or on subscription depending on the status of the user.

Other services could include the provision of software and data archives and datasets as well as support, at a higher level of sophistication, for graphics, visualisation and multimedia, and gateways to other networks and information sources on a global basis, although certain of these are best considered future developments. Gateways and hyper text links can be used to access such databases as the United Nations (UN) Food and Agriculture Organisation's (FAO) International Information System for Agricultural Sciences and Technology (AGRIS) and the Current Agricultural Research Information System (CARIS). A calender of events and meetings could also be provided.

Once the initial set of services has been established, based on the agreed core subject areas, further development can take place to provide these in other subject areas, such as economic research, based on a balanced analysis of demand, availability and processing elsewhere, and the economics of aggregating sources into the global coffee information network or continuing to rely on gateways and hyper text links. Based on similar analyses there may be some justification in parallel development, with cooperation, coordination and links, of such a network based on economic research of coffee. It is noted that some initial interest has been shown in this.

Probably one of the most beneficial overall attributes of any network is providing an efficient means for related organisations to disseminate and share information and knowledge within the community, irrespective of which of the above categories are used.

In the survey of the potential user population, none of the expected services listed scored under 50% in the desirability ranking as tabulated below. However databases occupied all the top positions in the priority order of research projects, researchers and research institutions. News services desirability ranking was newsletters, discussion lists and bulletin boards.

Research projects	80
Directory of researchers	69
Directory of research institutions	68
Newsletters	68
Discussion lists	66
Bulletin boards	55

NETWORK ENVIRONMENT

This project has already attracted the attention of the World Bank (WB) and the promised feedback is eagerly anticipated (Lodder, 1997). Also this project seems to fit well into the structure of the WB Information for Development (infoDev) program (http://www.worldbank.org/html/fpd/infodev) which accepts proposals for funding. A submission for this project seems appropriate in the infoDev Category 1. Consensus

Building and Awareness Raising, Subcategory B. Specialized Sectorial Networks. Further information about this program and the opportunities it offers can be found on an Internet site organised on behalf of WB by the United States of America (USA) Volunteers in Technical Assistance (VITA) (http://www.vita.org/technet/issue.html). Earlier topics have included information and ICT in relation to the environment and education. This can be placed in the context of a WB meeting held in June 1997 to accelerate the installation and spread of ICT in Africa. This was a follow up meeting to the G7 IT and Development Summit held in Johannesburg in April 1996 where the development of that continent away from irreversible marginalisation was linked with the potential of ICT to make knowledge networks available to African organisations linking them to the information resources centres of the world.

The above indicates one way in which any proposed global coffee information network can fit into the wider information network environment. It would certainly be unwise to develop such a network in isolation, particularly in relation to coffee itself. Hence such networks as the African Coffee Research Network (ACRN), together with the discussions in progress to regionalise coffee research in selected countries in Southern Africa following the successful and well established pattern for tea, and a proposed Research Network on Coffee for Asia Pacific Region should be investigated as to the role they can play, both as information providers and users, in any proposed global coffee information network. The written proposal for the latter fits very well with the ICO initiative.

The Consultative Group for International Agriculture Research (CGIAR), with particular interest in food crops, has a well developed and highly sophisticated network, CGNET, linking its centres and headquarters (HQ) through using international leased lines, Internet routers, terminal servers and a planned voice mail capability using local dial up at local rates to the CGNET Network Operating Centre (NOC) which acts as the hub of the network. Further information on this can be found through the Internet (http://www.cgnet.com). The high level of sophistication demonstrates the availability of the technology required and how successful implementation can take place if sufficient resourcing is provided. This system seems far in advance of what would suffice for the international coffee information community and is unlikely to be suitable as an overall model. However there may well be subsystems that can provide useful information and experience to any proposed global coffee network. Even better would be any opportunity, because of convenient geographical locations, of using the CGNET communications facilities if CGIAR was willing to make them available.

COSTS

There is no doubt that the introduction of electronic information transfer, for those that have the facilities to exploit it, has provided a method that can potentially reduce costs and is certainly the most cost effective method of carrying out such an activity when based on unit cost analyses. However overall costs can increase considerably because of the much larger volume of information that tends to be transferred once the facilities are available. Unfortunately even when this is a cost reducing activity, it is not all gain as the financial impact of this, although substantial, is reduced by the need for capital investment and training. Costs in this paper are concentrated on those of the network organisation, administration and management. The individual costs that have to be incurred by the user organisations in accessing the network are considered their own responsibility. As the results of the survey show, a considerable number already have the necessary facilities and

are using them to access other information sources. Other organisations will certainly need assistance in obtaining the required facilities and such actions will need facilitation by the network management team.

Which set of costs are incurred depends considerably on a cost benefit analysis of whether to use a dedicated in-house server for a WWW site, for example at the ICO as the hub of the network, or whether to use a third party host such as One World Online (OWO) (http://www.oneworld.org). However there are considerable costs in common with both arrangements. One of the advantages of using an in-house server is that more control can be exercised over the operation and development of the network, although there are usually associated higher costs, particularly capital ones. Indeed there may well be lower running costs with a third party host as well as providing additional skills, facilities and peripheral information sources within the same system. In addition, third party hosts, available on subscription, usually reduce the individual WWW site owner's legal responsibility (Smith, 1996). Occasionally a hybrid/dual system provides the optimal arrangement, but at this stage this is thought unlikely to be the initial arrangement best suited to any global coffee information network. Costs will also depend on where they are incurred. For the example, here costs are estimated in the European context.

A full costing for setting up a WWW site to act as the nucleus to any international coffee information network based on a third party host and covering staffing with full overheads including accommodation, technical support and consultancy, training and the subscription to the host, for example OWO, might be in the order of £50K in the first year, decreasing to under £40K in subsequent years and includes provision for a full time network coordinator. This takes into consideration the investment already made by the ICO in the first stage development to create a prototype WWW site purely for ICO purposes. However this estimate is tentative and a further detailed investigation into the costing of a specific network design using existing resources and possibly taking advantage of any local contributions, particularly the non financial ones, to be made by network members, will be necessary. In this very superficial costing, no account has been taken of the continuing rapid decline in the costs of ICT hardware and software, or of any savings that will occur in addition to increased efficiency and uncosted benefits. The further costing investigation should include a cost benefit analysis as part of any subsequent design stage, to identify the preferred option between using either a third party host or concentrating on in-house WWW site resources.

CONCLUSIONS

Based on the initial findings of this project it is seems likely that the earlier expressed hope that the ICO become the hub of an international information network at the service of officials in the agricultural services of governments, scientists and students engaged in basic[coffee] research (Lodder, 1996) can be realised. This would be in line with the suggestion from the recent ICO seminar on coffee and the environment that the ICO should act as a think tank for the dissemination of information on sustainable coffee production (Tropical Agricultural Association, 1996).

Research so far indicates that it is feasible and desirable to set up a global information network related to coffee. This is based on the operations of the many other networks in selective and restricted subject areas/disciplines, which are frequently global and

incorporate Third World user communities, giving both direct and indirect access to specific information and using gateways and hyper text links for additional information provision. Such implementation is not dependent on the technological developments, but the economic and political will to take advantage of facilities already available and develop them for the specific needs of the international coffee information user community. The potential user community survey indicates that there is a positive attitude to such a development and a commitment to become involved both as information providers and users. Access facilities are available and the desired services can be provided.

The findings of this feasibility study will be presented to the ICO in September 1997 and the final project report will include a detailed evaluation of the best options. It is hoped that interested parties will use this interim report as a guide to send further feedback to the author or to the ICO, which could be incorporated into the final report.

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The views expressed in this paper are those of the author alone and do not necessarily represent an official view of either the ICO or the IDS.

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ABSTRACT

A feasibility study into a global network for coffee research has been initiated by the International Coffee Organization (ICO) and an interim report is given here on the preliminary findings, placing them in a somewhat wider context within the general worldwide information and communications technologies (ICT) framework. The investigation is taking note of existing information sources and other resources that are already available. The potential benefits of such a network are being explored.

A survey of the potential user community is being carried out to obtain information on access facilities available, users' activities and potential commitment, and services desired. The initial results of early analyses of these data are provided. These are encouraging and positive, involving mainly the major players.

The levels of connectivity are being considered, particularly in relation to a potential network where a significant proportion of the user community is in the Third World. The range of services possible is discussed in relation to those now expected in the development of such a network, with reference to particular information products and other networks that are already fully operational. Initial costings are presented.

Research so far indicates that such a network is feasible in line with the desires of the international coffee information community. The best options are to be evaluated and presented in detail in the final project report to be presented to the ICO in September 1997.

APPENDIX A

INTERNATIONAL COFFEE ORGANIZATION

FEASIBILITY STUDY ON A GLOBAL RESEARCH NETWORK ON COFFEE

The International Coffee Organization, in accordance with a proposal approved by the International Coffee Council in May 1996, is conducting a feasibility study on the benefits of forming a global network covering scientific and technical research on coffee growing and processing. The project is further described in the document attached as Annex II. Institutions conducting significant research in coffee are therefore requested to complete the following questionnaire, designed to provide the basic background information for the study, and return it by 30 April 1997 to:

The Executive Director
International Coffee Organization
22 Berners Street
London W1P 4DD
United Kingdom **Fax: +44 171 580 6129**

QUESTIONNAIRE FOR COFFEE RESEARCH INSTITUTIONS

General

1. Name, address, telephone, fax and email (if any) of responding institution

.....
.....
.....
.....
.....

2. Name of Director or Chief Executive

.....

3. Name of contact person for the study

.....

4. Number of scientific research and technical staff employed

.....

5. Areas of coffee research undertaken (*please specify*)

.....
.....
.....
.....

6. Annual number of research projects in progress

.....

7. Annual volume of publications containing research findings on coffee

.....

8. Number of person/years assigned to coffee research per annum

.....

9. Names of publications (including periodical titles) issued with information on coffee

.....
.....
.....
.....

Information technology environment

10. Number and basic specification of computers
(e.g. 20 Pentium 100 PCs)

.....
.....

11. Do you have access to (*please tick*)

CD-ROM drives ..... Internet email ..... World Wide Web .....

12. Do you use (*please tick*)

Telnet ..... Gopher ..... FTP .....

13. Do you use any online information services (*please specify*)

.....
.....

14. Do you purchase information services on CD-ROM (*please specify*)

.....

15. Do you communicate outside your country by (*please tick*)

(a) Letters ..... (b) Fax .....
(c) email ..... (d) Others ..... (*please specify*)

.....

16. What is your main word processing software (*please specify*)

.....

17. What is your main spreadsheet software (*please specify*)

.....

18. What speed modems do you have (*please specify*)

.....

Contributions to a research network

19. What information could you make available to a research network

.....
.....
.....

20. Would you be in a position to act as a national or regional coordinator for a research network

.....

21. What resources (financial or other) could you make available to a research network (*please specify*)

.....
.....

Services expected from a research network

22. Please indicate the type of specific services you might expect from a research network and rank them between 1 (lowest) and 4 (highest)

(a)	Newsletters	.....	(b)	Directory of institutions	.....
(c)	Directory of researchers	.....	(d)	Electronic discussion lists	.....
(e)	Electronic bulletin boards	.....	(f)	Research databases	.....

23. Please indicate (using the same ranking) the importance you attach to

(a) Sharing of information .....
(b) Avoidance of duplication .....
(c) Harmonization of research .....
(d) Testing techniques in different environments .....
(e) Exchange of materials .....
(f) Other aspects (*please specify*) .....

.....

24. Please comment on any other aspects of the proposal to establish a global coffee research network which you consider of relevance

.....
.....
.....
.....
.....
.....

25. When returning this questionnaire please attach an organization chart of your institution and the latest Annual Report (if available)

Name: ..... Signed: .....

Title: ..... Date: .....

International Coffee Organization
Research Questionnaire 20 March 1997

ECONOMIC ASPECTS OF COFFEE BERRY DISEASE CONTROL IN TANZANIA

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GENERAL OVERVIEW

Pesticide usage in Tanzania is dominated mainly by the need to control CBD and CLR. Effective control of CBD has largely depended on rigorous fungicide spraying schedule to protect the developing crop when it is prone to infection (Bujulu et al 1977; Griffiths et al 1971). In year 1993 the world pesticide market was valued at approximately US $ 4120m and of this expenditure, about 30-35% was on fungicides. In contrast, fungicides accounted for nearly 65% of the Tanzania pesticide market valued at approximately US $ 38m in 1994. The need for this emphasis on fungicides in the Tanzania context is the need to control CBD, and if we look at the broad structure of pesticide usage in Tanzania we can clearly see that: (a) Coffee dominates the overall market structure accounting for nearly 50% of pesticide usage. (b) With the coffee market, fungicide usage predominant and accounts for approximately 55% of the total pesticide expenditure on coffee. Of the two major diseases in coffee, CBD and CLR, the former probably accounts for some 70% of the total expenditure on fungicides, and shows just how important the control of CBD is in Tanzania. The economics of CBD control can be dealt with from both the farmer and the national point of view. In terms of farm level economics, the relevant issues would be the exploration of alternative control measures open to the farmer their costs and benefits, existing practices. At the National level, the economics of CBD control would address itself to the impact of the currently practices control measures on the national level, their costs in terms of foreign exchange.

FARM LEVEL ECONOMICS

Coffee is a major source of income to over 420,000 small scale producers in the country (Noah 1988). It is fully recognised today that Coffee Berry Disease can adequately be controlled at the farm level through efficient application and timely use of tested and approved fungicides. In Tanzania, the use of these fungicides is prescribed to farmers through Kahawa News, leaflets and Lyamungu News letter. These news and leaflets specify the fungicide to use, the rate, frequency and timing of application. How ever the economics of these protection measures is often silent despite its central role in determining the worth whileness of the measures. To a coffee farmer, be an estate owner, a manager or a smallscale operator, the alternative courses of action with regard to CBD control are in the short run. (1) Not to spray at all (2) To spray for protection only. In the long run however, a third alternative exists ie. use of CBD resistant varieties (Fig 1). To evaluate among this

alternative measures, a farmer will consider each option in terms of: (1) Contribution to increased productivity or improved savings in crop losses. (2) Justification of the measures in terms of costs and benefits.

It follows therefore that any economic evaluation must start with the assessment of the possible magnitude of crop losses from not controlling CBD or crop against accruing from control measures. In practice this is a management function through the cropping cycle to perform repeated and periodic checks on the cropping level, the magnitude of the disease and the likely damage if control measures are not implemented.

LIKELY BENEFITS FROM ALTERNATIVES: EXPERIMENTAL EVIDENCE

Although difficulties have been voiced regarding the conclusive establishment of crop losses from CBD (Griffiths et al, 1969; Bock 1963; Huxley et al, 1969), various opinions on the subject have been recorded. Ngulu (1993). Kilambo et al (1995) reported that crop losses attributed by CBD at farm level ranges between 30-60%. Bujulu (1977) and Brownbridge (1984) reported that crop losses due to CBD in the northern coffee growing areas of Tanzania ranges between 4-50%. Griffiths (1971) has also shown that few and mistimed early spray will not only fail to control the disease but will result in substantial crop losses.

An early spray timing trial at Lyamungu shows that at today's prices of coffee and costs of inputs a farmer is likely to obtain in a flush years a benefit of four to five times the cost of controlling CBD by spraying both in short and long rains (Table 1). A look at the cost benefit variability over the period of experimentation shows that in a poor crop year a lower cost programme of spraying either during the short or long rains is favourable. The choice of when to spray and its frequency must obviously be dictated by the cropping level and the disease magnitude and the variability of the two over time and space.

CURRENT CBD CONTROL MEASURES WITHIN ESTATE AND THEIR LIKELY PROFITABILITY

Our survey of the large scale estates in Arusha Tanzania revealed that the vast majority spray for CBD protection and very few in deed do not spray. An examination of the estate practices reveals that farmers apply a minimum of seven recommended spray rounds a year. However, in severe CBD years they undertake an extended programme for crop insurance. Swai et al (1996) recorded up to 10 spray rounds in some farmers. It is difficult to justify any fungicide spray regime exceeding more than seven sprays per season. The fungicides used range from single to fungicide mixtures depending on availability and individual estate policy. To show a comparison between the non-spraying and well sprayed estates evidence was obtained from Tingatinga and Kibo coffee estates and the average yields from these estates compared with Burka Coffee estate. Tingatinga and Kibo did not use to spray up to 1994. The average yield for a five year production period for the two non-spraying estates was 960 kg/ha clean coffee with the worst two years averaging 250 kg/ha. On the other hand Burka estate on average yielded 1680 kg/ha of clean coffee with the average of the worst two years being 1350 kg/ha. The comparison in yields relates to the same period.

CURRENT CBD CONTROL MEASURES WITHIN SMALLHOLDING AND THEIR LIKELY PROFITABILITY.

Within the smallholder coffee producing community the recommendations issued by Lyamungu Coffee Research are highly compromised. Specifically in relation to CBD control the current practices range from non-application of any control measures to application of 4 - 5 spray rounds in a year concentrated in the long rains. This is in contrast to the seven recommended rounds per annum. The main fungicides used are Bravo 500 and copper (50% WP) with the latter predominating. The main reasons accounting for the observed practices are low profitability and saving, poor farmer liquidity fungicide availability, inadequate support credit, discouraging loan, repayment system, and the farmer strategies to minimise production constrains and risks (Swai et al, 1996). Nevertheless their low productivity due to production constraints in consequence reduces their credit worth and as a result receive less credit than that necessary to cover the full cost of control.

BREAK EVEN RESPONSES TO IMPLEMENTING THE SPRAY PROGRAMME.

The farmer alternatives within the spray programmes are to use either straight or combinations of fungicides. Table 2 shows the recommended spray programmes for CBD control. Programme I is designed for use in high altitude areas where CBD is severe but leaf rust is not significant problem. Programme II is an alternative to programme I and using copper instead of Bravo. The programme can control leaf rust in addition to CBD. With current prices of fungicides the annual cost per hectare of implementing programme I ranges from TAS 0.13m to 0.2m. This in consequence would require a response of between 120 to 230 kg of clean coffee from the same production area to cover only the cost of protection. The use of programme II would cost 0.11m per hectare at current prices and would require a response of 150kg of clean coffee to break the even. Programme III is more diversified and the annual costs of implementation ranges from 0.16 - 0.25m. This means that a farmer will have to salvage a crop loss between 160 - 250 kg/ha of clean coffee to cover his costs.

The implication of the above observations is that a farmer will have to tailor his expenditure on control measures to his best estimate of the potential crop losses being salvaged by spraying. In so doing the farmer will have no benefit of advance quantitative knowledge, as relied on in his paper, of the actual damage caused by the CBD in particular period of production. He therefore has to exercise his every best judgement and experience to forecast the magnitude of the crop requiring protection, the likelihood of bad weather causing severe CBD as well as the timing of the decision to spray relative to the expected losses and how well these hamonise with the resources at hand.

FUTURE PRACTICES

The price of fungicides has been increasing annually, as compared to coffee price therefore there is increasing economic rationale to shift from high cost technology to low cost methods of control. As a short term, the low cost methods will be: (1) Refining the use of spraying by relating the programme more rigidly to rainfall pattern. (2) Shifting to chemical mixtures (3) Increased use of cultural methods of control. In the long run, however, the release of resistant varieties and increasing its adoption to farmer will be answer to the CBD problem.

NATIONAL LEVEL ECONOMICS

It is true that a consequence of poor CBD control practices especially within smallholdings is a loss of national yield. In terms of foreign exchange, the importation of fungicides presents a drain in national monitory reserves with the share of coffee being nearly US $ 28.9M today. Considering the unfavourable balance of payments and increasing disparity between the unit prices for agricultural exports against those of manufactured imports, it is imperative that the Government should examine ways and means to minimise costs at the national level and maximise benefits. In the short term local formulation and manufacture of CBD protection chemicals presents an alternative Nevertheless this would be both technically and economically infeasible due to shortage of raw material and investment capital and in any case such an investment would in the long term be undermined by the new resistant varieties. In terms of feasible practical alternative, encouragement of good spray practices with support credit system present a practical and realistic alternative. Equally, encouragement of Research into low cost rapid uptake technology based on improved cultural practices would result in increased farmer and national benefits both financially and real terms.

In the long term the resistance varieties are the ultimate answer to the CBD problem especially to the small holders sector who are currently dominates the total national production. Nationally the implication of adopting the CBD resistant varieties is the reduction in annual fungicide importation costs by approximately 90% and a saving in foreign exchange valued at nearly US $ 26M. Where at the farm level the lowering in costs would translate into improved profitability and farmer incomes.

Figure 1: Farmer's choices for CBD control

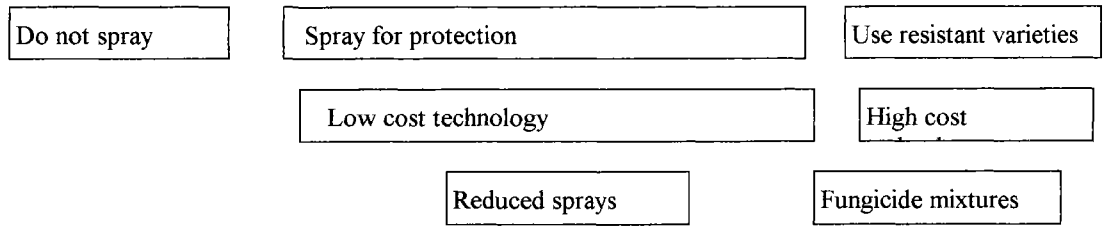

Table 1: Yields and Cost/Benefit analysis of various spray timings (Lyamungu, 1998 - 90)

Treatment	19 88		19 89		19 90	
	kg/ha	C.B.R.	kg/ha	C.B.R.	kg/ha	C.B.R.
T1	1010	1:3.6	822	1:4.8	605	1:3.5
T2	982	1:2.8	736	1:3.1	625	1:4.8
T1+T2	1425	1:5.2	1660	1:7.8	1720	1:8.0
Control	630	-	385	-	332	-

T1: Two sprays in the short rains T2: Four sprays in the long rains, T1 + T2: combined
CBR : Cost / Benefit Ratio N.B Labour costs not included

Table 2: Recommended Spray Schedule for CBD control

Sch.	J	F	M	A	M	J	J	A	S	O	N	D
I	-	-	**B**	**B**	**B**	**B**	**B**	-	-	-	-	-
II	-	-	**C**	**C**	**C**	**C**	**C**	-	-	**C**	**C**	-
IIIa	-	-	**BC**	**B**	**B**	**B**	**B**	-	-	**C**	**C**	-
IIIb	-	-	**BC**	**BC**	**BC**	**BC**	**BC**	-	-	**C**	**C**	-

B: Bravo 5 l/ha or Dyrene 6 l/ha and/or Daconil 4.4 kg/ha C: copper formulations (50% WP) 7 kg/ha
.BC: B + C combined in half rates

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Summary

Coffee is the most important cash crop for Tanzanian economy. The major disease affecting coffee industry in Tanzania is CBD. The control measures against the disease are: bu use of fungicides as a short term solution; and by use of resistant varieties as a long term solution. The choice of when to spray and its frequency must obviously be dictated by the cropping level, disease magnitude and variability of the two over time and space. The cost benefit pay off strongly favours reduced but well timed sprays and fungicide mixtures. The release of resistance varieties are the altimate answer to the CBD problem especially to small holder sector and nation as a whole.

COFFEE RESEARCH IN TANZANIA

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Lyamungu Coffee Research Station started in 1934 with the aim of improving coffee production through research on agronomic problems.

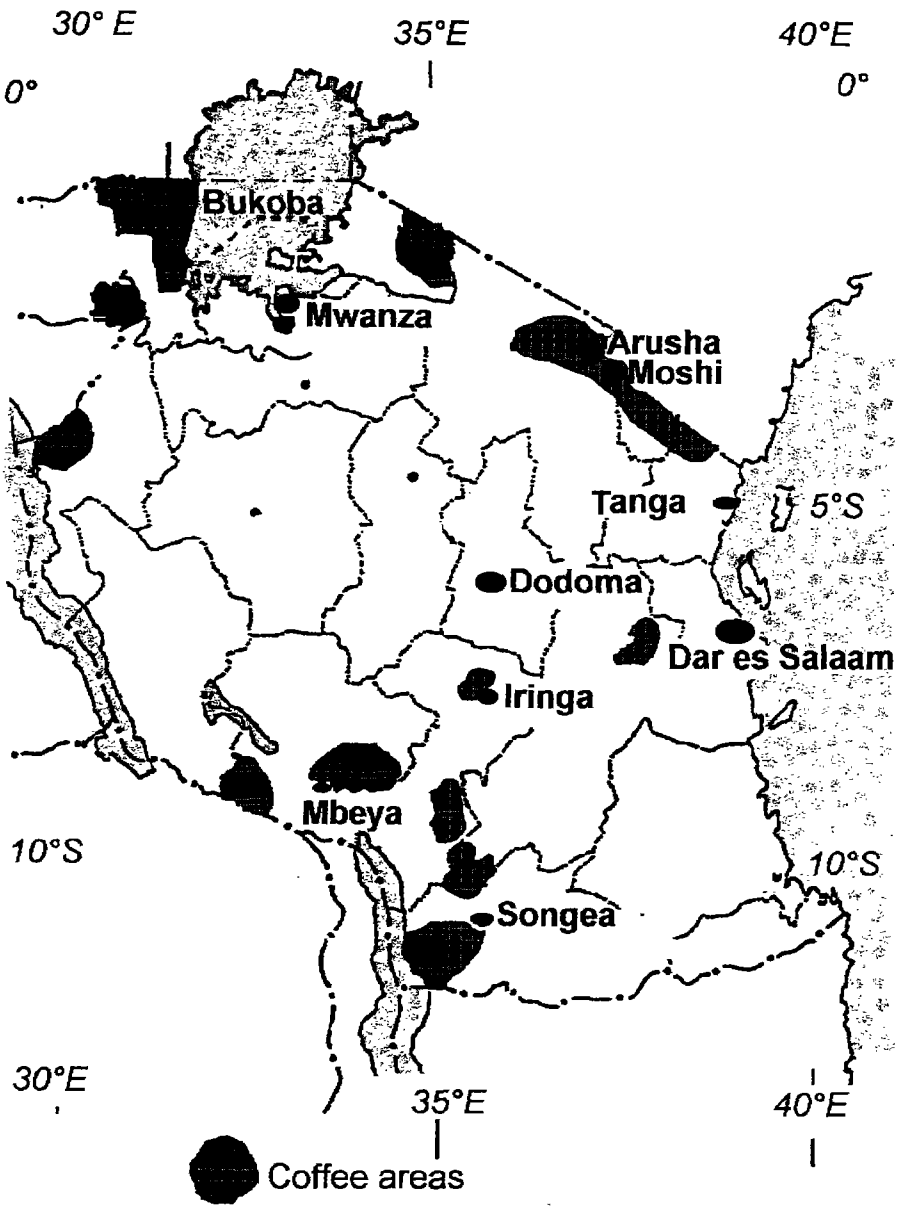
Initially a coffee research levy was imposed in every coffee sale. Research output was commendable since funds and research expertise was available. By 1962 most of the research work had conclusive results for problems encountered in coffee production. New coffee varieties were released together with agronomic packages. In addition coffee processing procedures were recommended. As a result quality coffee was produced. Tanzania Coffee Board was responsible for the coffee research institutions in the country.

By 1970, the coffee research stations and related activities were handed over to the Government of Tanzania. Additional research activities were given to Lyamungu. These included wheat research, maize, legumes and horticulture. These research programmes grew up to the national level. Horticulture research and training activities moved in 1976 to Tengeru. Wheat research moved in 1980 to Arusha while the Bean research was shifted to Selian Research Institute in 1994.

Agricultural Research in Tanzania was changed to a parastatal known as the Tanzania Agricultural Research Organisation (TARO) in 1981. Coffee Research was co-ordinated at the national level from Lyamungu Agricultural Research and Training Institute (LARTI). The European Community through the Coffee Development Programme (CDP) provided expert and financial support for a few years.

By 1989 TARO was dissolved and Agricultural Research was run by the Ministry of Agriculture. Inadequate funds dominated Coffee Research.
To date Coffee Research remains the main activity at Lyamungu. It is financed through a cess with the government backing, and temporarily by the European Union through EDF funds.

COFFEE GROWING IN TANZANIA



PLANT BREEDING

In the 50's Lyamungu proposed Bourbon (N39) and Bourbon x Kents (KP 423, KP 162, H66) varieties to the farmers after selection from local plantations.
CBD then spread in the country. A breeding programme was started with the aim of combining the good adaptation and quality of the above varieties with the resistance of Rume Sudan and Hibrido de Timor. Many interruptions slowed down this programme.
To date 16 hybrids have been preselected. They are multiplied through cuttings. Currently a multilocational trial with 27 sites throughout the coffee regions of Tanzania has been established. The release of the best hybrids is envisaged within two years. The use of in-vitro techniques for fast multiplication is envisaged.

AGRONOMY AND NUTRITION

The now 'traditional' pruning system which was developed in the 60's was aimed at reducing disease incidence by opening the canopy. The trees are capped several times in order to strengthen the root system, better resist drought and facilitate spraying and picking. spacing is 9'x9' (1300 tr. /ha).
Trials conducted at Lyamungu together with information from other countries indicate that much higher yields can be obtained with higher densities and free growth. This should further apply to disease resistant varieties. Investigation is going on. Present recommendation is 2.5 m x 1.5 m for new planting or 9'x4.5' for regeneration thus about 2700 tr./ha.
The most common practice, especially in the Northern Zone, is to interplant banana with coffee. First results of on-station trials clearly confirm that this practice must be rationally applied not to be detrimental to coffee production.

Soils surveys throughout the country and NPK reference trials will help to define the best fertilisation for traditional and new varieties.

PLANT PROTECTION

Coffee Berry Disease (CBD) is a major problem in most areas for *Coffea arabica*. It can be responsible for more than 80% crop loss. Coffee Leaf Rust (CLR) remains important in low areas.

Surveys and isolates collection are made in all regions every year in order to monitor the evolution of the disease. Breeders' material is screened on the station and in all the sites of the multilocational trial. Resistance of Hibrido de Timor and its derivatives (Catimor) to CLR is checked every season. Fungicides trials help to define the best molecules and spraying schedules for susceptible varieties.

Integrated Pest Management against the major insect pests (Antestia, stem and branches borers, berry borer and berry moth) is aimed at reducing pests incidence through rational use of chemicals. Efficiency of new pesticides against pests is being checked. Better knowledge of the pests and of their cycle, natural predators and parasites is also sought for.

Nematodes, especially root -knot, are widely spread in Tanzania. Research aims at a better knowledge of their distribution in the country and of their influence on growth and yield . Investigation on possible genetic resistance has been undertaken recently.

ROBUSTA (*COFFEA CANEPHORA)*

Robusta coffee was grown in Kagera region (Lake Zone) long before European colonisation from wild trees found in the forests. The local name is Mbona.

Robusta research was established at Maruku (Bukoba) in 1948. Clone selection was started from gerplasm introduced from Belgian congo, Uganda, and from farmers' fields. However only seeds have been distributed to the farmers up to now.

Two main types and intermediates can be found, according to growth habit: the erecta type (typical Central African Robusta type)and the spreading type (possibly crossed with the local type). Five outstanding clones of the spreading type are currently proposed for release. Three vegetative propagation units have been established.

The main problems for the expanding coffee areas are soil acidity, berry moth, fusarium diseases and the adoption of modern coffee growing techniques in a region with a strong growing tradition.

SUMMARY

Coffee Research started in Tanzania in 1934 when the coffee growers founded Lyamungu Research station, in the Kilimanjaro region.
The major achievements until 1960 where the selection of arabica varieties N39, H66, KP 162 and KP 423. Cultural practices were defined for these cultivars as well.
CBD and CLR became then major problems for the industry. Only genetic resistance could give a satisfactory reponse. Hybridisation and selection used Rume Sudan and Hibrido de Timor as main progenitors for resistance. Back crosses to commercial varieites provided adaptation and quality.
The final stage of selection is carried on before releasing the hybrids through vegetative propagation. An agronomic package is being studied fir this new planting material. This includes fertilisers requirements and pest control. Fungicides application for existing coffee are still under investaigation.
Clone and progeny selection of Robusta Coffee has been conducted at Maruku (Lake zone) since 1948. Five Robusta clones have been selected. They are currently being multiplied in Vegetative Propagation Units.

RESUME

La station de Lyamungu (Kilimandjaro Region) fut fondée en 1934 par les planteurs de café.
Ses principales réalisations jusqu'aux années 60 furent la sélection des variétés N39, H66, KP 162 et KP 423, et la proposition de systèmes de culture adaptés.
Puis la rouille orangée et l'anthracnose des baies devinrent des problèmes majeurs pour les planteurs. Seule la résistance génétique pouvait, à terme, y répondre. Rume Sudan et l'Hybride de Timor furent croisés avec les variétés commerciales pour allier résistance, adaptation et qualité.
La phase finale de la sélection est en cours, avant la distribution de boutures aux planteurs. Un paquet technologique est à l'étude simultanément pour ce nouveau matériel (fertilisation, techniques culturales, lutte contre les insectes et nématodes).

BREEDING FOR DISEASE AND PEST RESISTANCE, AND IMPROVED QUALITY IN COFFEE

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1. INTRODUCTION

Coffee is the source of one of the world's most popular stimulating beverage. It is at the same time, a major export commodity and an important source of income and employment for numerous developing countries in Latin America, Africa and Asia. It belongs to the genus *Coffea* which consists of about 100 or so species and the African centre is the origin of the genus.

Coffea arabica is the most widely grown species and produces coffee of the highest possible quality. It is also the only natural alloploypploid in the genus *Coffea* with 2n=4x=44 and is self compatible. It has its primary centre of genetic diversity in South West highlands of Ethiopia and the adjacent Boma plateau in Eastern Sudan. Arabica coffee is principally cultivated in South and Central America, the Central and Eastern African highlands and in some Southern African states. *Coffea canephora* is the second most important species. It is indigenous to all tropical lowland forests of Western and Central Africa, the centre of greatest genetic diversity being the Congo basin. It is grown mainly in Cote d' Ivoire, Uganda, Angola, and Cameroon in Africa, and Indonesia, Vietnam and India in Asia. Because of self-incompatibility, *C. canephora* is highly polymorphic as are other diploid *Coffea* species. *Coffea liberica* and *Coffea dewevrei* or *excelsa* are both indigenous to the dense forests of West africa. Both become large trees and on account of their inferior liquor quality, are of limited commercial value.

Arabica coffee up to now shows maximum genetic variability in Ethiopia. However, owing to the restricted nature of materials eventually introduced to the major coffee growing zones of the world (Wellman, 1961) genetic diversity outside Ethiopia has for a long time been extremely limited. In contrast, *C. canephora* shows immense genetic variability chiefly owing to its allogamous nature. This lack of genetic variability in *C. arabica* grown in the new world has had serious consequences in its long history of cultivation. Two diseases, Coffee leaf rust, *Hemileia vastatrix,* and Coffee berry disease, *Colletotrichum kahawae* have had the most devastating effect on its production. The former disease has world wide distribution while the latter is still confined to the African continent. Arabica coffee is also afflicted by numerous minor or localized diseases and of course, pests. Though *Coffea canephora* is similarlly affected, the severity in general does not approach that experienced in *C. arabica.*

Apart from yield, most breeding programmes in coffee have had the basic objectives of disease resistance and improved coffee quality. Indeed the history of coffee improvement is like a discourse in resistance breeding. As a consequence, it has necessitated continuous efforts in quality improvement to restore acceptable levels of coffee quality. This presentation reviews various aspects of breeding conducted in coffee to develop new varieties combining disease and pest resistance, with improved quality.

2. BREEDING FOR RESISTANCE TO DISEASES AND PESTS IN COFFEE

Of the various methods of disease control, breeding for disease resistance is always to be preferred in the long run. Not only does it cut down on production costs, it is also socio environmentally most attractive. However, considering the nature of perennial crops like coffee and the epidemic potential under the tropical environment where they grow, resistance always ought to be reliable and durable. In coffee, there are a few major diseases that have received most attention and the next sections will explore the impact of these diseases,the nature of resistance and strategies of breeding applied.

2.1 Impact Of Major Diseases And Pests Of Coffee

Coffee leaf rust caused by *Hemileia vastatrix* is a pathogen of the leaf, characterised by orange rust pustules on the under surface of the leaf. It causes severe losses as a result of loss of photosynthetic surface and premature leaf drop. The disease first noticed in Western Kenya in 1861, had the most devastating effect of any coffee disease known until then, when it wiped out Arabica coffee plantations in Sri Lanka in 1868/69 and then spread onwards to South East Asia causing similar destruction. In Indonesia, Vietnam and the Philippines, the coffee industry was only saved by Robusta coffee, a form of *Coffee canephora.* Since then, Coffee leaf rust has gradually spread through all Arabica coffee producing countries of the world. It was only in the 1970's that the disease was finally recorded in Latin America.

Coffee berry disease (CBD) caused by *Colletotrichum kahawae*, is a typical anthracnose of the green and ripening berries, first reported in Western Kenya in 1922 (Macdonald, 1926). It is thought to have originated from *Coffea eugenioides* a diploid *Coffea* species indigenous to the adjacent areas (Mogk, 1975; Robinson, 1976). By 1939 it had spread to the East of the Rift Valley and Kivu province in the Democratic Republic of Congo. From there on, it was reported in Angola, Comeroon , Moshi in Tanzania, and finally in Ethiopia, towards the end of 1960's and early 1970 (Mulinge, 1973). CBD is already well established in several South African countries which were until recently thought to be free from the disease.

Crop losses due to CBD can be extremely severe. In Kenya, losses exceeding 50% occur during excessively wet years and even with chemical control, losses estimated at between 20% - 30% may occur (Anon, 1988). Losses of similar madgnitudes have been observed in some Southern African countres. Overall losses in Ethiopia are estimated to be around 20% - 30%.

Other Diseases

Fusarium (Giberella) stilboides (Fusarium bark disease), the pathogen causes localized bark lesions which result from the damage of the vascular system. The vascular wilt often results in death of entire mature trees. The young suckers are frequently completely ringbarked. The most severe incidence of Fusarium bark disease ever reported was in Malawi (Siddiqi, 1980) where it almost destroyed completely the coffee industry. The disease also occurs in some parts of Kenya.

Fusarium xylarioides, Tracheomycosis or Fusarium wilt. It causes vascular wilt and sporulates on the bark of dead or dying trees. It is so far the most serious disease affecting *Coffea canephora* and other diploid species. In Central and West Africa, it practically wiped out *C. axcelsa* plantations and sussceptible *C. canephora* notebly Kouillou Coffee, which were replaced by resistant *C. canephora* types bred earlier in the former Zaire. It also affects *C. arabica.* Cases were reported in Ethiopia (Kranz & Mogk, 1973),

Southern Sudan (Lejuene, 1956) and Zimbabwe. Van der Graaff & Pieters (1980) reported considerable variability in resistance among Ethiopian Coffee.

Bacterial blight of coffee - *Pseudomonas syringae and P. syringae pv garcae*, is the most important bacterial disease ever reported in Coffee. It causes severe losses in parts of Kenya and Brazil. The pathogen attacks the shoot tips, leaves, young succulent branches, flowers and pin heads causing severe scorching and dieback. Varietal differences have been reported in Latin America, (Cordosa & Sera, 1983).

Cercospora coffeicola, the brown eye spot and berry blotch is of some importance in Zambia, Eastern Africa, Central America and some Asian countries. Differences in resistance have been reported among *C. arabica* varieties (Soto & Campos, 1971: Van der Vossen & Cook, 1975: Van der Graaff, 1981). In Robusta Coffee, the pathogen causes the red blister disease observed in Uganda (Leakey, 1970).

Coffee Pests

Unlike in many other crops, not much serious work has been reported on breeding and selection for resistance to insect pests and nematodes of Coffee. Reports of variation in resistance or tolerance have been observed in certain species of root knot nametodes including *Meloidogyne* and *Pratylanchus*, and for insect pests, leaf miner, *Leucoptera. Perileucoptera*, berry borer, *Hypothenemus hampei* and branch borer *Xyleborus morstatti.*

2.2 The Nature Of Resistance

Coffee leaf rust - Inheritance of rust resistance illustrates a classical case of the effects of vertical resistance i.e. race-specific major gene resistance and horizontal resistance, race-non-specific polygenic resistance. Up to now, 30 races of the rust pathogen have been identified. The genes SH_1, SH_2, SH_4 are of *C. arabica* origin, while SH_3 is derived from natural crosses between *C. liberica* and *C. arabica.* Hybrido de Timor, an interspecific hybrid between *C. canephora* and *C. arabica* appears to carry the SH_6 - SH_9 genes. It has been shown that resistance derived from *C. arabica* can easily be matched by the corresponding rust races (Ribeiro et al, 1971: Eskes. 1983a). The factor SH_3 however, is rather special (Visveshwara, 1979 Eskes, 1983a). *C. canephora*, contains vertical resistance that confers both complete and incomplete resistance (Eskes 1983 a, b). However, on basis of work done at the CIFC (Rodrigues, 1985), among the physiologic groups, group **A** derived from *C. canephora* which occurs in Hybrido de Timor is characterized by resistance to all known races of rust. Variety Catimor was selected on basis of this resistance.

The other type of resistance of the horizontal nature has been demonstrated in *C. canephora* and *C. Arabica.* (Eskes & Carvalho, 1983). Owour (1983) reported considerable variation for quantitative resistance and confirmed relatively high level of such resistance in variety Rume Sudan and a number of genotypes from Ethiopia. It is true that polygenetically inherited incomplete resistance may offer more durable resistance than the hypersensive type (Parlevliet, 1979). However, incomplete resistance to coffee rust does not always guarantee that such resistance is horizontal, it must be confirmed that the resistance is polygenic (Eskes,1983a).

Coffee berry disease - the resistance appears to differ markedly from that of coffee leaf rust. In the first instance, though differences in aggressiveness in the pathogen population have been demonstrated in Kenya (Masaba, 1980) and in Ethiopia (Van der Graaff, 1981) no substantial differential interactions were found. Secondly, histological evidence produced (Masaba & Van der Vossen, 1982), suggests that resistance depends to a great extent on formation of cork barrier, a mechanism often associated with race-non-specific

resistance. Studies by Omondi and colleagues on genetic diversity among isolates of *C. kahawae* to be presented later in these proceedings, may shed some light on expected behavior of this pathogen when confronted by resistant cultivars.

The mode of inheritance of resistance to CBD among *C. arabica* varieties studied in Kenya (Van der Vossen & Wàlyaro, 1980) indicated the presence of 3 genes with large effects (major genes) one of which had multiple alleles. The effects of underlying polygenic system was never discounted. In Ethiopia, Ameha and Belachew (1982) estimated gene differences of between 3 and 5 genes plus some minor genes.

In the breeding programmes for CBD in Kenya and Ethiopia, two different approaches were initially followed. In Kenya, the programme aimed at incorporating CBD resistance from exotic gemplasm was achieved through an elaborate scheme of single and multiple crosses followed by backcrossing. The reason was that productivity and the high quality of the typical Kenya coffee had to be maintained (Van der Vossen & Walyaro,1981). The Ethiopian programme involved individual line selection among the naturally occurring population within the centre of genetic diversity. Consideration was given to other characters including yield, quality anf other diseases but mainly within the framework of selection for CBD resistance. The resistance developed in both Kenya and Ethiopian programmes has remained fairly stable for over 25 years since the materials were first tested under experimental field conditions. And for the last 10 years since the new varieties were released countrywide, no cases of noticeable failure of resistance have been recorded.

Other Diseases And Pests

Active selection for resistance to other diseases have been reported for *Fusarium stilboides* in Malawi and *Fusarium xylarioides* in former Zaire and Ethiopia. In Brazil, selection for resistance has been reported for Bacterial Blight of coffee and the root knot nematode *Meloidogyne incognita.*

2.3 Breeding Strategies And Results Obtained

Coffee leaf rust

In recent years, considerable advances have been made in research on coffee rust and indeed results appear promising. On the question of durability, considering the past behaviour of the rust, only time will tell. Of particular interest will be the behaviour of the derivatives of Hybrido de Timor and other *C. canephora x C arabica* hybrids like Icatu when planted out as commercial varieties in extensive areas under diverse environments.

For the present moment, several approaches are being used to breed for rust resistance. These include, introduction of vertical resistance genes of *C. canephora* or *C. liberica* origin into C. *arabica* varieties, use of horizontal risistance genes originating from the same diploid species in *C. arabica* through backcrossing schemes or, selection among *C. arabica* populations, progenies which show transgressive segregation for increased horizontal resistance.

Various methods are available for selection for rust resistance. For qualitative resistance, field recording, glasshouse inoculation and leaf disc methods have been widely used. For quantitative resistance field observation is not so easy though quantitative methods have been proposed. A combination of field, green house and laboratory methods have to be used to differentiate quantitative resistance (Eskes, 1983a). Owour (1983) successfully used the leaf disc method to determine quantitative variation in rust resistance in Kenya

while in France, a comparable laboratory method using complete leaves is normally used (Muller & Letode, 1983)

Regarding the impact of breeding for resistance to Coffee rust, results have been rather mixed. While in South East Asia, the countries shifted from growing *C. arabica* to *C. canephora* in India the main varieties distributed are derived from *C. arabica x C. liberica* crosses which still show some level of rust resistance. Varieties derived from Hybrido de Timor have also been distributed. In Latin America, aftr the disasters experienced with varieties bred earlier, attention has shifted to resistance derived from Hybrido de Timor and *C. canephora*. The Colombia variety which derived its resistance from Hybrido de Timor, is an outstanding example of such breeding efforts. Another excellent variety developed in Kenya is Ruiru 11 which combines similar resistance derived from Hybrido de Timor with race-non-specific resistance of the Rume Sudan type in the background.

Coffee Berry Disease

Breeding for CBD resistnce appears to present less problems than coffee rust. The main reason being, no evidence has been recorded of vertical resistance to CBD. Variation in inheritance appears quantitative and genetic studies have shown the presence of a number of major genes showing additive, dominance or recessive effects. The effects of minor genes is also evident in mediating this variation.

The elucidation of the inheritance of resistance to CBD in the Kenyan breeding programme led to further refinement in the hybridization and selection programme such that all the 3 resistance genes identified in the study were to be represented in the new variety. Ruiru 11 is the result of this programme. It is a hybrid variety derived from two sets of parent population (Walyaro et el , 1982; Walyaro et al 1984, Agwanda & Owour, 1989). One population contains CBD resistance on 3 or at least 2 major gene loci combined with soem quantitative resistance to rust mentioned earlier. The other parent population contains 1 major gene for CBD resistance and rust resistance of Hybrido de Timor type. In Ethiopia, because of immense genetic variability available, it was possible to accumulate resistance rapidly through line selection from the already existing populations (Van der Graaff & Pieters, 1982). Hybridization and selection programmes were later conducted for genetic studies and also to derive new additional varieties.

Regarding selection methods for CBD resistance, several field and laboratory methods have been used. Field evaluation is enhanced where disease pressure is high. In Kenya a few sprays of fungicides are often applied to selection fields to increase CBD severity and thus allow more rigorous selection. Inoculation of attached berries (Van der Vossen et al, 1976; Van der Graaff, 1978, 1981) is another method of field evaluation that was used at some stage in breeding programmes in Kenya and Ethiopia. Two laboratory screening methods are usually used, the detached berry test (Bock, 1986; Firman, 1964) and the hypocotyl inoculation test (Van der Vossen et al, 1976). At the CRF, Ruiru, the latter test was found more reliable and has been used ever since as the standard procedure.

More advances in selection for CBD resistance derive from recent studies involving use of molecular markers to locate resistance genes in individual genotypes and at the same time enable one to select against donor background in early generations. The implications of such results are of immense value for breeding purposes. Details of this work are to be presented by Lashermes in these proceedings.

Unlike Coffee rust, results obtained in Arabica coffee breeding for CBD resistance have been more encouraging. Not only have new varieties been developed within a reasonably short scope of time, the resistance has proved fairly stable. The distribution of CBD resistant materials in Ethiopia began in 1978

(Van der Graaff, 1981). Since then, further improvement have resulted in the release of more additional lines.

In Kenya, variety Ruiru 11 was released in 1985. It combines disease resistance with compact growth and improved productivity. The introduction of this variety was a milestone in coffee improvement efforts in Kenya. From the national parspective, chemicals for the control of CBD and rust were dominating the pesticide market in Kenya (Njagi, 1982). Apart from the foreign exchange implications, such use of pesticides is known to have adverse effects on the environment and poses a health harzard. The advantages to the farmers using this variety are obvious. Apart from reducing the costs of production, disease control accounts for over 30% of those costs, the compact growth habit allows for more intensive coffee cultivation and entails easier pruning systems. The hybrid nature of the variety on the other hand, has a lot of merits including heterosis for yield and the benefits of coping with future changes or even unexpected hazards. (Walyaro, 1983; Van der Vossen, 1985; Ameha, 1982).

Regarding multiplication rate, initial problems were experienced to the extent that the targets set were not always attained. These however, were of logistical nature and prompted further research on logistics of mass F_1 hybrid production through artificial cross pollination. It should now be possible to achieve an annual planting rate of 2 - 3 % of the existing arabica coffee tree population. Other modalities of multiplication are also being considered.

Other Coffee Diseases

Fusarium stilboides - In Malawi, Siddiqi (1980) was able to select resistant material based on disease levels in young plants. Seedling tests have also been used in Ethiopia and inoculation of suckers and stumped plants is possible . *F. Stilboides* in Malawi caused such serious setbacks to a thriving coffee industry that even the availability of resistance material has not helped restore its previous position.

Fusarium xylarioides. Tracheomycosis in Arabica coffee still occurs in certain areas of Ethiopia though some selection for resistance was previously reported in (Van der Graaff, 1980). Regarding selection, one important way is the negative selection against unduly susceptible types. In *C. canephora* this approach was apparently successful as materials selected in former Zaire were more resistant and were used to replace susceptible types in the country and in West Africa. Since then and for the last 40 years, this disease has been unimportant in *C. canephora.* However, of late there appears to have been a re-emergence of Tracheomycosis. Widespread incidences have been reported in North - Eastern Zaire and Southwestern Uganda. This disease can also be a threat to all *C. arabica* throughout the world since many foreign varieties tested in Ethiopia were highly susceptible (Van der Graaff, 1981).

Pseudomonas syringae. The disease causes severe damage to *C. arabica* in parts of Brazil and Kenya. Selections of SH_1 gene occurring in variety Geisha also confer resistance to *P. syringae* (Carvalho, 1988). Derivatives of Hybrido de Timor have also been found to show resistance to Bacterial blight (Moreno, 1989). However, the nature of resistance is still unclear.

Cercospora coffeicola - is generally not a severe disease of *C. arabica* except in certain varieties e.g. Caturra and SL 28/34. Avoidance of undue suscesptibility may be an important way to improve resistance. It has also been found to be severe in some *C. canephora* types in Uganda.

Resistance To Pests

There is very limited information on resistance to pests in coffee and the nature of its inheritance. A few cases of sources of resistance reported are given below for root knot namatodes and a few insect species.

Nematodes - Resistance to *Meloidogyne exigua* occurs in some Ethiopian materials, selections of Catimor and some diploid coffee species. For the more serious nematode in parts of Brazil, *Meloidogyne incognito*, resistance was found in Hybrido de Timor, Icatu, some Catimor selections and the diploid species, *C. canephora* and *C. congensis.* Selection for resistance to *M. incognito* is being conducted in Campinas (Carvalho, 1988). Apparently the resistance is of the vertical nature, since at least 3 races have already been identified.

Insects - The leaf miner, *Perileucoptera coffeela* and *Leucoptera* spp have received the most attention among insect pests. Resistance to *P. coffeela* is absent in most *C. arabica* and *C. canephora* varieties except the C. *arabica* variety Mokka which shows some tolarance. Regarding *Leucoptera* spp, variation in resistance has been reported in some Arabica coffee varieties (Wanjala, 1980; Van der Graaff, 1978, 1981). Avoidance of susceptibility can be achieved through field observation at well chosen sites.

In *Coffea canephora,* variation in tolerance has been reported against the berry borer *Hypothenemus hampei* (Cramer, 1967) and against the branch borer *Xyleborus morstatti* (Coste, 1968).

3. IMPROVEMENT OF COFFEE QUALITY.

3.1 Importance Of Coffee Quality

Coffee quality is one of the most important factor that determines the desirebility and thus the relative prices obtained from given categories of coffee. Quality depends on bean size and the liquor . Studies on genetic improvement of quality have been relatively few and are mainly recent (Vishveshwara, 1971; Walyaro, 1983; Castillo & Moreno, 1988;Owuor, 1988; and Moreno et al, 1995). The main reason is that with the commencement of active breeding for disease resistance, hybridization schemes often involved materials of widely differing genetic backgrounds. As a consequence, selection for improved quality became unavoidable if the original quality of the traditional varieties was to be restored.

3.2 Coffee Classification

There exists a great deal of variation in systems used for quality assessment ranging from grading based on bean size and % defective beans in unwashed Robusta to the more comprehensive methods used for bean and cup quality determination evolved for the highest quality arabica coffees refered to as 'Colombian Milds' . In essence therefore, coffee classification differs from country to country depending on the consumer market being targeted.

Classfication of bean characteristics is determined through mechanical and pneumatic separation and thus is fairly objective. Liquor quality on the other hand, is assessed organoleptically, which may give room to subjectivity (Wooton, 1967; Kulaba,1979). However, in the absence of such procedures it is important for scientific purposes that liquor quality assessment is conducted under controlled conditions with replicated samples in order to assess consistency. Example of the grading systems used in Kenya which is similar to that used in Colombia is as follows:-

Bean Size:

PB - the fraction of beans retained by piano wire screen with 4.43 mm space.
AA - the fraction of heavy beans retained by no. 18(7.15mm) screen.
AB - the fraction of heavy beans retained by no.15 (5.95mm) screen.
TT - light beans separated from AA and AB.
C - the fraction of beans retained by piano wire screen with 2.90mm space.

Liquor quality: this is determined on basis of level of acidity, body and flavour. These three traits determine to a large extent the liquor (Devonhire, 1956). The overall standard or general standard however, indicates the overall appreciation of the quality of the coffee, taking into account visual assessment of general appearance of the green and roast bean.

3.3 Genetic Variability Of Quality Characters

A number of factors are known to influence the quality of coffee. These range from soil and climatic conditions, to agronomic practices, nutrition level and effects of certain diseases and pests. The stage of harvesting of cherries and mode of processing have a particularly great bearing on the final assessment of liquor quality. Considerable attention has been devoted to these aspects of quality improvement (Northmore, 1965; Wormer,1966; Cannel, 1971; Sivet, 1972; Charmentant & Leroy, 1985; Leroy et al, 1991).

Apart from these factors, it is known that the underlying variation in quality characteristics is genetic. Between Coffee species, these differences are particularly pronounced. For example, Robusta coffee beans are generally smaller than Arabicas. The average caffein content in Robusta is almost twice as high as in Arabica, while *Coffea euginioides* has even lower caffein content than Arabica coffee and several species of the *Mascarocoffea* have no detectable caffein in the beans. Within the same species, large valiability in quality characteristics can also be observed.

Initial studies conducted at the C R F, Ruiru (Walyaro,1983) involving a range of genetically diverse Arabica coffee varieties, confirmed large genetic variation for most quality characteristics and especially so, for bean characters. A later study (Agwanda et al ,1997 in the press) conducted over multiple environments, largely agreed with these findings. The variation for most quality characters was found to be mainly due to additive genetic effects, the dominance or genetic interaction components being relatively unimportant. It is clear from table 1 that most bean characters are highly heritable while most liquor quality attributes are less heritable except for the overall standard. For liquor quality characteristics, it is possible that due to the method of determination, it is often difficult to isolate the bulk of genetic effects from the non heritable causes of variation (Charrier, 1982). Nonetheless, even with these limitations, results obtained indicate that improvement of bean size and liquor quality (if selection is based mainly on %AA and the overall standard) is an objective that can be attained relatively easily. However, if analytical procedures were developed that relate specific chemicals components to cup quality even more rapid genetic response would be expected in quality improvement programmes.

Table 1.

Heritability estimates of quality characters in Arabica coffee from a diallel cross between 11 varieties (Walyaro, 1983)

Character	Heritability (h^2_w) estimates based on means of 4 trees	individual tree
Beans size 1)	0.74	0.41
100 - bean weight	0.67	0.35
% PB	0.70	0.36
" AA	0.64	0.31
" AB	0.25	0.08
" TT	0.73	0.40
" C		
Cup quality 1)		
acidity	0.15	
body	0.08	
flavour	0.23	
overall standard	0.48	

Note: 1) See under selection criteria for a description of beans size and cup quality characters.

However,of more concern is the effect of genotype - environment interactions on quality characteristics especially where trials are conducted over widely differing environments. In Walyaro's (1983) earlier study, though environments were rather restricted, these effects were evident for most of the quality characters but no where as important as those observed for growth and yield characters. Later studies however, have confirmed that given widely differing environments, genotype - environment interactions can be a major source of variation for most of the characters (Agwanda et al, 1997; Mawardi & Hulip,1995). This would indicate that for efficient selection for bean and liquor quality characters, environments should be chosen which maximise expression of such characters. However to be conclusive, more information is required on the nature of these interactions especially the relative magnitude of the linear and non-liner components of genotype - environment interactions..

In the selection programme at the CRF, emphasis was always placed on % AA and overall standard though other bean grades and the liquor attributes, acidity, body and flavour were always taken into account. The results so far obtained confirm that this method of selection was no doubt effective. It has also been suggested (Agwanda et al,1997) that combined selection by applying selection indices combining AA% and flavour within certain environmental restrictions may give equally effective results.

3.4 Selection For Improved Coffee Quality, The Kenyan Example

In the Kenyan breeding programme, the sources of CBD and rust resistance included varieties, Rume Sudan, Hybrido de Timor, Blue Mountain and K7 among others. The range of variability for quality characters among some of these varieties and others like Caturra and Padang in comparison to SL28 is given in Table 2. SL28 which is the standard cultivar, is characterized by large bean size (%AA) and excellent cup quality.

Caturra and Rume Sudan in contrast, have small bean size and relatively inferior cup quality while Hybrido de Timor has fairly good bean size but extremely poor cup quality. The Catimor ex Colombia at the bottom of the Table have quite satisfactory bean size and though somehow lacking in cup quality compared to SL28, their quality surpases the parents, Caturra and Hybrido de Timor. In the middle of the same Table, it is possible to appreciate improvements from the F_1 generations through the multiple crosses to the backcrosses, reflecting clear response to selection within the population.

Table 2 :- Examples of beans size and cup quality characters for varieties, single and multiple cross and and backcross in Arabic coffee (Van der Vossen & Walyaro, 1981)

Variety or cross	Bean grading %			Cup quality			
	PB	AA	AB	Acid	Body	Flav.	Stand.
SL28	14	**46**	18	1.0	1.0	2.0	**2.0**
Padang	15	37	20	1.8	1.8	3.6	3.6
Caturra	11	15	25	2.0	1.5	3.7	3.8
Rume Sudan	29	3	27	2.5	2.0	3.3	3.3
Hybrido de Timor	23	39	21	2.0	1.6	4.1	4.0
SL28 x Caturra	23	24	21	1.5	1.5	3.0	3.3
SL28 x Rume Sudan	35	15	27	1.5	1.0	2.5	3.0
SL28 x Hybrido de Timor	26	35	18	2.0	2.0	3.8	3.6
Caturra x Hybrido de Timor	31	29	19	3.0	2.5	4.5	3.8
a. (R. S. x SL28)(Bourbon x H.deT.)	17	15	40	1.0	1.0	3.0	3.0
b. (R. S. x K7)(H.deT. x SL34)	28	18	44	2.0	2.0	3.3	3.5
c. (SL34 x R. S.) x H de T.	30	10	46	2.5	2.5	4.0	4.0
SL28 x (a)	28	31	17	1.0	1.0	1.5	**2.0**
SL34 x (b)	29	**42**	18	0.5	0.5	1.5	**2.0**
SL28 x (c)	35	38	12	1.5	1.5	3.3	3.0
Caturra x (a)	26	21	21	1.5	2.0	3.3	3.3
" x (b)	40	18	20	2.0	1.5	4.0	3.8
" x (c)	23	14	31	1.5	2.0	3.5	3.3
Catimor ex Colombia F3 prog. 1	19	**48**	13	1.5	1.7	3.5	3.2
" " " 2	23	**47**	15	1.5	2.0	4.0	3.5
" " " 3	19	33	28	2.0	1.7	3.8	3.3

Note: - See under section on selection criteria for a description of bean and quality characters.
- Scores for acidity, body and flavour 0 - 4, for standard 0 - 7; 0 means very good, 4 respectively 7 is very poor.

The variety Ruiru 11 was a result of meticulous selection similar to that illustrated above. As a result, the quality in general is excellent. Some concern was however, expressed initially about the quality, especially as some liquorers were able to detect some tinge of foreign flavour while others complained that the variety was somehow lacking in acidity. However, from multiple samples submitted for blind liquoring to several companies, results have confirmed that the quality is not significantly different from the traditional varieties, SL28 and SL34. A more comprehensive assessment carried out by the ICO Coffee Test Unit (Njoroge et al,

1990), concluded that cultivar Ruiru 11 was similar in flavour quality to traditional *C. arabica* cultivar SL28. They further confirmed that the superior flavour qualities of Kenya Arabica coffee would not therefore be affected by the introduction of the new Ruiru 11 cultvar. In figure la, b, and c we reproduce the mean intensity scores for flavour attributes in Ruiru 11, SL28 and Robusta coffee, courtersy of Njoroge et al (1990). Of some interest for example (see Fig.l c,) the acidity of Ruiru 11 and SL28 are depicted as exactly identical.

TABLE 3

The important flavour attributes evaluated in coffee brews (aroma, taste and mouthfeel)
(Source: Anon,1988)

Aroma	Taste	Mouthfeel
Ashy		
Burnt	Acidity	Body
Caramel		
Cereal	Bitterness	Astringency
Malty		
Earthy	Sweetness	
Floral		
Fruity	Salty	
Citrus		
Grassy	Sour	
Nutty		
Rubber		
Winey		
Woody		

TABLE 4

The scale of intensities and quantitative scores of the coffee flavour attributes assessed.
(Source: I C O sensory evaluation scoresheet)

Intensity of attribute	Quantitative score
None	1
Extremely weak	2
Very weak	3
Weak	4
Slightly moderate	5
Moderate	6
Slightly strong	7
Strong	8
Very strong	9
Extremely strong	10

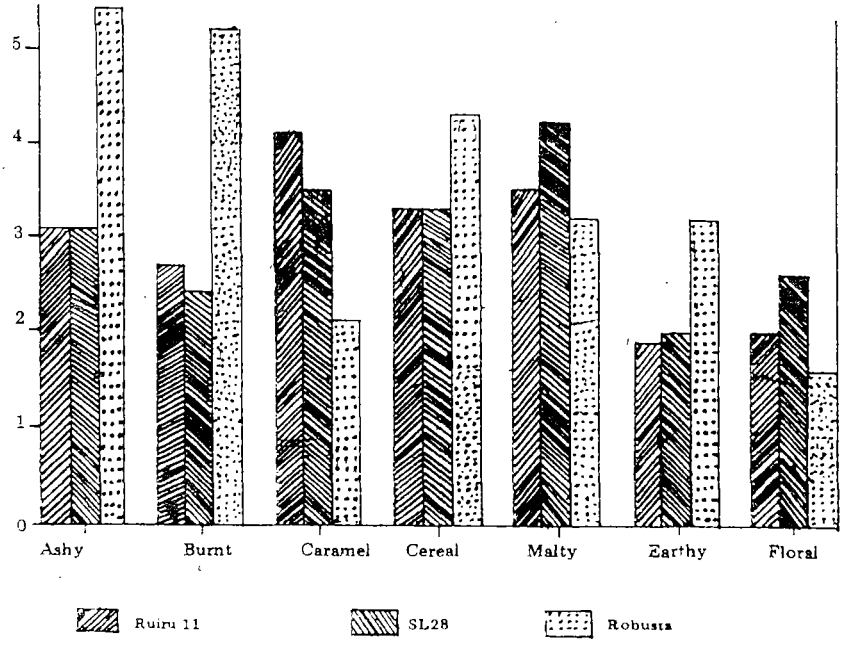

Fig. 1a:Descriptive flavour profiles of Ruiru 11, SL28 and Robusta coffees

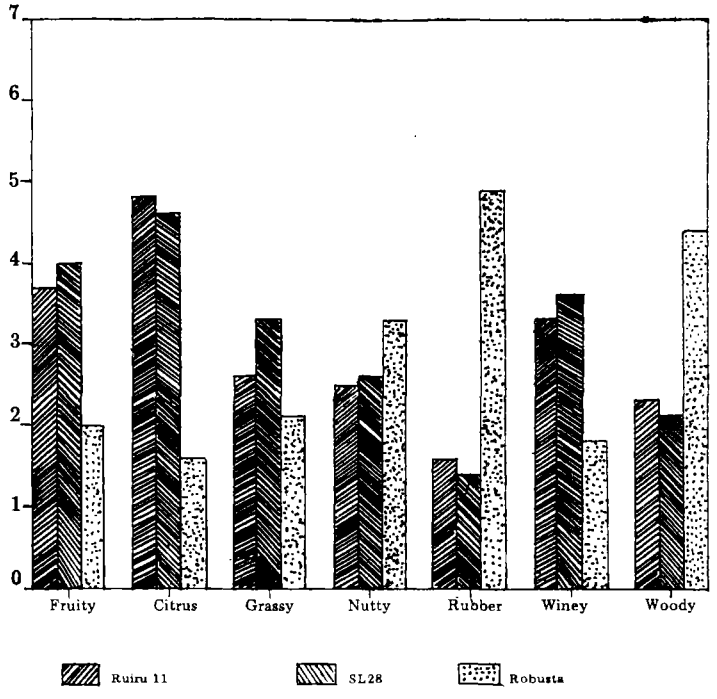

Fig 1b: Descriptive flavour profiles of Ruiru 11, SL28 and Robusta Coffees

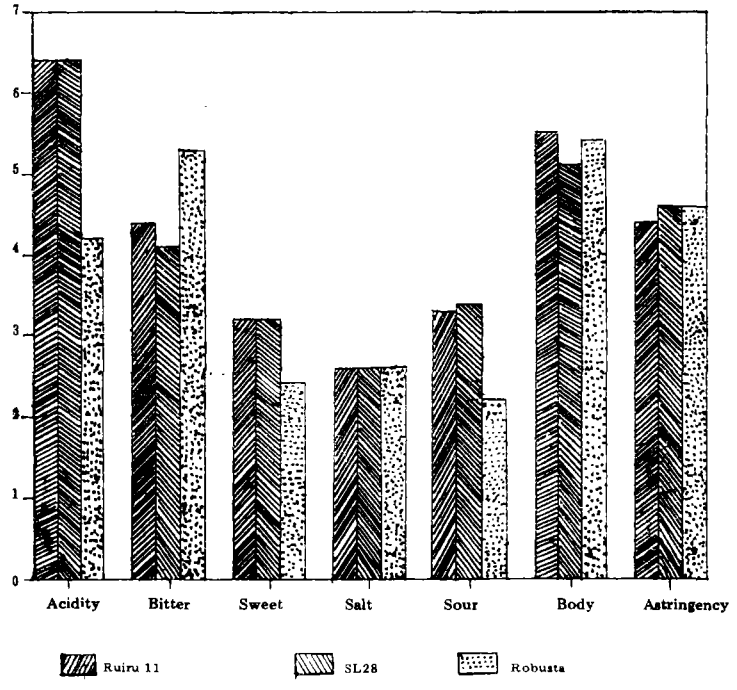

Fig 1c: Descriptive flavour profiles of Ruiru 11, SL28 and Robusta Coffees

CONCLUSIONS

Recent efforts in breeding for disease resistance are starting to have a major impact on coffee production worldwide, especially in those countries where two major coffee diseases ie Coffee leaf rust and Coffee berry disease have often caused enormous crop losses over the years. The resistance so far developed appears fairly stable; nonetheless, it is essential that a close watch be maintained on i) the behaviour of such resistance over time, and ii) the variability trends within the pathogen populations.

Of the other coffee diseases, Tracheomycosis, Fusarium wilt, which appears to be re-emerging in Eastern and Central Africa, and to some extent, Bacterial blight of coffee endemic in parts of Kenya and Brazil, will need urgent attention in future improvement programmes. Among coffee pests, attention should continue to focus on the more destructive species of the root knot nematodes.

Breeding for disease resistance normally also affects most of the coffee quality characteristics. For countries that are quality conscious, it is often neessary to conduct simultaneously, well planned quality improvement programmes in order to obtain varieties that closely match the established standards. It is encouraging that some of the recently developed varieties for example, Ruiru 11 and the Colombia variety do largely conform to these requirements.

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ABSTRACT

Apart from yield, disease resistance and quality are the major preoccupation of most improvement programmes in Coffee. The reason being, disease and pest control account for a large proportion of farming costs while coffee quality determines the desirability and thus the pricing of Coffee. In this presentation, the major diseases and pests of Coffee where attention is focused on development of resistance are discussed. In particular, an account is given of the major breakthroughs and strategies employed in breeding for stable and durable resistance to two of the most devastating diseases of coffee, Coffee berry disease (*Colletotrichum kahawae*) and Coffee rust (*Hemileia vastatrix*).

In most of these programmes , improved coffee quality attributes are invariably considered among the ultimate goals. Whereas determination of berry and bean characteristics is fairly straightforward, aroma and flavor attributes present difficulties. An account is given of breeding and selection for improved bean and cup quality, the latter relying mainly on conventional organoleptic evaluation. It is postulated however, that development of reliable laboratory procedures that relate specific chemical compounds to cup quality could have important bearing on genetic improvement of cup quality in coffee.

ARABICA COFFEE BREEDING IN ETHIOPIA : A REVIEW

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INTRODUCTION

Coffee (Coffea arabica L.) is the most important commodity crop of Ethiopia. More than 65% of the country's foreign exchange income comes from this single commodity crop. Arabica coffee, which is predominantly self-pollinating is grown in almost all administrative regions between altitude ranging from 550 to 2600m and under different management systems (2,11). The fact that Ethiopia is the center of origin and diversity, there is immense genetic variability that offers great potential for improvement of the crop.

A preliminary coffee improvement work was started in 1952 by the then called Jimma Agricultural Technical School (JATS) with the help of P.G. sylvain, FAO coffee specialist, who provided seeds of 50 arabica varieties, and 6 varieties of other species (1). Subsequently, the JATS had made additional collections of indigenous coffee types and international varieties. In 1965, the FAO coffee collection mission to Ethiopia (7) handed over 433 additional accessions to the technical school. The whole efforts of JATS in variety testing and agronomic activity trials were not very fruitfull probably due to organizational problems and shortage of skilled manpower.

A comprehensive research work on arabica coffee was started after the establishment of Jima Agricultural Research Center (JARC) of the Institute of Agricultural Research (IAR) in late 1967. Long - and short - term breeding programs were immediately launched and the major objectives were to: (1) collect and conserve coffee germplasm, (2) develop cultivars that combine high yield, disease resistance and good quality, and (3) multiply and supply improved seeds. The first breeding activity was started with 76 accessions recieved from French Coffee Collection Mission (ORSTOM) to Ethiopia in 1966 and 123 arabica varieties introduced from different countries (9). Since then, the JARC has made a number of research breakthrough in germplasm collection, finding a number of CBD resistant cultivars and heterotic hybrids, and improving management practices. The intension of the present paper is, therefore, to review the achievements made in arabica coffee breeding program and use these results as a base-line to plan more efficient breeding programs for the future.

THE BREEDING PROGRAM

COLLECTION

Diagramatic representation of arabica coffee breeding scheme in Ethiopa is given in Fig.1. Collection is the first and primary step. The collection program includes collections of both national (indigenous) and international (exotic) coffee genetic variability (9). In national collection program, areas were determined on priority basis and collection is being carried out annually since 1970 inorder to cover all coffee growing areas and capture maximum genetic variability for selection/breeding work and conserve for future use. The international collection program is conducted throught direct contact or correspondance with different countries. In international collection program known C. arabica L. varieties, rust differentals and diploid species were of special interest.

PROGENY TESTING AND SELECTION

The collections are planted in replicated trials at the main center or sub-centers and evaluated over years for yield, disease and pest resistance, and other desirable agronomic characters (step II). Those collections that showed superior performance over others for the characters of interest are selected and further tested under different environmental conditions (step III). The exotic collections by-pass step II (since they are improved materials developed else where) and directly tested for this adaptation under different environments.

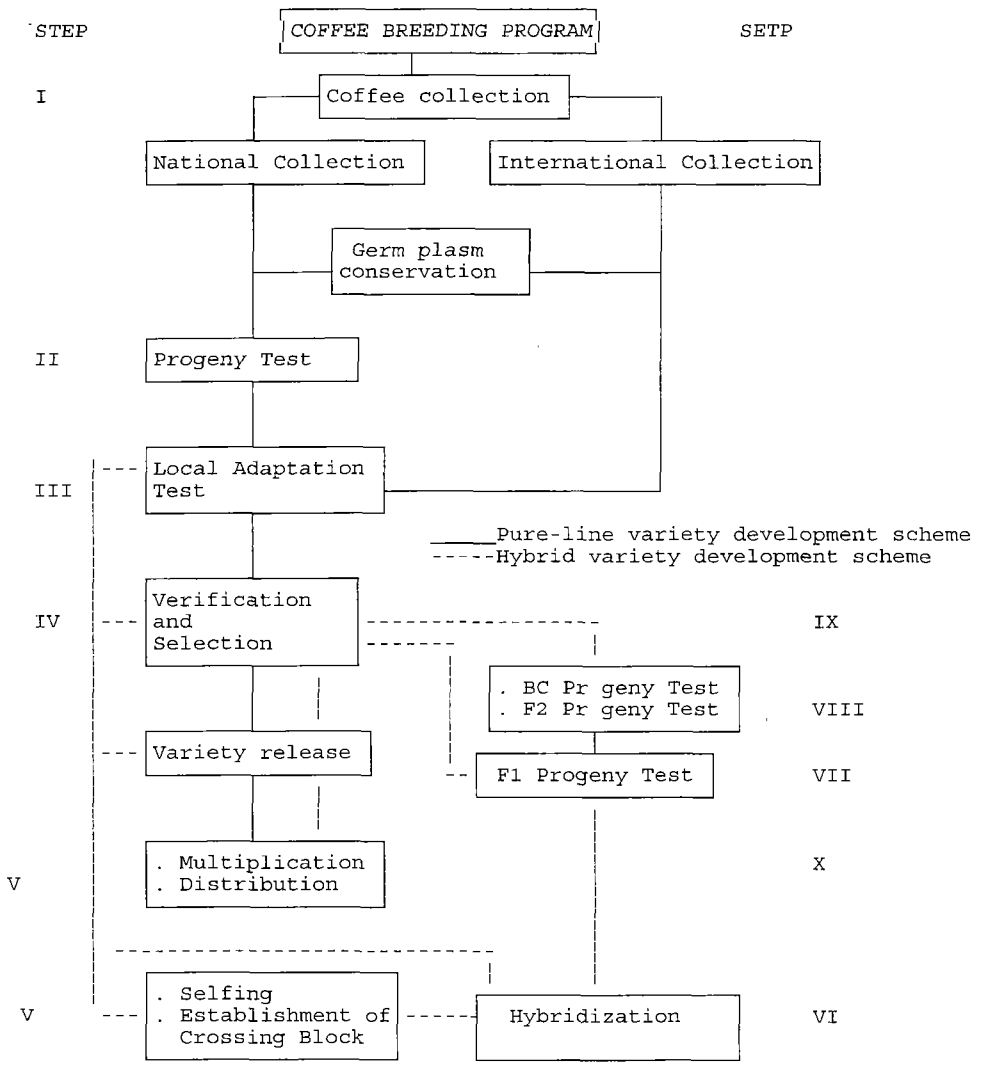

Fig. 1. A schematic representation of variety development process in arabica coffee breeding program in Ethiopia.

The top exotic and indigenous selections identified for each location are further verified on on-farm in the respective locality for final selection (step IV). The end selections are approved or disproved for release by the National Variety Release Committee (NVRC). Seeds of the approved selections are multiplied and distributed to growers with new variety name (step V).

HYBRIDIZATION

Hybridization is done for two main goals: (1) to study inheritance of important agronomic traits and (2) to develop hybrids that combine high yield, CBD resistant, good quality and other important characters. In this program, selections after local adaptation and verification tests (step III & IV) and the released pure-line varieties (step V) that can fit to the desired

objectives can be used as parental lines. The selected parental lines are established in a crossing block with 40-50 trees after selfing (step V). Based on specific objectives of the breeding program crosses are made among different parental lines in the crossing block mostly using Griffings half diallel fashion (8) (step VI). Some times the crossing can be made directly on local adaptation or verification plots to save time.

Similar to collections the F1 crosses under go a series of field trials to select the best hybrids for release (step VII-IX). The studies on F1 hybrids can be further advanced to F2 generation or under gone series of backcrosses to study inheritance of important agronomic traits or to transfer certain important traits that may be lacking in the F1 hybrid (step VIII). The hybrids with transforred trait(s) are further tested to verify their over all performance before recommendation is made for release.

RESULTS AND DISCUSSIONS

COLLECTIONS

The coffee collection program was started in 1966 using 73 accessions collected by French collection expedition to Ethiopia (9). Since the inception of collection program (1966 - 1996), 2789 indigenous and 190 exotic accessions were collected from different parts of the country and planted at Jima Agricultural Research Center (JARC) or its sub-centers (Table 1). Nearly half of the indigenous accessions were collected mainly for CBD resistance. CBD is the major coffee disease considerably threatening the Ethiopian coffee industry since its out-break in 1971 (19). The other accessions were collected for various agronomic characteristics of breeding interest. Many of the accessions were died due mainly to poor adaptation, root or stem diseases and over bearing die-back except that of 634 accessions from Harerge that were abandoned with Mechara sub-center as a consequence of the civil war in Ethiopia during 1991. At present, there are about 1927 indigenous and 128 exotic accessions availablie in the gene bank at JARC.

Table 1. Summary of coffee collections made from 1966-1996.

Type of collection	Areas of collection (origin)	No. of accessions	
		original	present
National coll.(1966-96)			
Yield and other characters	All coffee regions	1558	766
CBD resistance	Illubabor, Kaffa, Sidamo, Harerge	1231	1161
Sub-total		2789	1927
International coll. (1968-84)			
C. arabica L. variety	Brazil, India, Tanzania, Portugal, Cuba	183	122
Diploid spp.	Brazil	7	6
Sub-total		190	128
Total		2979	2055

The accessions currently available in the gene bank are too few to represent the high genetic variability available within the population in Ethiopia. On the other hand, due to deforestation of coffee forest owing to the increasing demand of land for food crops, replacement of the land races by few improved varieties, compition by other cash crops such as chat (Catha idulis), and many other reasons, the gnetic erosion is by far advancing the pace of our annual collection program. To combat this danger, a well organized, more systematic and intensive collection program should be carried out as a matter of urgency together with Ethiopian Bio-Diversity Institute (BDI), Coffee and Tea Authority (CTA) and Ministry of Agriculture (MOA). Besides, areas with natural coffee forest and high gnetic diversity should be identified through out the country and protected by law as in-situ conservation.

PROGENY TESTING AND SELECTION

Progeny testing includes three stages of field performance trials - screening, local adaptation and verification trials. During screening, about half of the total national collections (1966 - 1984 batches) were evaluated over 6-11 years for different agronomic characters at Jimma/Melko and Gera. There was highly significant variation within each batch of collections for yield CBD resistance, and growth and quality characters (3,10). The genetic advance through selection for yield at 20% selection intensity was up to 2.2 kg of fresh cherry per tree. These results well confirmed the presence of high genetic variability within arabica coffee population at its center of origin and the possibility to bring maximum improvement through selection.

Most of the selections obtained after screening were tested for adaptation under different environments. The results showed highly significant variations between locations genotype x environment interactions for yield and many other characters measured suggesting that the indigenous cultivars are location specific. Therefore, for good growth and economic yield return suitable varieties should be developed for each specific agro-ecological zones of the country. In other studies, selection was found to be more effective when cultivars were tested in their place of origin than when tested in other environments (4) supporting the above conclusion. At present there are 279 promissing selections identified for different locations representing different coffee ecologies (Table 2). The selections were primarly identified for yield and CBD resistance.

Table 2. Summary of promissing selections available for different locaitons representing high medium & low altitude area.

Location	Altitude (m)	Ecological class	High yield (>15 Qt/ha)		Medium yield (<15 Qt/ha)		Total No. of selections
			No. of selection	Yield range	No. of selection	Yield range	
Gera	1900	Highland	50	15.0-23.5	32	8.1-14.6	82
Wonago	1850	"	-	-	32	10.5-14.9	22
Melko	1750	Midland	63	14.6-24.6	52	6.3-14.0	115
Metu	1550	"	10	14.9-25.0	13	9.9-14.7	23
Tepi	1200	Lowland	2	14.8-18.1	10	6.4-12.2	12
Bebeka	1000	"	8	14.8-19.4	7	9.5-13.9	25
Total			133		146		279

Evaluation of some 123 introduced C. arabica L. varieties at Melko/Jima and Gera did not show better performance compared to local selections. It was only Rume Sudan that showed lower but comparable yield to our local selections probably because of its resistance to CBD (9). Such poor performance of the introduced varieties appeared to be due mainly to adaptation problem and their susceptibility to stem disease (Gibberella zylarioides) and coffee berry disease (CBD). Ten catimor hybrtid lines and eight C. arabica L. varieties tested at Bebeka and Tepi where the altitude is low (1000 - 1200m) and CBD is not present, however, showed superior performance (4,9). Currently Vr Geisha and four catimor hybrid lines that gave clean coffee of 15 - 19.4 Qt/ha are under verification.

HYBRIDIZATION PROGRAM

1. **Inheritance of Resistance to CBD**

A complete diallel crosses were made between six cultivars of resistant (R1, R2), intermediate (R3) and susceptible (R4, R5, R6) groups in their level of resistance to CBD to study the nature of genes controlling CBD (15). The crosses were evaluated both in the field and laboratory but since the results were essentially the same (r= 0.946 and 0.962 for 1981 and 1982, respectively), only the DBT result is presented (Table 3). The hybrids resistant x susceptible, were significantly susceptible over their respective mid-parent values except R_1 x R_6 and R_1 x R_5 in 1981 and 1982, respectively. Mean deviations of all the F_1's from the susceptible parents were not significant except for one cross suggesting that the hybrids were as susceptible as their respective susceptible parents. These results led to the conclusion that partial to complete dominance of the susceptible genes to the resistant genes were present in the population and resistance is controlled by recessive genes. It was also estimated that 3-5 major recessive genes of additive nature seemed to control resistance. The result further showed non-significant reciprocal differences suggesting that maternal effects are negligable. The study was advanced to F_2 generations through backcrossing and selfing of the F_1's to confirm the present results and precicely determine the sgregation pattern and number of genes involved in controlling CBD resistance. Data collection is completed and the analysis and summerization is under way.

2. Heterosis and Combining Ability

Some of the result of these studies are sammerized in Table 4-6. Perecentage mean heterosis of the hybrids over the mid-parent and better parent were positive for yield, some component of yield except over better parent for number of primary nodes and bearing primary branches (Table 4), and all seedling characters studied (Table 5). The degree of heterosis over the better parent was up to 60% and 69% for yield and seedling characters, respectively. Among the hybrids evaluated in teh field, 741 x F-59 and 7395 x F-59 were the highest yielders and exhibited high and positive better parent heterosis for all the characters measured.

Table 3. Hybrid and parental means susceptibilities to CBD and percentage suseeptibilities of the hybrids over the mid-parent (OMP) and susceptible parent (OSP) in 15 mean of reciprocal crosses after percent suseptibilities from deatched berry test (DBT) were classed and grades 1-5 were assigned.

Identification	F_1 mean value using 5 grades*		% susceptible			
			OMP		OSP	
	1981	1982	1981	1982	1981	1982
Resist. x Resist.						
$R_1 \times R_2$	1.72	2.17	34	39	29	30
Resist. x Interm.						
$R_1 \times R_3$	2.39	2.11	53	9	26	-14
$R_2 \times R_3$	2.50	2.72	55	32	32	11
Resist. x Suscept						
$R_1 \times R_4$	4.39	4.39	61**	41*	4	-8
$R_1 \times R_5$	3.73	3.50	46**	13	-4	-27*
$R_1 \times R_6$	3.61	3.84	33	38*	-4	-7
$R_2 \times R_4$	4.62	4.22	66**	31*	9	-12
$R_2 \times R_5$	4.95	4.22	51**	31*	2	-12
$R_2 \times R_6$	3.95	3.95	42**	37*	-6	-4
Interm. x Suscept.						
$R_3 \times R_4$	4.44	4.67	45**	29*	5	-2
$R_3 \times R_5$	3.89	4.17	35*	16	0	-13
$R_3 \times R_6$	3.89	3.56	27	9	-8	-13
Suscept. x Suscept.						
$R_4 \times R_5$	4.34	4.34	0	5	1	-2
$R_4 \times R_6$	4.23	4.67	0	5	0	-2
$R_4 \times R_5$	3.94	4.78	-3	7	-7	0
Parentals						
R_1	1.22	1.44				
R_2	1.33	1.67				
R_3	1.89	2.44				
R_4	4.22	4.78				
R_5	3.89	4.78				
R_6	4.22	4.11				

LSD 0.05 and 0.01 = 0.98 and 1.39 for 1981 columns, respectively, and 0.99 and 1.41 for 1982 columns, respectively

* Grade 1 = highly resistant, Grade 5 = highly susceptible,
source = (15)

Table 4. Three years average yield and heterosis as percentage over the mid-parent (OMP) and better parent (OBP) for yield, and some components of yield in coffee.

Hybrid	Yield (Qt/ha)	Girth		No. of flowers & fruits		Length of single prim. br.		No. of prim. nodes		No. of bearing prim. node		Yield	
		OMP	OBP	OMP	OBP	OMP	OBP	OMP	OBP	OMP	OBP	OMP	OBP
741 x 7332	22.3	6*	4	16	3	6*	5	2	-6	8	6	25*	5
741 x 2970	23.0	11**	6*	47**	15	13**	3	10**	6	13*	-4	69**	60**
741 x F59	23.7	16**	10**	50**	16*	16**	10**	15**	12**	24**	8	38**	18
741 x 7395	18.7	2	2	-15	-32**	5	-3	-3	-7*	-4	-13*	22	15
7332 x F59	23.3	5*	-2	10	-6	2	-2	2	-9**	-2	-16**	13	9
7332 x 2970	21.6	8**	2	24*	7	8*	-1	-4	-15**	3	-14**	27*	2
7332 x 7395	19.5	7**	5*	4	-8	2	-1	-1	-12**	6	-5	4	-8
7395 x 2970	22.2	11**	6*	31**	27**	7*	2	12**	12**	19**	11*	52**	36**
7395 X F59	24.0	18**	12**	30**	23**	9**	9**	12**	11**	17**	11*	32**	20**
Mean	22	9.3	5.0	21.9	5.0	7.5	2.4	5.0	-0.9	9.3	-1.8	31.3	17.4

*, ** significant at 0.05 and 0.01 probability levels, respectively.
Source = (12,14)

Table 5. Heterosis as percentage over the mid-parent (OMP) and better parent (OBP) for some seedling characteristics in coffee.

Hybrid	Girth		Height		Internode length		Shoot fresh wt.		Shoot dry wt.		Shoot volume		Leaf area	
	OMP	OBP	OMP	OBP	OMP	OBP	OMP	OBP	OMP	OBP	OMP	OBP	OMP	OBP
74110 x 74158	-1	-2	3	2	6	6	9	1	7	0	9	-1	15	12
74110 x 20071	7	5	12**	5	12	6	24**	12	33**	22*	24**	11	26**	16
74110 x 221A71	14**	6	25**	14**	33**	25**	65**	64**	58**	57**	69**	69**	44**	39**
74110 x 1371	13**	11**	23**	19**	29**	22**	59**	53**	58**	53**	49**	38**	52**	41**
74110 x 1571	12**	9*	20**	20**	25**	25**	52**	44**	44**	38**	49**	43**	54**	46**
74158 x 20071	9*	5	-4	-9*	-6	-11	-3	-5	-1	-6	-7	-8	6	-1
74158 x 221A71	15**	6	11*	0	20*	13	14	4	15	7	2	-7	15	14
74158 x 1371	1	-3	5	3	0	-6	9	4	9	5	2	-1	10	4
74158 x 1571	2	-2	10*	9*	6	6	19*	16	19*	17	7	0	23*	20*
20071 x 221A71	12**	7	24*	6	25**	11	18**	33**	44**	31**	52**	37**	36*	29**
20071 x 1371	5	5	9*	5	17*	17*	15	8	17	10	12	8	11	11
20071 x 1571	-2	-2	6	-1	6	0	6	2	5	0	-1	-8	13	9
221A71 x 1371	-7	-11**	-8	-18**	-19*	-28**	-7	-11	-8	-12	-14	-20*	-13	-17
221A71 x 1571	2	-3	6	-4	0	-6	12	5	9	3	6	2	13	11
1371 x 1571	11**	10**	18**	14**	24**	17**	39**	36**	39**	37*	40**	35**	32**	28**
Mean	6	3	11	4	12	6	24	18	23	17	20	13	22	18

*, ** Significant at 0.05 and 0.01 probability levels, respectively.
Source: (5)

Except that the GCA mean squares for yield and SCA mean squares for number of secondary branches, for all the rest 12 characters studied, both GCA and SCA mean squares were signidicant or highly significant (Table 6). This result suggested that both additive and non-additive (dominance & epistasis) gene actions are important in controlling inheritance of most of the characters studied. Variance ratio of GCA to that of SCA, however, showed that additive gene action is predominant only for numbers of primary nodes and secondary branches and all the rest characters were largely governed by non-additive gene actions.

Table 6. Mean squares for general combining ability (GCA) and specific combining ability (SCA) and ratios of variance due to GCA and SCA.

Character	Mean squares			Ratio+
	GCA	SCA	Error	
Field				
Yield	73694NS	570741**	116499	-0.0314
Girth	0.0216**	0.0213**	0.0029	0.3370
No. of flowers and fruits	233678**	198565**	26904	0.4015
Length of 1st prim. branch	37.3807**	19.8037*	6.1689	0.1481
No. of primary nodes	2618**	694**	173	1.564
No. of bearing prim. nodes	225.32*	569.74**	67.78	0.1046
No. of secondary branches	194*	29NS	52	-2.0578
Nursery				
Girth	0.0006**	0.0007**	0.0001	0.2083
Seedling height	2.14**	1.25**	0.12	0.4487
Internode length	0.06**	0.06**	0.01	0.2500
Shoot fresh wt.	0.37**	0.38**	0.04	0.2426
Shoot dry wt.	0.035**	0.034**	0.004	0.2600
Shoot volume	0.41**	0.41**	0.04	0.2500
Leaf area	5.23**	3.79**	0.66	0.3650

*, ** significant at 0.05 and 0.01 probability levels, respectively.
+ Ratio: As suggested by griffing (8)

The results of heterosis and combining ability studies clearly indicated the presence of considerable amount of positive heterosis in crosses among indigenous selections and the possibility to find maximum heterosis by continuous crossing among diverse parents. The results further suggested that selection and subsequent hybridization are proper breeding approach to exploit the advantage of both additive and non-additive genetic effects and the possibility to develop superior hybrids for yield improvement. Recently, two high yielding and CBD resistant hybrids were approved for disstribution to growers.

3. Breeding for Quality

Quality is the most important single factor dictating world market in coffee. Since there is high genetic variability in Ethiopia there is immense possibilities for quality improvement. Some quality assessement done on large number of selections and the high quality of Harerge, Yirgacheffe/Sidamo, Gimbi, Nekemte and Limu coffees that fetches premium price on the world market clearly confirm this fact (9,6).

On the other hand much work has not been done to exploit the available genetic potential and produce higher quality cultivars. This was due to problems of liquoring facilities and skilled man power. Despite these problems some 250 high yielding and CBD resistant selections had been tested and some cultivars that proved to be high or above average for quality (raw, roast,and liquor) were included in hybridization program to study inheritance of quality characters,

Table 7. Sets of crosses under evaluation for genetic studies and improvement of quality and yield result at Bebeka.

Set	Description	No. of crosses	7 years mean yield (Q/ha)		Heterosis (%)
			hybrid	chack/parent	
I	CBD resist. x High yielder	15	12.8	10.3	24.3
II	CBD resist. x Harer type High yielder x Harer type	13	20.4	11.8	72.9
III	CBD resist. x High quality + CBD res.	19	8.4	7.6	10.6
IV	CBD resist. + High quality x CBD resist. + High quality	10	12.3	5.0	146.0
V	Sidamo type x Sidamo type	15	-	-	-
VI	Sidamo type x Non-Sidamo type + CBD resist.	15	-	-	-

N.B Set V & VI are at seedling stage.

and the effect of environment on quality and develop hybrids with supprior qualtiy (Table 7). The hybrids were planted at Bebeka, Jima, Gomma and Gera, but only yield data from Bebeka is reported. From the yield result, it is apparent that there is a possibility to find high yielding hybrids from crosses between high quality selections. Therefore, systematic selection and hybridization program should be continued giving due emphasis to coffee areas that have already attained good reputation for their high quality on the world market in oder to produce very high quality cultigvars for special market.

IMPROVED CULTIVARS

The Ethiopian coffee ecology is so diverse that varieties suited to one location do not equally perform in other locations. This problem led to develop cultivars for each ecology. At present there are 18 released cultivars (2 hybrids and 16 pure-lines) developed for high, medium and low altitude areas (Table 8). Except two of the low land cultivars, all are resistant to CBD, but intermediate resistant or susceptible to coffee leaf rust (CLR).

Table 8. Summary of improved cultivars under production.

Ecology	Adaptation status	No. of released cultivars	Resistance level		Yield range (Q/ha)	
			CBD	CLR	on-station	on-farm
Highland (1750-2100m)	Suitable	5	R	IM	12 - 18	7 - 10
	H. suitable	8	R	IM	17 - 23	9 - 13
Midland (1550-1750m)	Suitable	7	R	IM	12 - 18	7 - 10
	H. suitable	8	R	IM	16 - 24	8 - 15
Lowland (1000-1550m)	Suitable	5	R	IM	8 - 15	6 - 9
	H. suitable	3	S, R	R, IM	10 - 15	8 - 13
Total		18				

R = Resistant, S = Susceptible, IM = Intermediate.

At the moment CLR is not a serious problem, but a breeding program is already under way as it appeared to be a potential disease in the future. Yield potential of the cultivars was as high as 24 Q/ha on research stations on 2500 tree basis, but produce nearly half of the full potential on farmers field simply because of improper management. Besides, there are several hybrids and pure-line selections currently under verification. The pace of variety release in general, is so slow probably becauses of the perennial nature of the crop and conventional way of the breeding method that we follow. Quick and efficient breeding method is necessary to produce varieties for the major environmental conditions prevailing in Ethiopia.

MULTIPLICATION PROGRAM

In a preponderanltly self-pollinated crops like arabica coffee, pure-line cultivars can be effectively multiplied through seed. Progenies produced from seed orchard established after selfing of the mother trees of all the released pure-line cultivars were sefficiently homogenous. Since 1977, the Jima Agricultural Research Center has supplied about 600 Qts of seeds to MCTD from 15 high yielding and CBD resistant cultivars for distribution to growers. Besides, MCTD is annually preparing some amount of seeds from demonstration plots and supply to growers to partly satisfy the needs of the growers.

Unlike pure-line cultivars, multiplication of F1 hybrids is difficult. It can be multiplied true-to-type only through hand-pollination or vegetative propagation (cutting, tissue culture, etc.). These methods are laborous and expensive. There are two coffee hybrids recently approved for release. The center has prepared a project document for multiplication of the hybrids through hand-pollination and tissue culture (13). However, this multiplication program is facing difficulties because of lack of agents resposible for multiplication and lack of tissue culture facilities.

FUTURE RESEARCH DIRECTION

1. The present conventional breeding method and the perennial nature of coffee have significantly retarded the rate of variety release. It is now high time to adopt modern plant breeding methods such as in-vitro selection, genetic engineering and tissue cultures possibilities so as to supplement the conventional method and shorten the breeding cycle.

2. At present because of various reasons coffee genetic erosion is increasing at an alarming rate than ever. It appeared that a well designed, systematic and intensive ex-situ and in-situ conservation system should be carried out in cooperation wtih Ethiopian Bio-diversity Institute as a matter of top urgency.

3. Quality is the most important factor dictating world market. Because of its endowment with high genetic variability, there is a great opportunity for Ethiopia to develop very high quality *cultivars for sale at exceptionally higher price. A systematic and effective selection* and breeding program for quality should be designed giving major emphasis to areas well known for their high quality coffee.

4. Indigenous coffee cultivars are location specific. Besides, there is considerable quality variations between regions. In order to minimize adoptation problem, and avoid blending effects of known quality areas with coffee from another area(s), the selection and breeding work should be done for each locality using coffee materials from the respective location.

5. Drought is becoming a problem in many areas. Besides there is an increasing demand to grow coffee where it does not grow before. Therefore, breeding for tolerance to drought, low management and marginal areas should be given due consideration.

ABSTRACT

Coffee (Coffea arabica L.) stands first in the Ethiopian economy. It's production, however, faces a serious threat mainly as a result of coffee berry disease (CBD) and lack of recommended cultivars for different localities. In Ethiopia, coffee breeding work in progress since 1978, was primarily aimed at developing cultivars which combine high yield with CBD resistance and good quality. Germplasm collection, selection and hybridization were the main activities to achieve the desired goal. About 2789 indigenous and 190 exotic accessions were collected. Some of these have died because of various reasons and currently 1927 indigenous and 128 exotic accessions are available in the gene bank. Rigorous screening of the first 1380 indigenous and all the exotic accessions for desirable traits resulted 279 promising selections of which 17 CBD resistant selections were released. Recently, 3 cultivars were approved for release and over 10 are in the pipe line. In crosses among the indignenous selections, inheritance of desirable traits and effects of heterosis were studied. CBD susceptible genes were dominant over resistance and three to five major recessive genes whose effects are additive were estimated to be involved in controlling resistance. The study was advanced to F2 through selfing and back crossing to confirm these results. Yield, and the growth characters studied were governed by

additive and non-additive gene actions, the non-additive being predominant. The F1 hybrids exhibited significantly high heterosis of up to 69% and 60 for seedling character and yield, respectively. The highest heterosis was obtained among those crosses whose parents distinctly differ in origin and morphology. Currently, two high yielding and CBD resistant hybrids were developed and approved for release. The implication of the present selection and breeding results and future prospects are discussed in detail.

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COMPORTEMENT D'HYBRIDES F1 de *COFFEA ARABICA* POUR LA VIGUEUR, LA PRODUCTION ET LA FERTILITÉ EN AMÉRIQUE CENTRALE

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1. INTRODUCTION

Les variétés de *Coffea arabica* cultivées en Amérique centrale ont une base génétique étroite. Il en résulte une grande sensibilité à la plupart des maladies et parasites. L'introgression des gènes de résistance à la rouille orangée, issus de l'Hybride de Timor, commencée au Portugal dans les années 60, a donné naissance aux nouvelles lignées Catimor / Sarchimor. Actuellement parmi les centaines de lignées créées et testées dans la région (Echeverri et Fernández, 1989), deux, les variétés IHCAFE90 et Costa Rica 95, donnent satisfaction pour leur productivité et leur résistance à la rouille (Aguilar,1995). La sélection de ce type de matériel se terminera peu après l'an 2000, ce qui clôturera le cycle de sélection.

Pour continuer à accroître la productivité et la qualité du café, PROMECAFE, le CATIE et la Coopération Française mènent conjointement un nouveau schéma d'amélioration génétique du caféier arabica, depuis 1992. L'originalité de ce programme réside tout d'abord dans la création de variétés hybrides F1, ce qui raccourcit le cycle de sélection à une dizaine d'années alors qu'un programme classique de sélection généalogique dure 25 ans. En outre, la technique de multiplication des hybrides retenue est la multiplication végétative *in vitro* par embryogenèse somatique, technique aujourd'hui bien maîtrisée (Etienne *et al.*, 1997).

Le schéma d'amélioration choisi exploite l'existence du phénomène d'hétérosis, rapporté chez cette espèce autogame par Srinivasan et Vishveshvara (1978), Charrier (1978), Araujo et Pereira (1980), Walyaro (1983), Ameha et Belachew (1985), Ameha (1990), Santacreo *et al.* (1992) et plus récemment Cilas *et al* (*in press*). Dans ce programme, nous cherchons à maximiser la vigueur hybride en croisant des origines spontanées ou subspontanées d'Ethiopie avec les variétés cultivées à port nain. L'analyse de la structuration de la diversité génétique chez l'espèce *C. arabica* grâce aux marqueurs neutres RAPD (Random Amplified Polymorphic DNA) a montré que ces deux types de matériel végétal appartiennent à deux pools génétiques differenciés (Lashermes *et al.*, 1996).

Les résultats préliminaires présentés ici contribuent à une meilleure compréhension de l'hétérosis chez le caféier arabica et à son exploitation pour la sélection de nouvelles variétés.

2. MATERIEL ET METHODES

Matériel végétal

Les lignées parentales utilisées en croisement sont :

- quatre cultivars (Caturra, Catuai, Catimor et Sarchimor), dont trois sont largement cultivées en Amérique centrale,
- 11 origines provenant des prospections en Ethiopie de la FAO (1968) et de l'ORSTOM (Charrier, 1978), respectivement E-156, E-416, E-531 et ET-5, ET-6, ET-15, ET-16, ET-25, ET-32B, ET-35B, ET-41,
- deux variétés originaires d'Ethiopie et du Soudan, Anfilo et Rume Sudan.

En 1991 et 1992, 32 familles F1 furent créées entre les quatre cultivars pris comme femelles et les 13 origines d'Ethiopie et Soudan prises comme mâles. Dans tous les croisements, le pollen provient d'un seul arbre. Les parents femelles sont également utilisés comme témoins.

Un premier essai comparatif des hybrides créés en 1991 et des variétés témoins a été planté à Turrialba (Costa Rica) (essai n° 1), dans une zone tropicale humide sans saison sèche marquée, à 600 m d'altitude. Le deuxième essai comparatifs des hybrides créés en 1992 et des variétés témoins a été planté à Heredia (Costa Rica) (essai n° 2), dans un climat tropical humide avec trois à quatre mois de saison sèche, à 1100 m. Le dispositif expérimental adopté est une randomisation totale des parcelles monoarbre, avec des effectifs par famille variant de 12 à 30 individus. Les distances de plantation sont de 2 m x 1 m, dans l'essai 1 et de 2 m x 1,5 m dans l'essai 2. Les parents mâles (origines éthiopiennes) ont été plantées dans des parcelles voisines des essais ; ils se développent lentement et leurs productions sont faibles.

Caractères observés

Depuis la plantation des deux essais comparatifs d'hybrides F1 en 1992 / 93, les caractères observés (Tableau 1) ont concerné :

- le développement végétatif des jeunes caféiers, à l'âge de 15 mois,
- les productions de fruits, pendant les deux premières années de récolte,
- la qualité de la fructification.

Quatre variables supplémentaires ont été calculées pour l'essai 2 sous forme des rapports suivants : production moyenne / diamètre au collet, production / hauteur, production / nombre de rameaux primaires et production / longueur moyenne des deux plus longs rameaux primaires.

Analyse des données

Les caractéristiques des hybrides F1 sont comparées avec celles des meilleures lignées parentales (cultivars). Leurs valeurs moyennes sont comparées par des analyses de variance à un critère de classification. Cette analyse est complétée par une comparaison des variances suivant le test F de Snedecor et par l'étude des corrélations entre les familles communes aux deux essais. Le taux d'hétérosis est calculé par rapport à la valeur du meilleur parent, par la formule classique suivante :

$$\% \text{ hétérosis} = [(\text{valeur de l'hybride} - \text{valeur du meilleur parent}) / (\text{valeur du meilleur parent})] \times 100$$

Tableau 1 : Caractères observés et variables traitées par essai.

Caractères observés	**Méthode, unité**	**Variables traitées**	
		Essai 1 (1992)	**Essai 2 (1993)**
Production par arbre	En g de cerises fraîches	Moyenne de 1994 et 1995	Moyenne de 1995 et 1996
Poids de 100 grains secs	En g, calculé sur 200 grains, à 0% d'humidité	En 1995	Moyenne de 1995 et 1996
Nombre de fruits observés sur les six noeuds les plus chargés		En 1995	Moyenne de 1995 et 1996
Pourcentage de fruits caracolis	Calculé sur les six noeuds les plus chargés	En 1995	En 1995, 1996 et moyenne
Pourcentage de fruits flottants	Calculé sur 200 fruits	En 1995	En 1995, 1996 et moyenne
Diamètre au collet	En mm, à 5 cm du sol		A 15 mois
Hauteur totale	En cm		A 15 mois
Nombre de rameaux primaires			A 15 mois
Longueur moyenne des deux plus longs rameaux primaires	En cm		A 15 mois
Longueur moyenne des entre-noeuds	En cm, calculée sur les deux plus longues primaires		A 15 mois

3. RESULTATS

Comparaison globale des parents et des hybrides

La comparaison des performances moyennes des hybrides et des lignées montre qu'il existe une différence significative entre les deux populations pour chaque caractère (Tableau 2). Les hybrides sont supérieurs aux lignées pour tous les caractères de croissance végétative et de production, à l'exception des caractéristiques de fertilité (fruits flottants et grains caracolis).

La vigueur hybride est plus importante pour la production que pour les caractères végétatifs. Le gain pour la production est de 31 % chez l'ensemble des hybrides. Le rapport production / vigueur (diamètre au collet, hauteur, nombre et longueur des primaires) met en évidence un avantage des hybrides par rapport aux lignées de 16 à 24 %. Les hybrides possèdent aussi plus de fruits par noeud. Les gains pour le poids de 100 grains sont significatifs mais faibles. Par contre, les pourcentages de fruits à un grain caracoli et de graines vides sont très supérieurs chez les hybrides, respectivement 11 % et 8 %.

Enfin, les rapports de variance montrent que les variances des lignées sont toujours supérieures à celles des hybrides, sauf pour les loges vides.

Tableau 2 : Moyenne des lignées et des hybrides F1 (Essai 2), % de gain et rapport des variances, avec la signification des tests : * (0,5 ≥ P > 0,01), ** (P > 0,001) et *** (P < 0,001)

Variables	Lignées	Hybrides F1	Analyse de variance	% de gain	Rapport des variances (1)
Production par arbre (g)	2079	2724	***	31	1,5 *
Poids de 100 grains secs (g)	16	17	*	4	3,1 ***
Diamètre au collet (mm)	27	31	***	12	2,1 ***
Hauteur (cm)	102	112	***	10	1,8 **
Nombre de primaires	57	61	*	6	2,5 ***
Longueur de deux primaires (cm)	62	69	***	12	1,4 *
Longueur des entre-noeuds (cm)	3	3	***	11	1,6 *
Nombre de fruits sur six noeuds	97	102	*	5	1,8 **
% de grains caracolis	7	11	***	- 49	1,7 **
% de fruits flottants	4	8	***	- 205	0,4 ***
Production / Diamètre	149	175	***	17	
Production / Hauteur	40	48	***	21	
Production / Nb primaires	72	89	***	24	
Production / L de deux primaires	68	78	**	16	

(1) (SCElignées/(n-1))/(SCEhybrides/(n-2)) testé par le test F de Snedecor

Comparaison des familles hybrides F1

Les performances de production et de fertilité des familles F1 sont données pour les deux essais dans les tableaux 3 et 4. Dans l'essai 1 à Turrialba, la famille 2 a une production supérieure aux variétés témoins Catimor / Sarchimor et Caturra, avec un gain de 53 %. Cinq autres familles (3, 4, 5, 6 et 7) ont une production statistiquement égale à celles de ces témoins. Les mauvais résultats obtenus par le témoin Catuai dans cet essai sont peu représentatifs des résultats moyens obtenus par cette lignée en Amérique centrale. En conséquence, les pourcentages d'hétérosis sont surévalués pour les familles qui en sont issues.

Dans l'essai 2 à Heredia, c'est la famille 19 qui a donné une production significativement supérieure à celle des variétés témoins. Le gain de production atteint 102 %. Neuf autres familles F1 (1, 11, 20, 21, 22, 23, 24, 25, 26) ont produit autant que la meilleure variété témoin (Catimor). Les gains sont compris entre 22 % et 85 %.

En règle générale, les hybrides F1 présentent plus de grains caracolis et de fruits vides que les variétés témoins. Certaines familles hybrides se distinguent par des pourcentages particulièrement élevés : par exemple les familles 4 dans l'essai 1 et 22 et 24 dans l'essai 2. Cependant, il est possible de sélectionner des familles ayant une fertilité et une production équivalente à celles des témoins (familles 6 et 7 dans l'essai 1 et 19, 25 et 26 dans l'essai 2).

Les corrélations calculées pour les neuf familles communes aux deux essais montrent que le pourcentage de loges vides est comparable d'un essai à l'autre (r = 0,90). Les coefficients de corrélation sont proches des seuils de signification pour les grains caracolis (r = 0,67), la production (r = 0,54) et le poids de 100 grains (r = 0,52).

Tableau 3 : Caractéristiques de production, fertilité et granulométrie dans l'essai 1. Classement des familles F1 et des lignées (en italique) d'après la moyenne de leur production en 1995 et 1996.

Matériel	**Production moyenne par arbre (g)**	**Vigueur hybride pour la production (%)**	**% de grains caracolis (1995)**	**% de fruits flottants (1995)**	**Poids de 100 grains secs (g)**
Famille 2	2998 a	53	12,0	8,3	17,2
Famille 3	2855 ab	37	19,0	2,0	19,6
Famille 5	2827 abc	36	19,5	5,0	19,0
Famille 4	2246 abc	(171)	17,0	13,8	18,2
Famille 6	2207 abc	32	12,0	3,0	19,5
Famille 7	2193 abc	(165)	12,7	4,0	19,0
Famille 1 *	2119 abcd	27	15,8	5,0	18,0
Sarchimor *	2086 bcd		13,0	3,0	18,3
Catimor *	1962 cd		8,3	2,5	18,2
Famille 8	1901 cd	- 9	19,6	8,6	18,1
Famille 9 *	1878 cd	(127)	14,5	5,0	17,5
Famille 10	1860 cd	(125)	11,0	2,4	16,7
Famille 11 *	1842 cd	10	12,0	3,0	18,5
Caturra *	1674 cde		8,4	2,4	17,2
Famille 12	1662 cde	0	9,0	10,0	16,5
Famille 13	1657 cdè	(100)	13,4	3,0	18,0
Famille 14	1653 cde	- 20	13,0	4,0	16,6
Famille 15 *	1519 de	- 9	11,0	12,4	17,4
Famille 16	1467 de	(77)	13,0	12,0	16,8
Famille 17 *	1258 de	(52)	8,6	7,8	16,0
Famille 18	1234 de	(49)	11,0	1,0	16,5
*Catuai**	828 e		10,0	2,7	16,0

* Familles F1 et lignées communes aux deux essais

( ) Vigueur hybride des familles ayant pour parent Catuai

Tableau 4 : Caractéristiques de production, fertilité et granulométrie dans l'essai 2. Classement des familles F1 et des lignées (en italique) d'après la moyenne de leur production en 1995 et 1996.

Matériel	Production moyenne par arbre (g)	Vigueur hybride pour la production (%)	% de grains caracolis (moyenne)	% de fruits flottants (moyenne)	Poids de 100 grains secs (g) (moyenne)
Famille 19	4034 a	102	7,9	5,8	17,6
Famille 1 *	3696 ab	85	11,7	6,5	18,4
Famille 20	3459 abc	83,4	13,8	5,9	20,5
Famille 21	3389 abcd	79,7	13,5	6,2	19,7
Famille 22	3385 abcd	22	11,7	13,8	17,6
Famille 11 *	3370 abcd	68,6	10,9	4,8	19,5
Famille 23	3324 abcd	66,4	9,8	14,6	17,9
Famille 24	2924 bcde	37,5	14,0	14,2	14,2
Famille 25	2921 bcdef	46,1	9,0	6,0	16,9
Famille 26	2821 bcdef	49,6	9,9	5,9	20,4
Catimor *	2770 bcdefg		7,3	2,9	16,3
Famille 27	2677 bcdefg	- 3,3	10,8	7,3	17,0
Famille 28	2638 cdefg	39,9	12,2	6,5	19,8
Famille 29	2512 cdefg	- 9,3	10,6	11,3	18,4
Famille 9 *	2370 defgh	11,5	12,0	6,6	17,3
Famille 30	2276 efgh	-17,8	12,6	10,9	17,0
Famille 31	2250 fgh	5,8	9,0	4,0	18,9
Famille 32	2241 fgh	5,4	9,0	4,0	18,0
Catuai *	2126 fgh		8,2	5,0	15,9
Famille 15 *	2103 fgh	5,2	11,2	8,3	16,5
Caturra *	1999 fgh		6,7	4,4	16,5
Sarchimor *	1886 ghi		7,7	4,3	20,0
Famille 17 *	1866 ghi	-12,2	10,6	9,4	17,3

(*) Familles F1 et lignées communes aux deux essais

Relations entre le rendement, la fertilité et la vigueur végétative

Le tableau 5 indique des corrélations phénotypiques positives et significatives entre la production et le diamètre au collet ou le nombre de fruits par noeud. Une régression multiple de la production sur ces deux variables (par ailleurs peu corrélées r = 0,31) permet d'expliquer 55% (r^2) de la production. La vigueur hybride est aussi liée à ces deux variables et se caractérise donc non seulement par une accroissement du volume de la plante, mais également par une augmentation du nombre de fruits par noeud.

Par contre, la baisse de fertilité observée chez les hybrides F1 n'est corrélée ni à l'hétérosis ni à l'augmentation de productivité (Tableau 5). En l'absence de liaisons physiologiques entre les caractères de production et de fertilité, il sera possible de sélectionner les familles F1 sur la variation observée pour les taux de grains caracolis et de loges vides. Les corrélations entre ces variables d'une année sur l'autre sont significatives (Tableau 6). On peut donc effectuer une sélection intra et inter-familles d'après les caractères de fertilité. Cette sélection sera plus efficace sur le taux de loges vides mais, en sélectionnant sur ce critère, on sélectionnera aussi sur le taux de grains caracolis.

Tableau 5 : Corrélations phénotypiques entre la production ou la vigueur hybride et quelques variables, dans l'essai 2 (492 données individuelles pour la production moyenne, 23 données familiales pour la vigueur hybride).

	Production moyenne	**Vigueur hybride**
Nombre de fruits par noeud	0,65 ***	0,51 *
Diamètre au collet à 15 mois	0,59 ***	0,57 *
% de grains caracolis (moyenne)	NS	NS
% de fruits flottants (moyenne)	NS	NS

Tableau 6 : Corrélations pour les caractères de fertilité, dans l'essai 2. (492 données individuelles pour la production, 23 données familiales pour la vigueur hybride).

	Corrélation sur les mesures individuelles	**Corrélation sur la moyenne familiale**
% de grains caracolis 1995 / 1996	0,32 ***	0,63 ***
% de fruits flottants 1995 / 1996	0,57 ***	0,89 ***
% de fruits flottants / % de grains caracolis	0,28 ***	0,50 *

4. DISCUSSION - CONCLUSION

En moyenne, la population des hybrides F1 est plus performante que les lignées parentales, résultat qui confirme ceux obtenus par Walyaro (1983), Ameha (1990) ou plus récemment Cilas *et al* (*in press*). La vigueur hybride (exprimée en % du meilleur parent) se traduit par une hausse de la productivité qui dépasse 30% sur les deux premières récoltes. L'analyse des prochaines récoltes renseignera sur l'existence d'un effet de précocité à fructifier chez les hybrides F1.

Cette valeur hybride est plus importante pour un caractère global comme le rendement que pour des caractères simples comme le diamètre au collet ou la hauteur de la plante ou encore d'autres composantes du rendement. Ceci est conforme aux études de Grafius (1960) sur le maïs, de Duarte et Adams (1972) sur le haricot ou de Lefort-Buson (1986) sur le colza.

La production est bien corrélée au diamètre au collet et au nombre de fruits par noeuds. Ce résultat est en accord avec ceux obtenus sur des caféiers arabica par Walyaro (1983) et canephora par Leroy (1993). Pour tous les caractères observés à l'exception des fruits flottants, la comparaison des variances des hybrides F1 et des lignées parentales montre une plus grande homogénéité des familles F1. On peut donc émettre l'hypothèse d'une plus grande stabilité des hybrides (homéostase) par rapport aux variations du milieu.

Cependant, l'ensemble des hybrides F1 présente une moins bonne fertilité (fruits flottants et grains caracolis) que les lignées parentales. Ce défaut a déjà été souligné par Santacreo (1992) pour le taux de fruits flottants. En Amérique centrale, les seuils maximum admis sont de 5 % à 10 % pour les taux de grains caracolis et de fruits flottants. Comme ces seuils sont situés dans l'intervalle de variation des familles F1, il sera d'entreprendre une sélection rigoureuse des hybrides pour ces deux composantes de la fertilité. Il devrait être relativement facile de sélectionner sur le taux de loges vides, qui semble être stable d'une année sur l'autre, et ne retenir que des individus élites se rapprochant de la fertilité des lignées. L'effet maternel trouvé pour le poids de 100 grains pourrait être en accord avec celui mis en évidence par Walyaro (1983) pour le taux de gros grains.

Les résultats obtenus montrent que la création d'hybrides F1 entre des lignées très productives et des origines spontanées d'Ethiopie, éloignées génétiquement, est très prometteuse. L'amélioration de ces hybrides sera poursuivie notamment sur la qualité du café produit.

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RESUME

Les variétés *C. arabica*, cultivées en Amérique centrale, sont issues d'une base génétique étroite. Depuis 1992, PROMECAFE, le CATIE et la Coopération française ont entrepris un programme original d'amélioration génétique, basé le croisement de variétés traditionnelles (Caturra, Catuai) ou des dérivés de l'Hybride de Timor (Catimor, Sarchimor) par des caféiers d'origine spontanée (Ethiopie, Soudan). Ce programme a pour objectifs

d'augmenter l'adaptabilité et la productivité des variétés, et de leur conférer une meilleure résistance aux principaux aléas de la région (rouille et nématodes), tout en maintenant ou en améliorant la qualité du café produit. Les hybrides F1 sélectionnés seront multipliés *in vitro* par embryogenèse somatique. L'utilisation de cette technique permet de raccourcir le cycle de sélection de 30 à 10 ans et d'évaluer plusieurs dizaines d'hybrides F1 pour les situations extrêmement variées de l'Amérique centrale.

Des observations concernant la croissance, la production et la fertilité ont été entreprises dans deux essais. Les premiers résultats montrent que les hybrides F1 sont plus vigoureux et plus productifs que les meilleures variétés. La vigueur hybride, calculée sur le meilleur parent, atteint fréquemment 30% pour le rendement. Les gains réalisés pour le poids des grains sont peu importants. Seuls les caractères de fertilité sont moins favorables que chez les variétés.

ABSTRACT

The varieties of *C. arabica* cultivated in Central America resulted from a very small genetic base. Since 1992, PROMECAFE, CATIE and the French Cooperation have started on an original program of genetic improvement, based on crosses between traditional varieties (Caturra, Catuai) or some Timor Hybrid derivatives (Catimor, Sarchimor), and spontaneous coffee trees (Ethiopia, Sudan). The objectives of this program are to increase the adaptability and productivity of varieties, and to improve the resistance to the main pests and diseases of the region (leaf rust and nematodes), without lowering the quality of produced coffee. Selected F1 hybrids will be multiplied *in vitro* by somatic embryogenesis. The use of this technique permits to shorten the selection cycle from 30 to 10 years and to propose many tens of F1 hybrids for the highly varied conditions of Central America.

Observations concerning growth, production and fertility were initiated in two field experiments. The first results showed that F1 hybrids are more vigorous and more productive that the best varieties. The hybrid vigor, calculated on the best parent, frequently reached 30% for the yield. The gains realized for the seed weight were poor. However, the F1 families generally presented a lower fertility than the varieties.

GENOTYPE-BY-ENVIRONMENT INTERACTION AND ITS IMPLICATIONS ON SELECTION FOR IMPROVED QUALITY IN ARABICA COFFEE (*COFFEA ARABICA* L.)

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Introduction

The wide environmental differences that occur in space and time has an important bearing on the performance of crop plants and hence on the plant breeding process. The interaction between genotypes and the environments modify the expression of genes; and may be manifested either as shifts in the relative ranking of genotypes across environments (Allard & Bradshaws, 1963), or as changes in the absolute differences in genotypic performance (Byth, 1977). This phenomenon, usually referred to as genotype-by-environment (GE) interactions, reduces the correspondence between the observed phenotype and the underlying genotype (Comstock & Moll, 1963). The prevalence of significant GE interactions necessitates extensive sampling of environments for variety testing purposes. Such an undertaking is however usually constrained by a number of factors including logistical considerations and limitations in research resources such as the availability of land, skilled personnel and finances (Hamblin *et al.*, 1980; Dhilon *et al.*, 1991).

In view of these constraints, a number of strategies have been adopted by plant breeders in order to accommodate the negative effects of GE interactions. Such strategies falls basically in two groups. The first approach involves stratification of environments based on the edapho-climatic characteristics into clusters within which GE interaction expected to be is minimal (Horner & Frey, 1957; Abou-El-Fitouh *et al.*, 1969; Crossa *et al.*, 1995). This approach in which a breeder 'avoids' the effects of GE interactions (Calighari, 1991), may be effective in situations where environmental differences well established and consistent, but offers little safeguard against unpredictable and transient environmental conditions. For a perennial crop like coffee, and for a trait like quality where regularity and consistency are desired, the technique of clustering may not be adequate despite its logistical appeal.

The second approach analyses GE interactions in order to identify crop genotypes which minimise the chance of poor performance and/or crop failure when subjected to variable environmental conditions (Finlay & Wilkinson, 1963; Eberhart & Russell, 1966; Hamblin *et al.*, 1980; Brennan *et al.*, 1981; Glaz *et al.*, 1985; Brennan & Shephard, 1985; Dhillon *et al.*, 1991; Kang & Pham, 1991; Calhoun *et al.*, 1994; Annichiarico & Perenzin, 1994). This strategy anchors on the breeder's ability to carefully identify the target population of environments, present and future, in which the improved cultivars are expected to thrive. The GE interaction structure of such population of environments is then determined and genotypes that minimise GE interaction identified for released as new varieties or used for further breeding work.

In recent times, more efforts have been directed towards understanding GE interactions in terms of their effects on growth, development and productivity. In this respect, identification of phenological stages most susceptible to GE interactions and the dominant environmental factors responsible for the observed interactions

has formed the subject of numerous studies (Cooper *et al.*, 1995; van Oosterom *et al.*, 1996a, 1996b). The aim has been to design testing strategies in which the behaviour of a genotype over varying environments could be predicted with greater precision and at minimum cost and time. This approach is particularly attractive when considering a perennial species like coffee and for a trait like quality whose development traverse a broad spectrum of possible environmental variations.

In this article therefore, the effects of GE interactions on the expression of bean and liquor qualities are discussed in relation to their influence on the efficiency of selection and testing of improved varieties of *Coffea arabica* L.. Results from a multi-location trial in Kenya involving 22 complex hybrid varieties and two check cultivars planted in five locations forms the subject of this discussion. The varieties were planted in a randomised complete block designs with three replicates per location. Each family was represented by eight plants per replicate out of which data was collected on five plants per replicate. Ripe cherries were collected on five plants per replicate and wet-processed according to standard procedures (Anon, 1983). The resulting parchment were then hulled using a hand operated hulling machine and graded according to size and shape into six grades (Walyaro, 1983), namely, pea berries (PB), AA, AB, TT, C, and T. Additionally, data was taken on single bean weight (SBNW).

Samples for liquor quality assessments were further selected with the help of an ultra violet sortex machine to remove beans with insect or mechanical damages. Assessment of liquor quality was carried out on blind samples by members of the Mild Coffee Association of East Africa (MCTA). Characterisation was done on the basis of two samples (one per replicate), each of 250g. Five attributes were considered (Devonshire, 1956) including the following: (1) Quality of roast beans (QRB) (2) Acidity (3) Body (4) Flavour and (5) Overall standard.

Results and discussions

Analysis of variance and GE interactions

1. Environmental effects on mean performance

The mean performance for bean and liquor traits and the within-location analysis of variance are shown in Table 1. Significant between-location mean differences were observed for all the traits considered. Location 1 had the best mean performance for AA beans whereas the mean performance for this trait was poorest in locations 4 and 5. Concerning liquor traits, locations 1, 2 and 5 did not show distinct difference in their level of performance. Location 5 however had the best performance for body , flavour and overall standard whereas location 4 showed the poorest performance for all the liquor traits.

From the mean performance for TT and AB in locations 1 and 5, it can be deduced that the conditions that prevailed in location 1 were optimal for both berry expansion and bean filling. Such conditions would lead to a reduction in TT (large light beans) and an increase in AA (large heavy beans) as was observed in this study. On the other hand, conditions experienced in location 5 were apparently optimum for complete berry expansion but were sub-optimum for bean filling. This is indicated by the unusually large proportion of TT beans and the low level of AA. The high proportions of AB still realised in this location however indicates that the stress factor limiting the formation of large bold beans was nonetheless not severe.

It is therefore apparent that both timing and intensity of such stress were important in articulating genetic differences between families for both bean and liquor traits. In this respect, the absence of stress during berry expansion and bean filling stages is necessary for maximum differentiation between genotypes for bean qualities. Moderate amounts of stress is however required to optimise differential expression between genotypes for liquor traits. These conclusions become evident when the results from locations 4 and 5 are compared. The two locations presented stressful conditions at the bean filling stages, with the exception that location four experienced a higher level of moisture stress. The observations were also coherent with physical climatic factors (rainfall amounts in this case) shown in figure 1. Optimum rainfall amounts during berry expansion and bean filling stages were observed in location 1 whereas precipitation was optimal during berry expansion but sub-optimal during bean filling in location 5.

2. Environmental effects on genetic variation

The analysis of variance results for family effects (also shown in Table 1) demonstrates that genetic variability for bean traits were maximised in location 1 whereas no variability was detected for liquor traits in the same

site. Genetic variation for liquor traits were on the other hand maximised in location 5. Appreciable variation for bean traits were also observed in the location. From these results, it appears that the two groups of traits were more or less antagonistic in terms of the environments necessary for their differential expression. These observations have an important bearing on both selection and testing of Arabica coffee varieties for improved quality. Concerning selection for improved bean qualities, batter progress will most probably be realised if selection is conducted under conditions similar to those which prevailed in location 1 whereas better progress from selection would be realised under conditions of location 5 when improving liquor quality. However considering that the economic value of coffee depends both on bean and liquor qualities, combined selection for the two traits would be advisable.

3.Environmental effects on selection efficiency

In view of the foregoing results, it is suggested that improved efficiency in selection and testing of *C. arabica* genotypes for bean and liquor qualities could be attained by taking into consideration both the stress factors associated with GE interactions in these traits and the developmental stages which are most susceptible to such factors. An optimum selection and testing regime could thus be designed using the following guidelines: 1)The elucidation of the physical environmental stress factors and their intensities necessary to evoke maximum expression and differentiation between genotypes 2) Identification of plant phenological stages which are most prone to environmental influence 3)Synchronisation of stress availability with the susceptible plant development stages

The use of off-season initiation of flowering thorough irrigation could be useful in creating different levels of stress even within a single locality and thereby reduce the need of extensive sampling of locations in an attempt to encompass more environmental variations. Considering the antagonism that exists in terms of environments that foster differential expression for the two groups of traits, combined selection for both bean and liquor traits may offer the best opportunity for improved selection efficiency.

Combined selection for bean and liquor qualities

The use of index selection in crop plants aims either at improving single or multiple traits of economic interest. In the former situation, secondary traits may be used in a attempt to increase the efficiency with which improvements in a target trait can be realised. Such application has found its way in the improvement of yield in Arabica coffee (Walyaro & Van der Vossen, 1979; Walyaro, 1983). In the events where more than one trait is of economic significance, index selection has been shown to be more efficient than individual trait selection procedures such as tandem selection or independent culling levels (Baker,1986). Its application in selection for improved bean and liquor qualities seems particularly appealing given the fact that maximum differentiation among genotypes for these two traits may require contrasting environmental conditions as was shown in this study. Table 2 compares the expected genetic gains and correlated genetic gains when selection is aimed at maximising improvements in each trait and when selection is directed towards simultaneously improving the two most important quality traits (AA and flavour in this case). The highest expected response for any of the traits occurred when selection was aimed at optimising the trait itself. Correlated response for bean traits were poor when response was optimised for any of the liquor traits. Similarly correlated response for liquor traits were low when response was maximised for bean traits. Conversely however, when both AA and flavour are combined into an index that simultaneously maximise response in the two traits, up to 84% of the maximum expected gains in AA and 88% of the maximum expected genetic gains in flavour could be achieved. Expected correlated response in other traits were equally high with the exception of AB for which a substantial reduction (74%) would be expected. It can thus be concluded that combined selection for bean and liquor traits present a more reasonable approach for improving the aggregate quality of Arabica coffee. The use of such indices should therefore form part of the "managed environment selection strategy" (Cooper *et al.*, 1995) as described above.

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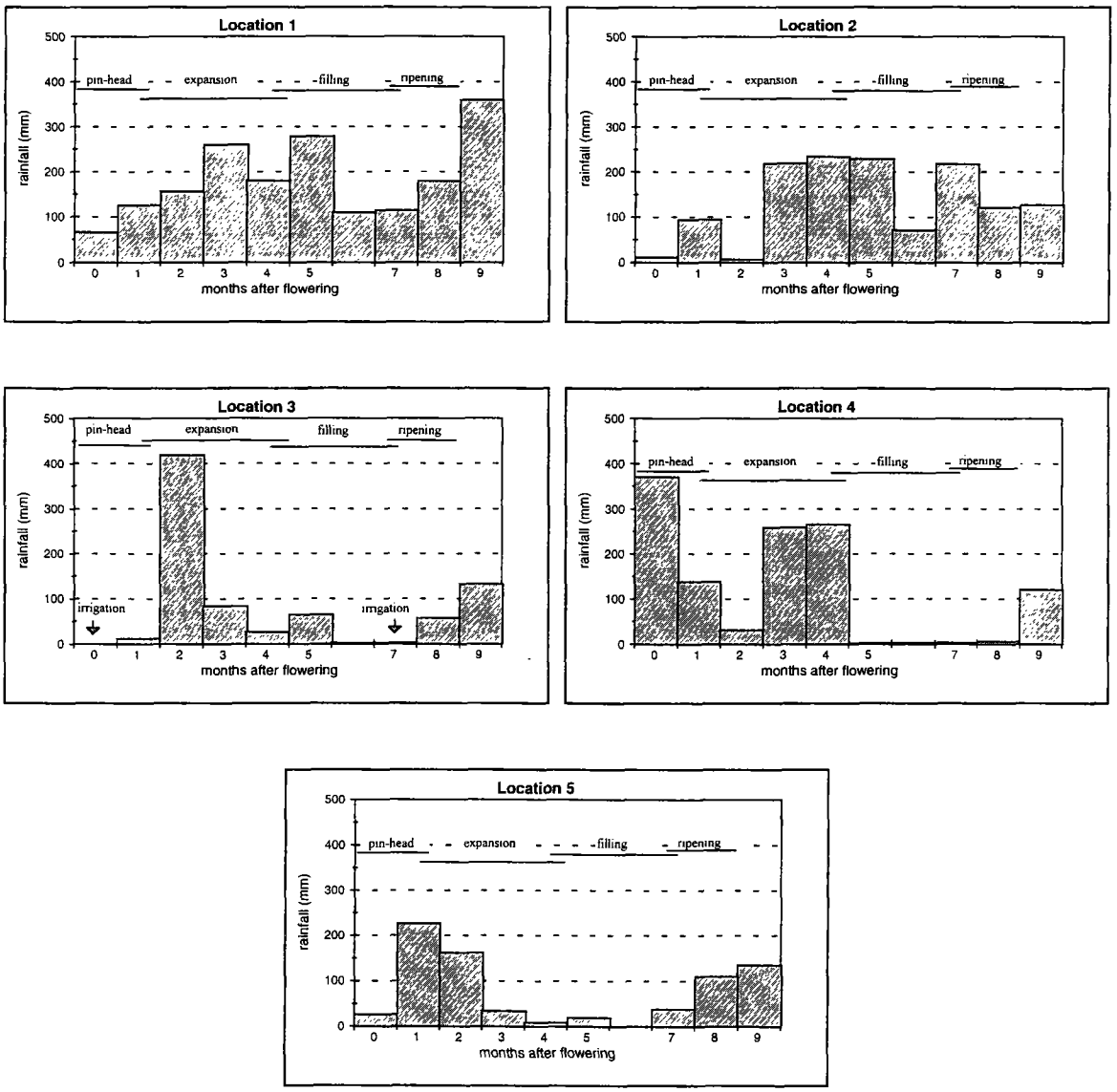

Figure 1 : Rainfall records and berry development phases in five locations in Kenya

Table 1. Mean performance[1] and genetic variations[2] for quality traits of *C. arabica* L. grown in five locations in Kenya

	Location 1			Location 2			Location 3			Location 4			Location 5		
Trait	Mean	Rank	MS_f	Mean	Rank	MS_f	Mean	Rank	MS_f	Mean	Rank	MS_f	Mean	Rank	MS_f
Acidity	2.41	3ab		2.22	5b		2.46	2ab		2.72	1a		2.35	4b	
Body	2.27	4a		2.29	3a		2.57	2a		2.64	1a	*	2.15	5a	*
Flavour	3.72	3b		3.64	4b		3.83	2b		4.11	1a		3.60	5b	****
Standard	3.72	4b		3.73	3b		3.83	2b		4.25	1a		3.69	5b	*
TT	19.14	5d	*	19.61	4c		23.18	3b		27.01	2a	*	27.46	1a	
PB	11.01	3ab	****	11.63	1a	**	10.15	4ab		11.56	2a	*	9.70	5b	
AA	27.63	1a	****	16.37	3b	***	16.72	2b		12.72	4c	*	11.08	5c	***
AB	36.51	5c	****	45.48	1a	***	41.14	3b	*	37.27	4c		41.60	2b	**
SBNW	0.18	1a		0.16	5c	*	0.16	4c		0.16	3c		0.17	2b	

[1]Ranks with similar letters were not statistically different

[2] *, **, ***, **** P value significant at 0.1, 0.5, 0.01 and 0.001 respectively

Table 2. Expected genetic gains[1] and correlated response on quality traits when response in one or more traits are optimise

	Response traits (5% selection intensity)						
Target traits	Body	Flavour	PB	AA	AB	SBNW	r (h,i)*
Body	10.62 (100.00)	5.12 (70.72)	6.50 (29.20)	15.36 (32.38)	-3.78 (-16.36)	1.92 (42.57)	0.73
Flavour	7.52 (70.81)	7.24 (100.00)	6.33 (28.44)	23.02 (48.52)	-10.61 (-45.93)	1.55 (34.37)	0.78
PB	3.10 (29.19)	2.06 (28.45)	22.26 (100.00)	44.55 (93.91)	-19.60 (-84.85)	4.37 (96.90)	0.84
AA	3.44 (32.39)	3.51 (48.48)	20.90 (93.89)	47.44 (100.00)	-19.27 (-83.42)	4.20 (93.13)	0.89
AB	-1.74 (-16.38)	-3.32 (-45.86)	-18.89 (84.86)	-39.57 (-83.41)	23.10 (100.00)	-3.35 (-74.28)	0.87
SBNW	4.52 (42.56)	2.48 (34.25)	21.58 (96.95)	44.25 (93.28)	-17.19 (-74.42)	4.51 (100.00)	0.90
Flavour + AA	6.50 (62.33)	6.37 (87.98)	15.26 (68.55)	39.97 (84.25)	-17.01 (-73.64)	3.24 (71.84)	0.85

[1]figures in parenthesis indicate the maximum expected correlated response relative to direct responsefor aech trait

QUALITY ASPECTS IN ARABICA COFFEE BREEDING PROGRAMMES IN AFRICA

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In response to heavy crop losses caused by coffee berry disease (CBD), breeding programmes were initiated some 25-30 years ago in a number of arabica coffee producing countries in Africa with the objective of developing disease resistant cultivars. Durable host resistance to such an important plant pathogen as CBD, preferably in combination with resistance to coffee leaf rust (CLR), would provide an economically and environmentally more attractive alternative to costly fungicide spraying, particularly to the resource-poor smallholder farmers who are the main coffee producers in Africa (Van der Vossen, 1985). Some programmes have been more successful in achieving their main objectives than others and it may be of interest to agricultural scientists and policy makers to learn from past experiences.

Any attempt of a critical analysis of possible reasons for these variable results may take into consideration the following quality aspects:

1. *Quality of breeding strategies and methodologies* : progress per unit of time, effort and financial resources leading to satisfactory results, i.e. locally adapted and productive coffee cultivars with effective and durable resistance to CBD and CLR.

2. *Quality of the new disease resistant cultivars* : in addition to yield and disease resistance, bean and liquor characteristics have to be at least similar to those of the traditional (disease susceptible) cultivars; this is a prerequisite in countries renown for the quality of their arabica coffees to avoid loss of share in the premium segment of the world coffee market.

3. *Quality of technology transfer* : promotional activities during the introduction phase leading to high farmers' acceptance; adequate technology and logistics for large-scale multiplication and dissemination of the new cultivars to all coffee growers within a reasonable space of time.

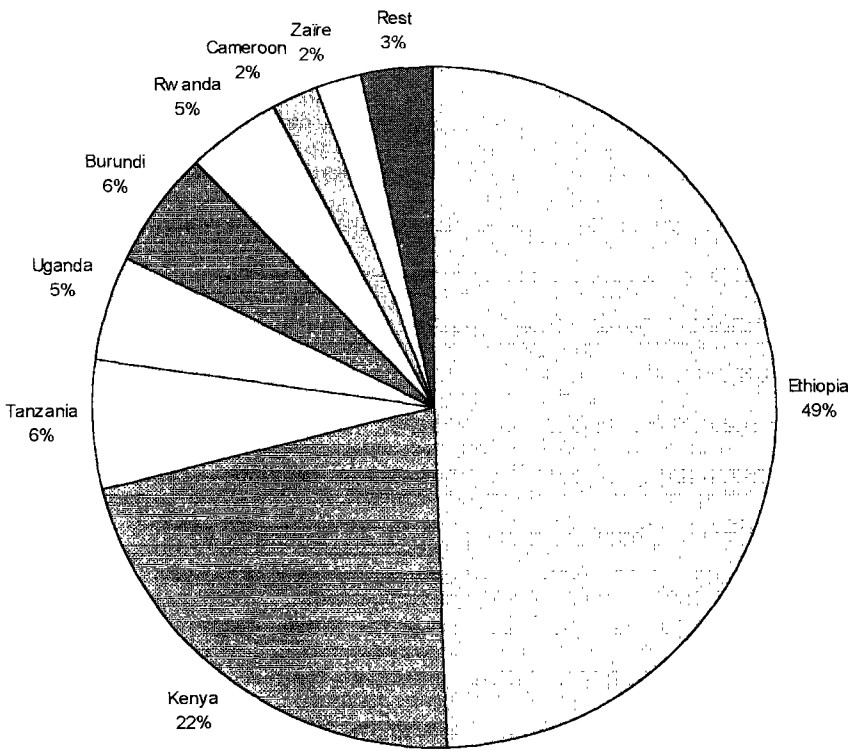
Production of arabica coffee in Africa

World production of arabica coffee for the 1995/96 season was estimated at about 3.5 million t (70% of total coffee production), of which only 16% or 464,000 t is produced in Africa (ICO, F.O.Light and USDA statistics, 1996). Fig.1 shows that almost half of that quantity is produced in Ethiopia and another 22% in Kenya. Tanzania*, Uganda*, Burundi and Rwanda each produce 5-6%; Cameroon* and Zaïre* each 2% and the remaining 3% of African arabicas is produced in Madagascar*, Malawi, Zimbabwe and Zambia. Most of the coffee is exported, except in Ethiopia where 50-60% of total production is consumed domestically. Coffee exports often constitute an important and sometimes

(e.g. Ethiopia, Uganda) the major source of foreign exchange earnings. Countries with an asterisk (*) are also important producers and exporters of Robusta coffee.

In the international coffee trade few types can match the quality, and with that the top prices, of well produced Kenyan and Tanzanian arabicas. Only 15% of the coffees exported from Ethiopia are washed and sometimes of excellent quality (e.g. Limu, Sidamo), but some unwashed Ethiopian arabicas (e.g. Harar, Gimbi) have distinctive (moka) flavours which also fetch premium prices in certain niche markets. Arabica coffee from other African countries resort mostly under the "milds" (washed) class, obtaining average arabica prices on the world coffee market.

Fig.1: Arabica coffee production in Africa



Note: total production in Africa for the 1995/96 season: 464,000 t (= 16 % of world arabicas)

CBD and its economic importance

CBD can be a devastating anthracnose of developing berries in *Coffea arabica*. It only occurs in Africa and is particularly serious at high altitudes, where CLR is usually less important and easily controlled by a few fungicide sprays. CBD may cause crop losses of 50-80% in years favourable to a severe disease epidemic (prolonged wet and cool weather). Control by frequent fungicide sprays is expensive (30-40% of total production costs), not always effective and usually beyond the means of the smallholder coffee growers.

CBD is caused by the fungus *Colletotrichum coffeanum*, renamed *C.kahawae* (Waller et al, 1994). It is a typical "new-encounter" disease, which probably spread from wild *Coffea eugenioides* populations in mountain forests in western Kenya to nearby arabica coffee plantations established

after 1910 (Robinson, 1976). During the next 50 years CBD found its way to all important arabica coffee producing countries in Africa, most likely through free movement of coffee plant material, and eventually it also reached the semi-natural arabica coffee populations in Ethiopia around 1971.

Although not equally disastrous in all countries - severe in Ethiopia, Kenya, Tanzania, Uganda and Cameroon, but apparently a minor problem in Rwanda and Burundi - CBD alone may be responsible for the destruction of at least one-third of the total arabica coffee crop in Africa. With present high world market prices, that would mean an annual loss of some $ 500-700 million in potential revenue to nations and several hundred thousands of coffee farmers.

An appraisal of selection and breeding programmes aimed at CBD resistance

Kenya

The breeding programme, which was initiated in 1971 at the Coffee Research Station (CRS) near Ruiru as a major project, could expand from previous selection and crossing (1966) work within local and exotic arabica coffee germplasm. A strategy was chosen of introgressing (by recombination- and back-crosses) the CBD resistance, earlier identified in certain arabica genotypes, into local cultivars while maintaining the productivity and high quality of the typical Kenyan coffees. Other characteristics like resistance to CLR and compact growth habit were also targetted in this breeding programme (Agwanda & Owuor, 1989).

It was realised that the development of a reliable screening test would be essential to achieving satisfactory selection progress in CBD resistance, since large variation in field infection caused by macro- and micro-climatic factors severely obscured genetic variation in host resistance. After disappointing results with the detached berry test (as routinely applied by coffee pathologists), an inoculation test on the hypocotyl stems of 6-week-old seedlings under strictly standardised conditions of inoculum type and concentration, as well as temperature and humidity during infection and incubation periods, proved to be a very accurate, fast and reproducible screening test with results closely correlated to mature plant resistance (Cook, 1973; Van der Vossen et al., 1976). Histological studies indicated that the resistance mechanism is to a large extent based on the effective formation of cork barriers preventing the pathogen from further invading host tissue. It was also observed that this mechanism was identical in berries and hypocotyl stems (Masaba & Van der Vossen, 1982).

In addition to its basic function of effectively screening large numbers of segregating breeding populations for CBD resistant genotypes, this preselection test offered also the opportunity for detailed genetic studies. Host resistance to CBD appears to be controlled by major genes on three loci, identified in different host genotypes (e.g. trees of Rume Sudan; Hibrido de Timor clone 1343; cv. K7) with respectively dominant, co-dominant and recessive gene effects (Van der Vossen & Walyaro, 1980). This genetic information has been applied in the Kenyan breeding programme to effect accumulation (pyramidisation) of all three genes in F1 hybrid cultivars and so enhance the level and possibly also the durability of resistance. Recent DNA molecular work by Agwanda (1997) points to the possibility of applying MAS (marker assisted selection) for early identification of such resistance genes and so accelerating gene pyramiding in new CBD resistant cultivars. F1 hybrid varieties would also exploit maximally the hybrid vigour for increased yield and yield stability found in crosses between genetically divergent genotypes of arabica coffee (Walyaro, 1983).

The first seed lots of the new F1 hybrid cultivar, called Ruiru II, were released by the CRS to coffee growers in 1986. In the meantime, almost 10,000 ha have been planted in Kenya with the CBD resistant hybrid cultivar and so far there have been no incidences of differential pathogenicity (break-down of resistance) in the field (D.M.Masaba & C.O.Agwanda, CRS Kenya, personal comm., 1996).

The Ruiru II variety is early and high yielding, also resistant to CLR and has the distinct compact growth habit comparable to Catimor. Claims by certain sections of the coffee trade, that the liquor

quality of Ruiru II would be inferior to typical Kenyan arabicas (e.g. cv. SL28), could never be confirmed in impartial liquoring tests (Njoroge et al., 1990; C.O.Agwanda, CRS Kenya, pers.comm., 1997). Altitude, agronomic practices and post-harvest handling may have been a larger cause of variation in liquor quality of coffee samples than intrinsic (genetically determined) differences.

Farmers' acceptance of the new disease resistant cultivar is high, but so far F1 hybrid seed production, although not difficult from a technical point of view, has remained below original targets, mainly attributed to logistic problems. The annual demand for seed is estimated at 12-15 million, while the CRS has been able to produce maximally 2-3 million seeds per year. One way of improving the seed supply could be decentralisation and privatisation of seed production gardens, provided strict inspection on quality standards for all operations of seed multiplication can be assured. Conventional methods of vegetative propagation (cuttings, grafting) may only aggravate the logistic problems of mass propagation and distribution, while advanced methods of micro-propagation (tissue culture; embryogenesis) are not yet sufficiently tested for large-scale application in arabica coffee.

Ethiopia

Prior to the first serious outbreaks of CBD in 1971, genetic research by the agricultural research station (now National Coffee Research Centre) near Jimma focused mostly on assembling a national coffee germplasm collection, which would be representative of the vast genetic resources of *Coffea arabica* found in the highland forests of south-west Ethiopia. It was basically a continuation of earlier international efforts (FAO 1964/65 Mission: Meyer et al.,1968; ORSTOM 1966 Mission: Guillaumet & Hallé, 1967) to collect and preserve this unique source of genetic diversity on a broad basis, before it would be permanently lost due to progressive deforestation and intensified production of coffee and food crops.

The threat of CBD to the coffee industry of Ethiopia made the plant pathologists and breeders at Jimma concentrate all their efforts in subsequent years on developing CBD resistant varieties. Prompted by the earlier observed genetic variation and the fact that arabica coffee is a self-pollinated tree species, they chose a strategy of mass screening within the indigenous coffee populations for CBD resistant parent trees, followed by one or two generations of line selection. In the years 1971-75 several thousands of trees - in existing germplasm collections and on farmers' coffee plots in areas with severe CBD incidence - were screened for CBD resistance by field observations, as well as by artificial inoculation tests of berries (detached and attached) and young seedlings (Robinson, 1974; Van der Graaff, 1981). Typical of a "new encounter" disease, only a very low ratio (0.5%) of highly CBD resistant trees was found within these natural coffee populations, but due to the large size of the operation more than 150 resistant trees could be selected for further screening in multi-locational progeny trials.

By 1978 some 15 lines, later to be reduced to 5, were finally selected and released as new CBD resistant varieties. However, these cultivars proved to be of very limited value to the coffee industry, despite their CBD resistance, because of unsatisfactory agronomic traits, such as low yield potential, inferior bean and liquor quality and poor adaptation to major coffee production areas outside the highland forests (Anon., 1986, 1989, 1995). Ameha (1983) reported considerable hybrid vigour for yield (25-120% increase over midparent values) and yield stability in F1 crosses between trees of different Ethiopian subpopulations and proposed to exploit this in a programme of hybrid seed production of cv. *Ababuna* and other CBD resistant F1 hybrids (Ameha, 1990).

In contrast to results obtained at the CRS in Kenya, the pathologists and breeders at Jimma assumed polygenic and recessive inheritance of CBD resistance in their material (Van der Graaff, 1981, 1992; Ameha & Bayetta, 1982). However, they based their observations mainly on field scores and on the (for genetic studies) unreliable detached berry inoculation test. In any case, their assumptions appear to contradict with the high level of resistance found in the F1 hybrid cv. *Ababuna*, which is a cross between resistant (accession 741) and susceptible (accession F59) parent genotypes. In that case CBD resistance must be controlled by dominant major genes in the

resistant parent. The CBD resistance present in Ethiopian coffee germplasm (ex FAO mission 1964 and ORSTOM mission, 1967) planted at the CRS in Kenya was also clearly controlled by one or at the most two major genes (Van der Vossen & Walyaro, 1979). Claims by Van der Graaff (1982) of statistical errors invalidating the analyses of the Kenyan CBD inheritance studies were refuted by Dancer (1986).

Tanzania

Arabica coffee production, which is mostly concentrated in the northern highlands, declined from 50,00 t in 1971 to less than 28,000 t per year in 1995/96, partly due to increased disease problems but especially also because of the disappearance of coffee estates (20% of the coffee land) which used to produce half the total crop. On the other hand, the production of robusta coffee, concentrated in the area west of Lake Victoria, increased from 5,000 t to 15,000 t per year over the same period.

The coffee research station at Lyamungu, established in 1934 on the southern slopes of Mt. Kilimanjaro, played a leading role in germplasm collection, selection and breeding of arabica coffee in East Africa until late 1960's. The first crosses were made in 1952/53 (the "Lyamungu hybrids") and earlier breeding objectives were mainly CLR resistance in combination with vigour and yield, while preserving the high quality of the Tanzanian coffee cultivars like N39 (Fernie, 1970).

After the first serious outbreak of CBD on Mt.Kilimanjaro in 1966, breeding efforts were redirected to include CBD resistance in crossing programmes with selections of Hibrido de Timor and Rume Sudan as progenitors of resistance. However, due to a change in research priorities - the station became a general agricultural research and training centre in 1969 - resources of manpower and funds became too restricted for a proper continuation of the coffee breeding programme. The programme is still at an early stage of recombination crosses without the necessary follow-up of back-crossing to improve on bean and liquor quality in particular (A.B.Eskes, CIRAD-CP, pers.comm., 1997). The planned release of CBD (and CLR) resistant clonal cultivars - after final selection based on multi-locational trials - may be premature and a disservice to farmers and the coffee industry, because such cultivars would not meet the minimum standards of quality for Tanzanian arabicas. . Presently, coffee breeding in Tanzania appears to be receiving the required support, partly also from the European Development Fund, for more satisfactory results in the longer term.

Uganda

Just 12% of the 200,000 t coffee produced in 1995/96 was arabica coffee, mainly produced by smallholder farmers on the slopes of Mt.Elgon in east Uganda. However, production of arabica coffee increased from 9,000 t in 1971/72 to the present 24,000 t per year, almost similar in quantity to the present Tanzanian crop. The main cultivars planted are typicas including Bugishu local, a typica similar to the Mibirizi variety of Burundi and Rwanda (Krug & De Poerck, 1968). Although marketed as washed coffee, the quality is generally inferior to the Kenyan and Tanzanian bourbon type coffees.

A collection of arabica varieties was planted at the Kawanda research station (north of Kampala) in 1935/38 and the first series of single crosses ("Pritchard hybrids") were made in 1954/55 with CLR resistance as main objective. All hybridization work since 1968 has been aimed at combining CBD and CLR resistance with yield and good liquor quality, including Hibrido de Timor and Rume Sudan as progenitors for CBD resistance (Millot, 1968-70; Kibirige-Sebunya, 1973). This programme was revived in 1984 during the Coffee Rehabilitation Programme, applying methods and strategies similar to the programme in Kenya, with breeding and selection activities concentrating on the high-altitude substation Buginyanya in the arabica coffee region on Mt. Elgon (Anon., 1984/85). Information on progress made to-date is not available, but no new cultivars with CBD resistance appear to have been released so far.

Cameroon

Production in 1995/96 consisted of 60,000 t robusta and 10,000 t arabica coffee. Arabica coffee is produced by smallholders in the highlands of the western provinces, usually under shade and average yields are only 150 kg/ha. The main variety is *Jamaïque*, a typica resembling Blue Mountain but highly susceptible to CBD. General neglect and heavy crop losses due to CBD, especially above 1,500 m altitude, have caused a decline in production over the past decade (20,000 t in 1987).

A detailed review of past selection and breeding work in arabica coffee by the institute of agricultural research (IRA) is given by Bouharmont (1995). Variety collections and trials with a total of almost 300 varieties/cultivars and accessions, including Ethiopian germplasm collected in 1966 by the ORSTOM mission, were established and recorded over several years on the Foumbot (1,100 m) and Santa (1,800 m) field stations. The variety *Java*, which is similar to the variety *Abyssinia* introduced onto Java in 1928 from Ethiopia (Cramer, 1957), was selected for its general vigour and tolerance to water stress, productivity, high resistance to CBD and also some CLR tolerance. Java was officially recommended as new cultivar in 1980 to replace the susceptible arabica coffee in Cameroon.

The Ethiopian collection contained several CBD resistant genotypes, but in general this material proved to be very intolerant to water stress. The Catimor lines from Costa Rica turned out to be highly susceptible to CBD, but very resistant to CLR. This is material derived from crosses between Caturra and Hibrido de Timor clone CIFC 832/1, which does not carry the CBD resistance gene as does the clone HdT CIFC 1343/269. The latter clone was used in the Catimor lines developed in Colombia, many of which were CBD resistant in Kenya (Van der Vossen & Walyaro, 1981).

Early data from a programme of some 180 single crosses made after 1984 revealed considerable hybrid vigour for yield, particularly in crosses of the cv. Java with ET 59 and some other Ethiopian accessions. Further breeding and selection will be required to improve bean and liquor quality. The longish bean shape of cv. Java may indicate defects in liquor quality, but there are no references to liquoring reports.

Conclusions

A summary of the appraisal of the five above described breeding programmes for arabica coffee, by applying three different sets of criteria for quality, is presented in Table 1.

The Kenyan programme appears to have scored remarkably well in the first two sets of quality criteria, as regards breeding strategies/methods and the various characteristics of the new cultivar. However, in regard to transfer of technology the programme has met some problems, more in particular the organisational and logistic aspects of F1 hybrid seed production. For that reason the multiplication of the disease resistant cultivar Ruiru II has remained below original targets, notwithstanding the high level of farmers' acceptance.

In most other arabica coffee breeding programmes the breeding strategies and methods followed have been less effective as well, with the result that new cultivars resistant to CBD and CLR are either not yet available (Tanzania, Uganda), or are not well adapted to local growing conditions and do not meet the required standards of bean and liquor quality (Ethiopia). The presently recommended cv. Java in Cameroon is not the result of a breeding programme as such and there may be some defects in bean and liquor quality.

There may have been many reasons for these unsatisfactory results, such as interruption of the programme due to the politico-economic situation of the country, lack of funds and infrastructure, frequent change of research staff, but sometimes also conflicting recommendations by external consultants on principles of breeding strategies and genetic interpretation of the inhertitance of disease (CBD in particular) resistance.

Table 1: **Summary of an appraisal of the quality of five arabica breeding programmes in Africa**

Criteria of quality	Kenya	Ethiopia	Tanzania	Uganda	Cameroon
1. Breeding strategies & methods	**5**	**3**	**2**	**2**	**2**
genetic resources	+	+	+	+	+
disease resistance screening	+				
selection	+	+			+
breeding	+	+	+	+	
genetic studies	+				
2. Quality of new cultivars	**5**	**1**			**3**
CBD resistance	+	+			+
CLR resistance	+				
Yield	+				+
bean & liquor quality	+				
local adaptation	+				+
3. Transfer of technology	**3**	**2**			**2**
methods of multiplication	+	+			+
organisation & logistics of multipl.					
on-farm testing		+			+
promotion & extension	+				
farmers' acceptance	+				

Note: sum of +'s (= adequate activity/results) gives score: 0 (low) - 5 (high)

Recommendations

1. The five countries together produce only 16% of the world arabica coffee and because of the high domestic consumption in Ethiopia the share in the world (arabica) coffee market is only about 10%, far too small a quantity to be of major influence on price fluctuations. In other words, there is no real justification for protectionistic attitudes of some countries on their arabica coffee research efforts and outputs. There would be much more to gain from close collaboration in coffee research.

2. Conservation of the *Coffea arabica* germplasm present in the Ethiopian forest coffees is essential for present and future breeding purposes. However, the recent experience from Ethiopia and other countries has shown that most of these coffee types are unsuitable for direct use as cultivars for more intensive coffee production. The whole arabica gene pool is definitely of Ethiopian origin, but some coffees have evolved gradually elsewhere, through many centuries of natural and man-made selection, to the more drought tolerant and productive cultivars (*typicas* and *bourbons*) often grown without shade in other countries. Such cultivars and also other varieties or advanced breeding lines are, therefore, less exotic to Ethiopia than has been suggested sometimes. Basically, there are no sound scientific reasons for restricting exchange of all arabica germplasm between breeders in different countries.

3. Recent advances in conventional and molecular genetics offer considerable opportunities also for arabica coffee breeders to increase selection efficiency and so develop disease resistant cultivars

which should improve the socio-economic situation of millions of smallholder coffee growers and secure also a stable position in the top-quality segment of the world coffee market for their countries.

4. Work in isolation is usually detrimental to research output and may have been one of the underlying causes of slow progress in some arabica coffee breeding programmes. Just as in other crops, such programmes would benefit greatly from effective networking between arabica coffee breeders at regional and international levels. Examples in other crops are: rice - IRRI in Asia; wheat - CYMMIT in Asia and Africa; potato - CIP in Asia and Africa.

5. The EU supported collaborative project - between CRS Kenya, IRA Cameroon, CIFC Portugal and CIRAD-CP France, on CBD resistance in arabica coffee and started in 1995 - is in that respect an excellent initiative, but should have naturally included also Ethiopia, Tanzania and Uganda.

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Summary

An appraisal has been made of the results of arabica coffee breeding programmes carried out in five African countries - Kenya, Ethiopia, Tanzania, Uganda and Cameroon - with resistance to coffee berry disease as main objective, by looking at : (1) quality of breeding strategies and methodologies, (2) quality of the new disease resistant cultivars and (3) quality of technology transfer. The Kenyan programme appears to have scored remarkably well in the first two points, but the multiplication of the new disease resistant cultivar Ruiru II has remained below original targets notwithstanding the high level of farmers' acceptance. However, in other arabica coffee breeding programmes above mentioned quality aspects appear to have been realized in a less satisfactory manner. New cultivars resistant to CBD and CLR are either not yet available, or are not well adapted to local growing conditions, or do not meet the required standards of bean and liquor quality. Recent advances in conventional and molecular genetics offer considerable opportunities also for arabica coffee breeders to increase selection efficiency and so develop disease resistant cultivars which should improve the socio-economic situation of millions of smallholder coffee growers and secure also a stable position in the top-quality segment of the world coffee market for their countries. Work in isolation is usually detrimental to research output and may have been one of the underlying causes of slow progress in some arabica coffee breeding programmes. Just as in other crops, such programmes would benefit greatly from effective networking between arabica coffee breeders at regional and international levels.

INTRODUCTION DE GÈNES D'INTÉRÊT AGRONOMIQUE DANS L'ESPÈCE *COFFEA CANEPHORA* PIERRE PAR TRANSFORMATION AVEC *AGROBACTERIUM SP.*

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INTRODUCTION

Le cycle biologique du caféier robusta *Coffea canephora* Pierre, plante pérenne allogame, est de cinq ans, et les programmes d'amélioration classique se déroulent sur plusieurs dizaines d'années. L'utilisation de la transformation génétique, pour l'introduction de gènes d'intérêt, constituerait donc un progrès important pour l'amélioration de cette espèce.

L'embryogenèse du caféier est bien maîtrisée pour la production en masse d'embryons somatiques (Yasuda *et al.*, 1985; Zamarripa *et al.*, 1991; Berthouly et Michaux-Ferrière, 1996), qui constituent des explants primaires intéressants pour la transformation génétique. La régénération d'embryons secondaires sur ces explants primaires est relativement facile et rapide.

Pour la plupart des grandes cultures, les insectes constituent une des causes majeures de perte de productivité. L'utilisation des gènes de *Bacillus thuringiensis* pour l'obtention de plantes transgéniques résistantes aux insectes est maintenant classiquement employée sur de nombreuses espèces végétales (pour revue, voir Estruch *et al.*, 1997).

Parmi les ravageurs du caféier, la chenille mineuse des feuilles *Perileucoptera coffeela* Guérin Méneville cause des dégâts importants de défoliation et de chute de rendement, en particulier au Brésil (Guerreiro *et al.*, 1990). Une étude récente menée dans les laboratoires du CIRAD a permis de mettre en évidence l'action d'une endotoxine de *B.thuringiensis* codée par le gène *cryIA(c)* contre cette chenille (Guerreiro *et al.*, 1993).

A ce jour, les seules expériences de transformation du caféier par *Agrobacterium* ayant conduit à la régénération de plantules transgéniques ont été menées au Centre de Recherches Nestlé -Tours (Spiral et Pétiard, 1993; Spiral *et al.*, 1993) avec *A. Rhizogenes*. D'autres travaux ont été menés sur la transformation du caféier par *A.rhizogenes* (Sugiyama *et al.*, 1995), par *A.tumefaciens* (Feng *et al.*, 1992; Freire *et al.*, 1994), ou par électroporation de protoplastes (Barton *et al.*, 1991), mais sans régénération de plantes transformées.

Sur la base des résultats obtenus, une coopération entre le CIRAD et le Centre de Recherches Nestlé-Tours a été initiée en 1994 pour créer des caféiers transgéniques résistants à la mineuse des feuilles. La présente communication décrit l'obtention de plantes transgéniques de caféier *C.canephora* contenant le gène d'intérêt agronomique *cryIA(c)*, en particulier grâce à *A.tumefaciens* désarmé.

MATÉRIEL ET MÉTHODES

Matériel végétal

Le génotype de *C.canephora* 126 a été choisi pour les premiers travaux en raison de sa bonne valeur agronomique et de la facilité d'obtention d'embryons somatiques *in vitro* pour ce génotype par culture d'explants foliaires sur un unique milieu solide (gelrite à 3 g/litre), contenant 5µM de BAP et 0,09 M de saccharose (Yasuda *et al.*, 1985). Les repiquages sont effectués toutes les cinq semaines pendant une période de trois à cinq mois, jusqu'à l'apparition des embryons somatiques à la périphérie de l'explant.

Matériel bactérien

Les souches d'*Agrobacterium* utilisées sont *Agrobacterium rhizogenes* A4 et la souche désarmée *d'A.tumefaciens* LBA4404. Les vecteurs binaires utilisés dérivent du vecteur pBIN 19 (Bevan, 1984) qui a été modifié de façon à éliminer le gène de résistance à la kanamycine et à optimiser l'efficacité du transfert du gène d'intérêt (Royer *et al.*, soumis). L'ADN-T qui correspond à la séquence d'ADN transféré par l'agrobactérie dans le génome de la plante comprend de la bordure droite (RB) à la bordure gauche (LB) un gène codant pour l'endotoxine de *B.thuringiensis (B.t.)* CryIA(c), un gène de sélection et un gène rapporteur. Le gène codant pour CryIA(c) est soit le gène natif *cryIA(c)* de *B.thuringiensis* (Adang *et al.*, 1985) ou soit un gène *cryIA(c)* modifié synthétisé à l'université d'Ottawa (Sardana *et al.*, 1996). Ce gène est sous le contrôle du promoteur EF1α (Curie *et al.*, 1991), qui s'est avéré fonctionnel chez le caféier en expression transitoire (Van Boxtel *et al.*, 1994). Le gène de sélection est le gène *csr1-1* (Brasileiro *et al.*, 1992) conférant la résistance à l'herbicide chlorsulfuron (Dupont). Ce gène est utilisé pour la sélection des cellules transformées. Il est placé sous le contrôle du promoteur p70S qui correspond à une forme modifiée du promoteur du gène 35S de la mosaïque du chou-fleur (CaMV) dans laquelle la séquence "enhancer" a été dupliquée. Le gène rapporteur utilisé est le gène gus-intron (Vancanneyt *et al.*, 1990), qui permet l'expression de la β-glucuronidase (GUS) exclusivement dans les tissus transformés et non dans l'agrobactérie. Ce gène est sous le contrôle du promoteur 35S du CaMV. Les différentes constructions ont été introduites dans les deux souches d'*Agrobacterium* par conjugaison triparentale en utilisant une souche de *E. Coli.* comprenant le plasmide "helper" pRK2013.

Protocole de transformation

Les embryons somatiques ont été prélevés sur les explants foliaires au stade torpille, puis ont été blessés au scalpel. Ils sont ensuite trempés dans un milieu liquide correspondant à celui utilisé pour la culture des explants primaires mais dépourvu de BAP, en présence de la bactérie. Les bactéries sont, quant à elles, cultivées pendant 16 à 20 heures pour atteindre une densité optique proche de 0,5 à 600 nm. Elles sont ensuite centrifugées et reprises dans le milieu de trempage. Celui-ci dure 20 à 30 minutes, les embryons somatiques sont ensuite cultivés à l'obscurité à 26°C sur le même milieu que les explants foliaires.

Après 2 ou 3 jours de coculture, les embryons sont rincés dans le milieu de culture liquide supplémenté en céfotaxime (Claforan, Roussel) à la dose de 1 g/litre pendant environ 2 heures. Cela permet de détruire les bactéries présentes sur les explants. Les embryons sont ensuite transférés sur le même milieu solide contenant 400 mg/litre de céfotaxime, et cultivés à l'obscurité. Après un délai de 21 à 28 jours, les explants sont transférés en conditions de luminosité faible (16 heures sur 24) et transférés sur un milieu sélectif contenant, en plus de la céfotaxime à 400 mg/litre, du chlorsulfuron (80 µ g/litre).

Test histochimique GUS

Le protocole de Jefferson et Wilson (1991) a été légèrement modifié par l'utilisation d'un tampon phosphate à pH 7 (0,2 M NaH_2PO_4, 2 H_2O et 0,2M Na_2HPO_4, 12 H_2O). Les sels K_3FeCN_6 et K_4FeCN_6 sont utilisés à 0,5 mM pour augmenter l'intensité de la réaction. Le méthanol (20% v/v de la solution finale) est utilisé pour réduire d'éventuelles expressions endogènes du gène GUS (Kosugi *et al.*, 1990). La lecture du test se fait après son incubation à 37°C pendant une nuit.

Analyses moléculaires

Pour les réactions de PCR ("Polymerase Chain Reaction"), l'ADN des échantillons est extrait à partir de

quelques mg de feuilles fraîches selon la méthode de Edwards *et al.* (1991) modifiée par l'addition de bisulfite de sodium 100 mM au tampon d'extraction. Afin d'éliminer les ARN et les protéines dans l'ADN extrait, on réalise ensuite une RNase et un phénol-chloroforme.

L'analyse de l'intégration des gènes dans le génome des caféiers a été réalisée par hybridation moléculaire après l'extraction de l'ADN d'environ 1 gramme de feuilles, et selon le protocole développé par Invitrogen (Easy DNA Kit). La digestion de l'ADN se fait par l'enzyme de restriction *SspI*. Après séparation des fragments de restriction sur un gel d'agarose, l'ADN est transféré sur une membrane nylon (Appligene), puis hybridé avec une sonde radioactive du gène *uidA*.

RÉSULTATS

A ce jour, plus de 30 expériences différentes de transformation d'embryons somatiques ont été menées. Quelques milliers d'embryons ont été cocultivés avec *A.rhizogenes,* et plusieurs dizaines de milliers avec *A.tumefaciens*.

Après la coculture, et le transfert sur le milieu sélectif (chlorsulfuron), les explants sont repiqués toutes les trois à quatre semaines, afin de maintenir une concentration en herbicide suffisante dans le milieu de culture. L'herbicide utilisé est en effet photolabile et thermolabile. Après quelques semaines sur milieu sélectif, les explants primaires se nécrosent fortement.

Six à dix mois après la fin de la coculture, des cals embryogènes apparaissent sur quelques explants. La fréquence d'apparition de ces cals est très faible, moins de 1% des explants présentent un développement. Ces cals sont alors isolés et maintenus sur le même milieu sélectif. Parallèlement, la céfotaxime est supprimée à ce stade. Après quelques semaines, des embryons somatiques se développent sur ces cals. Ils sont alors transférés sur un milieu de germination (macro et microéléments de Murashige et Skoog (1962), vitamines de Morel, BAP 1 µM, saccharose 0,03 M), toujours en présence de l'agent sélectif. Après une période de germination de 3 à 12 semaines, on obtient un embryon développé avec deux cotylédons étalés. Les embryons sont alors transférés sur un milieu d'enracinement identique au précédent mais dépourvu de BAP. Selon ce protocole, on a pu régénérer une soixantaine de plantules à partir de chaque cal embryogène. Ces plantes sont en principe identiques, car elles sont obtenues à partir d'un seul événement de transformation. Douze à dix-huit mois après la coculture, les jeunes plantules sont sevrées en serre.

Le test histochimique GUS a été réalisé sur les cals embryogènes de départ, sur les embryons obtenus, et enfin sur les racines et les feuilles des jeunes plantules. Suivant les expériences, 30 à 80% des cals qui se sont développés sur le milieu sélectif ont montré une coloration bleue caractéristique des tissus transformés. Les taux d'échappements (cals non colorés se développant sur milieu sélectif) sont donc importants. Le test histochimique GUS est suffisamment fiable, puisque, après les analyses moléculaires, il s'est avéré que tous les cals positifs avaient effectivement donné des plantules transformées, alors que les cals non colorés étaient non transformés. Il constitue donc un bon crible pour distinguer les transformants des échappements.

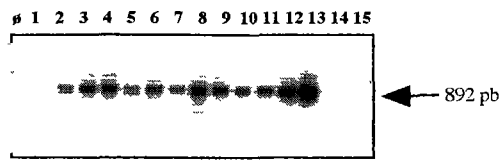
Les tests moléculaires par PCR ont été pratiqués sur de jeunes feuilles des plantules *in vitro*. Les électrophorégrammes présentés sur la Figure 1 montrent les produits d'amplification par PCR du gène GUS et du gène *cryIA(c)* de B.t. (natif ou synthétique) pour quelques plantes transformées. Quelques plantes non transformées et les témoins positifs et négatifs sont également présentés sur ces mêmes électrophorégrammes. Les plantes positives pour le test histochimique ont toutes présenté une amplification par PCR du gène GUS (fragment de 892 pb), du gène de B.t. natif (571 pb) ou du gène de B.t. synthétique (670 pb).

L'intégration effective de l'ADN dans le génome de caféier a été vérifiée par hybridation moléculaire pour quelques plantules de *C.canephora* avec le gène GUS (Figure 2). On observe, pour le transformant, les deux bandes attendues, à 1,0 kb et à 3,4 kb. Cette dernière est caractéristique de l'intégration de l'ADN-T dans le génome du caféier, et ne correspond pas au profil obtenu pour le plasmide bactérien non intégré. Parallèlement, un caféier non transformé ne présente aucune bande d'hybridation avec cette même sonde. L'intégration des autres gènes dans le génome a été montrée de la même façon. Comme attendu, toutes les plantes issues d'un même cal, et donc d'un même événement de transformation, présentent des bandes identiques par hybridation moléculaire.

Le Tableau 1 dresse le bilan des événements de transformation indépendants obtenus pour les deux

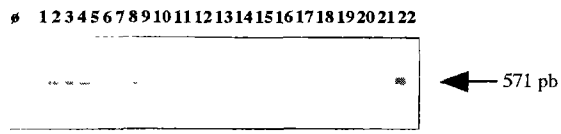
Figure 1: Electrophorégrammes obtenus après la migration des séquences amplifiées par PCR

Amplification du gène rapporteur GUS



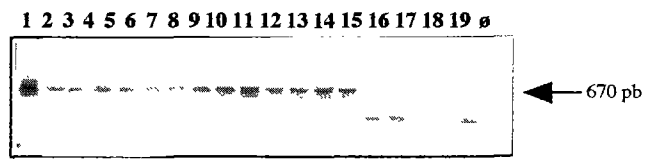
Légende:
ø: Marqueur de poids moléculaire øX174 digéré par *HaeIII*.
Puits 1-12: plantes transformées qui présentent une activité enzymatique GUS
Puits 13: amplification obtenue à partir du plasmide bactérien purifié (témoin positif)
Puits 14-15: ADN de C.canephora non transformé (témoin négatif)

Amplification du gène *B.t* natif



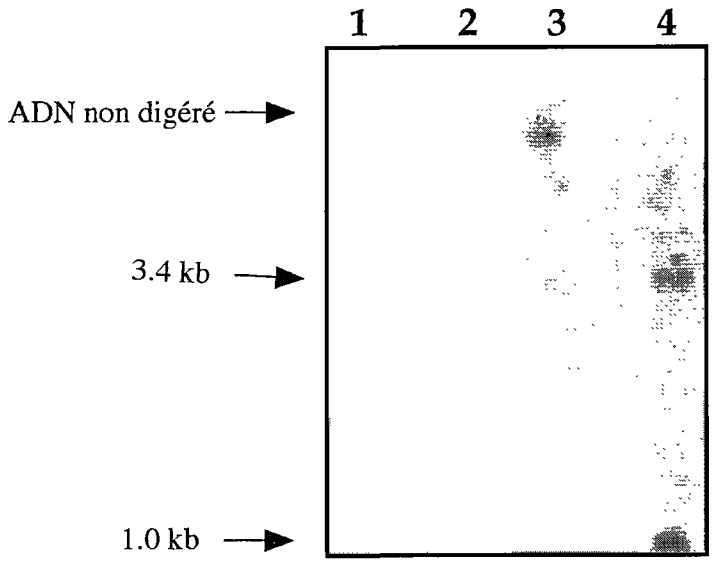
Légende:
ø: Marqueur de poids moléculaire øX174 digéré par *HaeIII*.
Puits 1-3, 6-9, 12-21: plantes transformées
Puits 4, 5, 10 et 11: ADN de C.canephora non transformé (témoin négatif)
Puits 22: amplification obtenue à partir du plasmide bactérien purifié (témoin positif)

Amplification du gène *B.t* synthétique



Légende:
ø: Marqueur de poids moléculaire øX174 digéré par *HaeIII*.
Puits 1: amplification obtenue à partir du plasmide bactérien purifié (témoin positif)
Puits 2-14: plantes transformées
Puits 15 -19: ADN de C.canephora non transformé (témoin négatif)

Figure 2: Autoradiographe obtenu après hybridation moléculaire de l'ADN génomique de différents caféiers avec la sonde GUS



Légende:

Puits 1: ADN de caféier non transformé et non digéré
Puits 2: ADN de caféier non transformé et digéré par l'enzyme de restriction *SspI*.
Puits 3: ADN de caféier transformé et non digéré
Puits 4: ADN de caféier transformé et digéré par l'enzyme de restriction *SspI*.

bactéries avec les gènes de B.t. natifs ou synthétiques. Sur la trentaine d'expériences effectuées, seules trois ont permis la régénération de plantules transgéniques. Ceci montre qu'il faut encore optimiser le processus de transformation d'embryons somatiques de caféier. L'optimisation des différents facteurs contrôlant la transformation est actuellement en cours de réalisation. Les efforts porteront surtout sur la transformation avec *A.tumefaciens* désarmé, parce que les plantes régénérées présentent un phénotype normal. En revanche, les plantes régénérées après transformation avec *A.rhizogenes* armé présentent, pour la plupart, un phénotype modifié ("Hairy root"), et ne sont pas agronomiquement utilisables.

Tableau 1. Bilan des expériences en cours sur la transformation d'embryons somatiques de *Coffea canephora par A.rhizogenes* armé et *A.tumefaciens* désarmé.

Bactérie	Construction	Expérience (nombre d'embryons traités)	Nombre d'événements de transformation indépendants
A.rhizogenes A4	Gène *cryIA(c)* natif	1 (300)	4
	Gène *cryIA(c)* synthétique	2 (500)	3
A.tumefaciens LBA4404	Gène *cryIA(c)* synthétique	3 (5000)	20

CONCLUSIONS

Pour la première fois chez le caféier *C.canephora*, un gène d'intérêt agronomique a été intégré dans le génome par la coculture d'embryons somatiques avec les agrobactéries *A.rhizogenes* et *A.tumefaciens*. Des plantes transgéniques ont été régénérées. L'intégration effective de ce gène a été vérifiée par des test moléculaires (PCR et hybridation moléculaire). Des tests vont maintenant se poursuivre pour détecter la production de la δ endotoxine de *B.thuringiensis* dans les plantes transformées, et pour tester leur résistance à la mineuse des feuilles par des bio-essais avec des insectes.

Les travaux sont également en cours pour transformer l'autre espèce de caféier, *C.arabica*. Un protocole de transformation optimisé devrait conduire dans un futur proche à l'introduction d'autres gènes d'intérêt agronomique ou technologique dans le caféier.

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Abréviations

GUS : β-glucuronidase
PCR : polymerase chain reaction
BAP : benzyl-6-aminopurine
pb : paires de bases

kb : milliers de paires de bases
CaMV : cauliflower mosaic virus (virus de la mosaïque du chou-fleur)
B.t. : *Bacillus thuringiensis*
ADNT : partie de l'ADN bactérien transféré dans la plante par transformation génétique

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RÉSUMÉ

Des plantules de caféier transgéniques ont été obtenues via *Agrobacterium rhizogenes* et *Agrobacterium tumefaciens*. Pour cela, des embryons somatiques de *C.canephora* Pierre ont été utilisés comme explants primaires. Ils ont été infectés avec les souches *A.rhizogenes* A4 et *A.tumefaciens* LBA4404 désarmée. Le plasmide binaire utilisé dérive du vecteur pBIN19, l'ADN-T comprend le gène rapporteur gus-intron, un gène de sélection conférant la résistance à un herbicide (chlorsulfuron) et un gène, natif ou synthétique, codant pour l'endotoxine de *Bacillus thuringiensis* CryIA(c) active contre la mineuse des feuilles du caféier, *Perileucoptera coffeela* Guérin-Méneville.

Une coculture de 2 ou 3 jours et une application différée de l'agent sélectif de 21 à 28 jours ont permis la sélection de tissus transgéniques puis la régénération de plantules. L'intégration de la construction a été vérifiée par PCR et hybridation moléculaire. La résistance à la mineuse des feuilles des plantes transformées va être testée, et les essais seront élargis à l'espèce *C.arabica*. De plus, la méthode de transformation ainsi développée pourrait être étendue à d'autres gènes d'intérêt.

Transgenic coffee plantlets were obtained after coculture with *Agrobacterium rhizogenes* strain A4 and disarmed *Agrobacterium tumefaciens* LBA4404 strain. Somatic embryos of *C.canephora* Pierre were used as primary explants. The binary plasmid used is derived from pBIN19 vector. The T-DNA contains the reporter gene gus with intron PIV2, a selective gene confering resistance against an herbicide (chlorsulfuron), and a native or synthetic gene encoding for a *Bacillus thuringiensis* CryIA(c) endotoxin, active against the coffee leaf miner, *Perileucoptera coffeela* Guérin-Méneville.

Transgenic tissues were selected after coculture during 2 or 3 days, and a delay of 21 to 28 days before applying selective agent. Transgenic plantlets were regenerated through somatic embryogenesis. Stable integration of T-DNA into coffee genome was confirmed by PCR and Southern blotting. Resistance against leaf miner will be studied later, and transformation of *C.arabica* genotypes is on progress. Developed method could allow stable integration in coffee of other genes of agronomical or technological interest.

BREEDING FOR QUALITATIVE TRAITS IN ARABICA COFFEE

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INTRODUCTION

Coffea arabica is the only tetraploid and self-pollinated species in the genus *Coffea*. All other species of *Coffea* are diploid and outcrossed (Carvalho *et al.* 1969; Medina Fo. *et al.* 1984). This genetic isolation has hindered the utilization in Arabica breeding programs of morphological, source of resistance and metabolic variation existing in the genus. A few Arabica improvement programs have relied on spontaneous or artificial doubling of *C. canephora* for synthesizing interspecific hybrids. Artificial Robusta tetraploids were created by colchicine treatment leading to the 'Icatu' and the 'Arabusta' populations; using a spontaneous Arabica x Robusta hybrid (so called Timor Hybrid), the 'Catimor' population was created.

The first interspecific cross leading to the 'Icatu' population was made in Brazil in 1950, using a *C. arabica* cv. Red Bourbon as female and a colchicine-duplicated Robusta as male. Successive backcrosses were made to *C.arabica* cv. Mundo Novo, and S_3 and S_4 plants derived from BC_2 and BC_3 crosses were selected for future planting (Carvalho 1988). Similar crosses were also made in Brazil by A. Carvalho with *C. arabica* cv. Mundo Novo with duplicated *C. racemosa* and S_2 and S_3 plants were selected for yield and disease resistance (unpublished data).

Somaclonal Variation is an *in vitro* technique that explores the naturally occurring (or in vitro-induced) variability of somatic cells following plant regeneration (Larkin & Scowcroft 1981). Most of this variability is due to chromosome alterations, e.g. breakage, translocations, deletions, aneuploidy, polyploidy, gene amplification, transposons, somatic crossing-over and point mutations (Evans & Sharp 1983). Somaclonal Variation is an excellent method for shortening the lime-lines of breeding programs of perennial species. It provide access to limited variability already existing in the genus. Somaclones carry few genetic alterations, so that the genetic integrity of the commercial variety is preserved (Evans & Sharp 1986; Sondahl & Bragin 1991).

With the availability of other breeding methods and a strong market demand for quality coffee, attention has being directed to breed for new varieties with enhanced aroma and taste. Green coffee quality is determined by Genetics, Environment and Processing. This paper reports progress on Arabica improvement efforts using a combination of Somaclonal Variation and standard breeding techniques with the primary aim to develop new varieties with stable genetic traits for cup quality.

MATERIAL AND METHODS

Plant material and growing conditions

Leaf segments of S_5 'Icatu' and S_3 Arabica x Racemosa hybrids were used to produce somatic embryos and plantlets (Sondahl & Sharp 1977; Sondahl & Bragin 1991). Due to their somatic origin, the resulting plants were called "somaclones". A total of 2,244 'Icatu' and 744 Ara./Rac. somaclones were raised to maturity under field conditions, at 3.5 x 2.0 m spacing, one plant per hill, at 21° latitude South and 1040 m altitude. Control plants of the donor genotypes were also established in the same Experimental Area for comparisons. Single plant selections of S_0 somaclones begun at the time of first flowering. From individual somaclones, ripe cherries were harvest and parchment coffee prepared for sensory and chemical evaluation. Seeds of the selected plants were used to establish S_1 and S_2 generations. The progeny of each selected S_0 somaclone were carried forward as a breeding line. The 2nd Experimental Area was established at 4.0 x 1.0 m spacing, one plant/hill, at 19° latitude South at 1,200 m altitude. In the 3rd Experimental Field (19° latitude South, 1,200m altitude), 'Icatu' somaclones were planted at 4.0 x 1.0m with two plants/hill and the Ara/Rac. lines were planted at 3.5 x 1.5 m with two plants/hill. The most advanced lines derived from 'Icatu' donor plants are being called the 'Naomi' population. The most advanced lines derived from the Ara/Rac. donor plants are being called the 'Aramosa' population.

Sensorial and Chemical analyses

After removal of the parchment, coffee beans from each S_0 somaclone were submitted to controlled roasting and coffee cups were prepared for sensory evaluation according with the standard procedures described by Petracco (1997). Green coffee samples were submitted to chemical analysis according with the standard procedures for each compound (Illy & Viani 1995).

Agronomic evaluations

The somaclones and their progenies were evaluated under normal coffee field conditions at the S_0, S_1 and S_2 generations. Any deviation from the phenotype of the original donor material was classified as a "variant". Seeds of the most interesting variant types were used to study the subsequent generations.

(a) *Resistance to Leaf Rust*

Field observations of leaf rust infection within plants of each breeding line has being made at the peak of the disease incidence; plants were classified as susceptible, partial resistance (horizontal) and totally resistance (vertical).

In addition, rust resistance was also evaluated under controlled laboratory conditions for some Aramosa lines. Leaf discs were excised from the 2nd or 3rd pair of leaves of lateral branches from 3-year old trees. Before cutting the discs, the leaves were washed and dried at room temperature at the laboratory conditions. An average of 2-4 discs of 10 mm diameter per leaf were produced, using ca. 20-30 leaves per plant, to reach a total of 60 discs per Aramosa line. The discs were incubated in 15x15x15 cm transparent plastic boxes (gerbox), using 20 discs/box. Inside each gerbox, the discs were placed on top of a nylon mesh which was supported by a humid sponge to secure at least 90% relative humidity inside the box. Artificial inoculation of rust uredospores (*Hemileia vastatrix*) race II were made with the aid of a very soft brush (camel hairs), using 1 mg of spores in the inferior (abaxial) side of each leaf disc. A mist of water was applied over the uredospores to facilitate germination. The gerboxes were closed and incubated at 22 °C, in darkness during the first 72 h, and a 16 h light/8 h dark thereafter. Evaluation was made 45 days after inoculation, using the following criteria: R= when all 60 leaf disks failed to show rust spores; and S= when at least one disk presented rust spores.

(b) *Resistance to leaf miner*

Plants were evaluated by visual inspection and scored as Resistant (V), Tolerant (H) or Susceptible (S). Only 'Aramosa' plants were scored for this disease using a total of 14,545 plants representing 48 breeding lines.

(c) *Resistance to drought*

Coffea racemosa has being described as an importance source of drought resistance (Medina Fo, 1984) and so, there was interest in evaluating the progenies of the Aramosa somaclones for this characteristic. A total of 14,545 plants representing 48 lines were scored as highly resistant (***), intermediate resistant (**), mild infestation (*) and infested (o).

RESULTS

1. Selection at the S_o level

A total of 2,244 somaclones derived from Icatu donor plants and 744 somaclones derived from Ara/Rac hybrids were established in the 1st Experimental Area (Somaclonal Library). After several rounds of selection, a total of 72 S_o Icatu somaclones were selected based on Sensory Notes (26 plants), Chemical properties (17 plants) and Agronomic traits (29 plants). In the Ara/Rac somaclone group, 47

lines were selected representing desirable Sensory Notes (23 plants), Chemical composition (3 plants) and Agronomic traits (21 plants).

Seeds of selected plants were used to established the 2nd and 3rd Experimental Areas (Progeny Fields).

2. *'Naomi' Population*

The data from the 2nd Experimental Area will not be presented in this paper.

A total of 57 lines of Icatu somaclones were carried to the 3rd Experimental Area based on Sensory, Chemical and Morphological properties. In this somaclone group it was found plants with the highest notes for cup quality: I-21 (3.0) and I-31 (3.0). Interesting that these plants displayed a combination of the sensory notes usually recognized in a typical Natural coffee (mild acidity & body) and notes from a highly aromatic Ethiopian coffee. The progeny of these lines will be evaluated for the persistence and stability of such cup characteristics.

Total oil content was evaluated for somaclones originated from many different plant donors, but the somaclones derived from Icatu donors had the most variability for oil: 18.8% (line I-35) to 11.2% (line I-13). Oil content did not influence the sensory scores and it was neither associated with any morphological character like Robustoid vs. Arabica types (Table 1).

Besides variation for cup quality and chemical parameters, plants of the 'Naiomi' population are also displaying variability for leaf rust resistance and morphology (Arabica vs. Robustoid; Tall vs. Short stature).

Field evaluation was made at the time of first fruit set and two morphological groups were quite visible: the Arabica type (39 lines) and the Robustoid type (18 lines). The Arabica phenotype displayed leaf, branch, flower and fruit morphology similar to Mundo Novo. The Robustoid phenotype has leaf, flower and fruits that resemble the Robusta aspect. Among the Arabica type, 36 lines had plants of tall stature and 3 lines plants of short stature; among the Robustoid type, tall and short stature plants were segregating within the same lines.

Field evaluation for leaf rust resistance has being made for 48 lines so far. Among the Arabica type, 32 lines were scored with a type of Vertical resistance (V), one line with Horizontal resistance (H) and eight lines were susceptible. Three lines had the majority of plants with Horizontal resistance reaction: line I-5 (50%), line I-6 (48%) and I-11 (64%). Line I-10 has shown 62% of the plants with Vertical resistance and the remaining plants are segregating as susceptible and/or Horizontal type of reaction (Table 1). Among the Robustoid type, six lines were found completely free of leaf spores (Resistant, V), two lines with Horizontal resistance and the remaining lines were susceptible. Again, there is no association of the morphological type with resistance or susceptibility to leaf rust.

Yield from the different lines are being evaluated; the projected yield for the 'Naoimi' population is being estimated at a level of 2,700 kg/ha, at a plant density of 2,500 hills/ha. However, yield varies greatly from line to line and among plants of the same line.

TABLE 1. Coffee Breeding Program: NAOMI POPULATION

SOMA. No.	SENSORY GRADE (So level)	CHEMICAL ANALYSIS (So level)	MORPHOLOGY (So level)	SEGREGATION (S1 level)	LEAF RUST RESIST. (S1 level)
1	1.0	12.7% oil			V
2	0.0	13.6% oil			V
3				Robustoid	S
4				Robustoid	S
5		1.0% caffe.			50% H
6				Robustoid	48% H
7	-2.0	13.3% oil	Short stature	Rob.; Tall/Short	98% S
8				Robustoid	84% S
9	-1.0	17.7% oil			V
10					62% V
11				Robustoid	64% H
12					98% V
13		11.2% oil		Robustoid	S
14	0.0			Robustoid	S
15				Robustoid	S
16	-1.0	13.4% oil		Robustoid	S
17	1.0	18.6% oil			V
18	1.0	12.5% oil			V
19					V
20		1.1% caffe.			V
21	3.0				V
22					V
23			Narrow Leaf		V
24	-1.0	18.0% oil			V
25				Robustoid	S
26				Robustoid	V
27					V
28					V
29					V
30			open branch.		V
31	3.0				V
32					V
33	1.0		Robustoid	Robustoid	V
34		17.8% oil			H
35	-1.0	18.8% oil		Robustoid	V
36	1.0	13.2% oil	Short stature		V
37	2.0			Robustoid	V

TABLE 1. Continue

SOMA. No.	SENSORY GRADE (So level)	CHEMICAL ANALYSIS (So level)	MORPHOLOGY (So level)	SEGREGATION (S1 level)	LEAF RUST RESIST. (S1 level)
38	1.0				V
39	1.0				V
40	-1.0				V
41	1.0	12.8% oil			
42	1.0		Short stature		
43	1.0		Robustoid		V
44	1.0		Robustoid		V
45	1.0		Short stature		V
46	-1.0		Short stature		S
47	2.0				V
48	1.0				V
49					V
50	1.0				
51	2.0				
52	1.0		Short stature		
53					
54			Short stature		
55	-1.0				
56	-1.0		LA type		
57					
58					
59	1.0				
60			Semi-erect		
61					
62			Erect		
63			Short stature		
64			Short stature		
65					
66					

3. 'Aramosa' Population

The highest sensory grade for cup quality in the 'Aramosa' population was found in the A-41 line (2.5). Many lines received cup scores of Merit 2.0, for exemple: A-23, A-25, A-26, A-33, A-36 and A-38 (Table 2). The remaining of the lines selected had sensory grades between 1.0 and 1.5.

'Aramosa' plants revealed to have a 30% reduced caffeine level from Arabica plants: average 0.85% vs. 1.2% (Table 2). While not exhaustive, many

caffeine analyses were made from 'Aramosa' samples and their values were always between 0.8 and 0.9%.

Among a total of 49 lines established in the 3rd Experimental Area, some progenies were uniform for stature (either Tall or Short), but the majority of the 'Aramosa' lines are segregating for stature within the same line. Among 2,147 plants evaluated so far, 66.1% of the plants were classified as Tall stature and 32.9% were classified as Short stature. Many Aramosa plants have a unique leaf morphology: flat, dark-green, leather-type texture. The majority of these plants are being observed to be resistant to both leaf miner and leaf rust attack.

Initial data for leaf rust resistance evaluated under controlled laboratory conditions are quite promising. From a total of 68 plants analyzed so far, 40% did not show a single disk with spores out of 60 disks inoculated and 22% had infection in less than 20% of the disks. The best results were found with line A-3. When leaf samples were randomly taken from eight plants of A-3 line, four plants revealed to be resistant to leaf rust race II (Table 2).

Initial evaluation were also made for Leaf Miner resistance. Among 2,147 plants evaluated, 3.9% were found totally resistant to leaf miner. The frequency of leaf miner resistance vary from line to line. The lines that revealed 20-21% of plants with leaf miner resistant reaction include the following: A-2, A-3, A-18, A-29, A-33, A-40 and A-43 (Table 2). Seeds of selected plants were taken for future screening under artificial inoculation and controlled conditions.

Resistance to drought was evaluated during the occurrence of an extended dry period last year (May-October/96). Plants were scored during the week of September 03/96 and 5.3% out of 14,545 plants examined were classified as drought-resistant plants. With the exception for three lines, all other lines had plants with drought resistant reaction. The lines with greater number of resistant plants include A-5, A-9, A-15, A-36, A-47 (Table 2).

In addition, these positive properties, the majority of the 'Aramosa' lines are very early maturing. While plants of 'Catuai' take ca. 220 days from flower to ripe cherries, 'Aramosa' plants ripe in about 180 days. In *C. racemosa,* it takes only 90 days from flowers to mature cherries and so, the short ripening period being observed in 'Aramosa' plants is influenced by the presence of Racemosa genes.

Productivity of the selected 'Aramosa' lines are being evaluated. So far, the projected yield is being estimated as 3,000 kg/ha, at a plant density of 2,600 hills/ha with two plants per hill.

4. Next Steps

The maintenance of superior cup tasting properties must be demonstrated among the progenies of the plants selected for high-sensory grades. In addition, the extend of the environmental influence on the cup quality must be evaluated to clearly demonstrate the genotypic effect of the traits controlling sensory notes.

Among the superior tasting lines, selection for positive Agronomic characteristics should be superimposed including high yield, short stature, disease and pest resistance, uniform ripening and drought resistance.

TABLE 2. Coffee Breeding Program: ARAMOSA POPULATION

SOMA. No.	SENSORY GRADE	CHEMICAL ANALYSIS	MORPHOL.	SEGREGATION	LEAF RUST RESIST.	LEAF MINER RESIST.	DROUGHT RESIST.
	(So level)	(So level)	(So level)	(S1 level)	(S1 level)	(S1 level)	(S1 level)
					(no. pls)	(% pls)	(no. pls)
1					1	8.7	9
2	1.0	0.9% caf.				20.0	12
3					5	21.0	13
4					1	5.5	3
5						4.9	40
6						11.0	8
7						13.5	25
8	1.5	0.8% caf.				12.8	3
9					3	9.0	33
10	1.5	0.9% caf.			2	17.0	20
11	1.5	0.9% caf.				4.9	11
12					2	18.0	7
13						7.6	
14						9.8	11
15	1.5	0.8% caf.			1	7.5	28
16						16.5	5
17						3.0	18
18	0.5	0.9% caf.			1	20.2	11
19						9.9	10
20	1.5	0.8% caf.				12.5	7
21	1.5	0.9% caf.				12.5	9
22	1.0	0.8% caf.				9.0	11
23	2.0	0.9% caf.				18.2	23
24	1.5	0.8% caf.				14.9	13
25	2.0					14.0	22
26	2.0				1	6.3	21
27	1.0	0.9% caf.			1	14.0	14
28						12.0	20
29	1.5	0.9% caf.				18.0	8
30					2	21.0	9
31					1	13.0	
32	1.5				1	6.2	7
33	2.0	0.8% caf.				16.2	21
34						22.6	20
35	1.5				2	15.0	8
36	2.0				1	11.0	38
37						19.0	19

TABLE 2. Continue

SOMA. No.	SENSORY GRADE	CHEMICAL ANALYSIS	MORPHOL.	SEGREGATION	LEAF RUST RESIST.	LEAF MINER RESIST.	DROUGHT RESIST.
	(So level)	(So level)	(So level)	(S1 level)	(S1 level)	(S1 level)	(S1 level)
					(no. pls)	(% pls)	(no. pls)
38	2.0						
39	1.5					7.6	9
40	1.0				1	20.0	14
41	2.5					17.6	9
42	1					7.5	13
43						21.2	9
44						10.0	3
45						18.0	12
46						19.0	
47					3	17.5	12
48	1.5				3	5.0	28
49	1.5	0.8% caf.				5.0	14
50						12.7	24

ABSTRACT

Arabica improvement programs relying on hybridization focused so far on agronomic traits like increased yield and resistance to diseases. Strong market demand for quality coffee has now directed attention to breed for new varieties with enhanced aroma and taste.

This paper reports progress on Arabica breeding, with emphasis on cup quality, obtained by Somaclonal Variation which captures naturally-occurring variability of somatic cells after plant regeneration.

The first population selected, being called 'Naomi', derives from Icatu donor plants. Evaluation of 2,200 somaclones under field conditions yielded 72 lines, some of which selected on sensory notes (26 lines), chemical data (17 lines) and agronomic properties (29 lines). Second and third generation plants are growing under field conditions for additional screening. Variability has been found for sensory attributes (-3 to +3), oil content (11.2% to 18.8%), soluble solids (25.6% to 34.1%), short stature, narrow to large robustoid leaves (and fruits) and horizontal resistance to leaf rust.

The second somaclonal population selected, based on *Arabica x Racemosa* hybrids, is being called 'Aramosa'. This 'Aramosa' population is highly resistant to drought and early maturing (ca. 180 days from flowers to ripe fruits). From 300 original somaclone plants, 47 lines were selected based on sensory evaluation (23 lines), chemical analyses (3 lines) and agronomical properties, like stature and resistance to disease and pest (21 lines).

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L'EMBRYOGENÈSE SOMATIQUE : UN OUTIL POUR L'AMÉLIORATION GÉNÉTIQUE DU CAFÉIER

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1. INTRODUCTION

Pour pouvoir améliorer significativement la productivité, la résistance et la qualité du café chez les variétés cultivées en Amérique Centrale, génétiquement très proches, il est apparu nécessaire d'utiliser la diversité génétique présente chez les origines sauvages ou sub-sauvages (Charrier, 1985). C'est dans ce but que PROMECAFE, le CATIE et la Coopération française (CIRAD, ORSTOM, MAE) développent un programme d'amélioration génétique de *Coffea arabica* qui présente deux originalités : i) la création et la sélection d'hybrides F1 (Bertrand *et al.*, 1997), entre les variétés locales (Caturra, Catuai, Catimor, Sarchimor) et des caféiers sauvages d'Ethiopie et du Soudan, et ii) le recours aux méthodes de culture *in vitro* par embryogenèse somatique pour la multiplication et la diffusion clonale des meilleurs hybrides.

A moyen terme, l'objectif est de diffuser les hybrides améliorés dans la région. La technique retenue pour la micropropagation est l'embryogenèse somatique haute fréquence (Berthouly et Michaux-Ferrière, 1995 ; Van Boxtel et Berthouly, 1996), qui présente un potentiel de multiplication trés élevé. L'embryogenèse somatique présente aussi des aptitudes à l'automatisation grâce à la friabilité et la robustesse du matériel embryogène ainsi qu'à la possible utilisation de milieux liquides. Ces qualités sont indispensables pour la production de plants élites à des coûts attractifs. Jusqu'à présent, l'utilisation de milieux liquides, en suspensions cellulaires ou en bioréacteurs, a permis d'effectuer la phase de prolifération du matériel embryogène et la régénération d'embryons somatiques jusqu'à des stades juvéniles, mais il n a pas été possible de réaliser la phasé de developpement des plantules prêtes à être acclimatées. Dans ces deux systèmes, le retour sur milieu solide pour le développement des embryons puis des plantes impose une main-d'oeuvre extrêmement lourde et une utilisation importante de produits et de surfaces de laboratoire difficilement compatible avec un développement commercial.

Cet exposé concerne les résultats obtenus avec un procédé d'embryogenèse somatique haute fréquence original puisque les deux phases de prolifération et de régénération (développement des plantules) se déroulent dans leur

totalité en milieu liquide, en utilisant respectivement les techniques de suspensions cellulaires et de culture en immersion temporaire (Figure 1). L'objectif est de developper et valider une méthode de propagation applicable à l'ensemble des hybrides sélectionnés.

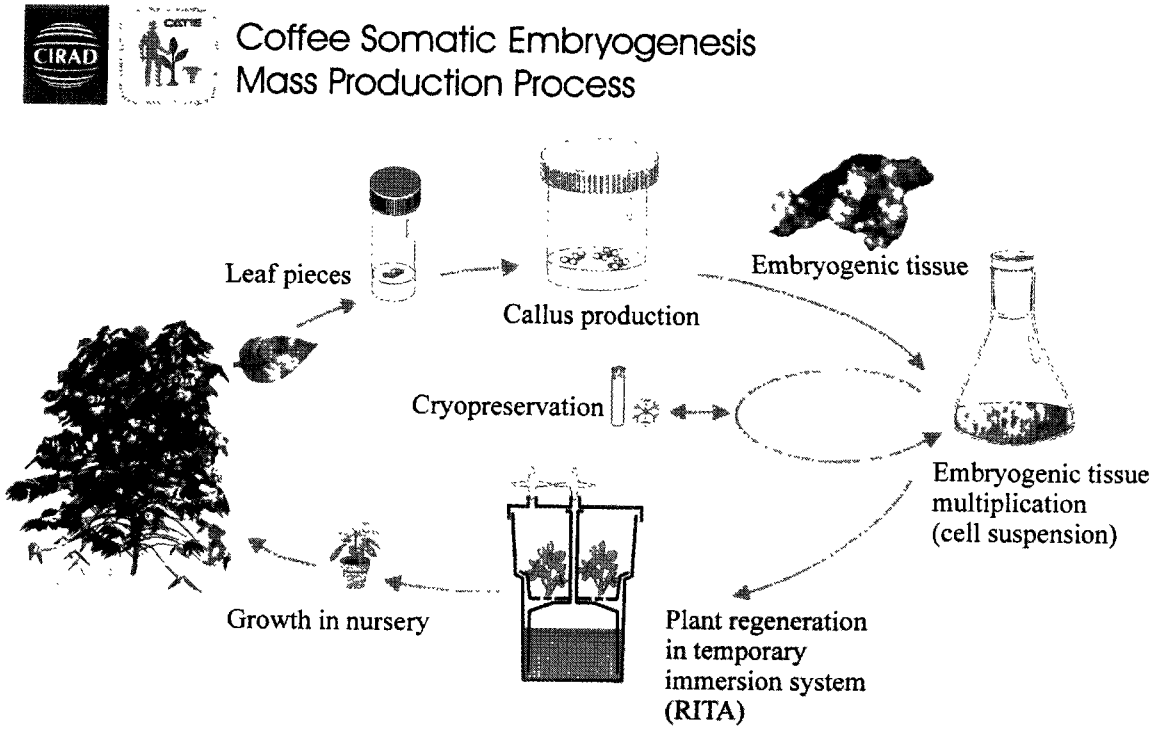

Figure 1 : Schématisation du procédé d'embryogenèse somatique utilisé pour la multiplication des hybrides F1 de *C. arabica*.

2. MATERIEL ET METHODES

Matériel végétal

Dix hybrides F1 de *C. arabica*, appartenant à quatre familles différentes, ont été utilisés : Famille 1 / hybride 1, Famille 2 / hybrides 1 et 2, Famille 3 / hybrides 1 et 2, et Famille 4 / hybrides 1, 2, 3, 4 et 5. Les familles d'hybrides proviennent de croisements entre des variétés cultivées en Amérique Centrale et des individus sauvages ou sub-sauvages, originaires d'Ethiopie ou du Soudan (Bertrand *et al.*, 1997).

1/. Callogenèse. L'explant de départ est un morceau de feuille juvénile d'environ 1 cm^2. Après désinfection, il est placé sur le milieu de callogenèse C (Van Boxtel et Berthouly, 1996) pendant un mois en tube à l'obscurité et à 27°C puis transféré en pots à la lumière indirecte sur le milieu E d'induction de l'embryogenèse jusqu'à apparition du cal embryogène haute fréquence (six mois).

2/. Suspension cellulaire. Les suspensions cellulaires sont initiées en cultivant le cal embryogène en boîtes multipuits pendant quatre semaines. Les agrégats embryogènes contenus dans trois puits sont alors rassemblés en Erlenmeyers de 125 ml. L'entretien des suspensions cellulaires est réalisé dans le milieu de prolifération CP (Van Boxtel et Berthouly, 1996). Après la phase d'établissement, la culture a lieu dans des Erlenmeyers de 250 ml

agités à 100 r.p.m et à 27°C. Des sub-cultures sont réalisées toutes les dix semaines. L'échelle de notation de la qualité des suspensions cellulaires est la suivante:

1 = suspension morte,
2 = suspension mourante, pas de croissance, milieu obscur,
3 = suspension mourante, croissance très faible, milieu obscur,
4 = agrégats de tailles très hétérogènes, présence de proembryons, croissance moyenne, milieu trouble,
5 = agrégats de tailles très hétérogènes, présence de proembryons, croissance moyenne, milieu plutôt limpide,
6 = agrégats hétérogènes, bonne croissance, milieu plutôt limpide,
7 = agrégats assez homogènes, forte croissance, milieu limpide,
8 = agrégats homogènes, jaune et denses, forte croissance, milieu limpide,
9 = agrégats très homogènes, jaune et denses, forte croissance, milieu limpide,
10 = agrégats très homogènes, jaune orangé, très denses, forte croissance, milieu limpide.

3/. Régénération en immersion temporaire. Le système d'immersion temporaire (Récipient pour Immersion Temporaire Automatique) utilisé pour l'ensemble de la phase de régénération, embryons somatiques et plantes, a déjà été décrit précédemement (Alvard *et al.*, 1993 ; Berthouly *et al.*, 1995 ; Teisson *et al.* (1995) ; Etienne *et al.*, 1997). Le récipient RITA^{R} (CIRAD, France) a été conçu spécialement pour la culture *in vitro*. Il s'agit d'un récipient d'un litre comprenant un compartiment supérieur dans lequel est déposé le matériel végétal et un compartiment inférieur dans lequel repose le milieu de culture. Les deux compartiments sont séparés par une toile à blutée de 400 µM. Les récipients sont connectés à une pompe à air qui, sous le contrôle d'une horloge, propulse de l'air dans le récipient inférieur et, lorsqu'il y a surpression, permet au milieu d'immerger le matériel végétal. Lorsque la pompe n'envoie plus d'air, le milieu redescend par gravité.

Toute la phase de régénération se déroule dans le même récipient et à la lumière directe. Les agrégats embryogènes issus de suspension cellulaire sont déposés dans le RITA, ainsi que 250 ml de milieu d'expression R pour permettre le développement des embryons somatiques. Le milieu d'expression est alors remplacé par le milieu DEV (Van Boxtel et Berthouly, 1996) utilisé pour la germination puis le développement des plantules. La germination se caractérise par l'allongement de l'embryon, un changement de couleur (jaune - orangé) et l'ouverture des cotylédons. La conversion des embryons en plantes se caractérise par le développement d'au moins une paire de feuilles et d'une pointe racinaire. Cette étape dure trois à quatre mois et nécessite un simple renouvellement du milieu après six semaines. Au terme de cette étape, les plantules qui possèdent une paire de feuilles peuvent être transférées à la serre pour aclimatation.

Analyse des résultats

La plupart des dispositifs peuvent être testés par des analyses de variance à un critère de classification, suivies de tests de Duncan (seuil P=0,05).

3. RESULTATS

Production de cal embryogène

La formation d'un cal primaire a éte obtenu chez tous les hybrides testés. (donnée non présentée). Les niveaux de contamination conséquents à l'introduction *in vitro* des explants oscillent entre 30 et 70 %. Le Tableau 1 montre que l'obtention de cal embryogène haute fréquence (Figure 2) a été possible pour tous les hybrides, à des fréquences relativement élevées variant de 17 à 79 %. Dans cet intervalle, les niveaux de réactivité diffèrent peu entre les familles d'hybrides mais ils peuvent varier beaucoup entre les individus d'une même famille. Les hybrides 2 et 5 de la Famille 4 sont les plus embryogènes. De plus, les quantités de cal embryogène récoltées pour chaque explant de disposer de suffisament de matériel embryogène pour initier les suspensions cellulaires même si l'hybride présente une faible réactivité.

Tableau 1 : Comportement des hybrides F1 de *C. arabica* pour la production de cal embryogène haute fréquence. Pour chaque hybride, entre trois et sept introductions d'au moins 100 explants ont été réalisées. Les valeurs obtenues pour la fréquence d'explants embryogènes (CE) et le poids de cal embryogène produit par explant (PCE) correspondent à la moyenne de différentes répétitions. Les taux d'explants embryogènes ont été calculés après neuf mois de culture. Les analyses statistiques sont effectuées sur des valeurs transformées : $Arcsin\sqrt{CE}$ et $\log(PCE+1)$.

Hybrides de *C. arabica*	**Nombre d'explants introduits**	**Nombre d'explants non contaminés**	**CE (%)**	**PCE (mg)**
Famille 1 / hybride 1	642	281	38 ^{c}	282 ^{a}
Famille 2 / hybride 1	504	260	6 ^{d}	72 ^{b}
Famille 2 / hybride 2	641	363	24 ^{cd}	179 ^{a}
Famille 3 / hybride 1	866	341	42 ^{bc}	224 ^{a}
Famille 3 / hybride 2	623	341	17 ^{cd}	117 ^{ab}
Famille 4 / hybride 1	369	74	52 ^{abc}	262 ^{a}
Famille 4 / hybride 2	384	162	74 ^{ab}	284 ^{a}
Famille 4 / hybride 3	385	133	33 ^{cd}	378 ^{a}
Famille 4 / hybride 4	382	98	32 ^{cd}	211 ^{a}
Famille 4 / hybride 5	285	84	79 ^{a}	325 ^{a}

Suspensions cellulaires embryogènes

Des suspensions embryogènes (Figure 3) ont pu être etablies pour l'ensembles des hybrides testés (Tableau 2). Toutefois, pour cette étape de culture, il existe des différences de comportement entre les familles.

Comme l'indiquent les notes sur la qualité morphologique des suspensions, le matériel embryogène obtenu avec la Famille 4 se comporte mieux en suspension cellulaire. Il faut noter que les deux hybrides ayant donné les plus mauvaises réponses au niveau de la production de cal embryogène (CE) (Famille 1 / hybride 1 et Famille 3 / hybride 2) sont aussi les plus récalcitrants pour l'initiation d'une suspension cellulaire.

Tableau 2 : Comportement des hybrides F1 de *C. arabica* au niveau de la prolifération des suspensions cellulaires embryogènes. Les notations sur la réusite de l'établissement et sur la qualité morphologique des suspensions sont réalisées sur dix Erlenmeyers. La réussite de l'établissement d'une suspension est jugée après deux mois. L'évaluation de la qualité morphologique, détaillée dans le matériel et méthodes, est réalisée sur des suspensions de plus de cinq mois.

Hybrides de *C. arabica*	**Taux de réussite pour l'établissement d'une suspension (%)**	**Qualité morphologique (1-10)**
Famille 1 / hybride 1	80	8,9 ± 1,3
Famille 2 / hybride 1	20	6,0 ± 1,4
Famille 2 / hybride 2	70	6,9 ± 1,8
Famille 3 / hybride 1	50	6,2 ± 2,4
Famille 3 / hybride 2	20	5,0 ± 1,1
Famille 4 / hybride 1	100	10 ± 0
Famille 4 / hybride 2	100	7,3 ± 2,3
Famille 4 / hybride 3	100	10 ± 0
Famille 4 / hybride 5	80	10 ± 0

Régénération d'embryons somatiques et de plantes en immersion temporaire

La phase de régénération a été étudiée sur cinq hybrides. La production d'importantes quantités d'embryons somatiques (Figure 4) et de plantes (Figure 5) est possible sur les cinq hybrides testés (Tableau 3). Elle est cependant variable d'un hybride à l'autre. Là encore, l'hybride Famille 1 / hybride 1 se révèle très efficace avec une production de plus de 9.000 embryons et plantules par récipient au bout de six mois de régénération. Ce sont à nouveau les deux mêmes hybrides (Famille 2 / hybride 1 et Famille 3 / hybride 2), qui donnent les plus faibles performances, respectivement 750 et 1.000 embryons par récipient. La totalité des embryons somatiques germent (donnée non présentée). Les taux de conversion des embryons en plantes sont élevés et assez similaires puisqu'ils varient entre 83 et 95 %. Comme pour les autres phases de culture, il existe un faible effet génotypique pour la conversion des embryons en plantes.

Tableau 3 : Comportement des hybrides pour la phase de régénération en immersion temporaire. La production d'embryons somatiques intervient après trois mois de culture en RITA^R et celle de plantes après six mois. Les valeurs présentées sont les moyennes d'au moins trois répétitions, correspondant à des récipients différents. Les analyses statistiques ont été effectuées sur des valeurs transformées de la manière suivante : $\log((\text{Nb emb somatiques}) + 1)$ et $\text{Arcsin}(\sqrt{\%\ \text{conv. plantes}})$.

Hybrides de *C. arabica*	**Nombre d'embryons somatiques produits par récipient RITA^R**	**Conversion en plantes en récipient RITA^R (%)**
Famille 1 / hybride 1	9647 [a]	95 a
Famille 2 / hybride 1	767 *	85 *
Famille 2 / hybride 2	2678 [b]	89 b
Famille 3 / hybride 1	2663 [b]	85 bc
Famille 3 / hybride 2	1038 [c]	83 c

* non analysé statistiquement (deux répétitions)

L'efficacité du système de culture en immersion temporaire a été comparée à celle d'une culture sur milieu gélifié pour la germination et la conversion des embryons en plantes, étapes généralement les plus lourdes dans les procédés d'embryogenèse somatique. Les données obtenues pour chaque système après obtention de plantes sont présentées dans le Tableau 4. Au niveau qualitatif, la germination s'avère plus lente dans le récipient en immersion temporaire que sur milieu gélifié. Cependant, elle est beaucoup plus synchrone, permet l'obtention d'un matériel extrêmement homogène (Figures 4 et 5) et une meilleure conversion des embryons en plantes (+ 10 %).

L'analyse des données techniques est largement favorable au système de culture en immersion temporaire. La production de 9.000 plantules est possible dans un seul récipient RITA^R alors qu'il faut utiliser 1.500 pots de verre de 200 ml avec un milieu gélifié pour obtenir le même résultat. La comparaison des surfaces utilisées pour produire 9.000 plantes est également révélatrice de la moindre efficacité du système de culture en milieu gélifié, 10 cm^2 pour l'immersion temporaire contre près de 6 m^2 pour le milieu gélifié. Trois transferts d'embryons et de plantes successifs ont été nécessaires sur milieu gélifié pour obtenir des plantes aclimatables, ce qui représente 76 h de travail. Le temps de confection des milieux de culture, également défavorable à l'utilisation de milieux solides, n'a pas été inclus. Deux changements de milieu de culture liquide ont été suffisants pour obtenir des plantules aclimatables, ce qui a représenté 10 mn de travail.

Tableau 4 : Comparaison de l'efficacité de la germination et de la conversion des embryons en plantes en immersion temporaire avec celle obtenue sur milieu de culture gélifié. Cette étude a été réalisée sur l'hybride Famille 1 / hybride 1 et porte sur un effectif de 9.000 embryons somatiques.

Caractéristiques de la phase de conversion des embryons en plantes	**Conversion des embryons en plantes en immersion temporaire**	**Conversion des embryons en plantes sur milieu solide**
Durée (semaines)	20 ± 1	12 ± 3
Densité de culture (nb embryons / récipient)	9.000	200 (4 sem)/ 40 (4 sem)/ 6 (4 sem)
Nombre de récipients	1	45 / 225 / 1.500
Surface occupée par les recipients (m^2)	0,001	0,2 / 0,9 / 5,8
Germination (%)	100 ± 0	97 ± 3
Conversion en plantes (%)	94,6 ± 2,9	84,3 ± 5,0
Développement	très homogène, synchrone	hétérogène, asynchrone
Interventions pour 9.000 plants	2 changements de milieu liquide	3 repiquages
Temps d'intervention pour 9.000 plants (h)	0,18	76

4. DISCUSSION - CONCLUSION

Les résultats présentés dans ce travail démontrent que le procédé d'embryogenèse somatique que nous utilisons chez le caféier est applicable dans le nouveau schéma de création et diffusion variétale d'hybrides F1 de *C. arabica*. En effet, tous les hybrides introduits ont répondu positivement aux différentes étapes du procédé. De plus, même si des effets génotypiques assez marqués ont été observés, les rendements biologiques obtenus à chaque étape sont suffisants pour envisager une production massale. Il est intéressant de remarquer que l'aptitude de chaque hybride à l'embryogenèse somatique, révélée par la fréquence d'explants embryogènes, conditionne l'efficacité des étapes suivantes. La qualité du cal embryogène pourrait ainsi être variable entre les "hybrides très embryogènes" ou "peu embryogènes". Des études histologiques sont en cours afin de confirmer cette observation.

La possibilité d'obtenir la prolifération de cal embryogène et sa régénération dans un système de culture en immersion temporaire similaire à celui-ci a déjà été rapportée chez des variétés de café (Berthouly *et al.*, 1995). Ce travail démontre que le RITA^R permet la production massive de plantes d'hybrides F1 de *C. arabica*. Chez la plupart des plantes, l'utilisation de suspensions cellulaires ou de bioréacteurs permet essentiellement la multiplication du matériel embryogène. Même si dans certains cas ces deux systèmes sont utilisés pour initier le processus de régénération, ils ne permettent pas d'éviter un étalement sur milieu gélifié ("plating") des agrégats embryogènes ou des jeunes embryons (Tautorus *et al.*, 1991 ; Zamarripa *et al.*, 1991 ; Neuenschwander et Baumann, 1992). L'utilisation de l'immersion temporaire pour la régénération de plantes permet de s'affranchir de toutes les opérations d'isolement et transferts manuels des embryons somatiques au cours du procédé *in vitro*.

Les plantes obtenues présentent peu ou pas de problème de vitricité aux fréquences d'immersion utilisées et des taux d'aclimatation supérieurs à 90 % ont été enregistrés (Etienne *et al.*, 1997). Trente autres candidats sélectionnés eux aussi pour leur comportement agronomique seront introduits par embryogenèse somatique. L'objectif est d'une part de connaître leur comportement *in vitro* de manière assez fine et d'autre part de les multiplier pour pouvoir établir des essais de comportement dans différents pays d'Amérique Centrale. Les recherches sont actuellement focalisées sur la validation du procédé à l'échelle de production pilote et sur la validation de la qualité agronomique du matériel végétal.

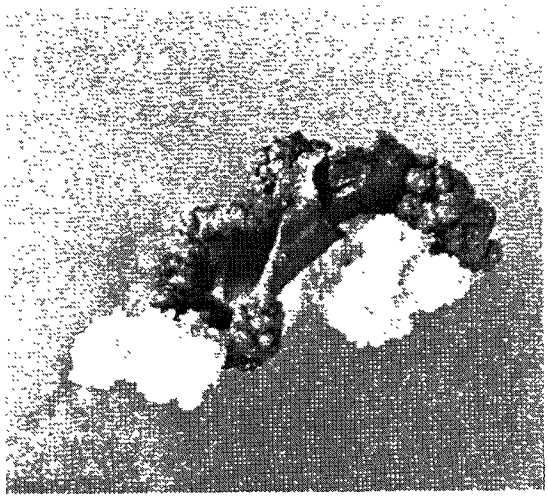

Figure 2 : Cal embryogène haute fréquence d'un hybride de *C. arabica.*

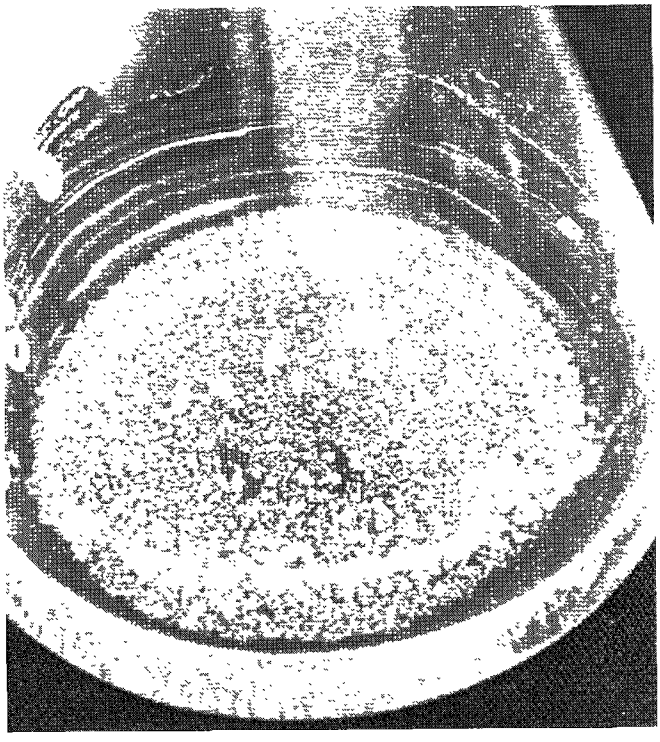

Figure 3 : Suspension embryogène entretenue en Erlenmeyer.

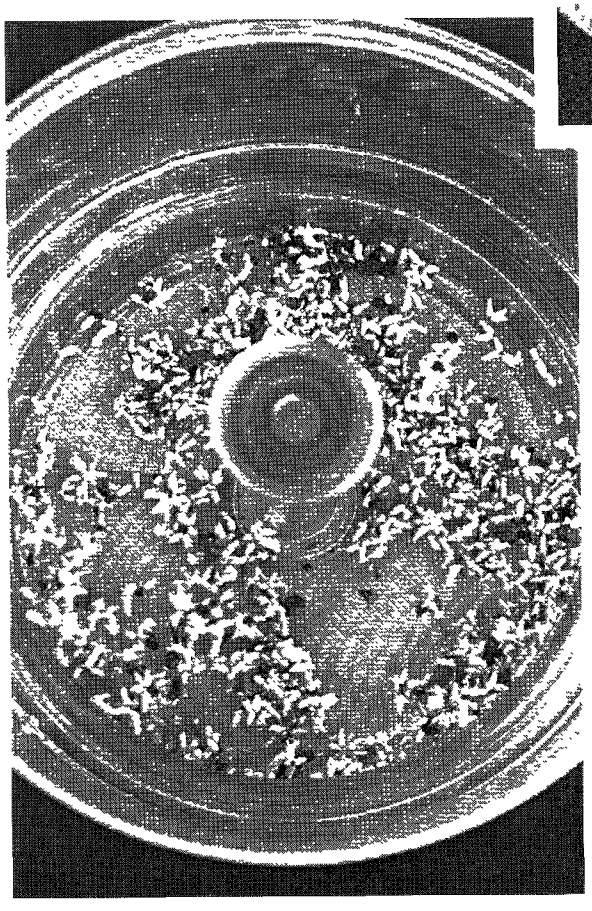

Figure 4 : Embryons somatiques d'hybrides F1 de *C. arabica* développés dans le récipient pour immersions temporaire RITA^R.

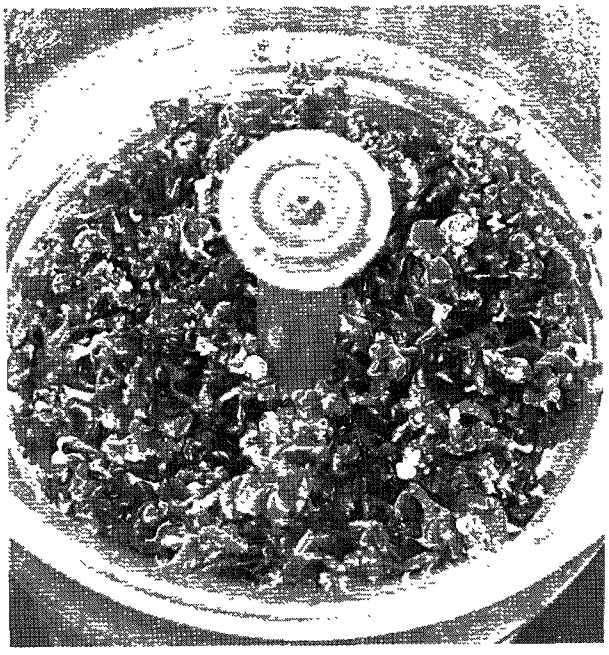

Figure 5 : Plantules d'hybrides F1 de *C. arabica* se développant dans le récipient pour immersion temporaire RITA^R.

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RESUME

Le schéma de création d'hybrides F1 du programme d'amélioration génétique du caféier Arabica en Amérique Centrale, entre variétés et individus Ethiopiens, devrait conduire à retenir une quarantaine de candidats sur des critères de production, de qualité et de résistance aux maladies. Seules les techniques de culture *in vitro* peuvent permettre une multiplication clonale à grande échelle de ce type de matériel. Pour cela, une méthode d'embryogenèse somatique haute fréquence été développée par le CIRAD, le CATIE et PROMECAFE. Pour se donner les meilleures chances de réussir une production massale de vitroplants à des coûts réduits, la plus grande partie du procédé se déroule en milieu liquide. Si le procédé reste classique pour la phase de multiplication des agrégats cellulaires embryogènes, il est totalement novateur pour la régénération des vitroplants. L'utilisation du système de culture en immersion temporaire (RITA^R) permet dans un même récipient et sans subculture ni changement de milieu d'aller jusqu'à la production de plantes aclimatables à partir des cellules embryogènes. Dix hybrides F1 candidats ont été introduits *in vitro* au cours de l'année 96 avec comme objectifs : i) réaliser l'évaluation technique et économique du procédé de micropropagation en vue du développement commercial, et ii) diffuser ces candidats aux pays d'Amérique Centrale, membres de PROMECAFE, pour une évaluation de leur comportement dans des essais multilocaux. Le procédé d'embryogenèse somatique utilisé a permis la multiplication à grande échelle des premiers candidats. Pour les dix premiers hybrides, il a été possible d'obtenir du cal embryogène haute fréquence, d'établir et d'entretenir à long terme des suspensions embryogènes, et de réussir la régénération de grandes quantités de plantes en immersion temporaire. Les premiers résultats confirment d'ores et déjà le potentiel de ce procédé et sa possible utilisation dans un schéma de sélection d'hybrides F1 chez le café.

ABSTRACT

The selection of F1 hybrids with improved yields and resistance to diseases is the goal of the Central America coffee genetic improvement programme. It should lead to the selection of about 40 improved F1 hybrids betwen varieties and wild genotypes from Ethiopia. *In vitro* micropropagation techniques is the only way to rapidly propagate these hybrids. For that purpose, a high frequency somatic embryogenic method was developped by CIRAD, CATIE and PROMECAFE. To reduce the production costs, most parts of the process are done with liquid medium. The use of immersion techniques with especially adapted culture vessels (RITA^R) allows the direct regeneration, in the same container and without subculture, of plantlets from cell suspensions. The objectives of the work are now the technical and economical evaluation of the *in vitro* process and the multilocal field evaluation of the plants. Ten F1 hybrids were introduced in the laboratory in 1996. High frequency embryogenic tissues, cell supensions and regeneration of plants with the immersion techniques were obtained for all of these hybrids. The results underline the interest of somatic embryogenesis for the large scale propagation of coffee improved F1 hybrids.

CRYOPRESERVATION OF COFFEE (*COFFEA ARABICA*) SEEDS

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Introduction

Coffee-trees belong to the tribe *Coffeae* in the family *Rubiaceae* (Bridson & Verdcourt, 1988) The genus *Coffea* L. is subdivided in two subgenera: *Coffea* and *Baracoffea*. Approximately 100 taxa have been identified so far in the subgenus *Coffea* (Charrier & Berthaud, 1985). All species are woody, ranging from small-sized shrubs to robust trees and originate from the inter-tropical forests of Africa and Madagascar. Commercial coffee production relies on two species only, *C. arabica* L. and *C. canephora* Pierre, but many *Coffea* species form a valuable gene reservoir for different breeding purposes (Berthaud & Charrier, 1988).

Though *C. arabica* seeds can withstand desiccation down to 0.06-0.08 g $H_2O.g^{-1}$ dw (Becwar *et al.*, 1983 ; Ellis *et al.*, 1990), they cannot be considered orthodox because they remain cold-sensitive and desiccation does not improve their longevity (Ellis *et al.*, 1990 ; Van der Vossen, 1977). *C. arabica* seeds are also characterised by their very short lifespan in the hydrated state (Couturon, 1980). Because of their intermediate storage behaviour, coffee seeds cannot be used for long-term conservation and coffee genetic resources are conventionally conserved as trees in field genebanks. However, significant problems appeared with the maintenance of these field genebanks: i) genetic erosion in some species due to their poor adaptation to the local environment and to attacks by pests and pathogens; ii) important labour costs and large space requirements. Thus, research for alternative methods to field conservation for coffee genetic resources became a priority (Dussert *et al.*, 1997a ; Dussert *et al.*, 1997b).

Different *in vitro* conservation methods are employed, depending on the storage duration requested (Engelmann, 1991). For short- and medium-term storage, the aim is to reduce growth and to increase the intervals between subcultures. For long term storage, cryopreservation, i.e. storage at ultra-low temperature, usually that of liquid nitrogen (-196°C), is the only current method. At this temperature, all cellular divisions and metabolic processes are stopped. The plant material can thus be stored without alteration or modification for a theoretically unlimited period of time. Moreover, cultures are stored in a small volume and requiring a very limited maintenance.

Whatever their water content, *C. arabica* seeds do not withstand direct immersion into liquid nitrogen temperature (Becwar *et al.*, 1983). However, successful cryopreservation of zygotic embryos, extracted from mature seeds, was achieved for *C. liberica* (Normah & Vengadasalam, 1992), *C. arabica* (Abdelnour-Esquivel *et*

al., 1992 ; Florin *et al.*, 1993), *C. canephora* and the interspecific hybrid arabusta (Abdelnour-Esquivel *et al.*, 1992). With all species, partial dehydration of excised embryos to 0.2 g $H_2O.g^{-1}$ dw was sufficient to obtain high survival rates after direct immersion in liquid nitrogen. This suggests that the endosperm and the embryo of *C. arabica* seeds differ in their response to desiccation and freezing. Desiccation duration, freezing and thawing rates, were thus investigated simultaneously in *C. arabica* to define conditions under which both the endosperm and the embryo survive (Dussert *et al.*, 1997c).

Materials and methods

Plant material

Fresh mature seeds of *C. arabica* var. Typica were provided from CATIE, Costa Rica. Seed water content upon receipt was 0.5 g $H_2O.g^{-1}$ dw.

Desiccation and cryopreservation

After the testa was removed, seeds were desiccated over 80 g silica gel for 0 to 16 h in 1160 ml glass vessels or by equilibrating them (down to 0.2 g $H_2O.g^{-1}$ dw) for 3 weeks under 78% RH. Water content (expressed in g $H_2O.g^{-1}$ dw) was estimated using 3 replicates of 10 seeds. Dry weight was measured after 2 days of desiccation in an oven at 105°C.

Before cryopreservation, seeds were hermetically sealed in 10 ml polypropylene tubes (50 seeds per tube). The initial temperature of the seeds was 25°C (room temperature). Rapid cooling (200°C.min^{-1}) was achieved by plunging tubes directly into LN. Slow cooling consisted of precooling seeds down to -50°C at 1, 2, 4 or 20°C.min^{-1} prior to immersion into LN. A second experiment consisted in precooling seeds down to 0°C, -20°C, -50°C and -100°C at 1°C.min^{-1}, then rewarmed directly (precooling controls) or immersed in LN before rewarming. The effect of precooling was assessed by thawing seeds directly after the precooling step. Some seeds were directly transferred *in vitro* or cryopreserved without precooling. Precooling was carried out using a programmable cooling apparatus (Minicool LC 40, L'Air Liquide, France). Cryopreserved seeds were stored for one week at -196°C before thawing. Thawing was carried out either rapidly (mean rate of 420 °C.min^{-1} between -196°C and 0°C) by plunging tubes in a 40°C water-bath for 2 min or slowly (mean rate of 70°C.min^{-1} between -196°C and 0°C) by placing tubes at room temperature (25°C) for 30 min. Cooling and thawing rates were measured separately using a K type thermocouple embedded in one seed and a data logger (Model 50, Electronic Controls Design, USA).

Culture conditions

After freezing, both seeds and zygotic embryos were inoculated and cultured *in vitro* for survival assessment. Before disinfection, seeds were washed with soap and tap water. Disinfection was achieved by soaking seeds in sodium hypochlorite (12%) for 15 min with continuous shaking on a rotary shaker, followed by 5 min under vacuum and 10 min again with shaking. Seeds were rinsed three times with sterile water before inoculation into test tubes (250 x 24 mm) sealed with Parafilm Ribbon on water gel (3 $g.l^{-1}$ agar).

Before excision of zygotic embryos, disinfected seeds were immersed for two days in sterile water for rehydration. Excised zygotic embryos were inoculated on the germination medium (20 ml) defined by Bertrand-Desbrunais and Charrier (1989) into test tubes (250 x 24 mm) sealed with Parafilm Ribbon.

All cultures were maintained in the dark until the hypocotyl stood upright. They were then transferred to light conditions (30 $\mu E.m^{-2}.s^{-1}$, 12h light/12h dark photoperiod).

Four month-old well-developed *in vitro* seedlings were transferred to the greenhouse and grown on an acidophilic peat compost.

Survival assessment

Both germination *sensu stricto* and development of normal seedlings were used to assess seed survival. Emergence of the hypocotyl and radicle was used as the criterion for estimating the germination rate after 1 and 4 months in culture. Seedlings which stood upright on the medium at those times of observation were considered normal. The ratio of normal seedlings after 1 month in culture to normal seedlings after 4 months was used to estimate the development rate of seedlings. After 3 months in the greenhouse, greening of cotyledonary leaves and

development of apical shoot was used as the criterion for assessing normal plants. Excised embryos were considered viable when they stood upright on the culture medium and the first pair of leaves was developed.

Results

Effects of desiccation, slow and rapid cooling

There was a low negative effect of desiccation on seed viability (Table 1). However, at the lowest water content (0.14 g $H_2O.g^{-1}$ dw), the proportion of normal seedlings recovered from desiccated seeds remained very high (79%). When cooled rapidly (200°C.min^{-1}) by direct immersion into LN, the emergence of hypocotyl and radicle was observed in 53% of seeds at 0.20 g $H_2O.g^{-1}$ dw, while at other water contents, germination was very low or nil. However, none of these germinated seeds produced normal seedlings and, after 4 months in culture, no further development had been noted. If seeds were precooled to -50°C at 4°C.min^{-1} prior to immersion into LN (slow cooling), the rate of emergence of hypocotyl and radicle was 4% at 0.28 g $H_2O.g^{-1}$ dw, 63% at 0.20 g $H_2O.g^{-1}$ dw and 35% at 0.14 g $H_2O.g^{-1}$ dw. Moreover, 12% of germinated seeds developed into normal seedlings after desiccation down to 0.20 g $H_2O.g^{-1}$ dw only.

Table 1. Germination (%) of *C. arabica* seeds at various water contents after desiccation, slow cooling or rapid cooling (200°C.min^{-1}) and development of normal seedlings (%). Slow cooling consisted of precooling seeds to -50°C at 4°C.min^{-1} prior to immersion in liquid nitrogen.

Water content (g $H_2O.g^{-1}$ dw)	Treatment	Germination (%)	Normal seedlings (%)
0.37	Desiccation control	97.8	93.5
	Slow cooling	0.0	0.0
	Rapid cooling	0.0	0.0
0.28	Desiccation control	97.0	91.5
	Slow cooling	4.4	0.0
	Rapid cooling	0.0	0.0
0.20	Desiccation control	95.2	83.0
	Slow cooling	63.2	12.2
	Rapid cooling	53.1	0.0
0.14	Desiccation control	91.5	78.7
	Slow cooling	35.4	0.0
	Rapid cooling	4.3	0.0

Effect of the precooling rate

There was a highly significant effect of the precooling rate on the germination rate and the production of normal seedlings from *C. arabica* seeds precooled to -50°C before immersion in LN (Table 2). No seedlings were produced from seeds precooled at 20°C.min^{-1}, whereas, for the lowest precooling rate tested, 1°C.min^{-1}, 30% of *C. arabica* seeds developed into normal seedlings.

Table 2. Development of normal seedlings (%) from *C. arabica* seeds at 0.20 g $H_2O.g^{-1}$ dw precooled to -50°C at different rates prior immersion into liquid nitrogen.

Precooling rate (°C.min^{-1})	Germinated seeds (%)	Normal seedlings (%)
1	70.2	30.1
2	68.4	24.2
4	62.8	13.0
20	60.0	0.0

Effect of the precooling temperature on seed viability

After 4 months in culture, there was no significant difference in viability between control seeds and precooling controls for precooling temperatures of 0, -20 and -50°C (Figs. 1 and 2). In all cases, around 95% of the seeds germinated and developed into normal seedlings. A drastic decline in viability was observed when seeds were precooled to -100°C, since only 55% of them germinated (Fig. 1) and 24% produced normal seedlings (Fig. 2).

For precooling temperatures above -50°C, viability of seeds immersed in liquid nitrogen after precooling was always significantly lower than that of precooling controls (Figs. 1 and 2). By contrast, when seeds were precooled to -100°C, there was no difference in survival between precooling controls and cryopreserved seeds.

The germination rate of cryopreserved seeds increased with precooling temperatures down to -50°C, from 26% without precooling up to 70% for a precooling temperature of -50°C, and then decreased down to 55% for seeds precooled to -100°C (Fig. 1). The percentage of normal seedlings produced from cryopreserved seeds followed the same trend (Fig. 2) and was highly correlated with the germination rate. It could be approximated by subtracting 40% to the germination rate : e.g. after precooling to -50°C and immersion in LN, the germination rate was 70% and the percentage of normal seedlings was 30%.

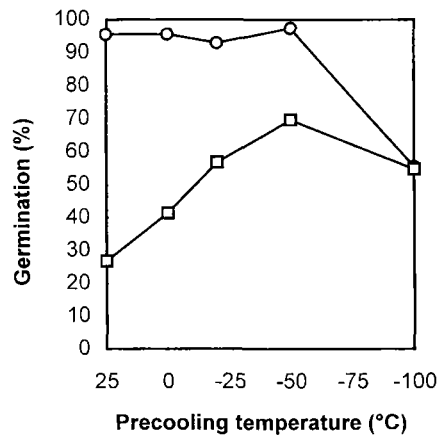

Figure 1. Effect of precooling temperature on the germination rate (%) of *C. arabica* precooling control seeds (**O**) and cryopreserved seeds (□).

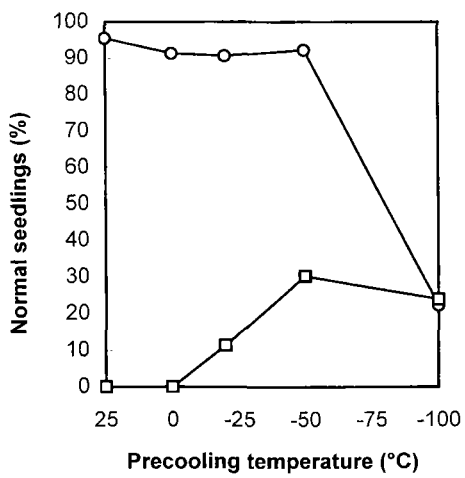

Figure 2. Effect of precooling temperature on the rate of normal seedlings produced (%) from *C. arabica* precooling control seeds (**O**) and cryopreserved seeds (□).

Effect of precooling temperature on zygotic embryo viability

Precooling had a significantly negative effect on the viability of zygotic embryos extracted from precooled and cryopreserved seeds (Fig. 3). However, this effect was very low and survival rates ranged from 83 to 97% for precooling controls and from 65 to 93% for cryopreserved seeds. The maximal viability (97%) was obtained with embryos extracted from seeds directly immersed into LN after desiccation, without any precooling treatment. All viable embryos developed into healthy plantlets which could be transferred to greenhouse conditions without any viability loss.

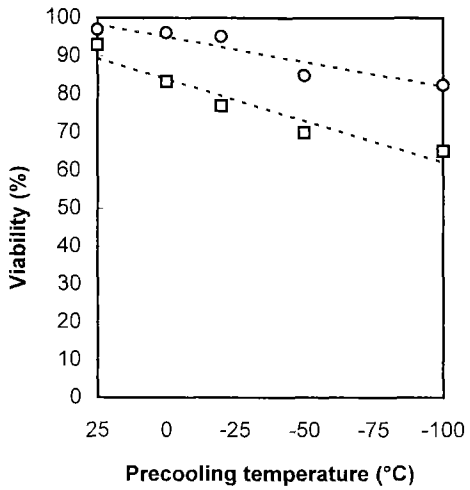

Figure 3. Effect of precooling temperature on the viability (%) of zygotic embryos extracted from *C. arabica* precooling control seeds (O) and cryopreserved seeds (□). Dotted lines correspond to linear regressions.

Effect of thawing rate

The thawing rate had no significant effect on the germination rate and the development of normal seedlings with seeds at 0.20 g $H_2O.g^{-1}$ dw, precooled down to -50°C at 4°C.min^{-1} prior to immersion into LN (Table 3). After both slow (70°C.min^{-1}) and rapid (420°C.min^{-1}) thawing, emergence of hypocotyl and radicle was observed in 55-70% of seeds and 13% of them developed into normal seedlings.

Table 5. Effect of thawing rate on germination (%) and development of normal seedlings (%) of *C. arabica* var. Typica seeds at 0.20 g $H_2O.g^{-1}$ dw precooled to -50°C at 4°C.min^{-1} prior to immersion in liquid nitrogen.

Thawing rate	Germination (%)	Normal seedlings (%)
Low	55.2	12.5
High	70.4	13.0

Discussion

When seeds of *C. arabica* at 0.20 g $H_2O.g^{-1}$ dw were cooled rapidly (200°C.min^{-1}), none of them developed into normal seedlings. This result is consistent with those of Becwar *et al.* (1983) who showed that *C. arabica* seeds did not survive after immersion in LN even if all freezable water had been removed from seeds. By contrast, when seeds were slowly precooled to -50°C prior to immersion into LN, 13% of them developed into normal seedlings. It is thus clear that slow precooling of *C. arabica* seeds had a dramatic effect on their survival and their capacity to develop normally. The development of normal seedlings was improved significantly if *C. arabica* seeds were precooled to -50°C at 1°C.min^{-1} but no survival was obtained with a cooling rate of 20°C.min^{-1}. Thus, precooling seems to be the key factor for successful cryopreservation of *C. arabica* seeds.

A drastic decline in survival was observed when seeds were precooled below -50°C (without liquid nitrogen exposure) and -50°C appeared to be the optimal precooling temperature for cryopreservation. If the importance of a low cooling rate for seed cryopreservation has been reported for different species (Stanwood, 1987 ; Vertucci, 1989), it is the first time that the importance of the precooling temperature was clearly demonstrated. A two-step freezing procedure has been widely used for cryopreservation of *in vitro* cultured material including cell suspensions, calluses, somatic embryos (Kartha & Engelmann, 1994 ; Withers & Engelmann, 1997) but has been employed in a limited number of cases only for zygotic embryos (Chmielarz, 1997 ; Fu *et al.*, 1993 ; Pence, 1991). With *in vitro* cultured material, optimal survival rates were generally observed for precooling temperatures between -30°C and -50°C which allow optimal freeze-induced dehydration of the samples (Kartha & Engelmann, 1994). With *C. arabica* seeds, the beneficial effect of precooling could not be associated with freeze-induced dehydration, since there was no more freezable water in seed tissues at 0.2 g $H_2O.g^{-1}$ dw (Becwar *et al.*, 1983). Vertucci (1989) has indicated that the cooling rate might modify the interactions between bound water and lipids at ultra-low temperatures. The beneficial effect on survival of a precisely defined precooling rate for *C. arabica* seeds could be related to the high lipid content of the endosperm of *C. arabica* seeds (Clifford, 1985).

In our study, very high survival rates were obtained with zygotic embryos extracted from seeds cryopreserved under all conditions used in the experiments. By contrast, with whole seeds, high germination and seedling development rates could be obtained for a limited set of conditions only. After precooling to -50°C and cryopreservation, 70% of excised embryos were viable and could develop into normal plantlets; 70 % of seeds germinated, but 30% of seeds only developed into normal seedlings after 4 months in culture. It is thus obvious that immersion into liquid nitrogen caused damages to the endosperm of about 40% of the seeds. In such cases, the endosperm was sufficiently intact to allow germination of all viable embryos but could not play its nutritional function and ensure normal further development of the embryos during the four months of culture.

For the first time, it was shown that whole seeds of *C. arabica* can be cryopreserved if they are slowly precooled to -50°C prior to their immersion in liquid nitrogen. Even if the percentage of seeds which developed into normal seedlings remained relatively low (30%) in comparison with that obtained from excised zygotic embryos, this protocol could represent a simple and efficient complementary option to field conservation for genebanks which cannot afford *in vitro* culture facilities. However, it should be first verified that cryopreserved seeds can germinate normally under greenhouse or nursery conditions. Moreover, this method might be simplified by using a -80°C freezer for precooling seeds to -50°C and could thus be more easily employed routinely in a large number of genebanks maintaining coffee genetic resources. However, additional research should be undertaken to determine the minimum number of seeds which have to be cryopreserved to guarantee the recovery of a minimum number of plants.

Cryopreservation protocols developed for zygotic embryos include their excision from seeds before the successive steps of disinfection, desiccation and freezing (Engelmann *et al.*, 1995). Rapid dehydration is generally achieved using the sterile air-stream of a laminar flow cabinet, air-tight containers with silica gel, compressed air stream or vacuum (Dumet *et al.*, 1997). Though the efficiency of these protocols has been demonstrated for numerous plant species (Engelmann *et al.*, 1995, Engelmann, 1997), including *C. arabica, C. canephora* and *C. liberica* (Abdelnour-Esquivel *et al*, 1997 ; Normah & Vengadasalm, 1992), they present several constraints and disadvantages: i) the need to work under aseptic conditions before cryopreservation ; ii) difficulty to achieve reproducible desiccation conditions when using the sterile air-stream of a laminar flow cabinet or silica gel: since the desiccation periods are generally very short, they need to be very precise and are highly dependent of the initial moisture content of the samples and of the characteristics of the air-flow or the silica gel used ; iii) impossibility to treat simultaneously large amounts of material since the time needed to extract one embryo (1 to 2 min for a single coffee seed) is very long compared with the optimal desiccation period (e.g. 30 min under the laminar flow for coffee embryos with an initial moisture content of 60% (fresh weight basis), as reported by Abdelnour-Esquivel *et al.* (1992)). We propose a new and simple approach which consists of drying seeds under controlled RH and of germinating *in vitro* whole seeds or embryos extracted from the seed after thawing only. This new method should allow to remove many of the problems encountered with traditional zygotic embryo cryopreservation protocols for all coffee species and, possibly, for other species which produce intermediate seeds

of relatively small size. Equilibrating coffee seeds under 78% R.H. allowed the seeds to reach optimal water content for cryopreservation without any viability loss, in a very easy and reproducible manner. This also allowed the processing of large amounts of seeds at the same time. Moreover, aseptic conditions were requested after thawing only. This method was very efficient since, when embryos were extracted after freezing, no loss in viability was observed after cryopreservation (97% survival) in comparison with unfrozen controls. The only drawback of this approach, compared with classical protocols, is that it would require a larger volume for storing seeds in liquid nitrogen containers, in comparison with excised embryos.

If whole seeds are germinated after cryopreservation, particular attention should be given to the possible occurrence of intra-accession genetic drift. Indeed, if the biochemical composition of the seeds is implicated in their tolerance to LN exposure and if this characteristic is genetically controlled, a 30% survival rate could lead to a genetic selection for 'LN exposure adapted' genotypes. When embryos are extracted from seeds after thawing and grown *in vitro*, no genetic deviation should be observed, in view of the very high survival rates obtained.

In conclusion, depending on the laboratory's or genebank's facilities, one of the two protocols proposed in this study could be easily applied for the establishment of a *C. arabica* germplasm cryobank. The application of this cryopreservation protocol to other coffee species is currently under investigation.

Abbreviations

CATIE : Centro Agronomico Tropical de Investigacion y Enseñanza ; dw : dry weight ; LN : liquid nitrogen ; ORSTOM : Institut français de recherche scientifique pour le développement en coopération ; IPGRI : International Plant Genetic Resources Institute.

Abstract. For the first time, success in cryopreservation of *Coffea arabica* seeds was obtained by extensive investigations on cooling and thawing processes. Desiccation sensitivity of *C. arabica* var. typica seeds was very low in the range of water contents studied (0.1 to 0.4 g $H_2O.g^{-1}$ dw). At 0.20 g $H_2O.g^{-1}$ dw, a very high percentage of *C. arabica* seeds germinated after direct immersion into liquid nitrogen (rapid cooling, 200°C.min^{-1}), but none of them could develop into normal seedlings. Normal seedlings could be recovered from cryopreserved *C. arabica* seeds only if they were desiccated to 0.20 g $H_2O.g^{-1}$ dw and cooled at optimal rate to a definite range of precooling temperatures prior to immersion in LN. The thawing rate had no effect on the survival of cryopreserved *C. arabica* seeds. Normal development of seedlings recovered from cryopreserved seeds was observed in the greenhouse. Moreover, very high survival rates were obtained as well for the cryopreservation of zygotic embryos using a new and simple technique. Long term conservation of *C. arabica* genetic resources can now be envisaged through cryopreservation of both seeds and zygotic embryos.

Résumé. Pour la première fois, des graines de *Coffea arabica* ont été cryoconservées avec succès grâce à des recherches poussées sur les processus de congélation et de réchauffement. La sensibilité à la déshydratation des graines de *C. arabica* var. typica était très faible dans la plage de teneurs en eau testées (0,1 à 0,4 g $H_2O.g^{-1}$ MS). A 0,20 g $H_2O.g^{-1}$ MS, un pourcentage élevé de graines ont germées après leur immersion directe dans l'azote liquide (congélation rapide, 200°C.min^{-1}), mais aucune d'entre elles n'a pu se développer en plantule normale. Des plantules normales ont pu être régénérées à partir de graines cryoconservées uniquement lorsqu'elles ont été pré-refroidies à une vitesse de congélation optimale jusqu'à une température de pré-refroidissement précise avant leur immersion dans l'azote liquide. La vitesse de réchauffement n'a eu aucun effet sur la survie des graines cryoconservées. Les plantes régénérées à partir de graines cryoconservées ont présenté un développement normal après leur transfert en serres. De plus, des taux de survie très élevés ont également été obtenus en extrayant les embryons zygotiques des graines après cryoconservation. Désormais, la conservation à long terme des ressources génétiques de *C. arabica* peut être envisagée à l'aide de la cryoconservation des graines.

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MOLECULAR MARKER-ASSISTED SELECTION : A POWERFUL APPROACH FOR COFFEE IMPROVEMENT

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1- INTRODUCTION

Molecular marker techniques have gained widespread applications in many fields of plant genetics and breeding. In coffee-trees, projects with the aim of introducing such techniques into breeding programmes through the development of molecular marker-assisted selection (MAS) have already been initiated. General principle of MAS is that if a gene(s) conferring a trait of interest is linked to an easily identifiable molecular marker, it may be much more efficient to select for the marker than for the trait itself. The development of marker-facilitated selection programmes promises to overcome present limitations of conventional breeding.

In this report, aspects regarding MAS and its potential usefulness in coffee breeding, mainly Arabica breeding, are presented. We also intend to present recent advances in the development and application of molecular marker techniques to coffee breeding.

2- GENETIC VARIATION AND COFFEE BREEDING

Commercial coffee production relies mainly on two species: *Coffea arabica* L. and *Coffea canephora* Pierre. *C. arabica* is the only tetraploid species (2n = 4x = 44) in the genus and is self-fertile while other *Coffea* species are diploid and generally self-incompatible (Bridson and Verdcourt 1988, Charrier and Berthaud 1985). Higher quality is associated with *C. arabica* which represents 70% of world production.

C. arabica is characterised by a low genetic diversity (Fig. 1) which is attributable to its allotetraploid origin and mode of speciation (Lashermes et al. 1996a, 1997). In addition, most cultivars are derived from the few trees which survived various efforts to spread arabica growing worldwide (van der Vossen 1985, Lashermes et al. 1996b). It is believed that the encountered agro-morphological variation which gave rise to so many named varieties, results from few major-gene spontaneous mutations conditioning plant, fruit and seed characters (van der Vossen 1985, Carvalho 1988). The cultivars present therefore, an homogeneous agronomic behaviour characterised by a high susceptibility to many pests and diseases, and very low adaptability.

Enlarging the genetic base and improvement of arabica cultivars have become a priority. Spontaneous accessions collected in the primary centre of diversity as well as wild relative *Coffea* species constitute a valuable

gene reservoir for breeding purposes. To date, *C. canephora* and its spontaneous interspecific crosses with *C. arabica* such as Hibrido de Timor, provides the main source of disease and pest resistance traits not found in *C. arabica* including coffee leaf rust (*Hemileia vastatrix*), Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* and resistance to root-knot nematode (*Meloidogyne sp.*). Likewise, other diploid species present considerable interests in this respect (Berthaud and Charrier 1988).

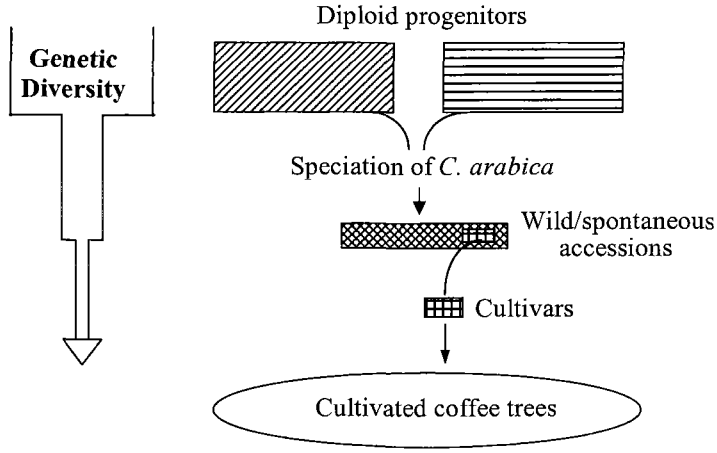

Figure 1. Genetic base of the cultivated Arabica coffee trees.

Most breeding programmes developed in the world focus on the use of Timor Hybrid populations which derived from a spontaneous interspecific hybridisation between *C. arabica* and *C. canephora* (Bettencourt 1973). Its exploitation have so far relied on conventional procedures in which a hybrid is produced between an outstanding variety and a donor genotype carrying the trait of interest, and the progeny is backcrossed to the recurrent parent. Undesirable genes from the donor parent are gradually eliminated by repeated backcrossing. In so doing, conventional coffee breeding methodology faces considerable difficulties.

Strong limitations are due to the long generation time of coffee-tree (5 years), the high cost of field trial, and the lack of accuracy of current strategy. One can estimate that a minimum of 25 years after hybridisation (five backcross-generations) is required to restore the genetic background of the recipient cultivar and there by ensure good quality of the improved variety. Combining various genes of resistance without reducing coffee quality appears therefore as an unrealisable task in an acceptable time-frame through traditional breeding approaches.

3-MOLECULAR FACILITATED SELECTION

3-1 Selection for major genes through linkage with molecular markers

An application of molecular marker in plant breeding is based on finding tight linkages between these markers and genes of interest. Such linkage permits one to infer the presence of a desirable gene by assaying for the marker. Large segregating progeny of plants can be therefore screened at the seedling stage for the presence of the gene(s) of interest. Major genes as well as quantitative trait loci (QTL; Lande and Thompson 1990) can be manipulated by MAS. Regarding Arabica coffee breeding, the most straightforward applications concern the introgression of pest and disease resistance genes. Benefits obtained from MAS depend on several factors such as the degree of linkage between the marker and the target gene, savings in time, and the relative costs of direct vs. marker-facilitated selection. However, MAS shows undoubtedly considerable interests for the transfer of resistance genes in a variety of circumstances (Melchinger 1990) such as:

* Quarantined pathogenes

If a virulent pathogen does not naturally occur in the test environment, artificial inoculation is prohibited for safety reasons. For instance, CBD is still restricted to the continent of Africa, and the availability of markers linked to the resistance gene(s) could allow pre-emptive breeding in countries (Asia, Latin America) where quarantine barriers are still effective.

* Reliability/limitation of direct testing for the resistance trait

Conventional selection progress could be hampered by the difficulty to ensure reliable test. Seedling test could also present strong inconvenient. For instance, the present test for evaluation for root-knot nematode is destructive leading to important difficulties in the utilisation of identified plant resistance sources. In addition, expression of many resistance genes can be strongly influenced by environmental conditions.

* Developmentally regulated character

Early selection based on the marker genotype of young seedlings would be particularly beneficial for late expressed traits.

* Transfer of recessive resistance genes

The classical procedure of transferring a recessive resistance gene includes a progeny test after each backcross generation to determine the presence of the desired allele. With MAS, the transfer can be accomplished without interruptions leading to an important time saving.

* Pyramiding of resistance genes/Combining valuable traits

Pyramiding of resistance genes has been suggested as a strategy to provide durable resistance. However, conventional breeding is complicated by the fact that, is difficult or often impossible to distinguish the various resistance genotypes. One the different genes conferring resistance to the same pathogen are tagged by tightly linked marker, they could be relatively easily be accumulated into a single genotype via marker-facilitated selection. Comparable advantages vs. conventional are procured when trying to combine simultaneously resistance genes to different disease/pests.

3-2 Molecular-assisted backcross breeding

Repeated backcrossing simultaneously accomplishes two essential goals: 1) allow segregation to remove donor parent chromosomes unlinked to the target gene and 2) allow recombination to remove donor parent segments which are linked to the target gene. Both objectives could be considerably facilitated by the use of molecular markers.

Genome selection

Beside the target trait, it is important to consider the complete genome of individuals. Chromosomal segments are segregating within backcross progenies and the individuals show various contents of the desired parental genome (Fig. 2).

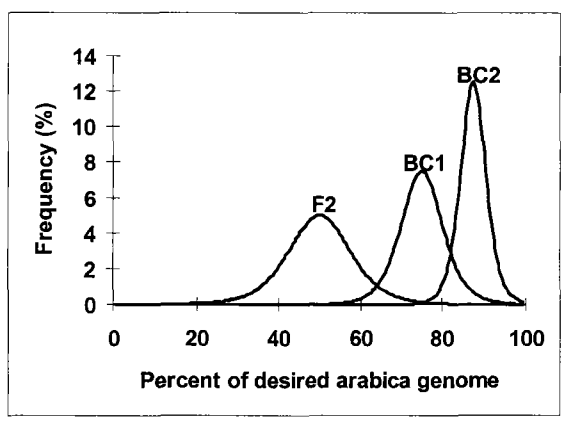

Figure 2. Frequency of individuals in F2, BC1, and BC2 having various contents (%) of the desired parental genome.

A genome selection could therefore be performed by the use of markers scattered throughout the genome resulting in a reduction of the number of backcross generations required to restore the genetic background of the recipient cultivar. Values were estimated (Fig. 3) for a hypothetical arabica genome of 22 chromosome pairs of, on average, 100 cM each (Total genome of 2200 cM) and using equations developed by Hillel et al. (1990) and Hospital et al. (1992). In the absence of selection, parental donor DNA is only removed by a factor of two in each generation. Simulations are given for MAS programme in which the either 10 or 2% best (in terms of percent recurrent parent genome) individuals in each generation were used as the parent for the next generation. Results equivalent to BC5 generation without selection are obtained after only two marker-assisted BC generations allowing an considerable

time saving. Table 1 presents the results of various levels of selection imposed on BC1 and BC2. It is apparent from this table that moderate levels of selection in both BC1 and BC2 result in individual almost identical to the recipient variety. Achieving a similar result by a unique genome selection in one of these two generations requires extremely intensive selection effort.

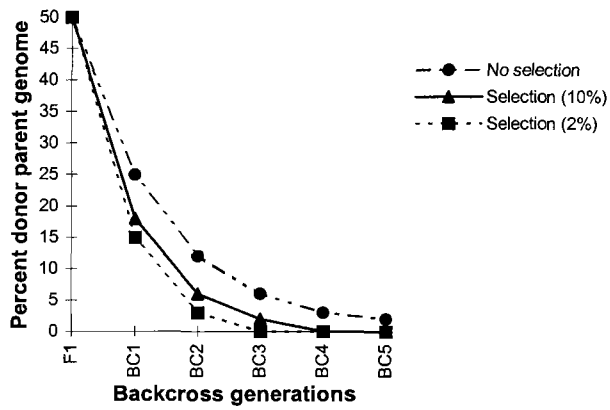

Figure 3. Average content (%) of the donor parental genome in backcross generations under various intensities of genomic selection.

	Selection in BC_1 (%)			
Selection in BC_2 (%)	100	30	10	2
100	87.5	89.7	91.0	92.0
30	90.9	92.4	93.2	94.1
10	92.5	93.6	94.5	95.1
2	94.1	94.9	95.5	96.1

Table 1. Percentage of the recipient arabica genome under various intensities selection in BC_1 and BC_2.

Reducing linkage drag

Removing of the linked donor segment could take many generations (Stam and Zeven 1981). Many examples of "linkage drag" are known in which undesirable traits that are closely linked to a target gene are carried out along during breeding programme (Zeven et al. 1983, Young and Tanksley 1989).

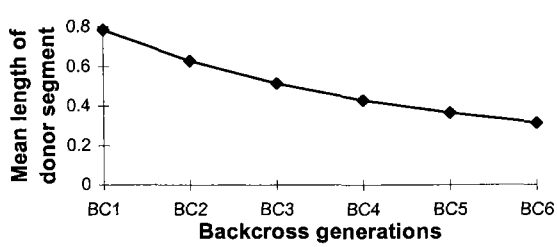

Figure 4. Mean length of donor segment surrounding the target gene after various numbers of backcross generations The length is expressed as a proportion of the carrier chromosome (chromosome of 100 cM long) (after Stam and Zeven 1981).

For instance in Arabica, even after 6 backcross generations, a region of 32cM flanking a target gene is expected to persist (Fig. 4). In most plant genomes 32cM is enough DNA to contain hundreds of genes. DNA markers can be used to eliminate, or at least significantly reduce, linkage drag by allowing the identification of rare recombinant individuals which are usually only selected by chance in classical breeding (Paterson et al. 1991). In approximately 150 backcross plants there is a 95% chance that at least one plant will have experienced a crossover

within 1 cM on one side or the other of the gene being selected. With one additional backcross generation of 300 plants, there would be a 95% chance of a crossover within 1 cM of the other side of the gene, generating a segment surrounding the target gene of less than 2 cM.

3-3 Genetic mapping

Some utilisations of MAS presuppose the existence of a detailed linkage map which represents the relative order of genetic markers, and their relative distances from one to another, along each chromosome of an organism (Paterson et al 1991). A genetic map of the coffee genome is being constructed using a random population derived from the clone IF200 of *C. canephora* (Paillard et al. 1996). So far, more than 150 markers have been placed on 15 linkage groups (Fig. 5). The development of this genetic linkage map will bring important informations on coffee genome and chromosomal organisation. In particular, one might use it to map important genes as recently done for the S-locus controlling self-incompatibility (Lashermes et al. 1996c).

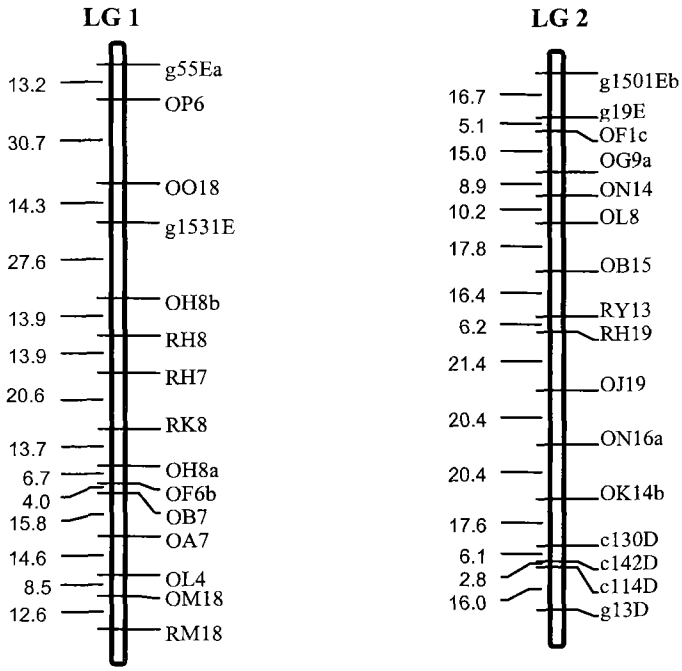

Figure 5. Linkage groups 1 and 2 of the genetic map of *C. canephora* (Paillard et al. 1996).
Map distances in cM are indicated on the left side of linkage groups and marker locus name are on the right side.

4- Example: Breeding for resistance to Coffee Berry Disease

Coffee Berry Disease (CBD) is the main constraint to sustainable and economical production of Arabica coffee in Africa. Based on the seedling inoculation method (Van der Vossen et al. 1976) and field expression of resistance on mature trees, three genes of resistance have been identified in the varieties Rume Sudan (*R* and *k* genes), Hibrido de Timor (*T* gene) and K7 (*k* gene) (Van der Vossen and Walyaro 1980). While K7 is a Kent type commercial variety, other resistant donors (Hibrido de Timor, Rume Sudan) correspond to exotic germplasm where the valuable resistant genes are associated with undesirable traits (Walyaro 1983). Efficiency of the seedling inoculation method becomes limited when a breeder is interested in accumulating a number of resistance genes into an improved cultivar, since this would require test crossing. Given the long generation cycle characteristic of Arabica coffee, the test cross approach is highly time-consuming and thus represents a real bottleneck to rapid development of varieties resistant to CBD. A study was therefore undertaken (Agwanda et al. 1997) to identify 1) molecular markers (random amplified polymorphic DNA, RAPD) associated with CBD resistance and (2) markers which could be used to select against the genetic background of the resistance donors. The initial evaluation involved 7 genotypes representing 5 varieties either resistant or susceptible to CBD. More

than 280 primers producing approximately 2200 distinct amplification products were assayed. The large number of identified marker-bands specific to the resistant genotypes but not concerned with CBD resistance may be used as basis for selecting against the genetic background of the donor parents. Three of the markers specific to resistant Catimor type were assumed to be tightly associated with the *T* gene based on their co-transmission with CBD resistance in the BC_1 and BC_2 generations. Attempts to identify markers associated with the *R* and *k* genes were less rewarding. Efforts are currently directed to map the T-gene on the Canephora genome. The availability of *T*-linked markers represents a starting point in the use of markers to enhance backcross programmes in Arabica coffee.

5- CONCLUSIONS AND PROSPECTS

The development of molecular markers in coffee trees, has opened new perspectives in breeding. The conventional selection of self or back-crossed coffee-tree progenies for further breeding is extremely laborious and time-consuming. The implementation of MAS is therefore very promising. In particular, the integration of MAS in coffee breeding, promises to drastically increase the efficiency of breeding programmes by 1) allowing for selection at an early stage and on a large number of breeding lines, 2) reducing the number of backcross cycles required to restore the quality of the traditional cultivars, 3) combining in one-step, selection for various traits or genes of resistance.

Monitoring of gene introgression from wild coffee species to *C. arabica* especially *C. canephora* and Timor Hybrid derivatives should be considerably facilitated. Regarding the utilisation of genetic resources offered by the spontaneous Arabica accessions collected in Ethiopia, a limitation in the use of MAS is the reduced polymorphism exhibited by the current molecular techniques. However, efforts are directed to resolve this problem through a recently EC-funded project (ERB3514PL961462), and it is anticipated that more cost-effective and simpler laboratory method will be developed.

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Summary

In recent years, the development of molecular markers techniques has allowed new opportunity in plant breeding. The conventional selection among breeding populations of coffee is extremely laborious and time-consuming, and Marker-assisted selection (MAS) could overcome most of the current limitation. In view of the genetic characteristics and breeding strategies of coffee-tree species (*Coffea arabica*, *C. canephora*) different MAS approaches can be implemented. Preliminary results obtained for Coffee Berry Disease (CBD) resistance in Arabica coffee are given as illustration.

ENVIRONMENTALLY FRIENDLY AND SUSTAINABLE COFFEE MANAGEMENT

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INTRODUCTION

The two most important species of coffea; arabica and canephora (robusta) comprise about 70% and 30% of the world productions, respectively. Other minor species of coffea are also cultivated which include *Coffea liberica* and *Excelsa*. Arabusta hybrids have also been introduced to several countries from the 1960's though on a limited commercial level. The world production trend between 1990/91 to 1995/96 is depicted in table 1. This high production leads to soil nutrient exhaustion through mining by the coffee which is not returned and possibility of serious enviromental pollution through pesticides use and processing effuluents and the byproducts.

Production of *Coffea arabica* in Africa has doubled since the 1960's due to emergence of new producers in Southern Africa such as Malawi, Zimbabwe and Zambia and growth in traditional Arabica countries of Burundi, Kenya and Rwanda. Production of Robusta however, stagnated during the same period due to production decline in Angola and currently due to the turmoil in Central Africa- the great lakes region. Some countries which were solely Arabica or Robusta have also started producing both such as Kenya, Uganda, etc. Increased production from the Asian countries such as Vietnam, Papua Newguinea has also been realised.

Coffee plays a vital row in the economic development of the producing countries, being one of the main foreign exchange agricultural commodity earner. Countries like Uganda, Rwanda and Burundi derive more than 80% of their total export earnings from coffee (Table 2). Therefore, coffee is an extremely important generator of employment and of an overiding importance to the social structure and development of the coffee growing countries. Furthermore, the small- Scale producers present a majority in the world and generate most of the jobs and manage most of the earth's natural resources

It is therefore, important that efforts should be made to sustain and improve the coffee industry in terms of increased production and quality. Towards this effort, coffee researh has

been intensified in areas of cultivation techniques, crop protection, primary processing, use of coffee by-products and improvement of genetic material towards disease and pest control and improved liquor quality in most of the countries. Coffee is grown in the high potential regions of the world suitable to other agricultural enterprises. This calls for efficient utilization of the land while at the same time avoiding environmental degradation. Increased production should therefore be coupled with environmental sustainability in this vastly small-holder business which hold most of the natural resources.

AGRONOMIC PRACTICES

Both Arabica and Robusta are tree crops which produce yields two to three years after planting with a long economic life beyond 30 years depending on local conditions and husbandry. Each requires different growing coditions with *C. arabica* preferring temperatures of 15-24^{0}C while *C canephora* prefers warmer conditions of 24-30^{0}C with less contrasting dry and rainy seasons. Both require an average rainfall of 1800 mm per annum for healthy growth and satisfactory productivity. The best coffee growing areas of Africa has more than 1500 mm of rainfall annually well distributed throught the year with a drier period of two to three months when growth slows, young wood harden and flower buds develop. The following is a brief mention of the various agronomic management aspects of *Coffea arabica* which to a large extent is also followed for the *Coffea canephora*:

Establishment

Coffee seedlings are usually raised from seed or as rooted cuttings in the nursery or through current advances in biotechnology like tissue culture and grafts. After germination or establishment of the cuttings/tissue culture material, they are transplanted (potted) into black polythene bags measuring 23 x17 cm to 30 x17 cm, previously filled with a mixture of top soil, manure, phosphatic fertilizer and a suitable insecticide. They are ready for field transplanting in 12-18 months after potting. The most enviromentally sound insecticides should be used avoiding insecticides like aldrin. Use of biological, cultural and physical control of insects should be encouraged at both nursery and the field. Insects like the giantlooper can be controlled physically.

In the field, seedlings should be planted in a well prepared land free of tree stumps in order to avoid soil born diseases such as *Armillaria mellea* which affect coffee trees and removal of difficult weeds such as couch grass for good management of the young seeedlings. Suitable soil conservation measures such as bench terraces whose terrace sides should be well protected with grass or suitable trees such as the multipurpose trees e.g *Leucaena, Sesbania, Caliandra*, etc. Planting holes are dug three months before planting along the contour to minimise soil borne diseases e.g *Fusarium spp* and soil erosion control, respectively, and then refilled one month before planting with top soil mixed with well rotted organic manure, phosphate fertilizer (type depending on acidity of the soil) and an appropriate insecticide. Agriculture lime can also be added depending on the soil reaction. The seedlings are planted after the onset of the rains. Application of mulch along the planted coffee row or around the seedlings helps to preserve moisture and suppress growth of weeds

apart from soil conservation and improvement of soil structure. The type of mulch material is important as continued use of one type may cause soil nutrient imbalance. Napier grass mulch can increase soil -k over along period of use thereby disterping the Ca+Mg/K ratio and may need soil amendments.The Ca=Mg/K. ratio is important in maintaining the coffee been quality. Table 3 gives nutrient analysis of some of the possible mulching material and organic manures. The challenge to the farmer is source of mulching materials as land suitable for planting mulch materials has become scarce.

The common coffee spacings for the traditional cultivars are 2.74 x 2.74 m (1329 trees per hectare), 2.74 x 1.37 m (2658 trees per hectare) and 1.37 x 1.37 m (5320 trees per hectare). For the compact cultivars like Ruiru 11, the spacing adopted is 2 x2m (2500 trees/ha) and or 2 x 1.5 m (3,333 trees/ ha) (Njoroge, 1991) or 1 x 1m for Catimor and Colombia cultivars in Colombia. Large coffee estates sometimes uses different spacings while maintaining the above tree densities, to facilitate efficient use of machinery for the different farm operations. High densities do assist in soil conservation especially through rain drop impact, control of run off through litterfall and weed suppression. Due to the good soil service cover, most of the solar energy is utilized by the coffee trees while the well distributed mass of roots utilize effectively the soil nutients. This means no increase of nutrient demand with increase in tree density. During the first two years of coffee establishment before the canopy closes up, nurse crops or intercrops can be introduced in the coffee inter-row spaces. These help soil conservation, weed suppression and an economic return to the farmer when an economic annual crop is used apart from the food security. Early maturing annual crops such as legumes, cereals and vegetables can be used such as non climbing beans, peas, tomatoes, Kales, carrots, irish potatoes, soya beans, millet, sorghum, etc depending on the ecological zone where the food crop can do well. The legumes and non-legumes should be alternated seasonally or on alternate coffee inter-rows. Cover crops such as Desmodium sp,sweet potatoes may strangle the young seeedlings though they protect the soil.

Nutrition

Most of the soils on which coffee is grown have low plant nutrients especially nitrogen. On average, one tonne of coffee beans can remove 46kgN, 8Kg P_2O_5 and 38Kg K_2O; parchment 2.3kg N, 0.3kg P_2O_5 and 1.9k$_2$O; Pulp 15.3kgN, 3.7kg P_2O_5 and 27.4kg k$_2$O. This means that Kenyan coffee soils producing about 100,000t/ annnum clean coffee are mined on average 4.6m tN, 0.8mt P_2O_5 and 3.8mt K_2O which is annually exported to the consuming countries ! This together with losses through parchment, pulp, erosion and leaching leaves the soils seriously exhausted. It is therefore necessary to return some of the losses through re-cycled prunings when used as mulch. Hence fertilizers are needed for both vegetative growth of tree and production of high quality coffee beans. In order to apply the correct type and rate of fertilizer and thus avoid toxicity and nutrient imbalances in the soil environment, fertilizer recommendations should best be based on soil and coffee leaf analysis results where the necessary facilities are available.

Nitrogen is the most limiting element, and arabica coffee

has responded positively to nitrogen application rates of 50-100 kg N/ha/year in kenya (Njoroge,1985). Responses of 300-400kg N/ha has also been recorded especially under very high yields and in soils with the right soil reaction for coffee. The proportion of large sized beans has been shown to decrease with increased nitrogen rates of application unless balanced with phosphate fertilizer (Njoroge, 1985). Better response to nitrogen have been observed with split application than with single dose application (Njoroge, 1985). This would also reduce leaching of nitrogen to underground water thereby polluting the surface and ground water.Studies in Kenya have also shown no positive yield increases to phosphorous application alone despite observed low soil phosphorous (Keter, 1974). The soils are however in most cases well supplied with potassium which is a major nutrient in coffee production especially during berry expansion period. The types of fertilizers used include straight, compound and foliar fertilizers. Studies on inorganic fertilizers should aim at minimising excess nutrients especially nitrogen in the soil to avoid it being washed or leached to surface or underground water.

Organic manures, mainly cattle manure has been used fore a long time in coffee. The organic manures increase the soil organic matter, thus imroving the water holding capacity and physical characteristics of the soil and release plant nutrients on decomposition (Oruko, 1977). Their use has been reported to lead to increased coffee yields and quality especially on very poor soils (Mitchell, 1970).

As the organic manures are formed from different sources they have varying nutrient composition, and their continous use may lead to nutrient imbalances which may affect the coffee bean quality (Northmore, 1965). However, because they may be cheap and readily available, organic manures can be used to substitute to some extent inorganic fertilizers and thus reduce production costs. More research should be geared towards evaluation of response of coffee to organic manures, organic to inorganic fertilizer substitution ratios and the use of green manures as sources of plant nutrition in coffee. This would also go towards reduction in over-reliance on imported fertilizers. Increased use of organic manures would improve soil fertility as a whole especially of the degraded and overused soils in the coffee growing zones. Studies in kenya has shown the possibility of substituting the inorganic fertilizer requirements in Kenyan coffee with two 13kg tins of well rotten cattle manure per annum. However, this would largely depend on the source of the manure and this calls for nutrient analysis of the source of the manure and this calls for nutrient analysis of the manures (chicken, pig, goat, etc) decomposed coffee pulp, sludge from methane gas plants, etc are increasingly being utilized . Results from kenya indicating possibilities of using green manures as source of coffee nutrients by use of plants such as lucerne (*Sativa medicago*) multipurpose trees such as *Lucerne spp., Sesbania spp, Caliandra spp*, etc

Green manure from permanent cover crops such as *Desmodium spp* appear to mineralize very slowly and may not be very useful in the short run but can be used to control soil erosion on slopy coffee farms. Planting the multipurpose trees for this purpose on the bench terraces, waste lands may also imrove sustainability of the soil environment. Most of the coffee zones are in the high potential agricultural land and has been extensively and

intensively cultivated such as Central Kenya leading to soil erosion and decline in cations and organic matter. Studies therefore need to be geared towards restoration of organic matter and improvement of the soil reactions.

Weed control

The weed species in coffee can be classified into annuals, perennial and sedges. Weeds have been shown to reduce coffee yields by over 50% as well as the coffee quality (Njoroge and Kimemia, 1989)as compared to clean weeding . Due to this reduction of yield by weeds, various weed control methods are used. The most common methods used are digging using forked hoes, slashing, mulching and the use of herbicides. There is a wide range of herbicides used in coffee (Njoroge, 1994) which include contact, systemic and soil acting herbicides. Some of those used in Eastern Africa coffee plantations are depicted in Table 4.

The use of low rates and volumes of recommended herbicides can be used effectively to control annual weeds in coffee at the 1-4 leaf stage using low volume nozzles (Njoroge and Kimemia,1992).Continued use of this technology will reduce the amount of herbicides released to the environment thereby reducing environmental pollution. The use of one type of herbicide has led to development of herbicide tolerance by some weeds like the tolerance of black jack (*Bidens pilosa L*) to paraquat observed in Kenya. Integration of the different herbicides and other methods of control is therefore recommended. Continued evaluation of new herbicide products especially against the difficult weeds such as *Oxalis spp., Cyperus spp.*, etc and diffferent methods of using the already existing ones is emphasised. slashing creates a carpet of weeds which may help reduce soil erosion at the middle of the rains while forking may encourage rain water acceptability reducing run off. Long term approach to weed management through integrated weed management (IWM) would be the best option for efficient weed management and reduction in environment degradation through soil erosion, nutrient leaching and environment pollution. Transfer of the correct control technologies to the farmer is a major component to this. This would entail eg. collect herbicide application technologies, rules of application etc to be applied leading to a reduced environmental pollution. Research should therefore be aimed at minimising weed tolerant to herbicides and avoidance of excess herbicides to the environment.

Mulching

Mulching is the covering of the soil with a layer of dry vegatation materials. The benefits are soil moisture preservation for better nutrient absorption by plant, prevention of soil erosion, thereby avoiding delivery of nutrient, pesticides, etc to surface and underground water, improvement of soil structure, supply of mineral nutrients on decomposition, minimizing use of inorganic nutrients, regulation of soil surface temperature, suppression of weeds leading to reduced herbicide use and reduction of thrips incidence thereby reducing insecticides use. Due to these benefits, mulch help to increase coffee yield and quality. The main mulching materials are napier grass, maize and banana stover, coffee prunings and any other dry vegetation material. However due to declining land sizes the area for growing mulch material has became less and hence use of mulch is

mainly limited to the large estates sector. The mulch material is applied in alternate coffee interrows and then alternated in the following years. There is no doubt that mulch does enhance environment sustainability. Use of life mulch such as *Desmodium spp.* may be very useful especially on slopy grounds with main shortcoming being low biomass production, possible moisture competition with coffee trees (Njoroge and Mwakha, 1982; Snoeck, *et al.*, 1994). Desmodium also mineralise very slowly.

Pruning

Coffee pruning involves removal of unwanted branches and removal of old items. The main reasons for pruning coffee trees are to maintain a suitable crop/leaf ratio for good cropping level and maintenance of a high proportion of large beans, open the tree centres to light, facilitate disease and pest control, as well as harvesting. This leads to efficient utilization of the sun's energy by the coffee plant and chemical used to control the pests thus avoiding excess pesticides in the environment.

The pruning system depends on the type of tree training adopted. Training is the modification of the natural habit of the coffee trees to suit the particular conditions under which they are grown. There are basically two training systems: single sytem system; and multiple stem system which is either capped or uncapped. The change of cycle to raise new stems is carried out after six or seven years. This improves coffee quality and less disease problems as the trees are healthier leading to less use of pesticides. Most smallholders allow their coffee to grow freely without capping, while most largeholders cap their coffee for ease of mechanisation. The compact cultivars like Ruiru 11 hybrid is currently recommended at single stem but require 'stumping' after every 5-7 years, to replace the old stems with new ones (Njoroge, *et al.*, 1992). Little pruning is carried out in some countries in the Southern Africa where foliar disaeases pose no major problem and they prefer replanting rather than raising new stems at the time of change of cycle. Prunings are best left in situ to act as mulch which would return nutrients to the soil. Hoewever where fuel energy is increasingly becoming scarce these prunings are used as firewood.

Intercropping and shading in coffee

Coffee is mainly grown as a monocrop in most countries, the main reason being that quality of coffee might be affected adversely if farmers ignore coffee in favour of intercrops. These could be due to competition for nutrients, water and light between coffee and the intercrops. However, coffee farmers particularly the small scale farmers have been intercropping their coffee various food, fruit and tuber crops especially at the establishment and change of cycle periods and even during the production phases. Large scale estates have also been observed to move in this direction.

Since coffee occupies a substantial amount of the high potantial land, available land for food crop planting is becoming limited and hence more intercropping is expected to occur in most countries as in Kenya. In indigenous home of coffee, Ethiopia, coffee is mostly grown in a multistorey cropping system with trees in the upper storey followed by coffee along with food crops such as maize, sorghum and legumes such as beans, peas and lentils while the ground floor is covered by root crops such as

yam, taro, vegetables such as cabbages and peppers, and spices such as ginger and cardamom(Awore, 1997). In kenya preliminary results have indicated that it is possible and economical to intercrop young arabica coffee with some food crops during the first two years after establishment (Njoroge, *et al.*, 1993). It is also possible to intercrop coffee with dry beans during the change of cycle phase (Mwakha, 1980). More studies are encouraged on this line in order to maximise on land available, improve food availability and higher incomes. This would also help to sustain the coffee and and farmers in periods of low coffee prices. Intercropping also assist in protecting the soil from vagaries of soil erosion before the coffee canopy closes up, better utilization of the sunlight energy and better weed management.

Several tree species have been grown in coffee mainly as shade trees or as wind break, such as *Cordia spp., Grevillea robusta, Albizia spp, Leucaena leucocephala*, and *Cypress spp* (Njoroge and Kimemia, 1993). In Ethiopia, indigenous trees such *Albizia gummifera, Allophylus abyssinica, Celtis africana, Cordia africana, Ekebergia capensis, Ficus sur, F. sycomorus, F.vasta, Milletia ferruginea, Macaranga Kilimandscharica, Croton machrostachys* are left as shade trees (Awoke, 1997). Use of shade trees have been shown to help even out erratic yields caused by periodic overbearing and also reduce crinkling of coffee leaves commonly known as 'hot and cold' disease apart from hail damage. Shade has also been shown to reduce infection of Bacterial Blight of coffee (BBC) due to reduced hail injury on the coffee trees thereby reducing pesticide usage. Further research is needed into suitable trees of economic value and the effect of shade on coffee trees as the original introduction of shade trees in coffee was not preceded by such studies. It was taken that coffee needed shade being an understrorey plant in the centre of origin. Apart from the above, shade trees help to recycle soil nutrient deep in the soil to the coffee rooting top soil through litter fall, leguminous trees fix atmospheric nitrogen, assist in controlling soil erosion, weed control and encourage rainfall. In a multistorey intercrop systems, most nutrients are held in the vegetative mass which is returned to the soil with litter fall. This system also may utilize the soil more effficiently.

Diseases and Pests

The major C*offea arabica* diseases are Cofffee Berry Disease (CBD) caused by Colletotrichum Kahawae in East Africa, Coffee Leaf Rust (CLR) caused by Hemileia vastatrix Berk et Br. in most of the coffee growing regions and to alimited extent Bacterial Blight of Coffee (BBC) caused by Pseudomonas syringae van Hall mainly in Kenya. There are other minor diseases which have tended to disappear with improved cultural practices on coffee farms (Masaba et al.,1986). All these diseases are controlled effectively by use of fungicides and bactericides. Control of BBC by use of copper based fungicides is not very effective and efforts are needed in searching for efficient control of this disease . the new arabica cultivar developed in Kenya, Ruiru 11 is resistant to CBD and CLR but prone to BBC. The recentLy introduced Colombian cultivar is resistant to CLR. The introduction of the disease resistant coffee cultivars have contributed significantly in reducing coffee production costs and environment pollution through reduced use of fungicides. Efficient spray technologies, appropriate chemicals and cultural

practices need to be well passed to the farmer to minimise excess chemicals to the environment. This is particularly so in the high CBD, CLR and BBC prone areas. The high annual amount of copper fungicides needed to control BBC and CBD may cause high copper accumulation in the soil rendering intercropping coffee with copper sensitive crops impossible. How much of these chemical go to the environment through the air and surface run off to river water need to be acertained. Most of the coffee farms are on slopy grounds leading to water streams whose water is consumed down stream. Studies on biological and cultural control of these diseases apart from breeding for resistance need to be intensified.

There are a number of coffee insect pests and most of these pests are controlled only when they exceed economic thresholds. The control methods range from sanitary, cultural, insecticides and biological control. Use of insecticides to control pests is not only expensive, but also insecticides destroy natural enemies of some of the coffee pests and the environment pollution. Attention has now been focused on Integrated Pest Management for many coffee pests. The control of coffee mealybug (Planococcus kenyae) by breeding and dissemination of its natural parasites, *Anagyrus spp.* is one of the show cases of biological control in Africa (Masaba *et al.*, 1986). Other cases include control of coffee scales by use of ladybird bugs, and control of giant looper (*Ascotis selenaria reciprocaria*) by *Macroharphis acuta*. Control of antestia bug by Antestia egg parasitoids and leaf miner by leaf miner parasitoids. More work is still in progress to find natural parasites and parasitoides of the common coffee pests in Kenya. Integrated Pest Management approach is recommended in Kenya (Anon, 1992). I think the main thrust of research should be directed towards biological and cultural control within the integrated pest management concept.

Processing practices

Majority of the Arabicas are wet-processed and to produce high quality coffee, careful processing should be practiced. This starts in the field where only the ripe cherry is picked. The harvested coffee is then sorted at the factory before pulping. The over and under ripe cherries, diseased, insect damaged cherry, foreign particals, plant debris are all removed. The coffee is then graded to cherry 1 (slight yellow to an overall red) and cherry 11 (yellow,draughted, under-ripe and over ripe cherry) and the two grades are pulped separately. The remainder which includes the green, rotten, dry, diseased and insect damaged cherry is dried as buni. The cherry is then pulped as soon as possible after harvesting. During pulping the coffee parchment is graded by density into three classes- firsts, seconds and lights.

The pulped coffee is put into fermentation tanks for 16 to 20 hours, to break the mucilage covering. Thereafter, the coffee is washed with clean water and then soaked in water for 16-24 hours. Coffee drying is very vital and has a profound effect on quality.

There are six distinct stages of coffee drying:

(i) Skin drying stage: In this stage moisture content is reduced from 55%-45%. This drying should take the shortest time possible (preferably 3 hours) otherwise the parchment develops sourness (onion flavour) which

lowers quality.

(ii) White stage: In this stage the moisture content is reduced from 44%-33% . It requires slow controlled drying to avoid cracking of the parchment.

(iii) Soft black drying stage: The moisture content is reduced from 32-22%, and MUST be done in the sun for 48 hours.The sun rays are believed to influence some chemical changes that improve liquor quality.

(iv) Medium black drying stage: The moisture content is reduced from 21%-16% . This is fairly a stable stage and the coffee can even be heaped together to 55mm or put in well ventilated bins to ease congestion at the drying tables.

(v) Hard black stage : The moisture content is reduced from 15% -12% .The coffee bean is now hard and can be dried quickly without any serious consequences.

(vi) Fully dry and conditioning . The coffee is dried on wooden floors or ventilated conditioning bins and the moisture content is reduced from 12% -10.5% . Dry air is fanned into the coffee to even up moisture. The coffee is then stored in well ventilated bins or wooden floors for about 4 weeks in order for the coffee to 'Mature', but not more than 6 months.

The parchment is put into sisal bags, in predetermined grades as parchment 1 or parchment 2. It is then delivered to hulling stations for hulling. The parchment is hulled to clean coffee loosing about 20% by weight.The clean coffee is graded by size and density into about 6 grades, namely AA, AB, PB, TT, C and T.

Samples of the graded coffee are taken for cup quality testing by liquorer and exporters also get samples for their own liquor tests before purchase. The coffee is sold to the coffee dealers in an auction or through direct sales. Coffee marketing in most of the countries is now getting liberalised with the development of more free markets in the region but at a controlled level to make sure the farmers leap maximum profits.

The byproducts of coffee processing are mainly coffee pulp, processing effluent and coffee husks . Due to the contribution of these byproducts to environmental pollution, effective environmentally friendly disposal methods and /or utilization of these byproducts to enhance economic returns to the farmer should be encouraged. Some of the alternatives of the byproducts usage include fertilizers, animal feed, alcohol, biogas, caffeine, sugar, pectines, chacoal, heat energy, mulching, wax, softboards, light weight structural materials, horticulture and acids.

In production of mild arabica coffee, coffee pulp, processing effluent and parchment husks are the major byproducts. Gemeil (1996) noted that processing 547,000 tonnes of green coffee in Central America generates 1.1 million tonnes of pulp per year resulting to water pollution equivalent to that generated by the urban areas of the major cities in the area!

In Costa Rica, coffee production is said to generate more pollution than any other sector in the economy as 57% of the coffee bean is made up of contaminants whose pulp do effect marine life in parts of the pacific ocean near the river mouths (Loria,1992). Pulp can be utilized as a fertilizer and soil conditioner. These assist soil water permeability,retention and addition of nutrients apart from animal feed, alcholic products,

caffeine extraction and pectines. The mineral and other constituents of the coffee pulp is given in tables 3 and 5

Water recirculation in the processing factories, treatment in seepage tanks before discharging it into river should be mandatory. Even after recirculation, treatment in seepage tanks, the polutants reaching the water streams need to be certained in order to re-assess the effficiency of this system. These enhance better coffee fermentation and reduction of water pollution apart from efficient water use. ln Central America, improved water treatment and recycling have reduced the amount of water use in cherry pulping and washing operations by two thirds (Geneil, 1996) while in Kenya, this reduction is more than 50% (Mburu and Mwaura, 1996). Pectines can also be extracted from the processing waste water.

Parchment husks can be disposed of by being used as heat energy for industrial boilers, domestic cooking, as mulch, other products such as soft boards, animal feed, wax, extraction of acetic acid. Coffee husks from dry processing can be used as mulch, and as chacoal which can assist in conserving the natural forests for protection of our bio-diversity

CONCLUSSION

Improved land preparation, coffee establishment, soil conservation measures, integrated pest management, increased use of organic nutrient sources, biocontrol of pests, diversification through multiple cropping, efficient use of processing water and appropriate utilization of coffee processing byproducts; appropriate and efficient technology transfer to farmers is of critical importance. lntroduction of environmentally friendly cultivars such as Ruiru 11, Colombia, etc. These would lead to reduced negative environmental impact and improved sustainable coffee management environment.

God created unpolluted earth and man was given the responsibility to protect and sustain the Gods intended creation through environmentally friendly and sustainable practices. Let us give our input through maintenance of sustainable coffee management.

ABSTRACT

The paper briefly gives an overview of agronomic practices from nursery to processing and tries to identify areas requiring attention in order to attain environmentally friendly and sustainable coffee management. Provision and transfer of efficient and appropriate crop protection techniques to farmers in order to minimise excess chemicals to the environment in an integrated crop protection system is emphasised. Farm practices leading to restoration and mainteinance of the soil organic matter, soil reaction, control of soil erosion such as, use of organic matter, green manuring, cover cropping, mulching, soil conservation, efficient processing, utilization of processing byproduct and intercropping systems .

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Table 1: World total production by group of coffee.

	Million bags					
	1990/91	1991/92	1992/93	1993/94	1994/95	1995/96*
All members	93.3	101.5	87.5	90.6	96.5	85.7
Colombia milds	16.0	19.8	16.0	14.2	15.1	14.4
Other milds	25.2	25.7	25.6	24.3	26.3	27.4
Brazilian and O.Arabicas	25.3	25.4	22.8	24.1	25.5	16.5
Robustas	26.8	30.6	23.1	28.0	29.6	27.4

*Preliminary estimates
60 kg = 1 bag
Source : ICO 1996. Coffee Newsletter No. 3

Table 2: Coffee as a percentage of total exports by value in some African countries, 1985-1989.

Producing country	Uganda	Rwanda	Burundi	Ethiopia	Tanzania	Madagascar	Cameroon	Kenya	Central Africa	Zaire
Average 1985-1989	94.9	90.7	81.9	59.2	42.5	31.1	29.5	29.0	27.1	20.8

Source: International Coffee Organisation (ICO)

Table 3: Nutrient content of organic manures and mulches.

	N	P_2O_5	K_2O	CaO	MgO	SO_3	B	Cu	Fe	Mn	Zn
			percent						ppm		
Boma manure	1.32	1.08	1.66	0.92	0.35	0.45	25	68	27500	916	99
Cattle manure	2.50	1.12	6.70	1.43	1.00	0.37	31	45	11200	1040	95
Coffee husks	0.48	0.07	0.40	0.31	0.08	0.15	4	96	100	32	52
Coffee pulp	3.73	0.40	6.51	0.99	0.30	0.85	18	35	880	226	18
Goat manure	2.66	3.89	4.87	1.36	1.17	0.37	45	30	1940	256	88
Maize stover	2.11	0.35	1.95	1.08	0.32	0.15	26	8	625	57	8
Napier grass	1.51	0.62	4.23	0.27	0.25	0.32	18	25	1300	157	132
Pig manure	2.34	5.27	0.96	4.23	1.55	0.52	25	211	7100	648	440
Pineapple tops	0.86	0.21	1.69	0.29	0.15	0.20	14	13	100	167	180
Poultry manure	3.54	3.24	1.55	5.06	0.98	0.57	30	190	6030	363	225
Rice husks	0.45	0.39	0.47	0.20	0.12	0.12	11	310	11500	326	142
Rice straw (immature)	1.04	0.39	1.49	0.55	0.52	1.05	19	14	4950	965	59
Rice straw (mature)	2.59	0.69	0.60	0.13	0.45	0.22	16	39	2750	902	51
Sawdust	0.36	0.11	0.16	0.24	0.03	0.10	15	70	5950	314	153
Sisal waste	1.15	0.28	1.52	5.81	0.98	0.22	36	242	1875	279	378
Sugarcane filter mud	1.34	2.38	0.73	3.27	0.36	-	45	60	4695	969	168
Vlei grass	1.39	0.30	1.06	0.66	0.25	0.32	19	72	12950	846	85

Source: Chemistry section, Coffee Research Foundation, Kenya

Table 4: Some commonly used herbicides in Eastern Africa Coffee.

Soil acting	Contact	Systemic
Atrazine (50 and 80 % wp)	Actril DS (70 % EC) (mixture of loxyil and 2,4-D)	Ametryne (80 % wp)
Candex (65 % Wp) (mixture of asulam and atrazine)	Amitrole (25 or 50 % Ml)	2,4-D amine
Diuron (48 % EC, 80 % wp)	Diquat	Asulam (40 % SL)
Flumeturon (80 % wp)	Paraquat	Dalapon (74 and 85 % wp)
Linuron (50 % wp)	Glufosinata- ammonium (20and 14 sl)	Fluazifop butyl (25 % EC)
Oxyfluorfen (24 % EC)		Glyphosate (various)
Simazine (50 and 80 % wp)		Haloxyfop ethoxyethyl MCPA (various) Tordon 101 (picloran plus 2,4-D)

Table 5: Mineral and other constituents of coffee pulp.

Mineral composition %		Other constituents %	
N	1.74	Ether extracts	0.48
P	0.10	Crude fibre	3.4
K	5.26	Crude protein	2.1
Ca	0.48	Reducing sugars	12.0
Mg	0.11	Non- reducing sugars	2.0
		Total pectic substances	6.5

Source ;Melich (1965)

Source; Adams and Douglas (1980)

EFFICACITÉ DE LA SÉLECTION DE GÉNITEURS ET TÊTES DE CLONES POUR L'AMÉLIORATION VARIÉTALE DE *COFFEA CANEPHORA* EN CÔTE D'IVOIRE

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Introduction :

La sélection de *Coffea canephora* en Côte d'Ivoire est basée sur l'utilisation de la vigueur hybride observée lorsque des géniteurs des deux groupes guinéens et congolais sont croisés ; c'est le schéma de sélection récurrente et réciproque (Leroy et al., 1993). Des estimations réalisées à partir du calcul de paramètres génétiques (Leroy et al., 1994) laissent entrevoir des possibilités de gains génétiques importants pour la productivité (jusqu'à 60 % par rapport au meilleur témoin clonal) à travers ce schéma de sélection (Leroy et al., 1997).

La réalisation effective de ces gains génétiques prévus dépend de la qualité de la sélection des géniteurs et des arbres têtes de clones au niveau des dispositifs statistiques et des méthodes d'analyses. Le choix des géniteurs repose sur la performance de leur descendance en croisement avec un ou plusieurs testeurs. La cohérence des performances des géniteurs en croisement avec plusieurs testeurs donnera une idée de l'efficacité de leur sélection. La qualité de la sélection des arbres têtes de clone est évaluée par la relation entre leur valeur individuelle et celle des clones correspondants.

I Sélection des arbres têtes de clones :

I.1 Matériel et dispositif statistique :

Il s'agit de comparer la valeur individuelle des arbres têtes de clone à la moyenne du clone obtenu à partir de chacun de ces arbres-mères.

Deux couples d'essai, dans deux stations différentes de l'IDEFOR/DCC, ont permis de réaliser une telle comparaison. Ces deux couples ne sont pas des répétitions multilocales et ne concernent donc pas les mêmes clones.

Chacun des couples est constitué d'un essai de descendances implanté dans le début des années 80 et d'un essai de comparaison clonale, composé des clones obtenus à partir du bouturage d'arbres identifiés dans l'essai de descendance. Dans chaque cas, le dispositif des essais était une randomisation totale de parcelles mono-arbre à raison de 30 à 55 répétitions par objet comparé (descendances ou clones).

Figure 1 - Comparaison de la moyenne du clone et de la valeur brute de l'arbre tête de clone (Expérimentation Abengourou) (Hg cerises fraîches cumulées sur quatre années de récoltes).

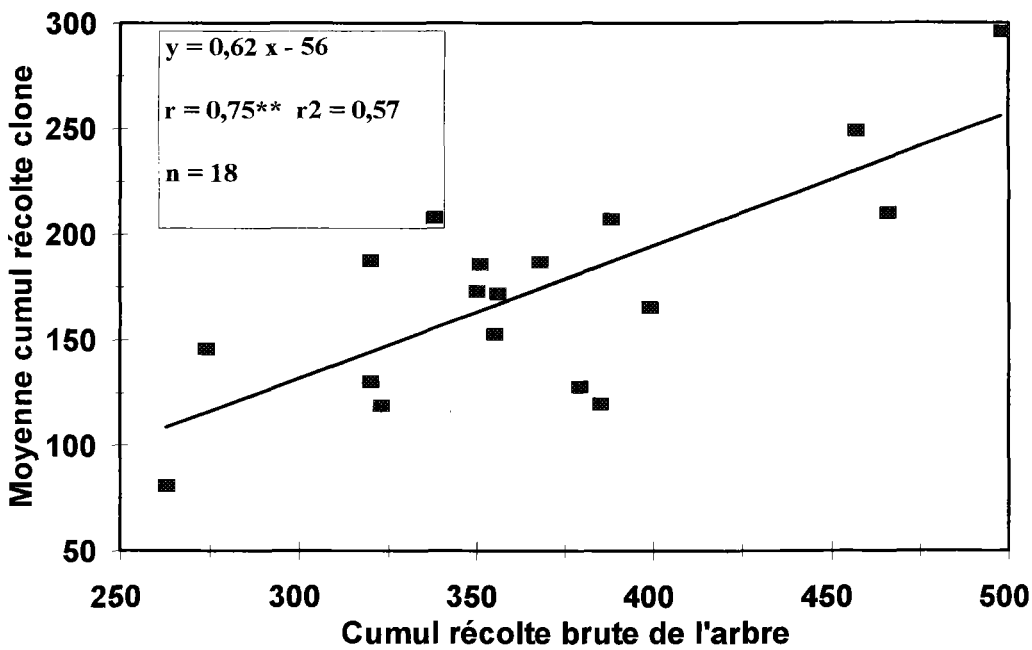
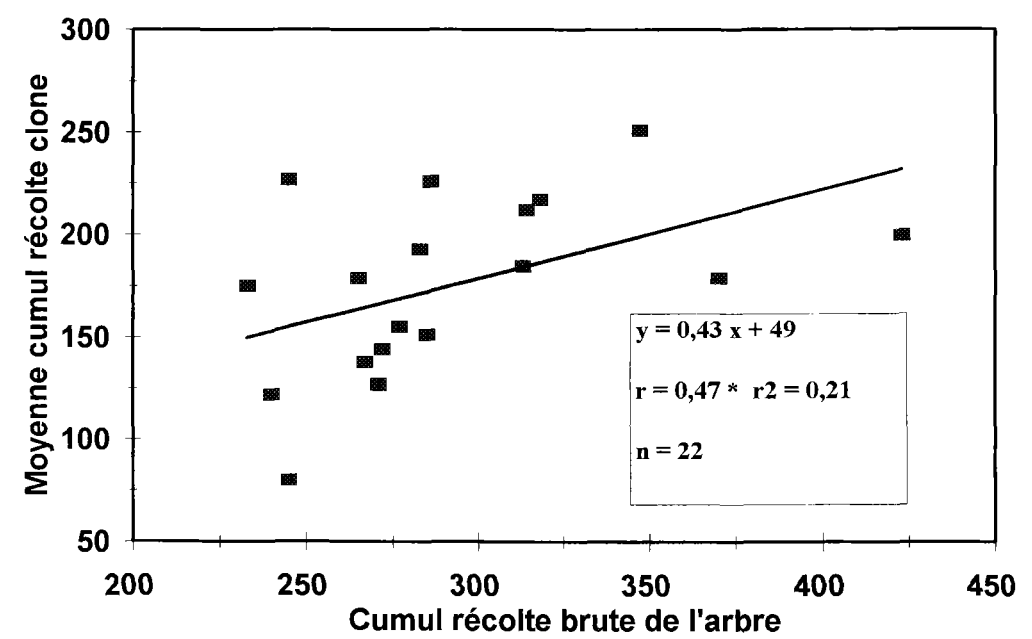


Figure 2 - Comparaison de la moyenne du clone et de la valeur brute de l'arbre tête de clone (Expérimentation de Divo). (Hg cerises fraîches cumulées sur quatre années de récoltes)



Les arbres têtes de clones ont été choisis après l'observation de quatre années de récolte.
Le classement des clones est également réalisé après quatre années de récoltes.

Les couples d'essais ont été implantés sur le même type de sol sur la même station de recherche. Les caractéristiques climatiques des années d'observations de l'essai de descendances et de l'essai clonal sont comparables.

Pour le couple d'essai de Divo, 22 paires clones - têtes de clones ont été comparées. A Abengourou, 18 paires sont considérées.

I.2 Résultats :

Les figures 1 et 2 illustrent la régression linéaire entre les têtes de clone et les clones. La corrélation est hautement significative (r = 0,75) dans l'expérimentation d'Abengourou et significative au seuil de 5 % (r = 0,47) à Divo.

A Abengourou, la relation entre la performance des arbres têtes de clones et les clones est donc très bonne.

A Divo, la faible corrélation s'explique en grande partie du fait que les deux meilleurs arbres têtes de clones ont donné des clones de valeur relativement moyenne. Excepté ces deux points, on constate que les meilleurs arbres têtes de clones donnent effectivement les clones les plus performants. Toutefois, certains arbres têtes de clones parmi les moins performants du groupe étudié ont donné malgré tout des clones classés parmi les premiers.
Ainsi, dans cette expérimentation, un arbre tête de clone performant est une condition suffisante mais pas nécessaire pour donner un clone performant.

Dans les deux cas, la sélection de clones à partir de l'observation de la valeur d'un arbre tête de clone est efficace. Au pire, le risque consiste à ne pas forcément sélectionner tous les clones performants. Cependant, le risque, plus préjudiciable, de sélectionner des clones à faible potentiel en choisissant les meilleures têtes de clones apparaît faible. Ceci est très important pour l'efficacité de la sélection.

La pente de la droite de régression est une estimation de l'héritabilité au sens strict . Les valeurs de 0,62 et 0,43 sont compatibles avec les résultats antérieurs (Leroy et al., 1994).

II Sélection des géniteurs:

II.1 Méthodologie :

Dans le cadre de la sélection récurrente et réciproque, tous les géniteurs des deux populations de base guinéenne et congolaise sont testés en croisement sur deux testeurs de la population réciproque. Les géniteurs sont classés en fonction de la performance de leur descendance en croisement avec les testeurs.

Pour des raisons matérielles, tous les croisements contrôlées ne peuvent être réalisés la même année, ni donc les descendances être plantées la même année dans le même essai. Les rendements sont donc exprimés en pourcentage du meilleur témoin clonal, dont le comportement est bien connu. Ceci permet la comparaison des descendances sur plusieurs années et plusieurs essais.

Bien que le choix des géniteurs soit essentiellement basé sur leur performance en croisement intergroupe, quelques tests ont été réalisés en test intragroupe. La comparaison des tests inter et intragroupes, possible uniquement pour les géniteurs guinéens, donne une idée de l'amélioration des populations en valeur propre par rapport à leur valeur en croisement.

II.2 Cohérence des tests intergroupes sur différents testeurs :

La performance des géniteurs congolais croisés sur l'un des deux testeurs guinéens est tout à fait cohérente avec leur performance croisé sur l'autre testeur (Figure 3). Seul un croisement, sur les trente-sept étudiés, s'écarte

Figure 3 - Comparaison du résultat du test des géniteurs congolais sur les deux testeurs guinéens (155 et 410) (Cumul cerises fraîches sur 4 ans)

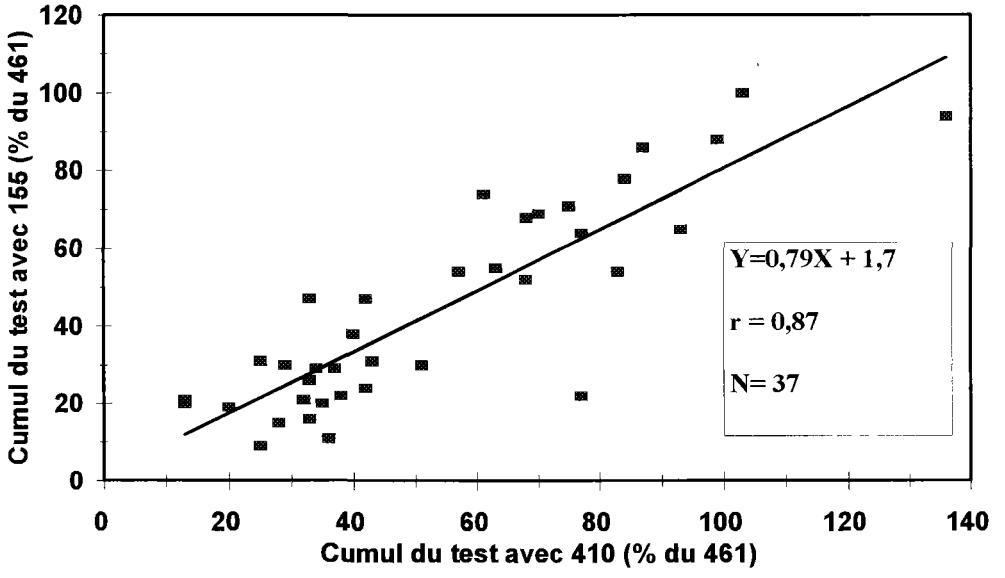
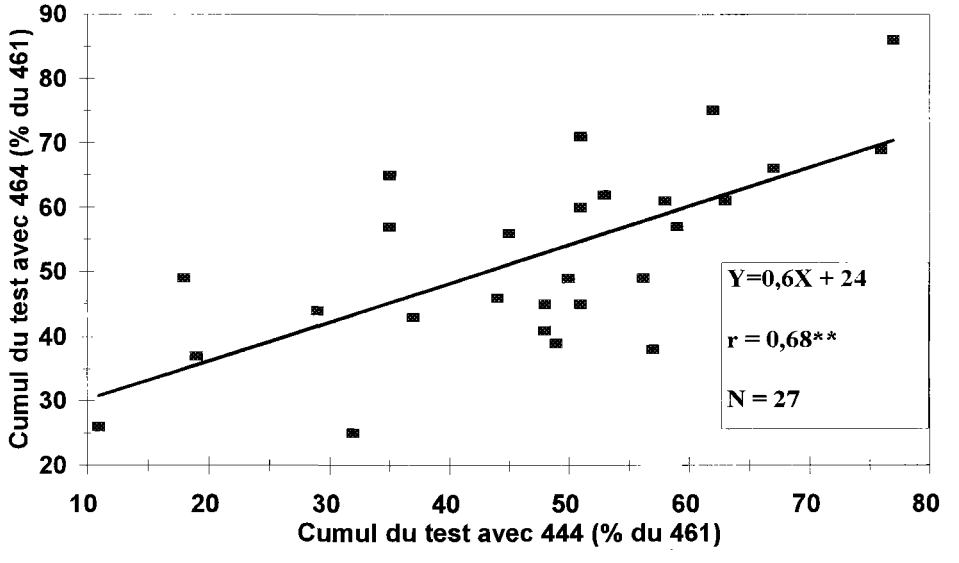


Figure 4 - Comparaison du résultat du test des géniteurs guinéens sur les deux testeurs congolais (444 et 464) (Cumul cerises fraîches sur 4 ans)



significativement de la droite de régression. La corrélation, égale à 0,87, est hautement significative. La pente de la droite de régression est égale à 0,79 traduisant la supériorité moyenne des croisements avec le testeur 410.

La corrélation entre les performances des géniteurs guinéens croisés sur l'un ou l'autre des deux testeurs congolais est presque aussi bonne que précédemment (Figure 4). La corrélation, 0,68, est toujours hautement significative. La baisse de la corrélation semble s'expliquer par le fait que certains géniteurs performants en croisement avec le testeur 444 ne le sont pas forcément avec le testeur 464. En revanche, tous les géniteurs performants avec le testeur 464 le sont également avec le testeur 444.

Remarques :

Le graphique montrant la relation entre la performance des géniteurs congolais sur deux testeurs guinéens (Figure 3) laisse percevoir une séparation des géniteurs en deux groupes. La séparation se situe au seuil de production de 60 % du témoin clonal 461. Une analyse fine de ces deux groupes montre qu'ils correspondent en réalité aux deux sous-groupes SG1 et SG2 du groupe congolais mis en évidence sur des bases électrophorètiques et phénotypiques (Montagnon et al., 1992). Le sous-groupe 1 se situe au-delà du seuil de 60 % du témoin clonal 461.

Les deux testeurs congolais ont été choisis avant la mise en évidence de ces deux sous-groupes. Ils appartiennent tous les deux au sous-groupe 2 (SG2). On remarque que seuls les croisements intergroupes du type guinéen par SG1 congolais atteignent voire dépassent 100 % du témoin clonal 461. Ce résultat réorientera le deuxième cycle en donnant plus de poids au SG1 congolais, en particulier, au niveau du ou des testeurs.

II.2 Cohérence des tests inter et intragroupes :

Les corrélations entre la performance des géniteurs guinéens sur testeur guinéen (intragroupe) ou congolais (intergroupe) sont nettement plus faibles que dans le cas précédent. La corrélation est tout juste significative au seuil de 5 % entre le testeur congolais 464 et le testeur guinéen 155. Elle est non significative entre le testeur congolais 444 et le testeur guinéen 155.

Ceci signifie que l'amélioration des populations de bases se traduira beaucoup plus au niveau des caractères à transmission additive tels que la taille des grains, les caractères architecturaux ou encore la résistance à la rouille orangée, qu'au niveau de la production. Ce type de résultat est classique pour la sélection récurrente et réciproque.

Conclusions

Pour les deux étapes de la sélection étudiées, la cohérence des résultats obtenus est très satisfaisante.

Le choix des arbres têtes de clone est efficace. L'application des techniques de lissage de données en fonction de l'environnement à travers l'analyse de la covariance (Papadakis par exemple) devrait améliorer la prédiction de la valeur des clones dans le cas de parcelles hétérogènes (Charmetant et Leroy, 1990).

La sélection combinée famille/individu devrait aussi faire progresser la technique dans le cas de descendances contrôlées. Ce n'était pas le cas dans les essais présentés ici (descendances de champs semenciers triclonaux).

Des essais en cours permettront de vérifier le progrès obtenu à partir de ces deux techniques (prise en compte de l'environnement et de la famille).

La qualité de la corrélation entre les performances des géniteurs testés avec deux testeurs différents de la population réciproque est très encourageante. Une conséquence directe pour le deuxième cycle de sélection est la possibilité de réduire à un seul par groupe ou sous-groupe le nombre des testeurs. Ceci est d'une importance capitale lorsque l'on songe que l'on peut réduire de moitié les surfaces d'essai et les manipulations liées aux croisements contrôlés sans altérer les gains génétiques escomptés.

Figure 5 - Comparaison du résultat du test des géniteurs guinéens sur un testeur congolais (444) et un testeur guinéen (155) (Cumul cerises fraîches sur 4 ans)

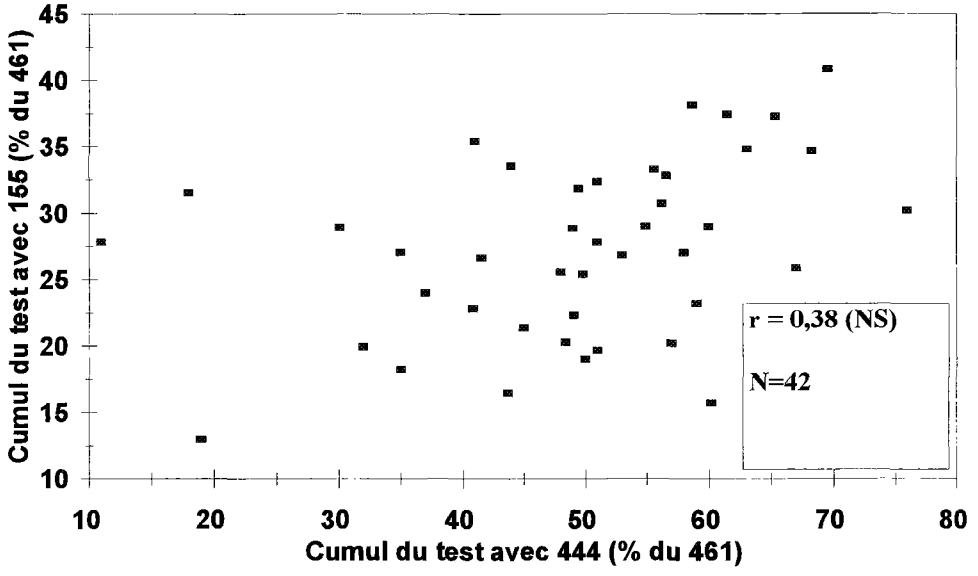
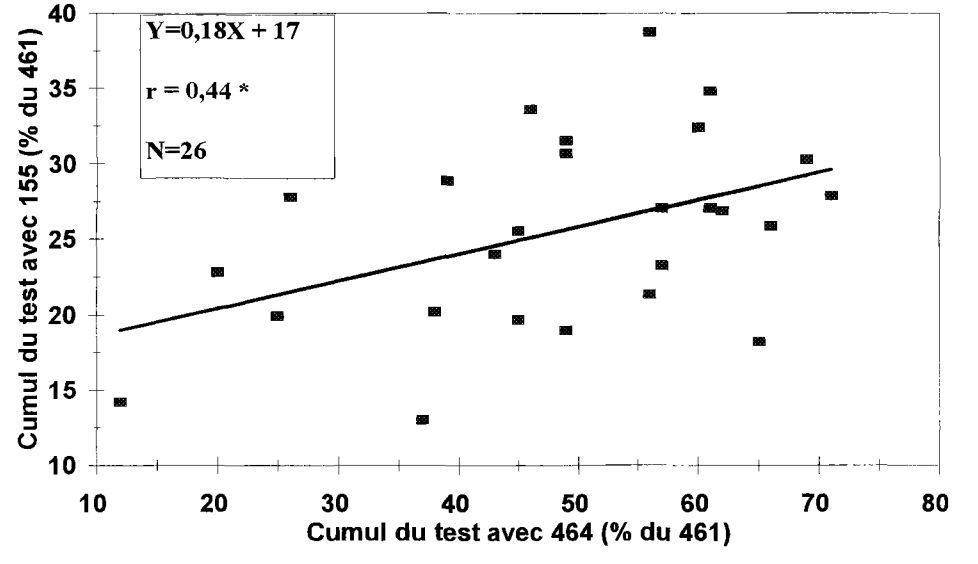


Figure 6 - Comparaison du résultat du test des géniteurs guinéens sur un testeur congolais (464) et un testeur guinéen (155) (Cumul cerises fraîches sur 4 ans)



En revanche, la corrélation entre les tests intra et intergroupes est plus faible voire non significative. Ceci n'est toutefois pas surprenant puisque l'on sélectionne les géniteurs sur leur Aptitude Spécifique à la Combinaison intergroupe, qui n'est pas forcément gage d'une bonne Aptitude Générale à la Combinaison intragroupe.

La sélection des géniteurs du nouveau cycle de sélection devra donc être très sévère au niveau des caractères à transmission additive lors du brassage intragroupe.

Enfin, la cohérence des résultats présentés donne *a posteriori* une garantie quant à la qualité des stratégies méthodologiques et statistiques utilisées pour la sélection de *Coffea canephora* en Côte d'Ivoire.

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Résumé

Plusieurs années de recherche permettent de tirer un premier bilan sur l'efficacité de la sélection de géniteurs et têtes de clone pour l'amélioration de *Coffea canephora* en Côte d'Ivoire. Les corrélations entre la production des têtes de clones et celles des clones correspondant, calculées pour deux expérimentations distinctes, s'élèvent à 0,75 et 0,47, traduisant l'efficacité de la sélection des têtes de clone. La corrélation entre la performance des géniteurs testés sur deux testeurs de la population réciproque est de 0,79 dans le cas des géniteurs congolais et de 0,68 dans le cas des géniteurs guinéens. En revanche, les corrélations entre les performances des géniteurs guinéens testés en inter et en intragroupe sont faibles. Les conséquences pour la sélection sont discutées.

IMPROVEMENT OF *COFFEA CANEPHORA* GERMPLASM IN TANZANIA : EXPLORATION AND COLLECTION OF NEW ROBUSTA MATERIAL FROM FARMERS' PLOTS

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INTRODUCTION

Robusta Coffee, *Coffea canephora* Pierre, in a diversity of forms, occurs wild in the equatorial forests from West Africa to Angola, Democratic Republic of Congo, Sudan, Uganda and North-Western Tanzania (Berthaud and Charrier 1988; Charrier and Berthaud 1988; Wrigley 1988).

Robusta coffee is cultivated in Tanzania mainly in Kagera Region, West of Lake Victoria and occupy an estimated area of 68,000 ha. Coffee production in the region is very low (260 kg/ha). mainly grown by small holder farmers with 0.3 to 2.5 ha and usually intercropped with banana and beans.

The major reasons for the low production of robusta coffee are complex but can be summarised as follows: 1) lack of improved superior genotypes/varieties with high yielding potentials, 2) lack of uniformity in many characters which determine yield and quality. 3) lack of genotypes/cultivars with resistance to diseases and insect pests, 4) late maturity in some cultivars which make the crop vulnerable to late season drought and pests, 5) poor responses of some local landraces to improved agronomic practices including manuring and fertilization, spacings and improved disease and pest management and 6) inadequate extension services and lack of a policy in the past to promote the crop.

In order to arrest the situation, it is necessary to have a co-ordinated crop improvement programme which includes above all, the improvement of the existing genetic variability base/ germplasm (consisting of 71 accessions of *C. canephora)* through introductions of material from outside, collection and evaluation of the available germplasm for required traits.

Early robusta cultivation and multiplication methods.

Traditionally the best robusta grown for chewing was selected from the wild (Burton 1860). It was not grown from seed, but vegetatively from large woody cuttings, a metre or more long, intercropped with banana near the house and trained to make large spreading trees (Speke 1863). By using vegetative propagation method, the Bahaya (the native people of Kagera Region) maintained the clonal material they had selected from the wild, thus maintaining the good quality flavour for chewing.

Kabwoto (1976) wrote that, in the 1880's the then colonial masters instructed chiefs to allow coffee growing in a wider scale for export. Seed beds were established from seed collected from selected indigenous trees to substitute the vegetative method. The method of selection shifted from the chewing types to those trees with precocious fruiting, outstanding yield, bean size and good liquoring. Both local 'spreading' and 'erect' types were planted together. The Bahaya people, having adopted the seed multiplication method, continued to select seeds from good trees in the neighbourhood. With the inception of the Maruku Research Station near Bukoba in 1948, more robusta coffee selections were collected from the region and also introduced from Kawanda Research Station, Uganda and the rest of the world. Seeds from selected 'spreading' and 'erect' types from Maruku were distributed to nurseries in the region. In the 1970's, seeds of the robusta cultivar, Erecta introduced from Kawanda, were distributed to the nurseries and to farmers. Multiplication by seeds continued by which time robusta coffee, being self-sterile and out-crossing, was genetically far removed from the original selection that Speke found cultivated in the early 1860s. Today, the robusta coffee plots in Kagera Region, Tanzania, contain a mixture of 'spreading', 'erect' and 'semi-erect/spreading' types with beans of varying sizes.

MATERIALS AND METHODS

From 2 - 20 June 1996, an exploration and collection mission was conducted in the Kagera Region, North-Western Tanzania. The main objectives of the mission were to search for outstanding and peculiar trees within the cultivated populations in farmers' plots and to collect cuttings from these trees for rooting at the Maruku nursery. The mission was organized to coincide with the main harvest time. Because of the relatively good accessibility of the villages and farms it was possible to explore a large area which covered the most diverse agroecological zones of the region. In order to hasten the exploration farmers were interviewed in their own plots and the identified/selected trees were tagged for immediate collection of cuttings. The selection criteria for 'spreading' and 'erect' types were:

1. More attention was given to trees more than 10 years old as these were considered to be well suited to local conditions.
2. Vigour including growth habit. Many farmers considered the spreading (bending) types to be ideal as they needed little or no pruning after initial training and are much more easier to harvest.
3. Plants which freely produce secondary branches on primaries.
4. Internode length : short and medium most preferred.
5. Presence of large or medium sized clusters.
6. Good sized beans.
7. Plants which give high yield consistently every year.
8. Early maturity.
9. Resistance to coffee leaf rust (*Hemileia vastatrix*).

RESULTS AND DISCUSSION

Figure 1 shows the villages visited during the mission. A total of 141 accessions were collected from 132 coffee growers/plots in 22 villages. Although morphological variation was visible among the samples, the local cultivars appeared, from a subjective assessment to have a common genetic base. Table 1 shows the general variability of the different components of vegetative growth, coffee productivity, maturity and field tolerance/resistance to coffee leaf rust. Most of the collected samples have a bush-like habit with primary and secondary branches and elongated leaves similar to the Kouillou type (cv, Nganda) found in Uganda (Charrier and Berthaud, 1988) but with a large proportion of big sized fruits.

CONCLUSION

Long term progress in Robusta coffee breeding programme depends upon the availability of an adequate genetic base to support breeding/selection for high yielding and agronomically acceptable genotypes resistant to the major diseases and insect pests. In order to achieve, it is important that collection, maintenance and utilisation of genetic resources are given due attention to avoid loss of any valuable material. Further exploration and collection expeditions and exchange with related institutions are recommended. All collected accessions will be multiplied, evaluated and used in present and future breeding and genetic investigations.

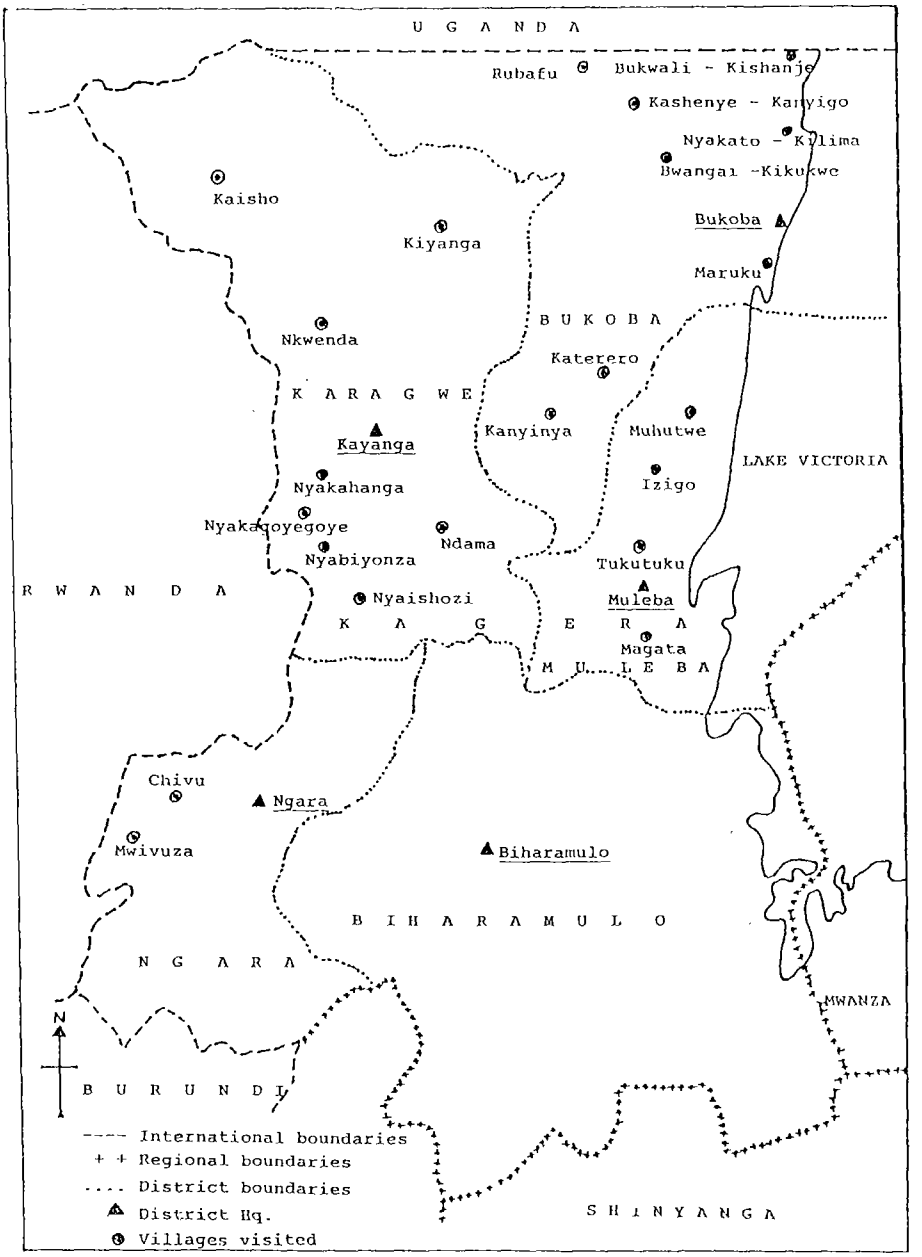

Fig 1: Map of the Kagera Region, Tanzania.

Table 1. General variability (%) observed in collected samples

Stem habit	:	Spreading	85.1
		Erect	1.4
		Semi-spreading/erect	13.5
Branching habit	:	Primary	17.7
		Primary + Secondary	78.0
		Primary + Secondary + Tertiary	4.3
Internode length	:	Long	5.7
		Medium	55.3
		Short	39.0
Cluster size	:	Large	56
		Medium	39
		Small	5
Cherry size	:	Big	76.6
		Medium	10.0
		Small	9.2
		Mixed	4.2
Maturity	:	Early	88.7
		Late	11.3
Leaf rust	:	Resistant	77.3
		Susceptible	22.7

ACKNOWLEDGEMENTS

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ABSTRACT

Successful development of plant breeding as a basis for crop improvement and sustainable agriculture, in any region depend on the availability of adapted, indigenous genetic resources. The genetic base of *Coffea canephora* is becoming narrow due to genetic erosion. To widen it, The Government of Tanzania, in 1996, supported an exploration and collection of robusta material from farmers' plots. As a result, 141 genotypes were collected from different agro-ecological zones in Kagera Region, Tanzania. Great genetic variability exist in the components of vegetative growth and coffee productivity. Related to the present situation, some urgent and concrete targets for preservation and evaluation of genetic diversity are foreseen.

VERS LA DIVERSIFICATION DE LA QUALITÉ DU CAFÉ MALGACHE : UN NOUVEAU CAFÉIER HYBRIDE, LE « RATELO » OU GCA

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1. INTRODUCTION

Les deux espèces de caféiers principalement cultivées dans le monde, le *Coffea arabica* et *Coffea canephora,* ont été introduits à Madagascar respectivement, après son implantation à La Réunion en 1715 et au début du 20ème siècle.

La production caféière malgache est largement dominée par le Canephora. Le café boisson produit par celui-ci est généralement considéré comme de qualité inférieure par rapport à celui de son concurrent Arabica La sélection visant l'amélioration de sa qualité organoleptique n'a pas offert jusqu'à maintenant des résultats satisfaisants.

La production arabicole ne représente que les 10% de la production nationale malgache. La qualité du produit obtenu varie peu et reste conforme à des normes commerciales de qualité, mais le matériel végétal, en raison de sa grande uniformité génétique, se prête difficilement à l'amélioration variétale et se montre très vulnérable aux agressions du milieu ou du parasitisme.

La plupart des auteurs s'accordent à dire que le *Coffea arabica* est un allotétraploïde, à la formation duquel *Coffea.eugenioïdes* aurait participé avec soit des canephoroïdes soit des liberioexcelsoïdes. De ces hypothèses d'apparenté, on pourrait estimer reproduire une combinaison intéressante par le biais de croisement entre ces espèces. Ainsi, un programme d'hybridation a été lancé à Madagascar, dans le but de créer un nouveau caféier dans lequel on espère combiner la faible teneur en caféine de *Coffea eugenioïdes,* la rusticité de *Coffea canephora* et la qualité organoleptique de *Coffea. arabica.*

2. LA CULTURE DES CAFEIERS A MADAGASCAR

Le *canephora* est exploité sur les plaines alluvionnaires de la bande côtière orientale, qui s'étendent sur environ 1.200 km entre les lattitudes 13° et 24° sud et dans la région Nord-Ouest de l'île. Cette espèce couvre la plus grande superficie des caféraies malgaches et donne les 90% de la production nationale.

L'*arabica* est cultivé surtout dans le Centre-Nord de l'île, où il donne les 50% de la production arabicole de Madagascar. Les plantations de la Haute Falaise orientale, de la Haute Terre centrale et du Moyen Ouest sont dispersées en de petites caféraies de case autour des habitations , dans des anciens parcs à boeufs ou dans des fossés entourant les villages.

Le *Coffea eugenioïdes,* originaire des forêts de montagne de l'Afrique de l'est, est conservé en collection dans les Stations de Recherches du FO.FI.FA.(Centre National de la Recherche Appliquée au Développement Rural) à Ilaka-Est et Kianjavato.

Le Ratelo est une nouvelle famille de caféiers hybrides tétraploïdes trois voies obtenus à Madagascar à partir de croisements entre *Coffea eugenioïdes, Coffea canephora et Coffea Arabica, d'*où le nom en malgache « Ratelo », signifiant "organisme à trois sang" qui lui est attribué.
Ils sont répartis dans des Stations de Recherche du FO.FI.FA, sur quatre sites caféicoles à vocation canephora et arabica.

3. MATERIEL

3.1 DESCRIPTION MORPHOLOGIQUE DES PARENTS DE GCA

3.1.1 LES PARENTS DIPLOIDES

les *Coffea. eugenioïdes*

Les pieds de Coffea. *eugenioïdes,* A16, sont issus de graines rapportées d'un jardin de Nairobi. Leur base génétique est restreinte.

Ils présentent des rameaux très grêles à ramifications nombreuses, feuilles petites, ovales et lancéolées, brusquement acuminées, portant en moyenne sept à neuf paires de nervures secondaires.

Une à trois inflorescences axillaires comportant jusqu' à six fleurs sur bois aoûté ou vert Les fleurs sont petites, présentant quatre à six pétales. Les fruits sont petits, subglobuleux, rouges à maturité. Les graines sont lenticulaires.

Le *Coffea. Canephora* :

Le K76 a été choisi dans la collection d'une station de recherche, et a été sélectionné pour sa productivité à partir de croisements étalés dans le temps. Sa base génétique est assez large.

Il présente des rameaux forts, des plagiotropes peu ramifiés, de grandes feuilles ovales allongées brusquement acuminées portant en moyenne onze paires de nervures secondaires, limbe internervaire papyracé, plat ou légèrement gaufré.

Les fleurs sont grandes, montrant cinq à six pétales

les fruits sont moyens et ovoïdes, rouges à maturité

Les hybrides F1 (*Coffea eugenioïdes x Coffea canephora)* GC:

Les rameaux primaires des GC F1 sont parfois grêles, souvent munis de plagiotropes secondaires importants.

Les feuilles ont une grandeur intermédiaire entre celles des parents, portant en moyenne dix à treize paires de nervures secondaires.

Les fleurs de tailles moyennes, sont en général pentamères, se rapprochant de celles de *Coffea. eugenioïdes.*

Les fruits, rouges à maturité, donnent des graines ellipsoïdes.

3.1.2. LES PARENTS TÉTRAPLOIDES:

La différence de ploïdie entre l'hybride GCF1 (2n=2x=22) et l'*arabica*(2n=4x=44) nécessite une duplication chromosomique préalable du premier avant son croisement avec l'*arabica;* cette opération consiste à décapiter l'axe orthotrope au niveau des bourgeons axillaires sériaux, et en l'application d'une émulsion de lanoline(49%), d'eau de coco(49%) et de colchicine(1%).

Les hybrides autotétraploïdes (GC T0)F1 ainsi obtenus présentent des rameaux forts à ramifications secondaires importantes, des feuilles larges et plus arrondies que celles des hybrides diploïdes, brusquement acuminées avec des limbes internervaires subcoriaces et plus ou moins gaufrés.

Les fleurs sont blanches, souvent pentamères et les fruits à pulpes souvent épaisses sont ovoïdes.

Les *C.arabica*

Ils présentent des rameaux longs, fluxueux et assez grêles; des feuilles ovales, acuminées et brièvement pétiolées, à bord ondulé et à surface luisante, légèrement gaufrée.

Fleurs blanches, attachées par un court pédicelle. Corolle formée d'un long tube qui s'épanouit en cinq lobes étroits. Etamines exsertes.

Fruits ovoïdes ou subglobuleux, rouge à maturité.

Les géniteurs utilisés sont ET6-18 et R23AA x ET6-18 et ET15-43 x B14

ET6-18, originaire de l'Ethiopie de la région de Wush-Wush, a été choisi pour sa vigueur, sa précocité de production et son bon comportement vis-à-vis de la rouille orangée. Outre leur bon comportement vis-à-vis de la rouille orangée, R23AA x ET6-18 et ET15-43 x B14 ont été choisis pour leur production.

A noter que R23 est un Babaca Kaffa originaire du Rubona (Rwanda), B14 est un cattura vermelho et ET15-43 provient de la région de Goré (Ethiopie)

4.OBTENTION DES GCA:

Le schéma suivant résume la mode d'obtention des hybrides Ratelo

C. eugenioïdes x C.canephora
!
!
GC F1 (diploïde)
!
polyploïdisation à la colchicine
!
GC T0 F1 (tétraploïde) x *C. arabica*
!
!
Ratelo (*GCA*) (tétraploïde)

Les premiers croisements entre l'hybride autotétraploïde (GC T0) F1 avec la variété de *C. arabica* d'origine éthiopienne ET6-18 d'une part et avec les deux hybrides inter-origines R23AA x ET6-18 et ET15-43 x B14 d'autre part, ont été effectués dans la Station de Recherche du FOFIFA d' Ilaka-Est.
Les hybrides trois voies de première génération ainsi obtenus, et implantés en champs d'expérimentation constituent la base à partir de laquelle ont été créées d'autres générations d'autofécondation et/ou d'inter-croisement (back-cross avec *arabica*, croisement entre Ratelo, croisement Ratelo x *Arabusta*).

Actuellement le FOFIFA dispose de plus de 5.000 individus Ratelo répartis en champ d'expérimentation dans ses Stations de Recherches.

5. CARACTERISTIQUES DES Ratelo .

5.1. CARACTERES MORPHOLOGIQUES

Port: les Ratelo (GCA) sont des arbustes de taille souvent réduite et à port variable: pyramidal, conique, souvent buissonnant.

Ramifications: les rameaux sont forts, semi-érigés, présentant des ramifications secondaires et tertiaires abondantes à entre-noeuds courts.

Feuilles: les feuilles à limbe internervaire subcoriace et peu gaufré présentent une certaine variabilité; de couleur vert-foncée, à acumen court, elles sont ovales, plus ou moins arrondies; leur taille est souvent intermédiaire à celles des parents.,

Inflorescences: trois à six inflorescences par axe florifère sur vieux ou bois vert; composées de deux à cinq fleurs par inflorescence. Les fleurs sont grosses de couleur blanches, composées de cinq pétales soudés à leur base, sur laquelle sont insérés les cinq étamines.

Fruits: Les cerises sont ovoïdes de couleur rouge sang à maturité, présentant généralement un disque moyen. L'épaisseur de la pulpe est variable suivant le génotype.

5.2. BIOLOGIE FLORALE:

Les Ratelo sont partiellement autogames.

Sur 2.344 fleurs isolées dans un manchon avant l'éclosion, on a pu observer 74% de nouaison et pour 1.326 fleurs castrées 70% se sont nouées.

Les grosses floraisons se situent au mois de septembre-octobre et le cycle de floraison-maturité est de neuf à dix mois.

L'intervalle pluie déclencheuse-floraison varie de 7 à 13 jours suivant les températures moyennes journalières et le génotypes. Une durée de 9 jours a été souvent observée.

5.3.CARACTERES AGRONOMIQUES:

Les entre-noeuds courts entraînent une réduction de taille chez les Ratelo et leur permettrait une forte densité de plantation. La présence de palmettes leur donne un potentiel floral assez élevé.

Initialement créés en basse altitude, où les Ratelo végètent et produisent normalement, leur mise en place en moyenne et haute altitude ne change pas pour autant leur comportement.

5.3.1. Résistance aux maladies et parasites:

Des notations faites en basses altitudes (Ilaka-Est) et hautes altitudes (Sahambavy), sur les divers maladies et parasites, en particulier la rouille orangée (*Hemileia vastatrix*), le tigre (*Dulinus unicolor*) et les foreurs de troncs et branches; on a pu constater que les caféiers GCA présentent une tolérance remarquable vis à vis de la rouille et des divers scolytes. Des attaques de tigres de caféier ont pu être remarquées en basses altitudes, sans toutefois causer de défoliation importante.

Quelques dessèchements de fruits, dûs à la présence de larves de *Ceratitis sp* dans la pulpe ont été remarqués en basses altitudes.

5.3.2. Production:

La potentialité de production est très variable mais on a pu noter que 2% des génotypes en collection produisent plus de 10kg de cerises par pied par an.

80% de la récolte sont groupés au mois de juin-juillet.

Le taux de conversion cerise/café marchand est très variable, allant de 7 à 12%, avec comme taux moyen de 9%; cette faiblesse du taux de conversion pourrait être attribuée à l'apparition fréquente de fructification anormale (loges vides ou incomplètes).

Sur un échantillon de 21.780 graines tout venant, 73% sont classées normales et 27% caracoli, taux variant de 14 à 43 % suivant les génotypes.

5.4.CARACTERES TECHNOLOGIQUES ET ORGANOLEPTIQUES

5.4.1.Granulométrie

La granulométrie (poids moyen de 100 graines), est variable suivant le génotype; allant de 12 à 21 g. avec une moyenne de 16 g.

5.4.2.Goût

Le café-boisson préparé à partir des GCA est apprécié par les dégustateurs.

5.4.3.Teneur en caféine

Le taux de caféine est aussi variable suivant le génotype. Les échantillons de graines récoltées sur 23 pieds et analysées au laboratoire du CIRAD-CP à Montpellier ont donné une teneur en caféine variant de 0,50 à 1,38 % MS (moyenne 0,80).

L'analyse de variance et la comparaison des moyennes de teneur en caféine entre les hybrides issus des croisements entre *Coffea. eugenioïdes* et caféiers cultivés ont donné le résultat suivant:

0,2	0,77	0,80	0,81	1,20	1,30	1,87
EUG	GCF1	GCA	ARA	AGH	DGC	CAN

(EUG: *Coffea eugenioïdes*, GCF1: hybrides *Coffea eugenioïdes x Coffea canephora*, GCA descendances GC T0 x *Coffea arabica*, ARA: *Coffea arabica*, AGH: autohexaploïdes d'hybrides F1 entre *Coffea arabica x Coffea eugenioïdes*, DGC: descendances d'hybrides F1 *Coffea eugenioïdes x Coffea canephora*, CAN: *Coffea canephora*)

Le GCA s'associe à *Coffea arabica* et se rapproche plus du *Coffea eugenioïdes* que du *Coffea canephora*.

6. CONCLUSION:

Le nouveau caféier hybride tétraploïde Ratelo, résultat de la combinaison des deux caféiers cultivés *Coffea arabica* et *Coffea canephora* et d'un caféier sauvage *Coffea eugenioïdes* présente des caractères agronomiques et organoleptiques intéressants.

Cette nouvelle famille de caféiers pourrait donc constituer un avenir meilleur pour la caféiculture malgache. La nouvelle orientation consiste ainsi à élargir la base génétique de départ à partir de nouvelles combinaisons diploïdes GC ou GC F1 et /ou par retrocroisements successifs avec *Coffea arabica* tout en continuant la sélection à l'intérieur de cette population existante qui présente une variabilité morpho-physiologique importante.

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RESUME

Le Ratelo est une nouvelle famille de caféiers hybrides trois voies créé à partir d'un produit de croisement entre *Coffea eugenioïdes* et *Coffea canephora* préalablement tetraploïdisé qui croisé avec *Coffea arabica* a donné les GCA de premières générations à partir desquels ont été constituées d'autres générations d'autofécondation et d'intercroisement. On espère combiner chez ce type de caféier la rusticité de *Coffea canephora*, la faible teneur en caféine de *Coffea eugenioïdes* et la qualité organoleptique de *Coffea arabica*. Les Ratelo présentent des ramifications secondaires et tertiaires abondantes à entre-noeuds courts. Ils sont partiellement autogames et ont une variabilité morpho-physiologique importante. Leur tolérance aux races locales de rouille orangée et aux foreurs de branches est remarquable.
L'exploitation agronomique de Ratelo est pour le moment handicapée par l'apparition fréquente de fructification anormale, toutefois la sélection clonale est permise car 2% des génotypes observés ont produit plus de 10 kg de cerises par pied par an. La teneur en caféine des échantillons analysés varie de 0,5 à 1,38 % M.S., pour une moyenne de 0,80. Le boisson préparé à partir de Ratelo est apprécié par les dégustateurs.

Summary

Ratelo or GCA, a new family of tetraploid threeway hybrids of coffee, was created through a prior chromosome doubling of F1 GC diploid hybrids between *Coffea eugenioïdes* and *C.canephora* . The resulting tetraploids were then crossed with *C.arabica* to give rise to the first generation of Ratelo, followed by other generations of selfing and intercrossing. This new type of coffee is expected to combine the adaptability of *C.canephora*, the low coffein content of *C.eugenioïdes* and the flavor characteristics of *C.arabica*. These hybrids are small trees of reduced height, characterized by a compact habit resulting from the branching pattern of numerous secundary and tertiary branches with short internodes. They are partially autogamous, showing an important morpho-physiological variability, a tolerance to stem miners and to the local strain of orange rust.
Although the existence of heavy flowering waves suggeres a high production potential, abnormal fruit development often results in unfilled locules which hampers the agronomic exploitation of «**RATELO**». However, clonal selection within the existing population yielded 2% of genotypes producing 10 kg of berries per year. The caffein content was found to vary from 0,50 to 1,38 with a mean of 0,80 and the flavor was much appreciated by panelists.

IMPROVEMENT IN THE PERFORMANCE OF ARABUSTA COFFEE IN GHANA

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Introduction

Coffee canephora (Robusta coffee) which is indigenous to the West African sub-region is the main coffee cultivated in Ghana. Even though this species is well adapted to the lowland tropical climate of Ghana the yields of varieties that were being cultivated by farmers were low and establishment was also poor. Attempts have been made in recent years to upgrade the varieties in these two attributes with significant success. Varieties which established easily and with yields of over 3.5 tons per hectare (Adu-Ampomah *et al* 1993) as against the previous yields of below 2 tons/hectare (Ofori and Afrifa, 1975) have been obtained. However, the problem of the poor quality in Robusta in relation to Arabica still persists. An interspecific hybridisation programme between Robusta and Arabica was initiated with the view to improving the quality of Robusta coffee in Ghana whilst retaining the easy establishment and high yielding capabilities of the recently developed varieties. The earlier interspecific hybridisation programme resulted in the production of progenies with low yields largely due to poor fertility of flowers resulting in high peaberry production and empty shells (Martinson, 1986).

Materials and Methods

Six Arabica coffee genotypes were introduced from Cote d'Ivoire in the mid ninteen seventies (1970s) and coded Arabica 1-6 (RIC1-6) (Martinson, 1984). Of these, Arabica 2, 3 and 5 were used in this programme. The Robusta parents used were five coded P10, P12, P13, P20 and P22. These are high yielding and drought tolerant clones selected from old robusta plots at CRIG, Tafo. **Crosses were made between the arabica and the robusta which had previously been induced to form tetraploids by colchicine treatment (Martinson, 1986) (Table 1).**

Table 1

Crosses made between induced tetraploid robusta and arabica

Cross		
1. Robusta 13	x	Arabica 3
2. Robusta 20	x	Arabica 3
3. Robusta 12	x	Arabica 5
4. Arabica 2	x	Robusta 22
5. Arabica 2	x	Robusta 10

Hybridity was established from leaf and fruit characters of the resulting crosses (Capot *et al.* 1968). In most of these the length to breadth ratio (1.99 $\pm$ 0.069) of the lamina were intermediate between those of the two parents (Robusta, 1.92 $\pm$ 0.092 and Arabica, 2.42 $\pm$ 0.043). The orange coloured berries of the hybrid between the red-berried Robusta and the yellow-berried 'Arabica 3' further indicated the genuiness of the crosses.

The Arabusta F1 hybrids developed had problems of poor fertility and pea-berry production. F2 seedling were, however, derived from the F1 hybrids and planted out in a field trial in July 1985 for observation and selection. Each plot had thirty plants and there were seven replications. Individual vegetative growth and yield data were used to select the most vigorous and best yielding individual F2 progenies.These were multiplied clonally and planted in comparison with the F1 hybrids raised from cuttings of plants previously planted in the field.

Results and Discussions

F1 hybrids

The F1 hybrids matured and flowered at an average of twenty months from sowing which was earlier than those of the tetraploid robusta (36 months) and the arabica (30 months). In most cases, the berries of the hybrid were distinctly pedicelled, like those of the Arabica. However, the hybrids were characterized by over production of peaberries as shown in table 2.

Table 2

Percentage peaberry of F1 hybrid seed of C. canephora var robusta and C. arabica

Cross	Range of peaberry (%)	Mean (%)
Robusta 13 x Arabica 3	63.27-81.66	72.76
Robusta 20 x Arabica 3	27.58-82.17	54.88
Robusta 12 x Arabica 5	27.38-50.09	37.13
Arabica 2 x Robusta 22	53.12-76.47	73.59
Arabica 2 x Robusta 10	-	78.76

Two crosses (Robusta 20 x Arabica 3 and Robusta 12 x Arabica 5) had markedly lower peaberry production than the rest. This is unexpected as the colchicine treatment to the Robusta female parents involved was expected to induce some meotic imbalance and thus are more likely to produce peaberries than in those where the female parents are Arabica which had not been treated with colchicine.

F2 progenies

In the F2 progenies, some individual trees within the crosses showed easy establishment, by virtue of their large girth and wide span of lateral branches. The progenies of Robusta 12 x Arabica 5 and Robusta 20 x Arabica 3 were however superior (Table 3) to the others.

Table 3

Vegetative growth of F2 plants derived from Robusta - Arabica hybrids

Percentage of plants exceeding one-and-a-half times the general mean for stem diameter and span of lateral branches

Cross	Stem diameter (%)	Span of lateral branches (%)
Robusta 13 x Arabica 3	28.78	8.72
Robusta 20 x Arabica 3	49.76	22.92
Robusta 12 x Arabica 5	37.8	24.63
Arabica 2 x Robusta 22	22.55	7.06
Arabica 2 x Robusta 10	52.78	10.00

Table 4 shows the peaberry production and the yield levels among the F2 progenies. It is clear that there has been a discernible reduction in the production of peaberries among the F2 progenies in comparison to the F1 hybrids previously shown in Table 2. The progenies with Robusta as the original female parent produced less peaberries (14.1, 16.2 and 22.8%) and higher yields when compared with those with Arabica (33.6 and 47.4 % peaberries) as the original female parent. This seems to suggest that for significant improvement of these attributes in interspecific crosses in the tropical lowlands, Robusta which is better adapted should be used as female parents in crosses.

Comparison of F1 and F2 progenies

Table 5 compares the initial yields of F1 and F2 progenies in the first three years. With the exception of the F1 hybrid of Robusta 13 x Arabica 3 which yielded higher than the F2, all the F2 progenies out yielded their F1 counterparts.

Table 4

Yield of open pollinated F2 progenies of Arabusta

Cross	Mean % peaberry	Range % peaberry	Mean berry wet weight per tree per year (kg)	Range mean berry wet weight per tree per year (kg)
Robusta 13 x Arabica 3	22.8	10.2-37.4	2.90	1.55-6.25
Robusta 20 x Arabica 3	16.2	6.7-24.2	3.06	1.43-6.30
Robusta 12 x Arabica 5	14.1	6.0-26.1	2.65	1.2-4.00
Arabica 2 x Robusta 22	47.4	24.3-56.0	1.52	0.3-3.78
Arabica 2 x Robusta 10	33.6	21.1-55.6	1.80	0.5-3.03

Table 5

Yield of Arabusta F1 and F2 crosses over a 3-year period

Cross	Year 1 Mean yield per tree (kg)	Year 2 Mean yield per tree (kg)	Year 3 Mean yield per tree (kg)	Mean plot yield per tree (kg)
Robusta 13 x Arabica 3	0.21(F1)	0.63	1.81	0.64
Robusta 13 x Arabica 3	0.08(F2)	0.00	0.72	0.05
Robusta 20 x Arabica 3	0.19(F1)	1.50	2.40	0.76
Robusta 20 x Arabica 3	0.59(F2)	1.60	3.46	1.51
Robusta 12 x Arabica 5	0.40(F1)	0.71	1.77	0.66
Robusta 12 x Arabica 5	1.05(F2)	1.90	3.79	1.58
Arabica 2 x Robusta 22	0.20(F1)	0.56	1.32	0.31
Arabica 2 x Robusta 22	0.86(F2)	0.82	2.38	0.42

The superiority of the F2 progenies over the F1 hybrids is further authenticated by the fact that of the ten top high yielding individual trees (i.e those with mean yields greater than four times the plot average), nine belong to the F2 progenies and only one is derived from the F1 hybrids (Table 6).

Table 6

Ten top high yielding F1 and F2 arabusta plants with mean yields greater than four times the plot average

Stand No.	*Mean yield of fresh berries per tree per year (kg)	Cross	Generation
209	5.51	Robusta 12 x Arabica 5	F2
210	4.48	"	F2
212	5.30	"	F2
213	5.11	"	F2
36	7.81	"	F2
16	12.82	Robusta 20 x Arabica 3	F2
227	3.67	"	F2
124	3.78	"	F2
201	4.87	Arabica 2 x Robusta 22	F2
39	3.57	"	F1

* Overall average of plot = 0.87 kg

Table 7 shows the general contribution to yield in respect of F1 and F2 progenies. Again it is evident that the F2 progenies are superior to the F1 hybrids. All the other qualities that contribute to make a good cup quality coffee have not been tested in these F2 progenies but on the basis of establishment ability and yield they can constitute a potential variety in Ghana in future.

Table 7

Contribution to yield of arabusta F1 and F2 generation plants

Generation	No. of trees	No. of trees bearing	Cummulative 3-year yield fresh berries (kg)	Mean fresh berry yield per tree per year (kg)
F1	150	136	255.83	0.57
F2	135	112	486.99	1.20

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ABSTRACT

The bulk of coffee produced in Ghana is Robusta but as is well known this is inferior to Arabica in terms of quality. Arabica yields in Ghana are extremely low due to the low elevation and high temperatures. To improve the quality of Ghana's coffee a programme was initiated to introgress some Arabica characters into Robusta. The F1 hybrids of crosses between selected Arabica and Robusta had poor establishment capabilities, as well as poor yields as a result of high number of pea berries and empty shells. F2 progenies developed from the F1 generations have exhibited low proportions of pea berries production. In comparison with the F1 generation the F2 progenies are also by far superior in terms of establishment and yield. Some of the selected F2 progenies are potential candidates as future varieties in Ghana.

INTROGRESSION OF QUALITY TRAITS FROM *C. PSEUDOZANGUEBARIAE* TO *C. LIBERICA* VAR. *DEWEVREI*

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Introduction

Evaluations of coffee genetic resources for different characteristics allowed the discovery of many wild species having interesting traits for breeding purpose (Berthaud & Charrier, 1988). Interspecific hybrids offer the possibility to transfer these traits from wild coffee species to cultivated ones (Carvalho & Monaco, 1967; Louarn, 1992). Nevertheless introgression success depends mainly on the genetic basis of the trait, possible linkage with other traits and the restoration of the fertility.

To evaluate these possibilities, a cross between *C. liberica* var. *dewevrei* (DEW) and *C. pseudozanguebariae* (PSE) was produced. DEW, native in Central Africa, was cultivated in Centrafrique and in Indonesia. PSE is a wild species native from South Kenya and North Tanzania and is the only African species without caffeine in beans. It is not commercially used because of its small beans, low productivity and presence of a specific heteroside diterpene (called heteroside after) which could be involved in the bitterness of coffee beverage (Rakatomalala, 1993).

In this study, the first and second generations of hybrids were investigated for their morphology, phenology, biochemical compounds of beans, chromosomic parental composition, recombination behaviour through molecular markers and fertility. The results from this study will be presented especially in reference to the use of restriction fragment length polymorphisms (RFLP) in order to determine the genealogy of hybrids, the development of a rapid technique to determine chromosomic parental composition and, the inheritance of caffeine and heteroside content in beans.

Plant material

Plant material was obtained and maintained by J. Louarn at the Agricultural Coffee Station ORSTOM-IDEFOR (Man, Côte-d'Ivoire).

PSE is a shrub in growth habit. Its flowers blossom seven to eight days after the rain, and produce small berries which turn purple on ripening. The duration between flowering and ripening is two months on the average. DEW, at the opposite, is a large vigorous tree with high productivity. It flowers six days after the rain producing large berries which mature within a duration of 10 to 11 months giving orange-red cherries.

ranges between 0.5 and 1.8 % dmb (Louarn, 1992) but are heteroside-free. The parental species are diploid with 22 chromosomes but differ for their genome size : 1.14 pg in PSE and 1.43 pg in DEW (Barre et al., 1996).

Thirty three F1 hybrids were obtained by hand pollination of PSE by DEW. The second generation of hybrids (G2 hybrids) were obtained by open pollination. Thirteen F1 hybrids gave a progeny. We investigated 80 non controlled G2 hybrids.

Genealogy of G2 hybrids

RFLP markers were developed on parents, F1 hybrids and G2 hybrids using 23 probes. Fifty three G2 hybrids (66 %) have at least one allele different from those of F1 hybrids (Fig. 1). This implies that these hybrids were produced by pollination of F1 hybrids by other coffee trees. For each probe, three genotypes can be distinguished : two alleles from PSE (PP), two alleles from DEW (DD) and the heterozygote with one allele from each parent (PD). On the basis of the number of these three genotypes for 20 probes, G2 hybrids with foreign alleles can be clustered in two groups : 40 coffee trees with more DD than PP should come from the backcross of F1 hybrids by a DEW (BCDEW) and 13 coffee trees with more PP than DD should come from the backcross of F1 hybrids by a PSE (BCPSE). The others 27 G2 hybrids (34 %) without foreign allele could come from the pollination of a F1 hybrid either by another F1 hybrid or by a coffee tree closely related to the parents.

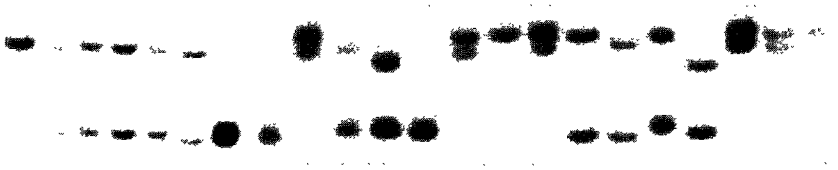

Figure 1: RFLP analysis with the probe CA 25 on the two parents (D : DEW and P : PSE), F1 hybrids (F1 and 9) and G2 hybrids (1-8 and 10-15) showing the presence of alleles absent in the F1 hybrids (b and c) in 9 G2 hybrids (2 ab, 3 bd, 4 cd, 6 ab, 8 ab, 10 bd, 12 cd, 13 ab, 14 ab). The genotype of 5 G2 hybrids can be determined : 1 is PP, 5 and 11 are DP and, 7 and 15 are DD.

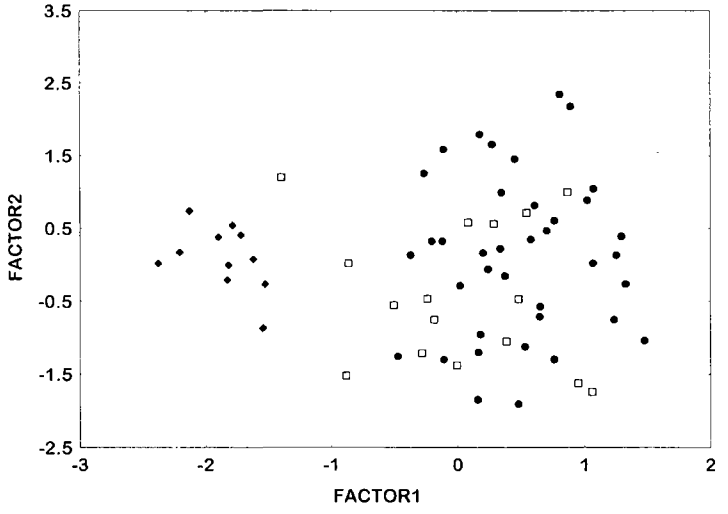

Figure 2: Principal component analysis on the G2 hybrids described by leave length, weight of 100 beans and the square root of caffeine content in beans. The first factor explains 78 % of variance. Symbols indicated G2 hybrids with foreign alleles from BCDEW (•), BCPSE (♦) and G2 hybrids without foreign allele ( ).

The first factor of a principal component analysis performed on the G2 hybrids described by leave length, 100 beans weight and the square root of caffeine content in beans explains 78 % of variance and discriminates two

are in the other. This two groups were confirmed by a discriminant analysis which allowed to clearly identify 13 G2 hybrids without foreign allele as belonging to the group BCDEW. Fourteen G2 hybrids were unidentified, three of them because they were intermediate and 11 due to missing values. These coffee trees were eliminated for further investigations.

Including the RFLP markers and the morphological characteristics, genealogy of G2 hybrids was two backcrosses on parental species : 54 BCDEW and 13 BCPSE.

Determination of parental chromosomic composition

Genomic *in situ* hybridisation (GISH) and flow cytometry were performed on 6 F1 hybrids and 7 G2 hybrids in order to determine their parental chromosomic contribution and their nuclear DNA content (qDNA), respectively. GISH was efficient to identify chromosomes from both species. Sequences homologous to PSE total DNA fluoresced red, resulting from the detection of the biotin-labelled total DNA from PSE with Texas Red. Sequences homologous to DEW total DNA fluoresced yellow-green, resulting from the detection of the digoxigenin-labelled total DNA from DEW with FITC. A double exposure, one using the FITC filter and one using the Texas Red filter, allowed to detect two types of chromosomes, one red-orange from PSE and one yellow from DEW.

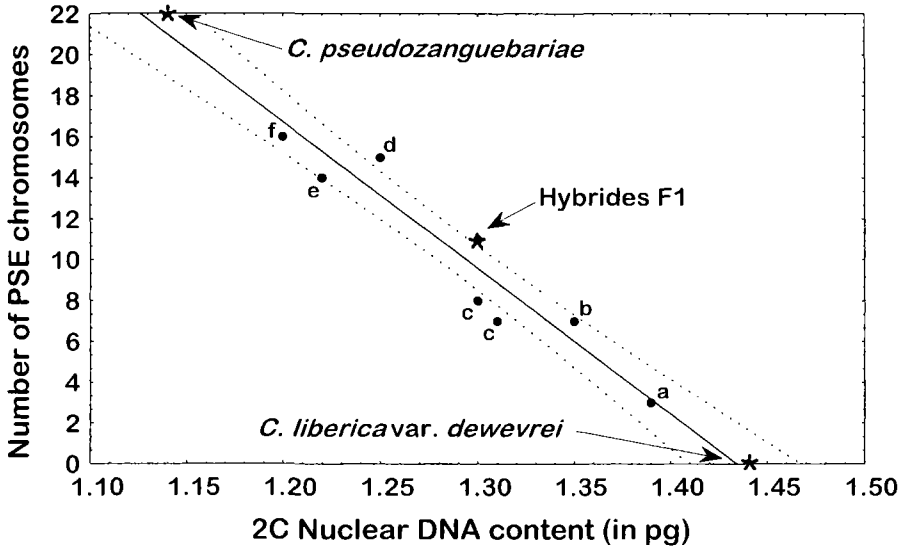

Figure 3: Linear relationship (y = -71.41 x + 102.42, r = 0.98***) between the nuclear DNA content and the number of chromosomes on DEW and PSE, F1 and G2 hybrids. Results of the Newmann & Keuls test on qDNA are indicated with letters.

F1 hybrids had qDNA intermediate between the parental species and as expected contained 11 chromosomes from each species. The 7 G2 hybrids had 22 chromosomes. Three BCPSE hybrids had more than 13 chromosomes from PSE and four BCDEW hybrids had less than 9 chromosomes from PSE. A linear relationship was emphasised between the number of PSE chromosomes *versus* qDNA in the species and their hybrids (Fig. 3). This result allows the use of flow cytometry to obtain a rough estimation of the parental chromosomic contribution in G2 hybrids.

Inheritance of caffeine and heteroside content in beans

In G2 hybrids, caffeine content varied from 0 to 1.2% dmb (Fig. 4). Caffeine content appeared to be under polygenic control with a strong genetic effect which explained 94.6 % of the variance between G2 hybrids (Tab. 1). Nevertheless, one major gene with two alleles seems to be involved in the control of presence/absence of caffeine. Indeed, 38 % of BCPSE hybrids were caffeine-free. Caffeine-free hybrids would be cc, whereas other

was 5 : 8 and did not differ from the expected 6.5 : 6.5. This result has to be confirmed on larger progenies.

Table 1: Results of the partly nested model of ANOVA used to test differences between 2 harvest years (fixed effect), between 6 progenies (each progeny is derived from a different F1 female parent) (random effect), and between 4 hybrids per progeny (random effect nested in progeny) for caffeine and relative heteroside content.

		Caffeine content		Heteroside content	
Effects	df	F	Prob.	F	Prob.
Harvest year	1	4	0.102	5.4	0.070
Progeny (Female parent)	5	2	0.133	0.9	0.490
G2 hybrid	**18**	**201**	**0.000**	**202**	**0.000**
Harvest year x Progeny	5	0.5	0.760	1.4	0.290
Harvest year x G2 hybrid	**19**	**7.3**	**0.000**	**9.4**	**0.000**
Error	240				

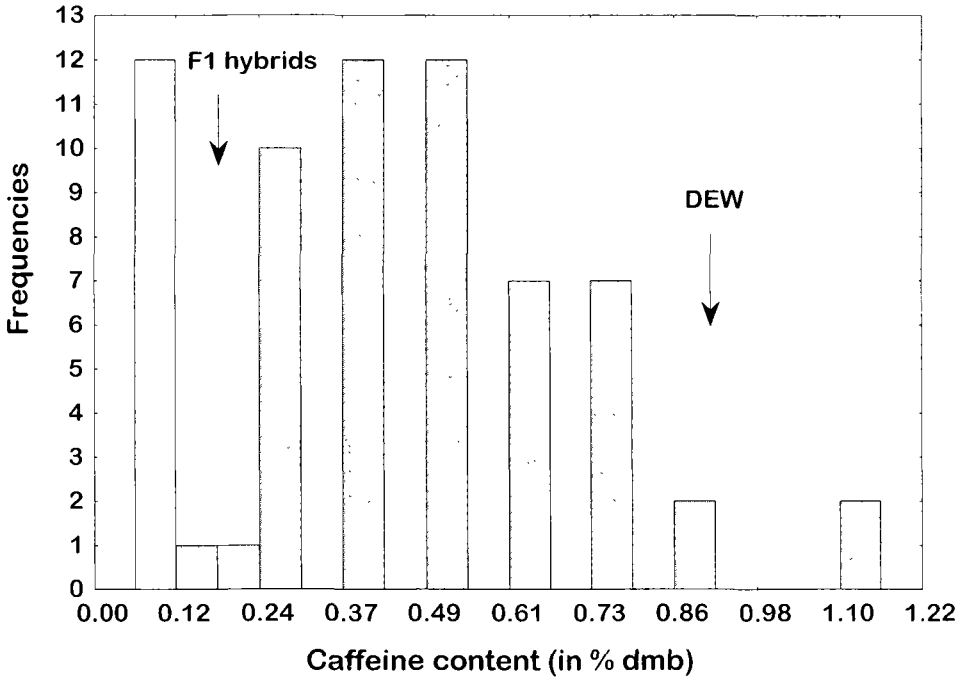

Figure 4: Distribution of caffeine content in BCPSE (white) and BCDEW (grey) hybrids.

Caffeine content on six DEW from Central Africa was 0.9 % dmb on average. Caffeine content was not additive in F1 hybrids and in G2 hybrids which contain caffeine. Indeed, caffeine content of F1 hybrids (0.2 % dmb) was lower than the parental average (0.47 % dmb). In BCPSE and BCDEW hybrids caffeine content was lower than the expected value with additivity : 0.0036 compared to 0.23 for BCPSE and 0.55 compared to 0.7 for BCDEW. Nevertheless, the square root of the caffeine content was additive in F1, BCPSE and BCDEW hybrids: 0.41 compared to 0.48 for F1, 0.16 compared to 0.24 for BCPSE and 0.73 compared to 0.72 for BCDEW.

Because of the absence of purified heteroside, the relative content of heteroside was calculated with regard to the content in PSE. Similarly as caffeine, heteroside content appeared to be under polygenic control with a strong genetic effect which explained 92.3 % of variance between G2 hybrids (Tab. 1). Nevertheless, the distribution of BCPSE hybrids showed two groups suggesting the control of this trait by one major gene with two codominant alleles (H et h) (Fig. 5). The first group would include HH hybrids (mean=1.30) whereas the second

differ ($\chi^2 = 0.69$; $P < 0.41$) from the expected 6.5 : 6.5.

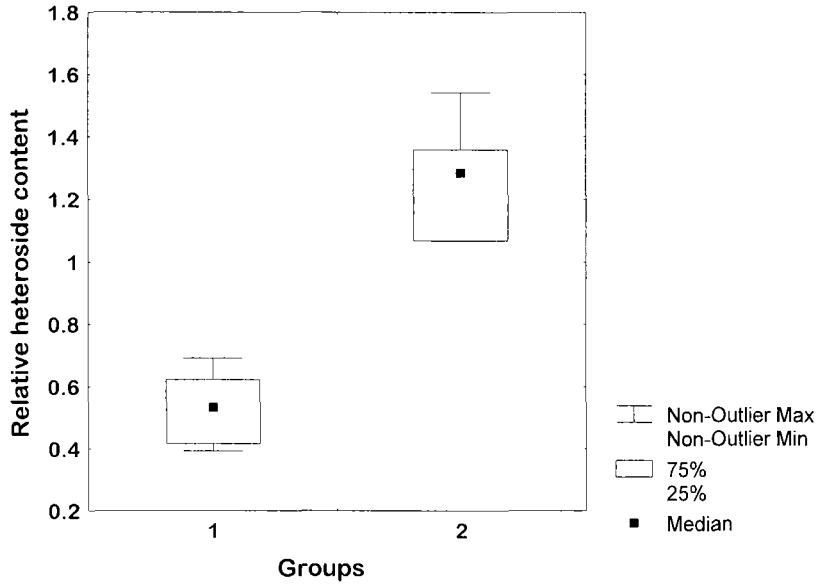

Figure 5: Relative heteroside content of two types of BCPSE hybrids: group 1 (8 genotypes) close to F1 average and group 2 (5 genotypes) close to PSE average.

The distribution of BCDEW hybrids suggested the presence of two groups: 30 hybrids without or with traces of heteroside (<0.05) and 23 hybrids with heteroside content comprised between 0.05 and 0.52 (Fig. 6). All hybrids with heteroside content lower than 0.05 were assumed to be hh and the others to be Hh. The hh : Hh distribution was 30 : 23 and did not differ ($\chi^2 = 0.92$; $P < 0.34$) from the expected 1 : 1 ratio. Nevertheless the relative heteroside content of Hh hybrids in BCDEW was 0.18 in average which was clearly lower than those of Hh hybrids in BCPSE and F1 hybrids.

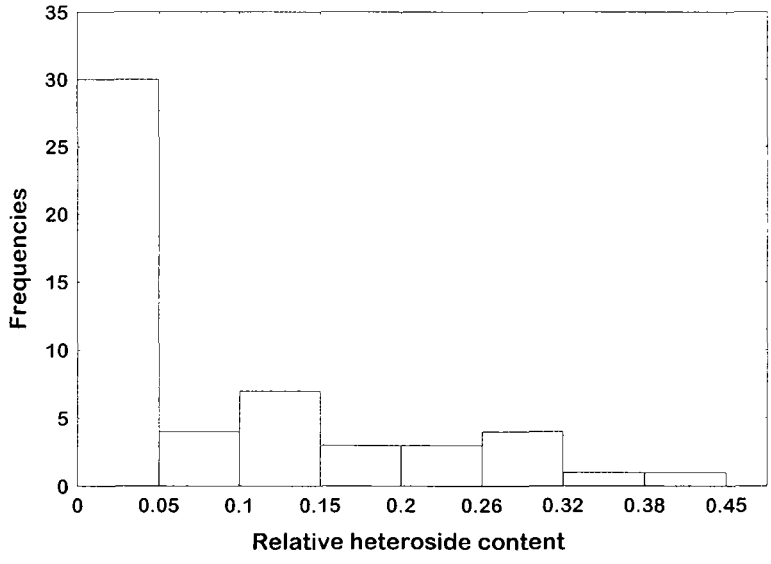

Figure 6: Distribution of heteroside content in BCDEW hybrids.

Conclusion

Open pollination is frequently use to increase the number of interspecific progenies. In this study RFLP were developed because they are codominant and they can be used for comparative mapping with *C. canephora*. Moreover, they were efficient to determine almost all G2 hybrids genealogy.

The presence of major genes for the control of caffeine and heteroside contents is encouraging for the transfer of absence of caffeine in cultivated species without transferring heteroside. Nevertheless, recessiveness of the absence of caffeine requires the help of molecular markers to monitor the c allele through generations of successive back-crosses. We are currently looking for marker linked with lack of caffeine should allow the distinction between hhCC and hhCc coffee trees and the selection of hhCc trees on seedling. In addition, the linear relationship between nuclear DNA content and parental chromosomic composition allows to use flow cytometry to select genotypes with the least chromosomes from the wild species.

In conclusion ,introgression of traits from one species to another unrelated species is based on rare events which implies the screening of large progenies. Seedlings can be both screened with molecular markers and flow cytometry which could be very relevant to help coffee breeding programs.

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Summary

Interspecific hybrids offer the possibility to transfer interesting traits from wild coffee species to cultivated ones. Success in introgression depends mainly on the genetic basis of the trait, on possible links with other unsuitable traits and on the restoration of the fertility. To evaluate these factors a cross between a wild caffeine-free species (*C. pseudozanguebariae* : PSE) and a species with 1 % dmb of caffeine content in beans (*C. liberica* var. *dewevrei* : DEW) was realised. PSE contains large amount of specific heteroside diterpene which is involved in the bitterness of coffee beverage. The first and second generations of hybrids were studied for their morphology, phenology, biochemical compounds of beans, parental chromosomic composition, recombination behaviour through molecular markers and fertility.

RFLP markers were efficient to determine the genealogy of the second generation hybrids which consisted of 54 F1 hybrids backcrossed by DEW and 13 F1 hybrids backcrossed by PSE. A linear relationship between the parental chromosomic composition, determined by genomic in situ hybridisation and the genome size, determined by flow cytometry, was emphasised. Analysis of caffeine content indicates that this trait is under polygenic control with a strong genetic effect. However, only one major gene seemed to be involved in the control of presence/absence of caffeine in beans. The specific heteroside diterpene seemed to be controlled by one major gene.

Associations between absence of caffeine in beans and unsuitable traits are still present and require further investigations. Nevertheless, the simple genetic inheritance of absence of caffeine allows to envisage its introgression into cultivated species. This transfer could be considerably facilitated by the use of molecular markers and flow cytometry.

Résumé

L'obtention d'hybrides interspécifique offre la possibilité de transférer des caractères intéressants des espèces sauvages vers les cultivées. Le succès de l'introgression dépend essentiellement du déterminisme

différents paramètres, un croisement entre une espèce sauvage sans caféine (C. pseudozanguebariae: PSE) et une espèce dont les grains contiennent environ 1 % ms (C. liberica var. dewevrei: DEW) a été réalisé. Les grains de PSE contiennent un hétéroside diterpène spécifique qui interviendrait dans l'amertume du café boisson. Les arbres issus des première et deuxième générations ont été étudiés pour leur morphologie, leur phénologie, leur composés biochimiques des grains, leur composition en chromosomes de chaque parent, leur recombinaisons grâce au marqueurs moléculaires et leur fertilité.

Les marqueurs RFLP ont permis de déterminer l'origine des hybrides de deuxième génération. On dénombre 54 arbres issus du rétrocroisement des hybrides F1 par l'espèce DEW et 13 issus du rétrocroisement des hybrides F1 par l'espèce PSE. Une relation linéaire a été mise en évidence entre la composition génomique des hybrides, déterminée par hybridation génomique in situ, et leur taille de génome mesurée par cytométrie en flux. L'analyse des teneurs en en caféine montre que c'est un caractère sous contrôle polygénique avec un fort effet génétique. Cependant, un gène majeur semble contrôler la présence/absence de caféine dans les grains. L'hétéroside diterpène spécifique semble aussi être contrôlé par un gène majeur.

Bien que l'absence de caféine soit toujours liée à d'autres caractères indésirables, l'existence d'un gène majeur pour le contrôle de l'absence de caféine permet d'envisager son transfert vers les espèces cultivées. Ce transfert pourrait être considérablement facilité par l'utilisation de marqueurs moléculaires et de la cytométrie en flux.

IMPROVEMENT OF COFFEE CUP QUALITY BY ETHYLENE

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SUMMARY

Arabica cherries in South of Brazil take 220 days from flowering to ripening. Fruit ripening is not uniform because of multiple bloomings during the flowering season. The presence of unripe beans after harvest has been associated with a "metallic taste". Previous studies have shown that ethylene causes accentuated abscission of young fruits (when applied early in the fruit development: Jan.-March) and accelerates cherry ripening.

This study evaluates the efficacy of early Ethrel applications (February) as a "pruning agent" of late flowers and late Ethrel applications (40 days before harvest) as a ripening regulator. Ethrel (2-chloroethane phosphoric acid solution) liberates ethylene soon after it is absorbed by plant tissues. Dosages must be around 500-1000 ppm to achieve effects with unshaded trees.

Two distinct field studies (1994 & 1996) involved two varieties (Yellow Catuai & Bourbon LC) and two locations (Cajuru & S.Sebastiao). It was possible to detect a positive influence of early Ethrel applications on cup quality, caused by differential pruning of very small pin-heads (late flowers) and evaluated by the average number of fruits on ground after 7 weeks of spraying: 325 @ 1000 ppm, 121 @ 500 ppm vs. 42 (control). While late Ethrel applications had effect of accelerating the apparent ripening process, no measurable difference on cup quality has being detected so far.

THE EFFECT OF SHADE, STEM NUMBER AND SPACING ON ROBUSTA COFFEE YIELD IN GHANA

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INTRODUCTION

Apart from genetic influences, yield in coffee may be affected by the environment as well as the management system used. The use of shade or no shade for coffee varies according to ecological conditions, local tradition and the level of management (Mitchell, 1988). Shade trees may be provided in coffee plantations to moderate light intensity and day time temperature, reduce weed growth as well as influence the physical and chemical properties of soils through their litter and root activities (Webster and Wilson, 1969).

Spacing determines the plant density per unit area and its subsequent effect on soil factors (Pereira and Jones, 1957), microclimate (Browning, 1977) and management pratices (Blandy, 1969). The spacing at which coffee trees are planted depends on the growth habit of the cultivar, the pruning system and other management practices (Mitchell, 1988). Whilst close planting may offer a high degree of ground cover at an early stage to suppress weed growth, management operations may be impeded.

Multiple stem production is a conventional practice in coffee cultivation (Anon, 1985). The number of stems for a coffee stand depends on the spacing and the method of pruning. The important factor is to get a crop with an open canopy so that fruit bearing and maturation are not impeded (Anon, 1985).

In Ghana, farmers often grow coffee with or without shade at various spacings and with different stem numbers. This study was aimed at investigating the effect of shade, coffee spacing and stem number on yield of improved robusta coffee in Ghana.

MATERIALS AND METHODS

The experiment was carried out at Cocoa Research Institute Sub-station, Afosu (06°23'N, 01°, OOW, 202m.asl). This station is

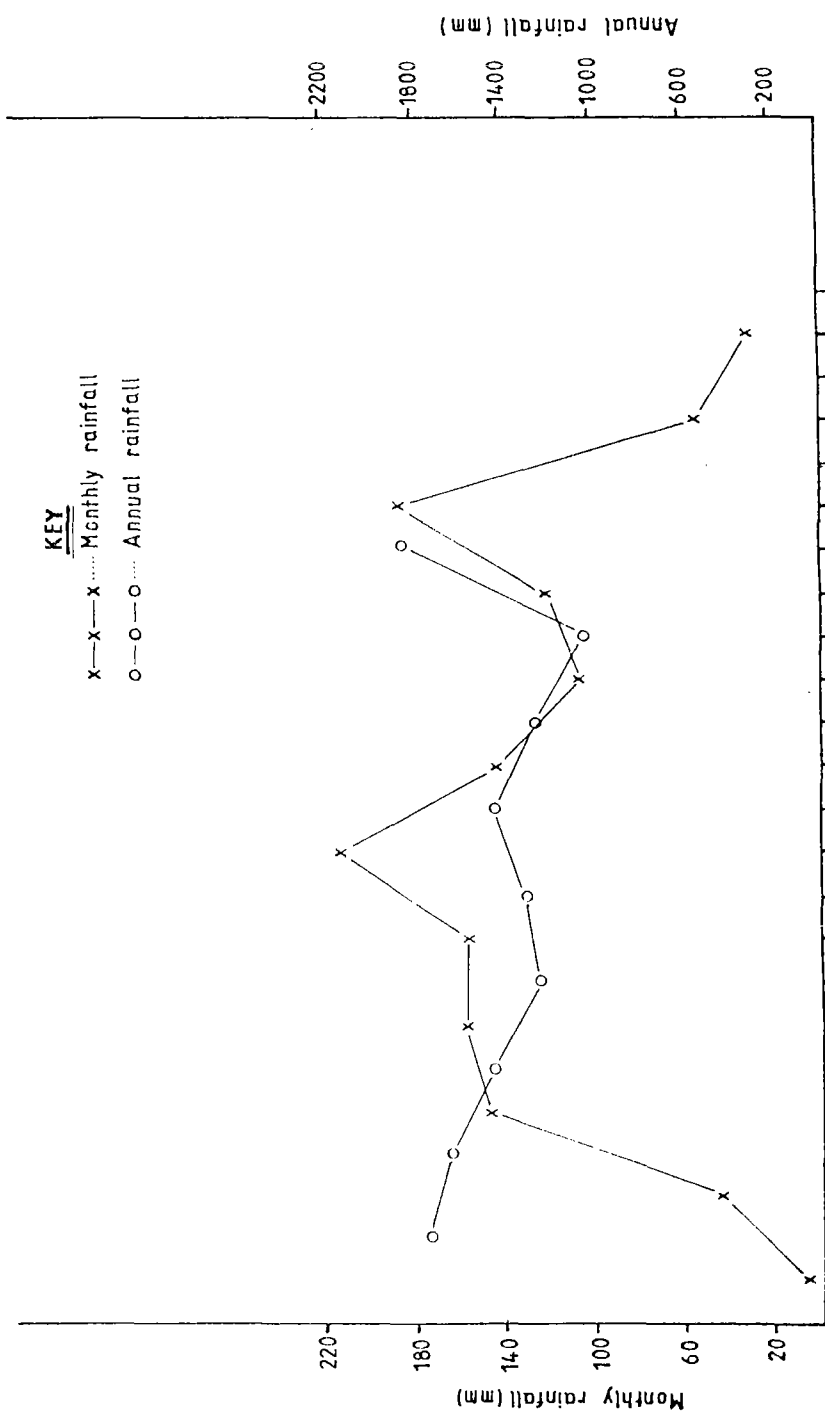

Fig. 1 Mean monthly and annual rainfall distribution (Afosu - 1987 to 1995)

normally described as marginal in terms of rainfall availability. The mean monthly as well total annual rainfall from 1987 to 1995 is shown in figure 1. This shows that over the experimental period, Afosu recorded a mean annual rainfall of 1436.8mm, a mean monthly minimum of 5.4mm in January and a mean monthly maximum of 213.0mm in June. The experiment was originally planted in 1981 but was devastated by bush fires in 1983. In June 1987, the trial was replanted with 8 months old seedlings of improved robusta coffee. The experimental design was a split-split plot in three replicates with shade as main plot, coffee spacing as sub plot and stem number as sub-sub plot. The shade treatments were (a) No shade and (b) Shade provided by *Glyricidia maculata* at 3m x 3m and thinned to 9m x 9m one year after planting. The coffee spacings were (a) 2.4m (b) 2.6m (c) 2.8m and (d) 3.0m triangular giving plant populations of 2400, 2200, 2060 and 1900 plants/ha respectively. The stem numbers used were (a) 1, (b) 2, (c) 3, (d) 4 stems per stool. To achieve the required stem number, seedlings were looped and pegged down with forked stakes. Orthotropic suckers emerged on the stem from which the required stem numbers were selected and trained. Temporary shade of plantain was planted in all plots at 3m x 3m three months before coffee planting and removed after two years.

Weed control was by high slashing with cutlass four times a year and sometimes supplemented with a herbicide (gylphosate) application, at 2 litres/ha. There was no major pest or disease problem and hence no control measures were undertaken. Desuckering was done every three months and the height of the coffee was restricted to about 2.2m by periodic topping. Owing to non-uniformity in the ages of the stem numbers, growth measurements could not be carried out. Ripe berries were harvested from mid October to mid January of each crop season and dried to unhulled state for yield recording.

RESULTS

The first crop was obtained three years after planting. Tables 1 to 3 show coffee yield obtained from 1990/91 to 1994/95.

The Effect of Shade on Yield

Unshaded coffee yielded significantly higher ($P \leq 0.05$) than the shaded coffee in each cropping season except 1993/94 when the difference was not significant (Table 1). The mean yield over the experimental period indicated that unshaded coffee yielded about 37% more than the shaded coffee. There was a biennial alternation in yield in the shaded coffee but this was absent in the unshaded coffee. On the contrary yield increased steadily for 3 years in the unshaded coffee after which yield began to decline.

Table 1: The effect of shade on coffee yield (kg dry beans/ha)

	No Shade	Shade	SED (48df)
1990/91	516.4	265.6	98.4
1991/92	2583.0	1852.8	110.6
1992/93	2615.3	823.1	500.4
1993/94	2394.1	2160.2	96.7
1994/95	1108.1	732.7	120.4
Mean	1843.7	1167.5	

The Effect of Spacing on Yield

Coffee planted at 2.4m and 2.6m triangular yielded significantly higher ($P \leq 0.05$) than coffee at 2.8m or 3.0m triangular under both shade regimes. There were no significant differences between the yield of coffee planted at 2.4m and 2.6m triangular and between coffee at 2.8m and 3.0m triangular (Table 2).

Table 2 **The effect of spacing on coffee yield (kg dry beans/ha)**
Coffee spacing (m triangular)

Cropping Season	2.4mΔ	2.6mΔ	2.8mΔ	3.0mΔ
1990/91	370.5	649.9	296.6	255.4
1991/92	2585.5	2724.0	1734.5	1819.9
1992/93	1998.6	2047.6	1392.3	1438.3
1993/94	2451.4	2782.8	1577.2	2297.5
1994/95	943.8	961.1	856.9	919.3
Mean	1670.0a	1833.0a	1171.5b	1346.1b

Means carrying the same letter are not significantly different at $P \leq 0.05$

The Effect of Stem Number on Yield

There were no consistent effect of stem number on coffee yield in the various cropping seasons (Table 3). However, the mean yield over the experimental period indicates that coffee with two or three stems per stool yielded better than coffee with either one or four stems. The lowest yield was obtained from coffee with four stems. There were no interaction effects of the factors on coffee yield.

Table 3: **The effect of stem number on coffee yield (kg. dry beans/ha)**

	Stem Numbers			
Cropping Season	1	2	3	4
1990/91	616.0	468.5	248.4	231.2
1991/92	1500.6	2567.0	2013.5	1972.1
1992/93	1570.9	2164.7	1683.8	1457.5
1993/94	2147.8	2456.1	2579.7	1926.2
1994/95	858.8	937.0	1110.8	774.9
Mean	1338.1b	1718.7a	1527.2a	1272.4b

Means carrying the same letter are not significantly different at $P \leq 0.05$

DISCUSSION

Unshaded coffee gave higher yields than shaded coffee in this study. This agrees with the observations by Webster and Wilson (1969) that when provided with adequate moisture and soil nutrients coffee shows a high rate of photosynthesis in high light intensity conditions. Though Afosu is generally referred to as a marginal area, the rainfall figures during the experimental period satisfied the minimum rainfall requirement of 1200mm per annum for robusta coffee

(Wellman, 1961) and probably contributed to the higher yields recorded from unshaded coffee. The level of shade provided in the shaded plots (123 trees/ha) may also have been too heavy, and probably contributed to the low yield. Amoah *et al* (1995) observed that sparse shade (70 trees/ha) gave better yield in ecologically suitable areas while moderate shade (90 trees/ha) was required for moderately and marginally suitable areas. Similarly Snoeck (1988) and Webster and Wilson (1969) observed a 34% decrease in coffee yield under dense shade (105 trees/ha) conditions owing to reduced photosynthetic activity. The biennial alternation in yield observed in the shaded plots is contrary to the results of other workers as moderate shade has been used to reduce erratic yields caused by over-bearing (Kimemia and Kaminchia, 1994). This may need further investigation.

Spacing determines the plant density per unit area and its subsequent effect on soil factors (Pereira and Jones, 1957). The poor yield performance of the widely spaced coffee could be related to the relatively lower plant densities. The best density guarantees optimum light interception, and this has been associated with coffee tree populations that produce complete ground cover (Kugura *et al*, 1978). A density of 5000 trees/ha was identified as a biological optimum for arabica coffee by Browning and Fisher (1976). Densities higher than 2400 trees/ha may therefore have to be investigated for robusta coffee in Ghana. Further investigations may also aim at studying the effect of plant density on the quality of clean coffee. This study involved dry unhulled coffee only. Njoroge and Kimemia (1994) observed that although overall yield increased with increasing plant density in arabica coffee, the proportion of large grade A coffee beans reduced with increasing tree density.

This study has demonstrated that robusta coffee may be planted with two or three stems with two stems per stool being optimum. This is in agreement with the results of other workers. Njoroge and Kimemia (1994) observed two stems per stool as being optimum for arabica coffee. Whilst coffee with two stems per stool were better in areas with good amount of rainfall, the one stem system was superior in the drier area (Njoroge and Kimemia, 1994). This suggests that coffee stem number per stool may affect the moisture relations of the trees. With increasing stem number, canopy size increases and hence a wider area is exposed for transpiration and when this is not balanced with good precipitation levels an aritifical moisture stress may be created which may consequently affect coffee yield (Wellman, 1961). Nonetheless, multiple stem production is a conventional practice in coffee cultivation and various stem numbers up to three may be maintained depending on the spacing and the method of pruning adopted (Anon, 1985).

CONCLUSION

Over a period of five cropping seasons, unshaded coffee yielded about 37% more than shaded coffee. Coffee yield increased with increasing planting density. Coffee at 2.4m (2400 trees/ha) and 2.6m (2220 trees/ha) triangular produced significantly higher yield than coffee at 2.8m (2060 trees/ha) and 3.0m (1900 trees/ha) triangular. Coffee may be cultivated with up to three stems per stool as this gives sustained yield over a longer period.

SUMMARY

Studies were carried out between 1987 and 1995 on the effects of shade, stem number per stool and the spacing of coffee on yield of improved robusta coffee in a marginal area in Ghana. The experimental design was a split-split plot with shade as main plot, coffee spacing as sub-plot and stem number as sub-sub plot. The shade treatments were (a) No shade and (b) shade provided by *Glyricidia maculata* at a final density of 123 trees/ha. The coffee spacings were 2.4m, 2.6m, 2.8m and 3.0m triangular giving plant populations of 2400, 2220, 2060 and 1900 plants/ha respectively. The stem numbers studied were 1,2,3 and 4 stems per stool.

Unshaded coffee yielded significantly higher than shaded coffee in all cropping seasons. Coffee planted at 2.4m and 2.6m triangular yielded significantly higher than coffee at 2.8m or 3.0m triangular under both shade regimes. There was no consistent trend in the effect of stem number on yield in the various cropping seasons. However the cumulative yield over the experimental period indicated that coffee at 2 stems per stool was superior to the other treatments. There was a biennial alternation in yield in the shaded coffee but this was absent in the unshaded coffee. There were no interaction effects of the factors investigated on coffee yield.

RESUMÉ

Recherche a été realisé entre c'année 1987 et 1995 sur l'effet d'ombrage, nombre de tige par une plant mère et l'espacement sur la production du café dans une région marginale du Ghana. Le plan expérience était la division d'une pièce de terre-une pièce avec l'ombrage comme la pièce principale, l'espacement du café comme sub-pièce et nombre dè tige comme sub-sub pièce. Le traitement d'ombrage etait (a) Sans ombrage et (b) ombrage provise par Glyricidia maculata à la densité finale de 123 arbres/ha. L'espacement étaient 2.4m, 2.6m, 2.8m et 3.0m triangulaire en donnant la population de 2400, 2220, 2060 e 1900 plantes/ha respectivement. Le nobre de tige réalisée étaient 1, 2, 3 et 4 tiges par une plante mère.

Le café sans ombrage ont produit plus nombreux que les uns avec ombrage dans tous les saisons. Café planté à 2.4m et 2.6m triangulaire ont produit plus beaucoup que les uns à 2.8m et 3.0m dans tout les deux conditions d'ombrage. Il n'y avait pas de la tendance consistante sur l'effet du nombre de tige sur la production dans tout les saisons. Cependant la production cumulatif avait indequé pendaat la période d'expérience que le café à tiges par une plante mére était supérieur. Il y avait une alternance bisannuel en production dans la piéce avec ombrage mais absent dans la pièce sans ombrage. Il n'y avait pas d'effects interactions des facteurs recherchés sur la production du café.

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THREE CYCLES OF COFFEE YIELDS FOR VARIETY x SPACING AND ECONOMICAL EVALUATION

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INTRODUCTION

Yield increase in coffee can be obtained as tree density increases above the conventional tree density of 1330 tree per hectare (Mitchell, 1976; Browning and Fisher, 1976; Gathaara, 1988).

Kiara (1981) suggested that the future testing of new varieties of coffee be within a range of 1300 to 5000 trees/ha on practical basis. In areas receiving more than 1250mm of rainfall or where coffee is grown under irrigation, coffee may be spaced with population density ranging from 2900 to 5000 trees/ha (Kaguru et al, 1978). In this case, trees should be brought up on single uncapped stem on the 1st cycle and on two stems in subsequent cycles. No pruning should be done except for desuckering and handling of secondary branches (Njoroge and Mwakha, 1989). Huxley, 1970 reported that the conventional pruning methods are not effective at higher plant densities. However, at higher coffee densities, the coffee should be trimmed on one or two heads depending on the density and most suitable system of pruning is free growth followed by stumping for rejuvenation after 5-6 years when yield declines are observed (Mitchell, 1975; Njoroge, 1989).

In every case, the cost of labour and material inputs increases as the number of coffee trees increase per hectare (Kessy, 1988). The lowest tree density of 1330 trees/ha at a conventional spacing (2.74m x 2.74m) always requires the least input and hence the least cost of production (Kiara and Njagi, 1982).
The practical implications are simply that farmer who chooses to plant coffee at higher density than the conventional spacing should be expect to have higher yield/ha but must be prepared to spend more money. Three commercial varieties available for farmers in Tanzania had not been tested for densities. Hence the objective of the experiment was to screen these varieties under different densities compared to the conventional (1330 plants/ha) at Lyamungu and look at the economical implications.

MATERIALS AND METHODS

The experiment commenced at Lyamungu Agricultural Research Institute in May, 1977 at an altitude of 1268 m a.s.l. and a geographical location of 3°14'S and 37°15'E.
The trial composed of three varieties of arabica coffee planted at four different densities. The design used was split plot with three replications.

Treatments

Variety (main plots)	Spacing (sub-plots) / Density (plants/ha)
	S_1 : 2.74m x 2.74m / 1330 (Conventional)
V_1 : KP 423 (Kent type)	S_2 : 3.00m x 1.00m / 3333
V_2 : N 39 (Bourbon type)	S_3 : 2.00m x 1.00m / 5000
V_3 : H 66 (Kent type)	S_4 : 1.83m x 0.76m / 7190

For the spacing S_1, S_2 and S_3 conventional planting holes were used while trenches of 60cm wide and 60cm deep were used to plant seedlings at a closest spacing (S_4). Coffee planted at a conventional spacing (S_1) was trained on the capped multiple stem pruning system and rest were raised on the multiple (two) stem-free growth; with stumping aimed at the end of successive growth cycles in 1982, 1987 and 1992. All other agronomic practices were applied as recommended.

Labour and material inputs were recorded in each spacing treatment from the time of field establishment in 1977 to the end of the experiment in 1992. The 1994 prices of parchment coffee, costs of labour and other material inputs were used for economical analysis. Partial budget was performed on the experimental data according to the CIMMYT economical manual (CIMMYT, 1988) to the variety x spacing interaction as they were statistically significant (P=0.05). 10% yield adjustments were made and a marginal rate of return of 50 to 100% was considered reasonable for a coffee farmer.

RESULTS AND DISCUSSION

The statistical analysis showed that the spacing and varieties treatments with their respective interactions had significant yield differences at P = 0.05 (Table 1).

Table 1: Significant levels for Variety (V), Spacing (S) and V x S.

Year	Variety (V)	Spacing (S)	V x S
1979	**	***	ns
1980	***	***	ns
1981	ns	***	*
1982	ns	*	**
1983	-	-	-
1984	**	**	***
1985	ns	**	*
1986	**	**	ns
1987	**	ns	ns
1988	-	-	-
1989	**	**	ns
1990	**	**	*
1991	**	***	**
1992	**	***	*

Table 2: Average yield (kg parchment /ha/year) from 1979 to 1992

	Variety			
Spacing	**KP423 (V_1)**	**N39 (V_2)**	**H66 (V_3)**	**Mean**
2.74m x 2.74m (S_1)	1158 a	632 a	806 a	865
3.00m x 1.00m (S_2)	1600 b	1058 b	1197 b	1285
2.00m x 1.00m (S_3)	1699 b	1273 c	1301 b	1425
1.83m x 0.76m (S_4)	1882 c	1372 c	1444 bc	1566
Mean	1585	1084	1187	

(a) Variety

Results are shown in table 2. KP423 in each of the four densities (1330, 3333, 5000 and 7190 trees/ha) had the highest yield and the least was N 39.

(b) Spacing

Results are shown in table 2. Coffee yield was observed to increase with increasing plant density above the conventional tree density of 1330 trees/ha. The highest yield was obtained with 7190 trees/ha.

(c) Interaction : Spacing x Variety

Table 3: Dominance analysis of Spacing by Variety Interaction (S.V.)

Net Benefit (T.Shs/ha)	Variable Costs (T.Shs/ha)	Density (S) (plants/ha)	Variety(V)	Type of Interaction
937,214	790,786	3333	KP423	S_2V_1*
773,368	1,061,432	5000	KP423	S_3V_1D
771,895	478,505	1330	KP423	S_1V_1*
597,563	1,435,237	7190	KP423	S_4V_1D
556,178	736,222	3333	H66	S_2V_3D
432,633	437,367	1330	H66	S_1V_3*
425,102	717,298	3333	N39	S_2V_2D
383,587	1,027,613	5000	H66	S_3V_3D
357,303	1,017,897	5000	N39	S_3V_2D
267,510	417,290	1330	N39	S_1V_2*
184,088	1,375,912	7190	H66	S_4V_3D
115,491	1,366,509	7190	N39	S_4V_2D

D=Dominated *=Undominated

Yields and economical results are shown in tables 3 and 4 respectively. For the whole experimental period (3 crop cycles), the KP 423 at the closest spacing (7190 trees/ha) had the highest yield/ha while the standard variety (N39) at the conventional Spacing (1330 trees/ha) had the least yield/ha. Figure 1 shows the yields of the three varieties for the different cycles. The conventional spacing had the lowest yields whilst the closest spacing had the highest yields for all varieties though not economical.

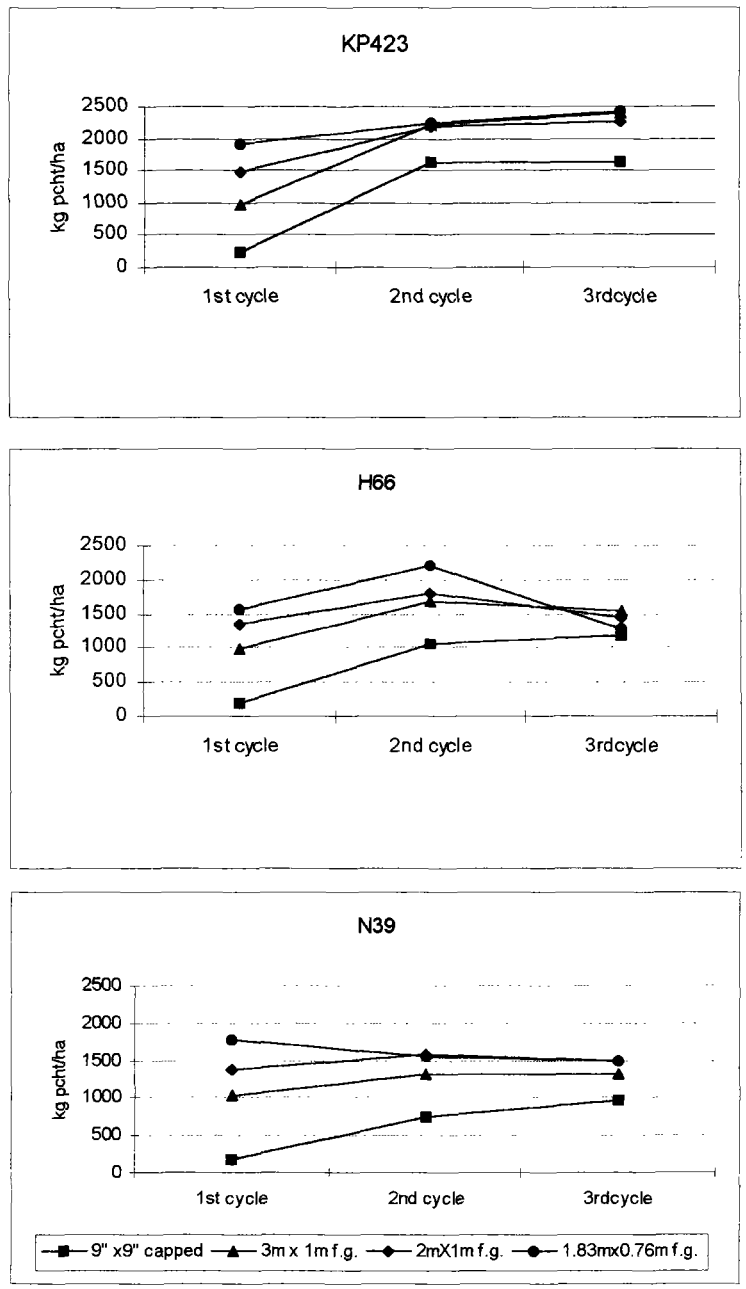

Figure 1: Response to spacing of 3 commercial varieties

Table 4: Marginal analysis of the undominated interactions per ha (Tanzanian Shillings)

Interaction			Change	from next	highest	benefit
Net Benefit	Pl./ha	Variety	Cost that vary	Marginal Net Benefit	Marginal Variable Cost	Marginal Rate of Return(%)
937,214	3333	KP423	790,786	165,319	312,281	53
771,895	1330	KP423	478,505	346,793	41,138	843
425,102	1330	H66	437,367	159,592	20,077	795
265,510	1330	N39	419,290	-	-	-

With respect to table 4, KP 423 at 7190 trees/ha had the highest gross income and variable costs while N39 at 1330 trees/ha had the least values of gross income and variable costs.

Table 3 of dominance analysis, shows that the S_2 V_1; S_1 V_1; S_1 V_3 and S_1 V_2 were the undominated interactions. Table 4 of marginal analysis of the above undominated interactions showed that the S_2 V_1 i.e: KP 423 at the spacing of 3.00 m x 1.00 m (3333 trees/ha) had the highest net benefit with marginal rate of return of 53% when compared with S_1 V_2 (conventional) which had the lowest net benefit and an intermediate marginal rate of return of 795 with respect to S_1 V_3.and S_1 V_1

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APPENDIX

Variable costs under different spacing

A. Field establishment

1. Marking out of holes: One manday marked 133 planting hole.
2. Digging of holes : One manday dug 20 holes.
3. Organic manure (FYM) : 1 hole required 1 debe of FYM @ T.Shs. 15.00.
4. Filling the holes and relining : One manday required for 20 holes.
5. Seedlings : One seedling cost T.Shs. 40.00
6. Planting : One manday plant 80 seedlings.
7. Re-filling : One manday required for the field with 1330 coffee plants.

B. Field maintenance

1. Capping : 100 trees/manday in the 3rd year after planting.
2. Pruning : 40 trees/manday x 3 rounds/year for the coffee planted at a conventional spacing (2.74m x 2.74m). Removal of skirt (lower primaries): 100 trees/manday.
3. Fertiliser: CAN 26%N applied at a rate of 170 g/tree x 3 rounds/year x 16 yrs. Labour: one 50 kg bag of fertiliser /manday.
4. Irrigation: Cost of 3 Mandays/ha x 3 rounds/year.
5. Stumping : 80 trees/manday at the end of 1982, 1987 and 1992 for the spacing S_2, S_3 and S_4.
6 Picking : 2 debes of 14 kg cherries/manday

C. Processing

1.Pulping and fermenting : 25 debes of 14 kg cherries/manday
2.Washing : 250 kg of fermented coffee/manday
3.Hand sorting and grading: 75 kg of wet parchment/manday
4.Drying : One manday required for 200 kg x 7 days.
5.Packing and storage: 500 kg of parchment/manday

One Manday: 8 hours work costing T.Shs. 500. Coffee price used was T.Shs.1200/kg parchment

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ABSTRACT

Three commercial varieties KP423, H66 and N39 planted since 1977 each at 1330, 3333, 5000 and 7190 plants/ha at Lyamungu Coffee Research Station are in discussion. Stumping was done at an interval of 6, 5 and 5 years for the first, second and third crop cycles respectively for the high densities. The 1330 plants/ha (conventional spacing) treatment was capped at multiple (two) stem pruning system. The rest were multiple (two) stem-free growth. KP423 significantly (P=0.05) out yielded the rest of the varieties. There is significant (P=0.05) yield increase with increasing plant densities. Significant (P=0.05) yield differences for variety x densities interactions are evident. The most economical practice to the farmer is to plant KP423 at 3333 plants per ha.

RESUME

Un essai comparant les variétés KP423, N39 et H66 aux densités de 1330, 3333, 5000 et 7190 plantes/ha est présenté. Il fut établi en 1977. Les 3 plus fortes densités, conduites en croissance libre, furent recépées après 6, 11 et 16 ans. Elles étaient en croissance libre sur deux tiges, alors que la densité conventionelle était écimée sur deux tiges. KP 423 fut significativement la productive. L'influence de la densité sur la récolte est positive et significative pour les trois variétés. Le traitement le plus économique est de planter KP 423 à 3333 plantes par hectare.

INTERCROPPING EFFECT OF SWEET POTATO ON THE GROWTH, DEVELOPMENT AND YIELD OF ARABICA COFFEE GROWN AT AIYURA, PAPUA-NEW-GUINEA

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INTRODUCTION

Whilst the initiative by Papua New Guinean (PNG) farmers to diversify the coffee-based agriculture is a direct response to declining coffee price, an effort to efficiently utilize scarce resources and maximise security and food supply, cash income, and minimise risks, the competitive presence of the annual crops in the garden will invariably affect the growth of coffee trees. It would seem that farmers are not necessarily concerned with maximising dual biological productivity of component crops, rather, an emphasis on intercropping seems to aim at maximising annual crop production without significantly reducing the growth of coffee. Whether this practice affects coffee growth and yield negatively or positively was investigated in the present study.

The specific objectives of the study were; a) to determine the effect of intercropping sweetpotato on the growth and development of tall arabica coffee; b) to determine and assess the potential and the ability of sweetpotato to suit agronomically for intercropping with coffee; and c) to assess economic benefits, viability and sustenance of intercropping such foodcrop with coffee.

METHODOLOGY

Site and Climate

The trial was conducted on Block 251 at the HAES Aiyura research farm (6^0 19'S, 145^0 55'E), at an altitude of 1640 masl.

The experiment site would receive some 2,000 mm of rainfall annually, three-quarters of which is expected during the months October to April. Mean minimum and maximum monthly temperatures range from 14-15°C and 23-24°C respectively; with little variation throughout the year. Relative humidity is high at 70 to 80%. Annual evaporation is high and estimated at 1,581 mm, and monthly rates of evaporation are similar throughout the year (McAlpine, *et al*, 1975).

The trial was conducted on a clay loam soil of moderate fertility, high in organic matter, nitrogen and base saturation. The pH in water was 5.2.

Experimental

The trial used a randomised block design (RBD) in which the five treatments were replicated 4 times.

Plant Spacings and plant populations

The coffee intra row spacing was constant at 1.5m but inter row spacings were varied from 2.5m (100% coffee density) to 3.5m (71%), to 5m (50%) and 10m (29 %) (Table 1). The sweetpotato densities were proportionately worked out.

Table 1. Coffee : Sweetpotato replacement densities, spacings and plant populations

T/ment Code	Density		Gross plot area (m^2)	No. of SP rows	Coffee spacing (m)	Coffee plant popl	Coffee harvest row
	%Coffee	%SP					
A	100	0	300	0	1.5*2.5	2667	mid 6
B	71	29	315	1	1.5*3.5	1905	4
C	50	50	300	3	1.5*5.0	1333	2
D	29	71	450	8	1.5*10	667	1
E	0	100	150	10	-	0	0

Two sole crop treatments (ie controls) at their respective optimum population densities (ie 2,667 pl ha^{-1} for coffee and 30, 000 pl ha^{-1} for sweetpotato) were included.

Planting Dates and Other Practices used

Eleven months old coffee seedlings were planted in April 1989 and harvest commenced in 1991. The first crop of sweetpotato was planted in 1991, the second in 1992 and the third crop in 1993. The sweetpotato crops were harvested after approximately 7 months of growth.

The sweetpotato (var wanmun) was planted on small raised mounds spaced at 1.0m each way ($1m^2$), the row nearest to the coffee was 1m away. Only the coffee was fertilized with NPK (12: 12: 17 + 2) at recommended rates based on densities (Harding, 1987).

Statistical Analysis

Two separate ANOVA's were computed, one for the coffee yield and growth parameters, and the other for sweetpotato. Mean yields were used to construct land equivalent ratios (LER) and yield reduction analysis.

The potential yield reduction (PYR) of a crop was calculated following the method of Njoroge *et al* (1993). Land Equivalent Ratios (LER) were calculated after Mead & Willey (1980). Monetary equivalent ratios (MER) were calculated after Adetiloye and Adekunle (1989).

The coffee growth data were subjected to simple regression analysis to determine correlations between yield and various growth parameters. The combined analysis of variance for coffee (1991-1994) and sweetpotato (1991, 1993, 1994) yield datas used the method of Snedecor (1946). The coffee vs sweetpotato (or *vice versa*)

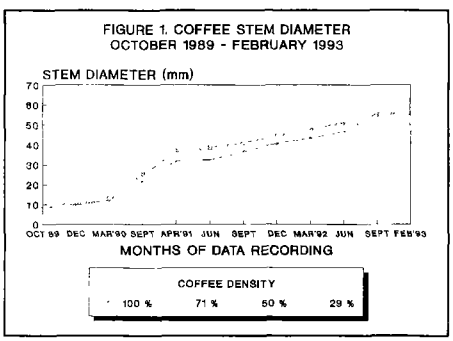
competitive relationships were determined by the Relative crowding coefficient (K_{AB}) after de Wit (1960).

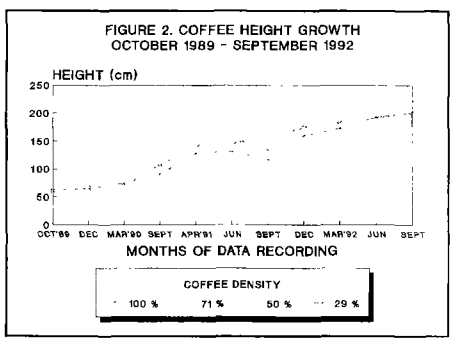
RESULTS AND DISCUSSIONS

Coffee yields for the first 4 years (1991-1994), together with data from the three sweetpotato crops are discussed.

Growth and Development of Intercropped Coffee

Coffee stem (Fig 1) and height (Fig 2) growth between October 1989 and February 1993 were chosen as relevant indicators of the intercropping effect on coffee.





Simple regressions determined to correlate between these and other growth parameters (Table 2) showed that, the growth and development of pure stand coffee was not significantly better or worse than the mixtures; coffee stem (Fig 1) and height (Fig 2) growth, and development seemed unaffected by intercropping it with sweetpotato.

Table 2: Selected simple regressions of various coffee growth parameters and yield

T/ment Code	Y	a + b x	X	Reg Coefficient
A	height	32.0 + 3.08x	diameter	0.98
B	height	28.76+ 3.25x	diameter	0.99
C	height	32.36+ 3.09x	diameter	0.97
D	height	30.58+ 3.13x	diameter	0.998
A	NOBPR	0.929+1.122x	diameter	0.99
B	NOBPR	1.506+1.125x	diameter	0.99
C	NOBPR	0.88+ 1.08x	diameter	0.99
D	NOBPR	1.19+ 1.114x	diameter	0.99
A	NOBPR	10+0.350x	height	0.98
B	NOBPR	11.268+0.345x	height	0.99
C	NOBPR	10.574+0.340x	height	0.99
D	NOBPR	11.946+0.355x	height	0.99
A	YIELD	0.043+0.027x	diameter	0.90
B	YIELD	0.274+0.034x	diameter	0.83
C	YIELD	0.113+0.0093x	diameter	0.75
D	YIELD	0.047+0.00146x	diameter	0.51

The calculated linear regressions (Table 2) shows that irrespective of coffee density the following parameters are highly correlated; height vs diameter growth (av.r = 0.99), number of bearing primaries vs diameter

(av.r = 0.99), number of bearing primaries vs height (av.r = 0.99), number of nodes vs diameter (av.r = 0.95) and number of nodes vs height (av.r = 0.95), (the last two variables not shown in Table 2).

When correlating yield with various growth parameters, in particular stem diameter, the correlation generally weakened as coffee density was reduced from the pure stand (Table 2), indicating that the usefulness of this method in predicting growth patterns and thereafter yield (e.g; Kiara 1996), is only valid at the biological optimum density of coffee.

Analysis of Variance for Each Years Data

Analysis of Variance for each year's coffee and sweetpotato yield datas used the split-plot principle after Little and Hills (1978: 125). The treatment plots were taken as main plots and the years as subplots.

Table 3: ANOVA of each years harvest of coffee green bean yield (t ha^{-1})

Source of variance	1991			1992		1993		1994	
	DF	SS	MS	SS	MS	SS	MS	SS	MS
Tot	15	5.81		9.53		5.31		0.77	
Block	3	0.85	0.28	0.81	0.27	0.26	0.09	0.04	0.01
T/ment	3	3.84	1.28***	7.87	2.62***	4.10	1.37***	0.61	0.20***
A+B vs C+D	1	2.536	2.536	5.641	5.641	3.861	3.861	0.273	0.273
A vs B	1	1.118	0.118	1.549	1.549	0.029	0.029	0.149	0.149
C vs D	1	0.186	0.186	0.696	0.696	0.205	0.205	0.120	0.120
Error	9	1.12	0.12	0.84	0.09	0.95	0.11	0.12	0.01
F-Values (A+B vs C+D) (A vs B) (C vs D)			 21.13*** 9.32* 1.55		 62.68*** 17.21*** 7.73*		 35.10*** 0.26 1.86		 27.30*** 14.90*** 12.00***

Tabulated F-value (DF= 1,9), P(0.05)= 5.12, P(0.01)=10.56

The analysis for coffee green bean yield, given in Table 3, indicated that the treatment differences (A + B) - (C + D) was statistically significant for all years, indicating real differences for these comparisons. The major portion of the variability among treatments was due to the comparisons, treatment A + B, leaving little doubt that treatment A and B are superior to C and D. However there is no evidence that A (100 % coffee) is any better than B (71 % coffee).

Treatment * Year Effects

In the combined analysis of variance, the treatment * years interaction was highly significant ($P<0.01$) for both coffee and sweetpotato yields (Table 4), indicating that the treatment effects were not consistent over the years.

The yield data given in Table 5 shows that when averaged over all treatments, coffee green bean yield was superior in 1992, followed by 1993, 1991 and 1994 in that order of decreasing yield. For sweetpotato, best yields were obtained in the 1991 crop, followed by the crop in 1994. The crop in 1993 gave significantly low yields. When averaged over the years, coffee yield at the 71 % coffee: 29 % sweetpotato gave consistently high yields, except in 1994 (harvest were not completed for the season).

Table 4: ANOVA of combined coffee green bean (4 yrs) and sweetpotato marketable tuber yield over three (3) years.

		Coffee			Sweet potato			
Source of variation	DF	SS	MS	F#	DF	SS	MS	F#
Total	63	2818.02	44.73		47	1111895.3	23657.4	
Yrs(Y)	3	874.175	291.39		2	564796.2	282098.1	
T/ment (T)	3	185.52	395.173		3	177996.8	59332.3	
Reps(R)	3	34.763	11.588		3	2362.3	787.48	
Y*T	9	414.017	46.00	7.453	6	335050.3	55841.7	60.130
T*R	9	22.20	2.467		6	5760.1	960.0	
Y*R	9	120.70	13.41		9	9805.9	1089.5	
Y*T*R	27	166.645	6.172		18	16723.8	929.10	

Tabulated F value 1 % level = 2.78 (df, 9,27); = 4.01, (df, 6,18).

Table 5: Mean coffee and sweetpotato marketable yield over 4 cropping years

Treatment Code	Coffee green bean yield (t ha^{-1})					Marketable sweetpotato yield (t ha^{-1})			
	1991	1992	1993	1994	Mean	1991	1992	1993	Mean
A	0.72	1.17	1.38	0.56	0.96	-	-	-	-
B	1.46	2.05	1.26	0.29	1.27	4.47	0.59	0.31	1.79
C	0.45	0.72	0.50	0.28	0.49	8.21	0.49	0.93	3.21
D	0.14	0.13	0.18	0.04	0.12	12.24	0.34	1.43	4.67
E	-	-	-	-	-	6.77	0.76	6.13	4.55
Mean	0.69	1.02	0.83	0.29	0.71	7.92	0.55	2.20	3.56
SE	0.25	0.22	0.23	0.08	0.195	1.36	0.10	0.72	0.73
LSD(P=0.05)	0.56	0.49	0.52	0.18	0.43	0.06	0.22	1.63	0.64
CV (%)	50.98	30.19	39.27	44.18	41.16	24.17	24.85	31.75	2.92

Land Equivalent Ratio (LER)

The LER values indicate that intercropping was advantageous during 1991/92 and 1992/93 (Table 6). In 1994 intercropping actually disadvantaged both coffee and sweetpotato yields. Despite this, when averaged over all the years, intercropping was advantageous at all intercropped treatments.

Monetary Equivalent Ratio (MER)

The average MER values over the four cropping seasons showed that only the contribution of intercropped yields at 71 % coffee: 29 % sweetpotato (ie, Treatment B) was economically superior (Table 7). At this density there was substantial economic gain in intercropping over both pure stand coffee and sweetpotato.

Potential Yield Reduction (PYR)

When averaged over all years of intercropping, there is a potential coffee yield reduction of 48 % imposed by 50:50 (coffee:sweetpotato) treatment and 88 % by 29:71 (Table 8). There were significant coffee yield gains at 71:29 treatment plot. The experimental data collected provides no evidence of a suppression of coffee yield at this density (Treatment B) by intercropping with sweetpotato.

Table 6: LER values for coffee/foodcrop intercropped at Aiyura

T/Code	SP 1991	Cof 1992	Totl LER	SP[1] 1993	Cof 1993	Totl LER	SP[2] 1994	Cof 1994	Totl LER	Mean LER		
										SP	COF	TOTL
A	-	1.00	1.00	-	1.00	-	1.00	1.00	-	-	1.00	1.00
B	0.66	1.23	1.89	3.08	0.49	3.57	0.11	0.54	0.65	1.28	0 75	2.03
C	1.12	0.43	1.64	1.11	0.38	1.49	0.30	0.27	0.57	0.87	0.36	1.23
D	1.81	0.08	1.89	0.43	0.40	0.82	0.70	0.03	0.80	0.98	0 19	1.17
E	1.00	-	1.00	1.00	-	1.00	1.00	-	1.00	1.00	-	1.00
SF	1 35	0.22	-	ns	0 23	-						
LSD (P=0 05)	3.10	0.49	-	ns	0.52	-						
CV %	24	30	-	22	4.5	-						

[1] sweetpotato planted mid 1992, harvested early 1993; [2] sweetpotato planted late 1993, harvested 1994.

Table 7: Calculated MER values for coffee/sweetpotato intercropped at Aiyura

T/ment	Plant Popl (%)		LER Coffee	LER SP	Monetary Equivalent Ratio (MER)			
	Coffee	SP			Totl	Cotf (ra/Ra)	SP (rb/Rb)	MER[*]
A	100	0	1.00	-	1.00	1.00	-	1.00
B	71	29	0.75	1.28	2.03	1.13	0.16	1.29
C	50	50	0.36	0.87	1.23	0.40	0.29	0.69
D	29	71	0.19	0.98	1.17	0.09	0.45	0.54
E	0	100	-	1.00	1.00	-	0.28	1.00

[*] MER = (ra + rb)/Ra, after Adetiloye and Adekunle (1989).

Table 8: PYR values for coffee and sweetpotato over 4 cropping seasons.

T/ment Code	% PYR of coffee					% PYR of sweetpotato			
	1991	1992	1993	1994	Mean	1991	1993	1994	Mean
A	0	0	0	0	0	100	100	100	100
B	-103	-75	9	48	-30	34	22	95	50
C	38	39	64	50	48	-21	36	85	33
D	81	89	87	93	88	-81	55	77	17
E	100	100	100	100	100	0	0	0	0

For sweetpotato, the mean yield figures indicate that no benefits were attained by intercropping at all intercropped plots. Yields were reduced by 50 %, 33 % and 17 % at treatment B, C and D respectively.

Interspecies Competitive Relationships

Calculated relative crowding coefficients (K_{AB}) of coffee with respect to sweetpotato are given in Table 9. Where $K_{AB} > 1$, coffee (A) was more competitive than sweetpotato (B), and *vice-versa*. Where a K_{AB} value was one would mean that there was no relative interspecies competition exerted against that specie.

When averaged over the 1991 and 1992 cropping seasons, sweetpotato was the most competitive species. In the 1993 to 1994 cropping season, coffee was the most competitive crop. This may indicate that during early stages of intercropping, annual crops are more aggressive or competitive than their perennial counterpart. This trend of competition changed during later years (Table 9).

Table 9: K_{AB} value for intercropped coffee (A) green bean with respect to sweetpotato (B) marketable yields over 4 cropping seasons

T/ment	Relative Crowding Coefficient K_{AB}						
	Crop1**	Crop2	Mean	Crop3	Crop4	Mean	Grand Mean
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00
B	1.25	0.92	1.09	7.37	4.18	5.78	3.43
C	0.52	0.95	0.74	2.39	3.30	2.85	1.80
D	0.26	0.59	0.43	1.36	0.75	1.06	0.75
E	1 00	1.00	1.00	1.00	1.00	1.00	1.00
Mean'	0.68	0.82	0.75	3.71	2.74	3.23	1.99

' means are for intercropped treatments B, C and D only

** Crop 1 - 1991 coffee/sweetpotato intercropping
Crop 2 - 1992 coffee/1993 sweetpotato intercropping
Crop 3 - 1993 coffee/1994 sweetpotato intercropping
Crop 4 - 1994 coffee/sweetpotato intercropping

The average competitive ability of the intercropped species over four cropping seasons at various coffee:sweetpotato densities showed that overall, coffee was the most competitive crop at treatment B and C, whilst sweetpotato was more competitive at Treatment D.

CONCLUSION

Several analytical approaches were used to quantify the intercropping effect on the growth, development and yield of coffee, and determine if there was agronomic and economic superiority of intercropping over monocropping coffee and sweetpotato. The results indicated that, 1) the intercropping pressure of sweetpotato on the growth, development and yield of tall coffee (variety Arusha) were minimal throughout the 5 years growing period. On average, intercropping was advantageous. 2) Overall, the 71 % coffee: 29 % sweetpotato densities gave substantial economic gains in intercropping. The experimental data provided no evidence of a suppression of coffee yield at that density. 3) Coffee is a less aggressive competitor for growth resources earlier in its life, making intercropping a risky practice during coffee establishment stage, as annual crops are more aggressive during that phase. However, the data indicated that permanent intercropping can be successfully practised throughout the coffee life by adjusting coffee density downwards, and by adopting other improved agronomic practices.

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ABSTRACT

Four coffee densities viz; 2,667, (pure stand), 1,905 (71%), 1,333 (50%) and 667 (29 %) plants per hectare were combined with sweetpotato in a replacement series experiment at Aiyura, Papua New Guinea between 1989 and 1995. A zero coffee plot (ie; 100% sweetpotato) was included in the trial. The treatments were replicated four times in a RBD.

The objective of the study was to determine the effect of intercropping sweetpotato on the growth, development and yield of tall coffee variety Arusha, and to assess the economic benefits, viability and prospects of permanent foodcrops vs coffee intercropping.

Simple linear regressions calculated between various parameters showed that irrespective of coffee density treatment, the height vs diameter growth (r= 0.99), number of bearing primaries vs diameter (r = 0.99), number of bearing primaries vs height (r = 0.99), number of nodes vs diameter (r = 0.95), and number of nodes vs height (r = 0.95) were highly correlated. When yield was correlated with various growth parameters, in particular stem diameter, the results showed that the correlation generally weakens as coffee density is reduced from the pure stand.

The LER values showed that when averaged over all the years, intercropping was advantageous at all coffee:sweetpotato intercropped treatments. The average MER values over the four cropping seasons showed that at 71% coffee: 29% sweet potato, there was substantial economic gain in intercropping, over both pure stand treatments of coffee and sweet potato. At that plot the relative potential yield reduction data for the component crops, averaged over all years of intercropping, gave no evidence of a suppression of coffee yield.

The Relative Crowding Coefficients indicated that during early stages of intercropping, the annual crop was more aggressive or competitive than the perennial counterpart. The trend of competition changed during later years.

INFLUENCE DE *ALBIZZIA SP.* SUR LA PRODUCTION DE *COFFEA CANEPHORA* VAR. *ROBUSTA* AU TOGO

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Introduction

Au Togo, la caféiculture occupe 43000 familles sur une superficie de 35000 hectares. Après le phosphate, le café est le deuxième produit d'exportation, il représente 20 à 25 % ad valorem des ressources exportées (Coste , 1989). La production caféière est à tendance intensive (mise en place de clones hauts producteurs de Robusta sélectionné et utilisation d'engrais minéraux souvent sur des cafeieres en plein soleil) ; elle est essentiellement localisée dans la région des plateaux au sud du pays.

La dévaluation du franc cfa a aggravé la situation assez précaire des caféiculteurs togolais à la suite de la chute des prix aux producteurs du début des années quatre vingt dix. Ceci a conduit à l'abandon du système intensif de production : les apports d'engrais sont devenus presqu'inexistents, ce qui s'explique par un rapport coût/bénéfice extrêmement faible, les entretiens sont rares. Les conséquences qu'on peut en attendre sont la baisse des rendements.La situation est pire pour les plantations en plein soleil (dix mille hectares) pour lesquelles le taux de disparition connaît un accroissement sensible.

Face à cette situation il est nécessaire de protéger les plantations par un léger ombrage. Celui-ci en limitant la production, permet d'éviter, les années où la pluviométrie est favorable, des pointes de productions très fortes, qui si elles ne sont pas soutenues par les engrais, risquent d'entraîner la mort de nombreux caféiers. Ce risque est d'autant plus élevé que si cette production est concomitante avec une saison sèche prolongée. L'ombrage limite aussi la croissance des mauvaises herbes. Lorsqu'il s'agit d'espèces légumineuses en particulier le sol est enrichi en azote par fixation de l'azote de atmosphérique, en matière organique par la chute des feuilles et des branchettes, et en minéraux par la remontée par les racines des éléments minéraux des couches profondes du sol.

Pour une récolte de 1000 kg de café marchand par hectare, les exportations d'éléments fertilisants sont relativement peu importantes 28.5 kg d'azote, 3.5 kg de phosphate et 36 kg de potasse. La simple mise en place d' espèces légumineuses d'ombrage doit permettre de compenser en grande partie ces exportations. Ceci est l'objet de la présente étude dont les premiers résultats sont ici exposés.

Matériel et méthodes

La zone de production caféière togolaise est divisée en 3 terroirs de rendement:
- Zone 1 :rendement faible: r < 400 kg/ha
- Zone 2 :rendement moyen: 400 kg/ha < r < 800 kg/ha
- Zone 3 :rendement élevé: r > 800 kg/ha

Les études sont réalisées dans deux cents plantations paysannes réparties dans les villages de la zone de production. Une enquête agroforestière suivie d'études plus approfondies sur deux légumineuses forestières les plus fréquentes dans six villages (deux par zone de rendement) : AR3, BE3 (villages de la zone 3) ; DA2, KP2 (villages de la zone 2) ; EZ1, SO1 (villages de la zone 1). Dans ces localités sont représentées des caféières à plein soleil et des caféières sous ombrage de ces légumineuses en croissance libre. Ces caféières sont en âge d'être recepées (plus de huit ans) et n'ont pas reçu d'engrais depuis plus de deux ans. Des parcelles élémentaires de vingt quatre caféiers sont délimitées en plein soleil et sous le houppier des légumineuses.

Les observations ont portées sur le cycle végétatif et la densité naturelle des légumineuses et la productivité des caféiers. Des appréciations visuelles sont faites sur l'état végétatif des caféiers dans les trois ambiances. Pour mesurer le rendement, la production en cerises fraîches est pesée sur chaque parcelle élémentaire et rapportée en kilogramme par hectare de café marchand. D'autres observations portent sur le nombre de plagiothropes productives, le nombre de glomérules sur les dernières plagiothropes inférieures de deux caféiers choisis au hasard, sur lesquels le comptage est fait sur deux caules opposées dans chaque parcelle élémentaire.

Résultats :

L'enquête agroforestière a révélé que quatre espèces de légumineuses font partie des espèces d'ombrage des caféières togolaises.Il s'agit de *Albizzia adianthifolia*, 50 % des cas *Albizzia zygia*, 30% des cas, *Albizzia ferruginea* 10% des cas, *Albizzia glaberima* 5% des cas, *Albizzia chevaleri* 1% des cas et *Erythrophleum guineense* 15% des cas.

Cycle végétatif des deux espèces les plus fréquentes

Albizzia adianthifolia est une espèce à grande expansion horizontale, ayant 6à 10 paires de pinnules petites et pubescentes. *Albizzia zygia* par contre est une espèce érigée (20 - 25 mètres de hauteur) avec deux à trois paires de pinnules.

L'observation pendant douze mois de l'évolution de l'appareil végétatif de *Albizzia adianthifolia* et *Albizzia zygia* permet de constater que :
ces espèces perdent leurs feuilles en début de saison des pluies (Mars - Avril); en ce moment elles libèrent leurs graines; un nouveau cycle recommence avec l'apparition de jeunes feuilles de nouvelles fleurs. Ce comportement biologique de la plante dépend des conditions particulières de chaque pied chez *Albizzia adianthifolia.*

Densité en milieu réel

Leur densité moyenne en milieu réel est de 13 pieds/ha pour *Albizzia zygia* et 16 pieds/ha pour *Albizzia adianthifolia*. Elle varie d'une zone de rendement à l'autre.(Tableau 1)

Tableau 1 : Densité des albizzia sp par zone de production.

	Albizzia zygia	*Albizzia adianthifolia*
Zone 1	33	16
Zone 2	7	8
Zone 3	-	25
Moyenne	13	16

Etat végétatif des caféiers dans les différentes ambiances

Les caféiers sous ombrage d'espèces d'*Albizzia* ont des feuilles vert-foncé,alors qu'en plein soleil les feuilles de caféiers sont verdâtres à jaunes.

Effets de Albizzia sp sur la production caféière

Effet sur les fructifères

Le rapport plagiothropes productives sur plagiothropes totales est de 0,74 pour les caféiers sous *A. adianthifolia*, 0,72 pour les caféiers sous *A. zygia* 0,54 pour les caféiers ensoleillés. Le tableau 2 ci- dessous indique les valeurs de ce rapport et celles des nombres de glomérules par zone de rendement.

Tableau 2 : Rapport des plagiothropes productives /plagiothropes totales et Nombres de glomérules sur la dernière plagiothrope inférieure.

Localités/zone de rendement	Rapport de plagiothropes productives/plagiothropes totales			Nombre de glomérules sur la dernière plagiothrope inférieure		
	Albizzia adianthifolia	*Albizzia zygia*	Soleil	*Albizzia adianthifolia*	*Albizzia zygia*	Soleil
AR3	0.75	0.68	0.65	4	4	3
BE3	0.72	0.63	0.58	5	4	6
DA2	0.78	0.67	0.45	5	4	6
KP2	0.72	0.76	0.54	9	6	3
EZ1	0.77	0.77	0.45	6	5	3
SO1	0.73	0.81	0.58	8	7	5
MOYENNE	0.75	0.72	0.54	6	5	4

Effet sur le rendement

L'analyse de la variance de la production de 1996 a révélé une différence significative entre les traitements sous Albizzia sp et le traitement en plein soleil.

Tableau 3 : Production de 1994-95 en kg cm/ha.

Répétitions	Caféiers sans ombrage	Caféiers avec ombrage d'*Albizzia*	Différences
AR3	869	1955	1086
BE3	627	1262	635
DA2	605	1044	439
KP2	447	1060	613
EZ1	266	844	578
SO1	238	744	506
Total	3052	6909	3857
Moyenne	508	1151	643

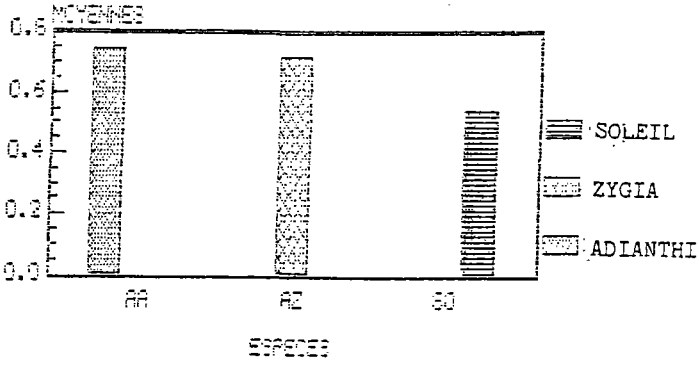
Tableau 4 : Production 1995-96 en Kgcm/ha.

Répétitions	Caféiers sous ombrage d'*Albizzia adianthifolia*	caféiers avec ombrage d'*Albizzia zygia*	Caféiers sans ombrage	Différence 1*	Différence 2**	Différence 3***
AR3	2877	1634	1238	1639	396	1243
BE3	3254	1921	1221	2033	700	1333
DA2	1877	1544	1150	727	394	333
KP2	1640	1388	988	652	400	252
EZ1	1455	1356	766	689	590	99
SO1	1221	1240	888	333	352	-19
Total	11826	9083	6509	6073	2832	3241
Moyenne	1962	1514	1042	1012	472	540

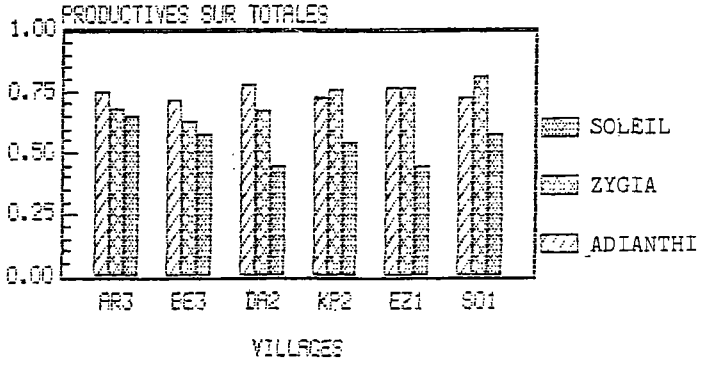
1 *: différence de production de caféiers sous *A. adianthifolia* et en plein soleil
2 **: différence de production de caféiers sous *A. zygia* et en plein soleil
3 ***: différence de production de caféiers sous *A.adianthifolia* et sous *A. zygia*

Le rendement moyen induit par la présence de *Albizzia adianthifolia* est 1012 kg café marchand à l'hectare et celui de *Albizzia zygia* est de 472kg café marchand à l'hectare. Les deux espèces étant le plus souvent associées leur effet moyen est estimé à 742 kg café marchand à l'hectare. Sur les deux campagnes elle est de 680 kg de café marchand à l'hectare.

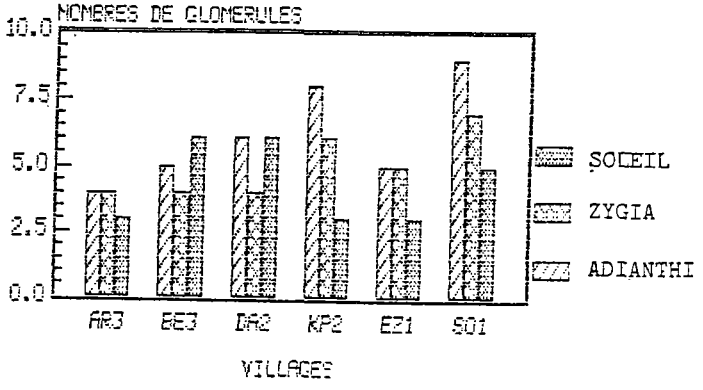
H I S T O G R A M M E 1 : RAPPORT DU NOMBRE DE PLAGIOTHROPES PRODUCTIVES SUR LE NOMBRE DE PLAGIOTHROPES TOTALES : MOYENNE PAR AMBIANCE



H I S T O G R A M M E 2 : RAPPORT DU NOMBRE DE PLAGIOTHROPES PRODUCTIVES SUR LE NOMBRE DE PLAGIOTHROPES TOTALES : MOYENNE PAR AMBIANCE ET PAR ZONE DE RENDEMENT



H I S T O G R A M M E 3 : NOMBRE DE GLOMERULES SUR LA DERNIERE PLAGIOTHROPE INFERIEURE : MOYENNE PAR AMBIANCE ET PAR ZONE DE RENDEMENT



Discussion-Conclusion

La fréquence élevée de la présence de *Albizzia adianthifolia* et *Albizzia zygia* dénote de leur importance dans la région caféière ou 60% des plantations sont ombragées, soit parce que les planteurs ont gardé des arbres d'ombrage au moment de la plantation soit parce qu'ils ont laissé repousser le recru forestier.

Les conditions particulières de perte des feuilles propres à chaque pied de *Albizzia adianthifolia* fait que dans une caféière couverte de cette espèce ou dans une forêt de cette espèce, cohabitent des arbres défeuillés et des arbres à feuillage, en conséquence la caféière a toujours de l'ombrage pendant cette période de repos végétatif de l'espèce d'ombrage. La structure en pinnules, rachis et foliolules de l'appareil végétatif permet de laisser passer une fraction des rayons solaires le jour. Et la nuit les foliolules ont la propriété de se racoler et laisser la rosée atteindre directement le feuillage des caféiers : ce qui constitue en saison sèche un apport d'eau appréciable. Aussi sa période de repos végétatif située au début de la saison pluvieuse est un caractère important pour sa fonction d'espèce d'ombrage et de brise-vent (harmattan). Par contre les pinnules peu nombreuses de *Albizzia zygia* et son port érigé limitent la qualité de son ombrage.

Les densités naturelles observées montrent que :

- l'espèce *zygia* est abondante en zone de faible rendement mais rare en zone de rendement élevé;
- l'espèce *adianthifolia* est confortablement présente dans ces deux zones.
- la zone de moyen rendement est marquée par le même niveau de présence des deux espèces.

Dans l'ensemble de la région de production, l'ambiance créée par les deux légumineuses entretient nettement mieux la présence des fructifères que l'ambiance ensoleillée. (Histogramme 1). Dans les zones de faibles à moyens rendements (DA2, KP2, EZ1, SO1) l'effet dépressif des conditions défavorables est plus prononcée en ambiance ensoleillée. Aussi l'espèce *zygia* paraît plus efficient dans ces conditions. (Histogramme 2)

Le nombre de glomérules sur la dernière plagiothrope indique l'intensité de décrépitude de la caféière : il est le niveau inférieur du nombre de noeuds encore fertiles sur la plagiothrope considérée. Son comptage représenté sur l'histogramme 3 montre que les nombres de glomérules les plus élevés s'observent généralement chez les caféiers sous *Albizzia sp.*

Dans la zone de moyens à forts rendements, il n'y a pas de différence entre les trois ambiances. Mieux encore l'ambiance ensoleillée est plus efficiente en conditions favorables de culture du caféier. Ce qui correspond à la zone constituée de la bande est des plateaux, caractérisée par la pluviométrie la plus forte et un pourcentage élevé de zone forestière.

Dans la zone de faible rendement *Albizzia adianthifolia* influence favorablement la production du caféier. Elle entretient la présence des plagiothropes. Cet entretien se manifeste au niveau de la formation et de la longévité des plagiothropes. Les fructifères âgées sont assez longues et présentent jusqu'à 20 à 30 noeuds. Seuls les 10 à 15 derniers noeuds portent des cerises. Les autres noeuds se caractérisent un tissus ligneux entièrement aoûté. Aussi ceux-ci sont moins exposés à la lumière solaire. Ceci allonge la duree du recepage cyclique en milieu paysan.

L'évolution de l'architecture des caféiers est fonction de chaque ambiance et chaque zone de rendement. Les caféiers ensoleillés se présentent en caules isolées avec une touffe de plagiothropes de dimensions réduites d'aspect végétatif vert pâle à jaunâtre en zone de faible à moyen rendement. Cette figure est atténuée en zone de rendement élevé. Les caféiers sous ombrage de légumineuses se présentent en caules verticales ou obliques en plus ou moins grande expansion, de grandes dimensions avec un aspect végétatif vert foncé dû

à l'effet fixateur de l'azote atmosphérique des legumineuses au profit des cafeiers.

En zone de faible rendement les deux espèces s'équivalent presque avec des variations en faveur de l'espèce *zygia* (Tableau 4, 3 ***), ce qui confirme les tendances observées sur la longévité des fructifères. Aussi la densité naturelle de *zygia* y est assez élevée (Tableau 1).

La production exprimée par les tableaux 3 et 4 sont similaires aux résultats d'essais menés par l'IRCC en Côte d'Ivoire, au Cameroun et en RCA sur des périodes de 7 à 10 ans avec un bon niveau d'entretien et de taille, les rendements moyens sans engrais variaient de 1200 à 2500kg de café marchand à l'hectare.

Ceci montre bien qu'avec une couverture de légumineuse fertilisante, un bon désherbage et une taille convenable, il est possible d'obtenir des rendements acceptables sur des longues périodes.

Cette étude est une première étape dans la recherche des alternatives visant à réduire les coûts de production en caféiculture togolaise, en utilisant au maximum les ressources locales en vue préserver l'environnement. Elle permet d'identifier *Albizzia adianthifolia* comme espèce à associer au caféier avec assez d'avantages dans toute la région de caféiculture. Mais il reste à préciser les densités optimales d'association. Aussi il sera utile de quantifier la biomasse et les éléments minéraux apportés par la légumineuse. D'ores et déjà c'est une légumineuse à conseiller aux planteurs.

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SUMMARY : With the purpose of reducing the productions'costs, the coffee tree is associated to the forest leguminous which are able in fixing nitrogen to diminish the use of mineral fertilizers and respond to the environnemental preoccupations. The method tested is free growth .

The results have permitted to identify a leguminous which can be recommanded to farmers : *Albizzia adianthifolia* ; it maintains the longevity of the fruitbearing branches and stimultes the production. The trials carried out in 1994-96 have shown that the interest of a fertilizer program using *Albizzia adianthifolia* was emphasized by an increase of the average production of 680kg cmha^{-1}.

KEY WORDS : *ALBIZZIA adianthifolia, COFFEA canephora* var Robusta, TOGO

RESUME : Dans le but de réduire les coûts de production, le caféier est associé aux légumineuses forestières fixatrices d'azote afin de diminuer l'apport des engrais minéraux et répondre aux préoccupations environnementales. Des essais mettant en oeuvre plusieurs espèces de légumineuses sont mis en place. Le mode de conduite testé est la croissance libre .

Les résultats ont permis d'identifier une légumineuse transférable aux planteurs : *Albizzia adianthifolia* ; elle entretient la longévité des branches fructifères stimule la production. Les essais conduits entre 1994-96 ont montré que l'intérêt d'un programme de fumure utilisant *Albizzia adianthifolia* fut mis en valeur par une augmentation de la production de 680kg cmha^{-1}.

MOTS CLEFS : *ALBIZZIA adianthifolia, COFFEA canephora* var Robusta, TOGO

CURRENT OBSERVATIONS AND FUTURE TRENDS IN ORGANIC COFFEE PRODUCTION IN KENYA

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1.0 INTRODUCTION

Organic farming may be defined as the growing of crops without the application of chemical inputs - inorganic fertilizers, pesticides and herbicides (Vereijken, 1989). It relies more on the use of organic and green manures, natural fertility of the land and biological control of pests. One of the advantages of organic farming is that it may reduce production costs although yields tends to go down. This aspect is particularly important to the Kenyan farmer where input prices have been increasing and consequently the use of fertilizers and other agro-inputs have decreased every year, leading to a production decline (Anon, 1991). Despite this decline in the use of chemicals a lot of money in foreign exchange is being spent, with fertilizers alone taking about KSh. 2 billion per year (Anon, 1991).

Coffee is one of the most important products of Kenya's agriculture. Currently it lies third after tourism and tea in terms of foreign exchange earning. It employs 30% of the national agricultural workforce (Anon, 1989). Most of the coffee is produced by small scale farmers (Whitaker, 1986).

The advantages of using organic manures and wastes in coffee in Kenya are have been documented Michori, (1981). They include increase in organic matter of soil and improvement of soil structure. Green manuring includes the incorporation of green leguminous plant to the soil. The decomposing plant material becomes a source of nutrients to the cultivated crop. The benefits are slow conversion of unavailable organic sources to more readily available plant nutrients, maintenance of organic matter content which indirectly affects soil structure, water holding capacity and infiltration, microbial diversity and soil porosity (Asenga, 1991).

Leguminous plants are preferred as a source of green manure because of their ability to fix nitrogen and release it to the soil (Agboola and Fayemi, 1972). They can be annual (Ojomo, 1981) or perennial plants (Wilson and Kang, 1981).

Due to the depressed coffee markets there is need to look into ways and means of improving earnings from coffee through reduced coffee production costs (Anon, 1989). Organically produced coffee, besides being produced at lower costs would also be bought at a premium prices (Anon, 1990). The system would be environmentally more advantageous.

In Kenya, one farmer in Kiambu has claimed to have increased her coffee yields from 6000 to 8000 kg cherry per annum by use of organic manure and intercropping with leguminous crops/trees, while controlling diseases and pests by using plant extracts (Ogana, 1991).

2.0 METHODOLOGY

2.1 Planting materials

The suitable planting material for organic coffee production must be resistant/tolerant to the major diseases and pests and be able to use efficiently the applied inputs like organic manures. The new Arabica variety Ruiru 11 is resistant Coffee Berry Disease and Coffee Leaf Rust the two most important coffee diseases in Kenya. It is also a compact variety allowing for high density planting. High density plantings results in better utilization of resources (manures, water and light) and reduces weed infestation. This coffee cultivar is therefore a suitable material for organic coffee production.

Due to its superior qualities research has intensified to produce adequate planting seedlings through the use of seed, vegetative propagation and in the near future through tissue culture.

2.2 Manures

The coffee nutrient requirements could be partially met through the use of coffee manure. Preliminary results have indicated that application of 26 kg per tree per year (2 - 20 litre container) would be adequate to maintain coffee production (Table 1). This helps to maintain a yield level of over 2000 kg/ha of clean coffee.

2.3 Green manures

Results have shown that the yield of Ruiru 11 coffee trees can be maintained at economical levels using green manures (Table 2). Application of green manures obtained from Leucaena Leucocepala resulted in the highest yield of clean coffee (Table 2). Over a six year period, no significant difference between the use of green manures and inorganic fertilizers, in terms of clean coffee yields and grade `A' sized beans. The use of green manure resulted in an improvement of soil bulk density and increase in soil organic matter and soil PH.

2.4 Crop protection

The pest and disease control should be primarily preventive rather than curative (Anon, 1989). Natural enemies of pests and diseases should be protected and encouraged through provision of conditions favourable to them. The use of synthetic pesticides is prohibited. Organic farming is carried out in a way that it reduces losses from pests. This includes use of crop varieties well adopted to the environment, disease resistant varieties like Ruiru 11, balanced manurial programme, active manipulation of the microclimate by regular pruning and continuous rejuvenation of the trees (IFOAM, 1992). Only in exceptional cases should copper and organic fungicides be used and which must be approved by the certifying organisation first.

The coffee pests could be controlled through proper cultural practices, biological control and use of natural pesticides like neem extract. In Kenya, biological control of some coffee pests is already recommended. Coffee mealybugs (Planococcus kenyae) are already being controlled by their natural parasite, Anagyrus spp, coffee scales by ladybirds and giant loopers (Ascotis Selenaria Reciprocaria) by macrorhaphis acuta (Anon, 1992).

3.0 PRODUCTION POTENTIAL

According to a survey carried out in 1988, about 22% of the small scale coffee farmers use manure instead of inorganic fertilizers and do not use pesticides (Nyoro,1988). These farmers may be developed to produce organic coffee from the traditional varieties. Currently there are 120, 523 ha of coffee under the small scale subsector. Approximately 20% of these can be developed to produce organic coffee. There is therefore a potential production of 8,000 tonnes of organic coffee assuming a low yield of 300kg/ha under organic farming conditions.

In addition to this, there are a number of coffee estates that are willing to convert to organic coffee production. When the production, processing and marketing procedures are put in place, these estates can start producing certified organic coffee.

4.0 FUTURE RESEARCH AND PRODUCTION TRENDS.

Since the production of organic coffee is not the norm in Kenya, there are no current recommendations on how to produce, process or market it . To be able to produce organic coffee economically, the following constrains will have to be addressed and resolved.

4.1 Coffee husbandly

Results have shown that application of 2 debes per tree of cattle manure (20 litre containers) would sustain coffee production at an economical level (Anon 1993). However the correction of soil pH and trace elements have not been thoroughly addressed.

Research is needed in the area, particularly the use of green colorganic manure particularly the decomposition and mineralization of organic and green manures used in coffee.

More suitable green manures plants which have first growth, high dry matter production and high decomposition rate need to be identified.

Research is needed to identify the active ingredient and proper dosage of natural pesticides.

With exception of Ruiru 11, the other coffee cultivars in Kenya are prone to Coffee berry disease (CBD) and Leaf rust. Intensify research into disease and pest management methods such as biocontrol, cultural and host plant resistance which will replace chemical methods.

4.2 Coffee processing

Organic coffee requires to be processed separately. This would pose a problem particularly in the cooperative sector, where organic and non organic farmers could be processing their coffee together. Modalities on how this can be done separately need to be addressed.

4.3 Marketing

There are no facilities of marketing organic coffee under the current coffee central auction system used in Kenya. Therefore a suitable marketing system need to be developed before farmers can produce organic coffee.

4.4 Certification

All organic products undergo certification by a recognized body under the auspices of International Federation for Organic Agriculture Movement IFOAM). No such body exists in Kenya. To be able to market organic coffee, a certification body need to be set up to

- develop local guidelines alongside those of IFOAM for producing coffee and
- Certify that the coffee has been produced organically.

5.0 CONCLUSION

It is assumed that if some of this practices are put in place Kenya can become a producer of high quality organic coffee.

6.0 ACKNOWLEDGEMENTS
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SUMMARY

Organic coffee is coffee produced and processed using natural products. Application of synthetic fertilizers and pesticides is not allowed. There has been an upsurge in interest and demand for this type of coffee particularly in Europe and the gourmet market of America. Although Kenya traditionally produces high quality coffee, it does not produce organic coffee. Results obtained from experiments indicate that there are good prospects of now producing organic coffee in Kenya. This include the use of the disease resistant Arabica hybrid, use of organic manures at the rate of 2 debes per tree per year, use of green manures from leguminous plants and use of biological control for the major Coffee insect pests. However there are certain areas in the production, processing and marketing processes that do require attention.

Table 1: Effects of green manure application to mature coffee, cv. Ruiru 1 on coffee yields and grade A beans (%) 1991- 1996.

Source of green manure	Clean coffee yield kg/ha	% Grade A beans
Leucaena intercrop	596	58.4
Leucaena purestand	1149	62.7
Sesbania intercrop	753	66.6
Sesbania purestand	915	66.5
Calliandra intercrop	771	60.5
Calliandra purestand	895	65.9
Desmodium intercrop	707	57.5
Lucerne intercrop	853	65.9
Pigeon peas intercrop	670	61.2
Beans intercrop	804	64.5
Soya bean	962	64.8
Cowpea intercrop	660	69.0
Napier grass mulch	745	70.6
Cattle manure	722	62.5
Inorganic fertilizer	702	65.1
Unfertilized control	898	57.3
Mean	800	63.7
SED	220.47	7.79
CV	33.7	15.0

Table 2. Fertilizer - Farm yard manure (FYM) substitution trial - effect of varying manure - N combination on yield on clean coffee (1992) and cumulative yield (1983-1992)

Treatment		clean coffee kg/ha	
M(debes/tree/year)	N(kg/ha)	1992	1983-92
0	0	2184 a	2782 a
0	35	2988 a	3212 a
0	70	2815 a	2936 a
0	140	2614 a	2975 a
		2650 a	2976 a
1	0	2552 a	2937 a
1	35	2791 a	2753 a
1	70	2887 a	3015 a
1	140	2866 a	3161 a
		2774 a	2967 a
2	0	2727 a	2980 a
2	35	2731 a	2939 a
2	70	2827 a	2998 a
2	140	2448 a	2698 a
		2683 a	2904 ab
4	0	2251 a	2880 a
4	35	2474 a	2770 a
4	70	2393 a	2734 a
4	140	2212 a	2575 a
		2333 a	2740 b

* 1 debe = 20 L tin

Note: Means followed by the same letter are not significantly different by DMRT ($p \leq 0.1$)

Source- CRF Annual report 1992/93

INTÉRÊT DE L'ENDOMYCORHIZATION DU CAFÉIER ARABICA POUR LA LUTTE CONTRE LES NÉMATODES (*PRATYLENCHUS COFFEAE* ET *MELOIDOGYNE KONAENSIS*)

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INTRODUCTION

En champ, les systèmes racinaires des caféiers Robusta (*Coffea canephora* P.) et Arabica (*Coffea arabica* L.) sont naturellement colonisés par les endomycorhizes à vésicules et arbuscules (MVA). L'intensité de la symbiose MVA varie avec les espèces présentes, le milieu, les pratiques culturales, l'âge des caféiers, et la fertilisation (Lopes *et al.*, 1983a).

Des expériences en serre et pépinière ont montré que la symbiose MVA augmente la croissance et améliore la nutrition de jeunes caféiers; certaines espèces MVA étant plus efficaces que d'autres (Lopes *et al.*, 1983b; Sieverding et Toro, 1986; Vaast et Zasoski, 1991). De ce fait, l'inoculation MVA en pépinière des jeunes semenceaux de caféiers est hautement recommandée afin d'obtenir des plants vigoureux en un laps de temps réduit.

Avec des plants préalablement mycorhizés en pépinière dans des sols non stérilisés, des essais en champ ont démontré que certaines espèces MVA augmentaient la survie à la plantation, la croissance et la précocité d'entrée en production du caféier Arabica (Sieverding et Toro, 1986; Siqueira *et al.*, 1993).

Les effets bénéfiques de la symbiose MVA sont dus à l'importante augmentation du volume de sol exploré par le réseau d'hyphes mycéliens externes des racines mycorhizées. Ce chevelu mycélien est souvent très dense, jusqu'à 10-15 m par cm de racine, et s'étend jusqu'à 8-15 cm de la racine. L'absorption du phosphore (P), particulièrement dans les sols acides tropicaux à fort pouvoir fixateur, mais également des oligo-éléments (notamment Zn, Cu), et de tout élément peu mobile (NH_4^+, K, Ca, S), est très significativement accrue par les hyphes externes puisant ces éléments au delà de la rhizosphère (qui s'étend sur 1-2 mm autour de la racine) dans des zones non explorées par les racines. Ces hyphes exercent un contrôle sur la flore microbienne de la rhizosphère permettant une meilleure

minéralisation et utilisation des formes organiques phosphatées et azotées (Marschner et Dell, 1994). De plus, ce réseau mycélien externe joue un rôle important dans le recyclage et la conservation des nutriments en les interceptant et en limitant leur lessivage vers les couches profondes du sol (Jeffries et Barea, 1994).

Outre des effets bénéfiques sur la croissance et la nutrition, la symbiose MVA peut augmenter la tolérance des systèmes racinaires vis-à-vis de champignons pathogènes (*Phytophthora, Pythium, Fusarium, Rhizoctonia*) et des nématodes (Azcon-Aguilar et Barea, 1996). La fertilité du sol, la combinaison plante-hôte - espèce MVA - agent pathogène, et les densités d'inoculum MVA et de l'agent pathogène influencent très fortement l'interaction mycorhize - agent pathogène.

Plusieurs mécanismes peuvent être responsables de cet effet bénéfique de la symbiose MVA dans la tolérance aux pathogènes racinaires (Azcon-Aguilar et Barea, 1996). Le mécanisme le plus souvent invoqué est l'augmentation de la vigueur de la plante mycorhizée, résultant d'une amélioration de son statut nutritionnel, qui renforce sa capacité à supporter les attaques du pathogène. La symbiose MVA peut également limiter les dégâts de l'agent pathogène au niveau des racines et ainsi maintenir leur capacité d'absorption. Elle peut aussi modifier la morphologie du système racinaire en stimulant sa ramification et le développement de radicelles. La prolifération des hyphes dans le sol permet aussi de compenser la perte de biomasse racinaire. La colonisation des racines par la symbiose MVA peut également restreindre, voire exclure, la pénétration et l'établissement de l'agent pathogène dans les zones racinaires où elle est pré-établie. La symbiose MVA peut provoquer une altération de l'exsudation racinaire (production de substances antibiotiques et nématicides) et une modification de la microflore rhizosphérique (stimulation de *rhizobacteria* et microorganismes antagonistes tels que *Trichoderma, Bacillus, Pseudomonas*). Ceci a pour effet de diminuer la sporulation et limiter la propagation des champignons responsables des pourritures racinaires, ou bien de réduire l'attractivité des racines envers les nématodes et de freiner leur cycle de reproduction.

Les nématodes constituent l'un des principaux problèmes parasitaires en arabicaculture. Les nématodes sédentaires à galles (*Meloidogyne* spp.) ou endomigrateurs (*Pratylenchus* spp.) induisent des pertes de production estimées entre 15 % et 50 % selon les régions productrices (Campos *et al.*, 1990). En attendant confirmation d'essais en cours sur porte-greffe Robusta et la création de variétés Arabica tolérantes ou résistantes (Bertrand *et al.*, 1995), l'application de nématicides reste le seul moyen de lutte. Cette lutte chimique est coûteuse, dangereuse pour l'environnement et, de surcroît, son efficacité est très limitée dans le temps.

Dans les régions productices, la pression foncière amène de plus en plus à replanter les caféières sur d'anciens vergers dont les sols sont apprauvis et où la parasitisme tellurique s'est accru. Dans ce contexte, la symbiose MVA présente un grand intérêt pour une approche intégrée de la nutrition et de la protection phytosanitaire du caféier, et pour une gestion durable de la fertilité des agrosystémes caféiers. Dans cette optique, 2 essais ont été menés en serre afin d'étudier les interactions entre nématodes et endomycorhizes et leurs effets sur la croissance et la nutrition d'un caféier Arabica sensible aux nématodes.

MATÉRIEL ET MÉTHODES

Dans les 2 essais, on a utilisé le même caféier Arabica cv Catuai rojo reconnu comme très sensible aux 2 genres de nématodes (*Pratylenchus* spp. et *Meloidogyne* spp.) les plus communs et dévastateurs en arabicaculture (Anzueto, 1993).

Une première expérience sur 11,5 mois a permis d'étudier les effets de 2 espéces MVA, *Acaulospora mellea* et *Glomus clarum*, originaires du Brésil et reconnues comme très efficaces pour la croissance de caféiers Arabica en pépinière et au champ(Siquiera *et al.*, 1993), et d'un nématode endomigrateur, *Pratylenchus coffeae*, à fort pouvoir pathogène et originaire d'une caféière du Guatemala (Anzueto, 1993). L'inoculation MVA a été faite à 2 époques :

* une inoculation MVA précoce au moment du repiquage des plantules en pot et précédant de 4 mois l'addition du nématode;
* une addition simultanée de MVA et du nématode aprés 4 mois de croissance des plantules en pot.

La récolte de cet essai a eu lieu 7,5 mois aprés l'addition du nématode.

Dans une seconde expérience qui a duré 13,5 mois, on a étudié les effets d'une espéce MVA, *A. mellea*, et d'un nématode sédentaire à galles, *Meloidogyne konaensis*, originaire d'une caféière du Guatemala (Anzueto, 1993). L'inoculation MVA a été effectué à 2 époques :

* une inoculation MVA précoce au moment du repiquage des plantules en pot soit 10 mois avant l'addition du nématode;
* une addition simultanée de MVA et du nématode aprés 10 mois de croissance des plantules en pot.

La récolte de ce second essai a eu lieu 3,5 mois aprés l'addition du nématode.

La croissance des plantes, la colonisation des racines par les espéces MVA, les densités racinaires de nématodes et la teneur foliaire en P ont été mesurées suivant des méthodes précédemment décrites (Vaast, 1995).

RÉSULTATS ET DISCUSSION

Effets des mycorhizes

La croissance des caféiers est significativement augmentée par une inoculation MVA quelle que soit l'espéce utilisée (tableaux 1 & 2). Il est à noter que *A. mellea* stimule plus fortement la croissance que *G. clarum* (tableau 1). Ces résultats confirment l'intérêt de l'inoculation MVA en pépinière du caféier Arabica et l'existence de différences en terme d'efficasité entre espèces MVA (Lopez *et al.*, 1983b; Sieverding et Toro, 1986; Vaast et Zasoski, 1991).

Il est évident que plus l'inoculation MVA est précoce et plus elle est profitable au développement végétatif et à la nutrition en P du caféier. En effet, seuls les plants précocement mycorhizés ont des teneurs foliaires en P supérieures à 0,08-0,10 %, seuil de carence (tableaux 1 & 2). Dans le sol utilisé, très acide et à fort pouvoir fixateur du P, la symbiose MVA permet au caféier-hôte de surmonter une disponibilité en P limitante. Dans la première expérience, la surface foliaire des plants mycorhizés avec *A. mellea* dès le repiquage est près de 9 fois supérieure à celle des témoins, alors que celle des plants mycorhizés aprés 4 mois d'élevage en pot n'est que de 2 fois supérieure à celle des témoins (tableau 1). Le même constat peut être fait avec *G. clarum* dans la première expérience (tableau 1) et avec *A. mellea* dans la seconde expérience (tableau 2).

Effets des nématodes

Aprés 7,5 mois, le nématode endomigrateur, *P. coffeae*, a provoqué une réduction de 70-80 % de la surface foliaire et de 50-60 % du poids racinaire des plants témoins et des plants tardivement mycorhizés (tableau 1). En revanche, les réductions des biomasses aérienne et racinaire sont de l'ordre de 25-35 % pour les plants précocement mycorhizés. L'invasion, la migration et les ponctions alimentaires du nématode endomigrateur provoquent une destruction des cellules corticales et l'apparition de nécroses qui ont pour

conséquence des pertes de biomasse racinaire (Sijmons *et al.*, 1994).

Sur une période de 3,5 mois, le nématode sédentaire à galles, *M. konaensis*, a provoqué des réductions de croissance de même ampleur (70-80 %) chez les plants témoins et tardivement mycorhizés (tableau 2). On observe de plus faibles réductions (30-50%) chez les plants précocement mycorhizés. La perte de biomasse racinaire est due au développement des nématodes femelles qui provoquent des modifications cellulaires (cellules géantes) et la formation de galles, et réduisent considérablement l'élongation des racines infestées (Sijmons *et al.*, 1994).

Ces fortes réductions de croissance confirment le fort pouvoir pathogéne de ces deux nématodes (Anzueto, 1993).

Interactions entre nématodes et espèces VAM

Il apparaît très nettement que l'époque de l'inoculation MVA par rapport à celle de l'exposition des plants aux nématodes est capitale. Lorsque l'addition de MVA et des nématodes est simultanée, la symbiose MVA n'a aucun effet prophylactique. Dans les deux expériences, la présence des nématodes aboutit à une réduction de croissance des plants tardivement mycorhizés, similaire à celle des plants témoins (tableaux 1 & 2). Cela est dû à la forte limitation par les nématodes de la colonisation des racines par les espèces MVA. En effet, quelle que soit l'espèce MVA (*A. mellea* ou *G. clarum*) l'intensité de la symbiose MVA est réduite de moitié lors de l'addition simultanée du nématode *P. coffeae* (tableau 1) et ne se développe que très peu avec le nématode à galles *M. konaensis* (tableau 2). En revanche, les nématodes ne réduisent que très peu la colonisation MVA lorsqu'elle est pré-établie même en présence des densités de nématodes très élevées (tableaux 1 & 2).

Avec *P. coffeae*, les densités de nématodes dans les racines des plants précocement mycorhizés sont de 5 à 10 fois supérieures à celles des racines des plants témoins ou tardivement mycorhizés (tableau 1). Cependant, la biomasse racinaire a moins diminué, les racines demeurent plus saines et les nécroses brunâtres, au point d'invasion du nématode endomigrateur, restent moins nombreuses et nettement plus localisées. Cette capacité de la symbiose MVA à limiter les dégâts d'un nématode migrateur et à maintenir la capacité d'absorption des racines préalablement mycorhizées a été observée sur d'autres plantes pérennes tels que les agrumes, le bananier, le pommier, le prunier (Pinochet *et al.*, 1996; Umesh *et al.*, 1988).

Avec *M. konaensis*, le nombre de masses d'oeufs dans les racines des plants précocement mycorhizés est de 2 à 3 fois supérieur à celui des racines des plants témoins ou tardivement mycorhizés (tableau 2). Cependant, la biomasse racinaire et surtout la proportion de radicelles, composantes du système racinaire les plus actives en terme d'absorption, ont beaucoup moins diminué chez les plants préalablement mycorhizés.

Indiscutablement, la tolérance des caféiers préalablement mycorhizés vis-à-vis des deux genres de nématodes est liée à leur vigueur au moment de leur exposition aux nématodes, mais surtout au maintien d'une capacité d'exploration du sol et d'absorption minérale suffisamment importante pour satisfaire les besoins de la plante. Sous l'effet d'un stress abiotique (sécheresse, faible fertilité) ou biotique d'origine tellurique (champignons, nématodes), les plantes tolérantes tendent à privilégier le développement de leur système racinaire (Marschner, 1986). Ces résultats montrent que la symbiose MVA pallie cette absence chez les plantes-hôtes sensibles en limitant les pertes de biomasse racinaire sous l'effet des attaques de nématodes et en compensant ces pertes par son réseau d'hyphes mycéliens. La contribution de ce mycélium a été estimé à 15-50 % pour les oligo-éléments (Zn, Cu, B), 10-25 % pour l'azote (NH_4^+ et NO_3^-), et 20-80 % pour le P (Marschner et Dell, 1994).

Tableau 1 : Effets de l'inoculation MVA avec *Acaulospora mellea* (Mellea) ou *Glomus clarum* (Clarum), 7,5 mois aprés l'addition de nématodes migrateurs (PC : *Pratylenchus coffeae*), sur la surface foliaire, le poids racinaire, le % de racines mycorhizées, la concentration foliaire en phosphore (P,) et la densité de nématodes des racines de caféiers agés de 11,5 mois.

MVA	PC	Surface Foliaire (cm^2)	Poids Racines (g)	% Racines Colonisées	P Foliaire (%)	Densité nematodes (nb/g)
Aucune	-	118 e	2,18 cd	-	0,058 c	-
	+	23 g	1,30 d	-	0,053 c	147 c
Précose[a]	-	909 a	12,88 a	72 ab	0,108 a	-
Mellea	+	671 b	8,72 ab	56 b	0,102 a	1689 a
Précose[a]	-	690 b	9,10 a	77 a	0,098 a	-
Clarum	+	462 c	6,08 b	66 ab	0,086 ab	782 b
Simul.[b]	-	215 d	2,36 c	53 b	0,085 ab	-
Mellea	+	65 f	1,30 d	23 d	0,059 c	209 c
Simul.[b]	-	176 de	2,68 c	63 ab	0.078 b	-
Clarum	+	33 g	1,32 d	37 c	0.050 c	184 c

[a]inoculation MVA au moment du repiquage des plantules en pot soit 4 mois avant l'addition des nématodes.
[b]addition simultanée de MVA et nématodes aprés 4 mois de croissance en pot.

Tableau 2 : Effets de l'inoculation MVA avec *Acaulospora mellea*, 3,5 mois aprés l'addition de nématodes à galles (MK : *Meloidogyne konaensis*), sur la surface foliaire, le poids racinaire, le % de racines mycorhizées, la concentration foliaire en phosphore (P), et la densité de masses d'oeufs des racines de caféiers agés de 12,5 mois.

MVA	MK	Surface Foliaire (cm^2)	Poids Racines (g)	% Racines Colonisées	P Foliaire (%)	Masses d'oeufs (nb/g)
Aucune	-	198 d	2,76 c	-	0,058 c	-
	+	29 f	1,12 d	-	0,051 c	15 b
Précose[a]	-	1487 a	17,36 a	67 a	0,094 a	-
MVA	+	791 b	7,51 b	58 ab	0,078 b	46 a
Simul.[b]	-	223 d	3,20 c	12 c	0,072 b	-
MVA	+	26 f	0,89 d	2 d	0,061 c	17 b

[a]inoculation MVA 10 mois avant l'addition des nématodes.
[b]addition simultanée de MVA et nématodes aprés 10 mois de croissance en pot.

Conclusion

Il apparaît qu'une inoculation MVA suffisamment précoce pour permettre un bon établissement de la symbiose confère à un Arabica, très sensible, une très forte tolérance vis-à-vis de 2 nématodes des genres *Pratylenchus* et *Meloidogyne*, les plus dévastateurs en arabicaculture.

Cette augmentation de tolérance est due au maintien d'une bonne vigueur et d'un statut nutritionnel adéquat des plantes préalablement mycorhizées. La symbiose MVA limite la perte de biomasse racinaire quel que soit le nématode, maintient les racines en état fonctionnel, stimule la formation de radicelles, et contribue par son réseau mycélien externe à la satisfaction des besoins minéraux de la plante, notamment en P. En revanche, la symbiose MVA n'affecte pas le cycle de développement et de reproduction des nématodes comme cela a pu être observé dans d'autres études.

Les recherches doivent être poursuivies selon deux axes. Le premier est de confirmer cette augmentation de tolérance vis-à-vis d'autres espèces ou populations de *Pratylenchus* et *Meloidogyne* à fort pouvoir pathogène et de mieux préciser les mécanismes responsables des effets bénéfiques de la symbiose MVA. Le second est de vérifier si la plantation, en sol infesté, de plants préalablement mycorhizés en pépinière peut être une alternative fiable, durable et peu coûteuse à la lutte chimique onéreuse, polluante et à efficacité limitée.

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RÉSUMÉ

Deux essais ont été menés en serre afin d'étudier les interactions entre nématodes et endomycorhizes à vesicles et arbuscules (MVA) et leurs effets sur la croissance et la nutrition du caféier Arabica cv Catuai rojo très sensible aux nématodes.

Une première expérience sur 11,5 mois a permis d'étudier les effets de 2 espèces MVA, *Acaulospora mellea* et *Glomus clarum*, et d'un nématode endomigrateur, *Pratylenchus coffeae*. L'inoculation MVA a été faite soit 4 mois avant l'addition du nématode soit simultanément à l'addition du nématode.

Dans une seconde expérience qui a duré 13,5 mois, les effets d'une espèce MVA, *A. mellea*, et d'un nématode sédentaire à galles, *Meloidogyne konaensis*, ont été étudiés avec une inoculation MVA soit 10 mois avant l'addition du nématode soit simultanément à l'addition du nématode.

Lors de l'addition simultanée, l'inoculation MVA n'a induit aucune tolérance vis-à-vis de *M. konaensis* ou de *P. coffeae*. Ces 2 nématodes ont fortement réduit la teneur foliaire en phosphore (P), la biomasse racinaire, la surface foliaire, et la colonisation racinaire par *A. mellea* ou *G. clarum* annihilant tout effet bénéfique de la symbiose MVA.

En revanche, l'inoculation MVA précoce a significativement augmenté la tolérance du caféier vis-à-vis *M. konaensis* et *P. coffeae*. Malgré la présence dans les racines de plus nombreuses masses d'oeufs (avec *M. konaensis*) ou de plus fortes densités de nématodes (avec *P. coffeae)*, la croissance des plants préalablement endomycorhizés a été de 20 à 30 fois supérieure à celle des plants non mycorhizés. De plus, la nutrition de ces plantes, notamment en P, a été peu (avec *M. konaensis*) ou pas affectée (avec *P. coffeae*).

En conclusion, il apparaît qu'une inoculation MVA suffisamment précoce pour permettre un bon établissement de la symbiose confère à un Arabica, très sensible, une très forte tolérance vis à vis de nématodes des genres *Pratylenchus* et *Meloidogyne*, les plus dévastateurs et communément observés en arabicaculture. Les recherches doivent être poursuivies selon deux axes. Le premier est de confirmer cette augmentation de tolérance vis-à-vis d'autres espèces ou populations de *Pratylenchus* et *Meloidogyne* à fort pouvoir pathogène et de mieux préciser les mécanismes responsables des effets bénéfiques de la symbiose MVA. Le second est de vérifier si la plantation, en sol infesté, de plants préalablement mycorhizés en pépinière peut être une alternative fiable, durable et peu coûteuse à la lutte chimique onéreuse, polluante et à efficacité limitée.

ABSTRACT

Two greenhouse experiments were undertaken to study the interactions between nematodes and vesicular arbuscular mycorrhizae (VAM) and their effects on the growth and nutrition of the nematode-susceptible Arabica coffee cv Catuai rojo.

The first experiment, lasting 11.5 months, studied the effects of 2 VAM species, *Acaulospora mellea* and *Glomus clarum*, and of 1 migratory endoparasitic nematode, *Pratylenchus coffeae*. Mycorrhizal inoculation was performed at two periods; a VAM inoculation preceding nematode addition by 4 months, and a simultaneous VAM and nematode addition.

In the second experiment lasting 13.5 months, the effects of one VAM species, *A. mellea* , and one sedentary endoparasitic nematode, *Meloidogyne konaensis*, were studied with VAM inoculation performed at 2 periods; VAM inoculation 10 months prior nematode addition, and a simultaneous VAM and nematode addition.

With simultaneous VAM and nematode addition, mycorrhizal inoculation did not enhance coffee tolerance to either *P. coffeae* or *M. konaensis*. Both nematodes strongly decreased foliar P concentration, root biomass, foliar area, and mycorrizal root colonization, negating the VAM symbiosis beneficial effects.

In contrast, an early VAM inoculation enhanced significantly coffee tolerance to *M. konaensis* and *P. coffeae*. Despite higher egg mass densities (with *M. konaensis*) and higher nematode densities (with *P. coffeae*) growth of coffee plants with well established mycorrhizal colonization was 20 to 30 times bigger than that of non-mycorrhizal plants. Nutrition of early mycorrhizal plants, particularly P, was slightly (with *M. konaensis*) or not affected (*P. coffeae*).

In conclusion, it appears that a VAM inoculation, early enough to allow a good VAM symbiosis establishment, results in an enhanced tolerance of an Arabica coffee cultivar highly susceptible to endoparasitic nematodes of distinct genera, *Pratylenchus* and *Meloidogyne*, that are highly damaging and widespread in Arabica coffee cultivation. Further researchs are worth undertaking to confirm that VAM symbiosis enhanced tolerance against other highly pathogenic *Pratylenchus* and *Meloidogyne* populations, and to determine more precisely the mechanisms involved in the beneficial effects of VAM symbiosis. It is also imperative to investigate whether plantation in nematode-infested field of coffee plants VAM-inoculated in the nursery can become a long-lasting, less costly alternative to chemical control not environmentally sound and only short-term effective.

INFLUENCE DES ENGRAIS SUR LA FOURNITURE DES FORMES D'AZOTE PAR LE SOL

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Introduction

Des études conduites en Côte d'Ivoire ont mis en évidence des liaisons très fortes entre les facteurs de rendement du caféier et les apports d'engrais minéral azoté. L'azote augmente chez le caféier, le nombre de noeuds fructifères sur les primaires, le nombre d'étages sur le tronc et favorise la floraison et la nouaison (12).
Généralement dans le sol, il est constaté que les engrais ne modifient pas, dans le temps et de manière significative, les quantités d'azote total du sol obtenues par la méthode Kjeldahl (1)(10)(13). Les processus de réorganisation et de transformation des différentes formes solubles et insolubles d'azote sont donc moins perçus.

La présente étude a pour objectif de caractériser les formes de l'azote total et de déterminer l'influence des engrais minéraux azotés sur ces formes. Trois méthodes d'analyse ont été utilisées avant et après incubation à humidité constante à 28°C: i)- l'hydrolyse acide 6N, technique dégradative pour l'analyse des protéines (7) a servi à doser les formes hydrolysables de l'azote; ii)- la solution de sulfate de potassium N pour l'extraction des formes minérales; iii)- et la méthode Kjeldahl pour doser l'azote total.

Matériel et méthodes

1- Sol

Les échantillons de sol proviennent de 2 essais: l'un installé en Côte d'Ivoire en Afrique Occidentale et l'autre, au Cameroun en Afrique Centrale sur des sols ferralitiques. Dans le cas de Côte d'Ivoire, l'essai est installé en 1977 sur la station de

Divo (D.10.2) et dans lequel on compare trois formes d'engrais azoté à un témoin: le sulfate d'ammoniaque, l'urée et le nitrate d'ammoniaque. La dose annuelle est de 100 kg d'azote par hectare en deux applications par an pour une densité de plantation de 1333 caféiers/ha (3x2.5m).
Le sol présente les caractéristiques physico-chimiques suivantes: A%=17,8 L%=8,5 ; S%=73,8 ; C%=1,35 ; N%=0,14 ; K meq/100=0,23 ; Ca meq/100=11,07 ; Mg meq/100=1,07 ; CEC meq/100=6,24 ; pH (eau)=7,6.

Pour cette étude, les échantillons de sol ont été prélevés 12 ans après l'installation aux profondeurs de 0-10, 10-20, 20-30, 30-50 et 50-70 cm.

Concernant le Cameroun, ce sont des échantillons composites de l'horizon 0-20 cm prélevés 13 ans après plantation. Ils proviennent d'un essai factoriel NPK à quatre niveaux installé en 1973 à Abong-Mbang à la densité de 1111 caféiers/ha (3x3m). Les échantillons correspondent aux traitements ci-après: N0PK = 0 kg N/ha ; N1PK = 90 kg N/ha ; N2PK = 180 kg N/ha et N3PK = 360 kg N/ha. Ces doses sont apportées sous forme de sulfate d'ammoniaque en trois fractions par an. Le sol des parcelles non fertilisées présentent les caractéristiques suivantes:
A%= 41,13 ; L%=9,49 ; S%=49,38 ; C%=2,71 ; N%=0,13 ; K meq/100=0,11 ; Ca meq/100=1,25 ; Mg meq/100=0,41 ; CEC meq/100=6,30 ; pH (eau)=5,70.

2- Analyses

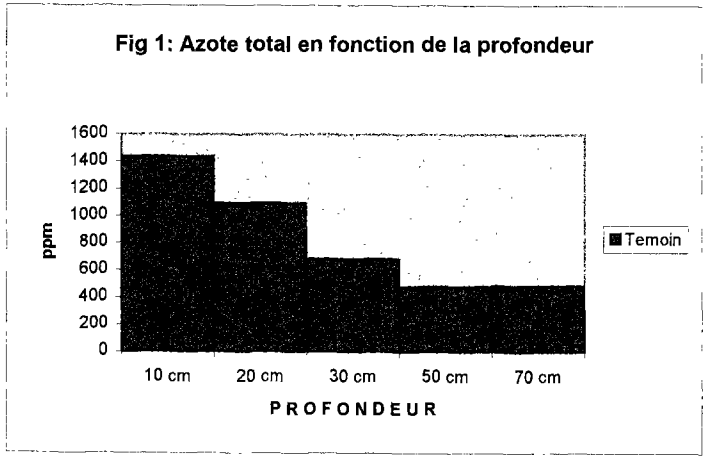
Les échantillons de sol ont été séchés à l'air et tamisés à 2 mm. Trois formes ont été dosées:

- L'azote total en distillant l'ammoniaque obtenu par une minéralisation Kjeldahl et en le dosant par une solution d'acide sulfurique N/50,

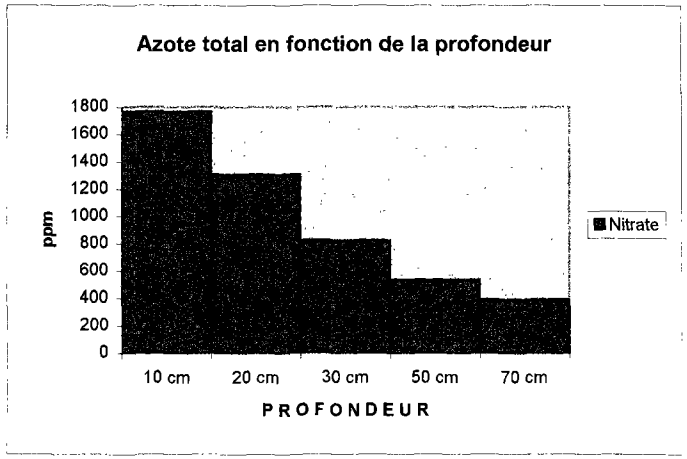
- Les formes minérales NH_4 et NO_3 sont extraites par une solution de sulfate de potassium N. Après filtration, l'ammoniaque est déplacée par de la magnésie calcinée et distillée. Les nitrates sont ensuite transformés en ammoniaque par l'alliage de Devarda et distillés. Une solution d'acide sulfurique N/100 est utilisée pour doser ces formes.

- L'azote hydrolysable obtenu par hydrolyse acide. L'hydrolyse acide se fait par ébullition à reflux pendant 16 heures d'un mélange sol et acide chlorhydrique 6N. Dans le surnageant obtenu par centrifugation, on dose l'azote hydrolysable total (Nht) après minéralisation et l'azote hydrolysable distillable (Nhd) par distillation d'une fraction alcalinisée.
L'azote hydrolysable non distillable (Nhnd) est obtenue par différence entre l'azote hydrolysable total et l'azote hydrolysable distillable. La différence entre l'azote total et l'azote hydrolysable total donne l'azote non hydrolysable (Nnh). Cette dernière forme peut être dosée en minéralisant le culot après centrifugation.

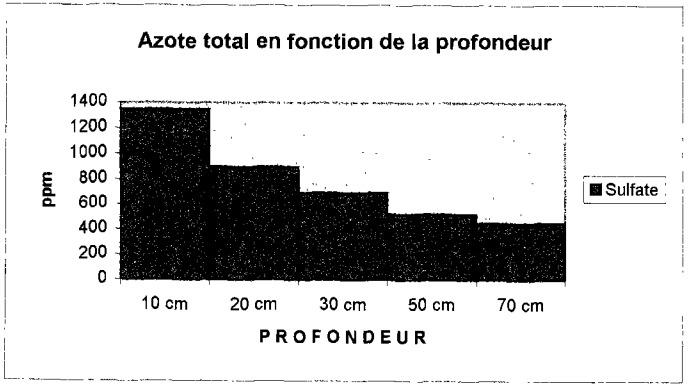
Toutes ces analyses ont été refaites sur des échantillons mis en incubation durant 6 semaines à 28°C et maintenus à une humidité constante correspondant au 2/3 de la capacité maximale de rétention



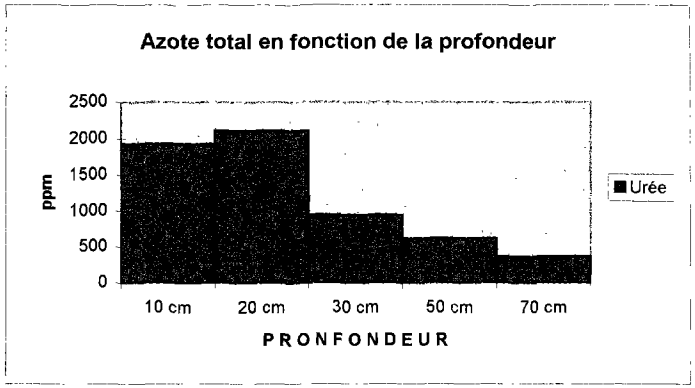
0 - 20cm =61% ; 0 - 30cm = 77%



0 - 20cm = 64% ; 0 - 30cm = 81%



0 - 20cm = 57% ; 0 - 30 cm = 75%



0 - 20 cm = 68% ; 0 - 30cm = 83%

Résultats et Discussions

1- L'azote total

Sur les échantillons de Côte d'Ivoire, les résultats des analyses indiquent que l'azote est fortement concentré dans les horizons de surface: en moyenne près de 60% dans les horizons de 0 - 20 cm, et 80% dans les horizons 0 - 30 cm (Fig1). Au delà de 30 cm de profondeur, l'activité biologique doit être moins intense. Dans les sols du Burundi, des observations similaires ont été faites par D. Snoeck (11).
Ces analyses montrent également que les quantités d'azote total seraient influencées par la nature des engrais azotés et par les doses apportées. En Côte d'Ivoire, l'urée aurait un effet positif après 12 ans d'application (Fig2). Au Cameroun, l'azote total augmente avec les doses du sulfate d'ammoniaque, observation faite également au bout de 13 ans d'apport de cet engrais. Ces résultats rejoignent ceux de Verlière sous caféier (15) mais divergent des travaux de Benac (1) de J. Snoeck (13) et de Raju et al (10).

L'effet des engrais sur l'azote total doit probablement se produire sur une longue période et dépendrait de la réaction des sols face aux engrais. En effet, l'action positive de l'urée sur l'azote total du sol a été observée par Oliver et al (8) en laboratoire . De même, Thomann et al (14) comparant l'urée et le sulfate d'ammoniaque, ont montré que sous panicum sur vertisol en Nouvelle Calédonie, la partie non consommée de l'urée est plus importante dans le sol par rapport au sulfate d'ammoniaque. Contrairement à la fertilisation minérale, l'application de matières organiques telles que des émondes de *Leucaena* et de *Desmodium* (11), du fumier et de la drêche (3) améliorent assez rapidement les teneurs en azote total.

2- Les formes d'azote hydrolysable

2-1 Avant incubation

Les formes azotées obtenues par hydrolyse acide montrent que:

- les quantités d'azote total hydrolysables sont plus élevées dans les traitements Urée et Nitrate que dans le Témoin et Sulfate dans le sol de Côte d'Ivoire. Pour les échantillons du Cameroun, elles sont plus fortes dans les parcelles fertilisées par rapport à celles du témoin. Cependant les proportions d'azote total hydrolysable (Nht) sont identiques et en moyenne de 79% de l'azote total du sol quelle que soit la nature des engrais et des doses utilisés (tableau 1). La partie non hydrolysable (Nnh) représente en moyenne 21% et corroborent des résultats sur d'autres types de sol (7)(16).

- les quantités d'azote hydrolysable distillable (Nhd) représentent en moyenne 23 à 30% de l'azote total du sol,

- les quantités d'azote hydrolysable non distillable (Nhnd) représentent 51 à 54% de l'azote total. Cette dernière fraction, constituée par des acides aminées et des protéines faiblement associées serait facilement utilisable par les plantes. Par contre, l'azote hydrolysable distillable (Nhd) provient de radicaux amides et de sucres aminés étroitement liés aux argiles et peu disponibles pour les plantes (2). On peut constater que

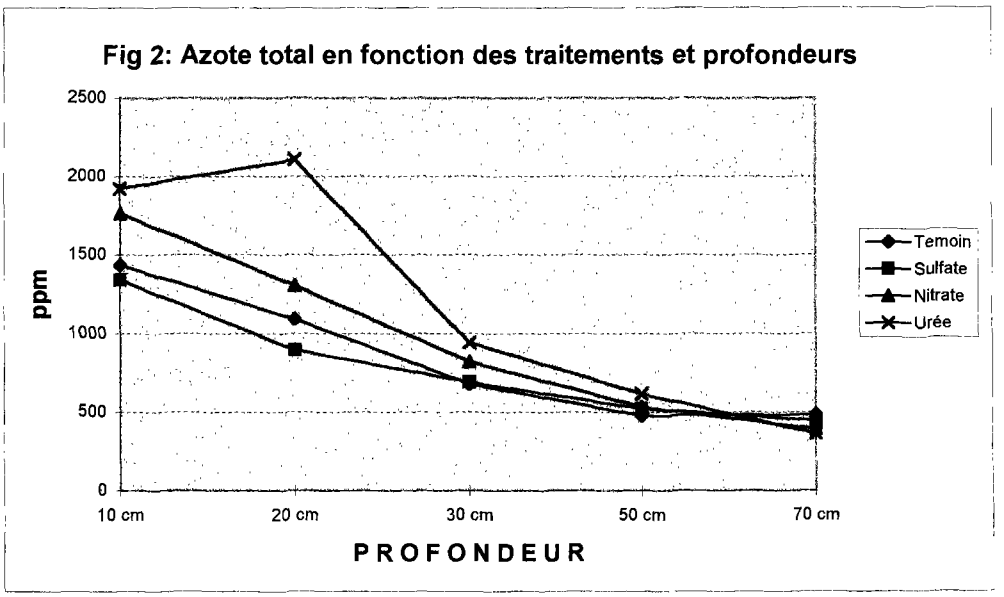


Tableau 1: Formes azotées obtenues par hydrolyse acide en ppm et en % . Profondeur 0 - 20 cm

	Traitements	N total	Nh total	Nh tot/N tot	Nhd	Nhnd	Nhnd/Nhd
COTE D'IVOIRE							
	Temoin	1265	992	79%	299 (24%)	693 (55%)	2,3
	Sulfate	1120	845	76%	272 (25%)	573 (51%)	2,1
	Nitrate	1536	1178	77%	356 (23%)	822 (54%)	2,3
	Urée	2016	1548	77%	436 (22%)	1112 (55%)	2,6
CAMEROUN							
	0 N/ha	1279	1021	79%	401 (31%)	620 (48%)	1,5
	90 N/ha	1503	1228	82%	410 (27%)	818 (55%)	2
	180 N/ha	1503	1250	83%	446 (30%)	804 (53%)	1,8
	360 N/ha	1708	1357	79%	515 (30%)	842 (49%)	1,6

l'apport des engrais se traduit par une augmentation des teneurs des formes Nhd et surtout celles de Nhnd aussi bien en Côte d'Ivoire qu'au Cameroun. Des résultats analogues sont obtenus par Egouminides et al (3) sur sol tropical ferrugineux du Burkina Faso.

Dans le sol de Cote d'Ivoire, le potentiel disponible pour les plantes est plus important dans les traitements Urée et Nitrate que le Sulfate où une baisse est observée par rapport au témoin. Concernant l'azote potentiellement minéralisable, on constate également d'après le tableau ci-dessous qu'il est nettement plus élevé après les apports de nitrate et d'urée, en particulier entre 10 et 30 cm. On enregistre, en revanche, une dégradation de ce potentiel minéralisable dans les sols non fertilisés ou recevant du sulfate d'ammoniaque.

N potentiellement minéralisable en ppm et en % de N total

Profondeur	Témoin	Sulfate	Nitrate	Urée
0- 10 cm	223 (15%)	158 (12%)	296 (17%)	391 (20%)
10- 20 cm	134 (12%)	62 (7%)	273 (21%)	370 (17%)
20- 30 cm	38 (5%)	28 (4%)	105 (13%)	117 (12%)

En outre, le rapport Nhnd/Nhd considéré comme indicateur d'azote disponible et d'activité microbienne dans le sol est légèrement plus faible avec le Sulfate.
Dans le cas du Cameroun, les résultats indiquent que les traitements avec les doses de 90 et 180 kg N/ha présentent un bon niveau d'azote hydrolysable et un meilleur rapport Nhnd/Nhd.

2-2 Après incubation

Le tableau 2 permet de constater que les quantités d'azote hydrolysable augmentent avec les apports de Sulfate dans les deux groupes d'échantillons. Dans les échantillons de Côte d'Ivoire, on note une faible extraction de l'azote hydrolysable total dans le traitement Urée et Témoin et corrélativement une diminution importante des quantités des formes Nhnd et une hausse des quantités des formes hydrolysables distillables Nhd. Il y a certainement eu des phénomènes de réorganisation avec accroissement de la fraction d'azote non hydrolysable Nnh. Des travaux notamment ceux de Gigou (5), de Oliver et al (8) et de Phuy et al (9) ont fait des observations similaires en mettant en évidence une incorporation de l'azote 15 dans les différentes fractions hydrolysable et non hydrolysable. Dans le traitement Urée, on assisterait à la formation de composés azotés plus complexes non hydrolysables. La réserve azotée du sol serait donc mieux préservée dans le cas d'utilisation de l'urée et du nitrate par rapport au sulfate.

Pour les échantillons du Cameroun, les teneurs en azote hydrolysable sont plus élevées dans les parcelles fertilisées que dans le Témoin. Les productions d'azote hydrolysable sont maximum à la dose de 180 kg N/ha, et une partie importante de l'azote non hydrolysable serait passée sous forme hydrolysable. Le stock azoté du sol serait moins conservé à cette dose par rapport à 90 kg N/ha. En outre, les fortes doses de sulfate d'ammoniaque exercent des actions néfastes sur le pH du sol. En effet, le pH a atteint respectivement 4,02 , 3,76 et 3,52 pour les doses de 90, 180 et 360 kg N/ha contre 5,7 pour le Témoin après treize ans d'application.

Tableau 2: Evolution des formes d'azote hydrolysable en ppm au cours de l'incubation (0 - 20 cm)

Côte D'Ivoire

Trait.	Incu-bation	Nht		Nhd		Nhnd	
Temoin	avant	992		299		693	
	apres	963	(- 29)	304	(+ 5)	660	(- 33)
Sulfate	avant	845		272		573	
	apres	867	(+ 22)	294	(+ 22)	573	0
Nitrate	avant	1178		356		822	
	apres	1174	(- 4)	369	(+ 13)	805	(- 17)
Uree	avant	1548		436		1112	
	apres	1485	(- 63)	446	(+ 10)	1039	(- 73)

Cameroun

Trait.	Incu-bation	Nht		Nhd		Nhnd	
0 N/ha	avant	1021		401		620	
	apres	1048	(+ 27)	428	(+ 27)	620	0
90 N/ha	avant	1228		410		818	
	apres	1299	(+ 71)	506	(+ 96)	793	(- 25)
180 N/ha	avant	1250		446		804	
	apres	1424	(+ 174)	546	(+ 100)	878	(+ 74)
360 N/ha	avant	1357		515		842	
	apres	1326	(- 31)	555	(+ 40)	771	(- 71)

3 - Les formes d'azote soluble

Avant l'incubation, les teneurs de formes d'azote soluble et organique sont plus faibles dans les traitements Témoin et Sulfate dans le sol de Côte d'Ivoire. L'azote total soluble représente près de 4% de l'azote total du sol dans tous les traitements.
Dans le sol du Cameroun, on observe une augmentation des quantités des formes minérales en fonction des doses de sulfate d'ammoniaque. L'azote total soluble représente 6 à 11% de l'azote total du sol. Ces teneurs sont plus élevées que celles dans le cas de la Côte d'Ivoire.

L'incubation modifie la proportion des formes de l'azote total soluble. En effet, la minéralisation au cours de l'incubation a produit essentiellement des nitrates dans le cas du sol de Côte d'Ivoire tandis que dans le sol du Cameroun, elle a produit une forte quantité d'azote ammoniacal et très peu d'azote nitrique, les témoins y compris (Tableau 3).

Tableau 3: Evolution des formes d'azote soluble en ppm au cours de l'incubation (0 - 20 cm)

Côte D'Ivoire

Trait.	Incu-bation	NH4		NO3		N org.	
Temoin	avant	13		7		22	
	apres	4	(- 9)	42	(+ 35)	15	(- 7)
Sulfate	avant	14		5		22	
	apres	5	(- 9)	56	(+ 51)	15	(- 7)
Nitrate	avant	15		12		28	
	apres	4	(- 11)	73	(+ 61)	16	(- 12)
Uree	avant	14		13		43	
	apres	7	(- 7)	59	(+ 46)	28	(- 15)

Cameroun

Trait.	Incu-bation	NH4		NO3		N org.	
0 N/ha	avant	13		10		48	
	apres	93	(+ 80)	13	(+ 3)	34	(- 14)
90 N/ha	avant	21		17		54	
	apres	86	(+ 65)	34	(+ 17)	56	(+ 2)
180 N/ha	avant	35		18		55	
	apres	100	(+ 65)	28	(+ 10)	44	(+ 11)
360 N/ha	avant	83		30		55	
	apres	136	(+ 53)	35	(+ 25)	56	(+ 1)

Dans le sol de Côte d'Ivoire, on note une diminution des teneurs de l'azote minéral NH_4 et de l'azote organique soluble dans tous les traitements. On peut également constater que les quantités de nitrate produites dans les traitements Urée et Témoin sont plus faibles que celles des traitements Sulfate et Nitrate. Ces deux derniers types d'engrais stimuleraient plus la minéralisation. Dans le cas du Cameroun, la production de nitrate est très faible par rapport à l'ammoniaque dans tous les traitements. A Maroua au Cameroun, Gigou (4) a observé une nitrification plus lente du sulfate d'ammoniaque par rapport à l'urée dans le sol. Il y aurait donc des facteurs qui limitent l'activité nitrifiante dans ce type de sol.
Ces résultats montrent que le caféier absorberait l'azote dont il a besoin sous forme nitrique ou ammoniacale selon le type de sol.

Conclusion

Dans l'ensemble, la présente étude indique que la fertilisation minérale ne modifie pas les proportions des formes de l'azote du sol quelle que soit la nature des engrais azotés. L'hydrolyse acide permet de dire que la réserve azotée du sol est mieux préservée en utilisant de l'urée et du nitrate dans les conditions de sol de Côte d'Ivoire par rapport au sulfate d'ammoniaque.

En effet, lorsqu'on fait une incubation à humidité constante, on observe que les quantités d'azote hydrolysable et soluble disponibles pour la plante augmentent avec le sulfate d'ammoniaque. Par contre , on assiste à la formation des formes complexes non hydrolysables avec l'urée. Il y a donc un appauvrissement des réserves azotées dans le sol suite à l'application du sulfate d'ammoniaque. Cet type d'engrais est également connu pour son effet sur la baisse du pH du sol.

Par ailleurs, les formes solubles produites au cours de l'incubation sont différentes selon l'origine des sols. Dans le sol de Côte d'Ivoire, la minéralisation a produit plus d'azote nitrique que d'azote ammoniacal contrairement au sol du Cameroun. Et ceci nous interroge sur les formes d'azote soluble absorbées par le caféier et les effets de ces différences formes minérales sur la qualité du café.

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RESUME

Deux methodes d'extraction de l'azote du sol, la solution de sulfate de potassium N et l'hydrolyse acide (HCL 6N) ont été utilisées pour apprécier l'effet des engrais minéraux sur les formes de l'azote du sol.Les échantillons de sol proviennent de deux essais sur caféier robusta conduits respectivement en Côte d'Ivoire et au Cameroun dans des sols ferrallitiques pendant une dizaine d'années.

L'hydrolyse acide permet de constater que la fertilisation azotée à base d'urée maintient la reserve d'azote du sol potentiellement minéralisable à un niveau plus élevé par rapport au sulfate d'ammoniaque. Le dosage des formes hydrolysables après incubation confirme l'action conservatrice de l'urée par la formation de complexes azotés non hydrlysables. Cependant, le nitrate et le sulfate d'ammoniaque provoquent une réorganisation inverse de la reserve tendant à mobiliser plus d'azote pour la plante.

Cette étude a également permis de montrer que le caféier absorberait indifféremment l'azote nitrique ou ammoniacal en fonction des ses besoins et des activités nitrifiantes du sol. En effet, les formes solubles produites à la suite de l'incubation sont différentes selon l'origne des échantillons.

THE EFFECT OF ORGANIC MATTER SOURCES AND NITROGEN FERTILIZER DOSES ON GROWTH OF ROBUSTA COFFEE IN EAST JAVA, INDONESIA

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Introduction

Organic matter is one of the important factors needed for increasing and sustaining land productivity, as well as for making inorganic fertilization more efficient. Soil organic matter content in most Indonesian coffee plantations is very low to low. To increase the content, many efforts have been done, i.e. by adding farmyard manure, compost, digested coffee pulp, urban waste, sugar factory waste and paper factory waste; but unfortunately the need for organic matter material still not been met yet (Baon & Soenaryo, 1988, 1989; Boopathy, 1987; Nur & Abdoellah, 1988). Beside it could not meet the need, supplying of organic matter as mentioned above is also facing some constraints. For supplying manure in plantations, many cows are needed to yield sufficient manure, but the carrying capacity of land is not enough to yield grass for feed. To bring about manure from outside plantation, the transportation cost is expensive.

One of many alternatives of organic matter sources in coffee plantation is cover crops which interplanted with coffee. Beside as organic matter source which are present *in situ*, leguminous cover crops are also can raise soil nitrogen content by symbiosis with bacteria. A research conducted by Indonesian Coffee and Cocoa Research Institute also showed that leguminous cover crops could raise the phosphorus availability of podzolic soils.

Beside problem to raise its content, organic matter conservation as a part of soil conservation in coffee plantation also need attention. In general, coffee is cultivated on mountainous land with steep slope. Soil conservation can be done by mechanical, chemical and biological methods. Among those methods, biological method, especially

vegetative method is a simplest and cheapest method. One of plant species which has prospective in vegetative soil conservation is vetiver grass (*Vetiveria zizanioides*). This grass have a deep roots and strong enough for decreasing run off and erosion.

Although leguminous cover crops and vetiver grass have many superiority, planting them in coffee plantations may have negative effects, i.e. raising air humidity under coffee canopy which may stimulate diseases, causing a difficulty in picking of dropped cherry (in harvesting period), and as a competitor of coffee for water and nutrient uptake.

This experiment was to test the superiority and weakness of interplanting of leguminous cover crops and vetiver grass as an alternative source of organic matter as well as nitrogen supplement in coffee plantations.

Materials and method

This experiment was arranged in split plot design, with the main plots were organic matter source and sub plots were nitrogen fertilizer doses. The main plots were 1)control (no organic matter), 2)with farmyard manure 20 dm^3/tree/year, 3)interplanted with leguminous cover crop of *Calopogonium caeruleum*, and 4)interplanted with vetiver grass. Sub plots were 1)control (no nitrogen fertilizer), 2)with a half of recommended dose of nitrogen fertilizer (10 g Urea/tree/year in 1st year and 20 g Urea/tree/year in 2nd year), and 3)with full recommended dose of nitrogen fertilizer (20 g Urea/tree/year in 1st year and 40 g Urea/tree/year in 2nd year). Each treatment combination was replicate three times. Plant materials were robusta coffee of BP 42, BP 234, BP 409 and BP 936 clones, with *Gliricidia sepium* and *Leucaena leucocephala* as shade plants. Parameters observed were number of productive internodes of coffee (in 2nd year after transplanting), soil moisture content in dry season, water saturation deficit in dry season, as well as carbon, nitrogen and phosphorus content of soil. Data were analyzed by variance analysis and among treatment means were analyzed by Tukey test.

Results and discussion

There was no interaction between main plots (organic matter sources) and sub plots (nitrogen fertilizer doses). Number of productive internodes at 2nd year after transplanting presented in Table 1.

Table 1. Number of productive internode of coffee at 2nd year after transplanting

Treatments	Number of productive internodes
Main plots	
Control	105.6 a
Farmyard manure	112.9 a
Calopogonium caeruleum	82.9 b
Vetiver grass	116.9 a
Sub plots	
Control	95.3 a
A half of N fertilizer dose	109.0 a
Full of N fertilizer dose	109.4 a

Note : Values for each treatment group if followed by same letters is not significantly different according to 5% Tukey test.

The main plot data showed that there was no significant different among farmyard manure application, vetiver grass and control; but interplanting of *C. caeruleum* with coffee inhibit internode growth. Inhibition of internode may decrease yield, as coffee cherry grew at internode. *C. caeruleum* is a creep plant, so if its growth was not controlled, its stems twist the stem and branches of coffee, and it inhibited the growth of coffee. So, the inhibition of *C. caeruleum* is more due to the physical disturbance than the other factors. When the growth of *C. caeruleum* was fully controlled, that disturbance did not take place. On the other hand, although number of internode statistically was not affected by farmyard manure and vetiver grass treatments, their value tend to be higher than control. In future, it is expected that their effects will be significant, because this observation is still on the 2nd year after transplanting, and in general organic matter have slow and long time effect. Soil treated with 30 dm^3 paper sludge/m^2 nursery bed results a better growth of arabica coffee seedlings than control (Nur & Abdoellah, 1988); while applying of 25 kg filter press cake/tree/year significantly increase coffee yield (Baon & Soenaryo, 1988, 1989). A study over a period of 12 years conducted in Kenya showed that addition of organic manures (cattle manure, compost, liquid manure and sludge) did not significantly increase clean coffee yields (Njoroge, 1988). Nevertheless, the author believed that organic fertilizers were essential for coffee production.

Beside as a source of soil organic matter, leguminous cover crop and grass are also used as mulch materials. The use of mulch in coffee is required in steep slopes, long dry season and leached soils, but the common sources are difficult to find. *Leucaena*, *Flemingia* and *Desmodium* are alternative sources of mulch materials (Snoeck *et al.*, 1993). By interplanting yielding mulch plant with coffee, problem of mulch material source was a little bit overcame.

Nitrogen content of the soil was below the optimum for coffee. It was shown from the data of nitrogen fertilization treatment that control plant tended to yield smaller number of

internode than nitrogen fertilization treatments, although until 2nd year after transplanting it was still not significant. As mentioned above, the lower the internode number, the lower the production will be reached. Njoroge (1988) reported that addition of NPK fertilizer significantly increased clean coffee yields in Kenya. Nevertheless, a half dose of recommended nitrogen for East Java is enough for two years old coffee.

Table 2. Soil moisture and water saturation deficit of leaves at dry season

Treatments	Soil moisture, %	Water saturation deficit, %
Main plots		
Control	22.73 a	18.41 a
Farmyard manure	23.17 a	23.18 a
Calopogonium caeruleum	25.40 a	25.78 a
Vetiver grass	25.67 a	26.81 a
Sub plots		
Control	23.63 a	22.16 a
A half of N fertilizer dose	24.63 a	26.94 a
Full of N fertilizer dose	24.47 a	21.54 a

Note : Values in the same column for each treatment group if followed by same letters is not significantly different according to 5% Tukey test.

Applying of farmyard manure, planting of *C. caeruleum* and vetiver grass did not affect soil moisture depletion in dry season, but their values tended to be higher than control. It means that when the organic matter is enough accumulated in the soil, its effect on water conservation will be significant. The higher soil moisture content in dry season of *C. caeruleum* and vetiver grass treatments were caused by the role of their pruned materials as mulches.

Coffee is more sensitive to weed in dry season due to the competition of water than in rainy season when weeds are growing rapidly (Friessleben *et al.*, 1991). Although the soil moisture content of *C. caeruleum* and vetiver grass treatments tended to be higher than control, the water saturation deficit of leaves also tended to be higher. It means that there was a competition in water between coffee and the crops, in spite of soil moisture was available. Nevertheless, results of the test statistically was not significant.

Although they were not significant statistically, treatments of farmyard manure and *C. caeruleum* tended to increase organic carbon and total nitrogen of soils. On the other hand, vetiver grass tended to decrease those parameters. Farmyard manure and leguminous crops were easily decomposed by soil microorganisms, but vetiver grass was more resistant due to its high silica content. That is why the first two treatments increased carbon and nitrogen content of soils but the last treatment did not.

Table 3. Contents of organic carbon, total nitrogen and available phosphorus of soil

Treatments	C, %	N, %	P, ppm
Main plots			
Control	1.740 a	0.217 a	155.0 a
Farmyard manure	1.807 a	0.227 a	149.0 a
Calopogonium caeruleum	1.877 a	0.233 a	128.6 a
Vetiver grass	1.687 a	0.210 a	153.3 a
Sub plots			
Control	1.830 a	0.218 a	141.8 a
A half of N fertilizer dose	1.750 a	0.218 a	146.8 a
Full of N fertilizer dose	1.752 a	0.230 a	151.0 a

Note : Values in the same column for each treatment group if followed by same letters is not significantly different according to 5% Tukey test.

Nitrogen fertilization tended to lower organic carbon of soils. The higher the nitrogen availability in soils, the higher the activities of soil microorganisms. In their activities, soil microorganisms used carbon to supply their energy need, so it caused in decreasing of soil carbon content.

Use of organic manures will be beneficial in making the nutrients especially phosphorus available to soil by the plants (Jayarama *et al.*, 1994), although in the present experiment it has not been proved yet.

Conclusion

Until 2nd year after transplanting, application of farmyard manure and interplanting with vetiver grass did not increase the number of productive internode of coffee. Interplanting with *C. caeruleum* retarded the growth of coffee internode. There was no increase in internode number by nitrogen fertilizer application. There were no effect of farmyard manure application, interplanting with *C. caeruleum* and vetiver grass, as well as nitrogen fertilizer application on soil moisture and leaf water saturation deficit of coffee in dry period, on the contents of organic carbon, total nitrogen, and available phosphorus of soils. This experiment will be continued until five years of coffee harvestings.

Summary

An experiment to study the effect of organic matter sources and nitrogen fertilizer doses on growth of robusta coffee had been conducted in Kaliwining Experimental Station of the Indonesian Coffee and Cocoa Research Institute, Jember, East Java, Indonesia. The experiment was arranged in randomized complete block, split plot design with three

blocks. The main plot was organic matter sources, i.e. control (no organic matter added), farmyard manure (20 kg of air dried per tree per year), *Calopogonium caeruleum* (planted inter rows of coffee and its leaves as well as its stem were used as mulch), and *Vetiveria zizanioides* (planted inter rows of coffee and its stem was used as mulch). The sub plot was nitrogen fertilizer dose, i.e. no nitrogen, a half of recommendation dose, and full of recommendation dose. The results on 2nd year after transplanting showed that the number of productive nodes of farmyard manure and vetiver grass treatments were higher than that of *C. caeruleum* treatment, but all of treatments were not significantly different to control. There was no effect of organic matter sources on water saturation deficit of coffee leaves in dry season; as well as on moisture-, C-, and N-content of soil; although those values of cover crop treatments tended to be higher than the other two treatments. Nitrogen fertilization is still needed for better growth although high N content organic matter has been added.

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LUTTE INTÉGRÉE CONTRE LES RAVAGEURS (IPM) ET APPROCHE INTÉGRÉE DU PATHOSYSTÈME / *COFFEA ARABICA*

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A. Lutte intégrée contre les ravageurs (IPM)

1) Introduction.

Pour situer la complexité des phénomènes étudiés et introduire cette revue, on peut rappeler quelques notions générales sur l'**Epidémiologie**. Cette discipline a forgé peu à peu, en moins de 30 ans, théorie, concept, et méthodologie spécifiques (Rapilly 1991). De descriptive et explicative, elle est devenue quantitative et prospective. Cette science concerne l'étude des populations de pathogènes au sein des populations de plantes-hôtes, ainsi que les maladies qui en résultent sous l'influence des interférences dues à l'environnement et à l'homme (Kranz, 1990). Les épidémies, c'est-à-dire le développement des maladies dans le temps et dans l'espace, sont des phénomènes complexes, impliquant de nombreux facteurs, comparables aux systèmes écologiques auxquels elles sont d'ailleurs intimement liées.

Les maladies et leurs épidémies doivent être considérées comme des **systèmes imbriqués de processus** caractérisés eux-mêmes par de multiples mécanismes réciproques de cause à effet, qui sont régulés par des phénomènes aléatoires, des fluctuations et des récurrences aussi bien que par des seuils et des discontinuités (Watt, 1966). Aussi, cette complexité conduit-elle à une grande diversité de schémas évolutifs qui, au sein du "carré de maladie" intégrant hôte, environnement, maladie et interférence humaine (Van der Plank, 1963), doivent être mesurés et analysés, ce qui génère généralement un important volume de données multivariées.

L'Epidémiologie concerne les maladies tant au niveau des populations que des communautés. Il s'agit donc d'une science éminemment quantitative qui requiert l'usage des mathématiques et des modèles afin de parvenir à analyser les effets et la pertinence des variables mesurées à partir des deux populations imbriquées que sont l'hôte et le pathogène et dont la résultante génère à son tour une population de lésions. Le but recherché est d'obtenir des résultats concrets utilisables efficacement en protection des cultures.

2) Diversité des agents nuisibles.

A cette évidente complexité, s'ajoute la multiplicité des agents nuisibles. Le mot anglais "pest", assez souvent réservé aux insectes ravageurs, intègre en fait tout organisme nuisible, gênant ou destructeur par rapport à une plante ou aux produits végétaux (Holliday 1989). La diversité des "agents nuisibles" est impressionnante puisqu'elle comprend les mauvaises herbes, les animaux (rongeurs, escargots), les insectes et les pathogènes (champignons, bactéries, virus, mycoplasmes). En outre, il est désormais admis que des désordres abiotiques tels que la pollution de l'air, la sécheresse, le pH du sol, ou des problèmes trophiques peuvent être adjoints à cette liste dans la mesure où ils provoquent, chez les plantes, des lésions ou des dysfonctionnements qui se traduisent par des "symptômes" et donc des "maladies" (Bos & Parlevliet 1995). Ces différents facteurs interagissent entre eux, ce qui constitue un des aspects de la complexité de l'agro-écosystème.

Devant cette diversité de catégories d'agents nuisibles, il est clair que les principes modernes de l'agriculture, comme par exemple l'implantation de cultures très homogènes au plan génétique et/ou l'usage immodéré d'engrais azotés (qui augmentent souvent la sensibilité des plantes aux maladies), sont autant de facteurs qui aggravent le potentiel de destruction des maladies (De Waard et al. 1993).

3) Contrôle chimique des maladies.

Devant cette variété de maladies menaçant l'agriculture, les méthodes de lutte, devenues totalement indispensables, demeurent très dépendantes des pesticides chimiques qui contribuent effectivement à une réduction significative des pertes de récoltes.

Malgré une efficacité démontrée, (James et al. 1993) leur contribution positive est contrebalancée par de réels effets négatifs sur l'environnement. Les plus récentes recherches en chimie analytique sur ce point ont démontré (i) que les pesticides avaient la capacité de se répandre, parfois sur de longues distances, dans le sol et dans l'eau, (ii) qu'ils avaient souvent une rémanence inquiétante, (iii) que des résidus étaient détectables dans les produits récoltés. Face à ces dangers, les industries chimiques mettent en oeuvre de gros efforts de recherche pour d'une part, tenter d'élucider le cheminement suivi par les pesticides pour se disséminer dans l'environnement, de comprendre d'autre part leurs mécanismes de dégradation et enfin pour en identifier les résidus (De Waard et al. 1993). La perception de plus en plus négative du public quant aux produits chimiques, conjuguée aux réels efforts des industries de fabrication font espérer que les nouvelles formulations atteignent des standards acceptables au plan toxicologique et environnemental.

4) Approches alternatives.

De nouvelles approches ont donc été recherchées puis adoptées pour apporter une alternative à cet usage exclusif des pesticides et pour minimiser leur impact sur l'environnement : ce sont les stratégies de "gestion intégrée des ravageurs" ou **Integrated Pest Management** (IPM), également nommée "Low Input Sustainable Agriculture" (LISA).

Selon une définition de la FAO, l'IPM représente un système de gestion des cultures qui, dans le contexte d'un environnement et d'une évolution des populations de ravageurs, utilise toutes les techniques de lutte disponibles, de la manière la plus cohérente possible, afin de maintenir le niveau des ravageurs en dessous du seuil de nuisance économique. Cette gestion rationnelle des ravageurs doit faire en sorte qu'il n'y ait pas de dépendance totale à une unique méthode de contrôle et prendre en compte les conséquences économiques, sociales et environnementales des stratégies de lutte. On comprend alors aisément que ceci ne peut être obtenu qu'au travers d'investigations résolument pluridisciplinaires pour acquérir des connaissances dans les domaines suivants :

- effets de l'homme et de l'environnement sur la culture et sur la dynamique des populations de ravageurs,
- effets des ravageurs sur la production, aux différents stades de développement de la culture,
- rentabilité économique de l'usage des méthodes de lutte,
- existence de procédures permettant l'acquisition, la synthèse et la diffusion des informations pratiques, sous une forme et des délais acceptables.

La finalité de ces approches intégrées réside dans la possibilité d'obtenir des réponses concrètes et rapides pour gérer les populations de pathogènes, si possible avant même que ne soient causés les premiers dommages.

5) Concepts et méthodologies.

Actuellement à la mode, les concepts de pertes de récoltes et de lutte intégrée ainsi que la terminologie correspondante ont subi d'importantes évolutions.

D'un point de vue historique, Zadoks (1987) rapporte que les premières enquêtes épidémiologiques nationales et internationales datent du début de ce siècle. Mais c'est sous l'impulsion des conditions dramatiques imposées par les deux guerres mondiales que la recherche agricole fut obligée de devenir efficace. Quelques chercheurs comme Moore (1952), Strickland (1953), Large (1966) *inter alia* jetèrent alors les bases des premiers véritables travaux sur les pertes de récolte et définirent des méthodologies dont beaucoup demeurent encore en vigueur. Leur contribution majeure fut de concevoir des schémas de réflexion sur les productions et sur les pertes en récolte qui établissaient les premières réglementations en matière d'agriculture à l'échelle nationale ou régionale. C'est à compter de 1967, lors du Symposium sur les pertes de récolte organisé par la FAO à Rome, que des méthodologies adaptées furent développées et que des mesures réellement pratiques furent édictées (e.g. recours aux pesticides ; timing de traitement ; conditions de récolte, de transport et de stockage).

Ultérieurement, furent développés les concepts épidémiologiques de dynamique des populations de ravageurs, de seuils de mise en oeuvre des méthodes, de prédiction de pertes, qui constituent les fondements des principes de la lutte intégrée contre les maladies et ravageurs (Stern et al. 1959, i.a.). En complément, les notions de dommage (injury), de dégâts (damage) et de pertes (loss) furent enfin clairement définies (Bos & Parlevliet 1995).

D'un point de vue méthodologique, différentes tendances sont à prendre en compte successivement (Kim & MacKenzie 1987).

- Une première période voit l'essor de **méthodes "réductionnistes"** qui, en résumé, tendent à maintenir toutes les variables constantes pour que seul le facteur étudié varie, ce qui permet d'en tester la signification. C'est alors l'analyse de variance qui fut utilisée massivement. Par exemple, pour évaluer l'effet d'engrais azotés sur la production de maïs, on s'efforce de contrôler tous les paramètres mais on en exclue les maladies. Comme le niveau d'azote affecte également la croissance des mauvaises herbes et le développement des pathogènes, le niveau de production est alors indirectement affecté, ce qui biaise l'expérimentation. Bien évidemment, pour traiter ces questions complexes, il importe de prendre en compte d'une part, plus de variables, et d'autre part, leurs interactions : ceci est inaccessible aux analyses mono-factorielles.

- De ce fait, sous l'impulsion de précurseurs comme Watt (1970), Zadoks (1971), Kranz & Hau (1980), est apparue une seconde manière de penser qui constitue la base du concept de la **science des systèmes**. Celle-ci postule que "le tout est plus que la somme des parties". Pour cerner un problème complexe, ce type d'approche prend en compte plutôt une combinaison de facteurs (interactions) que des facteurs individuels. En outre, cela permet d'intégrer tout le savoir existant ou, en tout état de cause, d'identifier les lacunes dans les connaissances afin de guider les recherches (Teng & Kropff 1995). Les principaux outils de la science des systèmes sont d'une part, les méthodes statistiques multivariées (Hau & Kranz 1990 ; Savary et al. 1995 ; i.a.) et d'autre part, la modélisation et la simulation qui permettent de tester les conséquences des variations sur une représentation du monde réel (Kranz 1990).

- Les fonctions de ces **modèles** sont diverses. Jeger et Tamsett (1983) distinguent les trois types suivants : (i) descriptif, (ii) de fonctionnement (fonction exploratoire), (iii) de prévision. La complexité d'un modèle et ses limites viennent du fait que le pathogène est influencé par l'environnement, que le statut de l'hôte est à son tour influencé par le pathogène et que l'hôte et le pathogène interagissent. Au sein d'un pathosystème, il est indispensable de spécifier les variables à étudier et de parvenir à comprendre leurs inter-relations.

Cela étant, il est important de garder à l'esprit les propos de Patten (1971) selon lesquels un modèle ne représente qu'une construction artificielle, une abstraction du monde réel, une approximation simplifiée de la réalité. Ceci implique donc qu'un modèle est rarement complet, achevé ou un objectif à lui seul. Sans ambages, Tomassone (1987) considère que tout modèle est faux et qu'il faut choisir le moins faux par rapport aux objectifs fixés.

- Une majorité de travaux de recherche se consacre encore, et uniquement, au pathogène le plus dommageable d'une culture, alors qu'une "**approche synoptique**" considérant, dans leur ensemble, les effets combinés des différents pathogènes sur la production est fortement recommandée (Shane & Teng 1987). Cette démarche, proposée pourtant par Stynes dès 1980, caractérise justement la voie retenue pour les recherches sur le caféier en Nouvelle-Calédonie.

Un bon exemple de l'ampleur du dispositif requis pour une approche réellement intégrée et synoptique est fourni par les travaux de Wiese (1982). Celui-ci a échantillonné 100 champs de pois aux USA en évaluant dans chacun, les ravageurs (insectes, mauvaises herbes, maladies, nématodes), le climat (grêle, gel, pluies, température), les caractéristiques du sol (nutriments, pH, topographie, texture, humidité, température), pratiques culturales (type de cultivar, rotations, pesticides, engrais, entretien). Des relevés furent effectués toutes les deux semaines pendant 4 mois. Les données obtenues ont permis la mise au point d'un modèle globalement interactif qui, sur la base de 12 variables, parvenait à expliquer 82% de la variabilité constatée *in situ*. Ces résultats n'ont pas été réellement reproductibles d'une année sur l'autre en raison de la variabilité dans le temps de la nature et de l'intensité des infestations parasitaires. Cependant, les travaux de Wiese ont eu le mérite de définir l'amplitude et la diversité des contraintes de la production en conditions d'exploitation agricole et non en station expérimentale. De ce fait, ils constituent une approche de référence pour la gestion et la lutte intégrée contre les maladies.

- Le concept d'**agriculture durable** (sustainability) ou de viabilité des agro-écosystèmes constitue désormais une composante importante dans les nouvelles approches IPM. Tous les experts s'accordent sur la nécessité de développer les recherches en "veillant à un accroissement régulier de la production agricole, tout en garantissant le maintien ou l'amélioration des ressources" (Fry 1982). La viabilité des agro-écosystèmes est en effet mise en péril par l'uniformisation des pratiques culturales, la mise en place de monocultures sur de très grandes surfaces, la mauvaise gestion des pesticides.

- Pour aboutir, il est clair que des recherches sur un thème aussi vaste ne peuvent se concevoir que dans le cadre d'**approches pluridisciplinaires** qui associent agro-pédologie, phytopathologie, physiologie végétale, amélioration des plantes, biologie moléculaire, bioclimatologie, socio-économie...

6) Caractéristiques des approches IPM

Assez généralement, la réalisation d'une stratégie d'IPM suit les étapes suivantes :

- définition de la situation agricole en intégrant tous les facteurs biotiques, physiques et socio-économiques,
- définition du profil des ravageurs, évaluation des pertes de récolte, chronique des pathogènes et analyse de leurs interrelations,
- résultats d'enquêtes et/ou d'expérimentations spécifiques visant à démontrer tel ou tel postulat suggéré par les observations de terrain,
- modèles de simulation (quantification du risque potentiel) & outils de gestion pour une mise en place rationnelle de stratégies de lutte.

L'objectif est de mettre au point des outils de gestion des contraintes agricoles. Assez généralement, de telles recherches sont réalisées tout d'abord en stations expérimentales afin de quantifier, en milieu contrôlé, les pertes de récoltes ; puis intervient une phase de terrain qui permet d'évaluer le risque en parcelles traditionnelles. Enfin, on réalise la simulation des dégâts sur la base des données acquise dans ces deux contextes. L'exemple des recherches sur le caféier dans le Pacifique tend à montrer que l'on peut aussi réaliser, en milieu traditionnel, des investigations souvent cantonnées aux stations expérimentales.

7) Diversité des techniques de lutte intégrée contre les ravageurs.

Par comparaison à l'usage exclusif des pesticides, on ne peut nier que les méthodes alternatives pour contrôler les maladies selon un concept d'IPM sont plus délicates à développer (De Waard et al. 1993). Cependant, tout en respectant les contraintes liées à l'environnement, à la santé publique et à l'économie mondiale, des approches de plus en plus sophistiquées se diversifient (Teng & Yang 1993). Il s'agit essentiellement (i) de la lutte biologique (Cook 1993), (ii) de la résistance induite, (iii) de l'engineering génétique ciblée soit sur les plantes (transgénie), soit sur les micro-organismes (transfert de gènes et biopesticides). Certaines de ces techniques sont déjà utilisées dans le cadre du caféier (Eskes et al. 1991 ; Martins & Moraes 1996 ; Quesada-Chanto & Jimenez-Ulate 1996 ; i.a.). De façon exhaustive, Reuveni (1995) fait l'inventaire des diverses techniques utilisables dans le cadre des programmes d'IPM :

- recours aux marqueurs biochimiques pour évaluer la résistance des plantes aux pathogènes,
- détection de gènes impliqués dans la résistance aux maladies,
- utilisation de l'hybridation somatique pour le transfert interspécifique d'une résistance aux maladies,
- utilisation des méthodes d'activation des mécanismes naturels de défense des plantes,
- utilisation de rhizobactéries comme fertilisants naturels,
- recours aux ennemis naturels des ravageurs, en liaison avec les pesticides,
- usage de biopesticides tels que les baculovirus, des champignons (pour le contrôle des mauvaises herbes).

8) Systèmes de prédiction.

La finalité majeure des recherches liées à l'IPM réside dans l'élaboration de systèmes de prédiction qui ont pour but d'évaluer le niveau prévisible de maladie, permettant au planteur d'initier, de manière raisonnée, une stratégie de contrôle (Johnson 1987). Sur quelles bases reposent ces systèmes de prédiction ?

- Par le passé, **l'Environnement** a été de loin le composant le plus souvent utilisé (Hyre et al. 1959). En effet, son incidence sur la maladie se fait à la fois de façon directe et indirecte. Les effets directs sont dus aux conditions physiques (température, humidité, présence d'eau libre sur les feuilles) qui influent sur la germination des spores, la pénétration puis l'infection. Plus indirectement, l'Environnement agit aussi bien sur les conditions de survie du pathogène, de sa dissémination, que tout particulièrement, sur la sensibilité de l'hôte à l'égard des agressions.

- Encore récemment, une majorité de modèles était basée sur la quantification du **pathogène,** privilégiant des relations par exemple, entre la densité d'inoculum et la maladie. Des méthodes telles que le piégeage de spores ou la détermination de la densité de pathogènes dans le sol furent très utilisées (Fry et al. 1983 ; Kushalappa & Lagesse 1981 ; i.a.).

- Il était également admis que les variations dans le temps de **l'hôte** étaient singulièrement plus faibles que celles du pathogène et de l'environnement. De ce fait, rares sont les modèles construits autour de l'hôte. Malgré tout, il est souvent intéressant de prendre en compte la phénologie de l'hôte car elle peut refléter des changements, soit du microclimat, soit de la réceptivité de l'hôte avec l'âge, soit enfin une sensibilité différentielle de la culture en fonction des périodes climatiques (Pscheidt & Stevenson 1983 ; Van der Plank 1982).

- En fait, pour optimiser la représentation d'un système biologique, on perçoit intuitivement qu'il est nécessaire d'**intégrer les trois composants** simultanément, afin de ne pas se contenter de postulats invérifiables. Pour aboutir au stade de la prédiction, trois phases sont nécessaires : analyse, synthèse et gestion du système. Sachant que l'agro-écosystème peut être subdivisé en 4 sous-systèmes (biologique, environnemental, socio-économique et technologique), que chacun d'entre eux doit être considéré à la fois individuellement et sous l'angle de ses interactions avec les autres, la complexité augmente ainsi rapidement ; seul le recours à des ordinateurs de plus en plus performants a rendu possible cette évolution (Johnson 1987). *In situ*, le choix des nombreuses variables pertinentes à retenir dépend à la fois de l'expérience et de l'intuition, comme de la capacité à pouvoir acquérir les données. Il est ensuite nécessaire de recourir à des méthodes statistiques adaptées pour hiérarchiser les variables.

L'étape finale pour la mise en oeuvre d'un système de prédiction est la synthèse de l'information au travers d'un **algorithme** afin de parvenir à une aide à la décision. Compte-tenu de la nature des facteurs régulant la maladie, du degré d'interaction entre les composants du carré de maladie et du degré de sophistication requis pour la mise en oeuvre de la méthode, quatre approches sont envisageables (Johnson 1987) :

- la prévision de maladie, qui détermine si les conditions biologiques et météorologiques favorables à l'apparition et au développement d'une maladie sont réunies (e.g. BLITECAST, MacKenzie 1981),
- la prédiction d'une infection qui évalue à l'avance, le devenir d'une maladie en se basant sur les relations

entre densité d'inoculum et intensité prévisible de la maladie (Fry 1982),

- la prévision du risque, qui prend en compte un grand nombre de facteurs pour évaluer quelle est la probabilité de développement d'une maladie. Au delà d'un seuil prédéfini, il importe d'adopter des mesures culturales ou de lutte particulières (Young et al. 1978),
- la prédiction épidémique, qui s'applique surtout à des épidémies polycycliques. Grâce à des évaluations séquentielles de la sévérité de la maladie, on prévoit l'évolution probable d'une épidémie (e.g. EPIPRE, Zadoks 1982).

9) Conclusions

Par comparaison avec la situation qui prévalait encore il y a 20 ans, il est clair que la compréhension des maladies et que les techniques pour les combattre se sont grandement améliorées. La recherche ayant généré de nouvelles stratégies et des méthodologies performantes, la lutte intégrée contre les ravageurs et les maladies représente désormais la démarche à préconiser pour concilier efficacité et innocuité.

Il faut donc s'interroger sur le peu d'utilisations pratiques des préconisations résultant de ces différentes approches... S'agit-il d'un "problème de fond" lié au manque de validation et à la défiance des professionnels quant aux prédictions issues de ces systèmes ? Ou s'agit-il plutôt d'un "problème de forme", lié à la mauvaise diffusion des informations techniques sur les services qu'ils peuvent rendre ? En tout état de cause, de nouveaux efforts doivent être entrepris afin de réaliser la compatibilité de ces systèmes avec les aspects économiques de la production et du type de dommages subis par la culture.

De façon beaucoup plus globale, il importe absolument d'améliorer l'efficience du transfert des résultats de la recherche vers le monde paysan. Sur ce point, la situation alarmante en matière de caféiculture a été stigmatisée par Muller (1995) lors du précédent Colloque ASIC à Kyoto <<... le fossé entre une caféiculture productive donnant l'accès légitime aux biens matériels et une caféiculture rudimentaire, apanage de la pauvreté, ne fait que se creuser... Dès lors, faire de la recherche utile c'est donc assurer l'application des résultats en milieu paysan>>. Ainsi que le soulignent Nyambo et al. (1996), ceci ne peut se faire qu'au travers d'une indispensable prise en compte de la composante socio-économique, trop souvent absente de nos programmes de recherche.

Conjoncturellement, le problème devient crucial car la demande en produits des cultures de rente, et particulièrement en café, est en pleine explosion. Pour y faire face, on ne peut malheureusement pas espérer d'accroissement substantiel des disponibilités en terres cultivées. Il n'est donc pas possible de faire d'exclusive quant aux approches et aux outils concernant le contrôle des ravageurs et des maladies. Ceux-ci doivent être optimisés pour accroître la productivité des terres disponibles afin de répondre à cet accroissement des besoins. Mais en tant que scientifiques, nous ne pouvons nier que les produits et/ou les stratégies de lutte préconisés ont ou auront des effets non prévus (James et al. 1990). Il nous revient donc la responsabilité de les contrôler, en cumulant science efficace et déontologie.

B. Approche intégrée du pathosystème/*Coffea arabica*

1) Introduction

Du fait de l'importance économique du café à travers le monde et des contraintes phytosanitaires qui affectent sa culture, de nombreux travaux de recherche ont abordé la pathologie fongique du caféier sous différents aspects : biologie des agents pathogènes, épidémiologie, génétique du parasitisme, méthodes de lutte, etc. Pour *Coffea arabica,* les recherches sont consacrées dans leur grande majorité, à l'étude de la rouille due à *Hemileia vastatrix* Berk. et Br., pathogène particulièrement dommageable dans de nombreux pays producteurs (Shieber 1972 ; Eskes et al. 1991 ; Sierra Sanz et al. 1994).

En matière d'épidémiologie, de nombreux auteurs se sont intéressés aux effets des conditions environnementales sur le développement de la rouille (Pedro 1983 ; Oseguera 1985) en focalisant leurs études sur un paramètre particulier comme l'altitude des plantations (Avelino et al. 1991 ; Whan et al. 1994), l'intensité de la lumière incidente (Eskes 1982), le temps d'eau libre sur les feuilles (Nascimento et Tubelis 1980), les amplitudes extrêmes des températures au cours du cycle cultural (Schrödter 1965 ; Brown et al. 1995), l'état physiologique de l'hôte (Eskes et Toma-Braghini 1982 ; Coutinho et al. 1994), etc. Pour l'essentiel, ces recherches ont été réalisées en laboratoire et/ou en station par quantification des phénomènes épidémiques, soit dans leur globalité (Zheng Fuchong et al. 1991 ; Farrera 1994), soit en analysant successivement les différentes phases du processus épidémique (pénétration, germination, sporulation et dissémination) (Nutman et Roberts 1963 ; Villegas 1988 ; Loaisiga et al. 1996). Les acquis de ces recherches permettent de mieux comprendre le développement des épidémies de la rouille dans les zones de caféiculture.

A partir de ces travaux, des modèles prédictifs réalisés par différents auteurs (Kushalappa et al. 1984,

Becker-Raterink 1985 ; Montoya et Sierra 1993 ; i.a.) se sont attachés à intégrer les principaux paramètres influant sur le développement de la rouille. Atteignant souvent un niveau élevé de complexité pour définir les différentes étapes du processus épidémique, ils semblent difficilement transposables hors station, dans des exploitations traditionnelles.

Les recherches épidémiologiques menées depuis 1991 en Nouvelle-Calédonie (NC) sur le caféier arabica se démarquent sensiblement des travaux cités précédemment. En effet, la prise de conscience de la complexité des agro-écosystèmes tropicaux et le développement de nouvelles méthodes de traitement des données ont fait émerger un thème de recherche qui, prenant en compte les principes énoncés plus haut quant à la nécessité d'une gestion intégrée pour contrôler les maladies des plantes, privilégie l'aspect fonctionnel des relations biocénotiques existant au sein d'un "pathosystème". A ce titre, Savary et al. (1995) confirment l'intérêt d'une approche holistique d'un pathosystème intégrant des caractéristiques aussi diverses que les variations spatio-temporelles de l'intensité de maladie, des informations précises sur le sol et le climat, les itinéraires culturaux et les aspects socio-économiques inhérents à la culture ; des outils statistiques appropriés contribuent à l'analyse de ces divers paramètres.

Les recherches développées sur des plantations de caféiers en NC, consistent à étudier le fonctionnement d'un pathosystème multiple associant, dans des contextes écologiques contrastés, le caféier arabica, ses principaux pathogènes fongiques (*Hemileia vastatrix, Colletotrichum gloeosporioides, Cercospora coffeicola*) et l'Environnement. L'intégration des caractéristiques pathologiques et environnementales des sites d'enquête, puis la modélisation de leurs interrelations permettent d'identifier et de hiérarchiser les paramètres mésologiques qui régissent l'émergence puis le développement des maladies du caféier. Cette caractérisation du déterminisme des évolutions pathologiques puis la prévision du risque épidémique sont les objectifs affichés pour l'optimisation des techniques de lutte.

2) Matériels & Méthodes

Méthodologies des enquêtes

Le dispositif d'enquête pluri-local est constitué par des sites traditionnels de caféiculture, choisis selon des critères de diversité aussi larges que possible. La distribution et la sévérité des attaques provoquées par chacun des pathogènes sont très contrastées en NC, ce qui a constitué un facteur favorisant cette étude. La démarche épidémiologique est subordonnée à deux étapes dont la rigueur conditionne la capacité ultérieure à utiliser les données acquises *in situ*.

La première consiste à réaliser un "état des lieux" le plus détaillé possible dans chaque site. Il s'avère en effet indispensable d'intégrer, dès le début des enquêtes, les multiples caractéristiques nécessaires à l'obtention, à terme, d'interprétations diversifiées. A titre d'exemple, il est nécessaire de caractériser globalement les classiques paramètres physico-chimiques d'un site ainsi que d'en quantifier l'hétérogénéité édaphique (mesures de pH et pédocomparateurs). En effet, la croissance d'un arbre sur un substrat particulier peut se traduire, par rapport à ses voisins, par un comportement particulier au plan physiologique et/ou, pathologique. De même, la position précise dans le site et la pérennité des arbres et des rameaux, la description précise de chaque rameau se révèlent ultérieurement comme des indicateurs précieux pour étudier l'évolution des mécanismes.

La seconde étape a consisté à réaliser un suivi mensuel durant tout le cycle cultural du caféier et ce, pendant plusieurs années consécutives ; ce continuum des relevés permet d'analyser les situations épidémiologiques dans la durée en dégageant les grandes tendances et en minimisant l'effet des impondérables conjoncturels. Des procédures particulières d'enquêtes (suivi feuille-à-feuille,...) et de caractérisation de l'environnement par acquisition automatique de données météorologiques ont été mises au point ou adaptées (Lamouroux et al. 1995). A ce titre, l'importance du facteur humain dans la fiabilité des observations de terrain doit être absolument prise en compte dès l'origine, faute de quoi, la variance inter-observateurs peut masquer la variance biologique recherchée.

Gestion des données

Ces données brutes caractérisant les contextes étudiés ainsi que les informations épidémiologiques accumulées au cours du temps génèrent des fichiers informatiques de taille importante (p.m. plus de 40.000 données par mois dans le cadre de cette étude). Le stockage et la gestion des données pathologiques ou environnementales ont été réalisés avec la base de données ORACLE. Grâce à un ensemble de requêtes SQL (Structured Querry Language), on obtient alors des tableaux de données synthétiques. Ces requêtes permettent de moduler le niveau d'analyse à une échelle de précision croissante : position des feuilles sur le rameau, sélection de patrimoines de feuilles de même âge (afin de servir de bases à des analyses rigoureuses de durée de cycle infectieux, d'évolution de la gravité des symptômes durant la période d'infection,...), position et/ou âge des rameaux, études interarbres (intrasite) et intersites, etc.

L'interprétation statistique de ces données est réalisée avec le logiciel ADE-4 (Analyses multivariées et expression graphique des Données Environnementales) développé à Lyon par Thioulouse, Chessel, Dolédec & Olivier (1997) ; les nouveaux modules de statistiques multivariées complexes se sont révélés particulièrement bien adaptés à la dimension spatio-temporelle de la problématique "caféier".

3) Résultats

Typologies & Costructure Pathologie/Environnement

La répétition dans le temps des observations génèrent des fichiers de données de type cubique (variables x sites x dates d'observation) dont l'analyse simultanée (et non, date par date) est assez particulière (Pellegrin et al 1995). Pour de tels fichiers constitués de tableaux successifs, le module Statico établit, entre autre, un "compromis" révélant les structures communes aux différentes dates pour obtenir une typologie claire des sites d'enquêtes en fonction, soit de leurs caractéristiques édaphiques, soit des dommages pathologiques. Il est important de signaler que, malgré des climatologies variant d'une année à l'autre, la position statistique de ces sites sur le plan du "compromis" évolue peu. Ceci entérine d'une part, la fiabilité des observations mensuelles et confirme d'autre part, la stabilité, dans chaque site, de l'expression des interactions entre le caféier et ses pathogènes.

De façon complémentaire, il est possible d'établir la costructure de ces deux "cubes" de données Pathologie et Environnement, en maximisant à chaque relevé leur covariance, puis en estimant la stabilité de la relation entre ces deux groupes de variables. Cette analyse recherche les combinaisons de variables (ou axes de co-inertie) qui expriment la co-variation temporelle (succession des observations) et la costructure spatiale existant entre les nuages de points représentant les données. La projection de ces variables sur des axes définit des plans précisant les liens entre les deux cubes de données. Les combinaisons de variables ainsi identifiées mettent en évidence des tendances significatives originales (Pellegrin et al 1995) : on retiendra par exemple que la rouille s'exprime préférentiellement dans des sites caractérisés par des sols mal structurés, peu fertiles, un pH faible, par de faibles pluies, des températures minimales relativement basses, un taux d'ombrage important et une altitude élevée. Malgré de fortes variations, d'une année à l'autre, de la pression parasitaire caractérisant certains sites et malgré la diversité des conditions climatiques enregistrées depuis 1992, les analyses de fichiers pluriannuels (sur 4 années) indiquent le rythme d'expression de ces liaisons et confirment bien la réalité de ce déterminisme.

Les tendances majeures résultant des précédentes interprétations ont servi de base à la réalisation d'une première modélisation visant à prédire le niveau de maladie. Bien que des valeurs significatives aient été obtenues, cette première approche statistique (analyse discriminante & bootstrap, sur moyennes annuelles) n'a pas été retenue car elle faisait abstraction de la cinétique épidémique.

Optimisation du modèle

Au terme de cette première phase des recherches, on pouvait considérer que les grandes tendances régissant le fonctionnement du pathosystème caféier étaient identifiées. Il est apparu cependant nécessaire de détailler ces mécanismes épidémiques en étudiant leur variabilité, qui s'exprime à la fois par une certaine hétérogénéité du comportement des arbres d'un même site et par des fluctuations annuelles des cinétiques épidémiques de certains sites. Aussi, pour renforcer la cohérence des approches épidémiologiques, des améliorations ont été recherchées à trois niveaux :

a) Un premier niveau a été consacré à l'évaluation de la part de variabilité imputable à : (i) la diversité génétique des populations d'agents pathogènes, afin de savoir si les différences d'infestations constatées *in situ* reflètent des potentialités différentes de l'agent causal, (ii) une diversité génétique des arbres, avec pour conséquence une plus ou moins forte sensibilité aux maladies, (iii) une possible infestation des sols en nématodes phytoparasites, susceptibles dans ce cas d'affecter gravement la physiologie du caféier.

- **Analyse des populations d'*H. vastatrix***. En premier lieu, grâce au concours du Dr. Rodrigues (CIFC, Portugal), la distribution spatiale des races a été précisée en NC : la race II est prédominante dans la majorité des sites étudiés, mais une cohabitation avec la race I ou III existe dans certaines parcelles.

De façon complémentaire, des travaux de biologie moléculaire sont en cours afin de prendre en compte les fluctuations spatio-temporelles du pathogène. Le caractère microcyclique de cette Urédinale strictement biotrophe, qui ne possède pas de reproduction sexuée connue et dont seules les urédospores semblent participer au cycle de développement, a nécessité des mises au point techniques particulières. Les différentes voies explorées (PCR-RFLP, RAPD) ont globalement infirmé l'existence d'une diversité entre les sites étudiés. En revanche, les premiers essais d'amorces amplifiant des régions extragéniques répétées (REP) du génome indiquent un certain polymorphisme intersite. Ces expérimentations se poursuivent pour parvenir à la mise au point d'un outil pratique de typage rapide des rouilles collectées sur le terrain.

- **Analyse des populations de *C. gloeosporioides*** (isolé de lésions foliaires). Cette approche, initialement pluri-méthodologique (zymogrammes, VCG, RAPD), a été recentrée sur la seule RAPD. Une structuration des populations a été mise en évidence alors que des souches intrasites provenant d'arbres voisins sont globalement homogènes. L'hypothèse retenue pour justifier cette importante diversité génétique intersites conduit à envisager un taux élevé de recombinaisons obtenu par reproduction sexuée.

- **Diversité intra- et interspécifique des caféiers.** Grâce au concours de P. Lashermes & P. Trouslot (LRGAPT, ORSTOM-Montpellier), une analyse systématique du génome des caféiers des sites de l'enquête a été réalisée par RAPD. Au sein des arabica suivis depuis 1992, la présence simultanée des variétés Bourbon et Typica ainsi que l'existence d'hybrides intraspécifiques de ces deux variétés ont été démontrées. Ce typage génétique des arbres est désormais pris en compte pour préciser les modalités de comportement particulier de ces catégories.

Les caractéristiques climatiques très particulières de NC autorisent la culture simultanée de plants d'arabica

et de robusta à la même altitude, voire dans une même parcelle. L'existence de nombreux arabica ayant acquis des caractères "robustoïdes", particulièrement la résistance à la rouille, a été démontrée par le CIRAD et l'ORSTOM. Ces hybrides "Timor-like" représentaient, en matière d'épidémiologie, une source potentielle d'hétérogénéité qu'il importait également d'évaluer. Les analyses par RAPD ont démontré l'absence d'hybride interspécifique au sein des arbres de l'enquête. Ceci confirme que la moindre sensibilité à la rouille de certains de ces arbres n'est pas due à une acquisition de caractères "robustoïdes".

- **Physiologie des caféiers.** Outre l'aspect strictement métabolique qui est abordé, pour partie, au travers des pédocomparateurs et de l'évaluation de l'hétérogénéité édaphique de chaque site, différents facteurs externes peuvent influer sensiblement sur la physiologie des arbres et partant, sur leur sensibilité aux maladies fongiques.

En particulier, l'infestation du système racinaire par des nématodes phytoparasites est connue - particulièrement en Amérique latine- pour constituer une contrainte majeure affectant le caféier arabica. (Bertrand et al. 1995). Grâce à la collaboration de J.L. Sarah (Nématologie, CIRAD-Montpellier), un inventaire nématologique a pu être réalisé pour tous les sites du dispositif. Au terme de deux échantillonnages réalisés à 6 mois d'intervalle, les comptages réalisés sur des prélèvements de terre et de racines révèlent une infestation généralement réduite dans la grande majorité des sites. Globalement, il apparaît donc légitime de s'affranchir, dans le cas des sols calédoniens, de la contrainte nématologique.

b) Le second niveau concerne l'optimisation des données épidémiologiques et mésologiques en élaborant de nouvelles variables plus discriminantes et en analysant des sous-fichiers catégoriels plus homogènes.

- Grâce aux capacités de gestion d'ORACLE, il a ainsi été possible de calculer, dans chaque site, la durée moyenne de vie des catégories de feuilles saines ou infectées par tel ou tel pathogène. Pour les feuilles malades, ce paramètre -lié à la prise en compte (feuille par feuille...) des périodes d'infection initiale puis de mortalité- est précieux puisqu'il reflète le pouvoir pathogène, mesuré directement *in situ*, en s'affranchissant d'expérimentations de stations ou en serre. Bien que la variance intersites soit forte, ceci donne néanmoins un aperçu original quant aux potentialités réelles d'agression de chacun des trois pathogènes. Ainsi, par comparaison avec un ensemble de feuilles saines (250 jours de vie, en moyenne), *C. coffeicola* apparaît nettement comme le plus dommageable des trois pathogènes (durée < 60j.). Pour la rouille, la durée moyenne de cycle infectieux sur les feuilles est de 80 jours alors que pour l'anthracnose, la survie des feuilles infestées est d'au moins 170 jours, ce qui dénote une faible agressivité à l'égard de l'hôte. Dans le même ordre d'idée, il a été possible d'établir que les co-infections impliquant rouille puis anthracnose (ou l'inverse), ne contribuent pas réellement à accentuer le processus parasitaire. Enfin, comme ces durées moyennes de cycle infectieux sont relativement stables d'une année à l'autre, il y a lieu de penser que ces valeurs sont représentatives du comportement pathogénique.

- Après une procédure de tri, on obtient des informations détaillées sur l'état sanitaire et la production de chacun des arbres d'un site, ainsi que sur l'évolution de ces deux indicateurs au cours de cycles culturaux successifs. Ces paramètres pouvant être croisés à leur tour, par exemple, avec la nature de la variété de caféier utilisée, on obtient alors différents sous-fichiers de plus en plus homogènes dont l'analyse peut améliorer la description des mécanismes du pathosystème.

- De même, les informations climatiques enregistrées depuis 1992 sont optimisées en recherchant aussi bien les périodes de contraintes bioclimatologiques pour chaque pathogène (e.g. extrêmes de température, abondance des pluies ayant un pouvoir lessivant sur les spores, ou déclenchant au contraire la germination) que les contraintes physiologiques de l'hôte (e.g. effet du pF, anoxie racinaire ou stress hydrique ; ...).

c) Le troisième niveau a trait à l'optimisation des outils statistiques. A cet égard, l'évolution du logiciel (ADE-3, puis ADE-4) et la création de modules spécifiques comme "Statico" sont allées de pair, grâce au soutien de l'équipe de D. Chessel (Univ. Lyon1), avec la définition des problèmes biométriques. Cette adaptabilité constitue sans aucun doute une caractéristique et un atout majeurs d'ADE-4.

Sur la base des facteurs édaphiques et climatiques identifiés impliqués dans le fonctionnement du pathosystème, les efforts se portent depuis 1996, en collaboration avec l'Université de Lyon1, sur la conception d'un nouvel outil statistique de prédiction, dans un contexte donné, du niveau de sévérité des maladies et du type de cinétique correspondant.

Pour ce faire, plusieurs pistes théoriques ont été explorées et particulièrement, une adaptation de la méthode de régression PLS (partial least squares) proposée en chimiométrie et dont elle est devenue un standard méthodologique (Tenenhaus et al. 1995). Sous le nom de "PLS de deuxième génération" ou PLSgen2, cette régression multivariée particulière permet : (i) de prédire l'ensemble des variables à expliquer par l'ensemble des variables explicatives, soit de façon univariée (PLS1) soit de façon multivariée (PLS2), (ii) d'analyser les relations entre deux tableaux de données même quand le nombre d'observations est inférieur à celui des variables, (iii) de conserver dans le modèle toutes les variables explicatives importantes même lorsque celles-ci sont fortement corrélées entre elles, (iv) d'obtenir des équations de régression cohérentes avec les coefficients de corrélation. Appliquée aux données du pathosystème caféier, la PLSgen2 a donné des résultats satisfaisants en terme de prédiction du niveau de maladie sur caféier. Toutefois, les résultats des différentes régressions PLS semblent plutôt orienter la modélisation vers des processus univariés, avec d'éventuelles interactions entre les différents pathogènes.

4) Conclusions

Les résultats de ces modules complémentaires concernant le diversité de l'hôte et de ses pathogènes, la définition de nouvelles variables pathologiques et bioclimatiques, l'homogénéisation des fichiers de données par focalisation sur des sous-ensembles plus homogènes sont autant de facteurs de clarification, qui après agrégation à l'ossature du modèle, permettent désormais d'augmenter aussi bien les coefficients de corrélation entre les caractéristiques pathologiques et environnementales, que le taux de résolution des prévisions du niveau de sévérité des attaques fongiques sur caféier. L'intégration de ces divers éléments débouche maintenant sur la phase de synthèse finale de ce programme.

Pour une gestion intégrée des maladies fongiques du caféier, ces études ont été résolument conçues de façon synoptique, systémique, trans-disciplinaire & pluri-méthodologique. Elles ont fait l'objet de multiples collaborations qui ont contribué à la cohérence du développement du programme.

Cet ensemble de procédures épidémiologiques et d'outils informatiques fait maintenant l'objet d'une diffusion et de formations à l'échelle régionale. Mais il devrait pouvoir également intéresser d'autres équipes qui, travaillant sur d'autres pathogènes (e.g. CBD) ou sur des ravageurs du caféier (scolyte, nématodes), souhaiteraient appréhender, dans de bonnes conditions, la complexité biologique de leur propre pathosystème.

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CORRECT IDENTIFICATION OF THE PATHOGEN *COLLETOTRICHUM KAHAWAE* CAUSING COFFEE BERRY DISEASE (CBD)

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1. INTRODUCTION

The imperfect fungus *Colletotrichum kahawae* mainly causes sunken black lesions on berries of *Coffea arabica* during expanding and filling stages of development. The fungus also attacks flower buds and ripening berries just before picking (late blight). Occassionally, the fungus invades young leaves and parts of the maturing bark.

Climatic conditions such as high humidity, wetness and temperatures of around 18 - 20° C favour conidia production and the distribution of the spores by splash. Therefore higher altitude coffee suffers more of CBD than coffee grown in altitudes below 1600 m.

Crop losses in Arabica growing countries of Africa reach 20 - 30%, but can exceed 80% in extremely wet years. Many efforts have been made in controlling the disease by fungicide sprays but this is not sustainable in the long term. Recently there exists great hope in breeding resistant varieties at various research centres. The variety "Ruiru 11" of the Kenyan Coffee Research Foundation has proved in field experiments to be outstanding for resistance to CBD and leaf rust, high production, good quality and may fetch good prices for ecological coffee in the world market. This paper presents most important conventional and modern methods for identifying the pathogen from other *Colletotrichum* spp. invading coffee and contributes to the theme of the conference on "the use and transfer of modern technology to improve coffee quality".

2. HISTORY OF TAXONOMIC DEVELOPMENT OF THE PATHOGEN

Worldwide the *Colletotrichum* population on *Coffea arabica* consists of several more or less well defined botanical species. *C. gloeosporioides* was described by PENZIG late last century invading a large number of fruiting crops in areas of tropical, sub-

tropical and temperate climatic conditions. In 1903 SCHRENK and SPAULDING described the perfect stage *Glomerella cingulata*, an ascomycete previously detected by STONEMAN and then named as *Gnomoniopsis*. In 1901 NOACK found a host specific species on coffee in Brazil and described that fungus as *C. coffeanum*.

After detecting the Coffee Berry Disease (CBD) in 1922 in Kenya, McDONALD (1926) adopted the name of the Brazilian isolate, not knowing that the latter was not pathogenic to green coffee berries. In the early 1950s scientists became aware that, using the same taxonomic name for isolates differing in pathogenicity would cause confusion among those involved in CBD work. RAYNER (1952) concluded his investigations with a differentiation of the CBD causing pathogen into *C. coffeanum* var. *virulans*. GIBBS (1969) divided the *Colletotrichum* population into four groups including the CBD pathogen strain. HINDORF (1970) established a scientific name for the pathogen but continued with the historical, taxonomically recognized name *C. coffeanum* Noack adding sensu Hindorf for the East African pathogenic isolates. Finally WALLER et al. (1993) decided that the CBD pathogen has distinct morphological and biochemical characteristics and therefore can exist as an independent species, *C. kahawae* (the name originates from the Kiswahili word for coffee = kahawa). But so far, some authors prefer to keep the name of *C. gloeosporioides* adding var. or f.sp. *coffeanum* (SREENIVASAPRASAD et al. 1993, BIRATU 1995).

3. DIFFERENTIATION OF THE *COLLETOTRICHUM* POPULATION WITH MORPHOLOGICAL CHARACTERISTICS

The fungus produces in its imperfect stage mycelium and conidia. Conidia are borne on conidiophores and produced in specific fruiting bodies, the acervuli. The pathogen can be characterized by a few morphological features *in vitro*. On malt extract agar the fungus develops grey aerial mycelium becoming black in the substrate. The growth rate in general is slower than that of other *Colletotrichum* spp. **(Fig.1).**

Temperature affects the growth of mycelium of the fungus. In 24 hrs, the growth rate is 3 mm at 15° C, 6 mm at 20° C, 7 mm at 25° C and 4 mm at 30° C. Mycelium growth rate *in vitro* does not vary greatly for different isolates of the pathogen and can be taken as a systematically important criterium. **(Fig. 2).**

In vitro, the fungus does not produce acervuli. Conidia are borne individually on single hyphae. The one-celled, hyaline conidia, become pinkish in masses, forming cylindrical shapes with rounded tips, but are highly variable and not suited for identification. Conidia sizes are in the range of 15-19 µm x 3-5 µm. Conidia production *in vitro* is much less than *in vivo* on expanding coffee berries. Pathogenicity tests on hypocotyls or detached berries are of major importance for an exact diagnosis of the pathogen.

4. PRECISE IDENTIFICATION BY USING BIOCHEMICAL METHODS

Today several biochemical and molecular methods make identification of micro-organisms in general and of the *Colletotrichum* population in particular easier and more precise. In a horizontal starch gel electrophoresis, the protein and isozyme patterns can be used to differentiate *Colletotrichum* spp. BIRATU (1995) obtained representative results from 35 isolates collected mainly from Ethiopia. *C. kahawae*

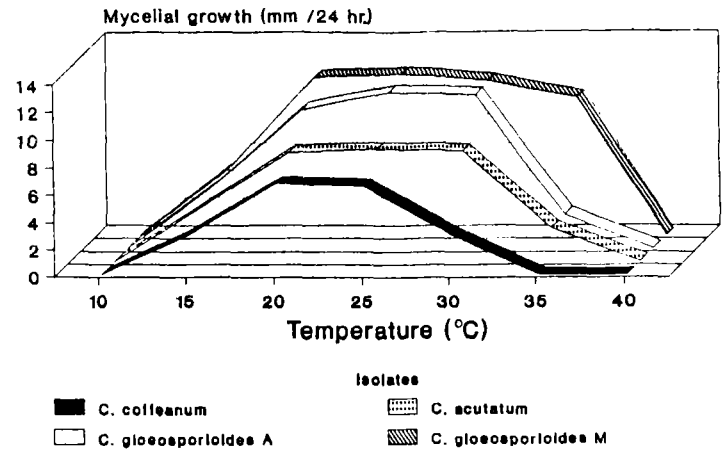

Fig. 1: **Mycelium growth rate of *C. kahawae* (= *C. coffeanum*), *C. gloeosporioides* (A - acervulus form, M = mycelium form) and *C. acutatum* at different temperatures.**

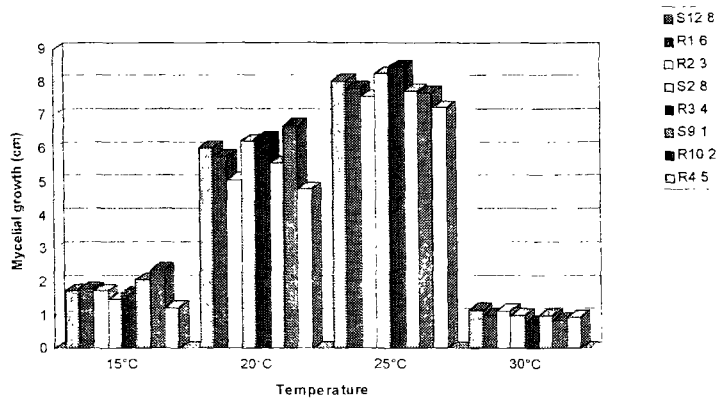

Fig. 2: **Mycelium growth rate of isolates of *C. kahawae* at different temperatures.**

produced 68 - 76 closely migrating protein bands with better differentiation in molecular weights of kDa 21 - 94 than in higher weights. Some of the disadvantages are the difficult laboratory preparation methods and larger variations in different isolates of the same species. Comparing different *Colletotrichum* spp. he found many similar bands occuring in independent species.

Isozyme patterns such as esterase, leucine aminopeptase, isocitrate dehydrogenase, malate dehydrogenase, shikimic acid dehydrogenase, peroxidase and aconitase have been studied but promising results were obtained with malate dehydrogenase and esterase only for the differentiation of the *Colletotrichum* population. For instance in the malate dehydrogenase pattern 3 peaks occur for isolates of *C. kahawae,* 1 - 2 peaks for *C. gloeosporioides* and 1 peak for *C. acutatum*, respectively. **(Fig.3).**

Genetic diversity of the pathogen can be differentiated by using Random Amplified Polymorphic DNA (RAPD) with specific primers. The advantages of this method is the constant occurrence of informative bands of the investigated fungal species that do not vary largely (OMONDI 1997). Primers are commercially available but eperimental procedures need to be standardised to produce stable results.

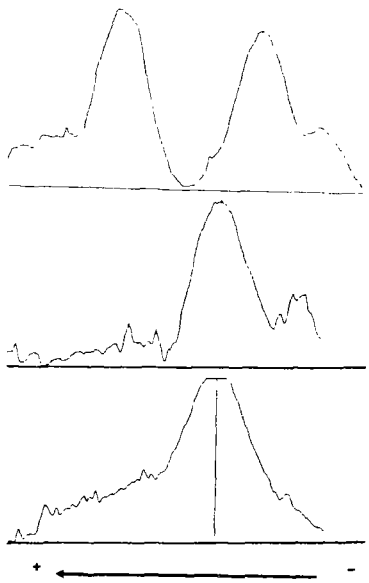

Fig. 3: **Malate dehydrogenase activities of *C. kahawae* (above) and *C. gloeosporioides* (middle = mycelium form, below = acervulus form)**

ABSTRACT

The CBD causing fungus *C. kahawae* can be identified most precisely by taking morphological and biochemical characteristics into consideration. For identification one still needs isolation of the fungal population, detecting morphological features *in vitro* such as mycelium colour and growth rate, conidia production and testing the pathogenicity on hypocotyls or berries.

Molecular-biological methods are assisting experts in handling the large number of pathogenic isolates. Protein and isozyme pattern can be analysed by a horizontal starch gel electrophoresis. The malate dehydrogenase and esterase are well suited for identification of species of the genus *Colletotrichum*. More precisely, Random Amplified Polymorphic DNA (RAPD) techniques provides a helpful means of identifying *C. kahawae*.

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OBSERVATIONS SUR LA DIVERSITÉ DE LA POPULATION DE *COLLETOTRICHUM KAHAWAE* AGENT DE L'ANTHRACNOSE DES BAIES DU CAFÉIER ARABICA

Implications pour l'amélioration génétique

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1 - INTRODUCTION

L'anthracnose des baies du caféier Arabica est apparue pour la première fois au Kenya à proximité de la frontière Ougandaise (Mac Donald, 1926). Cette maladie s'est progressivement étendue à l'ensemble des pays producteurs de l'Afrique de l'Est. Elle est signalée dès 1930 en Angola et en 1958 au Cameroun à plusieurs milliers de kilomètres du foyer primaire. Actuellement, cette maladie reste limitée au continent africain. L'agent pathogène *Colletotrichum kahawae* (Waller *et al.*, 1993) provoque une pourriture humide sur baies vertes, entraînant leur chute. Les pertes de production sont de l'ordre de 50 % à 80 % dans les zones de forte pression infectieuse.

Etant donné l'importance économique de cette maladie, la recherche de variétés résistantes a été entreprise depuis de nombreuses années dans différents pays et selon des schémas différents. En Ethiopie, Vermeulen (1979) et Van Der Graaf (1982) ont montré que cette résistance était de nature polygénique et qu'elle présentait un caractère quantitatif. Les génotypes repérés ont ainsi été intégrés dans un programme d'amélioration variétale par sélection massale. Au Kenya, des génotypes tolérants ont été repérés notamment dans des populations de Rume Sudan et de l'hybride de Timor. Un programme de sélection basé sur l'exploitation de cette résistance a abouti à la production de la variété Ruiru 11. Van Der Vossen et Walyaro (1980) suggèrent que la résistance serait sous la dépendance de 3 gènes. Au Cameroun, la sélection massale de populations existantes a permis d'identifier un génotype tolérant à la maladie, la variété Java (Bouharmont, 1994). En plus de ces génotypes, d'autres tels que le Blue Montain, l'hybride de Jackson et le "Local Bronze" expriment des niveaux de résistance intéressants.

En 1992, Rodrigues *et al.* émettent l'hypothèse de l'existence de réactions différentielles. Cela suscite des interrogations sur les différents schémas de sélection à mettre en oeuvre, plus particulièrement pour obtenir une solution durable. L'emploi de la lutte génétique nécessite notamment une connaissance précise de la structure de la population pathogène ainsi que de la variabilité et de la stabilité du pouvoir pathogène ; notre travail avait pour objectif d'analyser la diversité de la population de *C. kahawae* en vue de définir les isolats représentatifs de cette population qui seront pris en compte dans l'évaluation de la résistance et de sa caractérisation, sur du matériel végétal sauvage ou en cours de sélection.

Tab. 1 : Provenance et origine géographique des isolats de *Colletotrichum kahawae* étudiés.

	SOUCHES	ORIGINES	PROVENANCE
	CM675A	BAHAM	IRAD [b]
	CM 708	SANTA	"
	CM712 B	SANTA	"
	CM 712 Bs	SANTA	"
	CM728	SANTA	"
	CM732A	BAGADJOU	"
	CM740	SANTA	"
	CM818	MELING	"
	CM821	BALI NYONZA	"
	CM822	TATUM	"
	CM823	NDU	"
	CM826	NKAMBE	"
	CM829[a]	BATSIET	"
CAMEROUN	CM833	BAMENYAM	"
	CM834	KUMFUTU	"
	CM854	PINYIN-KONGSA	"
	CM863[a]	FOUMBOT	"
	ACA 2[a]	KRIBI	"
	CM901	AWING VILLE	"
	CM902	AWING BANDING	"
	CM903	SANTA POYSAN	"
	CM904	SABGA	"
	CM905	MELIN KUMBO	"
	CM906	NDUTOWN	"
	CM907	NKAMBE	"
	CM908	KUMFUTU	"
	CM909	BELO-ASHING	"
	CM910	ACHONG	"
	CM911	BELO-ASHING	"
	CM835	WUM	"
	BU002	GASHI KANWA	ISABU [b]
	BU003	"	ISABU
	BU007[d]	MUYANGA	ISABU
BURUNDI	BU010[a]	GITAGA	"
	BU012	GASOGWE	"
	BU015	FUHORORO	
	BU019	?	IMI[b]
	KN001B	-	CRF[b]
	KN002	-	CRF
KENYA	KN006		CRF
	KN007	KIAMBU	CRF
	KN008	RUIRU	CRF
	KN009	BUNGOMA	CRF
	TZ001		SCRI[b]
TANZANIE	TZ005		SCRI
	TZ006		SCRI
ETHIOPIE	ET001		IMI
	ET0015		"
MALAWI	MW007		IMI
RWANDA	RW002	RUBONA	CIRAD[b]
ZIMBABWE	ZW001	E.N. HARARA	CIFC[b]
ANGOLA	AG001		CIFC

2 - EVALUATION DE LA DIVERSITE DU PATHOGENE

2-1 Matériels et méthodes

2-1-1 Matériel phytopathogène

Les 49 isolats monoconidies de *Colletotrichum kahawae,* ont été prélevés sur des baies vertes infectées ainsi que sur des baies momifiées de caféier Arabica. Les isolats ont été collectés dans différents pays producteurs regroupés en deux grandes zones (tab.1). La population de l'Afrique de l'Ouest est constituée de 27 isolats du Nord-Ouest du Cameroun et un isolat d'Angola ; celle d'Afrique de l'Est est composée de 20 isolats dont 7 du Burundi, 5 du Kenya, 3 de Tanzanie, 2 d'Ethiopie (Centre de diversification de l'hôte), un du Malawi, un du Rwanda et un du Zimbabwe. Trois isolats de *C. gloeosporioïdes* ont servi de témoins, deux ont été prélevés sur des baies de caféier Arabica et un sur feuilles d'hévéa.

2-1-2 Les groupes de compatibilité végétative

Colletotrichum kahawae est un champignon imparfait ; il est toutefois sujet à des phénomènes de parasexualité qui consistent à l'appariement de filaments mycéliens et en la fusion dicaryotique de cellules issues de mycélia différents. Deux souches capables de former des hétérocaryons sont dites végétativement compatibles.

L'hétérocaryose est la base de la technique des GCV, décrite par Cove (1976) et modifiée par Puhalla (1985). La formation d'hétérocaryons est mise en évidence en appariant des mutants spontanés dont la mutation porte sur un ou plusieurs gènes de la voie d'assimilation des nitrates, et qui par conséquent, sont incapables d'utiliser le nitrate comme source d'azote (mutants "nit"). Ces mutants, mis sur un milieu approprié, ont une croissance rase alors que les colonies sauvages développent un mycélium abondant et aérien. Le phénomène d'hétérocaryose entre deux mutants complémentaires est donc visualisé par la formation de mycélium aérien dans la zone de rencontre des deux mycélia mutés.

La classification des isolats en Groupe de Compatibilité Végétative a été faite suivant les concepts associés de Leslie (1991), de Katan et *al.* (1991) et Puhalla (1985) :

1 - Les isolats qui sont végétativement compatibles entre eux sont décrits comme appartenant à un GCV ;
2 - Les souches auto-incompatibles ne peuvent en aucun cas former d'hétérocaryon avec d'autres souches ;
3 - Une souche ne peut être attribuée qu'à un seul GCV. Afin de préciser la classification des GCV obtenus, la notion d'intensité et de rapidité de formation des hétérocaryons a été utilisée pour définir des sous-groupes ;
4 - L'attribution des souches à des sous-groupes à l'intérieur d'un GCV est basée sur leur capacité à former un hétérocaryon fort avec des souches d'un sous-groupe tandis que leurs interactions avec des souches d'un autre sous-groupe sont faibles et lentes.

Avant de tester l'intercompatibilité des différentes souches entre elles, leur autocompatibilité doit être vérifiée par la confrontation de deux mutants différents d'une même souche ; selon Leslie (1993), les souches auto-incompatibles ne peuvent en aucun cas former d'hétérocaryons avec d'autres souches.

La caractérisation des mutants a été réalisée en trois étapes :

- la sélection des mutants chlorate résistants ;
- la caractérisation phénotypique des mutants nitrates (nit 1, nit 3 ou nit M) ;
- la confrontation deux à deux des différents types de mutants.

Pour simplifier la méthode, seules les confrontations nit 1/nit M ont été réalisées. Les confrontations ont été faites dans des boîtes de Pétri par dépôt de deux explants des deux types de mutant à deux centimètres l'un de l'autre. Les cultures ont été incubées à 25°C à l'obscurité pendant 30 jours. Lors de chaque confrontation, le délai d'apparition de l'hétérocaryon et la densité du mycélium aérien ont été caractérisés selon l'échelle suivante :

Date d'apparition de l'hétérocaryon :	1 : entre 0 et 10 jours
	2 : entre 11 et 15 jours
	3 : entre 16 et 30 jours
Type d'hétérocaryon après 20 jours :	a : bande continue très dense supérieure à 5 mm de largeur
	b : bande continue dense inférieure à 5 mm de largeur
	c : bande continue fine avec un mycélium peu aérien
	p : zone discontinue.

Les isolats dont les confrontations nit 1/nit M ont donné naissance à l'hétérocaryon sont considérés comme compatibles. Toutefois, l'absence d'hétérocaryon entre ces deux types de mutants ne permet pas de conclure en l'absence de compatibilité du fait que toutes les combinaisons de mutants n'ont pas été testées.

2-2 Résultats

Les résultats des confrontations nit 1/nit M des 40 isolats étudiés sont présentés dans le tableau 2.

Les isolats CM829 et CM863 prélevés sur baies infectées, mais non pathogènes et l'isolat de *C. gloeosporioides* (ACA-2) prélevé sur une feuille d'hévéa n'ont réagi avec aucun des isolats pathogènes.

L'isolat d'Angola AG001, pathogène et autocompatible, n'a réagi avec aucun des autres isolats.

Sur les 17 isolats du Cameroun, 15 sont autocompatibles et tous sont intercompatibles. La majorité des hétérocaryons présente des intensités fortes (type a ou b) ; ils apparaissent dans les 15 premiers jours (type 1 ou 2). Six isolats du Cameroun se sont complémentés avec ceux de l'Afrique de l'Est. Toutefois ces hétérocaryons sont de faible intensité (type c et p) et sont d'apparition tardive (type 3).

Dans la population de 20 isolats d'Afrique de l'Est, 16 sont autocompatibles. Au sein de cette population, la majorité des isolats se montre intercompatible, à l'exception de 4 isolats (KN008, KN009, TW001, TZ005) qui ne réalisent des hétérocaryoses qu'avec une partie de la population.

Hormis AG001, tous les isolats pathogènes forment un GCV. Ce GCV est subdivisé en deux sous-groupes :
- un sous-groupe composé majoritairement des isolats originaires du Cameroun plus un du Burundi (BU019) ;
- un sous-groupe composé des isolats de l'Afrique de l'Est.

Tab. 2 : Résultats des confrontations nit1 / nit M

SOUCHES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1-CM675A	1a	1a	1a	1b	2b	1a	1a	1a	1b	1b	1c	1b	1a	-	1c	2c	2p	-	-	-	-	-	-	-	-	2b	-	-	-	-	-	-	-	-	-	-	-	-	-
2-CM708		1a	1a	1a	1a	1a	1a	1a	2b	1a	1b	1a	1a	-	1a	2b	1a	-	-	3p	2c	2c	3p	3p	-	1a	3c	3c	2c	2b	2b	3c	2c	2c	2c	2c	-	2c	3p	-
3-CM712B			1a	1a	2b	1a	1a	1a	2c	2c	1a	1a	1a	-	1a	2b	2p	-	-	-	-	-	-	3p	-	1a	3p	-	3p	-	-	-	-	-	3p	3p	-	1c	-	-
4-CM712Bs				1a	1b	1a	2b	1a	1b	2b	2p	1a	1b	-	1b	2b	2b	-	-	-	-	-	-	3p	-	1a	3p	-	-	-	-	-	-	-	3p	3p	-	2c	-	-
5-CM714A					1b	1b	1a	1b	3p	1c	1c	2c	1b	-	2p	-	2c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6-CM728						1a	1a	1a	2c	1b	1b	1a	1a	-	1b	2b	1b	-	-	-	2c	3p	-	-	-	2b	-	-	2c	-	-	-	-	-	3p	3p	-	3p	-	-
7-CM732A							1b	1a	1c	2b	2c	1a	2b	-	1a	1b	2c	-	-	-	-	-	-	-	-	1b	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8-CM740B								1a	1a	1a	1a	1a	1b	-	1b	1a	1a	-	-	-	-	3p	3c	-	-	1a	-	-	3p	3p	-	2c	-	2c	3p	3p	-	2c	-	-
9-CM818									-	2p	-	1b	1a	-	-	2p	3p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10-CM821										1c	1a	1b	1a	-	1b	1b	-	-	-	-	-	-	-	-	-	2b	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11-CM822											2c	2p	2c	-	2c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12-CM823												1a	1b	-	1a	1a	3p	-	-	-	-	-	-	-	-	1a	-	-	2p	-	3p	-	-	-	-	-	-	1b	-	-
13-CM826													1b	-	1a	1a	1b	-	-	-	-	-	-	-	-	1b	-	-	3p	-	-	-	-	-	-	-	-	2b	-	-
14-CM829														*	-	-	-	*	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15-CM833															1b	2c	-	-	-	-	-	-	-	-	-	1b	-	-	-	-	-	-	-	-	-	-	-	-	-
16-CM834																1b	3p	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17-CM854																	-	-	-	-	-	-	-	-	-	3p	-	-	-	-	-	-	-	-	-	-	-	-	-
18-CM863																		*	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19-ACA2																			*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20-BU002																				2b	-	3c	3c	2c	-	-	3p	2b	1b	3p	2c	3c	2c	2b	-	-	3p	2b	-	-
21-BU003																					2c	2b	2c	2c	-	-	2c	2c	1b	1b	1b	2c	2c	1b	1b	2b	1b	1b	1c	-
22-BU007																						2c	2c	1b	-	-	2b	2c	1b	2c	-	2p	-	-	2c	2p	2c	1b	2c	-
23-BU010																							-	2c	-	-	2c	2c	2c	2c	-	2c	-	2b	2c	2c	2c	2c	3p	-
24-BU012																								1b	-	-	2b	1b	1b	1b	-	1b	2c	1b	1c	2c	1b	1a	1b	-
25-BU015																									-	-	-	-	3p	-	-	3p	-	-	2c	2c	-	2c	-	-
26-BU019																										-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27-KN001B																											2b	2b	2c	1b	-	1c	-	1b	2b	2b	1b	2b	2c	-
28-KN002																												-	1b	-	-	-	-	1b	2c	1c	2c	1b	2c	-
29-KN007																													1b	1b	1b	1b	2c	1b	1b	1c	1b	1b	1b	-
30-KN008																														2c	2c	-	2c	2c	1b	2c	3p	1b	2c	-
31-KN009																															2b	1b	-	1b	1b	1b	2b	1b	1b	-
32-TZ001																																2c	-	2c	2c	2c	1c	1c	1b	-
33-TZ005																																	2c	-	2b	2c	2b	2b	2b	-
34-TZ006																																		2b	2b	1b	2b	1b	1b	-
35-ET001																																			1a	1b	1a	1b	2b	-
36-ET001s																																				1b	1b	1b	1b	-
37-MW007																																					1b	1b	1b	-
38-RW002																																						1b	1b	-
39-ZW001																																							1c	-
40-AG001																																								3c

Légende : Date d'apparition de l'hétérocaryon :

1 : entre 0 et 10 jours
2 : entre 10 et 15 jours
3 : entre 15 et 30 jours

Type d'hétérocaryose après 20 jours :

a : bande continue très dense supérieure à 5 mm de largeur
b : bande continue dense inférieure à 5 mm de largeur
c : bande continue fine au mycélium peu aérien
p : zone discontinue

3 - EVALUATION ET CARACTERISATION DE LA RESISTANCE

3-1 Matériels et méthodes

3-1-1 Matériel végétal

Le pouvoir pathogène de 24 isolats, dont 11 ont été analysés avec la technique des GCV, représentatifs des pays producteurs a été évalué sur dix génotypes à l'aide de la technique d'inoculation sur semenceaux déracinés décrite par Cook (1973) et Van Der Vossen (1976).

Les 20 isolats du Cameroun ont été analysés sur 9 génotypes : ET6, ET19, ET21, Caturra, Java, CE, KE1, Mi6 et ET37 tandis que les 4 isolats des pays de l'Afrique de l'Est (Kenya, Zimbabwe, Rwanda) ont été testés sur 5 génotypes : ET4, ET19, ET21, Caturra et Java.

3-1-2 Technique d'inoculation

Les inoculations ont été réalisées par trempage des hypocotyles de semenceaux jusqu'au collet dans une suspension conidienne à 2.10^{-6} conidies/ml. Pour chaque isolat, 25 semenceaux au moins ont été inoculés par variété. Après inoculation, ils ont été placés dans un phytotron à 20°C, à l'obscurité, avec une atmosphère saturée en humidité, pendant 48 heures ; ils ont été ensuite soumis à une deuxième inoculation suivant la même procédure avant d'être replacés dans ce phytotron dans les mêmes conditions pendant 24 heures. Après ces deux inoculations, les semenceaux ont été placés sous photopériode de 12h/12h à 20°C.

La lecture des symptômes, trois semaines après la première inoculation, est effectuée d'après l'échelle de Van Der Graaf (1982) modifiée. Les données obtenues ont permis de définir un index d'intensité de la maladie (IIM), compris entre 0 et 100 (Bieysse *et al.*, 1995), et sous certaines conditions, de déterminer l'indice moyen de pathogénicité de l'isolat (IMP), et l'index de sensibilité de l'hôte (IS).

3-2 Résultats

Plus de 50% des lignées présentent un Index d'Intensité de la maladie supérieur à 70 (tab. 3) ; les résultats ne permettent pas de mettre en évidence des réactions de type spécifique.

Les IIM les plus élevés ont été obtenus avec les isolats CM 902 et CM 907. Les isolats CM 826, CM 834, CM 854 induisent les IIM les plus faibles. Des réactions de sensibilité intermédiaire sont notées avec les isolats CM 708, CM 818, CM 835, CM 901, CM 910, KN 001, KN 006, RW 002 et ZW 001.

Les génotypes ET4 et ET19 présentent un bon niveau de résistance vis à vis de la gamme d'isolats testés .

Des résultats complémentaires ont été obtenus avec 4 isolats d'origine camerounaise représentatifs des niveaux d'agressivité moyen à élevé vis à vis de génotypes identifiés en collection et en plantations paysannes pour leur tolérance au champ (tab. 4).

Cette expérimentation a permis de confirmer en inoculations artificielles la résistance observée au champ chez certains de ces génotypes : DG5, JK3, BB1.

Tab.3 : Indices d'intensité de la maladie (IIM) obtenus avec 10 génotypes inoculés avec24 isolats et indices moyens de pathogénicité des isolats (*IMP*).

	GENOTYPES										
ISOLATS	**ET4**	**ET6**	**ET19**	**ET21**	**ET37**	**CAT.**	**JAVA**	**CE**	**KF1**	**MI6**	***IMP***
CM708	54	81	58	56	95	87	78	59	56	78	**70**
CM732	58	80	74	79	95	98	85	83	71	99	**83**
CM818	-	81	70	62	89	92	87	61	76	91	**79**
CM821	-	54	51	56	38	92	58	13	16	22	**44**
CM822	81	95	86	81	99	99	88	64	88	98	**88**
CM826	-	19	25	18	39	35	33	11	19	35	**26**
CM834	-	29	31	36	-	50	38	10	32	18	**30**
CM835	-	39	38	43	88	58	55	30	48	61	**51**
CM854	31	48	49	44	76	68	44	32	34	33	**46**
CM901	-	50	49	68	53	73	61	42	52	67	**57**
CM902	-	94	91	93	-	100	98	99	100	100	**97**
CM903	-	95	83	-	97	95	96	72	86	96	**90**
CM904	-	79	74	79	100	97	77	70	90	100	**85**
CM905	-	84	67	-	86	94	94	63	96	94	**85**
CM906	-	98	71	-	80	91	99	68	82	99	**86**
CM907	-	100	87	-	-	100	99	96	98	94	**96**
CM908	-	93	70	-	93	89	91	40	65	82	**79**
CM909	-	85	76	98	77	93	88	83	77	83	**84**
CM910	-	68	58	28	-	-	95	55	96	89	**70**
CM911	-	100	92	-	88	-	99	-	91	100	**95**
KN001	61	-	61	40	-	87	61	-	-	-	**62**
KN006	57	-	38	-	-	88	62	-	-	-	**61**
RW002	79	-	42	-	-	88	59	-	-	-	**67**
ZW001	56	-	61	58	-	99	67	-	-	-	**68**

Tab.4 : Indices d'intensité de la maladie (IIM) obtenus avec 16 génotypes inoculés avec quatre isolats.

GENOTYPES		ISOLATS			
Var.	**Caract.**	**708**	**732**	**822**	**854**
ET11	L210C2	-	87	-	60
ET17	L217C6	67	69	-	44
Ab1	L1C3	96	98	-	69
AM	L1C3	99	98	-	84
BO	L247C2	86	95	-	41
CO	L13C3	84	71	-	40
Dg5	L84C2	88	62	-	24
JK2	L23C2	93	95	-	59
JK3	L24C3	67	57	-	32
KF9	L32C1	47	84	90	33
Lb1	L33C1	-	77	79	-
SC3	L206C4	-	80	75	-
Ab1	L1C1	59	95	97	40
Bb1	L175C6	55	52	-	37
ET2	L275C2	75	99	-	37
ET26	L220C1	57	70	-	-

4 - DISCUSSIONS - CONCLUSION

L'étude de la structure de la population de *Colletotrichum kahawae* montre qu'il existe une certaine diversité génétique ; avec l'étude des GCV, deux sous-groupes ont pu être mis en évidence : Afrique de l'Est et Cameroun. Ces deux sous groupes ne sont toutefois pas indépendant l'un de l'autre, des "souches pont", selon la définition de Katan, (CM708, CM732, CM740, KN001) forment des hétérocaryons entre les deux sous-groupes. Ceci met en évidence l'existence d'une relation entre ces deux populations géographiques dont la nature et l'origine doivent être précisées.

L'évaluation du pouvoir pathogène d'une partie de ces isolats n'a pas permis de mettre en évidence de façon claire la présence d'interactions hôte/isolat. Toutefois, une variabilité a été observée dans la composante agressivité : la plupart des isolats du Cameroun montrent un niveau d'agressivité supérieur aux isolats d'Afrique de l'Est. L'expression du niveau de résistance de l'hôte apparaît pour de nombreuses confrontations liée au niveau d'agressivité des isolats. Le criblage des divers génotypes vis à vis d'une large gamme d'isolats a permis d'identifier des génotypes présentant un bon niveau de tolérance, il s'agit de ET 4, ET19, DG5, JK3 et Bb1.

L'état actuel des connaissances acquises lors des expérimentations montre que dans le cadre de l'anthracnose des baies du caféier Arabica nous pourrions être en présence d'une résistance partielle de nature non spécifique. La présence d'une variabilité dans l'agressivité du pathogène conduit à souligner l'importance du choix des isolats dans les tests d'évaluation de la résistance, au champ et en conditions contrôlées, qui doivent être aussi représentatif que possible de la population pathogène.

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ENQUÊTE-DIAGNOSTIC SUR LA ROUILLE ORANGÉE DU CAFÉIER ARABICA AU HONDURAS

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1. INTRODUCTION

Les recherches menées en Amérique centrale sur la rouille orangée du caféier appartiennent à deux grandes catégories. Un premier groupe d'études s'est centré sur la recherche des facteurs climatiques qui expliquent le développement de la maladie. Simultanément, des études de lutte chimique ont été établies, parfois en station, et souvent dans des conditions agronomiques non représentatives. Ces recherches ont abouti à des recommandations de lutte très homogènes et indépendantes des situations de production. En général, on recommande trois pulvérisations cupriques par an, à intervalle de un ou deux mois, à partir du début des pluies. Il apparaît cependant que les niveaux de rouille orangée sont très variables, non seulement d'une zone climatique à l'autre mais aussi dans une même zone climatique, ce qui permet de penser que les recommandations de lutte actuelles sont inadaptées, et dans certains cas excessives. D'autres facteurs jusqu'alors rarement pris en compte en Amérique centrale, et qui sont peu ou pas liés au climat, comme les pratiques culturales, la structure de la plantation, la charge fructifère ou la nature chimique du sol, peuvent certainement expliquer la variabilité d'infection observée. Ces facteurs ont été considérés dans une enquête que nous avons menée au Honduras sur caféiers Arabica de port nain. La méthodologie de l'enquête s'est avérée très utile en épidémiologie. Elle a été employée par Savary (1987), Savary *et al* (1994) respectivement sur les maladies de l'arachide en Afrique de l'Ouest et du riz en Asie tropicale, et plus récemment par Lamouroux *et al* (1995) sur les maladies du caféier Arabica de port élevé en Nouvelle Calédonie.

2. MATÉRIELS ET MÉTHODES

2.1. Durée de l'étude, localisation des parcelles et taille de l'échantillon

Les résultats présentés ci-après concernent la période juillet 1994-février 1996. Vingt-cinq parcelles localisées dans trois régions du Honduras (lac de Yojoa, Santa Bárbara, El Paraíso) ont été enquêtées en 1994-1995. Nous avons observé de nouveau ces mêmes parcelles en 1995-1996. Cette année-là, dix plantations supplémentaires, dont neuf dans une nouvelle région (Comayagua), ont été examinées. L'enquête concerne donc soixante individus (25+25+10). Les coordonnées géographiques des parcelles ont été déterminées à l'aide d'un GPS (Global Positionning System).

2.2. Caractéristiques des parcelles

Elles sont constituées de treize lignes de treize caféiers, dont une bordure de deux plants de chaque côté. Cinq caféiers ont été marqués en zigzag à l'intérieur du cadre central formé de quatre-vingt un plants. Sur chaque plant marqué, nous avons identifié trois branches: une en bas, une au milieu, et une en haut de l'arbre. Celles-ci ont servi aux relevés de rouille orangée. Dans 73% des cas, les parcelles enquêtées n'ont reçu aucune pulvérisation de fongicide.

2.3. Observations

2.3.1. Environnement

2.3.1.1. Données climatiques

Les parcelles ont été caractérisées climatiquement à partir des données historiques de plus de deux cents stations météorologiques réparties dans le pays. Certaines d'entre elles ont des registres de pluviométries et de températures sur plus de trente ans. La pluviométrie annuelle des plantations en étude a été obtenue par kriging. Les quatre régions échantillonnées sont très tranchées du point de vue des précipitations. La zone du lac de Yojoa est la plus humide (moyenne annuelle de 2389 mm). Les zones de Comayagua et Santa Bárbara viennent ensuite (moyennes annuelles respectives de 1357 mm et 1371 mm). Enfin, la région de El Paraíso est la moins pluvieuse (987 mm). Par ailleurs, un modèle polynomial qui considère l'altitude et les coordonnées géographiques a permis d'estimer la température moyenne des parcelles étudiées (Osorio, publication en cours).

2.3.1.2. Sol

Pour chaque parcelle, un échantillon de sol composite (dix sous échantillons), prélevé peu avant le début de la saison des pluies, dans l'intervalle entre les lignes et à proximité des plants marqués, a été analysé. Le pH a été mesuré dans l'eau. Le pourcentage de matière organique a été évalué par la méthode de Walkey et Black. L'extraction du phosphore, potassium, fer, cuivre, manganèse et zinc a été réalisée avec la solution de Mehlich 1 (double acide). Le phosphore a été quantifié par colorimétrie, le potassium par photométrie à flamme, le fer, le cuivre, le manganèse et le zinc par spectrophotométrie atomique. L'extraction du calcium, du magnésium, et de l'aluminium a été effectuée à l'aide d'une solution de KCl, 1N. Ces éléments ont été quantifiés par titrage au NaOH.

2.3.1.3. Autres caractéristiques

Nous avons aussi considéré l'altitude et la topographie de la parcelle. Le pourcentage de la pente a été estimé à l'aide d'un clinomètre. Son orientation a été déterminée au moyen d'une boussole.

2.3.2. Production et vigueur des plants

Plusieurs variables relatives à la production des plants ont été mesurées peu avant la récolte sur les caféiers marqués. Le nombre d'axes orthotropes en production a été compté. Nous avons également réalisé un comptage du nombre de noeuds fructifères portés par chaque tige en production. Nous avons considéré qu'une tige était en production quand celle-ci avait plus de vingt noeuds fructifères. Le nombre de cerises par noeud a été évalué ensuite en s'inspirant de la méthode de Upreti *et al* (1991). Sur chaque tige, nous avons d'abord identifié dix branches réparties sur l'ensemble de l'axe orthotrope. Puis, nous avons compté le nombre de fruits porté par le noeud fructifère central de chacune de ces branches. Le nombre total de fruits porté par les arbres en observation est évalué à partir de ces trois variables (nombre de tiges en production, nombre de noeuds fructifères par tige, nombre de fruits par noeud). Nous avons enfin mesuré la taille des plants et la circonférence de la tige principale au niveau du sol. Le rapport de ces deux variables a été considéré comme une mesure de la vigueur des caféiers.

2.3.3. Itinéraire technique

2.3.3.1. Structure de la plantation

Certaines données, comme la variété et l'âge de la plante, ont été fournies par le producteur. D'autres, comme la distance entre les plants et la distance entre les lignes de caféiers, ont été mesurées autour des plants marqués. Nous avons par ailleurs identifié les arbres d'ombrage présents sur la parcelle. Le pourcentage d'ombrage a également été évalué à l'aide d'un densiomètre sphérique (Lemmon, 1956). Nous avons réalisé quatre observations pour chaque caféier marqué, deux parallèlement à la ligne de caféiers, dans les deux sens, et les deux autres perpendiculairement, dans les deux sens aussi. Cette donnée a été mesurée une seule fois en 1994 (peu avant la récolte) et deux fois en 1995 (en début de la saison des pluies et pendant la récolte). Pour les analyses nous avons considéré les moyennes par parcelle et par an. Finalement, le nombre de plants par trou, la présence de plants recépés, et l'orientation de la ligne de caféiers ont aussi été relevés.

2.3.3.2. Pratiques culturales

Les activités effectuées par le producteur sur la parcelle à partir du début de l'étude ont été documentées. Le nombre de désherbages (chimiques, mécaniques), le nombre de fertilisations (au sol, foliaires), le nombre d'amendements, le nombre de tailles de l'ombrage et des caféiers, le nombre de pulvérisations d'insecticide

ou de fongicide, et le nombre de passages de récolte constituent les principales variables de ce type.

2.3.4. La rouille orangée

La rouille orangée a fait l'objet de deux à trois observations suivant les parcelles en 1994-1995 (peu avant la récolte, pendant la récolte, en fin de récolte). En 1995-1996, les observations ont été réalisées à quatre reprises (en début de saison des pluies, peu avant la récolte, pendant la récolte, en fin de récolte). Nous n'avons considéré dans les analyses que le pourcentage de feuilles de l'année malades en fin de récolte, dans la mesure où cette donnée était disponible pour toutes les parcelles et que dans 87% des cas, elle correspondait à l'incidence maximale observée.

2.4. Analyses

Les analyses ont été effectuées d'abord par groupe de variables (environnement, vigueur/production, itinéraire technique). Dans chaque groupe nous avons transformé les variables quantitatives en variables qualitatives. La taille relativement petite de l'échantillon, nous a conduit, dans la plupart des cas, à choisir les limites de classes, sur le seul critère du nombre de parcelles présentes dans les classes formées. Ces dernières sont donc souvent équilibrées. Le test du $\varkappa^2$, effectué à partir de tableaux de contingence, a permis ensuite de sélectionner les variables liées à la rouille orangée. Parmi celles-ci, certaines étaient clairement redondantes. Dans ce cas, les variables moins explicatives de la maladie ont été exclues de la suite de l'analyse. La segmentation a enfin été utilisée pour traiter les meilleures variables décrivant l'infection et définir des conditions de risque vis à vis de la rouille orangée.

3. RÉSULTATS ET DISCUSSION

3.1. Environnement

3.1.1. Caractéristiques chimiques du sol

Le tableau 1 montre que la rouille orangée est liée à l'acidité du sol, mesurée par son pH, et sa teneur en aluminium. Les pourcentages d'infection les plus élevés sont obtenus quand le pH est faible et la teneur en aluminium quantifiable (supérieure à 0,01 cmol(+)/L). Une légère liaison semble exister aussi entre les teneurs en manganèse, les teneurs en zinc et la maladie. Une relation entre l'acidité du sol et la rouille orangée a été citée récemment par Lamouroux *et al* (1995). Dans ce cas cependant, les niveaux d'infection les plus élevés ont été trouvés sur les sols dont l'acidité était adéquate pour le caféier (entre 4,7 et 6,5). Par ailleurs, à la différence de ce qui a été trouvé en Nouvelle Calédonie, nous n'avons pas observé de liaison entre le pourcentage de matière organique et la rouille orangée ($\varkappa^2$=2,6 ; p=62,3%). Pour la suite des analyses nous n'avons conservé que la teneur en aluminium dans la mesure où celle-ci est associée au pH, au manganèse et au zinc (tableau 2).

Tableau 1 : Meilleures liaisons observées entre la rouille orangée et les caractéristiques chimiques du sol

Tableaux de contingences (nombre d'individus)		Pourcentage de feuilles jeunes avec rouille orangée en fin de récolte			$\varkappa^2$	p* (%)
		[9,2 , 31,9]	]31,9 , 57,7]	]57,7 , 85,1]		
[Al] (cmol(+)/L)	0,01 (à peine quantifiable)	14	4	6	13,7	0,9
	]0,01 , 0,62]	2	10	6		
	]0,62 , 6,38]	4	6	8		
pH	[4,0 , 5,3]	5	7	8	13,2	1,0
	]5,3 , 5,8]	2	8	9		
	]5,8 , 7,7]	13	5	3		
[Mn] mg/kg	[8 , 22]	3	8	10	6,1	18,9
	]22 , 44]	8	6	6		
	]44 , 147]	10	6	4		
[Zn] mg/kg	[0,5 , 1,0]	3	6	4	5,4	24,6
	]1,0 , 4,0]	6	8	11		
	]4,0 , 16,0]	11	6	5		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

Tableau 2 : Liaisons observées entre les teneurs en aluminium, manganèse, zinc et le pH

Tableaux de contingences (nombre d'individus)		[Al] (cmol(+)/L)			$\varkappa^2$	p* (%)
		0,01 (à peine quantifiable)	]0,01 , 0,62]	]0,62 , 6,38]		
pH	[4,0 , 5,3]	0	3	17	50,3	0,0
	]5,3 , 5,8]	8	10	1		
	]5,8 , 7,7]	16	5	0		
[Mn] mg/kg	[8 , 22]	4	6	11	9,6	4,7
	]22 , 44]	11	5	4		
	]44 , 147]	9	7	3		
[Zn] mg/kg	[0,5 , 1,0]	3	2	8	17,9	0,1
	]1,0 , 4,0]	7	8	10		
	]4,0 , 16,0]	14	8	0		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

3.1.2. Climat et topographie

Les incidences de rouille orangée les plus importantes ont été trouvées à des températures moyennes annuelles comprises entre 21,8°C et 23,4°C (tableau 3). En dessous de 21,8°C et au-dessus de 23,4°C, les pourcentages d'infection sont plus faibles. Cela correspond assez bien à ce que l'on connaît du comportement de la maladie en fonction de la température. Plusieurs auteurs mentionnent en effet que l'optimum de température pour la germination des spores se situe entre 22°C et 23°C (Nutman et Roberts, 1963 ; Kushalappa, 1989). Par ailleurs, les altitudes entre 650 m et 995 m semblent les plus propices au développement de la rouille orangée. En ce qui concerne les précipitations, il est bien connu qu'une forte pluviométrie favorise la maladie. Les incidences de rouille orangée les plus fortes ont été observées au niveau du lac de Yojoa qui se caractérise par une pluviométrie annuelle comprise entre 2329 mm et 2441 mm en moyenne. Enfin, un léger effet de la pente a pu être observé. Les parcelles planes paraissent avoir subi des infections moins sévères que les parcelles présentant une pente. Pour la suite de l'étude, nous n'avons gardé que la température. L'altitude n'a pas été conservée car elle est évidemment liée à la température (tableau 4). La pluviométrie, quant à elle, est associée à la teneur en aluminium dans le sol (tableau 5). On sait en effet que dans les zones très pluvieuses, les sols s'acidifient facilement suite à des phénomènes de lixiviation des bases (calcium, magnésium et potassium) qui sont remplacées par l'aluminium. Dans l'état actuel de l'étude, il n'est donc pas possible de dissocier les effets de l'acidité du sol de ceux de la pluviométrie sur la maladie. Dans une troisième phase de l'enquête (1996-1997), nous avons tenté de rechercher des sols à acidités contrastées dans une même zone climatique. Les pratiques culturales en particulier peuvent induire des modifications de l'acidité du sol soit parce que le producteur administre des amendements calcaires, soit au contraire par l'abus de fertilisants azotés.

Tableau 3 : Meilleures liaisons observées entre la rouille orangée et les variables de climat et de topographie

Tableaux de contingences (nombre d'individus)		Pourcentage de feuilles jeunes avec rouille orangée en fin de récolte			$\varkappa^2$	p* (%)
		[9,2 , 31,9]	]31,9 , 57,7]	]57,7 , 85,1]		
Température moyenne annuelle (°C)	[21,2 , 21,8] ou]23,4 , 24,2]	14	11	5	8,4	1,5
	]21,8 , 23,4]	6	9	15		
Altitude (m)	[595 , 650] ou]995, 1140]	10	9	3	4,5	6,2
	]650 , 995]	10	11	17		
Pluviométrie annuelle (mm)	[957 , 1000] ou [1190 , 1566]	17	14	11	4,3	11,5
	[2329 , 2441]	3	6	9		
% pente	0	8	6	3	3,1	20,8
	]3 , 71]	12	14	17		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

Tableau 4 : Liaison observée entre l'altitude et la température moyenne annuelle

Tableau de contingence (nombre d'individus)		Température moyenne annuelle (°C)			κ^2	p* (%)
		[21,2 , 21,8]	]21,8 , 23,4]	]23,4 , 24,2]		
Altitude (m)	[595 , 650] ou]995, 1140]	12	0	10	36,5	0,0
	]650 , 995]	2	30	6		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

Tableau 5 : Liaison observée entre la teneur en aluminium et la pluviométrie annuelle

Tableau de contingence (nombre d'individus)		[Al] (cmol(+)/L)			κ^2	p* (%)
		0,01	]0,01 , 0,62]	]0,62 , 6,38]		
Pluviométrie annuelle (mm)	[957 , 1000] ou [1190 , 1566]	23	13	6	19,2	0,0
	[2329 , 2441]	1	5	12		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

3.2. Vigueur et production des plants

Le tableau 6 suggère que les plants les plus vigoureux sont moins infectés par la rouille orangée. Par ailleurs, il est vérifié que les caféiers se sensibilisent à la rouille orangée quand ceux-ci ont de fortes productions (Avelino, 1991) mesurées en nombre de fruits total ou en nombre de tiges en production. Ces deux variables sont liées (tableau 7). Pour la suite de l'étude, le nombre total de fruits et la vigueur des plants ont été conservés.

Tableau 6 : Meilleures liaisons observées entre la rouille orangée et les variables de vigueur production

Tableaux de contingences (nombre d'individus)		Pourcentage de feuilles jeunes avec rouille orangée en fin de récolte			κ^2	p* (%)
		[9,2 , 31,9]	]31,9 , 57,7]	]57,7 , 85,1]		
Hauteur/Circonférence	[5,7 , 16,0]	17	10	13	5,6	6,1
	]16,0 , 20,7]	3	10	7		
Production totale pour les 5 plants marqués (en nombre de fruits)	[181 , 5913]	6	10	4	8,4	7,7
	]5913 , 9000]	10	4	6		
	]9000 , 37385]	4	6	10		
Nombre de tiges en production sur les 5 plants	[1 , 7]	5	9	4	3,3	18,7
	]7 , 25]	15	11	16		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

Tableau 7 : Liaisons observées entre le nombre total de fruits estimé et le nombre de tiges en production

Tableau de contingence (nombre d'individus)		Production totale pour les 5 plants marqués (en nombre de fruits)			κ^2	p* (%)
		[181 , 5913]	]5913 , 9000]	]9000 , 37385]		
Nombre de tiges en production sur les 5 plants	[1 , 7]	11	2	5	10,0	0,7
	]7 , 25]	9	18	15		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

3.3. Itinéraire technique

Des liaisons entre les incidences de rouille orangée, les distances entre plants sur la ligne, et les distances entre les lignes ont été observées (tableau 8). La densité de plantation paradoxalement n'est pas liée à la rouille orangée (κ^2=1,3, p=52,2%). En fait, il existe une relation entre les distances entre plants et les distances entre les lignes (tableau 9). Le producteur semble faire varier ces distances tout en conservant approximativement la densité recommandée pour ses variétés naines. Cela permet de penser que les faibles pourcentages de feuilles jeunes malades ne sont liés qu'en apparence aux petites distances entre les lignes. En réalité, cette liaison est vraisemblablement due

aux grandes distances entre caféiers sur la ligne qui leur sont associées. Par ailleurs, une relation assez bien marquée existe entre le pourcentage d'ombrage, le type d'arbres d'ombrage et les infections de rouille orangée. Les pourcentages d'ombrage inférieurs à 54 % sont liés à la fois aux incidences de rouille orangée les plus faibles et les plus élevées. On sait qu'en conditions de faible ombrage la production est nettement supérieure, ce qui expliquerait les fortes infections observées. Les faibles incidences, quant à elles, peuvent être attribuées à la diminution de l'humidité, l'augmentation de la température et la pénétration des rayons du soleil dans les parcelles qui présentent un ombrage réduit, surtout si celles-ci sont composées de jeunes plants ou au contraire de plants âgés en phase non productive. Les infections de rouille orangée semblent être plutôt faibles quand l'ombrage est uniquement constitué de légumineuses (*Inga spp.*) (tableau 8), mais c'est aussi dans ces conditions que le pourcentage d'ombrage est le moins élevé (tableau 10). Enfin, les incidences de la maladie semblent bien liées au nombre de passages de récolte (tableau 8). Deux hypothèses peuvent être formulées. La première, déjà mentionnée antérieurement (Avelino *et al*, 1991), est que les récolteurs favorisent la dissémination de la maladie dans la parcelle. La seconde réside sur le fait

Tableau 8 : Meilleures liaisons observées entre la rouille orangée et les variables d'itinéraire technique

Tableaux de contingences (nombre d'individus)		Pourcentage de feuilles jeunes avec rouille orangée en fin de récolte			$\varkappa^2$	p* (%)
		[9,2 , 31,9]	]31,9 , 57,7]	]57,7 , 85,1]		
Distance entre plants sur la ligne (m)	[0,84 , 1,15]	7	14	8	5,7	5,5
	]1,15 , 1,79]	13	6	12		
Nombre de passages de récolte	[2 , 4]	11	12	6	4,1	12,4
	]4 , 13]	9	8	14		
Distance entre les lignes (m)	[1,33 , 1,75]	9	6	4	6,8	14,5
	]1,75 , 1,94]	6	4	10		
	]1,94 , 2,31]	5	10	6		
% ombrage	[6 , 54]	15	10	15	3,8	15,1
	]54 , 83]	5	10	5		
Type d'ombrage	Légumineuses seules	6	4	4	5,5	23,6
	Légumineuses et bananiers seulement	6	10	4		
	Présence de fruitiers ou d'espèces forestières	8	6	12		
Nombre de désherbages mécaniques	[0 , 2]	8	13	11	2,5	28,0
	]2 , 6]	12	7	9		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

Tableau 9 : Liaisons observées entre la distance entre les lignes et la distance entre les plants sur la ligne

Tableau de contingence (nombre d'individus)		Distance entre les lignes (m)			$\varkappa^2$	p* (%)
		[1,33 , 1,75]	]1,75 , 1,94]	]1,94 , 2,31]		
Distance entre plants sur la ligne (m)	[0,84 , 1,15]	8	7	14	4,6	10,1
	]1,15 , 1,79]	11	13	7		

* Seuil de probabilité au-dessus duquel les variables sont considérées dépendantes

Tableau 10 : Liaisons observées entre le type d'ombrage et le pourcentage d'ombrage

Tableau de contingence (nombre d'individus)		Type d'ombrage			$\varkappa^2$	p* (%)
		Légumineuses seules	Légumineuses et bananiers seulement	Présence de fruitiers ou d'espèces forestières		
% d'ombrage	[6 , 54]	13	9	18	8,6	1,3
	]54 , 83]	1	11	8		

* Seuil de probabilité au dessus duquel les variables sont considérées dépendantes

que le nombre de passages de récolte est lié au nombre de floraisons. Or, ce dernier est plus grand quand la saison sèche est peu rigoureuse, et une saison sèche moins marquée permet de conserver une plus grande quantité d'inoculum résiduel. Pour finir, une très légère liaison entre la maladie et le nombre de désherbages mécaniques semble aussi se présenter (tableau 8). Pour la suite de l'étude nous n'avons conservé que la distance entre les plants, le pourcentage d'ombrage, et le nombre de passages de récolte.

3.4. La segmentation

Cette technique, proche de la régression multiple, permet de mettre en relation une variable à expliquer qualitative ou quantitative et plusieurs variables explicatives qualitatives. La segmentation hiérarchise les variables en fonction de leur pouvoir explicatif. La meilleure variable explicative sépare la population initiale en deux sous populations qui elles-mêmes peuvent être séparées par d'autres variables explicatives. La succession de dichotomies conduit à la formation d'une arborescence. Chaque branche de l'arborescence est traitée de façon indépendante. Les variables les plus explicatives retenues, celles qui sont responsables d'une dichotomie, ne sont donc pas forcément les mêmes dans les différentes branches de l'arbre. De ce point de vue, cette méthode semble être mieux adaptée à la réalité que la régression multiple. Cette dernière se limite en effet à inclure dans le modèle les variables qui sont les meilleures en moyenne, ce qui a peu de sens quand on souhaite aboutir à des recommandations adaptées à chaque cas particulier (Perrier et Delvaux, 1991). Par ailleurs, la représentation graphique des résultats, en raison de sa relative simplicité, pourrait être utilisée aisément dans la pratique par les producteurs ou techniciens. La figure 1 montre l'arborescence que nous avons obtenue avec la rouille orangée comme variable expliquée quantitative, et les

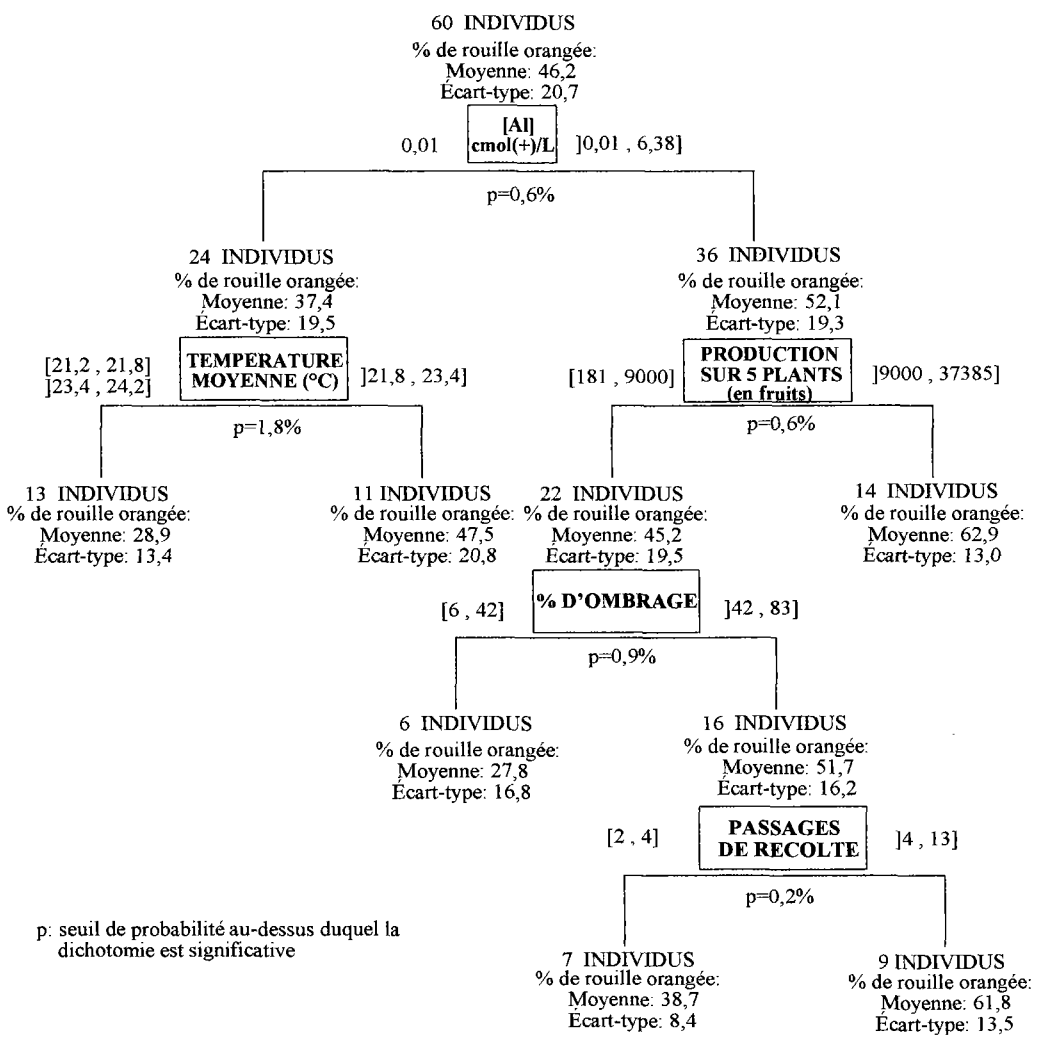

Figure 1 : Segmentation à partir des données d'une enquête-diagnostic sur la rouille orangée

variables explicatives qualitatives suivantes : teneur en aluminium, température moyenne annuelle, nombre de fruits total, rapport de la hauteur des plants sur leur circonférence, distance entre plants sur la ligne, nombre de passages de récolte, pourcentage d'ombrage. Nous avons fixé un effectif minimum de 6 individus (10% de l'effectif total) pour chaque groupe formé. Toutes les dichotomies sont significatives à 2%. Les niveaux d'infection les plus faibles sont obtenus quand la teneur en aluminium est inférieure ou égale à 0,01 cmol(+)/L et quand les températures moyennes sont inférieures ou égales à 21,8°C ou supérieures à 23,4°C. De faibles incidences sont aussi observées quand la teneur en aluminium est supérieure à 0,01 cmol(+)/L, la production faible à moyenne (entre 181 et 9000 cerises pour 5 caféiers) et l'ombrage faible (inférieur ou égal à 42%). Les incidences les plus importantes sont observées quand la teneur en aluminium est supérieure à 0,01 cmol(+)/L et la production importante (supérieure à 9000 cerises pour 5 caféiers), ou quand la teneur en aluminium est supérieure à 0,01 cmol(+)/L, la production faible à moyenne (entre 181 et 9000 cerises pour 5 caféiers), l'ombrage supérieur à 42%, et le nombre de passages de récolte élevé (supérieur à 4). Les deux dernières branches conduisent à des niveaux de rouille orangée intermédiaires. Il est remarquable de voir qu'une parcelle peut se trouver dans des domaines de risque bien distincts en fonction des productions attendues.

4. CONCLUSION

Les résultats qui ont été présentés ne sont encore que préliminaires. En 1996-1997, nous avons enquêté soixante-treize individus supplémentaires, qui, nous l'espérons, permettront de définir des domaines de risque plus précis et fiables. Pour que le modèle conduise à des applications pratiques, certaines variables, comme la production ou l'ombrage, devront être définies avant la date du premier traitement. Des ajustements seront donc nécessaires. Il n'est pas envisageable pour l'instant de recommander des pratiques culturales qui permettent de passer d'un domaine de risque élevé à un domaine de risque plus faible. Nous pensons que les domaines de risque doivent surtout servir à déterminer le nombre de pulvérisations cupriques nécessaires. Pour les niveaux élevés de rouille orangée, trois ou quatre pulvérisations cupriques pourraient s'avérer indispensables. Pour les niveaux intermédiaires, deux pulvérisations au maximum devraient suffire. Pour les niveaux faibles, une seule pulvérisation serait recommandée.

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RÉSUMÉ: L'objectif principal de cette étude est de caractériser non seulement les conditions du milieu (climat, topographie, nature chimique des sols), mais aussi celles d'itinéraire technique, de vigueur et de production des plants favorables ou défavorables au développement de la rouille orangée du caféier. Ces différents groupes de variables sont évalués à travers une enquête/diagnostic couvrant 25 parcelles suivies en 1994-95 et 1995-96, et 10 parcelles additionnelles observées seulement en 1995-96, dans 4 régions du Honduras. Des liaisons entre les incidences de rouille orangée et le climat, l'acidité du sol, le pourcentage d'ombrage, la distance entre les plants sur la ligne, la production, la vigueur des plants, le nombre de passages de récolte ont été mises en évidence. La segmentation est finalement utilisée dans le but d'élaborer une première ébauche des domaines de risque dû à la maladie.

RE-EMERGENCE OF *FUSARIUM* WILT OF COFFEE IN AFRICA

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Introduction

Vascular wilt or tracheomycosis was first reported in *Coffea excelsa* in the Central African Republic (Guillemat, 1946) although Steyaert (1948) reported that he had received diseased specimens of *C. excelsa* in August 1939 from which he had isolated a *Fusarium* spp. which he called *Fusarium xylarioides*. He was confident that he had isolated the pathogen which had been causing great destruction of plantations of *C. excelsa* in French Equatorial Africa - an epidemic so serious to make the culture of *C. excelsa* impractical. Subsequently, *Fusarium* wilt became a serious problem of Robusta coffee in several countries of West Africa including Ivory Coast and the Congo (Zaire / Democratic Republic of Congo) during the 1940s and 1950s but the establishment of effective breeding programmes in these countries reduced its impact to that of a minor disease. *Fusarium xylarioides* has also caused serious problems on *C. arabica* in Ethiopia (Lejeune, 1958; Van der Graff & Pieters, 1978) and in Zimbabwe (Clowes & Hill, 1981). As with Robusta coffee, the development of screening methods for *C. arabica* in Ethiopia has aided effective breeding programmes (Pieters & Van der Graff, 1980).

However, in 1986 (Anon, 1995), a disease of coffee which manifested itself as a wilting and defoliation of the trees was again reported from around the town of Isiro (North East Zaire / Democratic Republic of Congo). The incidence of this disease increased in the many abandoned plantations in that area and yields decreased dramatically. OZACAF (Office Zaireois du Cafe) prepared a detailed report (Anon, 1995) for the International

Coffee Organisation (ICO) which outlined the extent of the problem and its effects on the economy of N.E. Zaire. The International Mycological Institute (IMI) was approached by ICO in March 1996 to send a plant pathologist to Zaire to confirm the pathogen as *F. xylarioides* and conduct a survey and prepare a report on the nature and extent of the problem (Flood, 1996).

In Uganda, a wilt disease of coffee was also reported in Bundibugyo District in 1993 and in Rukungiri District in 1994; both these districts border Zaire. In 1995 a further outbreak of the disease was reported in Mukono District close to Kampala where 40% of Ugandan coffee is grown. Consequently, IMI were invited by UCDA (Ugandan Coffee Development Authority) to conduct a similar survey in Uganda in early 1997 (Flood, 1997).

Field Observations

Much of the area around Isiro (North-east Zaire) consists of plantations which were abandoned following nationalisation in the late 1970's. Smallholders had initially taken over some of these areas but they too abandoned the land to the bush as the disease affected more and more of their trees and they felt unable to prevent the disease spreading. However, one commercial plantation claimed some success with their programme of control measures which had been recommended by OZACAF and which included uprooting of the affected bushes and burning *in situ*.

In North Kivu province, the disease began to appear around the town of Beni in 1988-89, and by 1992 had become very widespread. The disease is thought to have spread to Beni from Isiro via a corridor of land cultivated with Robusta coffee which stretches from Mambasa to Komada. In contrast to Isiro, where the disease had passed its primary phase in Beni the full extent of its destructive capabilities were easily seen. In each of several plantations visited, over 90% of the trees were dead or dying.

In Uganda, the disease was observed to be widespread on smallholder plots to the south and east of Fort Portal (Kabarole District) and in the Rukungiri District which borders Zaire (Democratic Republic of Congo). In a very remote region just 12 kilometres from the Zaire border, a farmer was interviewed who had first observed the disease in 1993 but did not report it until 1994. In one field, virtually every tree was affected and the symptoms and the devastation seen were reminiscent of observations from Zaire. The field had since been turned into a cattle paddock and, in an adjacent field, he had already replanted with bananas. However, the affected trees had not been uprooted because of the sheer scale of the operation. Previously, in November 1996, a task force consisting of members from UCDA, COREC (Coffee Research Institute) and MAAIF (Ministry of Agriculture in Uganda) had visited this site and had reported no evidence of the disease on his coffee on the otherside of the road, but two months later 70% of his trees there were showing symptoms.

In Bushenyi District, the disease is confined to one village (Kamirundi) and was only reported in July 1996 yet 6 months later over 80% of the trees were affected. Similarly, in Mukono District, the symptoms at one small holding were first seen in 1995 and currently about 900 trees are affected. Subsequently, several other outbreaks have been reported in Mukono District and in several other districts throughout Uganda (Lukwago & Birikunzia, 1997).

In contrast, no *C. arabica* was observed to be affected in either country and in Uganda, clonal coffee has not shown symptoms even in areas of high inoculum pressure such as in Rukungiri District.

Disease Symptoms

In both countries and at all sites visited, symptoms were typical of those in a vascular wilt disease of coffee and were, in general, similar to those described by Fraselle (1950). Fraselle (1950) studying the disease in the Congo reported a generalised chlorosis of the leaves as one of the first symptoms. The leaves then became flaccid, desiccated and abscised from the branches. Chlorosis was also consistently observed in Zairean coffee material in this study but was never observed in Uganda where the leaves wilted and curled inwards before desiccation and abscission. Dieback often appeared from the top, spreading progressively downwards and the branches may blacken. Where there were multiple stems on a coffee bush, stems were affected in sequence - one after another until the entire tree died.

The bark of the trunk was observed to become hypertrophied with numerous vertical or spiral cracks and inspection of wood under the bark especially around the collar revealed characteristic blue-black staining of the wood. Black fruit bodies (perithecia) of the sexual form of the fungus (*Gibberella xylarioides*) were observed in the cracks in the bark at the base of the trees. Ascospores were observed in perithecia from bark samples collected in Mpigi and Mubende Districts at the end of the rainy season (May -June).

Isolation of the pathogen.

Tissue samples from trees were taken back to IMI for isolation and identification of the causal agent. The bark was aseptically removed and the wood surface sterilised in 30% hydrogen peroxide (100 vols) for 2 minutes, rinsed with sterile distilled water (SDW), plated onto 3% Tap water agar (TWA) and incubated at 20°C in diffuse daylight. After 2-4 days incubation the plates were observed daily for colony growth.

All wood samples taken from diseased trees in Zaire and Uganda revealed the presence of *F. xylarioides* and no other fungus apart from *F. xylarioides* was consistently present. In many samples it was the only fungus isolated from lesion tissues, while in some samples other *Fusarium* species were also detected, typically from older tissues. These included *F.*

solani (Martius) Sacc., *F. pallidioroseum* (Cooke) Sacc., and occasionally *F. oxysporum* Schlecht. and *F. decemcellulare* Brick. Since *F. xylarioides* tended to be slower growing than other species, it had to be subcultured promptly to avoid being overgrown. Conidia direct from the isolation plates were spread thinly on 3% TWA plates, incubated overnight and germlings seen to originate from single spores were isolated using a modified microscope objective marker and a fine tungsten wire. Strains were maintained on slants of SNA + filter paper (Nirenberg, 1976). So far, more than 100 strains have been obtained from 25 localities in Zaire and Uganda.

Characterization of the pathogen

F. xylarioides was readily identifiable on the isolation plates since it sporulated quickly, forming abundant distinctive, strongly curved / hooked micro- and macroconidia. On SNA (= Spezieller- Nährstoffarmer Agar) mycelial growth was sparse, with little aerial mycelium or pigmentation, but good production of typical conidia. This medium is recommended for routine growth purposes since it reduces cultural degeneration which occurs on rich agar media. After several weeks incubation, dark-blue / black stromata were formed on the filter paper. These differentiated into clusters of protoperithecia, but no ascospores were observed even after several months incubation. On potato sucrose agar (PSA) colonies were initially pink with floccose aerial mycelium. As the cultures aged they developed a violet-purple pigmentation (purple slate *sensu* Rayner, 1970), eventually darkening to violaceous black (Rayner, 1970). Clusters of protoperithecia were formed in the aerial mycelium. Similar structures also developed on other media, including oatmeal agar, malt agar, potato dextrose agar and Czapek-dox agar (CZ). The violet pigment was less evident on CZ.

At IMI, comprehensive molecular analysis using several DNA-fingerprinting techniques is underway using strains from diseased plants from all localities in Uganda and Zaire. These strains were also compared with a strain from Ethiopia (ex *C. arabica*) and a strain isolated from *C. excelsa* in Ivory Coast. The results so far indicate apparent homogeneity in the Zaire and Uganda strains isolated in the present study whilst the other two strains from *C. arabica* and *C. excelsa* gave distinctive patterns (Brayford & Flood, in preparation).

Discussion

All the tissue samples removed from diseased trees in Uganda and Zaire revealed the presence of *F. xylarioides* - often growing in pure culture which would seem to indicate its role as a primary colonist / pathogen. Thus, it would appear that following several decades when it was considered to be a minor pathogen of coffee in Central Africa, tracheomycosis or vascular wilt disease has re-emerged as a serious pathogen of seedling-derived Robusta coffee over the last ten years. For example, coffee yields for Haut- Zaire (around Isiro) have been declining steadily for the last decade since the reappearance of the disease and by 1995 had been reduced by more than 50% from the 1987 yield (Anon.1995).

In contrast to robusta coffee, *C. arabica* in Zaire and Uganda would as yet appear to be unaffected. Molecular analysis of an isolate from *C. arabica* and one from *C. excelsa* indicated that these isolates were distinct from each other and from those strains isolated from *C. robusta.* However, many more isolates from *C.arabica* and *C. excelsa* need to be collected and analysed before definite conclusions can be drawn on the adaptation of this pathogen to different host species.

Clonal coffee in Uganda, which was originally developed for high yields but also screened for resistance to Red Blister Disease (*Cercopora coffeicola*) may also be resistant to *Fusarium* wilt. Thus, in Rukungiri District, clonal coffee has been planted since 1982 and has exhibited no symptoms despite being in areas of high inoculum pressure and where seedling-derived material has been severely affected. However, controlled tests should be undertaken as soon as possible to confirm these observations.

The reason(s) for the re-emergence of tracheomycosis in *C. robusta* is as yet unclear. One hypothesis is that the coffee has become predisposed to infection due to old age. Many trees in N E Zaire are very old (40 years) but, in Uganda, although many old trees are affected, even 10 year old trees have been observed to have symptoms. There would also appear to be no correlation with overall standards of coffee management, since reasonably well maintained blocks would also appear to be affected. However, the role of specific management practices in the control of this disease does require further study.

Changes in the environment may also have a role in the re-emergence of vascular wilt - environmental stress such as water logging can damage the physiology of the plant and *F. xylarioides* is transmitted over short distances by rain splash (Jacques-Felix, 1954), so wetter years may have contributed to an increase in the incidence of the disease. Certainly, annual rainfall data on both sides of the Zaire /Uganda border has been unusually high in some years during the last decade. Rainfall data for Isiro from 1987-1995 revealed that 1988 was a wet year and this would have certainly encouraged the spread of *F. xylarioides* which was said to have been commonly observed around Isiro from 1986. Unfortunately, pre-1986 data were not available in Isiro but 1985 was shown to be a very wet year in Beni and the same may have occurred in Isiro. Data from 1988-95 revealed 1988 was also a very wet year in Beni which is consistent with the first appearance of the disease there (1988-89). Above average rainfall was also recorded in Western Uganda which is the other side of the Rwenzori Mountains. Rainfall data for the Fort Portal area from 1988-1995 indicated a sharp rise in annual rainfall since 1993, while data from Bundibugyo District (1993-96) indicated high rainfall especially in 1995. When interviewed, farmers generally reported that many recent years had been much wetter than usual. A similar pattern was observed in the Mukono district - rainfall data revealed that 1994 was a very wet year which is consistent with the first reports of the disease in Mukono in 1995.

Another explanation for the re-emergence of the disease is that there has been a change in pathogen population - a new, more aggressive strain of the fungus may have arisen, and the present epidemic is caused by a particular genotype within the wider gene-pool of the pathogen species. This explanation may account for the observed molecular homogeneity of strains from the present epidemic (Brayford & Flood, in preparation). Such homogeneity would otherwise be surprising in a reportedly heterothallic species which regularly produces its teleomorph in nature; ascospores of *Gibberella xylarioides* were frequently observed in perithecia on bark samples collected at the end of the rainy season. Unfortunately, very little is currently known about the basic biology of *F. xylarioides*, such as its infection mechanism, details of its distribution in host tissues, ability to survive in soil or infected tissues, the significance of ascospore production for infection and gene flow in populations and the role of possible vectors. Insects such as stem borers may be responsible for spread of this pathogen over considerable distances. Such information about the pathogen is vital to understanding of the re-emergence of this disease and to future control measures for sustainable coffee production in Africa.

Summary

Tracheomycosis or *Fusarium* wilt was a serious problem in many parts of West and Central Africa during the 1950s but was eventually controlled through effective breeding programmes. Consequently, for decades it was considered a minor disease but has now re-emerged in North East Zaire and Uganda and is again causing widespread concern.

Field survey data suggest that the disease appears to spread rapidly and has caused very severe losses. The fungus *Fusarium xylarioides* was consistently isolated from lesions in samples from trees showing characteristic symptoms of a vascular wilt, suggesting it has a primary rôle in disease causation.

The significance of host vigour, environmental factors (especially rainfall) and of variation within the pathogen populations in causing the present re-emergence of the disease remain unclear and urgently require further study in order to safeguard sustainable coffee production in Africa.

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Incidences in Uganda

Districts Affected	Localised/???
Mukaro	L
Lawerro	W
Mpigi	L
Mutunde	L
Liboga	W
Kibale	W
Majindi	W
Hoina	W
Kaberole	W
Dukunjin	W
Boshenyi	L
Buddibiyo	W
Aabai	N
Masuka	N
Misale	N
Mbaraboru	N
Kasese	L
Ntungamo	N
Kisoro	Not surveyed?
Kabale	Not Surveyed

Plan of Discussion

1) Found it was fx - why has it re-emerged?
Possible ???? in pathogen - molecular ???? thwarted - increased rain
Host - old etc - not ????

Control - breeding of ??? - effective in the past
New programme needed to be ????
Widely spread in localised areas - cultural different
Review stumps - fire wood - transmitting (Beri)

Little know about transmission, survival - ????

Populations - L ?????

INTEGRATED PEST MANAGEMENT IN COFFEE : NEEDS, LIMITATIONS AND OPPORTUNITIES

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INTRODUCTION

Coffee is one of the most valuable export crops of the third world countries. South and Central American countries produces 60% of total exported coffee, Africa 30%, while Asia and Oceania contributes 10%. Among the major factors limiting increased coffee production worldwide are losses due to pests (insects, diseases, nematodes and weeds), both indigenous and exotic (Table 1). Losses due to coffee pests are estimated to be 13% worldwide (Bardner, 1978) with more serious losses occurring in Africa, the home of *Coffea* spp.

Coffee pests can cause high yield losses and/or affect the cup quality of coffee either individually or in combination. It is difficult to quantify losses due to different pests worldwide because coffee is grown under wide agro-ecological and management practices, this affecting the economic pest status of the same in different countries. For example, the coffee berry borer has been reported to cause up to 80% damage in Uganda, 5-20% in Cote d'Ivoire, 84% in Congo, 96% in Tanzania, 90% in Malaysia and 60-80% in Brazil (Waterhouse and Norris, 1989). The coffee berry disease caused by *Colletotrichum kahawae* sp. Nov. (Waller and Bridge), a major pest of coffee in Africa, causes 90% crop loss on arabica coffee in Tanzania and up to 20% loss in Kenya (E. Koinange, *personnal communication*; Masaba, 1991). The African coffee root-knot nematode, *Meloidogyne spp.,* can cause up to 20% yield loss in Tanzania (Bridge, 1984). Infestations by antestia bug, *Antestiopsis* spp., and the coffee berry borer, *Hypothenemus hampei*, directly lower the bean and liquor qualities of coffee (Wanjala, 1980).

Table 1: Distribution of major coffee pests and their control

PEST	COUNTRY	CURRENT CONTROL MEASURES
INSECTS		
Coffee berry borer, *Hypothenemus hampei* Ferarri	Africa and Latin America	Chemical/Biocontrol
African coffee leaf miner, *Leucoptera meyricki* (Ghesq.), *L. caffeina* (Wash) Neotropical coffee leaf miner, *L. coffeela* (Guer.)	East and Central Africa Asia	Chemical/Biocontrol
Antestia bugs, *Antestiopsis* spp. Hemiptera:Pentatomidae	East and Central Africa	Biocontrol
White stem borer, *Anthores leuconotus* (Pase.) *Xylotrechus quadvipes* Chevrolat	East and Central Africa India and S .E. Asia	Chemical/Cultural Chemical/Cultural
Citrus mealy bug, *Planococcus citri* (Risso) Kenya mealybug, *P. kenyae* (Le Pelley)	East Africa and India Kenya	Biocontrol Biocontrol
Green coffee scales, *Coccus* spp.	Kenya, India, Papua New Guinea and Cuba	Chemical/Biocontrol
DISEASES		
Coffee leaf rust, *Hemileia vastatrix* (Berk & Broome).	Africa, Asia, Central and South America	Chemicals/Resistant Varieties
Coffee berry disease, *Colletotrichum coffeanum kahawae* sp. Nov. (Waller & Bridge)	Africa	Chemicals/Cultural/resistant varieties
South American leaf spot, *Mycena citricolor* (Berk & Curl.)	South & Central America	Cultural/Chemical
Coffee bark disease, *Fusarium stilboides* Wollen	Africa	Cultural/Chemical
Vascular wilt (Tracheomycosis), *Gibberella xylarioides* Heim & Saccas *Fusarium xylarioides* Steyaert	East and Central Africa	Chemical/Cultural
Bacterial wilt of coffee, *Pseudomonas syringae* pv. *garcae*	Africa.India and S. America	Chemical/Cultural
NEMATODES		
African Coffee Root nematodes, *Meloidogyne* spp. & *Pratylenchus coffeae*	Africa, Asia	Resistant robusta root Stocks

CURRENT COFFEE PEST MANAGEMENT PRACTICES

Over the years, a combination of pest management strategies such as cultural, biological, chemical and use of resistant varieties, have been developed (Table 1). However, at farm level, the general attitude to pest management has been dominated by the adoption of simplistic and quick approaches such as use of chemical pesticides. In addition, recommended pest control measures have been developed for individual pests rather than the pest complex normally encountered in any one farm or farming system. The lack of the holistic approach to both the control measures and the pest complex has resulted in a number of detrimental effects. For example, increases in pesticide prices have led to the high cost of coffee production thus reducing the net returns from the coffee sales and leading to the negligence of the crop by small-holders. In Kenya chemical control of coffee diseases alone accounts for 30% of the total cost of production (Masaba and Waller, 1991).

Pesticide use has also been reported to disrupt natural biocontrol mechanisms thus creating increased pressure of pests, which were previously of minor status. Increased copper sprays contributed to increases in severity of the coffee leafminer (*Leucoptera meyricki*) (Abasa, 1975), and of coffee berry disease (*Colletotrichum kahawae*) in Kenya (Furtado, 1969).

A number of pests have in the past developed resistance to commonly used pesticides. Examples include *Hypothenemus hampei* (Brun *et al.* 1989; 1994; Agnihothrudu, 1991) *Leucoptera meyricki* (Bardner and Mcharo 1988), *Bidens pilosa* and *Parthenium* (Njoroge, 1991) and *Colletotrichum kahawae* (Okioga, 1976).

In addition, pesticide residues in soils and plant tissues pose potential environmental and health risks. To increase coffee production and improve on quality and income to farmers on a sustainable level, and to reduce the potential environmental and health risks associated with chemical pesticide usage in coffee production, there is a need to search for alternative pest management options. This is achievable through the development of appropriate integrated pest management (IPM) strategies.

INTEGRATED PEST MANAGEMENT

The integrated pest management (IPM) is a holistic approach to the control of the entire coffee pest complex which utilizes a combination of biological, genetical, cultural, physical and chemical measures to suppress pest populations below economically damaging levels.

Farming Systems

Coffee is grown under different agro-ecological and management practices and the pest complex is often influenced by these two major factors. While in Latin America coffee is grown as a monocrop, small-scale farmers in East Africa and Asia normally intercrop coffee with food crops and fruit trees (Njoroge and Kimemia, 1993). Several tree species are also grown in coffee as shade trees or windbreaks (Njoroge and Kimemia, 1993). It is anticipated that both intercropping and use of shade trees will be sustained in coffee because of the need to optimize land use and the shade effect which evens out erratic yield and moderates high temperature changes that may result into physiological deformities in the coffee plant. For this reason, there is need to work out an integrated approach of managing the pest complex within the various farming systems.

Pest Management Strategies

Biocontrol

The insect pest and disease complex occurring on coffee is subject to a wide range of natural biocontrol systems. The widespread occurrence of natural enemies of arthropod pests in coffee is well recorded in some coffee producing countries (Notley, 1948,; Crowe and Greathead, 1970; Greathead, 1971; Abasa, 1975; Ndungi, 1994; O'Dowd, 1994) and of major pathogens of coffee (Masaba, 1991). The existence and/or effect of these natural biocontrol systems only becomes apparent when they are disturbed or removed especially through the indiscriminate use of pesticides. Pesticides may cause direct reduction in populations of arthropod natural enemies and antagonistic micro-organisms or cultural conditions under which coffee is grown may favour pests without a corresponding increase in the activities of natural biocontrol agents. Knowledge on interactions between natural enemies, pests and environment will be essential in manipulating the components of the system to produce practical IPM approaches.

To date, classical biocontrol of coffee pests has had few successful results (Nyambo *et al.* 1994). This has been due to lack of appreciation of the total pest complex and the role of farming systems in pest control strategies particularly where chemical pesticides are commonly used on other crops in the system. Another important factor is the need for proper taxonomy of the target pest and its alternative host plants. The lessons from the case of *P. kenyae* (Abasa, 1975) is a good example of how much time and resources can be saved when the pest is identified correctly from the beginning. Proper studies on the biology as well as the ecological requirements of the potential biocontrol agent are equally important.

The perceived danger that some arthropod natural enemies can spread major coffee diseases in an area and therefore could pose phytosanitary risk is a subject which needs further research. Work done by Nemeye *et al.* (1992) has provided some evidence to show that *Heterospilus coffeicolla*, a potential biocontrol agent of *H. hampei*, could transmit the coffee berry disease. This shows the need for more strict quarantine and sanitation in the importation of natural enemies to minimize such risks. However, there is no reason to suppose that the addition of exotic natural enemies or the enhancement of existing ones will have any untoward effects to diseases. Since the pests of coffee, notably insects, diseases and nematodes, are closely adapted to the *Coffea* spp., the natural enemies are also expected to be host specific or nearly so, and therefore the probability of environmental damage from introduced natural enemies is remote. The successful control of *P. kenyae* in Kenya with *Anagyrus* spp from Uganda since 1938 to date is a good example of long term benefits of classical biocontrol in coffee when host specific natural enemies are used.

Host-plant resistance

The breeding and selecting for resistance to the major pests of coffee has been given limited emphasis, with only a few reported cases in the literature (Walyaro *et al*, 1984; Capot, 1972; Palanichamy, 1973; Koch, 1973; van der Graff *et al*, 1978; Anon, 1996). Resistance to diseases has been a favoured option and has been very successful in some cases e.g. the release of Ruiru 11 which combines resistance to berry disease and leaf rust in Kenya (Walyaro *et al*, 1984) and of hybrid coffee which combines high quality, high yield, vigorous growth with resistance to leaf rust and the beetle, *Xyleborus mortati* in Cote d'Ivoire (Capot, 1972).

Conflict between the commercial quality requirements and pest control priorities may prevent the release of a resistant/tolerant variety. Therefore, where available, partial resistance combined with important agronomic traits could be a valuable component in IPM.

The availability of the relevant breeding technology may be another limiting factor in many of the third world countries and collaboration with International Institutions may be necessary. However, if well funded, traditional methods of breeding are very effective. Even after the development of suitable pest resistant varieties, availability of adequate planting material to replace old and susceptible ones in a short time may be a limiting factor and the use of new biotechnology approaches could be the answer. Uganda has been able to propagate robusta coffee clones known to be high yielding and resistant to rust through somatic embryogenesis using tissue culture, and research in this field is well advanced (Anon, 1996).

Chemical pesticides

Chemical pesticides will continue to be used in coffee pest management. To optimize the benefits of pesticide use, farmers should be educated and encouraged to use them more judiciously. The chemical compounds to be used need to be evaluated for their effects on non-target micro-organisms, which may be components of the natural biocontrol mechanisms. The rates need to be minimal and the application exacting on the target and at carefully selected stages of the pest and host cycles that are pre-determined by biological and epidemiological studies.

Biopesticides

The future trend in pesticides use is aimed at developing biopesticides, which are considered environmentally safe, and with reduced health risks. The availability of effective formulations and cost effective application techniques is an area that needs investment. In Colombia and Mexico, a program to develop *Beauveria bassiana* an indigenous entomopathogen, as a component of IPM for the control of the coffee berry borer is underway (Moore and Prior, 1988). Elsewhere, investigations have been initiated into the control of *Hemileia vastatrix* with strains of *Bacillus thuringiensis* (Roveratti *et al,* 1989) and *Verticillium lecanii* (Carrion, 1988; Eskes *et al,* 1991).

Biopesticides may in some cases, be used to complement chemical pesticides to effect adequate control of a particular pest or pest complex. In such a situation, compatibility with synthetic pesticides would be desirable, as it is often possible to schedule both into a spray programme. Although biopesticides may be selective, and environmentally safe, judicious use is essential to prolong their effectiveness in the field. As with chemical pesticides, the potential of the target pest developing resistance to a biopesticide after continuous field use should not be overlooked. Already there is evidence from Hawaii and Japan that *Plutella xylostella*, a pest of brassicas, has developed resistance to strains of *B. thuringiensis* (Tabashnik *et al,* 1992).

Use of natural products

The use of natural products is perhaps the least researched control approach. This could be because traditionally coffee is not intercropped with non-crop plants. However, work done in Indonesia has shown that some weed plants, including *Tagetes patula*, have good nematocidal effects against the nematode, *Pratylenchus coffeae* (Saleh, 1971). There could be many other potential natural products that could be safely used as alternatives to chemical pesticides but research is needed before such plants could be incorporated in IPM strategies.

Cultural practices

A wide range of cultural practices (pruning, sanitation, mulching, spacing, fertilization, shading,) have been recommended in combination with chemical pesticides, biocontrol agents and resistant varieties. The cultural practices should be the foundation upon which any of the

other artificial pest management strategies should be added (Swynnerton *et al*, 1948) since it is uneconomic to apply pesticides on poorly grown coffee. However, some of the operations could be time consuming and their opportunity costs high and therefore not attractive to farmers. Awareness creation may help farmers appreciate the benefits of the recommended cultural practices.

Quarantine

The need to restrict the movement of planting material and unprocessed coffee beans to minimize the spread of damaging pests to new areas should be re-examined. Strict legislation was able to keep India free from the coffee berry borer until the late 1980s (Chacko, 1978). However, possibly due to laxity on the enforcement of the quarantine legislation, *H. hampei* was reported in India in Tamil Nadu in 1990 (Kumar *et al*, 1990). By the end of 1991 the pest had spread to Karnataka and Kerala, two major coffee growing areas of India (Krishnamoorty-Bhat, 1991).

The value and potential of effective quarantine in coffee pest management cannot be under-estimated and should therefore be emphasized as part of IPM strategies where and when appropriate to reduce the speed by which notorious pests move across the coffee growing countries.

Constraints to IPM

Farmer acceptance

Coffee farmers, particularly in East Africa, are used to a pest control system, which is both narrow and prescriptive, e.g. use of chemical pesticides applied on calendar schedule. To enable farmers make more rational decisions in response to specific and/or pest complex on coffee will require investment in the development of locale-specific IPM practices and farmer training. Farmer training could be achieved through farmer field schools (FFS). The Coffee Research Foundation of Kenya (CRF) in collaboration with CAB International Institute of Biological Control, Kenya Station and Kenya Institute of Organic Farming has demonstrated the value of FFS in Kenya small-holder coffee production systems (Nyambo *et al*, 1996b).

Extension

In Africa, the extension system is ill equipped in terms of adequate trained personnel and efficient transport to effectively transmit the IPM technology to farmers. In addition, the extension staff are not specialized in any one aspect of Agriculture. Training in IPM practices supported by the FFS approach mentioned above may help facilitate the implementation of IPM.

Research

Currently, many of the pest control recommendations are aimed at individual pests of coffee because they were developed unilaterally without an interdisciplinary approach to the coffee pest complex (Nyambo *et al*., 1996a). As a result, many of the recommendations appear impractical and/or antagonistic at the farm level and this results in poor adoption. There is therefore, a need for an interdisciplinary approach to coffee research and management and a greater need for farmer involvement in the development of appropriate IPM practices.

Research-extension-farmer linkage

A lot of the required information on coffee pest management practices in many parts of the world is available at research institutions, but only a small proportion of it has filtered through

to the extension service and the farmer. This is partly because the information has not been translated into a form which is meaningful to the farmer and partly because the technology does not directly address the farmers' problems. A deliberate effort to establish a favourable environment to facilitate farmer-research-extension dialogue and interaction is needed. A good linkage would facilitate the development of appropriate on-farm trials to address farmer needs, discuss with farmers how to package the IPM practices for ease of adoption and for sustainability, and to jointly identify further research problems to address farmer needs.

IPM policy

Whilst use of chemical pesticides to control coffee pests has been accepted by farmers, the policy makers and the general public as the best and quickest means of pest control, information on IPM is wanting in most countries. IPM was recommended and widely practiced by farmers in East Africa up to late 1940s when effective pesticides became easily available to farmers (Swynnerton *et al.,* 1948). The benefits of IPM practices are gradual and need to be practiced over a wide area to be noticeable. To enhance the development and implementation of IPM technologies, there is a need to sensitize and educate policy makers, donors, the general public and farmers on the benefits of IPM practices so as to formulate good national IPM policies. In addition, there is a need to educate policy makers and the general public about the long-term benefits of IPM practices.

CONCLUSIONS

A major aim in the development of IPM measures against coffee pests must be to develop a package of mutually supportive strategies. Techniques involving cultural methods and the enhancement of natural biocontrol, which disrupts the pest's cycle, will enhance the effectiveness and durability of resistance by reducing pest pressure and variability. There may still be a need to use chemical pesticides in some situations but selective compounds which are applied in minimal effective doses on the appropriate target, and at carefully selected stages during the pest cycle should have minimal disruptive effects on the natural biocontrol.

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ABSTRACT

Coffee is an important cash and export crop for third world countries. The crop suffers heavy yield losses due to damage caused by a wide range of pests (insects, diseases, weeds, and nematodes). Current recommended pest control measures include a combination of cultural, resistant/tolerant cultivars and the use of broad-spectrum chemical pesticides. Chemical pesticides are more popular at the farm level than any of the other recommended pest control options. Coffee pest control measures are often aimed at individual pests with little consideration of the implications for the total coffee pest complex and its agro-ecosystems. This unilateral approach has resulted in increased pest pressure on coffee and its companion crops, outbreak of new pests of coffee, development of pest strains resistant to commonly used chemical pesticides, increased environmental problems and health risk to man and his livestock as well as an overall increase in the cost of coffee production, thus forcing many farmers to neglect their coffee plantations. Measures to alleviate the above problems are needed to improve coffee production and increase income from coffee on sustainable levels. Integrated pest management (IPM) offers such a measure. Techniques involving cultural methods and natural biocontrol, which disrupt the pest cycle, will enhance the effectiveness and durability of resistance by reducing pest pressure and variability. There may still be a need to use chemical pesticides in some situations but selective compounds at minimal effective doses on appropriate target(s), and at carefully selected stages during the pest cycle, should have minimum disruptive effects on the environment. However, lack of national IPM policies, poor extension systems, inefficient research-extension-farmer linkages and the general acceptance by farmers will delay the development and implementation of appropriate, acceptable and sustainable IPM strategies. The needs, limitations and opportunities of IPM approach in coffee production system are discussed.

LE SCOLYTE DES FRUITS DU CAFÉIER *HYPOTHENEMUS HAMPEI* FERR. (COLEOPTERA, SCOLYTIDAE) AU TOGO. MÉTHODE D'ESTIMATION DU NIVEAU D'INFESTATION, DES PERTES ET DU SEUIL DE DÉGÂT ÉCONOMIQUE

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Introduction

Le scolyte des fruits du caféier *Hypothenemus hampei* Ferr. est reconnu comme un ravageur redoutable par tous les pays producteurs de café. Ce petit coléoptère décrit par Ferrari en 1867 et identifié pour la première fois au Gabon 1901 (Ticheler, 1961) est actuellement présent dans tous les pays où le café est cultivé exceptés la Papouasie-Nouvelle Guinée, les Iles Salomon, l'Australie et Hawaï (Mathieu, 1995).

La femelle du ravageur rentre dans le fruit par l'apex et creuse des galeries dans lesquelles elle pond. Les larves en se nourrissant de l'albumen des graines accentuent les dégâts.

Les fruits peuvent être attaqués à tous les stades. Les pertes sont essentiellement dues à la perte de poids des fruits à la récolte mais aussi à la chute des petits fruits scolytés. A ces deux pertes, s'ajoute celle provoquée par la dépréciation du produit marchand.

Parmi les nombreuses méthodes d'échantillonnage en vue d'estimer les niveaux d'attaque, Bruneau de Miré (1976), préconise la prise au hasard de 2000 fruits, Decazy, (1984) propose une taille minimum de 500 fruits. D'autres méthodes ont été expérimentées par Baker et al et Sanchez (1984) au Mexique.

Les niveaux d'attaques varient d'un pays à l'autre. Le Pelley (1973) rapporte que plus de 90% des fruits peuvent être attaqués et les pertes à la récolte se situeraient entre 40-80%. En Amérique du Sud, les taux d'attaque sont compris entre 20-60% (Castro, 1985 ; Baker, 1984 ; Barrera, 1992), entre 27-59% en Jamaïque (Reid et Mansingh, 1985), 38-97 % aux Philippines (Morallo-Rejesus et Baldos, 1980) et sont de 50% en Nouvelle Calédonie et en Malaisie...

La lutte chimique est fonction du seuil de dégât économique. Ce seuil est évalué à 4 % par Lotode (1986) au Guatemala alors qu'en Afrique il est arbitrairement fixé à 5%.

Le but de cette étude est d'évaluer l'impact du scolyte sur la production caféière à partir d'une méthode d'échantillonnage qui prenne en compte en même temps que la perte de poids à la récolte, la perte induite par la chute des fruits scolytés. Cette méthode d'échantillonnage devra permettre au caféiculteur de prévoir les pertes réelles par un seul échantillonnage et de décider de l'opportunité du traitement insecticide.

1- Matériel et méthodes

Les études ont été conduites pendant 2 ans dans des plantations paysannes de 7 à 15 ans plantés de 6 à 7 clones de caféier Robusta en randomisation totale de 2,5 m x 3 m à la densité de 1333 pieds par hectare.

1.1- Méthodes d'échantillonnage

La caféière togolaise a été divisée en 5 zones agroéclimatiques Kpalimé, Kpélé, Dayes, Akébou, Akposso. Dans chaque zone, trois parcelles d'un hectare environ bien réparties ont été choisies.

Un mois après la grande floraison, 50 caféiers sont choisis au hasard dans chaque parcelle. Sur chacun des caféiers, un rameau bon producteur est retenu. Sous ce rameau, une gouttière en grillage moustiquaire est placée de façon à récupérer les fruits qui tombent (chute physiologique et chute provoquée par le scolyte).

Cette méthode permet de prendre en compte dans le calcul des pertes les fruits scolytés qui tombent entre deux observations. Ces fruits se reconnaissent par la perforation caractéristique près de l'apex.

Les observations ont lieu tous les 14 jours pendant deux mois puis après tous les 28 jours jusqu'à la récolte.

A chaque observation, les fruits sains et les fruits scolytés sont comptés séparément glomérule par glomérule. Dans le panier, les fruits scolytés tombés sont également dénombrés.

Le taux d'attaque à chaque observation, le taux d'attaque à la récolte, le taux d'attaque réel dans chaque parcelle et le pourcentage de chute des fruits ont été déterminés.

Pour calculer le pourcentage de perte à la récolte dû aux attaques d'*Hypothenemus hampei*, un lot de 10.000 fruits sains et un lot de 10.000 fruits scolytés sont prélevés à la récolte dans chaque parcelle. Les différents lots sont séchés, décortiqués séparément puis pesés après avoir ramené leur taux d'humidité à 12%.

1.2- Taux d'attaque et taux de chute

Les taux d'attaque :

Le taux d'attaque à la récolte correspond au taux d'attaque à la dernière observation (avant la récolte).

$$T1 = \frac{\text{Fruits scolytés comptés à la dernière observation}}{\text{Total de fruits comptés (sains + scolytés)}} \times 100$$

Le taux d'attaque réel de la plantation tient compte des fruits scolytés chutés.

$$T2 = \frac{\text{Fruits scolytés comptés à la dernière observat°} + \text{fruits scolytés chutés}}{\text{Total de fruits comptés + fruits scolytés chutés}} \times 100$$

Le taux de chute est exprimé comme suit :

$$TCH = \frac{(S_1 + P_1) - (S_f + P_f)}{S_1 + P_1} \times 100$$

S_1 et P_1 : fruits sains et fruits perforés à la première observation.
S_f et P_f : fruits sains et fruits perforés à la dernière observation.

Les taux de chute ont été calculés pour 14 parcelles scolytées.

A chaque observation, les fruits scolytés tombés sont comptés et leur pourcentage par rapport à la production totale déterminé.

1.3- Méthode de calcul des pertes à la récolte

Après décorticage des 10.000 fruits sains et des 10.000 fruits scolytés, le taux d'humidité de chaque lot est ramenée à 12%. Les lots sont ensuite pesés séparément.

Soient **M** et **N** les poids respectifs des lots de café sains et scolytés et μ le taux d'attaque de la parcelle considérée à la récolte, le poids de café grain issu de 100 fruits dont μ sont perforés est :

$$A = M\,(100 - \mu).\,10^{4} + N\,\mu\,10^{4}\ (Kg)\ (1)$$

La perte de poids pour 100 fruits dont μ sont scolytés est donc :

$$y_1 = \mu\,(M - N)10^{2}/M\ (\%)\ (2)$$

1.4- Perte due à la chute des fruits scolytés

Cette perte est absolue. Elle est estimée par rapport à la production moyenne (800kg/ha).

$y_2 = R.t$ où t = Pourcentage de fruits scolytés tombés. (3)
R = Rendement (kg/ha)

La perte totale occasionnée par le scolyte est égale à la somme des pertes à la récolte et celles dues à la chute des fruits scolytés tombés.

1.5- Seuil de dégât économique

Le seuil de dégât économique est la densité de population pour laquelle les mesures de lutte chimique doivent être prises pour éviter qu'une pullulation massive du ravageur n'occasionne un niveau de perte en café marchand supérieur au coût de traitement. C'est le niveau de perte en café dont la valeur correspond au coût du traitement insecticide (Decazy, 1989).

Il est exprimé de la manière suivante :

Y = C/R.p (%) (4) où
Y = Perte totale en café marchand (%)
C = Coût du traitement insecticide en F/ha.
$Y = y_1 + y_2$ (5)
y_1 = perte de poids à la récolte et y_2 = perte de poids provoquée par la chute des fruits scolytés.
R = Rendement/ha

Les taux d'attaque à chaque date d'observation, les taux d'attaque à la récolte et les pertes en poids à la récolte pour chacune des parcelles ont permis de déterminer la date à la quelle doit s'appliquer avec efficacité le traitement insecticide.

Le seuil de dégât économique correspond donc au taux d'attaque à cette date précise.

2- Résultats

Les niveaux d'infestation et les pertes

Les taux d'attaque réels sont compris entre 0,4 et 19,4% (Tab.1). Les pertes relatives à ces taux varient entre 0,16% et 11,6%/ha (Tab.2). Ces taux de perte correspondent à 13- 93 kg de café marchand par hectare.

Les taux de chute

L'étude de l'évolution des chutes dans 14 parcelles dans le temps révèle que les parcelles les plus attaquées ont en général les taux de chute les plus élevés.

Tableau 1 : Taux d'attaque totale par parcelle et par an

ZONES	Année 1			Année 2		
	PARCELLES					
	1	2	3	1	2	3
* Kpalimé	1.43	6.08	1.18	2.25	3.07	
* Kpélé	0.40	-	7.77	3.44	2.58	3.38
** **Dayes**	**6.35**	**7.23**	**19.43**	**13.37**	**6.40**	**11.35**
** Akposso	3.39	4.14	5.62	10.76	-	3.05
** Akébou	4.36	14.27	2.06	7.34	10.40	8.58

* Plaines
** Plateaux

Tableau 2 : Pourcentage de perte totale par parcelle et par an

ZONES	Année 1			Année 2		
	PARCELLES					
	1	2	3	1	2	3
* Kpalimé	2.95	2.32	0.62	0.40	3.31	-
* Kpélé	0.16	-	3.42	1.96	1.45	1.31
** **Dayes**	**2.30**	**6.27**	**11.60**	**7.27**	**3.18**	**6.26**
** Akposso	1.41	1.51	2.33	3.81	-	2.11
** Akébou	1.05	5.05	0.78	5.31	4.39	1.28

Seuil de dégât économique

La valeur du seuil économique est le niveau d'attaque dû au déprédateur correspondant à une intensité précise de la population dont les dégâts sont prévisibles ainsi que les frais totaux occasionnés par la lutte préventive (Fröhlich, 1975).

$$Y = \frac{C}{R.p} \times 100$$

$$Y = y_1 + y_2$$

y_1 = perte à la récolte

y_2 = perte due à la chute des fruits scolytés.

Le tableau de corrélation entre les taux d'attaque aux différentes dates d'observation et les pertes en poids a montré que le pourcentage de fruits scolytés tombés et le pourcentage de fruits perforés 4 mois après la floraison sont liés par la relation :

$$y_2 = 0{,}07\ \delta - 0{,}03 \qquad (R = 0{,}69) \quad (6)$$

y_2 = pourcentage de fruits perforés tombés.

δ = pourcentage de fruits perforés 4 mois après la floraison.

Le pourcentage de perte à la récolte est corrélé au pourcentage de fruits perforés à la récolte

$$y_1 = 0{,}40\ \mu - 0{,}31 \qquad (R = 0{,}60) \quad (7)$$ où μ est le taux d'attaque à la récolte.

Le taux d'attaque à la récolte μ et le taux d'attaque 4 mois après la floraison sont corrélés. $\mu = 1{,}7\ \delta + 2{,}6 \qquad (R = 0{,}80)$ (8)

$Y = y_1 + y_2$ ---- > $Y = 0{,}766\ \delta + 0{,}7$ (9)

La relation (4) devient :

$$\frac{C}{Rp} = 0{,}766\ \delta + 0{,}7 \qquad (10)$$

où δ représente le taux d'attaque 4 mois après la nouvelle floraison, il représente également le seuil de dégât économique c'est-à-dire le niveau de populations qui est corrélé avec les pertes.

Ainsi, à titre d'exemple, le calcul de la valeur du seuil économique pour l'endosulfan, l'insecticide le plus efficace contre le scolyte des fruits (Brun et Ruiz, 1987 ; Brun et al, 1989) dans les conditions togolaises est le suivant :
Les frais correspondant à une seule application s'élèvent à environ 15.000 F/ha, le prix d'achat au producteur du café marchand est de 600000F/ha,le seuil de dégât économique $\delta = 2,5$. *

Discussion et conclusion

La méthode d'échantillonnage utilisée permet de prendre en compte les deux types de perte (perte de poids à la récolte et perte de poids occasionnée par la chute des fruits perforés).

Les différentes méthodes d'échantillonnage déjà signalées négligent dans le calcul des taux d'attaque, des pertes et des seuils de dégât économique, les pertes occasionnées par la chute des fruits scolytés or ces pertes sont importantes car c'est tout le fruit qui est perdu.

Les taux d'attaque enregistrés au Togo sont faibles par rapport au taux habituellement signalés pour les pays africains : (40-80%) (Ticheler, 1961).

Le faible niveau d'attaque au Togo serait dû à la présence d'une faune parasitaire constituée par trois Hyménoptères : *Cephalonomia stephanoderis*, *Prorops nasuta* et *Phymastichus coffea*. Les deux premiers sont des ectoparasitoïdes Bethylidae des stades immatures et le troisième découvert pour la première fois au Togo par Borbon-Martinez, 1989 et décrit par La Salle, est un endoparasitoïde Eulophidae des adultes de scolyte.

Le seuil de dégât économique moyen pour le Togo est donc de 2,5% 3-4 mois après la grande floraison. Il est inférieur au seuil de 5% arbitrairement choisi pour tous les pays d'Afrique : (Remond, 1992).
Ce seuil varie suivant les zones de production et doit être actualisé en fonction du prix d'achat au producteur et du prix du produit insecticide.

La méthode ci-dessus décrite permettra une meilleure estimation des dégâts pour une gestion plus économique de la lutte chimique.

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- Résumé -

Le scolyte des fruits du caféier *Hypothenemus hampei* Ferr.est reconnu par tous les pays producteurs du café comme un ravageur redoutable. Présent dans la plupart des pays producteurs, ce petit coléoptère provoque des pertes de production importantes . Les nombreux travaux réalisés sur le ravageur ont porté sur l'estimation des pertes, la bioécologie, les méthodes de lutte...

Au Togo, l'échantillonnage de 50 rameaux (sous lesquels sont placées des gouttières en grillage moustiquaire destinées à récupérer les fruits scolytés qui tombent entre deux observations) choisis sur 50 caféiers eux-mêmes retenus de façon aléatoire par hectare a permis de déterminer le niveau d'infestation compris entre 0,4 - 19,43% ce qui correspond à 0.16 à 11.6 % de perte en café marchand par hectare.

Les plantations situées en hauteur (400 - 700 m) ; (Plateau de Dayes, Plateau Akposso-Akébou) sont plus attaquées que celles des plaines.

Les parcelles les plus attaquées ont les taux d'infestation les plus élevés. *Hypothenemus hampei* provoque des pertes non négligeables dues à la chute des fruits scolytés.

Le seuil de dégât économique est calculé par la relation $C/Rp = 0.766\ \delta + 0.7$ où C est le coût du traitement insecticide, **R** le rendement par hectare, **p** le prix du Kg de café marchand et δ le seuil de dégât économique qui est égal à 2.5%.

- Summary -

The coffee berry borer is the most important pest in all coffee producing countries. This small coleoptera which is present in almost all the producing countries causes heavy losses. A number of works have been done on the subject.

In Togo, 50 branches of coffee selected on 50 coffee trees per hectare was study to estimate the rate of infestation which is between 0.4 - 19.43% corresponding to 0.16 - 11.6% loss of commercial coffee per hectare.

The plantations situated on high lands (400 - 700m), (Dayes Plateau; Akposso-Akébou Plateau) are more attacked than those which are in plain.

The most attacked plots had the highest rate of infestation. *Hypothenemus hampei* provokes significant losses due to the dropping of small fruits.

The control decision index is calculated by the relation $C/Rp = 0.766\ \delta + 0.7$ where C is the cost of insecticide treatment, R is the yield, p is the Kg's price of the commercial coffee and δ is the decision index which is equal to 2.5% in our case.

BIOLOGICAL CONTROL OF COFFEE INSECT PESTS IN KENYA

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INTRODUCTION

Indiscriminate use of insecticides especially the broad spectrum and persistent ones for instance DDT and Dieldrin in control of pests in the early years, resulted in changes in pests status. Pests that were of minor importance turned to be major coffee pests. Examples of these include leafminer, Giant looper, Green Scales and Brown Scale whose natural enemies were reduced drastically leading them to multiply exceedingly (Crowe, 1994; Bedford and Bohler, 1982). In addition to this , there is pest resistance to insecticides and the increasing cost of the same that has necessitated the adoption of different strategies for control of insect pests.

In light of the foregoing, the use of toxic insecticides has been scaled down by:

(1) Use of of selective insecticides thereby preserving the natural enemies which in turn help to check the pest population.
(2) Use of thresholds for spray programmes ie. spray only when economical damage is imminent.
(3) Introduction of natural enemies to pest areas to keep them below economical thresholds.
(4) Conservation of existing natural enemies by manipulating the environment to suit their survival and multiplication.
(5) lastly periodic release of local natural enemies to improve their impact on the pest.

At coffee Research Foundation (CRF) several activities are taking place in the field of biological control and these include:

(1) Search and identification of new natural enemies of the various coffee pests.
(2) testing the efficacy of natural enemies both in the laboratory and field.

(3) rearing of natural enemies.
(4) Study of the biology of natural enemies in relation to their hosts for biocontrol purposes.
(5) study of dispersal of natural enemies in the field once released.

These activities and their success are described for six major coffee insect pests in this paper.

PESTS AND THEIR BIOCONTROL
ANTESTIA BUG (*Antestiopsis spp.*) (Hemiptera:Pentatomidae)

The bug is a major pest of coffee throughout Africa (Le pelley 1968). In Kenya it is a major pest in all coffee areas especially in the west of the rift valley (Anon, 1993). Damage caused include; blackening of flower buds, fall of immature berries, rotting and zebra stripping of beans. A 24% loss in bean weight and 7-35 % berry drop can be caused by 2 and 2-8 bugs respectively (Wanjala, 1980).

There are several scelionidae that parasitize Antestia bug and these include *Asolcus mopus* , *Asolcus suranus*, *Baryconus sp.*, *Hadronotus antestiae* and *Asolcus seychellensis* (Le pelley, 1968). Other egg parasites of Antestia, *Anactatus antestiae* and *Acrodisoides africanus* are described by Ferriere (1940). Adult parasites *Bogosia rubens* and *Bogosia antenorii* are mainly found in western Kenya.

Bogosia rubens which parasitizes adult Antestia is very difficult to rear in the laboratory. Moreover Antestia females parasitised by them are still fecund (Wanjala, 1982). Due to this shortcomings, more attention at the Coffee Research Foundation has been focused on egg parasites. The egg parasites that have been worked on considerably are *Asolcus seychellesis* and *Hadronotus antestiae*. The former is more efficient at low host densities and hence better for biocontrol.

Egg parasitism can go up to 90 % in the field (Dry,1921) and one female of Asolcus can parasitize up to 40 eggs(Le Pelley, 1968).

Laboratory rearing of egg parasitoids, *Asolcus seychellensis* is done in 2.5x15 cm test tubes in which eggs of Antestia stuck on cards by glue are inserted. Parasitoids are then released into the tubes for parasitization and fed on undiluted honey provided as a smear in the tubes. The parasitised eggs are then kept for emergence of parasitoids. Emerged adult parasitoids are released in the field for control purpose. A average of 120,000 parasitoids are released every year for augmentation of field population. Level of parasitism in the field and parasites dispersal rates are monitored in order to evaluate the efficacy of the egg parasites in keeping levels of Antestia below economic threshold.

COFFEE BERRY BORER (*Hypothenesmus hampei* Ferri)(Coleoptera: Scolytidae)

Coffee Berry Borer (CBB), *Hypothenemus hampei* Ferri originally reported in France in 1860s has become a serious pest of Coffee in several Coffee producing areas in the World and particularly in Kenya. The first CBB incidence in Kenya was reported in 1928 (Wilkinson, 1929) with infestation levels

ranging from 0.7% to 7.8%. CRF Annual report of 1975/76 reported localised outbreaks of CBB in some estates near Thika. A survey conducted in Kenya, especially in the Western region revealed an infestation level of as high as 80% occurring during the peak season with insignificant parasitism level of 18% (Murphy *et al* IIBC unpublished report, 1987).

Coffee Berry Borer control involve use of chemicals, cultural, and biocontrol approaches. However, insecticides used are very expensive while recommended cultural practises are rather tedious and labour intensive. The biocontrol practised involve use of pathogens and parasitoids. Le Pelley (1968) listed the major natural enemies that occur in Africa. A survey conducted in Kenya by Murphy *et al* (1986,1987, unpublished) reported two parasitic wasps; *Prorops nasuta* Watson and *Heterospilus Coffeicola* Schmied as parasitizing the borer with highest parasitism level of 18% caused by *P. nasuta*. An attempt to mass rear *P. nasuta* is being undertaken at CRF.

Thicheler (1961) from Ivory Coast, West Africa recorded a parasitic wasp, *Cephalonomia stephanoderis* (Betrem) as causing upto 50% parasitism. Thus the classical biocontrol using *C. Stephanoderis* may be applicable in Kenya to control the CBB. However, to aid parasitism by the parasitic wasps, well pruned Coffee is recommendable. This provides good breeding environments for these agents.

GIANT LOOPER (*Ascotis selenaria reciprocaria*)(Lepidoptera: Geometridae)

Giant looper, *Ascotis selenaria reciprocaria* (Walk) is one of the most important pest of Arabica coffee in Kenya. Its emergence as a major coffee insect pest has occurred as a result of excessive use of insecticides especially the organophosphates (Le pelley, 1968).

The Giant looper larval and young caterpillars chew circular holes right through the leaf or at the margin of the leaf, leaving a jagged edge. All stages prefer fresh leaves and sucker growth.

Biological control attempt of Giant looper in CRF has mainly utilized *Macrorhaphis spp.* and *Rhinocoris spp.* The above predator posses the piercing-sucking mouthpart with which they pierce and suck the fluid in the Giant looper caterpillar rendering the pest immobile and thus the subsequent death.

The two predators are successfully utilized in the Biological control of Giant looper in Kenya.

COFFEE BERRY MOTH (*prophantis smaragdina*)(Lepidoptera:Pyralidae)

Berry moth, *P. smaragdina* is one of the major insect pests that attack coffee fruits. The larva of berry moth bores into small and half grown berries, eats one or more seeds then passing to another in the same cluster, webbing the berries together. The berry cluster are suite readily noticed in the field through the webbing of silk containing dried hollow berried of different sizes.

Four hymenopteran parasitoids that attack the larval stage of *Prophantis smaragdina* viz. *Apanteles coffeae*, *Cractocnema sp.*, *Macrocentrus sp. Priestomerus sp.*, and an egg parasitoid-

Phanerotoma sp. have successfully been used for the control of the pest in CRF.

The larval parasitoids drill a hole into the healthy Berry Moth larva and lay an egg. Upon pupation, the parasitized pupa can be noticed by its white colour as opposed to the normal golden colour. A larval parasitoid emerge from the parasitized larva.

CRF mass rear the parasites in the laboratory and release in the farmers coffee farms.

COFFEE SCALE INSECT

Nine (9) Coffee Scale Insect pests in the order Homoptera attack Kenyan Coffee (Njeru, 1985). These include Green Scales (*Coccus alpinus)*, Kenya Mealy bug (*Planococcus kenyae*) and Fried egg scale (*Aspidiotus sp*) among others. Of these, *C. alpinus* and *Aspidiotus sp.* are of major economic importance (Mugo, 1994).

GREEN SCALES *(Coccus Alpinus)* (Homoptera : Coccidae)

Green Scale is a common pest of both mature and immature Arabica Coffee. Natural control of Green Scale include many species of ladybird beetles. Le Pelley (1968) listed *Coccophagus nubus* and *C pulvinariae* as parasitic wasps, and *Chilocorus angolensis, Hyperapsis Senegalensis* as Green Scale predators. The ongoing biocontrol of Green scale at Coffee Research Foundation (CRF) has realised some more locally available parasitoids and predators with potential efficiency namely *Metaphycus baruensis* (Moyes), *M. Swierhii* (Amanyn), *Lamennaisia sp.*, *Coccidoxenoides peregnus* (Tims) and *Olivershineruus sp.* among the parasitoids, and *Chilocorus nigripes* (Mader), *C. quadrimaculatus* (Weise) and *Hippodamia Variegata* (Goeze) as the predators (unpublished). Mugo (1996) studied the predation efficiency of three most common ladybird beetles namely, *C. nigripes, C. angolensis* and *H. senegalensis* against the Green Scales. The *C. angolensis, C. nigripes* and *H. senegalensis* had mean Green scales predation of 6.02, 5.84 and 3.85 per day, respectively. These predators are presently mass reared, and released to Coffee Farmers. However, the efficiency of these biological control agents is promoted by keeping away the attendant ants from the Green scale through banding.

FRIED EGG SCALE *(Aspidiotus sp)* (Homoptera Diaspididae).

This is a new scale insect that has attained pest status in Coffee (Anon, 1989). It was first reported in 1977 (Waikwa and *et al*, 1983). *Aspidiotus sp.* is recognized by its Circular Crustily white with yellow brown centre resembling fried egg. Unlike other scales, such as Green scale, Kenya mealybug, it's infestations are neither accompanied by sooty mould nor by any attendant ants.

Investigations carried out at CRF have revealed several local potential predators and parasitoids being associated with the scale. These includes *Encarsia fusca* (Carycre), *Asphytis sp* and *Marietta leopardia* (Mobch), among the parasitoids, and *C. angolensis, C. nigripes, H. senegalensis* and *Exochomus havipes.* Thumb, the ladybird beetles (Predators) (unpublished). The

studies on efficacy of *C. angolensis*, *C. nigripes* and *H. senegalensis* on Aspidiotus showed that these predators had mean Fried egg scales predation of 8.73, 4.31 and 3.20 per day, respectively (Mugo,1996). Both the parasitoids and the predators are being augmented and released to the Coffee fields.

YELLOW HEADED BORER (*Dirphya nigricornis)* (Coleoptera cerambycidae)

The borer is one of the most important coffee pest in Kenya causing large crop losses (Wanjala, 1985). The larval of this pest enter the tip of stems and bore downwards punching holes for frass emition as it moves down into the main stem. The tunnel get bigger as the larval grow thus causing heavy destruction of the stem.

In Kenya, *Iphiaulax varipalpis* (Hymenoptera: Braconidae) is the main parasite that participate in the regulation of *D. nigricornis* (Wanjala 1985). Apparently no egg, pupa or Adult parasitism or predation had been observed earlier than 1985. The new parasites recorded by Wanjala, 1985 included *Microplitis sp.*, *Camptotypus (Hemipinpla) sp.* and *Ectopsocus sp.* These occurred commonly in most areas. Other natural enemies found in abundance included ants (Formicidae) which attack larvae and pupae (Wanjala, 1985). Several birds were also found predating on flying adults.

As the pest is getting more important, the search for more natural enemies has intensified. This involves culturing of damaged stems with larvae of yellow headed borer. The rearing of *Iphiaulax varipalpis* is also being tried using the natural host ie larvae of *D. nigricornis*.

FUTURE PLANS OF MANAGEMENT

Future programmes intended to contain the major coffee insect pests referred, require intensification of biocontrol method through :-

* population studies of both the hosts and their natural enemies to determine conditions under which the natural enemies can be most effective.
* Formulation of collaborative classical biocontrol projects
* Augmentation of identified potential natural agents and releasing them to the farmers.
* Assessing the post - release impact of biocontrol agents.
* Integration of biocontrol with chemical management practices.

ACKNOWLEDGEMENT

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ABSTRACT:

Biological control of major Coffee pests pursued in Kenya are highlighted. The pests in question are Antestia bug (*Antestiopsis spp*), Coffee Berry Borer (*Hypothenemus hampei*), Giant Looper (*Ascotis Selenaria reciprocaria*), Berry Moth (*Prophantis smaragdina*), Scales (*Coccus alpinus* and *Aspidiotus sp*) and Yellow Headed Borer (*Dirphya nigricornis*).

The natural enemies (N.E) of different Coffee insect pests feeding or parasitizing on their host as in the case of *Ascotis Seleneria reciprocaria* and *Antestiopsis sp* eggs by *Asolcus Seychellensis* are discussed.

Two parasitoids that attack the larvae stages of *Prophantis Smaragdina* viz *Apanteles Coffeae* and *Prestomerus sp* are presented. A wasp natural enemy of Coffee Berry Borer and a ladybird species that predate on *Coccus alpinus* and *Aspidiotis sp* are also presented besides the larval parasite of *Dirphya nigricornis*.

Information on the efficacy and the status of the natural enemies of the coffee insect pests referred are also discussed.

PHYLOGEOGRAPHIC PATTERNS OF INTRODUCED POPULATIONS OF THE COFFEE BERRY BORER *HYPOTHENEMUS HAMPEI* (FERRARI) (COLEOPTERA : SCOLYTIDAE) INFERRED FROM MITOCHONDRIAL DNA SEQUENCES

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The coffee berry borer, *Hypothenemus hampei* (Ferrari), is the major insect pest on coffee. This beetle has now been recorded from most of the major coffee growing regions of the World (Le Pelley, 1968). The recent finding of endosulfan resistance in the South Pacific island of New Caledonia is thus a major threat to the international coffee industry (Brun, Marcillaud, Gaudichon, & Suckling, 1989). *H. hampei* has spread from equatorial Africa, the putative ancestral origin, during the last two centuries. *H. hampei* was first reported from Gabon in 1901, later in Central African Republic, Tchad and Congo 1902-1904. In Uganda attacks were noticed in 1908 and 1909. The first reference on introductions to the Far East is Java 1909. Subsequent findings were New Caledonia 1948, Philippines 1960, Tahiti 1961 and Fiji. It's introduction to the Western Hemisphere can be traced through Brazil 1924, Puerto Rico 1946, Surinam 1960, Peru 1962, Nicaragua and El Salvador 1969, Guatemala 1971, Mexico 1978, Jamaica 1978 and Ecuador 1981.

Mitochondrial DNA (mtDNA) data in population genetic studies are advantageous in revealing historical, and phylogenetic perspectives on intraspecific population structures, because of the maternal nonrecombining mode of mtDNA inheritance and rapid evolution in mtDNA sequences (Avise et al., 1987). The molecule often provides multiple alleles or haplotypes that can be ordered phylogenetically within a species, yielding intraspecific phylogenies (gene genealogies) interpretable as a matriarchal component of the organismal pedigree. Furthermore, mtDNA clones and clades within many species have proved to be geographically localised. Phylogeography refers to the study of the principles and processes governing the geographical distributions of gene genealogical linages at the intraspecific

level. We propose here to make use of the phylogenetic approach in order to understand the recent history of the CBB and then track the dispersal routes. The phylogeography of this species could be revealed by comparing the geographic distribution with the intraspecific phylogeny based on variation in mtDNA sequences.

We examined 17 populations of *H. hampei* collected world-wide by using PCR amplification and direct nucleotide sequencing. A total of 1,200 base pairs from the mitochondrial locus cytochrome oxidase I (Fig 1.) showed 21 variable positions or 1.8% variation between populations. Three other species were assigned as outgroup, *Hypothenemus obscurus*, *Cryphalus sp.* and *Coccotrypes dactyliperda.*

Examination of the most parsimonious tree of the mitochondrial data (Fig 2.) suggests that outside Kenya, the putative ancestral origin of the species, the world has been colonised by only two inbreeding lines of *H. hampei*. One encompassing Mexico, Central and Southern America (Columbia, Honduras and Mexico) and the other all strains from South East Asia and the South Pacific plus populations from Jamaica and the Ivory Coast. This data along with reports on introduction, support a hypothesis of the recent global spread of a few inbreeding coffee berry borer lines.

Conclusions and Future Directions

We have examined the nucleotide variation in the mitochondrial loci of *H hampei.*

Several broad conclusions can be made. There have been at least two introductions of CBB to the American continent, Jamaica and the mainland population. Jamaica has not been colonised from the American mainland as previously thought. East African populations are the origin of the South East Asia and the South Pacific populations, or an recolonisation to Africa

Our future work in this area will therefore focus upon more detailed comparison between populations by using more variable genetic markers, in order to reveal more about *H. hampei's* evolutionary history. Particularly important will be sampling native (central African) populations of *H. hampei*, since nothing is known about the genetic composition of natural populations

Summary

The coffee berry borer, *Hypothenemus hampei* (Ferrari), is one of the most serious pests in commercial coffee production. The species is thought to have originated in equatorial Africa but has now spread to most of the major coffee growing regions of the World; it was first recorded from the Far East (Java) in 1909 and the Neotropics (Brazil) in 1924. The genetic variation of 17 widespread populations of H. *hampei*, was assessed by sequencing a 1200bp regions of the mtDNA genome. A cladistic analysis resolved the haplotype sequences into three main lineages: 1) Mexico, Central America and South America, 2) Central Africa, 3) an unresolved group including the populations from Asia, Ivory Coast, Jamaica and New Caledonia. These results give us an insight into both the genetic relationships among far-flung populations and the main patterns in the dispersal. Three of the populations from New Caledonia are resistant to endosulfan and other cyclodiene-type insecticides. We suggest that this sort of analysis may serve in risk assessment of potential resistance in closely related populations.

Figure 1: Diagram showing the relative location of the region sequenced from the single mitochondrial locus cytochrome oxidase I/II (COI/COII). PCR products were sequenced directly using an ABI 373 automated sequencer (Applied Biosystems).

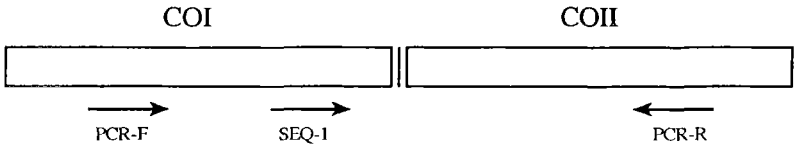
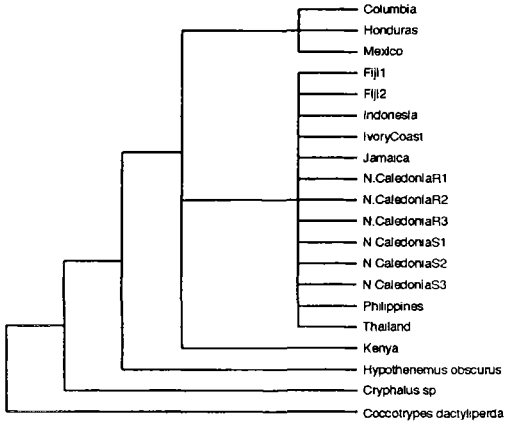


Fig. 2 Strict concensus tree of the most parsimonious trees (PAUP vs. 3.0)



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L'ÉCHANTILLONNAGE DES DÉGÂTS CAUSÉS PAR LE SCOLYTE DU FRUIT DU CAFÉIER (*HYPOTHENEMUS HAMPEI* FERR.) : Définition de procédures de sondage sur le terrain

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1. Introduction

Depuis le début du siècle, le scolyte, *Hypothenemus hampei* Ferr., est responsable de pertes considérables en caféiculture. Le préjudice est important tant au niveau de la quantité que de la qualité du café produit :
- Les attaques des scolytes ont souvent été incriminées dans la chute des jeunes fruits. Si Borbon-Martinez (1989) constate effectivement un effet du scolyte sur cette chute, Barrera (1994) ne trouve pas le même résultat. Il constate que les taux d'attaque par le scolyte des jeunes fruits ayant chuté ne sont pas différents de ceux des jeunes fruits restés sur l'arbre.
- La perforation réalisée par le scolyte constitue une porte d'entrée pour les maladies. Les attaques du scolyte vont donc favoriser l'apparition de fruits pourris.
- Le développement des larves de scolyte à l'intérieur du fruit se faisant au dépend de la graine c'est à dire de la partie commercialisable, il y aura donc une perte de poids des grains attaqués.
- La perforation du grain de café constitue une cause de dépréciation de la qualité du café. Le producteur doit soit trier les grains, soit vendre du café non trié qui subit alors des pénalités financières en fonction du taux d'attaque.

Ochoa-Millian *et al* (1989) estiment que les pertes totales peuvent atteindre 30 à 35 % de la production. D'un point de vue économique, le scolyte est le ravageur qui provoque les pertes les plus importantes en caféiculture.

Diverses méthodes de lutte contre le scolyte sont utilisées. Actuellement, la lutte est encore essentiellement chimique et culturale, mais la lutte biologique se développe grâce. Il n'existe pour le moment aucune lutte génétique. L'expérience montre que la lutte chimique ne permet pas d'éradiquer cet insecte, une partie des individus se disséminant par des vols qui peuvent être relativement longs (Decazy, 1990). La seule solution est donc de tenter de maintenir les populations, et donc les dégâts, à des niveaux acceptables.

La lutte contre cet insecte nécessite une bonne connaissance des dégâts qu'il occasionne. Les échantillonnages doivent donc permettre aux scientifiques de mieux évaluer l'action des mesures de contrôle et au producteur de décider de l'opportunité de la mise en place des méthodes de lutte et notamment de la libération des parasitoïdes, des quantités à lâcher et des meilleurs sites de lâcher. Il s'agit donc de définir des procédures d'échantillonnage pour estimer au mieux les attaques du scolytes en optimisant le choix des arbres dans les parcelles à évaluer ainsi que le choix des fruits dans les arbres échantillonnés.

De précédents travaux ont permis de mettre en évidence la supériorité de l'échantillonnage systématique pour le choix des arbres dans les parcelles d'étude (Rémond *et al*, 1995). L'échantillonnage de fruits "au hasard" entraîne toujours un biais important dans le sens de la surestimation du taux d'attaque. La meilleure fiabilité de l'échantillonnage systématique a été montrée expérimentalement, probablement en raison de la répartition agrégative des attaques (Decazy *et al*, 1989 ; Rémond *et al*, 1993). Il s'agit maintenant de quantifier les précisions obtenues pour différents systèmes d'échantillonnage et cette quantification a été possible grâce aux techniques de simulation.

2. Matériel et méthodes

Les données utilisées proviennent de trois sources :
- un relevé exhaustif des fruits sains et attaquées sur une parcelle du Salvador,
- une campagne de mesures effectuée en 1993 sur une parcelle du Guatemala. Dans cette parcelle, d'environ 400 arbres, 1/4 des glomérules sont échantillonnés sur tous les arbres
- des mesures identiques effectuées sur une parcelle au Nicaragua.

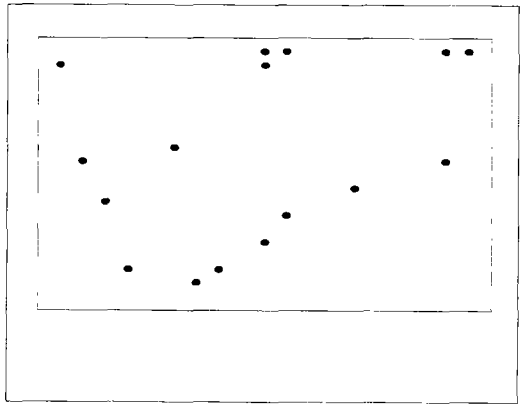
A partir de ces données il est possible de simuler différents systèmes d'échantillonnage d'arbres et de calculer des intervalles de confiance pour différents taux de sondage. Les simulations se heurtent aux problèmes des arbres manquants. Il a été montré qu'il est préférable d'augmenter le taux de sondage en fonction du nombre d'arbres manquants plutôt que de remplacer un arbre absent par son plus proche voisin (Rémond, 1996).

Quatre types d'échantillonnage d'arbres sont testés par simulation : les échantillonnages aléatoire, stratifié, systématique et systématique en quinconce (Figure 1). Des simulations d'échantillonnages aléatoires sont effectuées sur les trois parcelles ; 4000 répétitions sont effectuées pour chaque taille d'échantillon. L'objectif de la stratification est d'améliorer la répartition des arbres dans la parcelle par rapport à un échantillonnage aléatoire en évitant d'échantillonner deux arbres proches donc corrélés entre eux. Cinq découpages de la parcelle du Salvador, qui est la plus grande des parcelles à notre disposition (21 lignes de 40 arbres), ont été étudiés : 2 strates d'environ 10×40 arbres, 4 strates d'environ 5×40 arbres, 4 strates d'environ 10×20 arbres, 8 strates d'environ 5×20 arbres et 16 strates d'environ 5×10 arbres. L'échantillonnage systématique consiste à sélectionner des arbres à intervalles réguliers, facilitant le travail de repérage. La disposition en quinconce permet de maximiser les distances entre sites d'échantillonnage.

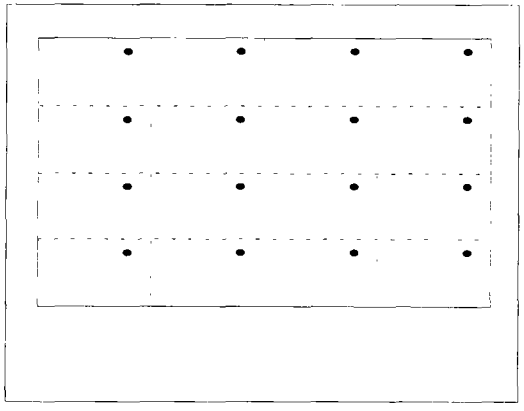
Pour le choix des fruits dans les arbres échantillonnées 3 techniques de sondage ont été testées expérimentalement : échantillonnage de 100 fruits au hasard, échantillonnage de 30 glomérules au hasard et échantillonnage systématique de 1 glomérule sur 7 (Figure 2). La confrontation entre les taux d'attaque réels et les taux d'attaque estimés par ces 3 techniques permet de juger de la fiabilité de ces 3 types d'échantillonnage.

Figure 1 : Les différentes techniques d'échantillonnage testées pour le choix des arbres dans les parcelles

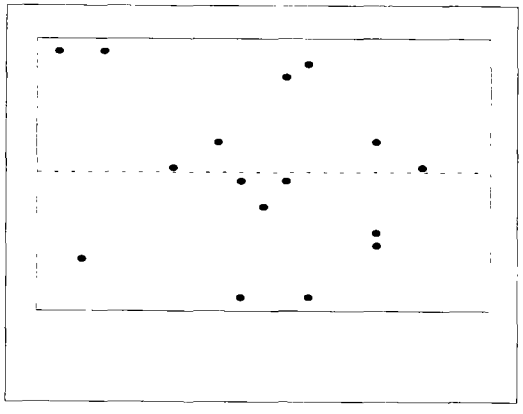
Aléatoire



Systématique



Stratifié (4 strates)



Systématique en quinconce

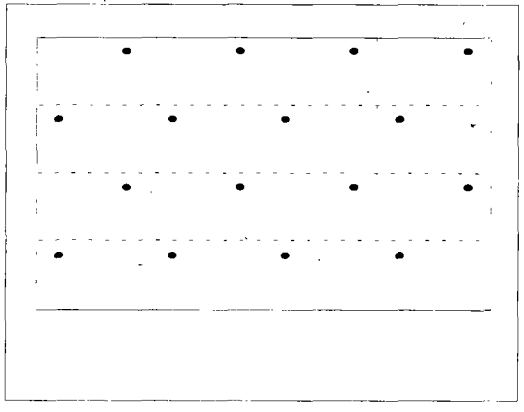
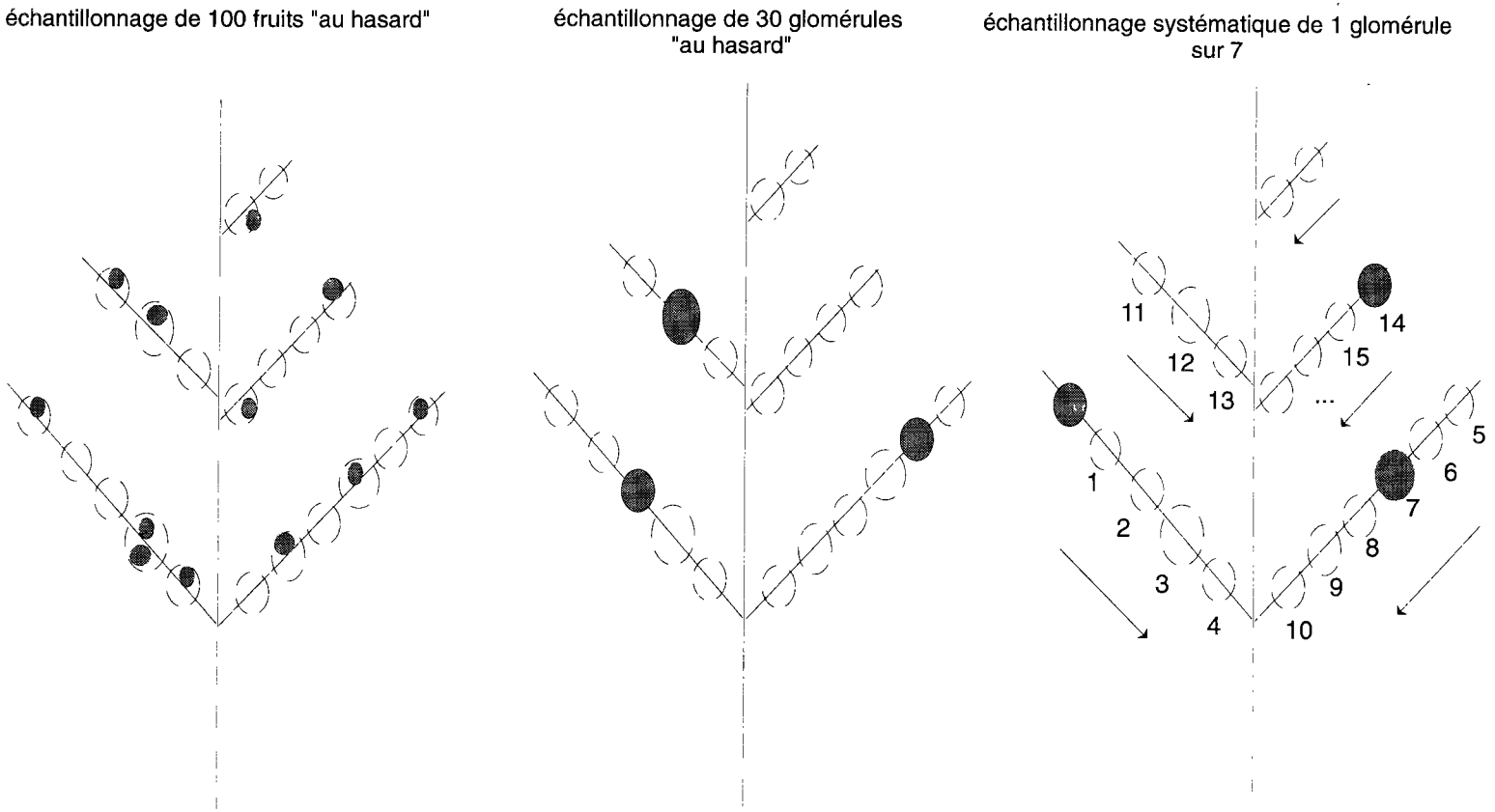


Figure 2 : Représentation schématique de différentes techniques d'échantillonnage des fruits sur un caféier



3. Résultats

Choix des arbres dans les parcelles

Pour chaque parcelle, les intervalles de confiance à 90 % sont estimés pour chacun des types d'échantillonnage et pour 2 tailles d'échantillon (Tableau 1).

Il apparaît que les échantillonnages systématiques (normal et en quinconce) sont en général meilleurs que les échantillonnages reposant sur des sélections aléatoires. Ils sont plus précis et nécessitent donc des tailles d'échantillon inférieures. Sur la parcelle du Salvador, où les différences sont les plus nettes, il faut 93 % de sites d'échantillonnage supplémentaires en échantillonnage aléatoire pour obtenir la même précision que l'échantillonnage systématique en quinconce.

Tableau 1 : Intervalle de confiance du pourcentage de fruits attaqués (p) à 90 % sur les 3 parcelles en fonction du type d'échantillonnage.

parcelle	Guatemala		Salvador		Nicaragua	
taille de l'échantillon / type d'échantillonnage	12 arbres	18 arbres	14 arbres	20 arbres	12 arbres	20 arbres
aléatoire	[0.2 ; 0.74]	[0.22 ; 0.67]	[2.5 ; 10]	[3 ; 9]	[8.4 ; 16]	[9.2 ; 15]
stratifié (strates de 10x5 arbres)	[0.2 ; 0.72]	[0.24 ; 0.68]	[2.75 ; 9.3]	[3.25 ; 9.25]	-	-
systématique normal	[0.2 ; 0.72]	[0.24 ; 0.66]	[2.6 ; 9.75]	[3 ; 8.8]	[8.8 ; 15.5]	[9.5 ; 14.3]
systématique en quinconce	[0.21 ; 0.74]	[0.22 ; 0.65]	[2.6 ; 8.8]	[3.2 ; 8.5]	[8.9 ; 15.6]	[9.8 ; 14.6]

Combien d'arbres échantillonner ?

Les conclusions des simulations amènent à préconiser d'échantillonner sur un minimum de 12 arbres pour une parcelle d'environ 400 arbres. Il n'est pas sûr qu'il soit possible de descendre sous la limite des 10 arbres pour des parcelles plus petites. Cela représente un taux de sondage d'environ 3 %. Au delà, le gain induit par l'échantillonnage d'un arbre supplémentaire diminue. Lorsque la parcelle est plus importante, il est possible de réaliser d'importantes économies d'échelle. Nous de disposons pas de données sur suffisammant de parcelle pour pouvoir conclure de manière définitive. Il parait néanmoins raisonnable de diminuer de 25% le taux de sondage de la parcelle pour 50 % d'arbres supplémentaires. La relation entre le nombre d'arbres présents sur la parcelle (n) et la taille minimale de l'échantillon (n_{min}) serait donc :

$$n_{min} = 0{,}033 \,.\, (0{,}75)^{k} \,.\, n \qquad \text{avec} \quad k = [\, \ln(n) - \ln(400) \,] \,/\, \ln(1{,}5).$$

C'est à dire que pour une parcelle de 600 arbres, la taille minimale de l'échantillon doit être de 15 arbres. Pour une parcelle de 10000 arbres, la taille minimale de l'échantillon serait de 3,3% x $(0{,}75)^{7{,}9}$ x 10000, soit 34 arbres. C'est évidemment une relation approximative d'autant plus qu'elle ne tient pas compte de la moyenne et de la variance du pourcentage d'attaque sur la parcelle.

Sur une grande parcelle d'environ 10000 arbres, 72 sites systématiques de 5 arbres ont été examinés. On simule sur ces données des échantillons systématiques (normaux ou en quinconce) de 36 arbres. L'intervalle de confiance obtenu est de [0,51 ; 1,85] pour un pourcentage d'attaque estimé à 1,03. Il semble donc que ce soit cohérent avec la formule donnée.

Choix des fruits dans les arbres

Les résultats des 3 types d'échantillonnage sont présentés dans le tableaux 2.

Tableau 2 : Moyennes des taux d'attaques et variances d'échantillonnage pour les 3 techniques testées.

Méthodes d'échantillonnage	aléatoire (100 fruits au hasard)	en grappe (30 glomérules au hasard)	systématique (1 glomérule sur7)
moyenne des taux d'attaque	6.414	3.977	3.404
Taux réel	3.446		
Variance d'échantillonnage	13.26	4.45	6.50

L'échantillonnage aléatoire provoque un biais important dans le sens d'une surestimation du taux d'attaque et sa variance d'échantillonnagee est supérieure à celle des autres techniques. L'écantillonnage systématique est le plus fiable car il donne une estiamation sans biais du taux d'attaque. L'échantillonnage en grappe demeure une alternative possible à l'échantillonnage systématique qui est plus lourd à mettre en oeuvre.

4. Conclusion

Pour échantillonner les arbres dans les parcelles à évaluer, il semble préférable d'utiliser un échantillonnage systématique en quinconce, ou à défaut, un échantillonnage systématique classique. Sur certaines parcelles, le gain de précision obtenue est très important. Le taux de sondage minimal à utiliser varie en fonction de la taille de la parcelle entre 3,2 % (Guatemela, 373 arbres) et 2,23 % (Salvador, 611 arbres). Des économies d'échelles importantes sont donc réalisables. On choisira de préférence un échantillonnage systématique en quinconce qui diminue l'effet éventuel de corrélations entre les rangs et maximise les distances entre sites. Le premier arbre sera choisit aléatoirement dans tous les cas.

Les arbres à échantillonner étant choisis, il reste à déterminer quels fruits examiner dans les caféiers. Le problème se pose dans les mêmes termes que pour l'échantillonnage des arbres: toute méthode laissant la responsabilité du choix à l'observateur est empirique. L'échantillonnage de fruits "au hasard" sur un arbre est à éviter. La sélection de glomérules au lieu de fruits semble réduire le biais mais, pour les mêmes raisons que précédemment. Dans le cadre de protocoles réalisés par des chercheurs, l'échantillonnage systématique de glomérules semble être la meilleure solution. Un taux de sondage d'un glomérule sur 7 est satisfaisant.

La solution retenue : échantillonnage systématique des arbres et des glomérules, demande un travail très important. Ce n'est donc sûrement pas la solution la plus économique d'autant plus que, nécessitant un découpage de la parcelle, elle ne permet pas une optimisation globale. Cependant c'est la seule technique dont les résultats soient fiables. L'échantillonnage de glomérules au hasard peut être une alternative intéressante pour décider de l'opportunité des traitements dans les parcelles de production ; cette méthode amène un gain de précision important par rapport à l'échantillonnage de fruits au hasard.

L'utilisation des statistiques spatiales permet de mettre en évidence un modèle de répartion des attaques de type markovien (Rémond, 1996). Il existe une contagion vraie entre arbres voisins. Ces constations amèneront à proposer des procédures d'échantillonnage adaptées à l'étude de la répartition et de la dynamique des scolytes. Dans cette perspective, un échantillonnage systématique de groupes de deux arbres semble apporter une information plus précise.

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Résumé :

Les protocoles d'échantillonnage, utilisés pour évaluer les dégâts des scolytes dans les parcelles de caféiers, ne sont généralement pas satisfaisants. En effet, les méthodes employées sont souvent basées sur un choix "au hasard" de fruits, ce qui entraîne un biais dans le sens de la surestimation des taux d'attaque. De plus, ces méthodes ne tiennent pas compte des corrélations existantes entre les arbres voisins ou entre les fruits ou glomérules d'un même arbre.
A partir d'une étude de la structuration des attaques observées dans plusieurs caféières d'Amérique centrale, des recommandations sont proposées pour évaluer au mieux les dégâts causés par cet insecte. Des procédures d'échantillonnages systématiques ont été testées expérimentalement et par simulation. Cette systématisation intervient à 2 niveaux : au niveau du choix des glomérules dans l'arbre échantillonné et au niveau du choix des arbres dans les parcelles. Cette procédure systématique à 2 degrés permet de minimiser le biais de l'estimation du taux d'attaque et donne l'intervalle de confiance le plus étroit sur cette estimation. Elle est donc recommandée pour les essais concernant le contrôle de cet insecte. Des tailles d'échantillons sont également proposées pour différents niveaux de précision souhaitée.

Abstract :

Sampling methods, used to evaluate coffee berry borer (CBB) in the field, are not generally precise enough. Indeed, these methods are often based on a random choice of berries, and this results in an overestimate of the percentage of attacks. Furthermore, these methods do not take into account the existing correlations between neighbouring trees or between fruits or fruiting nodes on a tree. Following field experiments in central America studying the spatial repartition of CBB, we are able to recommend sampling methods providing an accurate estimation of damages.
Systematic sampling methods were recommended. These sampling procedures were tested for confirmation in the field as well as via mathematical simulations. This systematic method involves two levels of sampling : at the individual tree level (for the choice of the berries) and at the plot level (for the choice of the trees). This sampling procedure allows to minimize the bias in estimating the percentage of attacks and gives narrower confident interval for the estimate. Sample sizes are also recommended in relation to the desired precision.

COFFEE BERRY BORER : THE GLOBAL IMPORTANCE OF THE PEST AND POTENTIAL FOR ECOREGIONAL RESEARCH COLLABORATION TOWARDS ENVIRONMENTALLY-FRIENDLY STRATEGIES FOR ITS MANAGEMENT

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ABSTRACT

The Coffee Berry Borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), originally reported in France in the trade coffee during the 1860s, has become a serious pest of coffee in most of the cropping areas in the world, especially in the tropics. This pest (CBB) is known to be important in southern/central America (Colombia, Brazil, Jamaica, Sri Lanka) and Africa (Kenya, Uganda, Gabon, Congo). Research has so far been mainly focused on the evaluation of chemical insecticides for CBB control, insecticide resistance monitoring and biological control with parasitoids and fungal pathogens. While these approaches have shown potential for appreciable control of CBB in southern America, there is scope for exploring the use of additional eco-friendly and sustainable options like semio-chemicals, botanicals and habitat management strategies. ICIPE seeks to undertake collaborative research with interested regional and national partners in CBB research in Africa (starting with Kenya) and in southern America (such as Colombia) and Asia (such as India) - and Pacific. Interestingly, in Kenya resort to beneficial cultural practices, and the use of effective insecticides for CBB control have in the interim been regarded as satisfactory, but more recent assessment indicates that there is need to involve additional components for sustainable long term management. This paper discusses the background for the different IPM components and highlights the potential role of such collaboration towards developing more holistic strategies for CBB management globally.

INSECTICIDE RESISTANCE IN THE COFFEE BERRY BORER : STATE OF CURRENT KNOWLEDGE

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Introduction

Hypothenemus hampei (Ferrari) is a major pest of coffee worldwide and is highly resistant to endosulfan in New Caledonia (Brun et al. 1989, 1992). Resistance has been mainly confined to four regions on the East Coast, where the existence of resistance is significantly more frequent in the newer sunny plantations that had been treated with endosulfan during the preceding 12 months than in fields that had not been treated recently or in older fields under native forest canopy (Brun et al. 1990). Parkin et al. (1992) related variation in resistance levels within fields to operational factors such as application from truck-mounted sprayers and the type of field. Cessation of endosulfan use led to a reduction in resistance frequency, whereas continued use increased the resistance frequency (Brun & Suckling 1992).

Although much has been learned about the evolution of resistance in *H. hampei* through monitoring, several other important factors, such as the effective dominance of resistance (Roush & McKenzie 1987, Roush & Daly 1990), cannot be studied without determining the mode of inheritance of the trait. We therefore sought to determine the degree of dominance and mode of inheritance of endosulfan resistance in *H. hampei* by using backcross methods on strains reared on semi-artificial diet (Brun et al. 1992).

Field Control Problems

From 1985 to 1987, the Coffee Board of New Caledonia reported increasing problems in controlling coffee berry borer populations (CBB), with the spray programme which had been used successfully for many years. ORSTOM was invited to become involved at that point, in order to determine whether endosulfan resistance was present. Preliminary results indicated a very large difference in response between samples from different regions, and a major research effort was commenced, developing into a multidisciplinary team from several countries. The aim of this research was to develop an insecticide resistance management programme for CBB, in the context of an integrated pest management approach.

Development of a resistance detection method

Preliminary investigations were conducted through a Potter tower method (Potter, 1952, Brun *et al.* 1989), but this method is expensive and requires specialised facilities. An alternative method was therefore developed (Figure 1), based on the vapour action of endosulfan (Brun *et al.* 1989), in the event that other laboratories might need to conduct resistance monitoring at some point in their Integrated Pest Management programmes (IPM).

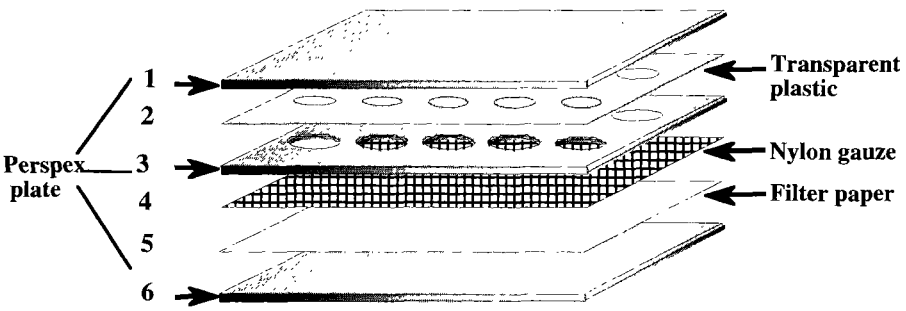

Fig. 1. Components of the ORSTOM-FAO test metho of Perspex, filter paper and gauze. The layers are assembled as shown. Insects are caged for 6 hours in Perspex chamber (above layer 3).

In this rapid, inexpensive and easy method, the insects are confined, for 6 hours, in a chamber, above treated filter paper. They cannot contact the treated surface, due to a nylon gauze layer, which prevents beetles from burrowing into the filter paper. This method was also tried without the gauze, as a residue contact and vapour action method, and very little difference in response either with or without the gauze was found (Brun *et al.* 1991), which suggested that vapour action is a primary means of the dose reaching the insects.

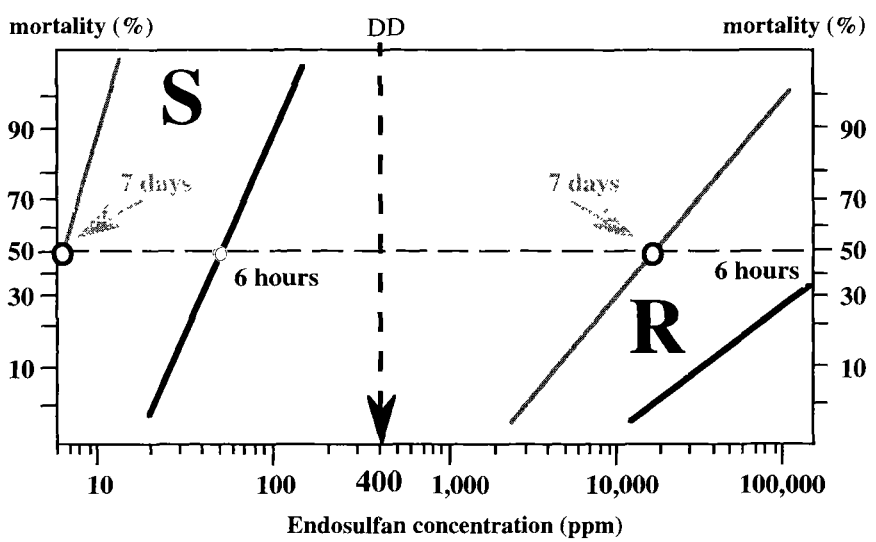

Fig. 2. Concentration-mortality responses for resistant (R) and susceptible (S) adult female *H. hampei* from New Caledonia to endosulfan after 6 hours exposure and 6 hours or 7 days assessment. 400 ppm is the discriminate dose (DD), that gives 99.95% mortality of susceptible females.

The effects of temperature and time on the ability of the method to distinguish between resistant and susceptible phenotypes was investigated (Brun *et al.* 1991) (Figure 2). Tests were conducted at 5 temperatures, from 22-34°C, with assessments every hour from 2-10 hours. It was clear that the combination of 25 °C, 400 ppm of endosulfan and 6 hours, which was the $LC_{99.95}$ of susceptibles, was reliable at detecting the presence of

resistant insects in samples. No survivors were detected in samples from the West Coast, or from other regions with no reported field control problems, in tests of over 4,000 beetles. While longer assessment times can be useful for measuring the ultimate mortality, little change in the resistance factor was found with assessments after periods of up to a week.

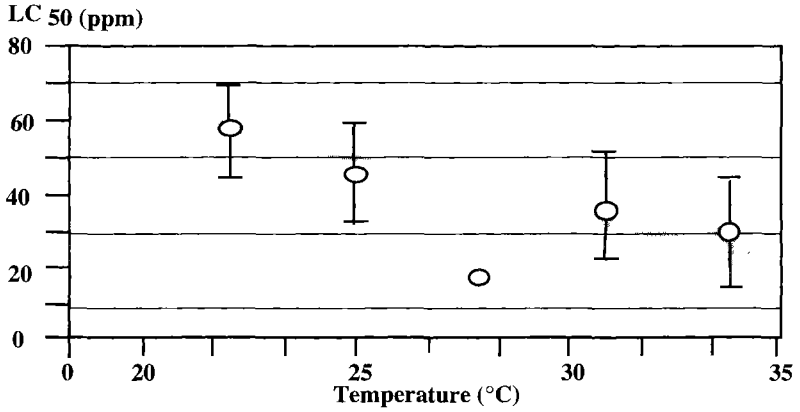

Fig. 3. Median lethal dosage estimate (LC_{50}) values of susceptible *H. hampei* caged above endosulfan-treated filter paper for 6 hours at five temperatures. Vertical lines indicate 95% confidence limit.

As shown above, the lethal concentration killing 50% of individuals (LC_{50}) declined with increasing temperature (Figure 3). Endosulfan was less toxic to susceptible insects at 22°C than at 34°C after 6 hours ($P<0.05$), according to likelihood ratio tests on pairs of concentration-mortality regressions. This negative correlation between temperature and LC_{50} for endosulfan has also been reported for *Sitophilus granarius* L. (Knauf, 1982).

Resistance Survey in New Caledonia

A large scale survey operation was then commenced with the two methods, in which samples were taken from the roadsides of over 200 fields from 15 regions of New Caledonia (Table 1).

	Potter Tower			ORSTOM-FAO method		
Location	Field Pop. tested	*H. hampei* tested	Resistant Pop. (%)	Field Pop. tested	*H. hampei* tested	Resistant Pop. (%)
WEST COAST						
Moindou	3	180	0	-	-	-
La Foa	16	1020	0	1	60	0
Bourail	5	300	0			-
Poya	1	60	0	1	60	0
Pouembout	1	60	0	1	60	0
Koné	1	60	0	1	60	0
Koumac	1	60	0	1	27	0
EAST COAST						
Canala	13	780	0	11	660	0
Kouaoua	7	480	0	5	300	0
Houaïlou	36	2319	8	32	1980	10
Ponérihouen	32	2503	97	30	2060	97
Poindimié	23	1853	100	12	1040	100
Touho	44	2915	63	35	3923	63
Hienghène	17	1005	12	17	1170	6
Pouébo	3	180	0	-	-	-
TOTAL TESTED	203	12 323		150	10 122	

Table 1. Geographic distribution of endosulfan resistance in *H. hampei* in New Caledonia.

The majority of samples were tested with both Potter tower and indirect exposure techniques, and included lindane as well. The precision of the two methods was similar, for fields with resistance present. Resistance was present in 5 of the 15 regions, and was very widespread in two regions, with 97-100% of samples containing resistance (Brun *et al.* 1990).

Effect of Management History

The possible impact of the technique of directional application of insecticides from the roadsides on the resistance frequency within fields was then investigated. Samples were taken in transects perpendicular to the roadsides, and showed a clear trend of more frequent resistance near the point of insecticide application (Brun and Suckling 1992). This was particularly evident for sunny fields (Table 2).

Distance from treatment point	**125 m.**	**100 m.**	**75 m.**	**50 m.**	**25 m.**	**0-10m.**	Detection technique
Resistance Frequency (%)	8.3	36.7	68.3	78.3	93.3	93.3	(Potter Tower)
	18	30	75	85	86.7	98.3	(Indirect exposure)

Table 2. Distribution of resistance frequency in *H. hampei* populations after directional treatment with endosulfan. A rapid increase in resistance frequency (phenotypic cline) near the point of insecticide application was shown.

Field application techniques used in New Caledonia, and their potential to select for resistance were also investigated. We used tracer dyes to determine the deposition characteristics of various sprayers under different conditions. Most of the deposition occurred within 10-20 m from the point of application (Parkin *et al.* 1992). Bioassays also indicated the greatest effect near the roadside (Figure 4).

We used filter paper packets to indicate the mortality which could be expected of free-living beetles, and infested green and dry coffee berries to indicate the mortality of beetles inside berries. The vapour action of endosulfan was clearly indicated by the mortality shown well beyond the point at which deposits were detectable.

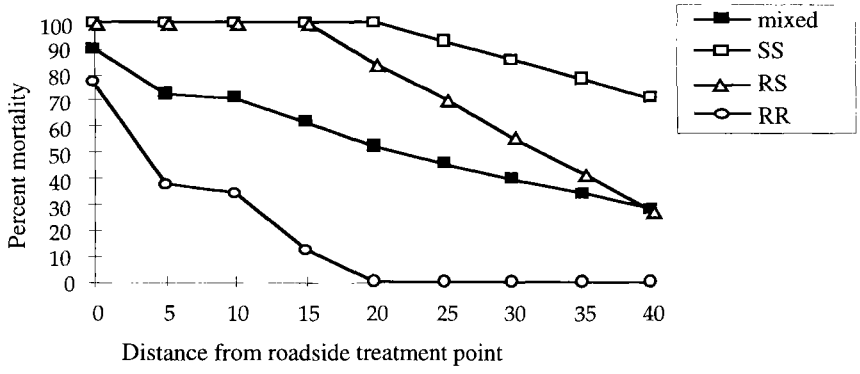

Fig. 4. Field experiment conducted at La Foa, using packet bioassay and *H. hampei* from different phenotypes : susceptible (SS), resistant (RR) or mixed populations (RS). Decreasing mortality of (SS) populations 20 m. away from the roadside endosulfan application point show the efficacy limit of the spraying technique used.

In these bioassays, we compared susceptible and resistant CBB strains, and found that a significant difference in mortality was evident between strains as far as 80 m away from the roadside. This difference in mortality relates to the selection pressure for resistance, since mortality of susceptibles favours the development of resistant beetles.

Resistance was present at a significantly higher proportion of sunny fields compared to the traditional type of fields, and the actual frequency of resistant insects was also higher at sunny fields. Incidentally, the higher frequency of endosulfan resistance in sunny fields is attributable to the higher temperatures during January and February when spray applications are made. Measurements made using a data logger indicate an average of 3° difference in sunny fields, which amounts to a 20% decrease in the LC_{50} of susceptibles (Unpublished data). Warmer temperatures therefore would lead to a higher kill of susceptible beetles, and enhanced selection for resistance.

Interestingly, resistance was also present at a proportion of fields which had not been treated in the last year. Two of the fields with resistance present were known to have never been treated with insecticides. This finding lends strong support to our idea that the resistance was spread during the harvest season, as trucks loaded with berries moved between fields. The trend of more resistance near the coast compared to higher valleys also supports this hypothesis.

Changes in resistance frequency between years were examined, in fields receiving continued endosulfan tratment, and fields receiving replacement of endosulfan by fenitrothion or no treatment. Continued endosulfan use caused a rapid increase in the resistance frequency within one year, at fields with initially low resistance frequencies present (Figure 5). In Ponérihouen valley, a switch to fenitrothion has induced a decrease in resistance level.

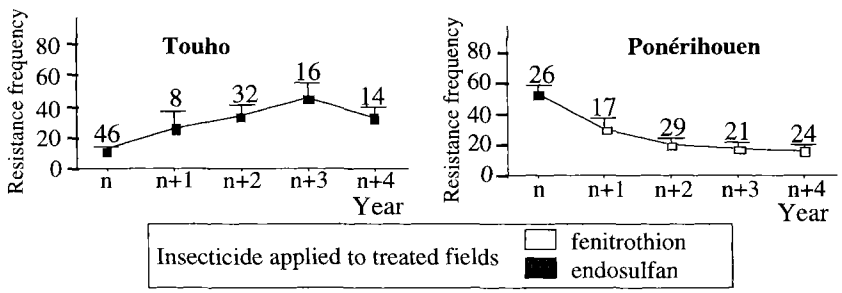

Fig. 5. Evolution of resistance frequency under two treatment regimes. *H. hampei* populations from Touho were endosulfan treated while Ponérihouen populations were treated with fenitrothion. The number of field populations is marked above error bars.

Genetics of Endosulfan Resistance

The genetics of endosulfan resistance in *H. hampei* was studied through classical crosses to determine the degree of dominance and the number of genes involved. After adult beetles were sprayed, mortality was recorded at 6 h and 7 d. Responses of F_1 females after 6 h indicated degrees of dominance of -0.38 ± 0.03 and -0.25 ± 0.03 for RRXS and SSXR crosses, respectively. In contrast, dominance after 7 d was -0.17 ± 0.02 and -0.02 ± 0.02, apparently indicating a trend toward codominance over time. Responses of backcrosses of the F_1 generation to both parental lines and of F_2 progeny were inconsistent with results predicted when assuming simple Mendelian inheritance. In contrast with females, we detected clear differences in responses of the F_1 males that

depended on which parent was resistant, implying that resistance is sex-linked or that paternal chromosomes are inactive in the sons.

A female-biased sex ratio is also known in *Metaseiulus occidentalis* (Nesbitt) (Roush & Hoy 1981) where males are haploid because of the loss of the paternal set of chromosomes (Nelson-Rees et al. 1980). But in contrast, in *H. hampei*, we have documented a functionally haplodiploid mode of inheritance of resistance although males possess two sets of chromosomes (Brun et al. 1995).

Results from a series of genetic crosses using males of differing insecticide resistance genotype indicate that progeny fail to inherit paternally transmitted resistant or susceptible insecticide resistance alleles. We therefore investigated the inheritance of resistance in crosses, using molecular markers, examining the cytology of *H. hampei* and determining the phenotypical status of males and females. Insecticide bioassays and molecular PASA analysis of specific resistance alleles indicate that resistance is determined solely by a single point mutation in Rdl and that its inheritance is consistent with haplodiploidy. Interestingly, cytological examination indicates that males are diploid but effectively shut off the expression of the paternally derived set of chromosomes by condensing those chromosomes in prophase.

Implication for the spread of resistance

These recent studies allow a better understanding of the mode of inheritance of endosulfan resistance in *H. hampei* and underlying genetics. Our findings demonstrate that the father's maternally derived chromosomes are completly transmitted to his offspring. Thus, in the presence of insecticide selection, the unique combination of this functional haplodiploidy, high inbreeding (Gingerich et al. 1996) and interesting mating system (brood predominantly female, pre-dispersal mating, female-initiated colonisation) may explain the rapid spread of resistance described in New Caledonia (Brun and Suckling 1992, Brun et al. 1990).

As resistance is semidominant, a single maternally derived resistance mutation can easily be exposed to selection in a functionally hemizygous male. That male, mating with his sisters, would then perpetuate and amplify the resistance allele within all of his female progeny. Thus, before a new resistant case is detected and documented, endosulfan treatment will induce resistance selection. Within only a few generations, a large number of homozygous resistant females can be produced. Those females will then disperse to begin purely homozygous resistant inbreeding lines.

We therefore highly recommend that a large scale resistance survey be initiated in countries where endosulfan treatment has been regularly used to control *H. hampei* populations. Reversion in endosulfan resistance levels would indicate that this resistance-associated mutation may be associated with some loss of fitness. Therefore, early detection of new resistance case may allow for insecticide resistance management to be developed as part of Integrated CBB Management strategies.

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ABSTRACT

Coffee production in New Caledonia is threatened by the coffee berry borer, *Hypothenemus hampei,* the major pest of coffee worldwide. If uncontrolled, this pest can reach very high infestation levels. In New Caledonia, ten years of endosulfan use have led to control failures due to the evolution of resistance to cyclodiene insecticides in the coffee berry borer.

A Potter tower direct spray technique and an indirect exposure method were used to map the distribution of the resistance and the changes over time. Resistance was found in five of 15 regions, but the resistance frequency declined after endosulfan use was discontinued. Transects across endosulfan-treated fields indicated higher resistance levels near the roadside. This phenomenon was probably due to air-blast application from roadsides across fields, in combination with limited gene flow. Bioassays at different distances from the roadside corroborated this hypothesis. A GABA receptor gene, which appears to be homologous to the *rdl* gene in *Drosophila*, shows a single amino acid substitution in resistant *H. hampei* at the same site as in other cyclodiene-resistant insects.

Crosses between resistant and susceptible genotypes indicated intermediate dominance, with functional haplo-diploidy. Gene or chromosome inactivation occurs in males, through loss of the paternal set of chromosomes. Indeed, cytological investigations showed that only the maternally derived complement is transmitted and expressed while it is condensed and non functional when paternally derived.

RÉSUMÉ

Le scolyte du café, *Hypothenemus hampei,* est, en Nouvelle-Calédonie comme ailleurs dans le monde, le ravageur majeur de cette culture. Sans contrôle approprié, le niveau d'infestation des cerises peut être très élevé. Après environ 10 ans d'utilisation d'endosulfan, il a été établi que les difficultés de lutte étaient dues à l'apparition de résistance aux cyclodiènes chez cet insecte.

Deux techniques complémentaires on été utilisées pour étudier la répartition des populations résistantes et pour en suivre l'évolution. Ces populations ont été détectées dans 5 des 15 régions prospectées et leur niveau de résistance décroît après arrêt de l'usage de l'endosulfan. L'étude de transects à travers certains champs montre une décroissance du niveau de résistance quand on s'éloigne des routes à partir desquelles sont pratiquées les pulvérisations d'insecticides. Au niveau d'un gène récepteur GABA, identique au gène *rdl* de la drosophile, on note la substitution d'un amino acide par un autre, comme démontré chez d'autres espèces d'insectes résistants aux cyclodiènes.

Des croisements pratiqués entre génotypes sensibles et résistants montrent que cette résistance est semi-dominante et de type "haplo-diploïde fonctionnelle". En effet, des études cytologiques montrent que la moitié du matériel chromosomique paternel dégénère alors que celui hérité de la lignée maternelle est totalement transmis.

EFFECT OF SOAKING PARCHMENT ON COFFEE FACTORY CAPACITY

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Introduction

The processing capacity of a coffee factory is impaired mainly by constraints in fermentation, skin and final drying of coffee besides labour availability and management. Drying creates the biggest problem as it is Labour consuming and requires a large area. Removal of the congestion normally experienced at this stage can facilitate the preceding stages of processing.

Although, the existing processing capacity in all coffee growing areas still suffices, constraints in processing have always been experienced and attempts made to expand the affected factories and establishing new ones. However, the existing factories should also be modernised in order to handle a bigger input with the existing facilities. Construction of new factories should also be avoided if possible since due to scarcity of land together its prohibitive cost and that of a new factory, this can decrease member payment and will sometimes create labour problems.

A primary coffee processing factory should always operate at its optimum capacity to avoid delayed coffee picking. The factory should also be in perfect condition to execute every

stage of processing precisely and to avoid breakdown during processing in order to cope with the anticipated input. The existing factories should also be modernised in order to handle a bigger input. Bottlenecks in the processing should be traced and steps taken to avoid them and never to be used as a temporary storage.

The circumstances that influence the efficiency of the process include the efficiency of all the processing equipment; labour availability and management. Incase fermentation and drying are too slow (Ilsley, 1973 Kamau, 1989 and) respectively the enhancement measures should be effected. It has been reported that, although pro-longed drying time can be caused by climatological circumstances, long rain spells and poor management, it is still easily possible to dry parchment, in such adverse conditions, in 14 days or even less (Kamau, 1989) by adhering to the recommended drying practices.

As there is also an increasing trend of coffee establishment, the expected corresponding increase in coffee production, will require extra processing facilities. Increased coffee production is therefore likely to aggravate this problem particularly where the farmers are unable to raise adequate funds to invest in additional new land for drying and processing machinery and equipment. Consequently, this may well be an already inherent constraint to production yet unnoticed.

In view of the processing constraints in coffee factories, a method capable of increasing the current coffee factory capacity without extra facilities and adverse effects on quality is therefore necessary. Such a procedure should facilitate the processing of coffee in dull weather characterised by very slow natural drying without affecting coffee quality. By way of modifying some stages of processing, like fermentation, soaking and drying the capacity of a coffee factory can perhaps be raised.

In the two stage wet coffee processing procedure, parchment is soaked in clean water after fermentation to primarily improve the raw appearance of the coffee bean while undesirable chemical substances also diffuse out of the bean which is an important

attribute to the final quality of coffee. Soaking also allows the breakdown of mucilage to be carried out as rapidly as possible, employing physical, chemical, or enzymatic means without noticeable adverse effects on the quality of the final product (Kulaba, 1979 and Wootton, 1965). This aspect has also been conveniently used, though to a limited extent, to hold parchment at this stage in order to overcome temporary congestion at the subsequent drying stage especially in dull weather.

Parchment can be soaked under water for 24 hours and according to Wootton (1965), there is nothing to be gained in terms of quality by extending the soaking period further. However, to avoid interfering with other factory procedures, soaking of parchment for a minimum of 16 hours has been an acceptable limit without an appreciable loss in quality (Wootton, 1965). The need to avoid congestion at the drying stage by creating extra coffee processing capacity through prolonged soaking, may have eminent but unquantified potential benefits. This work was undertaken to establish the additional processing capacity that can be created by pro-longed parchment soaking using the existing processing facilities and that will not hurt coffee quality.

Materials and methods.

A sample of 215 kg of clean parchment grade one (1) was drawn immediately after the final washing of the fermented parchment. 140 kg of the sample was separated and kept under clean water in a soak tank for 28 days. Every morning, the parchment was washed, drained and a 5 kg sample drawn for drying in the sun in accordance to the normal practice. The balance of the parchment was soaked in fresh clean water.

Another 75 kg sample of parchment was divided into 15 samples of 5 kg each. The samples were soaked separately in plastic basins under clean water which was renewed every day. During the first day and after every 7 days, 3 replicate samples were taken out for drying and repeated for 3 years. The trials were also conducted under different agro-ecological zones (Table 1).

At the end of each experiment the dry samples were prepared and submitted for immediate liquoring test to determine their final cup quality. Coffee cup quality results from the test samples were compared to those attained by the respective coffee factories from which samples were drawn.

While the soaking trials were in progress, 28 samples of parchment weighing 2 kg each were separately subjected to under water soak as recommended. This was to establish whether there was dry matter loss in the course of soaking. Every day a sample was drawn, skin dried and weighed before final drying. On completing drying, the weight of the dry parchment samples was taken. The samples were then hulled and the weight of clean coffee recorded.

RESULTS AND DISCUSSIONS

The capacity of a primary coffee factory can be determined using the method presented by Kamau in 1987. For parchment coffee soaked in soak tanks and decreasing in amount, the average drop in quality after 28 days of soaking was 2 with a maximum drop of 3 classes after 21 days of soaking. The drop in quality with prolonged soaking duration in all cases was rather erratic possibly due to the varying inherent coffee attribute with time though from the same source.

From table 2, it can be observed that, there was a general improvement in coffee quality for coffee soaked for less than 7 days. Otherwise, coffee could retain constant quality for up to 14 days. The quality changed from class 4 to 6 in between 10 and 15 days. Class 5 coffee took between 10 and 20 days to change to class 6 except for Azania early crop (EC) and Kisii in 1994 where the change took 7 and 5 days respectively. The results for Meru late crop (1994 LC) indicated that a rather poor sample of class 7 improved in quality to class 6 in the first 3 days and reverted back to class 7 later after 18 days from the beginning. All the samples took between 21 to 26 days to deteriorate to class 7 with class 5 and 6 recording 26 days in Koru (1995 EC) and Kisii (1993 LC) respectively. The only anomaly was observed in Azania (1994 EC) and Kisii (194 EC) where it took as short as 14 and 9 days

to deteriorate to class 7. In general, the poorer the initial quality of coffee, the less sensitive it was to the impact of pro - longed soaking. This was perhaps because it was already in too poor a state to be affected by mere soaking.

As for parchment soaked in buckets as discrete samples (ie Constant amounts), there was an average change in quality of 1.5 classes after 28 days of soaking. A maximum 3 classes deterioration was observed in 2 out of 13 experiments. The quality of the parchment dropped by at most one class after 7 days but in an unpredictable manner with respect to soaking duration.

The results of soaking constant weights of parchment in 1993 are presented in table 2. At Azania, comparison of results of the 2 seasons show that, the effect of soaking duration was highly significant ($p \leq 0.01$) during the late crop. The trend however, showed only slight drop between 1 and 7 days. A marked drop in quality was observed between 7 and 14 days followed by a small change between 14 and 21 days which thinned further between 21 and 28 days.

At Rukera, soaking had no significant effect ($p \leq 0.1$) on coffee quality. The overall quality of coffee was also not affected significantly ($p \leq 0.1$) by soaking duration. However, slight changes in quality can be observed. From day 1 to 7 of soaking, the quality dropped insignificantly, followed by slight deterioration when soak duration was increased.

At Meru, a comparison of the season effects revealed no significant effect ($p \leq 0.05$) on coffee quality. However, a slight improvement in the late crop was recorded. The treatment effects irrespective of the season had a slight improvement in quality between 1 and 7 days followed by a rapid deterioration up to 14 days followed by less deterioration.

Soaking at Kisii had a highly significant ($P \leq 0.01$)

effect on quality. Only slight deterioration occurred between 1 and 14 days with a marked subsequent drop between 14 and 21 days levelling towards the end.

At Koru, 1993 early crop gave significant shift in quality brought about by soaking ($p \leq 0.05$) that flattened off towards the end of soaking. An insignificant deterioration in quality was observed between 1 and 7 days which steadied thereafter.

Results of similar trial conducted in the same site from 1994 to 1996 indicate that the early crop of 1994 had significantly better quality results ($p \leq 0.05$) at Meru than the late crop. There was no significant difference in quality between 1 and 7 days. Deterioration again took effect between 7 and 14 days. The overall effect of the different soak duration was significant ($p \leq 0.01$) and in agreement with 1993 soaking results.

ln 1995, soaking yielded significant differences in quality. A slight drop in Quality was observed between 1 and 7 days but recovered from 7 and 14 days only to be followed by a big drop thereafter.

At Kisii, paired results in 1994 indicated that, seasons had no significant effect($p \leq 0.05$). However the soaking treatment had significant effect ($p \leq 0.01$). There was only a slight drop in quality between 1 and 7 days followed by a steady deterioration. Soaking of early crop in 1995 did not give significant ($p \leq 0.01$) drop in the overall quality. The 1996 early crop had significant ($P \leq 0.01$) shift in quality results with an improvement between 1 and 7 days of soaking followed by a steady deterioration.

At Koru, the early crop soaking in 1995 had a significant change with a big drop recorded between 7 and 14 days. However,

for the 1996 early crop, there was no significant change ($p \leq 0.01$) with very minimal drop from 7 to 14 days. A big drop was recorded after 21 days.

In regard to dry matter loss during soaking, the sample weight of skin dry and completely dry parchment and clean coffee expressed as a percentage of the original weight of fermented and washed parchment (Figure 1) show that there was no loss in dry matter during soaking of parchment.

CONCLUSION

The results confirm that coffee can generally be soaked for 7 days without significant loss in quality at all the sites. The higher the initial quality of coffee the more susceptible it was to the impact of prolonged soaking. At the initial stages of soaking, the coffee generally improved in quality in the first 7 days with a drop occurring between 7 and 14 days. Thereafter, there is rapid deterioration up to 21 days. From 21 to 28 days, the deterioration slows down, probably because the coffee attains an immunity stage to soaking.

Based on the daily capacity of primary coffee factory of about 7,000 kg per disc. The ability to soak coffee for even a single day has enormous benefits in terms of: (i) expanding the factory to a higher capacity, and (ii) relieving the normally congested drying stage particulary at the peak of coffee harvesting in a season. As the drying stages, normally take up a large area in the absence of mechanical driers and conditioning facilities, soaking can contribute towards scaling down the factory size as well. It can now be appreciated that soaking contributes to a great extent to improving the primary coffee processing capacity by up to 7 days.

Although the coffee harvesting season lasts for 12 weeks, with 20% of the coffee cherry ripens within 2 weeks of the peak, at which period the factory experiences capacity limitations.

However the possibility of soaking parchment for an extra day during the peak makes the season similarly longer. This is particularly vital at the peak where the cherry intake would

increase and the processing of the soaked coffee translated to post peak period. Thereafter as the ripening drops down towards the end of the season the need for soaking assumes less importance. Soaking however makes it possible to process as many days a week as possible during the peak since then the factory can handle much more coffee while the management is easier.

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Abstract

Coffee factories in Kenya have in the past experienced serious processing constraints. This has particulary occurred at the drying stage where a bottleneck normally arise. Alternative methods of increasing the capacity of a coffee factory, by modifying some stages of processing, without extra facilities are hence being considered.

As a first step, a soaking stage which has been conveniently used, though to a limited extent, to hold parchment in order to overcome temporary congestion at the drying stage has been under investigation as one of the prime options. Parchment grade one (1) was subjected to prolonged soaking in different agro-ecological zones, for up to 28 days, in clean water changed every day. The results show that over and above the normal practice, parchment can be soaked for 7 days without adverse effect on

coffee cup quality or loss in dry matter. The implications of prolonged soaking on the factory capacity are further illustrated. The coffee cup quality given in the outturn reports for the farms in which the trials were sited compares favourably.

Table 1

Location of Sampling sites

Sites	Altitude	Latitude	Longitude	Rainfall
Rukera	1620 m	1.06° S	36.45° E	1055 mm
Azania	1520 m	1.2° S	37.00° E	950 mm
Meru	1620 m	0.00°	37.35° E	1817 mm
Kisii	1700 m	0.41° S	34.47° E	2012 mm
Koru	1554 m	0.07° S	36.16° E	1732 mm

Table 2

Quality of coffee soaked in 1993 in different trial sites

SD (Days)	Koru	Rukera	Meru	Azania	Kisii
1	4 4 4	5 5 5	4 4 4	4 4 4	5 5 5
7	4 5 5	5 6 5	4 4 4	4 4 4	5 5 6
14	5 5 5	5 5 6	5 5 5	6 6 6	5 5 6
21	5 6 4	5 5 6	5 5 6	7 7 7	6 6 6
28	5 5 5	6 5 5	6 6 6	7 7 7	6 6 6

Table 3

The effect of soaking duration in days on coffee quality

Year	Trial site	Season	Days of soaking to achieve the respective class				
			Initial Class	Class 4	Class 5	Class 6	Class 7
1993	Azania	EC	4	5	7	15	-
	Rukera	EC	4	-	7	10	21
	Meru	EC	5	-	-	10	21
	Meru	LC	5	-		12	22
	Kisii	LC	5	-	-	12	26
1994	Azania	EC	5	-		7	14
	Azania	LC	4	-	7	15	21
	Meru	EC	7	2	7	13	21
	Meru	LC	5	-	-	5	18
	Kisii	EC	5	-	-	5	9
	Kisii	LC	5	-	-	3	21
1995	Meru	EC	5	-	7	20	-
	Kisii	EC	5	1	4	6	21
	Koru	EC	5	2	2	12	26
1996	Rukera	EC	5	3	12	19	-
	Kisii	EC	5	3	14	-	-
	Koru	EC	5	4	13	-	-

Key
(+) Signs in Parenthesis indicate the actual Class attained eg (-) in the class 4 column means 4-.
SD Soaking duration
EC Early crop
LC Late crop

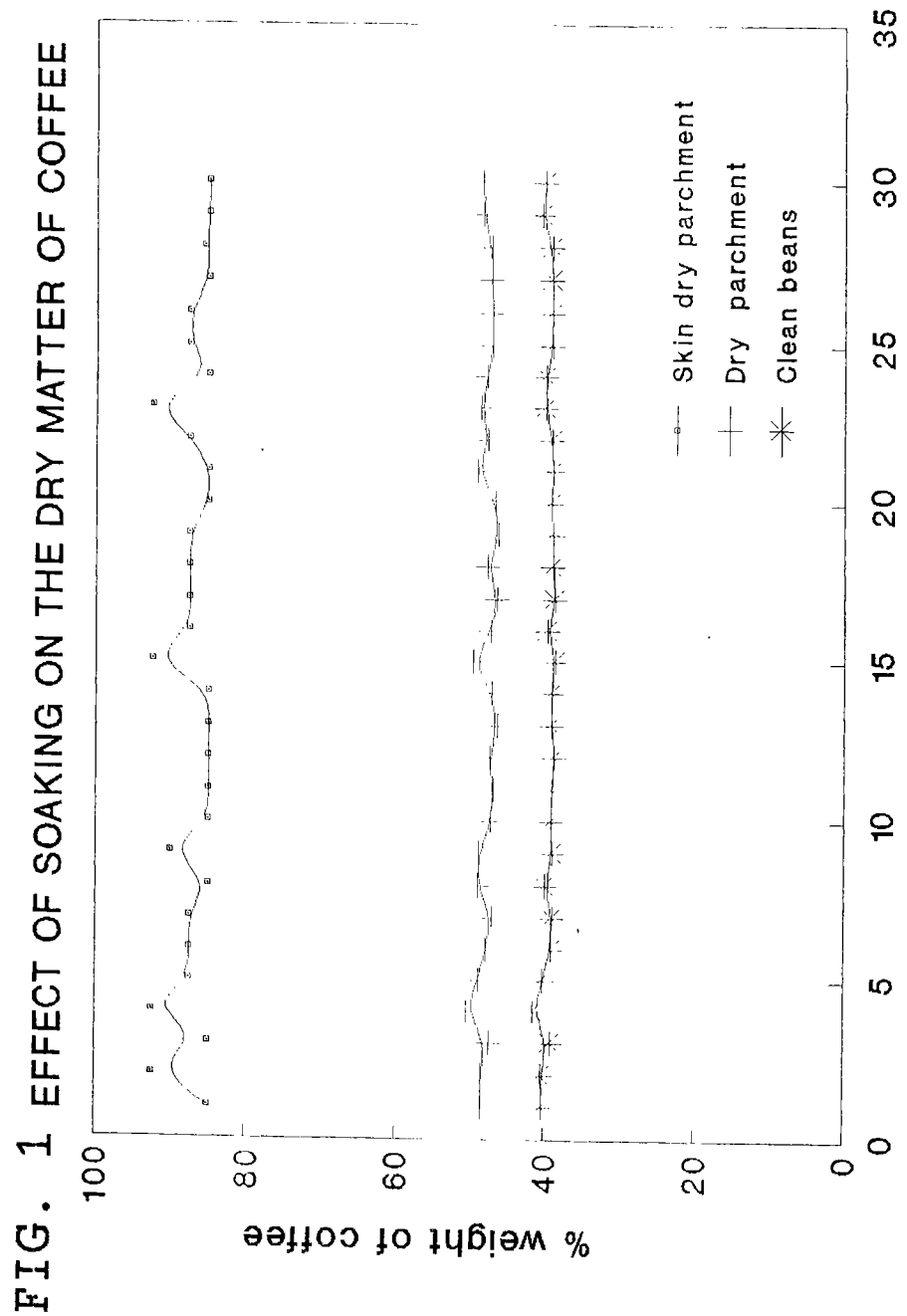

FIG. 1 EFFECT OF SOAKING ON THE DRY MATTER OF COFFEE

DEVELOPMENT AND ASSESSMENT OF A SOLAR COFFEE PROCESSING CENTER FOR COOPERATIVE USE IN INDONESIA

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INTRODUCTION

Indonesia has produced a quite significant volume of coffee beans amounted to 450.000 tons. The acceleration of coffee production has increased substantially since the third five years plans was initially launched in 1974. Foreign revenue from sales of coffee bean reached to be approximately US $ 850 million in the end of 1996 (AEKI, 1996). However, the government and related counterparts are continuously making considerable efforts toward the improvement of the existing coffee quality to gain an additional premium from exporting good quality coffee. All fresh picked coffee berries have to go through post harvest processes in which drying is the key operation and the major factor affecting the final coffee quality.

There are three groups in coffee drying,

The first is farmers who dry coffee in the first stage from the initial harvested moisture content (65 % w.b) to about 20 - 22 %. This group is the most decisive factor that influences the quality of the whole Indonesian coffee beans regarding that 80 % of the total area of coffee belong to smallholder.

The second is cooperative, middle traders and exporters. The first two run their business in small city nearby the farm, whereas the later perform the drying process mostly in the city. They receive partially dried coffee berries or parchment coffee from farmers and dry it at the second stage from 20 - 22 % to the final moisture content appropriate for storage (11 - 12 % w.b). This group does not contribute very much to the improvement of coffee beans; since the incoming raw coffee from farmers has deteriorated due to insufficient handling on the farm gate.

The third is coffee industry, generally big estates, who produce high quality coffee beans by wet process. Drying of parchment coffee is mostly in a single step from initial moisture content of 55 % (w.b) to the storage moisture content. The total production of this group is less than 20 %. The middle traders, exporters and coffee industry operate the mechanical dryer powered either by oil burner or wood furnace both in dry and wet seasons complemented by the drying floor. Mishandling of mechanical dryer in some cases may adversely affect the final coffee quality (Thome, 1991; Sri Mulato *et al.*, 1994).

Farmers definitely prepare their coffee for the market by traditional dry process. Very often, due to immediate needs for cash, farmers harvest their coffee when the berries are still green or partly yellow in color. Farmers do not implement selection and separation of red-colored picked berries from green one and

foreign materials as recommended by standard procedure (Thome, 1992; Buana & Hermansyah, 1990; Wayan *et al.*, 1993). The farmers apply sun drying regardless the weather may cause significant quality losses such as moldy beans, black and brown beans and off-flavor. Moreover, the beans being dried in the sun is commonly unprotected that is susceptible to insect and animal damage or unexpected contamination (Thome, 1991; Wayan, 1995).

Recent approach in coffee handling operation to anticipate the acceleration of coffee production is to centralize the drying in a processing unit operated by a group of farmers. The drying process in bulk capacity may produce uniform and consistent product as well as to prevent the outflow of deteriorated coffee bean from farm gate. In the past, the drying center used fossil fuel as heating sources and diesel engines to drive to drive fans. Since the price of fossil energy has increased steadily during the past 20 years, the intensive use of oil fuel was the most decisive factor to the drying cost. Consequently, the operation of that kind of drying system seemed to be expensive and become a financial burden to the farmers.

Proposed concept in coffee drying is therefore shifted toward the utilization renewable energy available in rural area. Solar energy or firewood that is sufficient amount produced from regular pruning of shed trees seems to be promising alternative energy. Low cost drying system as well as a simple design are urgently required. There have been numerous works in the area of coffee drying using solar energy (Trims et al., 1984; Sri Mulato *et al.*, 1993; Kamaruddin, 1996). Little work directed toward utilization of a continuous drying regime of coffee using solar collector during the day and firewood furnace during night.

This paper summarizes the technical and financial analysis of a model of solar processing unit as a newly proposed model of on-farm coffee drying facility. The model will cover the product from about given area of 100 - 150 ha of smallholder farm.

MATERIALS AND METHOD

Raw material

The location of the processing center is in a such representative location that no farmer participating in the scheme is too far way from the center. The center receives fresh picked coffee cherries or wet parchment coffee from farmers within the effective collection area. Farmers organize two or three day's rotations of harvesting and bring their fresh berries or wet coffee parchment to the collection point nearby. The collector will select and separate both defect beans and undesirable materials from healthy one and weigh the selected beans. Each day a truck picks of selected fresh berries or wet parchment up from a group collecting to the processing center in the same day of harvesting to keep the transportation efficient. The drying of incoming raw berries is under the supervision of well-trained personnel.

The drying steps conducted during the assessment were as follows,

1. Intermittent drying regime using solar energy.

During the day time, the heat source was solar collector by operating the blowers, whereas the drying process during night time did not take place and switching-off the blowers. The depth of fresh coffee berries or parchment coffee in the drying chamber should not exceed 35 cm. The superficial airflow was about 0,30 m per second. Regular stirring during drying is necessary to ensure uniform drying rate.

2. Continuous drying regime using solar energy and firewood furnace.

The drying process was by solar collector in the first day and then followed by the drying using firewood furnace at constant air temperature of 40 °C during night time. In the following morning, the expected moisture content of coffee is about 25 %. The drying process resumed by solar drying again. In the second night, the drying process continued by implementing a constant hot air of 60 °C produced from the furnace for about 12 hours until the final moisture content of coffee decreased to 12 %.

Technical construction of the unit

The building

The processing building has a dimension of 8 m wide and 2 m long erected on the middle part of a 15 m time 15 m square concrete platform (Figure 1).

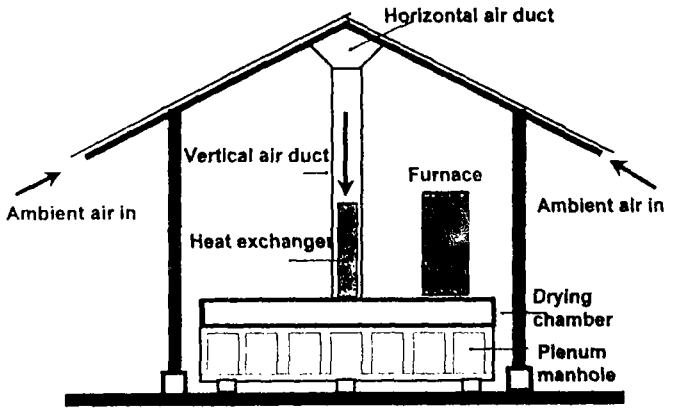

Figure 1. Front view of the Solar Processing Center with solar collector incorporated in the roof.

The structure of the building consists of four pairs of double T profile steel frame (15 cm) with 400 cm height connected using bolt-nuts. The distance between two adjacent frames is 300 cm. Both roofs of the building have function as solar collectors facing North and South at 25 ° inclination. Total effective surface area of the solar collector is 144 m^2. The solar collector uses conventional building materials, such as wood beams, C steel profiles, plywood, sheet metal and fiberglass. Each collector has rectangular wood beam frame with dimension 76 cm wide and 600 cm long.. Black-painted galvanized iron sheets as an absorber covered the upperside of frame. The entire backside of the absorber surface is insulation materials such as 2,5 cm thick layer of glasswool and a 0.6 cm thick aluminum foil supported in 8 mesh of wire screen (Figure 2).

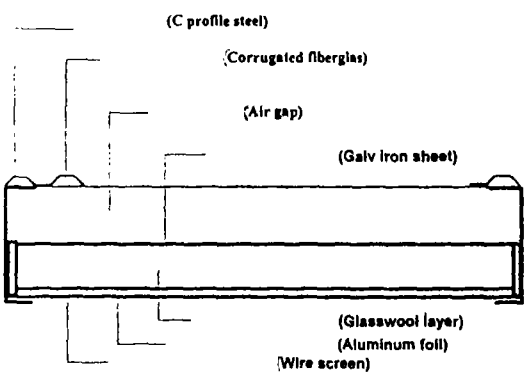

Figure 2. Cross section of a solar collector.

Ambient air enters by forced convection over the entire surface of absorber. The hot air generated from the collector flows through the horizontal and vertical channels connected to the drying chamber (Figure 3). The cross-sectional area of all air channels is 1 m^2 resulting in maximum air velocity of 2 m per second. The airflow rate ranges between 2.500 to 20.000 m^3 per hr depending on the number of blowers in operation.The maximum drying air temperature produced by solar collector is 40 °C to prevent significant losses in cup quality of the beans. This mode of operation is much lower than the drying air temperature commonly used in mechanical dryer which reaches 80 C.

The drying chamber

The drying process take places in a flat bed type dryer which is divided into seven compartments (Figure 3). Each has an individual axial blower equipped with an air valve and driven by a 1/4 HP motor. Each compartment has perforated drying floor made of 0,3 cm aluminum sheets supported by 100 x 250 cm rectangular wood plank walls, 3 cm thick. The main frame of the drying chamber is U profile steel 5 cm wide. An air space beneath the perforated floor of each compartment, called plenum, 60 cm in height, serves to distribute the drying air evenly over the whole cross area of the beans.

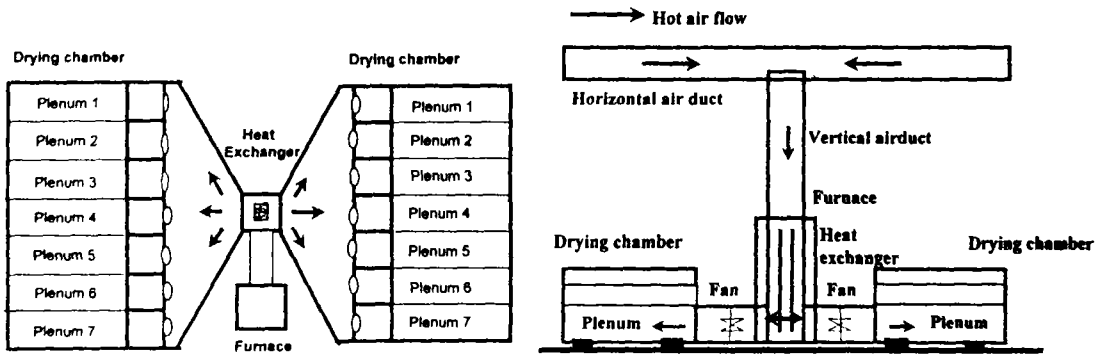

Figure 3. Side and overview of drying compartments.

The furnace

Figure 4 shows the side-view of the furnace that connected with a bundle of tube heat exchanger. The furnace has rectangular basement of 90 cm x 110 cm. The total height of the furnace is 240 cm. The combustion chamber is about 75 cm above the basement. The fuel stack on a V type steel wall havine a rectangular aperture of 12 cm x 80 cm to exhaust combustion gas. Thick fire bricks cover the V walls to prevent overheating.

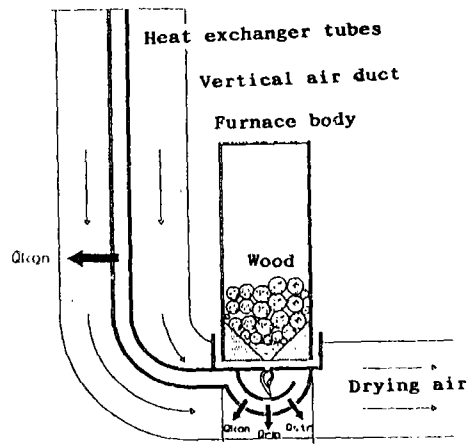

Figure 4. A side-view of the firewood furnace

A centrifugal blower installed in the outlet header of heat exchanger sucks both primary and secondary combustion air. The primary air enters into valves and flows down through the fuel bed, whereas secondary combustion air passes through the perforated steel tube located nearby the aperture. Secondary air is necessary to complete combustion as the exhaust gases travel across the aperture and reverse to the inlet zone of tubes heat exchanger and finally out to surrounding air through a chimney.

Short wood logs amounted to about 200 kg are loaded into the furnace through a charging door. Small amount of flammable wood chips or used-papers is laid just above the aperture prior to first loading of wood log to assist initial ignition. A tiny torch of flint match is inserted into the aperture to ignite wood chips or papers for a 5 minute period of start-up.

Cost analysis

The analysis was carried out to determine the production cost which is directly spent for processing of coffee beans based on the optimum annual use of the unit. The costs involved in operating or owning the unit were calculated at annual production of 100 tons dried coffee berries and coffee parchment.

Fixed cost consisting of depreciation and interest was calculated based on the estimation commonly used in routine production of the existing plantations. Variable cost which included labor cost, energy cost and repair or maintenance cost were estimated by the real expenditure spent during experiments.

RESULTS AND DISCUSSION

The drying air temperature profile

The thermal efficiency of the solar collector is usually evaluated by the energy balance within the collector and between the collector and its surroundings (Kreider and Keith, 1981). The thermal efficiency of the test solar collector used in this unit ranged from 40 - 45 % depending on the airflow rate (Sri Mulato *et al.*, 1995). The efficiency increased substantially from 30 % up to 40 % at corresponding increased of air flow rate from 3.500 m3/hr to 7.000 m3/hr respectively.

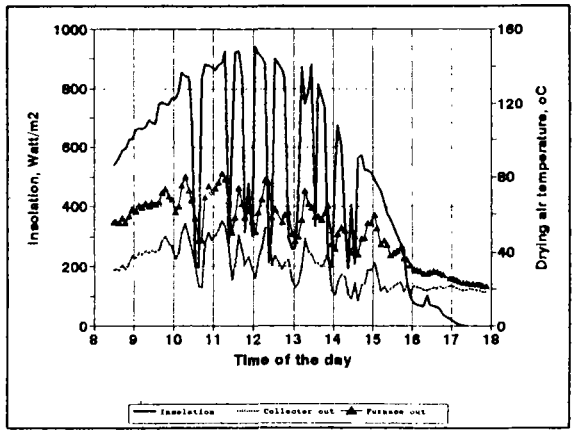

Figure 5. Temperature profiles of drying air at the outlet of solar collector and heat exchanger.

The higher air flow rate improved heat transfer from the absorber surface to the transport fluid. However, the predicted efficiency was essentially constant for an air flow rate above 9.000 m3/hr. This effect is typical of most solar plate collectors because a higher operating air flow rate tended to increase the heat loss through the whole surface of the collector to the surrounding area. The effect is larger than it is in the heat transfer within the absorber plate. The collector efficiency approached a maximum 45 % at an operating air flow rate of 14.000 m3/hr. It meant that 45 % of the solar radiation striking the absorber surface was convertible to heat up the ambient air suitable for drying process. Figure 5 shows the temperature profile of drying air at the outlet of solar collector and the outlet of heat exchanger after consecutive heating.

Figure 5 indicated that the drying air varied linearly with the amount of solar radiation shining during the day. The drying air temperature is heated up gradually from low temperature in the morning and to the higher one in the following hours corresponding with the increased of insolation. The attainable maximum temperature rise of air at 7.000 m3/hr flow rate was 55 °C when the peak insolation was about 900 Watt/m2. The profile of the drying air temperature within the plenum slightly decreased due to the heat loss during the heated air was flowing inside the entire air ducting system (15 m long). During the first stage of drying, the satisfactory drying of coffee used moderate air temperature below 40 °C. The mean drying air temperature produced by the collector as previously indicated was sufficient particularly during low harvesting season.

At the operating airflow rate of 14.000 m3/hr required for drying at full capacity during peak harvesting season, the possible maximum air temperature produced by solar collector decreased to less than 40 °C. The additional heat from furnace supplied to reheat the drying air up to the desired temperature.

The indirect heating of drying air with the exhaust products of wood combustion took place inside the tubes of heat exchanger. Heat combustion of air dried fire-wood used in this experiment ranged from 3.500 - 3.900 kcal/kg. Heat output from furnace varied between 50 - 100 kW depending on the amount of primary air introduced into the combustion chamber. During day time, the heat output was maintained at a constant value of 50 kW. It was proportional to primary airflow of 100 m3/hr to produce the average drying air temperature rise of 35 to 40 °C. A desired temperature rise ranged from 10 to 50 °C could be achieved by controlling the air supply to the furnace through adjustable gate valves. The furnace was regularly recharged with fresh wood log every three hours to maintain a relatively stable flame temperature.

The stable combustion rate was more essential particularly during the operation of the furnace during night time. Low ambient temperature and high relative humidity required more heat output from the furnace to produce the same drying air temperature as it was during day time. Consequently the amount of primary air should be increased up to 200 m3/hr to rise flame temperature to more than 800 °C. The product of combustion afterward traveled through the inner part of heat exchanger tubes. The drying air flowing on the outer of tubes absorbed large amount of heat energy carried by exhaust gases resulting a substantial increase in temperature up to 80 °C.

Drying rate

Freshly picked coffee consisted of about 80 % red berries and the rest was mixture between yellow and green ones. The initial moisture content of berries was about 60 - 65 % (w.b); whereas the average moisture content of parchment coffee was slightly lower that was 55 %. The final moisture content should be reduced to 11 - 12 % for safe storage and to comply trade regulation. The drying curves of coffee berry and parchment coffee dried with solar energy and combination of both solar energy and wood furnace are presented in Figure 6 and 7 respectively.

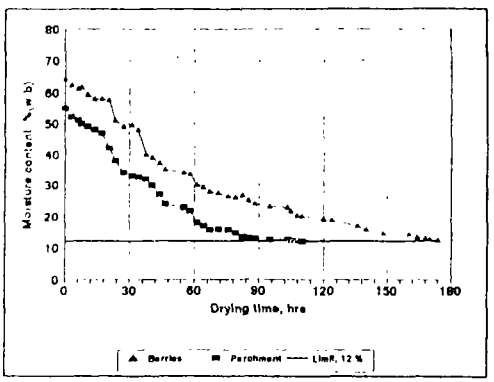

Figure 6. Decreasing moisture content (w.b basis) of coffee berries and parchment coffee and the drying time dried with solar energy.

At the beginning of drying process, both surfaces of coffee berry and parchment were covered with high moisture content. When the heat was introduced into the drying chamber, rapid water evaporation took place at a relatively high rate so that the surface began to dry out. Though both drying mechanisms were the same, the drying rate between them were significantly different. To reach a final moisture content of 12 %, parchment coffee consumed only about half of the drying time required for coffee berry, i.e, 96 and 212 hours respectively.

Intermittent operation of solar drying did not affect significantly on the overall drying time. Running the fan overnight was only useful during the first period of drying, in the first and second nights. The ambient air forced into the coffee bed was able to extract the surface moisture of both cherries and parchment causing slight decrease in moisture content. On the following night, when the surface moisture had dried out. Blowing of ambient air through the coffee bed gave adversely effect on grain wetting because the partially dried coffee is highly hygroscopic (Sivetz & Foote, 1973; Rothfos, 1980). An intermittent regime was implemented particularly during low harvesting season since stopping the fan overnight contributed on substantial reduction of drying cost. However, continuous operation of the dryer (day and night) was required when prevailing weather during peak harvesting period was not sufficient to provide heat for drying process.

The additional heat to resume drying process during night was generated from a firewood furnace. A wide range temperature control was suitable to dry coffee bean from an initial moisture content of 65 % to a final moisture content of 12 %. Using drying air temperature of 50 °C on wet coffee cherry or wet parchment coffee, a continuous drying regime reduced the drying period quite considerably. It was evident that continuous drying process could increase the drying rate almost double. The drying time to reach the final moisture content of 12 % was about only half of the intermittent drying that was 96 hours for berry and 50 hours for parchment (Figure 7).

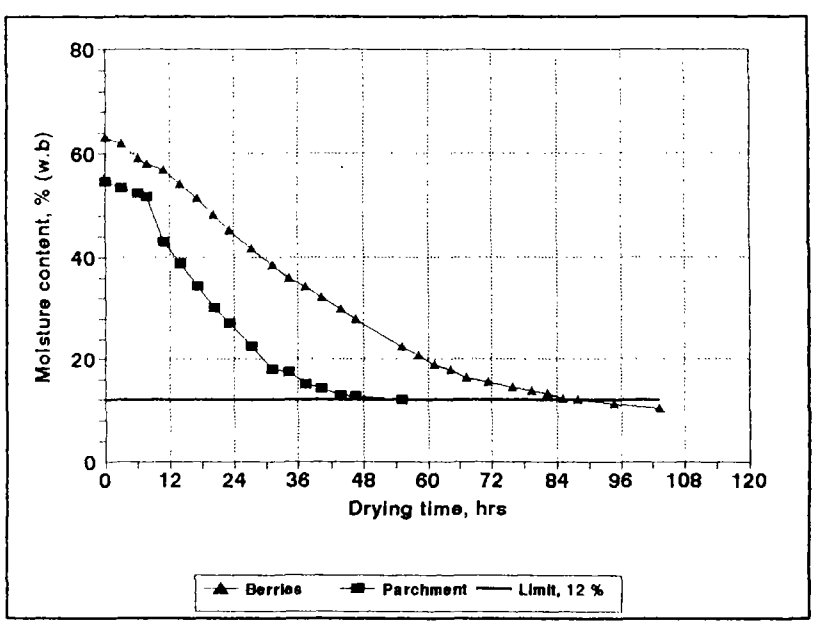

Figure 7. Decreasing moisture content (w.b basis) of coffee berry and parchment coffee and the drying time dried with the continuous drying operation.

Product quality

It is obvious that the drying air temperature is one the decisive factors to the final coffee quality in terms of physical appearance and cup taste. In general practice, the temperature of drying air should not exceed 35 °C in the first stage of drying. Parchments become dry more rapidly than the bean causing parchment to crack. The solar collector is designed to generate heat at medium level to keep the rate of coffee drying at moderate speed and to prevent loss of flavor in the dried beans.

Solar drying differed from conventionally mechanical drying in the mechanism of heating method employed. The first was characterized by steady heating rate according to the insolation. Thus, the bean's temperature rose gradually from ambient air temperature at the early morning to reach the peak temperature below 35 °C in the middle day. During continuous drying regime, the additional heat from furnace was introduced at the second night to maintain the drying air temperature at 45 - 50 °C. The bean temperature never rose beyond 38 °C. This drying mechanism was implemented to avoid adversely the physical appearance, color as well as the balance of acidity, body and flavor of the dried coffee bean. The same results reported by Kamau (1980) and Sulistyowati *et al.*, (1996) on the drying of coffee parchment using combination drying regime i.e. sun drying table and mechanical dryer.

The continuous dried coffee beans appeared to have better storage property because of the shorter drying period compared to a period of more than 14 days drying under the sun. As a result, the risk of coffee spoilage by microbial infestation was substantially reduced particularly during the drying process and in subsequent coffee storage. Moreover, the product being dried were protected against dust, soil, insects and other undesirable materials. All of these factors contributed to a significantly improved and more consistent product quality.

Cost analysis

The technical efficiency in any factory is reflected by the unit processing cost achieved. The estimated processing cost per kg dried coffee is presented in Table 1.

Table 1. The component of processing cost

Component	Rp x 1.000
A. Fixed cost	
1. Depreciation	2.000 (16,20 %)
2. Credit and deposit	3.000 (24,30 %)
B. Variable cost	
1. Maintenance and repair	2.500 (20,24 %)
2. Electricity	1.250 (10,12 %)
3. Fuel	1.300 (10,52 %)
4. Labor	2.300 (18,62 %)
Total cost	12.350
Cost per kg dried coffee	123,50

The costs involved in operating the unit was calculated with the following assumption; annual production of 100 tons dried parchment coffee; government soft loan scheme for cooperative assistance of 6 %, investment cost of Rp 65.000.000,- and economic service live of the unit of is 25 years.

Fixed cost was around Rp 5.000.000,- /year. It consisted of depreciation and interest were calculated based on the estimation commonly used in routine production of the existing factory which both contributed almost 40 % of the total cost. Variable cost which included labor cost, energy cost and repair or maintenance cost were estimated based in the real expenditure spent during experiments. The number of labor required during peak harvesting season of 4 months was one person (permanent) assisted by 2 seasonal workers, whereas during low season only one person was required. Based on the regional waging system, the labor cost involved to the direct processing was estimated about to Rp 2.300.000,-. The main tasks of the workers were to load and to level incoming berries or parchment in the drying chamber which

consumed about 1 - 2 hours. Additional works during drying were stirring the beans in the drying chamber every 3 hours, charging wood into the furnace and making operational record of the system.

Maintenance cost contributed 20 % of the total cost which was mainly allocated to repair the solar air heater. The estimated economic service live is around 8 years. This expenditure will be deposited every year amounted to about Rp 2.000.000,-. This unit was equipped with low energy blowers constructed from a locally available materials. This type of blower was simply designed so that village workshops were able to produce it. The electrical energy consumption was about 65 kWh per ton dried coffee. Moreover, the dryer applied multi fanned system instead of a single high powered electric-blower as commonly used in conventional drying system (Sri Mulato *et al.*, 1994; Sri Mulato, 1994). The electrical cost involved to drive fans and lighting was about Rp 1,200.000 per year.

Fuel cost was mainly to buy fire wood which was available in sufficient amount surrounding the coffee farm as by product resulting from pruning of shed trees. The consumption of fire wood per ton dried coffee was calculated about 2 m3. The price of wood per m3 at delivered to the unit was Rp 6.500.

The total cost of production per kg dried bean was computed by summation both fixed and variable cost at annual production level of 100 tons that was about Rp 123,50. This amount is substantially lower than of the conventional production cost which achieve to around Rp 350.

CONCLUSION

A solar processing unit was effective to dry about 9 tons of parchment coffee and has potential use as an on-farm centralized coffee drying facility. The has 144 m^2 solar collector and a fire wood furnace of 100 kW for heating sources. Technical and economic evaluation conducted started from last year experiment showed that,

1. The solar collector was able to generate drying air temperature up to 60 °C and relative humidity of 12 %; when air flow rate was 9.000 m^3/hr and average a daily insolation was more than 5 kWh/m^2. The average drying temperature during fair weather condition was 35 - 40 °C.

2. The time required to dry to moisture level of 12 % was 168 hours for coffee cherry and 144 hours for parchment coffee respectively compared to more than 218 hours for sun drying. An intermittent drying regime using solar collector was only effective during low season harvesting.

2. A high efficient wood furnace equipped with a smoke-free heat exchanger was integrated into the model enabling it to be operated in the coffee growing area having high rain fall intensity. The furnace had average thermal output of 100 kW which was sufficient to heat the drying air up to 80 °C at volumetric rate of 15.000 m^3/hr. A continuous drying regime using both solar collector during the day time and the furnace at night time could reduce significantly the drying time to 72 hours (3 days). This operational regime is recommended during peak harvesting season.

3. The risk of coffee spoilage by microbial infestation was reduced both during the drying process and in subsequent storage. The product being dried were protected against dust, soil, insects and other undesirable materials. All of these factors contributed to a significantly improved and more consistent product quality. The continuous drying operation did not adversely affect on the final coffee quality in term of acidity, body and flavor.

4. The production cost, at an annual production of 100 tons of dried parchment coffee beans collected from 100 ha smallholder coffee field, was Rp 123,50 per kg dried beans. The investment cost for constructing the model was Rp 65 million and the service life is about 25 years.

5. Intensive field assessment in some coffee growing regions now is being conducted jointly with extension services and the association of coffee exporters.

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Abstract,

Since the government of Indonesia launched a policy to enhance coffee productivity, proposed concept in coffee handling operation is to centralize the harvest in a processing center covering a given acreage. A farmer cooperative, bean collectors or by a private company can operate the unit according to their interest. The Indonesian Coffee and Cocoa Research Institute has developed a model of middle scale coffee processing unit since the past three years. The processing building has one hundred forty four (144) meter square of forced convection type plate solar collector as a roof. The collector produces heat for a flat bed type dryers having capacity of about nine tons of fresh coffee parchments. The results showed that the optimum air flow through the collector ranged between 1.000 - 1.500 m^3 hr a ton of wet parchment. When the daily insolation rate was 4.50 kWh/m^2 the collector was able to heat the drying air up to 60 °C at relative humidity of 12 %. The time required to produce dried coffee bean at marketable moisture level of 11 % was 144 hours (six days), whereas sun drying was more than 218 hours (nine days). A high efficient wood furnace equipped with a smoke-free heat exchanger provided additional heat for drying process during rainy season. The heat output of the furnace ranged from 50 to 100 kW that was sufficient to heat the drying air up to 80 °C at volumetric rate of 15.000 m^3 hr. The combination operation of both solar collector and the furnace alternately during the day and night time could significantly shorten the drying time to 72 hours (three days). The improvement in the rate of drying could reduce the risk of spoilage and microbial infestation to the coffee beans both during the drying process and in subsequent storage. The product was free against dust, soil, insects and other undesirable materials due to protected drying operation. All of these factors contributed to a significantly improved and more consistent product quality. The cost analysis showed that the processing cost, at an annual output of 100 tons dried coffee beans was Rp 123,50 kg dried bean. The investment cost was Rp 65 million at 25 years expected service life.

PRODUCTION AND NUTRITIONAL EVALUATION OF COFFEE PULP SILAGE

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Introduction

Coffee pulp is the first by-product produced during the coffee fruit (coffee berry) wet processing and it represents around 42% of its total fresh weight. Large quantities of coffee pulp accumulate at the processing factory causing a serious disposal problem. The high water content of the pulp obtained from the wet processing makes costly and difficult its handling and preservation. One way to get around this problem is to dehydrate it by solar energy or by hot air (8). Since the coffee berry producer or processor has all available physical facilities for drying purposes busy at the harvest time, another way to storage and preserve the large amounts of coffee pulp produced is by the process of ensiling. In this process the material being ensiled is kept in an air- and water-tight container which provides conditions to prevent its decay or oxidation. By means of this process the ensiled material keeps its nutritive value and can be used or dehydrated following the silo opening. The silaging of coffee pulp has been done mostly in an empirical way and there was a need for a better knowledge of the factors that favourably affect this process. Numerous attempts have been made to use coffee pulp as ingredient in the diet of farm animals (3,5). These attempts have had a limited success due presumptively to the presence of antiphysiological factors in the coffee pulp which affects animal weight gain and feed conversion when it is consumed even at relatively low levels (5). Caffeine, potassium and phenols have been pointed out as probable antiphysiological factors(4). The low palatability of the diets containing coffee pulp has been linked to the presence of condensed tannins. The possibility of removing these antiphisiological factors by microbial action has been explored and some progress has been made in this respect (9,12). In the case of the silaging of wet processed coffee pulp it allows at least its storage without decay and loss of its nutritional value.

In this paper preliminary information is presented on the determination of colony forming units in MRS culture medium, pH, and caffeine and condensed tannins contents in coffee pulp ensiled in minisilos. It is also presented some preliminary results on the assays carried out with pigs and fish using coffee pulp silage as a diet complement.

Materials and methods

Materials

Coffee pulp was obtained from wet processing coffee berries plants immediately after depulping. The minisilos were made with pieces of double strength wall PVC pipe 35 cm in length and 10 cm in dia and PVC end caps 10 cm in dia. As silage inoculant was used AGROS, a product consisting of Lactobacillus plantarum bacteria mixed with cellulase and hemicellulase (silage inoculant) produced by Interprise Limited, U.K. Peptone water and MRS broth were purchased from Merck. Sugar cane molasses was purchased from a local suplier.

Minisilos preparation

3-kg portions of either treated or non-treated (control) coffee pulp were placed into minisilos and pressed by hand with wood pestles. A small hole was made in the center of the bottom cap of the minisilos to allow drainage. For each treatment and controls 20 replicas were run. After incubation for 1,3,5, 9 and 21 days at ambient temperature, four minisilos from each treatment were selected at random to be analised. Samples were taken for the following determinations: microbial colonies forming units (CFU) in MRS medium, dry matter, pH, sugars, low weight organic acids, caffeine, condensed tannins and simple phenolic compounds.

Large silos preparation

Six cylindrical silos were prepared with a diameter between 210 and 220 cm, an initial height between 84 and 94 cm and an average capacity of 3.0 cubic meters of coffee pulp. The six large silos were loaded as follows: two as controls with wet processed coffee pulp alone, two with wet processed coffee pulp treated with 5% molasses and two with wet processed coffee pulp treated with 5% molasses plus 0.001% silage inoculant. Each silo was kept closed for more than 100 days. The coffee pulp silage was dried in a fixed coffee bean dryer up to a moisture content of 10%. The dried coffee pulp silage was milled with the aid of a hammer mill provided with a 4 mm screen.

Pigs growing and finishing silage feeding trials

Rations containing four levels (0, 10, 15 and 20%) of coffee pulp ensiled with 5% molasses (CPS-M) were formulated according to the specifications established for swine growing and finishing (1). A commercial feed locally available was also used for comparison purposes. The aleatory experimental design included five treatments with four replications per treatment. Castrated pigs (12-18 kg live weight) coming from the offspring of crossbred (Yorkshire x Landrace) sows served by Yorkshire or Landrace boars were used for the eight weeks growing period and the following seven weeks finishing period. Each animal was placed in a separate pen (0.90 x 1.40 m) provided with a feeder and a drinking water source. Equal portions of feed were supplied daily to all the animals and the amounts of feed left over were recorded. Animals weighing was made every two weeks.

Fish growing silage feeding trials

The productive response of Cachama fish hybrids (Colossoma macropomum x Piaractus macropomum) to coffee pulp silage (CPS) added to their diets was studied during consecutive periods of 202 and 172 days. This study was carried out in a warm water fish pond at UNET's Aquaculture Experiment Station located at San Antonio de Caparo in the south of the State of Táchira, Venezuela. The experimental work was done using a bifactorial design completely randomized. Specimens of Cachama hybrids with initial weights between 29 and 43 g were held in one-meter sided cubic cages placed in a stationary water pond. A 2600 kcal metabolisable energy/kg-feed diet with four complementation levels of CPS (0, 10, 15 and 20%) was used. A locally available commercial feed for fish growing was used for comparison purposes. There were four replications for each treatment making a total of 20 cages each one with 3 fish specimens.

The diets were formulated according to the specifications established for fish growing (Nutriment Requirement of Warmwater Fishes. The National Research Council, Washington, D.C., U.S.A., 1977). The fish were fed twice a day (in the morning and in the afternoon) beginning with an amount of feed equivalent in weight to 10% of the biomass which was adjusted every three weeks according to the fish weight obtained. Body length measurings and weighings were performed every three weeks.

Laboratory analyses

Coffee pulp silage samples were analysed to determine lactic acid bacteria counts, dry matter, caffeine, low molecular weight organic acids and condensed tannins. For those diets complemented with coffee pulp silage analyses of dry matter, total protein, total ash, crude fiber, ether extract, calcium and phosphorus were also performed.

Results and discussion

The results presented in this preliminary report are referred only to coffee pulp treated with 5% molasses and 5% molasses plus silage inoculant (bacteria-enzyme mix) and their accompanying controls. Fig. 1 shows the graph of lactic acid bacteria colony forming units (CFU) plotted as a function of incubation time. It can be seen that for the 5% molasses treatment and its control (C1) the highest CFU value was obtained at the 5-days incubation time. Afterwards the CFU values decreased and at 21-days incubation time reached compatible values a little above 1.0E+7. On the other hand the 5% molasses plus silage inoculant treatment and its control (C2) showed their highest CFU value at 1-day incubation time which implies a very high initial lactic acid bacteria load in the coffee pulp used (100 times higher than in C1). At 3- and 5-days incubation times there was a notorious decrease of the C2 CFU value but it levels off to the CFU value of 5% molasses plus silage inoculant treatment at the 9- and 21 days incubation times.

The difference in the initial lactic acid bacteria load of the coffee pulp influences the behaviour of the fermentation process taking place in the minisilos. It was found that in C2 the pH values were lower than in C1 at the majority of the incubation times and in C2 all the lactic acid content values were higher than in C1. The lowest pH value in coffee pulp ensiled with molasses and molasses plus silage inoculant was obtained at 5-days incubation time, whereas in C2 the lowest pH value was reached at 3-days incubation time and in C1 reached slowly its lowest

value at the end of the 21-days incubation period. Thus it is clear that the presence of an initial high load of lactic acid bacteria and/or molasses favours a rapid lowering of the pH which is a desirable feature in a silage processing to control the development of clostridia.

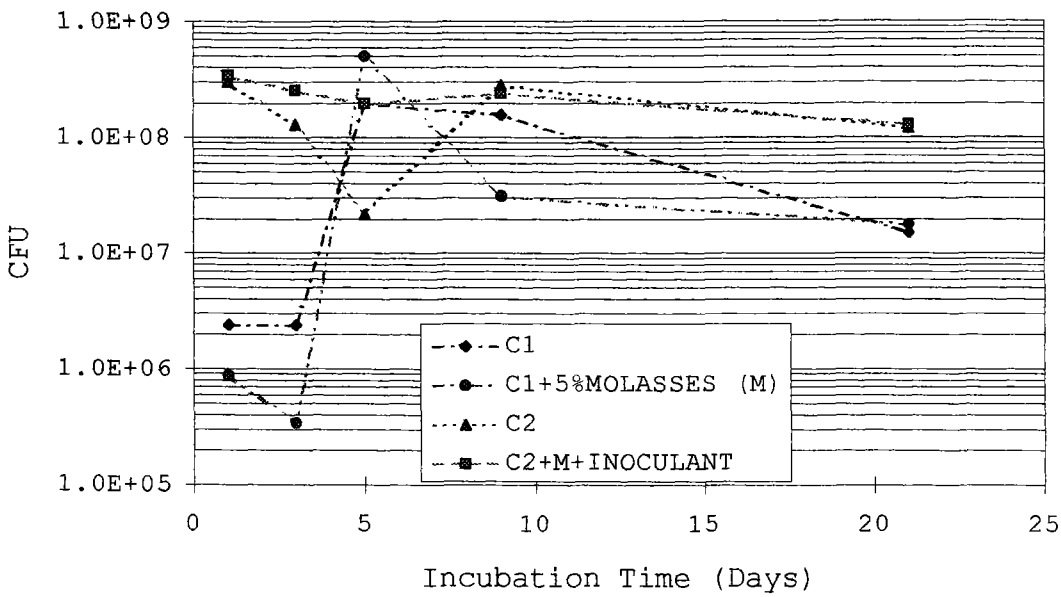

Fig. 1. Colony forming units (CFU) in MRS culture medium per gram of coffee pulp silage

Undoubtedly the adittion of molasses to the coffee pulp favours the production of acids and especially of lactic acid. The benefitial effect of the addition of silage inoculant it is not at all clear. From both the fresh (pulped by hand) and wet processed coffee pulp a bacterium similar to Lactobacillus plantarum was isolated in our laboratory (unpublished results). The addition of silage inoculant seems to be not warranted since in the treatment with molasses alone the pH reached values below 4.0 early and lactic acid production was relatively satisfactory (*cf* 10).

With regard to caffeine content in coffee pulp silage there was not a significant change along the incubation period in each treatment and among treatments. On the other hand condensed tannins content tended to increase along the incubation period in the coffee pulp ensiled with molasses and its control (C1) whereas in the coffee pulp ensiled with molasses plus silage inoculant and its control (C2) this value varied little during most of the incubation period. Porres *et al.* (12) claimed that the ensilage of coffee pulp significantly reduces the content of caffeine and tannins in long term (more than 100 days) ensiling periods.

It should be pointed here that no water soluble sugars and very little amounts of simple phenolics other than chlorogenic acid were detected in the wet processed coffee pulp used in the treatments above described. Overall results obtained from the production of coffee pulp silage in cylindrical minisilos (8 treatments and 190 minisilos) led to the conclusion that silaging with 5% molasses and with 5% molasses plus silage inoculant were the only treatments worthy of being tried to do nutritional assays with some farm animals. Table 1 shows that in the first

three large silos opened after an ensiling period of more than 100 days the coffee pulp ensiled with 5% molasses had a larger content of both lactic and acetic acids than the control (coffee pulp alone) and the control plus 5% molasses and silage inoculant (bacteria-enzyme mix).

Table 1

LOW MOLECULAR WEIGHT ORGANIC ACIDS, TANNINS AND CAFFEINE CONTENT (%DMB) IN DRIED AND GROUND COFFEE PULP SILAGE

COFFEE PULP SILAGE TYPE	LACTIC ACID	ACETIC ACID	BUTYRIC ACID	TANNINS	CAFFEINE
CONTROL (C)	0.19	0.56	0.10	0.36	0.39
C+5% MOLASSES (M)	4.91	1.45	0.21	0.28	0.50
C+M+INOCULANT	0.32	0.42	*	0.15	0.22

(*) Not detected

Table 2 shows that growing pigs (first 56 days) fed with rations containing CPS-M had equal (20% CPS-M) or better (10 and 15% CPS-M) total weight gain than those fed with commercial feed. There was no significant difference among the experimental treatments. With regard to total feed consumption there was no difference between the commercial feed ration and the experimental rations containing 0, 10 and 15% CPS-M. However the total feed consumption for 20% CPS-M was significantly lower ($p<0.1$) when compared to the rest of the treatments. It can be also observed that the experimental rations 0, 10, 15 and 20% CPS-M had a better feed conversion efficiency than the commercial feed. An accepted feed conversion efficiency for growing pigs is 2.7 which is quite close to those obtained with the experimental rations containing 0, 10, 15 and 20% CPS-M.

Table 2

TOTAL WEIGHT GAIN, TOTAL FEED CONSUMPTION AND FEED CONVERSION EFFICIENCY OF GROWING PIGS (FIRST 56 DAYS) AND FINISHING PIGS (NEXT 47 DAYS) FED WITH RATIONS COMPLEMENTED WITH 5% MOLASSES COFFEE PULP SILAGE

DAYS	PARAMETER	COMMERCIAL FEED	COFFEE PULP SILAGE COMPLEMENTATION IN %			
			0	10	15	20
FIRST 56	TOTAL WEIGHT GAIN (kg)	26.35	28.47	28.27	27.97	26.37
	TOTAL FEED CONSUMPTION (kg)	78.70	78.70	78.51	78.43	73.91
	FEED CONVERSION EFFICIENCY	2.99	2.76	2.78	2.80	2.80
NEXT 47	TOTAL WEIGHT GAIN (kg)	32.75	36.25	35.25	33.00	30.87
	TOTAL FEED CONSUMPTION (kg)	114.71	116.30	115.90	116.30	112.32
	FEED CONVERSION EFFICIENCY	3.54	3.21	3.29	3.52	3.64

Table 2 also shows that finishing pigs (next 47 days) fed with rations containing up to 15% CPS-M had equal total weight gain and total feed consumption to those fed with commercial feed. Finishing pigs fed with the experimental ration containing CPS-M had better (10%) or equal (15%) feed conversion efficiency than those fed with commercial feed. A feed conversion efficiency of 3.20 is considered satisfactory for finishing pigs. The economic analysis of producing one kg of live weight showed that the lowest cost was obtained with 15% and 10% CPS-M for pig growing and finishing, respectively. Overall results agree with most of the reports on the use of coffee pulp to complement the feeding rations of growing and finishing pigs(6,7,11).

Table 3 shows the total body weight and length gains of Cachama fish hybrids fed with the diets complemented with CPS. During the first 202-days growing period the diets containing 15 and 20% CPS gave higher total body weight and length gains than the 0 and 10% CPS, whereas during the next 172 days growing period those of the diet containing 10% CPS was better than the 0, 15 and 20% CPS. The highest total body weight gain for the sum of the two growing periods was obtained with the diet complemented with 10% CPS and it is comparable to that one obtained with the commercial feed. With regard to the total body length gain during the whole growing period there were no significant differences. In the CPS experimental treatments most of the weight gain (around 66%) occurred in the second growing period (172 days) whereas most of the length gain (around 68%) took place during the first growing period (202 days). It was also observed that fish density in the cage affects body weight and length gains.

Table 3

TOTAL BODY WEIGHT AND LENGTH GAINS OF CACHAMA HYBRIDS FED DIETS CONTAINING COFFEE PULP SILAGE

DAYS	PARAMETER	COMMERCIAL FEED	COFFEE PULP SILAGE COMPLEMENTATION IN %			
			0	10	15	20
FIRST 202	TOTAL WEIGHT GAIN (g)	426.86	379.10	407.46	489.37	438.09
	TOTAL LENGTH GAIN (cm)	11.85	12.63	13.58	14.25	14.78
NEXT 172	TOTAL WEIGHT GAIN (g)	893.33	805.00	957.50	795.38	750.00
	TOTAL LENGTH GAIN (cm)	8.00	5.87	7.12	6.44	6.17
TOTAL 374	TOTAL WEIGHT GAIN (g)	1320.19	1184.10	1364.96	1284.75	1188.09
	TOTAL LENGTH GAIN (cm)	19.85	18.50	20.70	20.69	20.95

From the data presented on complementation of Cachama fish diets with CPS it is concluded that CPS can be safely used to substitute part of expensive ingredients. García and Bayne (1974), cited by Braham (2), reported that Tilapia aurea fed with a diet suplemented with 30% coffee pulp showed a better growth than an accompanying control. It seems that fish can tolerate higher levels of coffee pulp in their diets than other farm animals. Some fish species are a source of high quality protein which can be grown in stationary warm water ponds and the inclusion of coffee pulp silage in their diets could contribute to lower the production costs,

especially in underdeveloped countries. The use of coffee pulp silage in the rations of chicks to produce seven weeks broilers and in lambs rations proved not to be economically viable under conditions prevailing in Venezuela. Rabbits fed with rations complemented with coffee pulp silage tolerate its presence well

In conclusion the results obtained showed that the composition of wet processed coffee pulp is substantially different from that of fresh coffee pulp. The difference is due to the leaching of sugars, caffeine, phenolics and other soluble compounds. The composition of the coffee pulp silage produced will depend largely on the origin and handling of the coffee pulp to be ensiled and this fact explains the controversial results oftenly reported about it. From the data presented here we may also conclude that coffee pulp silaging represents a valid alternative way to handle and store the huge amounts of coffee pulp produced in the coffee berries processing factories around the world.

Acknowledgements

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Summary

Cylindrical minisilos were prepared with a capacity of 3 kg of wet processed coffee pulp. The experimental and control treatments, replicated four times, were incubated during 1, 3, 5, 9 and 21 days. Results obtained from the minisilos incubation led to the conclusion that only the treated with 5% molasses and idem plus 0.001% silage inoculant showed pH values and lactic acid content adequate to insure a satisfactory coffee pulp ensilage process. Six large cylindrical silos each with a capacity of three cubic meters were prepared to produce the amount of silage required for animal feeding trials: two with coffee pulp alone, two treated with 5% molasses and two treated with 5% molasses plus 0.0001% silage inoculant. They were kept closed for more than 100 days and opened when the animals feeding trials were ready to commence. Pigs, fish and rabbits fed with rations complemented with coffee pulp silage tolerate its presence well. It was found that it is advantageous to replace expensive feed ingredients with coffee pulp silage in the rations supplied to pigs and fish.

Resumen

Se prepararon minisilos cilíndricos con una capacidad de 3 kgs. de pulpa de café obtenida por vía húmeda. Los tratamientos experimentales y controles, replicados cada uno cuatro veces, se incubaron por períodos de 1, 3, 5, 9 y 21 días. A partir de los resultados obtenidos de la incubación de los minisilos se concluyó que solamente los tratados con 5% de melaza y con 5% de melaza más 0,001% de inóculo de ensilaje mostraron valores de pH y contenido de ácido láctico adecuados para asegurar un proceso de ensilaje satisfactorio. Seis silos cilíndricos grandes, con una capacidad de 3,0 metros cúbicos, se prepararon para producir la cantidad de ensilaje requerido para realizar ensayos de alimentación animal: dos con pulpa de café sóla, dos tratados con 5% de melaza y dos tratados con 5% de melaza más inóculo de ensilaje. Los silos se mantuvieron cerrados por más de 100 días y se abrieron cuando los ensayos de alimentación estaban listos para comenzar. Cerdos, peces y conejos alimentados con raciones complementadas con pulpa de café ensilada toleran su presencia bien. Se encontró que es ventajoso reemplazar ingredientes costosos con pulpa de café ensilada en las raciones que se suministran a cerdos y peces.

THE SELECTION OF AN APPROPRIATE WASTEWATER TREATMENT SYSTEM FOR THE COFFEE INDUSTRY IN KENYA

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Abstract

The coffee industry in Kenya produces large volumes of high strength effluent on a seasonal basis. Many of the processing sites are on hills with little land available for treatment. A further difficulty arises because of a lack of resources within the industry both in terms of finances and skilled staff. This paper reviews the requirement for wastewater treatment in the light of recent research that has suggested the impact of pollution from coffee processing is minor, compared with other pollution sources.

The options for wastewater treatment together with the results of research showing their removal efficiencies are reviewed. Treatment methods include; anaerobic ponds, anaerobic contact processes and upflow anaerobic sludge blanket reactors (UASB's).

The COD and BOD_5 removal efficiencies reported for these systems are; anaerobic lagoons (50% and 96%), upflow anaerobic contact filters (90% and 90%) and the UASB (55% and 74%).

The selection of an appropriate treatment system is made using multiple criteria decision analysis (MCDA) which incorporates important decision making factors such as land availability and technical sophistication and produces an hierarchy of appropriate systems.

The decision making process is broken down into two stages; the first a screening stage and the second a more detailed weighted analysis. In the second stage, decision making factors are assigned weighted importance on the basis of the specific requirements of the Kenyan coffee industry. The use of this selection method is demonstrated with a case study of the Jacaranda Estate, Ruiru, Kenya.

ÉTUDE DES EFFETS DU pH, DE LA TEMPÉRATURE ET DE LA DISPONIBILITÉ EN NH_4^+ ET NO_3^- SUR LA CAPACITÉ D'ABSORPTION RACINAIRE D'UN CAFÉIER ARABICA, DANS LE BUT D'OPTIMISER SA NUTRITION AZOTÉE

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INTRODUCTION

L'azote (N) est l'élément le plus souvent limitant dans les plantations de caféiers avec des exportations estimées à 50-90 kg N par tonne de café marchand, des pertes annuelles par lixiviation, érosion et volatilisation s'élevant jusqu'à 100 kg N en sol non protégé. Pour le renouvellement de biomasse, les besoins annuels du caféier sont d'environ 30 kg N. Les engrais azotés constituent la principale source de N dans les agrosystèmes caféiers. Suivant le degré d'intensification, des apports de 30 à 300 kg N par hectare et par an sont recommandés. En systèmes de culture intensifs, de fortes fertilisations azotées entraînent une acidification du sol (Vaast, 1995), une contamination des nappes phréatiques par le nitrate (Reynolds-Vargas *et al.*, 1994), et ne sont pas toujours rentables.

Une modification des pratiques culturales doit être menée afin d'améliorer l'efficacité de la fertilisation azotée en diminuant les apports d'engrais et en réduisant les pertes. Au cours de ces dernières décennies, le fractionnement des apports de N au cours du cycle de production et une meilleure synchronisation avec les besoins du caféier ont apporté des améliorations sensibles. Des études récentes sur la minéralisation, la nitrification, et les pertes par lixiviation et volatilisation (Babbar et Zak, 1994 & 1995), ainsi que sur la contribution de la fixation biologique de légumineuses associées (Snoeck, 1996) devraient permettre de mieux doser la fertilisation azotée en relation avec la disponibilité saisonnière en NH_4^+ et NO_3^- du sol.

Parallèlement à une meilleure compréhension du cycle de N dans les agrosystèmes caféiers, un approfondissement des connaissances sur l'influence de l'environnement racinaire sur l'absorption du N par le caféier devrait permettre d'optimiser sa nutrition azotée. L'incidence des caractéristiques du sol telles que l'acidité, la température, l'aération, et la disponibilité en NH_4^+ et NO_3^-, sur la capacité et la vitesse d'absorption des racines de caféier n'a pas fait l'objet d'études détaillées.

L'objectif de cette étude est de mesurer l'absorption de NH_4^+ et NO_3^- par des racines de caféier en solution hydroponique en faisant varier le pH, la température, l'aération, la disponibilité en NH_4^+ et NO_3^- dans les limites des valeurs rencontrées au champ.

MATÉRIEL ET MÉTHODES

Deux groupes des semenceaux d'Arabica (*Coffea arabica* L.) cv Catuai rojo, âgés de 4 mois, ont été acclimatés pendant 4 jours en solution hydroponique contenant de l'azote sous forme de NH_4^+ ou de NO_3^-.

Avec chaque groupe de semenceaux, 4 expériences d'une durée de 3 heures ont été menées afin de mesurer l'absorption de NH_4^+ et/ou de NO_3^- en faisant varier :

* le pH de 2,75 à 7,25 par intervalle de 0,75
* la température de la solution de 4°C à 40°C par intervalle de 6°C
* le rapport NO_3^-/NH_4^+ ou NH_4^+/NO_3^- (en m*M*) de 0:1, 0,5:1, 1:1, 2:1, 3:1
* l'aération (injection de 50 ml/min de N_2 en solution) ou en inhibant la respiration racinaire (ajout de 50 µ*M* de 2,4 dinitrophénol (DNP)).

Dans chacune des expériences, les traitements ont été répétés 5 fois. Les quantités de NH_4^+ et de NO_3^- absorbées et les poids frais des racines ont été mesurés suivant les méthodes précédemment décrites (Vaast, 1995).

RÉSULTATS ET DISCUSSION

Effets du pH

Les pH bas (<4,25) et élevés (>6,50) influencent plus fortement l'absorption de NH_4^+ que celle de NO_3^- (figures 1a&b). L'absorption de NH_4^+ et de NO_3^- semble peu sensible aux pH intermédiaires (4,25 à 5,75), les plus courants en caféiculture. Cela indique que le caféier Catuai est bien adapté aux sols acides et a la capacité d'utiliser la forme minérale prédominante. En sol très acide (pH < 5,0) la nitrification est faible et le caféier satisferait ses besoins en N par l'absorption de NH_4^+. En sol moins acide favorisant la nitrification, il s'approvisionnerait principalement à partir de NO_3^-.

Effets de la température

Entre 16°C et 4°C, l'absorption de NH_4^+ est beaucoup moins diminuée que celle de NO_3^- (figures 2 a&b). Ceci constitue un avantage pour le caféier Catuai en zone d'altitude, notamment en saison froide durant laquelle la nitrification est plus fortement ralentie que l'ammonification.

Entre 16°C et 22°C, l'absorption du NO_3^- augmente très fortement pour devenir équivalente à celle du NH_4^+ entre 22°C et 34°C. Le Catuai est donc apte à utiliser indifféremment la forme de N disponible. A 40°C, les absorptions s'infléchissent ce qui milite pour l'adoption de pratiques culturales favorisant le maintien des couches superficielles du sol à des températures intermédiaires.

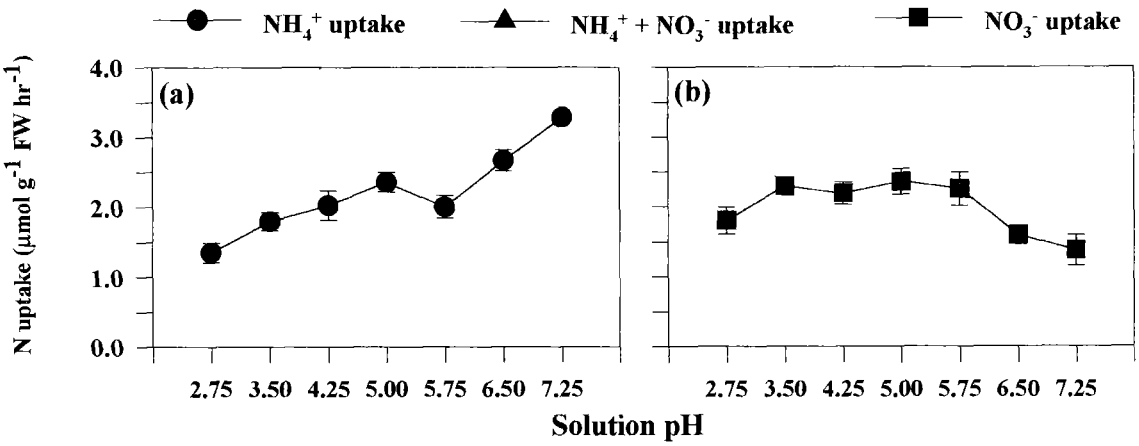
Effets des rapports NO_3^-/NH_4^+ et NH_4^+/NO_3^-

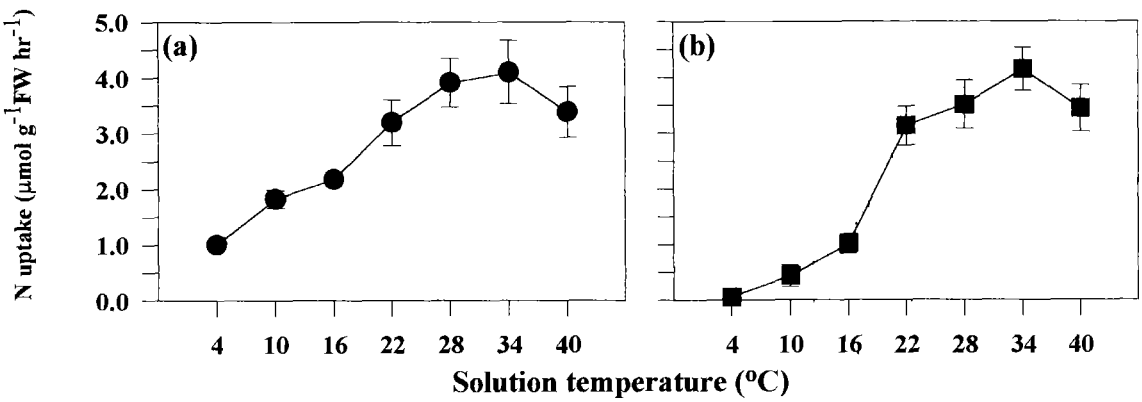
La présence de NO_3^- en solution n'a aucune influence sur l'absorption du NH_4^+ (fig. 3a). En revanche, l'absorption du NO_3^- est significativement réduite par la présence de NH_4^+ (fig. 3b), mais uniquement à la concentration la plus élevée (3 m*M*). L'absorption est plus forte en présence d'une solution azotée mixte (NH_4^+ + NO_3^-) que lorsqu'une seule forme (NH_4^+ ou NO_3^-) est disponible.

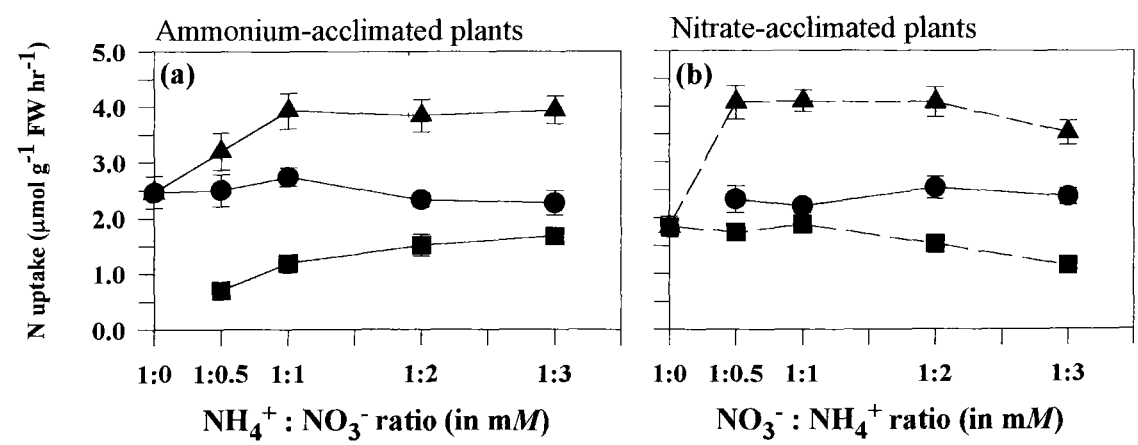
Effets du DNP et de conditions anaérobies

Le DNP bloque presque toute absorption de NH_4^+ (-95 %) et de NO_3^- (-97 %). En conditions anaérobies (injection continue de N_2 en solution), l'absorption du NH_4^+ est moins diminuée (-30 %) que celle du NO_3^- (-50 %). Ceci est avantageux pour le caféier en saison des pluies, lorsque l'aération du sol est faible et les pertes de NO_3^- par lessivage et dénitrification sont élevées (Babbar et Zak, 1995).

Figures 1, 2, & 3: Effets du pH, de la température, et des rapports NH_4^+/NO_3^- et NO_3^-/NH_4^+ sur l'absorption de NH_4^+, NO_3^-, et $NH_4^+ + NO_3^-$ par les racines du caféier Arabica Catuai rojo.







Conclusion

Ces résultats montrent que le caféier Arabica Catuai est bien adapté aux sols acides et qu'il est capable d'exploiter la ou les formes minérales d'azote disponibles suivant les conditions ambiantes. Ils suggèrent également que l'absorption azotée est sensible aux conditions anaérobies.

Sous réserve de confirmation avec d'autres cultivars, ces observations devraient permettre d'optimiser la nutrition azotée du caféier. Pour maintenir le système racinaire à des températures optimales pour l'absorption du NH_4^+ et du NO_3^-, des pratiques culturales telles que le paillage et l'ombrage aménagé sont recommandées. Afin d'obtenir un équilibre entre NH_4^+ et du NO_3^- dans le sol qui induise une absorption maximale, il est conseillé d'appliquer la ou les formes azotées déficitaires suivant les variations saisonnières.

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Résumé

Des semenceaux d'Arabica cv Catuai ont été utilisés pour déterminer les effets du pH, de la température, de la disponibilité en NH_4^+ et NO_3^- et de conditions anaérobies sur l'absorption de NH_4^+ et de NO_3^- dans des expériences de courtes durées (3 heures) en solution hydroponique.

Les résultats montrent que l'absorption de NH_4^+ et de NO_3^- par le Catuai est peu sensible aux pH allant de 4,00 à 6,00, les plus fréquents en arabicaculture. Par contre, l'absorption est réduite de 50 % pour NO_3^- et de 30 % pour NH_4^+ par des conditions anaérobies.

Entre 4°C et 16°C, le Catuai absorbe plus de NH_4^+ que de NO_3^-. Entre 16°C et 22°C, l'absorption de NO_3^- augmente très fortement pour devenir équivalente à celle du NH_4^+ entre 22°C et 34°C. A 40°C, les absorptions de NO_3^- et de NH_4^+ s'infléchissent.

L'expérience sur les rapports NH_4^+/NO_3^- montrent qu'aucune des 2 formes n'a d'effet inhibiteur marqué. Au contraire, l'absorption est plus forte en présence d'une solution azotée mixte ($NH_4^+ + NO_3^-$) que lorsqu'une seule forme (NH_4^+ ou NO_3^-) est disponible.

Ces résultats montrent que le caféier Catuai est bien adapté aux sols acides et qu'il possède une grande capacité à utiliser la ou les formes azotées minérales disponibles suivant les conditions ambiantes. Néanmoins, la fertilisation azotée du caféier peut être optimisée par le maintien dans l'environnement racinaire de températures intermédiaires et d'une disponibilité équilibrée entre NH_4^+ et NO_3^-.

MISE AU POINT DE PROTOCOLES DE TRAITEMENTS CONTRE L'ANTHRACNOSE DES BAIES (CBD) DU CAFÉIER ARABICA AU CAMEROUN

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1. Introduction

Le caféier Arabica, dans sa zone de culture au Cameroun (hauts plateaux des Provinces de l'Ouest et du Nord-Ouest) est sujet à des attaques d'anthracnose sur baies, dues à *Colletotrichum kahawae,* qui occasionnent des pertes de rendement considérables.
Les variétés locales (principal cultivar : Jamaïque) sont assez sensibles à l'anthracnose ou CBD (Coffee Berry disease). Cette maladie, apparue au Cameroun en 1958 (Muller, 1980), toujours localisée au seul continent africain, peut causer jusqu'à 80% de perte des récoltes dans les plantations villageoises. L'absence de variétés résistantes dans ces plantations, a fait de la lutte chimique la principale méthode de contrôle de la maladie.
Les résultats présentés dans cet article proviennent d'un essai de traitements chimiques contre la maladie mené durant 2 années consécutives. L'objectif est d'analyser globalement l'action des fongicides sur 2 années, et de comparer l'efficacité des traitements réalisés en 1994 en tenant compte des arrières effets des traitements de 1993.
Le cycle du caféier étant souvent bisannuel, cette étude est une première approche permettant de relier la maladie au cycle du caféier.

2. Matériel

Les données analysées proviennent d'un essai de traitements fongicides contre le CBD, réalisés dans une plantation villageoise du Nord-Ouest du Cameroun. Cette plantation est constituée de 1200 caféiers Arabica de la variété Jamaïque.
Le dispositif utilisé est un plan en randomisation totale avec des parcelles mono-arbre, chaque traitement étant répété 100 fois. Les observations se font sur 5 branches tirées au hasard sur chaque caféier.
Les traitements fongicides appliqués en 1993 sont : Octave 50 WP (50 % de prochloraze manganèse), Tilt CT (6.25 % de propiconazole + 37.5 % de chlorothalonil), Mirage 50 WP (50 % de prochloraze) et Pronader 450 (45 % de prochloraze) en référence à un témoin non traité. Les traitements se font en 5 applications espacées comme suit : 1er traitement : 26 Avril, 2ème traitement : 17 Mai, 3ème traitement : 14 Juin, 4ème traitement :12 Juillet et 5ème traitement : 2 Août.
Les traitements les moins efficaces en 1993 n'ont pas été retenus. Quatre unités (100 arbres) traitées en 93 ont été divisées en 4 sous unités en 1994 ; sur chacune de ces 16 sous unités, 3 traitements (Octave, Tilt et un traitement avec 4 applications d'Octave) ont été mis en essai face à un témoin. Le rythme des traitements est : 1er traitement : 3 Mai, 2ème traitement : 24 Mai, 3ème traitement : 23 Juin, 4ème traitement : 11 Juillet et 5ème traitement : 1 Août.

Les fréquences de traitement retenues permettent d'assurer une protection des baies jusqu'à la 23ème semaine après la floraison.

3. Méthodes

Les données sont issues des dénombrements des baies saines et malades aux 4ème et 27ème semaines après la floraison. Les récoltes ont été enregistrées par arbre en fin de période de fructification.
Deux variables ont été étudiées :

* le pourcentage de perte de baies, calculé de la manière suivante :

$$\%\ perte = \frac{BT4 - BT27}{BT4} * 100$$

avec: BT4= nombre de baies total à la 4ème semaine après floraison
BT27= nombre de baies total à le 27ème semaine après floraison

* le poids de cerises récoltées sur chaque arbre à la fin de la période d'observation.

Deux modèles d'Analyse de Variance (ANOVA) ont été utilisés :

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_{ij} + \varepsilon_{ijk}$$

avec : Y= variables étudiées (% perte et poids de cerise)
μ= moyenne générale
α_i= effet de l'année, i=1993,1994
β_j= effet des traitements fongicides, j=1,2,3
δ_{ij}= interaction entre les années et les traitements
ϵ_{ijk}= résidus

Le deuxième modèle est de la même forme :

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_{ij} + \varepsilon_{ijk}$$

avec : Y= variables étudiées (% perte et poids de cerise)
μ= moyenne générale
α'_i= effet des traitements de 1994, i=1,...,4
β'_j= effet des traitements de 1993, j=1,...,4
δ'_{ij}= interaction entre les traitements de 1993 et 1994
ϵ'_{ijk}= résidus

L'ANOVA réalisée sous SAS grâce à la procédure GLM (SAS user's guide), permet de déterminer les effets des différents facteurs et de leurs interactions.
Des comparaisons multiples de moyenne sont réalisées pour différencier les niveaux de traitement. Pour le premier modèle, l'option LSMEANS *modèle* /TDIFF dans la procédure GLM est utilisée. Pour le deuxième modèle, l'analyse est suivie d'un test de Newman et Keuls (Dagnélie, 1970) qui classe les moyennes significativement différentes par ordre croissant.
L'égalité des variances est testée par un test de Bartlett (Snedecor et Cochran, 1971).

4. Résultats

Dans un premier temps, l'objectif est de comparer les traitements sur deux années consécutives. En conséquence, seuls les traitements communs aux deux années sont conservés : Octave, Tilt et le témoin.
Dans un second temps, l'efficacité de l'ensemble des traitements de 1994 est étudiée en tenant compte des arrières effets des traitements de 1993.
Les moyennes et écarts-types des différentes combinaisons de traitements sont donnés dans le tableau I.

Tableau I : Pourcentage de perte, récolte par arbre, somme des récoltes par arbre sur les 2 années.

an	trait de l'année	arrière effet	n	% perte	sd	n	récol. en g.	sd	rec93 + rec94
93	Témoin		100	84	1.406		969	950	
	Octave		100	16	0.744		4785	2562	
	Tilt		100	31	1.387		3169	1574	
94	Témoin	Témoin	25	79	3.650	25	1335	1099	2515
		Octave	25	78	4.550	25	579	411	4561
		Tilt	25	72	4.627	26	783	545	4918
		Pronader	25	78	3.922	25	928	663	5020
	Octave	Témoin	25	40	3.896	25	3869	3387	5629
		Octave	25	29	9.168	24	3347	2149	8790
		Tilt	25	16	3.634	26	3075	1670	6412
		Pronader	25	28	5.213	24	3124	2299	6946
	Tilt	Témoin	25	40	7.333	26	4158	3249	3356
		Octave	25	27	6.414	25	1997	1184	6418
		Tilt	21	21	3.952	21	4405	2467	7668
		Pronader	25	23	4.434	25	2377	1559	6933
	Octave 4 appl	Témoin	25	32	2.746	25	3876	3663	5900
		Octave	25	22	8.653	26	2566	2071	7657
		Tilt	25	14	3.550	24	3298	2394	7091
		Pronader	25	19	5.438	24	2587	1153	7357

** Analyse des pertes de fruits :*

Le pourcentage de perte a été analysé après transformation des données en arcsinus racine carrée afin que la distribution de la variable soit normale.

Les résultats de l'analyse de variance mettent en évidence des effets significatifs des traitements et des interactions, mais aucun effet année n'est détecté au seuil de 5 % (tableau II). Dans l'ensemble, les traitements induisent les mêmes réponses durant les deux années. Les témoins perdent plus de fruits que les arbres traités. Le fongicide Octave assure une meilleure protection des baies. Le test de comparaison de moyenne indique que le Tilt engendre des réponses identiques les deux années. Il exite une interaction entre traitement et année qui s'explique par un effet différent de l'Octave entre les deux années. Le classement des traitements n'est toutefois pas modifié d'une année sur l'autre.

Tableau II : ANOVA des pourcentages de perte sur les 2 années.

SOURCE	DDL	SCE	F	Pr>F
année	1	0.15	3.76	0.0531
traitement	2	50.21	589.73	0.0001
année*trait	2	1.64	19.25	0.0001
résiduelle	586	24.95		
total	591	77.09		

L'analyse de variance du pourcentage de perte indique que les traitements 94 et les arrières effets 93 sont significativement différents (tableau III). Le test de Newman et Keuls différencie le témoin du traitement à l'Octave (4 applications) d'un 3ème groupe formé des 2 traitements Tilt et Octave (5 applications) lesquels perdent moins de fruits. Quant aux arrières effets, ils sont tous nettement différenciés.

Tableau III : ANOVA des pourcentages de perte en 1994.

SOURCE	DDL	SCE	F	Pr>F
trait 94	3	24.39	172.56	0.0001
trait 93	3	2.48	17.55	0.0001
trait 94*93	9	0.55	1.29	0.2387
résiduelle	380	17.90		
totale	395	45.29		

* *Analyse des récoltes :*
L'analyse de variance du poids de cerises récoltées par arbre montre que les effets "année", "traitements", et leurs interactions sont significatifs (tableau IV).

Tableau IV : ANOVA sur les récoltes des deux années.

SOURCE	DDL	SCE	F	Pr>F
année	1	41227124	10.97	0.0001
traitement	2	1035201326	137.69	0.0001
année*trait	2	61784934	8.22	0.0003
résiduelle	595	2236637365		
totale	600	3376437183		

Toutefois, la réponse aux traitements n'est pas identique chaque année. Il est à noter que les récoltes de 1993 ont été supérieures à celles de 1994 (713 kg/ha de café marchand en 1993 contre 588 kg/ha en 1994). Sur les deux années, les arbres traités ont une production supérieure à celle des témoins. Le traitement à l'Octave permet une récolte nettement plus abondante : environ 4 kg contre 3 kg avec Tilt et seulement 1 kg avec le témoin. Les comparaisons multiples ne différencient ni les témoins ni les traitements au Tilt de chaque année : la production est quasi identique. Néanmoins, il se trouve que les traitements à l'Octave ne sont pas comparables entre les deux années, en 1994 le fongicide induit un différentiel de production moins important : ce qui explique que l'interaction est significative.
L'ANOVA des récoltes de 1994 met en évidence une différence entre les traitements de l'année et entre les arrières effets dus aux traitements de l'année précédente (tableau V). Dans le premier cas, 2 groupes se séparent avec d'un côté le témoin sur lequel on a récolté peu de baies, de l'autre l'ensemble des traitements qui sont équivalents. Les arrières effets sont également divisés en 2 groupes : le témoin, le Pronader et l'Octave, le Tilt étant intermédiaire.

Tableau V : ANOVA des récoltes de 1994.

SOURCE	DDL	SCE	F	Pr>F
trait 94	3	407287979	30.47	0.0001
trait 93	3	92634916	6.93	0.0001
trait 94*93	9	63137251	1.57	0.1210
résiduelle	380	1693335515		
totale	395	2251448609		

On a pu mettre en évidence, en faisant la somme des récoltes par arbre sur les deux années, que l'optimisation de la récolte globale était obtenue lorsque les arbres étaient traités les deux années avec l'Octave (8790g de café récolté). Le plus faible rendement est obtenu pour les arbres témoins en 1993 et en 1994. La différence de rendement est donnée par un coefficient de 3.5. De plus le test de Newman et Keuls différencie nettement un premier groupe constitué d'arbres traités les 2 années et un second groupe constitué d'arbres traités une seule fois.

La corrélation entre le pourcentage de perte d'une année et la récolte de la même année est négative (tableau VI). Il semble en effet normal qu'un fort pourcentage de perte engendre une faible récolte. Toutefois cette corrélation est moins forte en 1994 puisque les récoltes de 1994 ont été moins importantes. Il existe une corrélation positive entre le pourcentage de perte en 1993 et la récolte de 1994 ; il est possible que des pertes importantes de fruits favorisent la production de l'année suivante. La corrélation entre la récolte de 1993 et le pourcentage de perte en 1994 est négative. Il faut également noter que les pourcentages de perte de la première année et de la deuxième année sont corrélés.

Tableau VI : Corrélation entre pourcentage de perte et récolte.

		ANNEE 1993		ANNEE 1994	
		% PERTE	RECOLTE	% PERTE	RECOLTE
ANNEE 1993	% PERTE	.	**- 0.65147**	**0.19881**	**0.17403**
	RECOLTE	**- 0.65147**	.	**- 0.17598**	0.00602
ANNEE 1994	% PERTE	**0.19881**	**- 0.17598**	.	**- 0.37662**
	RECOLTE	**0.17403**	0.00602	**- 0.37662**	.

Les corrélations en **gras** sont significatives au seuil 1/1000.

5. Discussion et conclusions

Les analyses réalisées sur l'effet des fongicides permettent de conclure que l'Octave est significativement plus efficace : c'est le traitement qui fournit le plus faible pourcentage de perte et la meilleure récolte. Toutefois, son efficacité est moindre en 1994. En effet, il a été récolté 4.8 kg en moyenne de café cerise sur ces arbres en 1993, contre seulement 3.3 kg en 1994. Il est possible d'expliquer ces résultats par une production initiale légèrement plus faible en 1994, et par des chutes dues à la maladie plus importantes.
Le témoin donne des récoltes comparables entre les deux années (970 g en 1993, 905 g en 1994) alors que le pourcentage de perte est plus élevé en 1994.
Le traitement au Tilt donne des résultats comparables d'une année sur l'autre. En effet, le pourcentage de perte est de l'ordre de 32% et environ 3.1 kg de café sont récoltés sur chacun des arbres bénéficiant de ce traitement les deux années.
En résumé, le fongicide Octave assure une meilleure protection des baies ce qui permet une bonne récolte. L'analyse des données de 1994 indique également que 4 applications de ce fongicide sont suffisantes (Regazzoni, 1996).

On peut se demander si l'optimisation des récoltes ne pourrait pas être la conjonction d'une production initiale de baies importante et d'un traitement efficace. Pour cela, des traitements en alternance pourraient être testés pour rechercher une séquence économiquement rentable.

Bien que l'effet des traitements sur les récoltes ne soient pas semblables d'une année à l'autre, le classement ne varie pas entre les deux années. On pourrait donc se contenter de la récolte pour la comparaison de l'efficacité des différents fongicides. Ceci permettrait de réduire les opérations de comptage et de saisie du nombre de baies. L'économie réalisée pourrait servir à mettre en place des études multilocales, ou encore à améliorer le système d'échantillonnage.

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Résumé :

L'anthracnose des baies du caféier, due à *Colletotrichum kahawae*, provoque d'importantes pertes de fruits dans les plantations africaines d'arabica. Au Cameroun, cette maladie peut causer jusqu'à 80 % de pertes de récoltes dans les parcelles villageoises des zones les plus atteintes. Bien que des programmes d'amélioration génétique aient pour objectif d'accroître la résistance des variétés cultivées, la lutte chimique demeure, pour le moment, la principale méthode utilisée pour contrôler cette maladie.
Dans cette étude, des traitements fongicides sont comparés durant deux années consécutives. Les traitements de l'année 1994 sont appliqués sur les arbres ayant été traités en 1993, suivant un dispositif croisé. Ce dispositif original permet d'évaluer les arrières effets des traitements sur les taux d'attaque et sur les productions. De plus, des séquences de traitements peuvent ainsi être recommandées pour optimiser la lutte contre cette maladie. Les analyses indiquent que, sur les deux années, le traitement à base de prochloraze-Mn est efficace pour réduire les pertes et augmenter les productions. Cependant, des arrières effets sont détectés, avec une diminution de la production initiale de fruits chez les arbres traités l'année d'avant. Un protocole de traitement en alternance pourrait optimiser la lutte contre cette maladie.

Abstact :

Coffee Berry Disease (CBD), caused by *Colletotrichum kahawae*, produces significant fruit losses in the african arabica plantations. In Cameroon, this disease can induce to 80 % of losses in farmer's plantations in the most damaged areas. Although breeding programmes were developped to increase the coffee resistance to CBD, at present, the chemical struggle is the principal method used to control the disease.
In this study, fongicidal treatments were compared during two consecutive years. The treatments of 1994 were applied on the trees treated in 1993 according to a cross design. This design allowed to evaluate the fongicide effects of 1993 on the percentage of disease and the production of the year 1994. It is also possible to recommand a treatment sequence to optimize the disease control. The analyzes indicate that the treatment with prochloraze-Mn was efficient to reduce the losses and to increase the yield. Therefore, back effects were detected, with a reduction of the initial yield of the trees treated the previous year. An alternating treatment protocol could be used to optimize the disease control.

UTILISATION D'UN PIÈGE À ATTRACTIF KAIROMONAL POUR LE SUIVI DES POPULATIONS DU SCOLYTE DU CAFÉ EN CHAMP

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Introduction

Le scolyte des grains, *Hypothenemus hampei*, est le ravageur majeur du caféier dans toutes régions du monde qu'il a colonisées (Waterhouse et Norris 1989). Au cours de la dernière décade ce ravageur a connu une extension importante de son aire de répartition en envahissant tous les pays producteurs d'Amérique Centrale (excepté le Costa Rica), ainsi que la plupart des plantations de Colombie.

De nombreux travaux récents ont porté sur les principaux aspects de la biologie et de l'écologie de ce ravageur, ainsi que sur la dynamique de ses populations. Parallèlement, des efforts considérables ont été consacrés à la mise en place des différentes composantes d'une lutte biologique par utilisation de parasitoïdes d'origine africaine et de souches de Beauveria bassiana.

Cependant, malgré les moyens mis en oeuvre, la lutte biologique est parfois insuffisante pour contenir les populations de H. hampei. C'est en particulier le cas quand les infestations initiales sont précoces. Dans ces situations difficiles, le recours rapide à des traitements insecticides demeure généralement la seule solution efficace à la disposition des planteurs pour tenter d'enrayer l'infestation.
Du fait de son activité contre ce ravageur, l'endosulfan est le composé le plus employé dans la majorité des pays producteurs. Cependant, le risque d'apparition d'une résistance à un insecticide quelconque est directement lié à la pression de sélection exercée sur les populations. Aussi est-il essentiel, pour assurer la pérennité des divers moyens de lutte, d'utiliser d'une façon aussi rationnelle que possible les insecticides actuellement disponibles, et en particulier l'endosulfan.
Les travaux présentés ci-après ont donc pour objet d'étudier si le piégeage peut être un outil supplémentaire dans la prise de décision sur l'opportunité de traitements localisés aux seuls foyers d'infestations ou sur la généralisation à l'ensemble du champ.

Matériel et méthode

Parcelle d'étude :
L'expérimentation s'est déroulée sur la côte Ouest de la Nouvelle-Calédonie, sur une parcelle d'environ 0,4 ha. Une prospection rapide du champ a permis de localiser un seul foyer d'infestation, situé sur l'une des bordures. Un transect d'une vingtaine de mètres de large a été tracé à partir de ce foyer initial d'infestation et quatre zones ont été délimitées le long de cet axe. Dans chaque zone, 6 à 7 arbres présélectionnés ont été régulièrement suivis, tant pour la phénologie des plants que pour la caractérisation des populations de scolytes présentes (Fig. 1)

Phénologie des caféiers :
Sur chacun des 19 arbres suivis mensuellement, 5 branches marquées ont été échantillonnées et le nombre de cerises vertes, rouges ou sèches a été noté. A partir de ce dénombrement régulier et du nombre de branches de chaque arbre, une évaluation des fruits de chaque catégorie a pu être calculée pour chacune des zones. Ainsi, l'état de maturation moyen de chaque groupe de plantes-hôtes a été apprécié.

Populations de *H. hampei* :
Le nombre moyen de stades pré imaginaux (larves, prénymphes et nymphes) ainsi que d'adultes (mâles et femelles) a été déterminé par dissections mensuelles, au laboratoire, de

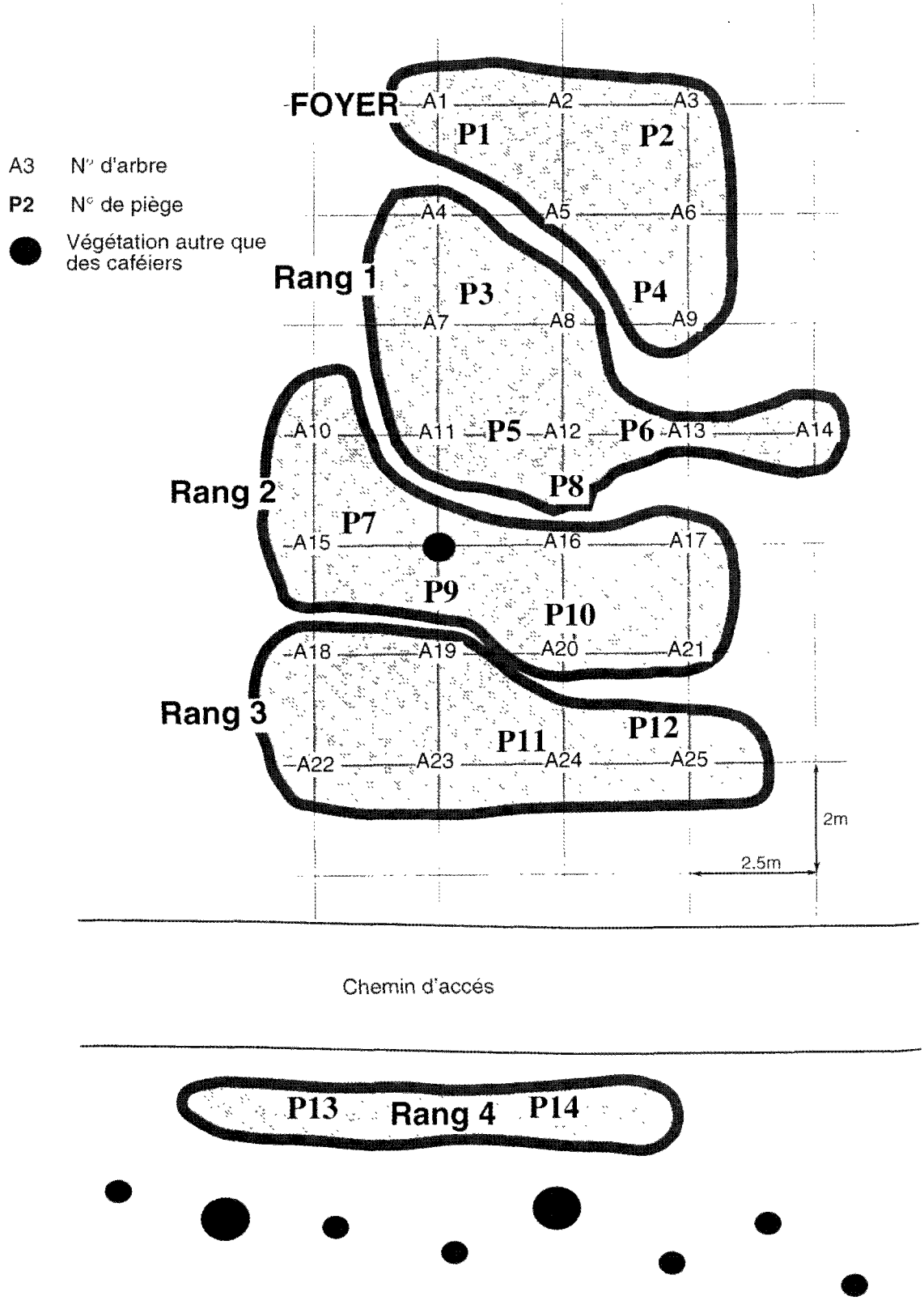

Fig. 1: Organisation de la parcelle d'étude

cerises provenant de chaque zone d'étude.
La population de scolytes de ces zones a ensuite été calculée en tenant compte du nombre de cerises de chaque catégorie et du taux d'infestation de chaque catégorie de cerises.

Captures de *H. hampei* :
Les adultes femelles volant dans le champ ont été capturés à l'aide de douze pièges rouges à entonnoirs multiples adapté du piège de Lindgren (1983) (Mathieu et al., sous presse). Un mélange à partie égale d'éthanol et de méthanol a été utilisé comme attractif dans ces pièges. En effet, ce mélange a été démontré comme attractif pour l'insecte en olfactomètre de laboratoire et en piégeage (Mathieu, 1995)
Deux pièges ont également été placés à l'extérieur de la parcelle afin d'évaluer l'importance des vols de colonisation à l'extérieur du champ.

Résultats

Les données présentées concernent la période d'Octobre à Janvier qui correspond à la fin de la période de maturation des cerises et à la récolte qui se déroule généralement en Novembre.

Phénologie des cerises :
Le premier graphique sur l'évolution du taux d'infestation indique l'évaluation du nombre total de cerises vertes et rouges dans la zone d'étude. Les cerises vertes diminuent pendant toute cette période du fait de leur maturation alors que les cerises rouges non récoltées deviennent des cerises sèches dans lesquelles la multiplication des scolytes se poursuit, ou tombent au sol (Fig. 2).

Infestation des cerises :
L'étude comparative du niveau d'infestation au foyer et à une vingtaine de mètres de celui-ci montre que des différences très importantes d'attaque existent au sein d'un même champ. Lors du premier relevé d'octobre, les cerises vertes récoltées au foyer ont un taux d'infestation de 20% alors que les cerises rouges de ces quelques arbres les plus infestés présentent un taux d'infestation d'environ 50%. Trois mois après, si la récolte n'a pas été faite au fur et à mesure de la maturation des fruits, le taux d'infestation des cerises, quel que soit le type de fruit, est de 100% (Fig. 2).

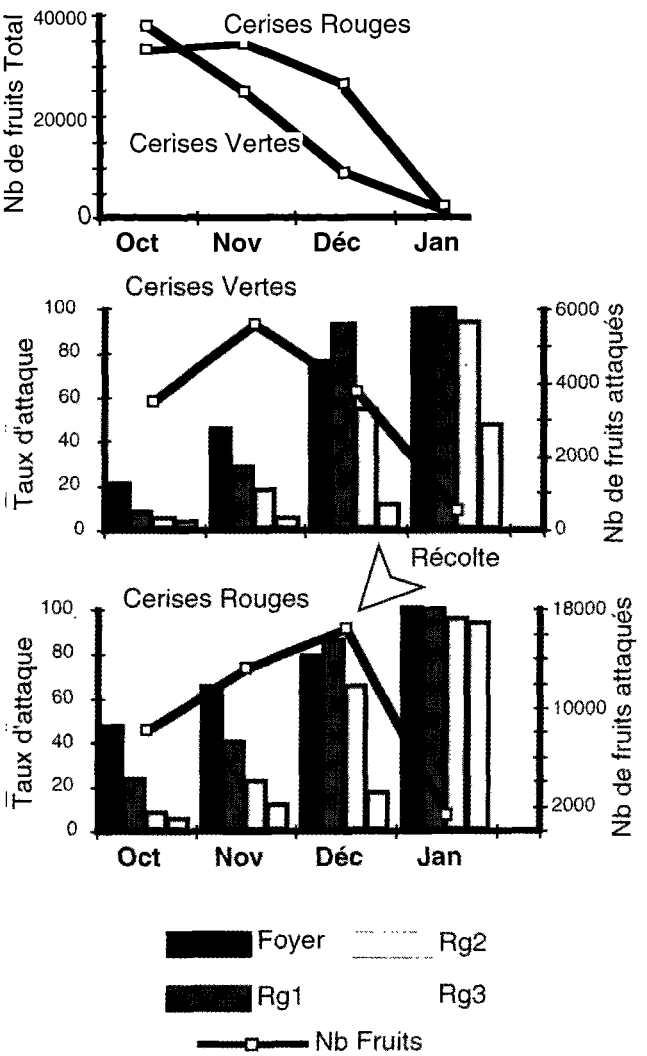

Fig. 2: Evolution de la production de cerises et du taux d'infestation
en fonction de la date du relevé et de la zone (Foyer, Rg1,Rg2, Rg3)

Dans le même temps, dans la zone située à une vingtaine de mètres du foyer, le taux d'infestation des cerises vertes ou rouges passe d'environ 3 à 5% à moins de 20%, trois mois plus tard. Par contre, si la récolte n'est pas pratiquée à temps, le taux d'infestation noté en Janvier est très rapidement proche de 100% (Fig. 2).
Ces données soulignent à nouveau le caractère très agrégatif des infestations dues à H. hampei et les difficultés à proposer un échantillonnage représentatif de la sévérité des attaques dans les différentes zones d'un champ.

Piégeage des scolytes :
Pendant toute la durée de l'étude, 73.168 femelles ont été capturées à l'intérieur du champ alors que seulement 196 femelles étaient récoltées par les deux pièges situés à sa proximité. Un maximum de femelles furent capturées dans la zone située au foyer initial d'infestation qui correspond également au dénombrement le plus important de population de scolytes dans les cerises.
Ainsi cette étude démontre qu'il existe une étroite corrélation entre les captures par piégeage des femelles au cours de leur vol de colonisation et le niveau d'infestation des cerises situées à proximité des pièges.

Conclusions

La difficulté de réalisation d'un échantillonnage représentatif d'une infestation par le scolyte du café a été soulignée par Decazy et al (1989). Les organismes de recherches qui s'intéressent l'amélioration de cette culture et au contrôle de ce ravageur proposent généralement un échantillonnage aléatoire de quelques dizaines de cerises prélevées au hasard sur des arbres contigus situés en divers points d'une même parcelle. La décision de déclencher une opération de lutte dépendra alors du taux moyen d'infestation noté au niveau de la zone échantillonnée.

Les résultats présentés ici démontrent l'efficacité du piégeage comme outil complémentaire d'identification et de surveillance des premiers foyers d'infestation dans une parcelle. Dans les situations où ces foyers sont limités à quelques arbres, ce qui est parfois le cas du fait du caractère très agrégatif de la répartition de *H. hampei*, il peut être économiquement souhaitable de faire un traitement précoce limité aux seules zones concernées par ces foyers.
Ces traitements ciblés auraient également l'avantage de diminuer la pression de sélection et par conséquent les risques d'apparition de résistances. Cette stratégie assurera une meilleure longévité des composés les plus efficaces contre le scolyte du café.

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L'ANTHRACNOSE DES BAIES (CBD) DU CAFÉIER ARABICA : Aspects microscopiques des interactions hôte-parasite

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Introduction

Une maladie attaquant les drupes de caféiers Arabica a été signalée pour la première fois au Kenya en 1922; très vite, cette maladie a pris une importance considérable, autant par son extension vers les pays environnants que par son impact sur les récoltes. Cette maladie, restée jusqu'à présent limitée au continent africain, appelée "anthracnose des baies du caféier" ou "Coffee Berry Disease" (CBD) est spécifique des fruits. Elle présente un aspect typique sur jeune fruit vert caractérisé par des lésions brunes, en légère dépression, présentant une évolution rapide ("forme active") en début d'épidémie, qui conduit à la pourriture de la pulpe et des jeunes graines.

Afin de caractériser le déroulement du processus infectieux, l'étude des aspects microscopiques des interactions hôte-parasite a été entreprise. Des observations en microscopie à balayage ont été réalisées pour visualiser les premiers phénomènes de reconnaissance intervenant à la surface de la drupe, en comparant une souche pathogène et une souche non pathogène. Puis, un suivi histologique des événements postérieurs à la phase de pénétration a été conduit à la fois sur drupe et sur semenceaux ; ceci a permis de décrire différentes étapes de la colonisation des tissus dans le cas d'une réaction compatible.

Matériels et méthodes

- Etude de l'interface hôte-parasite.

L'étude de l'interface hôte - parasite sur baie est réalisée avec un isolat pathogène CM732 *(C. kahawae ;* prélevé au Cameroun) et un isolat non pathogène CLB (*Colletotrichum* sp. ; prélevé en Colombie). Des drupes vertes et mûres ainsi que des hypocotyles de semenceaux de *C. arabica* ont été infectés.

Deux techniques d'inoculation des fruits ont été utilisées :

* Inoculation par goutte : les drupes sont disposées dans des bacs humides placés en chambre de culture à 20° C. Sur chaque fruit est déposée une goutte de 20 μl de suspension calibrée à 1.10^6 conidies/ml. Après 24 heures de contact à l'obscurité, la goutte est évaporée puis les fruits sont placées en photopériode 12 h/12 h.

* Inoculation par trempage : on plonge les drupes ou les semenceaux (au stade petit soldat) dans une suspension calibrée à 1.10^6 conidies/ml. La suite des événements est identique.

Les échantillons, après différents temps de contact avec l'isolat, sont placés dans du fixateur puis déshydratés par la méthode du point critique et enfin métallisés.

- Suivi histologique.

L'étude de la colonisation des tissus est réalisée avec l'isolat CM732, connu comme très agressif. Les réactions obtenues sur drupes vertes et sur semenceaux de deux variétés de *C. arabica* (variétés Caturra et Java) ont été comparées à celles obtenues lors de l'inoculation de *C. congensis*.

La technique d'inoculation par trempage, décrite précédemment a été utilisée. Les prélèvements des semenceaux ont été effectués entre 0 et 21 jours. Les échantillons, après différents temps de contact avec l'isolat, sont placés dans du fixateur puis déshydratés par bains successifs d'alcool de concentrations croissantes et enfin inclus dans de la résine, afin de permettre des coupes de 3 μm au microtome (LKB).

Résultats

- Etude de l'interface hôte-parasite sur drupe.

L'isolat *C. kahawae* CM732 (figure 1, photo A) forme sur fruit un tube germinatif 6 heures après l'inoculation et pénètre dans l'épiderme dans les 8 à 16 heures suivantes. On note une dépression des appressoria caractéristique du développement d'un hyphe infectieux de pénétration (figure 1, photo B). La colonisation des tissus conduit à la dégradation des parois pectocellulosiques des cellules sous épidermiques de la totalité du mésocarpe (figure 1, photo C). Dix jours après inoculation, on observe des acervules en surface des drupes. Ces acervules, dont le nombre augmente au cours du temps, libèrent après deux semaines de grandes quantité de conidies (figure 1, photo D).

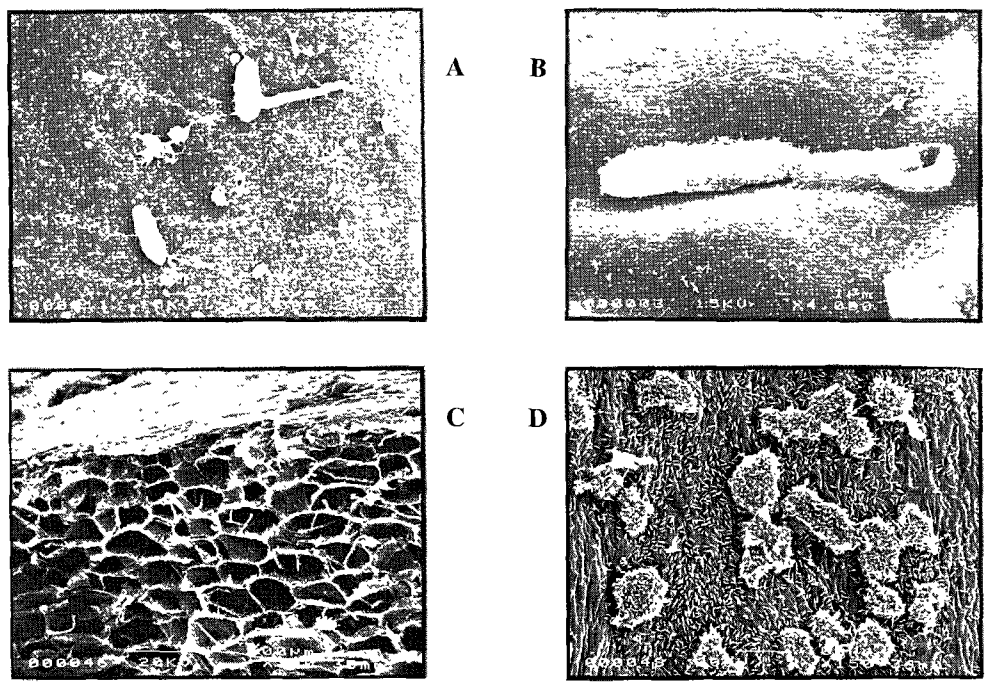

Fig.1 : Différents stades de développement d'un isolat (CM 732) de *Colletotrichum kahawae* sur des baies vertes de *C. arabica* entre 6 heures (Photos A et B), 10 jours (Photo C) et 16 jours (Photo D) après inoculation.

L'isolat (CLB) de *Colletotrichum* sp, non pathogène, forme des tubes germinatifs et des appressoria dans un laps de temps comparable à l'isolat pathogène CM732 mais aucune pénétration n'est observée (figure 2, photos E et F). Les tubes germinatifs sans appressoria s'allongent à la surface du fruit et produisent en 8 à 10 jours, de courtes ramifications portant quelques conidies isolées à l'extrémité (figure 2, photo G). En surface de la drupe, 16 jours après inoculation, un réseau dense d'hyphes est observé (figure 2, photo H).

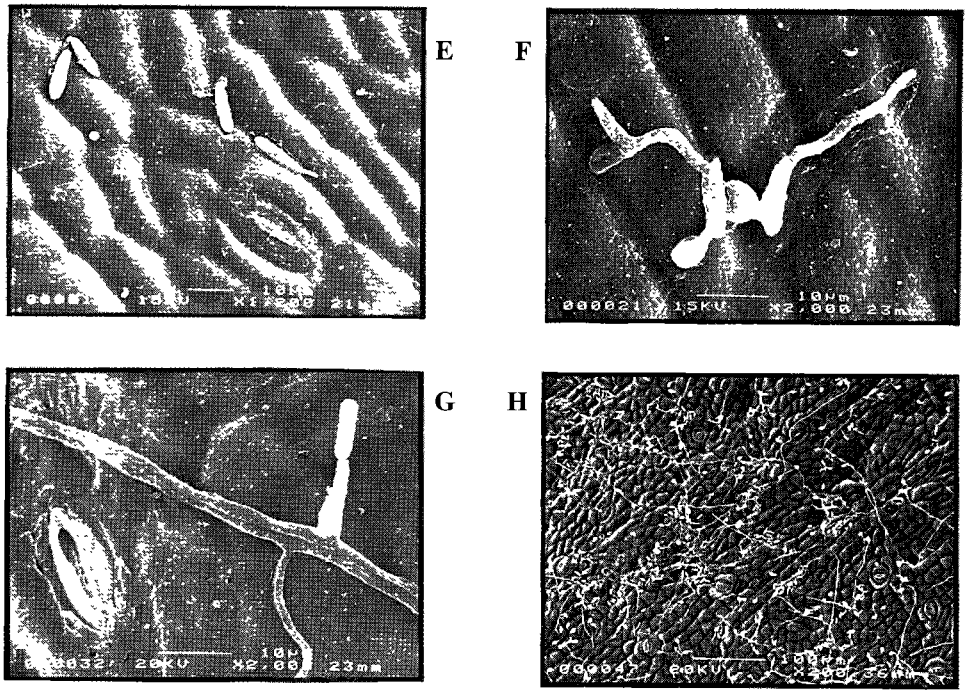

Fig. 2 : Différents stades de développement d'un *Colletrotrichum* sp non pathogène (CLB) sur baies vertes de *C. arabica* entre 6 heures (Photos E et F), 8 jours après inoculation (Photo G) et 16 jours après inoculation (Photo H).

- Etude de la colonisation des tissus.

Sur des **drupes** de génotypes sensibles, aucune phase de latence n'est observée en inoculation artificielle ; la colonisation est totale au bout de 10 jours et s'étend jusqu'à la graine. En surface de ces cellules se forment de nombreux acervules d'où émergent des conidies (Figure 3, Photo I). De même, sur **hypocotyles de semenceaux** de génotypes sensibles (*C. arabica* variété Caturra) les cellules sont détruites dans les zones de pénétration du pathogène au bout de 10 jours.

Avec la **variété Java**, considérée comme résistante au champ, il faut 20 à 25 jours pour détruire les semenceaux. Contrairement aux réactions obtenues avec des génotypes sensibles, on observe avec la variété Java une grande variabilité de réaction entre semenceaux : la moitié environ des semenceaux ne présente aucune lésion. Dans les zones infectées, la majorité des réactions histologiques obtenues (figure 3, photo J) sont comparables à celles observées sur du matériel sensible. Dans les tissus qui ne montrent aucun symptôme après inoculation, on observe spécifiquement la présence d'amyloplastes et la formation de cloisonnements cellulaires (figure 3, photo K). Ce type de réactions est également observable sur des hypocotyles de génotype sensible, dans les tissus non colonisés par le parasite, en bordure des lésions.

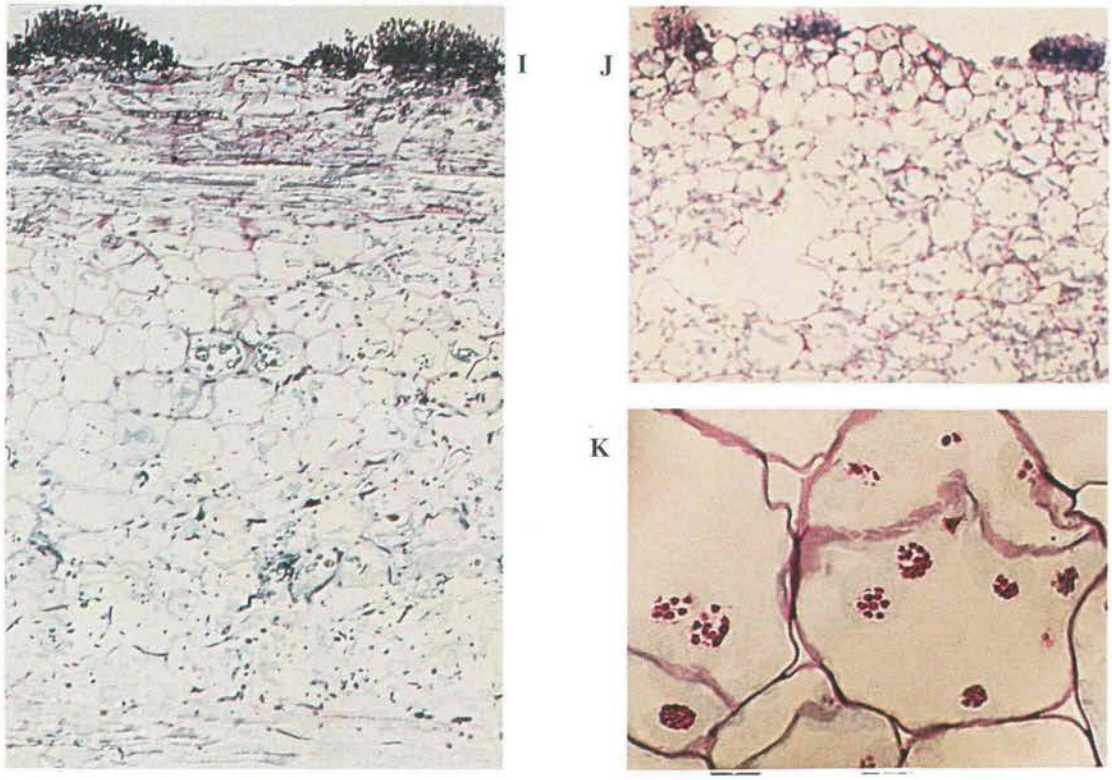

Fig. 3 : Coupe longitudinale d'une drupe verte de Caturra inoculée depuis 10 jours avec l'isolat CM732 (photo I). Coupes transversales de semenceaux de la variété Java inoculés avec l'isolat CM732 : semenceau infecté avec formation d'acervules en surface (photo J) ; semenceau inoculé n'ayant pas développé de symptôme et montrant la formation de cloisonnements cellulaires et la présence d'amyloplastes (photo K).

Sur les hypocotyles de ***C. congensis***, espèce non affectée au champ par l'anthracnose des baies, la majorité des semenceaux n'a présenté aucune nécrose après 20-25 jours. Dans les cas où une pénétration du parasite est observée, on retrouve une dégradation des tissus avec formation d'acervules d'aspect typique en surface (figure 4, photo L) ; toutefois la dégradation est beaucoup plus superficielle que sur les génotypes sensibles. Sur les hypocotyles où aucune pénétration n'est observée (figure 4, photo M), on note la présence de phénols (flèche) qui pourrait correspondre à un possible phénomène de résistance.

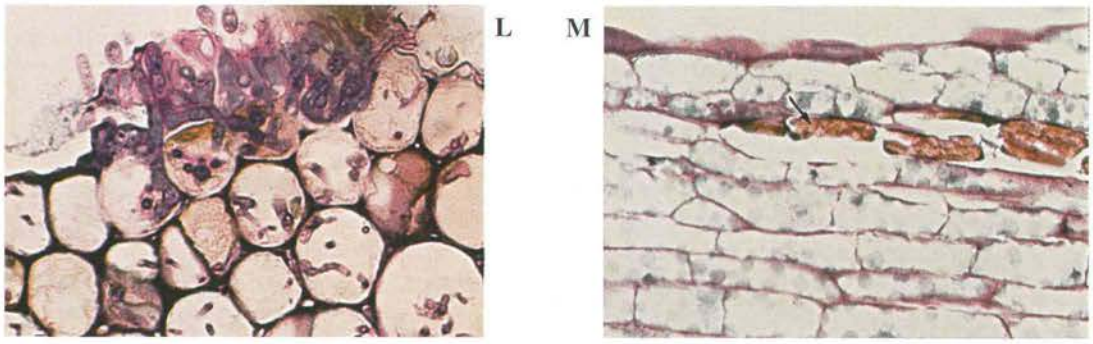

Fig. 4 : Coupes longitudinale et transversale de semenceaux *C. congensis* inoculés avec l'isolat CM732 montrant la formation d'acervules typiques en surface sur semenceau infecté (photo L) ou la présence de phénols sur les semenceaux infectés n'ayant pas développé de symptôme (photo M).

Conclusions et perspectives

Avec des génotypes sensibles, sur baies ou hypocotyles, la colonisation des tissus par un isolat pathogène se traduit par une dégradation des cellules de la totalité du mésocarpe et la formation de nombreux acervules. L'isolat non pathogène est quant à lui incapable de coloniser les tissus et forme un réseau d'hyphes à la surface des tissus.

Lors d'inoculations artificielles, les hypocotyles de semenceaux de génotypes sensibles présentent des modifications histologiques comparables à celles observées sur baies. Avec des génotypes résistants on observe, sur fruit ou semenceaux, la production de phénols qui pourrait correspondre à un possible phénomène de résistance.

Ces travaux doivent être poursuivis en étudiant les conditions de réalisation des inoculations artificielles qui pourraient influer sur les réactions du matériel végétal testé ainsi que les processus susceptibles d'être impliqués dans la résistance des génotypes utilisés actuellement dans les schémas d'amélioration (Rume Sudan, Catimor, Java, Ethiopiens, ...).

Résumé

L'anthracnose des baies du caféier Arabica est une maladie due au *Colletotrichum kahawae*, signalée pour la première fois au Kenya en 1922. Cette infection s'est répandue ensuite à toutes les zones d'Arabica culture du continent africain. Elle reste jusqu'à présent confinée à ce continent. C'est une maladie spécifique des fruits. Les cerise malades présentent un aspect typique avec principalement des lésions brunes, en légère dépression, à évolution rapide ("forme active"), provoquant une pourriture humide de la pulpe et des jeunes graines, avec formation d'acervules libérant un grand nombre de conidies.

La recherche de variétés de caféiers résistants à l'anthracnose des fruits nécessite d'acquérir de nouvelles connaissances sur les relations hôte-parasite, notamment par une étude fine du déroulement du processus infectieux. Des observations en microscopie à balayage ont permis de visualiser les premiers phénomènes de reconnaissance intervenant à la surface de la baie, en comparant une souche pathogène et une souche non pathogène. Le suivi histologique des événements postérieurs à la phase de pénétration, conduit à la fois sur drupe, organe cible de la maladie, et sur semenceaux infecté utilisés dans les tests précoces d'évaluation de la résistance, a permis de décrire différentes étapes de la colonisation des tissus dans le cas d'une réaction compatible.

Sur fruits, l'isolat pathogène forme un tube germinatif 6 heures après l'inoculation et pénètre dans l'épiderme dans les 8 à 16 heures suivantes. La colonisation des tissus se traduit par une dégradation des parois pectocellulosiques des cellules sous épidermiques puis de la totalité du mésocarpe. Les premiers acervules sont observables après 7 jours. Par contre, l'isolat non pathogène forme un tube germinatif et un appressorium dans un laps de temps équivalent mais aucune pénétration n'est observée. En revanche, un réseau plus ou moins dense d'hyphes est observé en surface de la cerise et produit en 8 à 10 jours, de courtes ramifications portant quelques conidies isolées à l'extrémité.

Sur hypocotyles de semenceaux infectés appartenant à des génotypes de résistance différente, une description de la cinétique de colonisation et des modifications tissulaires associées, ainsi que des facteurs susceptibles de limiter l'envahissement des tissus, a été entreprise. Sur les hypocotyles de semenceaux de génotypes sensibles, nous avons observé la présence d'amyloplastes et la formation de recloisonnements cellulaires. Sur des génotypes résistants, ces modifications sont accompagnées par la production de phénols. Ceci pourrait illustrer un possible phénomène de résistance.

Compte tenu de l'utilisation de l'inoculation d'hypocotyles comme test précoce de mesure du niveau de résistance, il est important de compléter ces observations par une étude précise des conditions d'inoculation des semenceaux qui pourraient influer sur les réactions du matériel végétal.

COFFEE DISEASES AND THEIR SIGNIFICANCE IN ETHIOPIA

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INTRODUCTION

In Ethiopia, coffee (Coffea arabica L.) grows in almost all administrative zones under conditions ranging from semi-savannah climate of the Gambella plain to the wet forest zones of the south west, east and northern regions (500-2600 m.asl). The annual rainfall in the regions varies from 750 to 2400 mm (Tewolde, 1988). Coffee is the major cash crop of Ethiopia. It contributes 5% to the GDP, 12% to the agricultural output, 70% to the foreign exchange earnings, 10% to the total government revenues and employs 25% of the labour force (Mesfin, 1989). The crop is prone to a number of diseases which attack fruits, leaves, stem, trunk and roots reducing yield and marketability. Yield losses caused by coffee diseases remain among the major constraints to production in many parts of Ethiopia. Previous estimates of average yield losses, due to coffee berry disease (CBD) alone, amounted to some 20-25% of the total crop (Vander Graaf, 1981). It is not enough to just believe that coffee diseases cause an economic loss, the magnitude of the disease must be evaluated from time to time so that it can be related to the yield gain obtained through control. The timely assessment of coffee diseases and their status in the country is a very important step, which helps both the policy makers and farmers, in the determination of an appropriate integrated pest management strategy. This study was conducted to determine, whether or not there was a shift in the status of Coffea arabica diseases in Ethiopia.

MATERIALS AND METHODS

The survey covered sites, in coffee growing regions that were truly representative of the regions under consideration and was of sufficient size to obtain estimates of the desired level of accuracy. Systematic sampling technique (Joseph, 1984) was employed to estimate severity on CBD, leaf blight, brown eye-spot and thread blight. W-shaped path was used because it gives better coverage of the entire field. From each site 100 coffee trees were taken and disease severity was calculated as:

$$\text{Disease severity} = \frac{\text{Area of plant tissue affected by disease}}{\text{Total area}} \times 100$$

Dead coffee trees were uprooted and investigated for root-rot and Thracheomycosis. The number of dead trees was expressed as a percentage of the total coffee trees.

Coffee leaf rust severity was determined using graphical methods (Rayner, 1961), where squares 2 x 2 cm were drawn on a transparent paper and by placement methods all squares that occupied the whole leaf and also areas of leaf rust sori were counted and calculated as:

$$\text{Rust severity} = \frac{\text{Squares bearing rust sori/180 leaves}}{\text{Total squares/180 leaves}} \times 100$$

The data were transformed before being analyzed.

RESULTS AND DISCUSSION

A number of coffee diseases have been recorded on Coffea arabica in Ethiopia (Table 1).

Table 1. Occurrence and status of coffee diseases in Ethiopia.

Disease	Causative agent	Status
Coffee berry disease	Colletotrichum kahawae (syn. Colletotrichum coffeanum)	Major
Tracheomycosis	Gibberella xylarioides	Minor
Coffee leaf rust	Hemilleia vastatrix	"
Brown eye-spot	Cercospora coffeicola	"
Brown blight	Colletotrichum gloeosporioids	"
Bean discolouration	Pseudomonas syringae	"
Leaf blight	Ascochyta tarda	"
Fruit-rot	Fusarium sp.	"
Root-rot	Armillaria mellea	"
Damping off	Pythium and Rhizoctonia sp.	"

Most of the diseases are minor and endemic in Ethiopia. This endemicity might have resulted from a process of coevolution between Coffea arabica and the pathogens or could be attributed to the presence of hyperparasites. Coffee berry disease (CBD) occurred in almost all major coffee growing areas (Table 2) and was severe at high altitudes in Oromia region and along valleys in the southern peoples region. The severity varied from region to region and from season to season. The loss incurred due to the disease varied. According to five years mean result (1987-91) between sprayed and unsprayed plots at Gera the loss exceeded 40% (Eshetu & Girma, 1992). On the other hand, Merdassa (1985) reported yield losses of 51% at Melko and 81% at Wondo Genet. In Harerge the loss may be estimated to range from 37 to 100% (Tefestewold and Mengistu, 1986).

However, currently the overall national loss due to CBD is estimated at 24 - 30% (Table 2). The recent increase in severity could be attributed to lack of appropriate control measures.

Table 2. Mean prevalence of CBD on garden coffee in Oromia and southern people regions during 1995-96 crop seasons

Region	District	Altitude (m.asl)	Mean % CBD
Oromia	Limu	1980	33.0
	Bedele	1980	44.3
	Metu	1770	24.2
	Gore	2000	34.8
	Gera	1900	27.8
	Hageremariam	1980	30.04
	Anfilo	1960	15.8
	Nopa	1700	9.1
Southern peoples	Yirgachefe	1910	34.12
	Dilla	1530	16.00
	Aleta Wondo	1840	15.00
	Yirgalem	1810	16.00
	Fiseha-Genet	1720	15.00
Mean			24.1
Sd			10.26
C.V%			42.5

Leaf blight severity was high in Sidamo and was mostly observed on flushes of recently stumped coffee trees. The disease was the most prevalent minor disease in the region. Very high coffee leaf rust severity was recorded in Harerge. Severity of brown eye-spot was also high in Harerge when compared with other coffee growing areas (Table 3). This prevalence seems to be related to geno types. The coffee geno type from western Ethiopia being practically unaffected by the pathogen have adequate resistance levels under prevailing growth condition (Vander Graaf, 1981). Thread blight was recorded at Metu and Limu, but the prevalence was negligible. Tree death induced by Tracheomycosis was observed at Gera, Tepi and Bebeka. The disease mainly occurred in plantations and was rarely observed on individual farms. This could be ascribed to the fact that most individual farmers scarcely use slashing and pruning tools, by which the disease is mechanically transmitted.

Table 3. Mean prevalence and distribution of minor coffee disease in major coffee growing regions of Ethiopia.

Disease	Locations								
	Metu	Tepi/Bebeka	Gera	Mugi	Sidamo	Harerge	Mean	S.D	C.V%
Leaf blight	1.2	3.0	7.6	1.02	7.9	-	4.10	3.33	81.0
Coffee leaf rust	1.5	2.6	6.6	5.8	1.8	21.00	6.55	7.39	112.0
Brown eye-spot	TR*	TR	TR	TR	3.1	7.6	1.85	3.06	165.0
Thread blight	1.5	-	-	-	-	-	1.5	-	-
Tree death	-	32.09	35	-	-	-	33.55	2.06	6.0

*TR < 1%

Currently only coffee berry disease (CBD) is economically important in Ethiopia. However, this should not give one the impression that the minor diseases will remain so indefinitely because these diseases may gain economic importance if the host-parasite relationship is disturbed (Eshetu, 1993). Irregular damage of coffee leaf rust was observed on garden coffee in some pocket areas in Sidamo and on released CBD resistant selections at Tepi and Bebeka coffee state farms. However, the later could be attributed to the

occurrence of large areas of genetically uniform host plants which constitute an ideal medium for infection. Occurrence and distribution of the rest of the minor diseases in the country is insignificant.

Although, there was no major shift in the status of coffee diseases in Ethiopia Tracheomycosis in plantations and coffee leaf rust in Harerge area should get due attention.

SUMMARY

Yield losses caused by coffee diseases remain among the major constraints to increased production in many parts of Ethiopia, where a number of diseases have been recorded. Follow-up studies were necessary to determine whether or not there was a major shift in the status of coffee diseases. The study covered sites that were truly representative of all the coffee growing regions, where the prevalence and distribution of diseases was determined. Severity was determined as the area or volume of plant parts. Trachemycosis was severe (32%) in plantations while high leaf rust severity (27%) was observed in Hararge region. However, the severity in garden coffee in the south and south western region was very low. The prevalence of other minor diseases was insignificant. Currently only coffee berry disease is economically important, the overall national loss due to CBD is estimated at 24-30%, but this should not give one the impression that the other diseases will remain minor indefinitely because these diseases may gain economic importance if the host parasite relationship is disturbed. From the results of the study there was no major shift in the status of coffee diseases. However, trachemycosis in plantations and coffee leaf rust in Hararge region should get due Attention.

ACKNOWLEDGMENT

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EFFECTS OF CAFFEINE ON COFFEE BERRY DISEASE (*COLLETOTRICHUM KAHAWAE*)

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Introduction

One of the most important coffee production limiting factors in east Africa is coffee berry disease (CBD) or anthracnose, caused by *Colletotrichum kahawae* (WALLER et al. 1993) (= *C. coffeanum* Noack sensu Hindorf 1970). CBD causes over 47% annual national yield loss in Ethiopia. *C. gloeosporioides* is a saprophyte mostly associated with the pathogen and competes for similar niche on coffee tree.

Caffeine is one of the phytotoxic purine alkaloid substances extracted in higher concentration from coffee fruits, young seedlings, fallen leaves and roots, than theophylline and theobromine (WALLER and CHOU 1980, MAZZAFERA et al. 1991).

Synthetic caffeine produced lower mycelial growth and inhibited aflatoxin on a number of *Aspergillus* and *Penicillium* species (BUCHANAN et al. (1981), strains of *A. ochraceus* (TSUBOUCHI et al. 1985) and on *A. parasiticus* by MOSS et al. (1990). In contrast to caffeine, theobromine and theophylline were of less effective (MOSS et al. 1990).

BUCHANAN et al. (1981) suggested that coffee and cacao beans having 2 mg or more caffeine/g should be relatively resistant to ochratoxin A contamination. MEDEIROS et al. (1990) had indicated a toxic effect of caffeine on *Hemileia vastatrix* and suggested that it may have a role in the mechanism of coffee leaf rust resistance.

Robusta coffee, which is a not a host for coffee berry disease causing *Colletotrichum kahawae* contains more caffeine, theophylline and theobromine than the susceptible *C. arabica* (KAPPLER and BAUMANN 1985, IARC 1991).

Works on coffee berry disease in particular, however, do not clarify effects of caffeine on the CBD pathogen. The objective of this study was to assess the in vitro effects of caffeine and other alkaloids on the behaviour of *C. kahawae* and *C. gloeosporioides*.

Materials and methods

Six isolates of *C. kahawae* (isolates Harar, 37, Kaffa, 46, 104 and 1152) and two isolates of *C. gloeosporioides* (isolates 123 fast growing and 124 slow growing) were identified from diseased green coffee berries and branches (isolate 18) in Ethiopia. *C. kahawae* isolates represented different coffee growing regions of Ethiopia, namely the former Kaffa (western), Sidamo (south) and Hararge (east) regions. Pathogenicity tests were conducted using the standard methods of VAN DER VOSSEN et al. (1976) and VAN DER GRAAFF (1981). Isolates were re-confirmed by Dr. H. Hindorf Isolated used were maintained in sterilised water or PDA slants/plates (Biratu 1995).

Media preparation
Caffeine (CAF., Merck), theobromine (THB., Aldrich) and theophylline (THP., Roth) at concentrations 0.10, 0.25, 0.50 and 1.0% (w/v) were stirred up with 3.9% potato dextrose agar (PDA, Merck) or 0.15% water agar (WA), autoclaved for 20 min. at 121 °C. 15 ml of the solution was poured in 85 mm sterilised plastic petri dish (Greiner). 0.15% Octave (Prochloraz), fungicide, was stirred up with PDA after the PDA was autoclaved and cooled down. Effects of these media on the rates of conidial germination, mycelial inhibition, branches proliferation and conidial production were compared with the control plates using the following procedures.

Mycelial growth rate
A 5 mm mycelial plug from actively growing (7 days old) culture of *Colletotrichum* isolates was inoculated at the centre of the plate. Unmodified PDA plate was used as control. The inoculated plates were incubated at 26 ± 1°C for 7 darkness. Each treatment had 4 replications. Mycelial growth diameter (mm) was measured on the 7th day and per cent inhibition rate was calculated. The mean results of the 5 isolates are presented in Tab. 1.. Inhibition rate of the control plate was considered as 0 or no inhibition, faster growth than the control plate or growth enhancement is indicated by negative integer inhibition, and lower growth rate than the control plate is indicated in positive integer.

Effects on the mycelial branching
Our preliminary studies indicated that mycelial branches of *Coletotrichum* sp. manifested irregularities if they were grown on fungicides amended media and/or incubated at higher temperatures over 32°C. Deformed and irregular mycelial branching were counted in the treated media. Number of mycelial branching were determined by adopting and modifying the methods of MORDUE et al. (1989). The number of mycelial branching were counted within 102 µm from the tip of the hyphae.

Conidial size
Conidia size varied on different media tested. 25-50 conidia/isolate were measured to see the effects of the from 7 days old culture. Conidia were stained with lactophenol toluene blue (JOHNSTON & BOOTH 1983).

Conidial production
7 days old culture were washed by flooding with 10 ml sterilised water, rubbed with iron rod, transferred to test tubes and the number of conidia/ml was counted. 2-4 cultures/isolate were used. Denser conidia suspensions were serially diluted before final count using a hemocytometer.

Results and Discussion

All six isolates of *Colletotrichum kahawae* were pathogenic on green coffee berries, detached leaves and hypocotyls. While *C. gloeosporioides* showed no effect on green coffee berries and hypocotyls.

All isolates maintained their characters and remained viable when stored in sterile distilled or ionised water for over 26 months at room and or 20°C.

C. kahawae and *C. gloeosporioides* reacted differently to purine alkaloids. Mycelial growth rate of the pathogenic isolates was faster on theobromine treated media than on the control plate (PDA). As the concentration of theobromine increased from 01.% to 0.5% growth rate between the pathogenic isolates became irregular. The rate of isolates of Harar, Kaffa and 1152 (Sidamo) was much faster. Isolate 37 showed slower rate followed by isolate 104 and 46. However, both isolates of *C. gloeosporioides* were inhibited by about 36 to 44% at 0.1% THB. and inhibition declined as the concentration was increased (Tab. 1).

Tab. 1: Per cent inhibitory effects of different levels (0.1-0.5% w/v) of caffeine, theobromine and theophylline on the mycelial growth rates of *C. kahwae* and *C. gloeosporioides*.

Media	Rates	*C. kahawae*							*C. gloeosporioides*		
		Hararge		**Kaffa**		**Sidamo**					
	(%)	**Harar**	**37**	**Kaffa**	**46**	**104**	**1152**	**mean**	**123**	**124**	**mean**
PDA	0.00	0*	0	0	0	0	0	**0**	0	0	**0**
THB.	0.10	-2**	1	-2	-2	-3	-3	**-1.8**	44	36	**40.0**
THB.	0.25	-15	-7	-11	-9	-5	-15	**-10.0**	31	27	**29.0**
THB.	0.50	-10	-2	-13	-9	-5	-15	**-9.0**	29	20	**24.5**
THP.	0.10	-7	-5	-2	-7	-12	-5	**-6.3**	36	29	**32.5**
THP.	0.25	29	28	27	28	20	26	**26.0**	45	49	**47.0**
THP.	0.50	56	50	61	51	51	56	**54.0**	56	61	**59.0**
CAF.	0.10	29	23	29	28	23	36	**28.0**	60	52	**56.0**
CAF.	0.25	93	87	95	88	90	97	**92.0**	95	90	**92.5**
CAF	0.50	100	100	100	100	100	100	**100**	100	100	**100**
OCT.	0.15	100	100	100	100,	100	100	**100**	100	100	100.0

* 0= no caffeine and no inhibition, standard plate, ** - = negative integer faster growth than the control plate. PDA = Potato dextrose agar, THB. = theobromine (0.1-0.50% w/v) plus PDA, THP = theophylline (0.1-0.5% w/v) plus PDA, CAF = caffeine (0.1-0.5% W/v) plus PDA. OCT. = Octave (0.15%).

Theophylline had mixed effect. At the lowest concentration (0.1%) growth rate of all pathogenic isolates was enhanced by 2-12%, however, inhibition effect was maximised from 20 to 61% as the concentration was increased from 0.25% to 0. 50%, respectively. The non-pathogenic isolates were also inhibited at the lowest concentration of THP.. In contrary to theobromine inhibitory effects of THP. improved by over 20% as the concentration increased to 0.5%.

Caffeine was the most effective purine alkaloid tested. Mycelial growth of the pathogenic isolates was inhibited by about 23-36% at the lowest concentration. Inhibition efficiency was maximised to 87-97% at 0.25% caffeine. Complete and permanent inhibition was observed at 0.5% caffeine. Caffeine double effective on the non pathogenic isolates, a mean of 56% inhibition was observed at 0.1% concentration of caffeine. The results agree with the previous reports of (BUCHANAN et al. (1981)and MOSS et al. (1990). In contrast to caffeine, theobromine and theophylline were of less effective on *Colletotrichum* sp. 0.5% caffeine was equally effective as 0.15% Octave However,

Octave produced 7-10 mm growth after about 15 days of incubation while 0.5% caffeine had no growth.

Higher branching frequency showed growth hindrance of the media. Results in Tab. 2 agreed to the results of Tab. 1. indicating that branching was less frequent on control plate (PDA) and THB.. More frequent branching 0.8 and 1.3 were recorded on THP. and CAF., respectively. The faster growing *C. gloeosporioides*, isolate (123) had more frequent branching on THP than on other plates. The slow growing isolate 124 had the least branching on all plates. THP. and THB.

Tab. 2: Effects of 0.1% theophylline, theobromine and caffeine amended PDA and PDA alone on the mean number of hyphal branching (within 102 µm from the tip of the mycelium).

Species	isolate	THP.*	THB.	CAF.	PDA
C. kahawae	Harar	1.0 ab	0.5 cdef	1.6 a	0.1 f
	1152	0.8 cdef	0.6 cdef	1.1 abcd	0.5 cdef
	Kaffa	0.6 cdef	0.3 ef	1.2 abc	0.5 cdef
	mean	0.8	0.4	1.3	0 4
C. gloeosporioides	123	1.5 ab	1.1 abc	1.2 abc	0.5 cdef
	124	0.3 ef	0.3 ef	0.8 cdef	0.4 def

* THP = theophylline, THB. = theobromine, CAF. = caffeine, PDA = potato dextrose agar. Means followed with the same letter have no significant difference ($P< 0.05$) according to DMRT.

Conidial production of both species was highly reduced on 0.1% caffeine by over 80%. The slow growing non pathogenic isolate produced no conidia (Tab. 3).

Tab. 3 Comparisons of number of conidial harvest (x 10,000)/ml of different isolates.

	C. kahawae	*C. gloeosporioides*	
media	1152	123	124
WA	8	32	-
0.1% CAF	1	3.5	-
0.5% CAF	0	0	-
1.0% CAF	0	0	-
PDA	252	1132	510

A similar study on 0.25% concentrations of the purine alkaloids indicated that conidial harvest of the pathogenic isolate on theobromine was twice and that of theophylline was three times higher that on caffeine.

Conidia size of both species varied on different media (Tab. 4). Conidia of the pathogenic isolates was 12% larger on theophylline than the control plate. All isolates produced the smallest size of conidia on caffeine. 0.25% caffeine which inhibited mycelial growth by about 92% (Tab. 1) reduced conidial size by 30% (Tab. 4). A similar concentration of theobromine enhanced mycelial growth of all pathogenic isolates by an average of 10% but the conidia size was reduced by 12%. The non pathogenic isolates produced no conidia on 0.25% caffeine. However, theophylline reduced the mycelial growth rate of isolate 123 by 45% (Tab. 1) and the conidia size by 26% (Tab. 4). The same isolate was inhibited by about 31% (Tab 1) and conidia size was reduced by only 15% (Tab. 4).

Tab. 4 Comparisons of conidia length (µm) on 0.25% purine amended PDA and control.

	isolate	THP.*	THB.*	Caf.*	PDA control
C. kahawae	Harar	20.1 ± 5.6	12.9 ± 2.5	10.2 ± 1.3	15.7 ± 3.1
	104	18.5 ± 5.3	17.3 ± 4.3	12.9 ± 2.3	16.2 ± 2.5
	37	19.1 ± 4.1	12.7 ± 1.0	12.4 ± 2.0	16.0 ± 3.3
	46	17.0 ± 4.8	14.2 ± 1.9	12.9 ± 2.0	17.5 ± 4.3
	mean	18.1	14.3	11.3	16.1
C. gloeosporioides	123	11.3 ± 1.0	12.9 ± 1.5	0**	15.2 ± 2.0
	124	13.5 ± 1.3	12.4 ± 0.6	0**	0**
	mean	12.4	12.6	0	-

* THP = theophylline, THB. = theobromine, CAF. = caffeine, PDA = potato dextrose agar. ** Conidia were not produced during recording time.

After 15 days of incubation theophylline remained with a pronounced coloured colony. Light bluish coloured pathogenic isolates on the control plate (PDA) became more white to greyish white to light olive green. Theophylline inhibited the mycelial growth rate of the pathogenic isolates by 13% but had frequent saltaion.

Theobromine favoured highly dense aerial mycelia and darker green to black colony after 15 days of incubation. All isolates completely covered the media, signifying the low control potential.

After 15 days 0.25% caffeine still inhibited mycelial growth of the pathogenic isolates by 84%, drying and cracking the media at the point of inoculum. The non-pathogen was controlled by 66%. The aerial mycelia of all isolates had reduced vigour and were compact. The pathogenic isolates became dark grey to black, and the non-pathogenic isolates were bluish grey. Thus caffeine can also serve in the discrimination and control of *Colletotrichum* sp. on coffee.

Studies of BIRATU et al. (1996) on coffee seeds indicated higher caffeine contents in CBD resistant coffee cultivars. Physiological significance of purine alkaloids in coffee needs more study to facilitate future researches in coffee pests control.

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Summary

In vitro effects of caffeine, theobromine, theophylline were compared with that of PDA and Octave (Prochloraz). Six isolates of *Colletotrichum kahawae* and two isolates of *C. gloeosporioides* were used in the study. 0.15% Octave and 0.5% caffeine completely and permanently inhibited mycelial growth of both species. The same amount of caffeine enhanced no sporulation. Lower rates of caffeine increased hyphal branching and reduced conidial size than theophylline and theobromine.

Conidia size of both species varied on different media (Tab. 4). Conidial length of the pathogenic isolates was 12% larger on theophylline than that of the control plate. All isolates produced the smallest size of conidia on caffeine. 0.25% caffeine which inhibited mycelial growth by about 92% (Tab. 1) reduced conidial size by 30% (Tab. 4). A similar concentration of theobromine enhanced mycelial growth of all pathogenic isolates by an average of 10% but the conidia size was reduced by 12%. The non pathogenic isolates produced no conidia on 0.25% caffeine. However, theophylline reduced the mycelial growth rate of isolate 123 by 45% (Tab. 1) and the conidia size by 26% (Tab. 4). The same isolate was inhibited by about 31% (Tab. 1) and conidia size was reduced by only 15% (Tab. 4).

Colony colours were light, darker and with different intracellular pigmentation on caffeine, theobromine and theophylline than on PDA, respectively. Aerial mycelial growth of both species was vigorous on theobromine amended media.

Seeds of CBD resistant coffee selections in Ethiopia and cultivars in Kenya had higher caffeine content than the susceptible ones.

THE EFFECT OF PRUNING, WEEDING AND FERTILIZATION ON THE YIELD OF ARABICA COFFEE IN SOUTH WESTERN ETHIOPIA

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INTRODUCTION

A definite cycle of physiological activity occurs in the coffee tree which should at least be understood by many coffee growers and development agents. The coffee tree has a certain period of growth which will vary according to the specific climate, soil, and altitudinal conditions. Hence pruning and management operations will vary slightly from one distinct ecological condition to another. In Ethiopia arabica coffee has adapted to wide range of ecological conditions ranging from about 550 m.a.s.l. (Gambella) to elevations up to 2200 m in parts of Jima, Ilubabor, Kefa and Sidamo.

In the lower altitudes where the conditions are warm and humid the tree grows very tall favoring vegetative growth, while in the higher elevations where it is cooler and humid the growth of the tree is stunted and less productive. Leaf rust and insect pests in the low elevation and coffee berry disease (CBD) in the higher elevations may threaten production unless suitable varieties are available (Cannell, 1971). Pruning is an operation to direct tree growth in a desired manner so as to regulate and optimize yield over a period of years.

Naturally, arabica coffee is an upright growing tree reaching to a height of over 10 m, particularly in the lower warm humid conditions. However, if the apex is mechanically removed, damaged, or killed by cold temperature, suckers in the lower point below this part are encouraged to grow. This will give rise to the growth of many suckers which when left uncontrolled are un-desirable of a productive tree.

Through proper pruning productive suckers and branches are selected for the coming seasons. Proper pruning has been found to respond favorably to improved management practices such as fertilization and weed control (Beaumont and Fukunaga, 1958).

Most of coffee plantations in southwest Ethiopia are under naturally growing forest which are selectively thinned. The population is also variable and other kinds of management practices are of low standard. Hence, this experiment was designed to combine pruning, weeding and fertilization practices to improve yield of coffee under different ecological conditions.

MATERIALS AND METHODS

A trial consisting of fertilization, weeding and pruning treatments in a randomized complete block design arranged in a split plot where weeding treatments were split plots on fertilizer treatments and pruning treatments were split plots on weeding treatments were laid down in several locations having distinct ecological variation. The treatments were replicated four times. Only experiments of Bebeka (1100 m), Agaro (1650 m) and Gera (1940 m) representing low, medium and high altitudes respectively are presented here.

The fertilizer treatments were N_0P_0 and $N_{150}P_{33}$ and the weeding treatments ware regular slashing versus regular slashing + hoeing once in March.

The pruning treatments were as follows: (1) single stem pruning system capped at every third node and also pruning the unwanted branches at the end of the crop season; (2) two-stem tree to be stumped when they reach maturity; (3) 2-4-6 year old stems on a tree where the 6 year old stem is to be stumped after crop; (4) 1-2-3-4 year old stem on a tree where the 4 year old stems is stumped every year and (5) three-stems on a tree where three stems or suckers were selected on a stump after stumping and retained until they get old. Other routine pruning is carried out in all plots at the end of the cropping season.

The plot size were 12 m x 12 m, 10 m x 10 m and 5 m x 10 m for Agaro, Bebeka and Gera respectively. The varieties were also different in the three sites. At Agaro a local unidentified variety, at Bebeka a uniform but local selection and at Gera 2 selections moderately resistant to CBD but sprayed against CBD were used.

Low level of shade trees were used at Gera and Bebeka whereas at Agaro the shades were not of the desired types and were girdled to dry on stand. In all the three stations the densities were adjusted to 3200 trees/ha. Although vegetative growth was measured during the trial period only coffee yield result is reported in this paper.

RESULTS

Effects of fertilizer treatments

Gera is cool and humid with approximately 1840 mm. annual rainfall and 182 rainy days; Agaro is moderately warm with an average annual rainfall of 1675 mm. and Bebeka is warm and humid having an average annual rainfall of 1726 mm. distributed into about 150 rainy days. Temperature and rainfall factors play important roles in the response of the coffee trees to the management practices subjected. The amount and distribution of the rain seems not limiting factor to the performance of fertilizer treatments in these areas. All of the locations are also medium to high in soil organic matter content (Paulos, 1994).

At Agaro significant response to fertilizer treatment was observed in the first five years followed by non-significant response in the following two years (Table 1). A positive response was again observed in the eighth year. Soil test from this area indicates low N and P in the highly managed plantation.

At Gera non-significant increase was observed to fertilizer treatment. Another fertilizer experiment carried out earlier at the same station also indicated no response to fertilizer application (Table 3). However, from the big difference in yield between the fertilized and non-fertilized plots it is advisable to apply a maintenance dose to protect the trees from exhaustion.

The response of coffee trees to fertilizer application was also non-significant at Bebeka (Table 2). Bebeka is an area where high organic matter content of the soil is reported (Paris-Ketting, 1987). In a separate trial on fertilizer study that was carried out there,

about 15 km away from this trial site it was indicated some low response to N and p application (Paulos 1994). However, the latter trial was under no shade and high yielding varieties were used. Soil nitrogen and available phosphorus are low under exposed areas (Paulos 1994, Paris-Ketting, 1987).

The effect of weeding treatments

In all of the three locations Digitaria abyssinica syn. D. scalarum is a common weed reducing coffee yield very badily. However, the sites were under shade and couch grass did not establish itself well when the trials were laid down. At Gera Cyperus spp were also the dominant weeds but these were effectively controlled during the first year of the trial and did not become a problem in subsequent years.

The effect of pruning treatments

Agaro: Mean yield of clean coffee for the first three years are presented here. The single stem capped at every third node was inferior compared to the other pruning treatments (Table 1). The yield obtained, however, is not considered low. The 2-4-6 and 1-2-3-4 years old stems pruning systems rotated on the same tree have also given high yield as the multiple system used as conventional. The single stem pruned trees gave low yield in subsequent years although the 1-2-3-4 year old stem gave lower yield in 1987. The effect of biennial bearing was also observed in some of the high yielding plots despite the exercise of pruning treatments. Yields collected and evaluated until 1990 indicated that the effect of biennial bearing habit has not been avoided by the pruning treatment under Agaro condition.

Gera: At Gera the single stem capped at every third node until a height of 2.0 m gave less than the yield of other pruning treatments (Table 3). Gera is a cool humid area where tree growth is slow. The low performance of the single stem capped is not un-expected. In the following two years yield was consistent and the single stem gave again low yield. No significant difference was observed in yield between the other pruning treatments.

Bebeka: Bebeka being warm, humid and a low land zone in the classification of the Ethiopian coffee growing areas, vegetative growth is very fast. Coffee yield is generally much lower than the medium and highland zones. The 2-stems and the 3-stems consistently gave higher yields than the single stem capped or the two partial stumping treatments (2-4-6 year old stems and 1-2-3-4 years old stems) (Table 2).

Summary

In Goma woreda, where Agaro is in the centre, the application of recommended level of N and P (N150 P33) is needed for high yielding trees. Once couch grass is brought under control through hoeing and frequent slashing, regular slashing will keep the weeds down in coffee plantation. Among the pruning practices the single stem capped is not recommendable for Goma area. Clean stumping and raising to 2 or 3 stems per tree or raising suckers of 1 to 6 years old on a tree and stumping the oldest 6 year old stem or the oldest 4 year old sucker are acceptable under Agaro condition.

This experiment also confirmed the need of low fertilizer for Gera as has been suggested in the past (Paulos, 1994). The Cyperus spp and D. abyssinica, the dominant weeds in area should be controlled by integrating cultivation and frequent slashing. Except the single stem the other pruning treatments tested gave similar results.

From this experiment the response to fertilizer application at Bebeka was low. However, this could be from the low yielding variety. For high yielding trees a maintenance dose is suggested. The weeding exercise has not been a critical factor in this trial. Among the

pruning treatments the 2- and 3- stem verticals were the high yielding ones at Bebeka and the 1-2-3-4 year old was probably the lowest yielding one.

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Table 1. Effect of pruning and fertilizer treatments on the yield of coffee at Agaro (mean of three crops). (kg ha^{-1}, clean coffee)

Pruning	N_oP_o	$N_{150}P_{33}$	Mean pruning
1. Single stem capped	1713	2091	1902
2. 2- stem	2552	3244	2898
3. 2-4-6 year old stem	2620	3747	3184
4. 1-2-3-4 year old stem	2791	3793	3292
5. 3- stem	2730	3503	3116
Mean	2481	3271	2876
LSD 0.05 between fertilizer mean			490
LSD 0.05 between pruning treatments			347

Table 2. Effect of pruning practices on the yield of coffee under unfertilized and fertilized conditions (clean coffee kg/ha) (3 years mean).

Pruning	N_oP_o	$N_{150}P_{33}$	Mean
1. Single stem capped	544	573	559
2. 2-stem	670	708	689
3. 2-4-6 year old stem	415	601	590
4. 1-2-3-4 year old stem	637	831	734
5. 3-stem	790	823	807
Mean	644	707	676

Table 3. The effect of pruning and fertilization on the yield of coffee at Gera (clean coffee kg/ha).

Pruning	Second crop			Third crop		
	N_oP_o	$N_{150}P_{33}$	Mean	N_oP_o	$N_{150}P_{33}$	Mean
1. Single stem capped	905	1034	970	1549	2991	2270
2. 2-stem	1840	2095	1968	2010	2549	2280
3. 2-4-6 year old stem	1228	2870	2049	2324	3546	2935
4. 1-2-3-4 year old stem	2162	2496	2329	2662	3260	2961
5. 3-stem	2120	3160	2640	2674	2852	2763
Mean	1651	2331	1991	2244	3040	2642
LSD 0.05 between fertilizer means			405			n.s
LSD 0.05 between pruning means			573			n.s

LA ROUILLE ORANGÉE EN CÔTE D'IVOIRE : IMPORTANCE ET FACTEURS IMPLIQUÉS DANS LA SENSIBILITÉ AU CHAMP DE *COFFEA CANEPHORA*

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1. INTRODUCTION

L'importance de la rouille orangée due à *Hemileia vastatrix*, a été évaluée sur *Coffea canephora* en Côte d'Ivoire. En terme de défoliation, les résultats montrent que sur certains génotypes l'impact de la maladie peut atteindre des niveaux comparables à *Coffea arabica*. Les observations multilocales, révèlent une forte interaction entre génotypes, localités et périodes d'observation ; ce qui laisse présager l'existence probable de races de rouille autres que la race II, seule race inventoriée jusqu'ici en Côte d'Ivoire.

Cependant, malgré cette probable variabilité de l'agent pathogène, bon nombre de génotypes ont montré des niveaux de résistance élevés, ce qui laisse entrevoir la possibilité de réaliser une sélection efficace contre cette maladie.

2. MATERIEL ET METHODES

Matériel végétal

- Génotypes guinéens et congolais implantés à Divo en 1988 .
- Essai de confirmation de clones regroupant 21 génotypes plantés entre 1986 et 1988 dans 5 localités de Côte - d'ivoire.
- 10 000 descendances issues de 161 croisements contrôlés à partir de 50 géniteurs différents. Plusieurs croisements intracongolais , intraguinéens et intergroupes

Tous les essais de clones ou de descendances sont plantés selon un dispositif en randomisation totale avec des parcelles élémentaires mono-arbre.

Méthodes

Dans les essais, la productivité est observée arbre par arbre et la vigueur est estimée par le diamètre au collet à 30 mois.

La sensibilité au champ a été notée selon une échelle de 1 à 5 sur chaque plant individuellement durant la période d'août à septembre qui correspond habituellement au pic des attaques de la rouille du caféier en Côte - d'Ivoire. Dans l'énoncé des résultats, les arbres qui présentent des taches sporulantes (note au moins égale à 3) sont considérés comme sensibles au champ.

L'incidence de la rouille a été évaluée en comparant des objets bénéficiant d'une protection fongicide (Bayleton Ec 25 : triadiméfon) et des objets non traités

3. RESULTATS

● Importance de la rouille orangée

L'absence de taches de rouille sporulantes sur les caféiers traités indique l'efficacité des traitements au triadiméfon.

Pour les plants non traités, le pourcentage de feuilles avec taches sporulantes atteint 58 % pour le clone 410 (guinéen sensible) et 14 % pour le clone 464 (congolais peu sensible). Le clone 410 non traité a subi une défoliation de 60 %

La sensibilité au champ à la rouille peut donc être un facteur important de défoliation chez *coffea canephora*.

L'étude de l'incidence de la rouille orangée sur la production a montré une augmentation de production de 40 % sur les caféiers bénéficiant d'une protection fongicide

● Sensibilité du matériel végétal

L'essai clonal de génotypes guinéens confirme la grande sensibilité de la plupart des clones de ce groupe. Quelques-uns montrent toutefois une résistance partielle (02189) ou complète (02116 et 02292)

Les essais multilocaux révèlent une variabilité importante du niveau de sensibilité des clones. Trois des 6 clones actuellement vulgarisés en Côte d'Ivoire sont fortement attaqués dans presqùe toutes les localités

Les résultats de l'évaluation de 161 descendances en sélection multilocale révèlent une forte influence de l'environnement sur les génotypes dont la résistance est de type intermédiaire.

TABLEAU 1

Sensibilité au champ à la rouille orangée de génotypes guinéens de *coffea canephora*. Essai clonal planté en 1988:

	Années			
Clones	**1989**	**1990**	**1991**	**Produc-**
02239	1.20 A	1.60	3.40 CD	3.30
155	1.33 A	1.33	3.20 CD	3.50
02169	1.60 AB	1.93	4.86 F	1.70
02189	1.73 AB	1.60	2.27 B	0.70
02116	1.80 AB	1.47	1.07 A	2.40
02292	1.80 AB	1.60	1.53 A	1.20
02121	2.33 B	1.73	2.27 B	7.10
410	2.93 C	1.53	3.40 CD	4.30
02286	3.33 CD	1.93	2.73 BC	3.00
02210	3.60 DE	1.27	3.73 DC	3.20
02138	4.07 E	1.67	4.20 EF	3.20
02244	4.69 F	1.92	4.69 F	4.20
02222	5.00 F	2.00	4.67 F	2.90
Test F**	HS	NS	HS	
Moyennes	2.73	1.88	3.23	3.1

Produc. = Production en kg cerises fraîches par arbre
HS = Hautement significatif
NS = Non significatif

Les moyennes suivies par la même lettre ne sont pas significativement différentes selon le *test* de *Newman et Keuls*

TABLEAU 2

Pourcentage d'arbres sensibles à la rouille orangée en 1991 dans cinq localités de Côte d'Ivoire

Clones	Localités et Années de plantation				
	Zagné 1989	**Soubré 1988**	**Binger 1986**	**Abeng 1986**	**Divo 1986**
107 +H	73	94	100	-	-
119 H	8	35	0	64	86
126 +H	0	3	0	7	0
182 +C	39	87	75	-	-
197 +R	4	47	37	-	-
202 C	0	16	0	0	0
305 H	0	3	0	43	21
461 +H	11	13	0	59	7
477 +C	0	0	0	-	-
503 R	0	0	0	12	7
512 H	7	77	0	0	46
513 H	0	6	0	20	32
526 H	0	19	0	25	0
529 H	0	48	0	46	27
539 C	0	0	0	0	7
586 R	0	0	0	0	0
587 R	0	0	0	43	20
588 R	0	0	0	6	0
589 R	0	0	0	0	0
594 R	0	0	0	13	0
609 C	0	0	0	41	7
H.P.(mm)	**1815**	**1472**	**1836**	**1410**	**1338**

C = Congolais, H = Guinéen x Congolais, R = Autre.

● **Existence de plusieurs races de rouille en Côte d'ivoire.**

L'incidence de la rouille orangée a été forte en 1989 et 1991 et faible en 1990, sur les clones guinéens. Trois clones (155, 02169 et 02239) particulièrement peu attaqués en 1989, ont sévèrement atteints en 1991. Une nouvelle race de rouille semble avoir surmonté la résistance de ces trois clones. (tableau 1).

Les observations réalisées en essai multilocal, renforcent cette hypothèse de l'existence de plusieurs races de rouille. Une sensibilité au champ variable selon le lieu d'observation apparaît (tableau 2). Certains clones sont sensibles partout et d'autres ne le sont qu'à certains endroits seulement.

Les résultats présentés ici semblent bien confirmer l'existence de plusieurs races de rouille qui restent à être identifiées

4. CONCLUSION

L'importance de la rouille orangée est démontrée par la défoliation qu'elle peut entraîner et par le grand nombre de clones et de descendances de *coffea canephora* sensibles au champ. Des études complémentaires sont nécessaires pour préciser les chutes de rendement consécutives aux attaques du parasite. Les premiers résultats indiquent que les dégâts peuvent être comparables à ceux observés sur *coffea arabica*

La forte interaction de la sensibilité au champ entre clones et années d'une part, et d'autre part, entre clones et localités, laisse présager l'existence de plusieurs races de rouilles en Côte d'Ivoire.

La présence présumée de plusieurs races du parasite doit être prise en compte dans le programme de sélection pour la résistance au champ

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ABONDANCE, DIVERSITÉ ET DISTRIBUTION GÉOGRAPHIQUE DES HÉMIPTÈRES NUISIBLES OU ASSOCIÉS AUX CAFÉIERS AU CAMEROUN

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L'ensemble des Arthropodes vivant dans le sol ou sur les parties aériennes des végétaux a été considéré dans le cadre des recherches particulières : le peuplement du palmier rônier (**Vuattoux**, 1968), les micro Arthropodes du sol (**Athias**, 1971), les peuplements de divers arbustes d'une savane préforestière (**Planquette**, 1972), les Arthropodes de la canopée (**Gagné**, 1979 ; **Erwin**, 1989), pour ne citer que quelques travaux. En ce qui concerne les insectes en particulier, certains groupes ont fait l'objet d'études quantitatives relativement recentes : les Carabiques (**Daget** et **Lecordier**, 1971 a), les Fourmis terricoles (**Levieux**, 1971), les Termites (**Josens**, 1972) etc. Bien avant cela, **Hargreaves** (1949), étudiant l'entomofaune des cultures tropicales, s'est penché sur les Hémiptères présents sur le cotonnier dans diverses régions du monde et a dressé une liste, qui comprend environ 94 espèces réparties dans 47 genres appartenant à 7 familles .

Au Cameroun précisément, de nombreux travaux ont été entrepris de longue date sur la faune entomologique des cultures. Déjà du temps de la colonisation allemande (1884 - 1917), des données sur les ravageurs des plantes avaient été réunies par quelques auteurs : **Haglund** (1894), **Preuss** (1903), **Aulman et la Baume** (1912), **Hageudorn** (1912). La période post-coloniale fut marquée elle aussi par d'importantes contributions d'entomologistes ayant effectué au Cameroun des séjours plus ou moins longs ou des missions de recherches de courte durée ; **Weidner** (1938, 1940) étudia les Coléoptères foreurs des cacaoyers dans la région de Tiko (Sud-Ouest) ; **Pascalet** qui séjourna très longtemps et dirigea en fin de carrière le Laboratoire de recherche de Nkongsamba dans le Littoral, publia en 1939, une note sur le Scolyte du caféier. Au cours de son séjour de 1951 à 1958 d'abord à la Station de Recherche Cacaoyère de Nkoemvone située près de la ville d'Ebolowa au Sud Cameroun et ensuite au Centre de Recherche Agronomique de Nkolbisson à Yaoundé, **E.M. Lavabre** entreprit un important travail sur les insectes nuisibles des cultures qui sera suivi d'une série de publications. **Descamp** qui était chargé des prospections antiacridiennes à Garoua au Nord Cameroun de 1951 à 1956, effectua des recherches sur les ennemis des cultures, notamment les parasites du riz dans cette partie du pays. **B. de Miré** et **P. Jacquemart (1966-1976)** entamèrent le premier dans les régions Centre et Sud du Cameroun, le second dans la zone Nord et Extrême-Nord, des travaux de recherche et d'expérimentation plus systématiques et plus amplifiés sur les ravageurs des cultures, principalement du cacaoyer, des caféiers et du cotonnier. S'agissant des Hémiptères, on trouve là aussi quelques contributions importantes dûes pour la plupart à des entomologistes de renom ; **J. Carayon** étudia au cours de la période 1947 - 1948 et 1954, les Hétéroptères du Quinquina et des caféiers, notamment les *Helopeltis* et les *Antestiopsis* ; **D. Matile Ferrero** (1979) procéda à l'inventaire des Cochenilles nuisibles aux cultures du

Cameroun. Mais si les résultats de ces recherches sont éparpillés dans des notes et revues diverses, il faut arriver à **G. Nonveiller** (1984) pour voir paraître un catalogue des Insectes du Cameroun d'intérêt agricole dans lequel se trouvent rassemblés de nombreux renseignements concernant aussi bien les ravageurs cités qui sont au nombre de 1300 espèces environ, que leurs parasites et prédateurs ainsi que les plantes hôtes. Un tel ouvrage de synthèse manquait en effet, et l'auteur qui séjournera au Cameroun pendant plus d'une douzaine d'années où il enseignait l'entomologie agricole, a eu le mérite de l'avoir réalisé. Cependant la part réservée aux Hémiptères des caféiers dans ce catalogue reste faible et très incomplète au regard des autres cultures notamment céréalières, légumières ou textiles. Des 118 espèces réparties dans quatorze familles d'Hétéroptères traitées dans l'ouvrage, le caféier en tant que plante hôte n'a été cité qu'à l'occasion de cinq espèces seulement : *Leptoglossus australis (L. membranaceus F.), Volumnus obscurus, Antestiopsis lineaticolis intricata, Cryotacrus comes, Coloborrhis corticina.*

Ayant pour ma part pu m'intéresser à cette faune, laquelle s'est révelée très riche, il m'a semblé utile de lister ici les différentes espèces recensées, d'étudier leur répartition et leurs relations avec les caféiers et de consacrer des études monographiques particulières à certaines d'entre elles.

1 - REGIONS D'ETUDE ET TECHNIQUES DE RECOLTE

La collecte des Hémiptèrs à été réalisée d'une part dans les plantations paysannes situées à proximité des stations de Santa dans le Nord-Ouest et de Foumbot dans l'Ouest du Cameroun et d'autre part dans les collections caféières des Stations de Nkolbisson et de Barombi-Kang. Ces régions se distinguent tant par leur situation géographique que par leurs sols ou leur régime climatique.

Santa Coffee - 5°48'N, 10°13'E se trouve dans un vaste cratère volcanique (Mont Bambui) à 1800 m d'altitude. Climat types de moussons montagnards et submontagnards « abrités » ; il pleut pendant une bonne partie de l'année, de mars à novembre, période pendant laquelle, tombe plus de 2000 mm d'eau. Les températures sont influencées par l'altitude : température maximale moyenne de l'année : 22°5C ; température minimale : 13°C ; décembre, janvier, février sont les mois les plus chauds. Humidités relative élevée : 70 à 90 % entre avril et décembre (**Suchel**, 1972).

Foumbot: 5°33'N, 10°35'E est située à une altitude d'environ 1100m, sur sol d'origine volcanique ; cette région est caractérisée par une pluviosité annuelle très modeste : 1100 à 1800 mm ; juillet, août, septembre et octobre étant les mois les plus pluvieux. La saison sèche est bien marquée et parfaitement en accord avec la latitude ; décembre, janvier, février sont les mois les plus secs. La température maximale enregistrée varie entre 33 et 39°C et la température minimale est comprise entre 14 et 18°C. L'humidité relative atteint 85° %.

Nkolbisson : 3°52'N, 11°28'E est située à une altitude d'environ 725 m. Le climat est de type subéquatorial à différenciation pluvio-thermique, caractérisé par quatre saisons de durée et d'intensité inégales : une petite saison de pluies (mars - avril - mai -juin) avec un total de 729 mm, une petite saison sèche (juillet - août) au cours de laquelle s'observent les températures les plus fraîches de l'année et le minimum d'insolation - une grande saison de pluies (septembre -octobre - mi-novembre : 654 mm) - une grande saison sèche (mi-novembre - décembre - janvier - février : 86,1 mm).

Barombi-Kang : 4°39'N, 9°27'E est située à une altitude de 278 m environ, sur un ancien plateau basaltique ; le régime climatique est équatorial à deux saisons : une longue saison des pluies de mars à novembre avec de très faibles ensoleillements de juillet à septembre et une saison sèche de novembre à mars. La pluviométrie annuelle est comprise entre 2300 à plus de 3000 mm. La température moyenne annuelle se situe entre 23 et 28°C. L'insolation est faible (3 à 6h par jour) et l'humidité relative est toujours très élevée et supérieure à 60 % (90 - 100 %).

Techniques de récolte

Deux techniques de récolte ont été utilisées ; la première technique : traitement des caféiers à l'insecticide est basée sur la méthode du fogging c'est-à-dire de traitement insecticide par nébulisation thermique ; développée par **de Miré** en 1965 pour étudier les populations de mirides du cacaoyer, cette méthode a été par la suite utilisée par divers auteurs : **Robert** (1973), **Majer** (1988), **Erwin** (1989), **Blanton**, 1990 etc. Elle consiste à étaler une bâche de toile au pied d'une vingtaine d'arbre choisis au hasard sur une superficie totale d'un hectare ; chaque bâche mesure 4m x 4m et comporte une fente jusqu'au milieu pour faire passer le tronc. On procède ensuite à la nébulisation thermique de la parcelle, suivie de ramassage des insectes tombés sur les bâches pendant 3 à 4 jours.. L'épandage du produit insecticide sur les caféiers a lieu tôt le matin, avant le levé du soleil, par temps calme ; le vent étant susceptible de provoquer la dérive du produit par rapport à la cible. Les insectes sont tués soit instantanément par effet de choc soit quelques heures, voire quelques jours après, grâce à la rémanence du produit

insecticide. Les traitements ont été réalisés chaque année pendant quatre ans, dans diverses localités de Foumbot et de Santa.

La deuxième technique : La récolte à vue a été utilisée dans les collections de caféiers de Barombi-Kang et de Nkolbisson où sont plantées plusieurs variétés de caféiers : *Coffea arabica, C. canephora, C. congensis, C. liberica, C. brevipes, Paracoffea* et *Psilantus.* Trois assistants du Laboratoire d'entomologie ont apporté leur contribution en capturant deux fois par mois, pendant 12 mois les Hémiptères présents sur l'ensemble de l'appareil végétatif des caféiers. Les captures ont eu lieu sur un échantillon de dix caféiers. Elles avaient lieu le matin entre sept heures et neuf heures, période pendant laquelle les insectes sont peu mobiles.

Identification des espèces.

La détermination des espèces a été réalisée par le laboratoire de faunistique et de taxonomie du Centre International en Recherche agronomique pour le Développement (CIRAD) à Montpellier en France et par le laboratoire d'entomologie générale et appliquée du Museum Natiional d'Histoire Naturelle de Paris sous la direction du Pr. **J. Carayon.**

2 - NOMBRE ET REPARTITION GEOGRAPHIQUE DES HEMIPTERES DES CAFEIERS

Environ cinquante quatre espèces d'Hémiptères reparties dans quarante trois genres appartenant à douze familles au total ont été récoltées dans la caféière.

Le tableau 1 ci-dessous donne la liste par famille de l'ensemble des espèces récoltées ; les familles sont classées suivant leur importance économique pour la culture caféière et les espèces en fonction de leur abondance, les plus fréquentes étant citées les premières ; une figure indique la présence ou non des espèces dans les différentes zones agro-écologiques étudiées.

L'examen du tableau montre que les familles 1, 2, 3, 4 sont celles où l'on rencontre les espèces les plus nuisibles aux caféiers, notamment au C. arabica. Cependant, c'est la famille des Coreidae qui comprend le plus grand nombre d'espèces trouvées sur les caféiers.

Il apparaît aussi que les différentes espèces sont très inégalement réparties dans les quatre régions d'étude. La grande majorité se trouve concentrée dans la région de moyenne altitude à Foumbot (1100 m) avec 46 espèces représentant 85 % du total, vient ensuite la région de basse altitude où on dénombre 28 espèces, quant à la région de haute altitude sa faune est moins riche que dans les deux précédentes : 26 espèces environ. Tous les Hémiptères récoltés en haute altitude à l'exception de deux espèces : *Saissetia coffea* et *Daclera punctata* fréquentent les caféiers cultivés en altitude moyenne ; ces observations ont permis de faire la classification suivante : (tableau 2) :

1 - Espèces de basse altitude (entre 10 et 750 m) : six espèces au total ; l'une d'elles, *Homoecerus pallens* a été récoltée en grand nombre sur le caféier robusta dans la région de Barombi-kang (278 m), où elle constitue l'espèce dominante.

2 - Espèces de moyenne et de haute altitude comprenant les espèces capturées entre 1000 et 1800 m : elles sont au nombre de 26 environ.

3 - Espèces communes aux sites de basse, et de moyenne altitude : ce sont environ 22 espèces plus ou moins abondantes suivant la région étudiée ; parmi elles vient en tête *Stenocoris (Erbula) southwoodi* très fréquente dans la caféière de basse altitude ; elle est suivie par une espèce du genre *Hydara : H.* tenuicornis.

3 - RELATIONS ENTRE LES ESPECES ET LES CAFEIERS HOTES

Parmi les 54 espèces d'Hémiptères capturées dans la caféière camerounaise 28 ont été trouvées en basse altitude sur les différentes variétés de caféiers de la collection variétale de Nkolbisson et de Barombi-kang. Leur présence sur chacune des variétés est notée ci-dessous et représentée par un petit triangle (tableau 3).

L'analyse quantitative des récoltes a permis de dresser le tableau 4 : les insectes sont classés suivant le nombre de spécimens récoltés. Les espèces les plus abondantes sont citées en premier. On observe qu'une douzaine d'espèces ayant à sa tête l'Alydidae *S. southwoodi* sont les plus représentées; Les variétés *C. congensis* et *C. arabica* sont celles qui abritent la population la plus élevée tandis que *C. liberica* apparaît comme la variété la moins attractive pour les Hémiptères.

FAMILLES ET ESPECES	LOCALITES		
	Nkolbisson & Barombi-kang	Foumbot	Santa
1 **Pentatomidae**			
Antestiopsis lineaticollis		◊	◊
Aspavia hastator	◊	◊	◊
Macrorhaphis acuta		◊	◊
Piezodorus rubrofasciatus		◊	◊
Parantestia immunda		◊	◊
Aspavia armigera	◊	◊	◊
Caura marginata	◊		
Caura bipartita	◊	◊	◊
Nezara viridula	◊	◊	◊
Nezara naspirus		◊	◊
Carbula melanacantha	◊	◊	◊
Lerida pugnax	◊		
Macrina Juvenca		◊	◊
Halyomorpha reflexa	◊		
2 **Miridae**			
Lycidocoris mimeticus		◊	◊
Lygus coffeae		◊	◊
Volumnus obscurus		◊	◊
3 **Jassidae**			
Coloborrhis corticina camerunensis		◊	◊
4 **Coccidae**			
Saissetia coffeae			◊
5 **Scutelleridae**			
Sphaerocoris annulus		◊	
Hotea subfasciata		◊	
6 **Coreidae**			
Hydara tenuicornis	◊	◊	
Cletus sp.	◊	◊	◊
C. lanciger	◊	◊	◊
C. unifasciatus	◊	◊	◊
Anoplocnemis curvipes	◊	◊	
A. tristator	◊	◊	◊
A. vidua	◊	◊	
Homoeocerus pallens	◊		
Leptoglossus australis	◊	◊	
Acanthomia histricodes		◊	◊
Elasmopoda falx		◊	
Daclera punctata			◊
Daladeropsis africana		◊	◊
Pseudotheraptus devastans		◊	
Rhyticoris sp.		◊	
Acanthocoris sp.		◊	
7 **Reduviidae**			
Rhynocoris hutsebauti	◊	◊	
Vestula lineaticeps	◊		
Rhinocoris carmelita		◊	
Hediocoris fasciatus		◊	
Vadimon sp.	◊	◊	
Ectrichodia rodhaini	◊		
8 **Pyrrhocoridae**			
Dysdercus voelkeri	◊	◊	◊
9 **Alydidae**			
Stenocoris southwoodi	◊	◊	◊
Riptortus dentipes	◊	◊	
Riptortus sp.	◊	◊	
Tupalus maculatus	◊	◊	
Tupalus sp.		◊	
10 **Dinidoridae**			
Coridius dubitabilis		◊	
C. xanthopterus		◊	
11 **Lygaeidae**			
Graptostethus servus	◊	◊	
Dieuches abundans	◊	◊	
12 **Tessaratomidae**			
Piezzosternum calidum		◊	◊

Tableau 1 : Liste des Hémiptères capturés sur les caféiers

Tableau 2 : Répartition des espèces selon l'altitude

Espèces de basse altitude	***Espècese de moyenne et de haute altitude***	***Espèces de basse, et de moyenne altitude***
Homoeocerus pallens	*Antestiopsis linéaticollis*	*Stenocoris southwoodi*
	Macroraphis acuta	*Hydara tenuicornis*
Caura marginata	*Piezodorus rubrofaciatus*	*Cletus sp.*
	Parantestia immunda	*C. lanciger*
Lerida pugnax	*Nezara naspirus*	*C. unifasciatus*
	Macrina juvenca	*Caura bipartita*
Halyomorpha reflexa	*Lycidocoris mimeticus*	*Carbula melanacanta*
	Lygus coffeae	*Aspavia hastator*
Vestula lineaticeps	*Volummus obscurus*	*A. armigera*
	Coloborrhis corticina	*Dysdercus voelkeri*
Ectricodia rodhaini	*Sphaerocoris annulus*	*Riptortus dentipes*
	Saissetia coffeae	*Graptostethus servus*
	Hotea subfasciatus	*Tupalus maculatus*
	Coridius dubitabilis	*Dieuches abundans*
	C. Xanthopterus	*Rhynocoris hutsebauti*
	Acantomia histricodes	*Anoplocnemis curvipes*
	Elasmopoda falx	*A. vidua*
	Daclea punctata	*A. Tristator*
	Daladeropsis africana	*Leptoglossus australis*
	Pseudotheraptus devastans	*Nezara viridula*
	Rhyticoris sp.	*Vadimon sp.*
	Acanthocoris sp.	*Riptortus sp.*
	Rhynocoris carmelita	
	Hediocoris fasciatus	
	Piezosternum calidum	
	Tupalus sp.	

Tableau 3 : Précense des espèces sur les différentes variétés de caféiers

Espèces	*C. robusta*	*C. arabica*	*C. arabusta*	*C. congensis*	*C. liberica*
1. Aspavia hastator	∇	∇	∇	∇	∇
2. Aspavia armigera		∇		∇	
3. Caura marginata	∇	∇	∇	∇	∇
4. Caura bipartita		∇	∇	∇	∇
5. Nezara viridula		∇			
6.Carbula melanacantha	∇	∇	∇	∇	∇
7. Lerida pugnax	∇	∇		∇	∇
8. Halyomorpha reflaxa	∇				
9. Hydara tenuicornis	∇	∇	∇	∇	∇
10. Cletus sp.	∇	∇	∇	∇	∇
11. C. lanciger		∇	∇	∇	∇
12. C. unifasciatus				∇	
13. Anoplochemis curvipes		∇			
14. A. tristator		∇		∇	
15. A. vidua					∇
16. Homoeocerus pallens	∇	∇		∇	
17. Leptoglossus australis	∇		∇		
18. Rhynocoris hutsebauti	∇	∇			
19. Vestula lineaticeps		∇		∇	
20. Vadimon sp.		∇			
21. Etrichodia rodhaini			∇	∇	
22. Dysdercus voelkeri	∇	∇	∇		
23. Stenocoris southwoodi	∇	∇	∇	∇	∇
24. Riptortus dentipes		∇		∇	∇
25. Riptortus sp.		∇			
26. Tupalus maculatus		∇	∇		
27. Graptostethus servus	∇	∇		∇	
28. Dieuches abundans		∇	∇	∇	

Tableau 4 : Fréquence des espèces récoltées sur les différentes variétés de caféiers

HEMIPTERES	***C. robusta***	***C. arabica***	***C. arabusta***	***.C.congensis***	***C. liberica***	***TOTAL***
Stenocoris southwoodi	88	54	52	155	47	396
Hydara tenuicornis	16	6	16	29	18	85
Cletus sp.	23	17	3	15	14	72
Caura marginata	7	5	4	26	8	50
Cletus lanciger	-	23	-	19	1	43
Caura bipartita	-	8	5	13	1	27
Carbula melanacantha	3	4	2	10	8	27
Aspavia hastator	2	1	2	10	10	25
Lerida pugnax	1	1	-	23	1	26
Riptortus dentipes	-	18	-	1	2	21
Homopeocerus pallens	6	1	-	3	-	10
Dysdercus voelkeri	2	4	1	-	-	7
Aspavia armigera	-	6	-	1	-	7
Anoplocnemis vidua	-	-	-	-	5	5
Graptostethus servus	1	3	-	1	-	5
Vestula lineaticeeps	-	1	-	3	-	4
Cletus unifasciatus	-	-	-	3	-	3
Ectrichodia rodhaini	1	-	-	2	-	3
Tupalus maculatus	-	2	1	-	-	3
Dieuches abundans	-	1	1	1	-	3
Rhynocoris hutsebauti	1	2	-	-	-	3
Anoplocnemis curvipes	-	3	-	-	-	3
Leptoglossus australis	1	-	1	-	-	2
Anoplocnemis tristator	-	1	-	1	-	2
Nezara viridula	-	1	-	-	-	1
Halyomorpha reflexa	1	-	-	-	-	1
Vadimon sp.	-	1	-	-	-	1
Riptortus sp.	-	1	-	-	-	1

CONCLUSION

Le présent inventaire constitue l'un des volets d'un projet plus vaste, de recherches entreprises sur les Hémiptères nuisibles ou associés aux caféiers au Cameroun ; cette étude a mis en évidence l'existence des rapports plus ou moins étroits entre les différentes espèces et les caféiers hôtes. Six espèces : *Antestiopsis lineaticollis, Coloborrhis corticina, Lycidocoris mimeticus, Sessetia coffea, Volumnus obscurus, Lygus coffea* vivent sur les caféiers et s'y nourrissent en occasionnant les dégâts. Par leur mode de nutrition, un bon nombre d'autres espèces sont susceptibles de causer des dommages aux caféiers mais elles ne constituent pas des fleaux. C'est le cas notamment du *Scutelleridea : Sphaerocoris annulus,* lequel peut entraîner l'avortement des fleurs aussi bien chez *Vernonia amygdalina* (Composée) sur laquelle il se nourrit et se développe que chez Coffea arabica (Mbondji et Pluot, sous presse). Un grand nombre d'espèces enfin séjournent et pondent à l'occasion sur les caféiers ; cependant, leur présence sur ces plantes ne semble être à l'origine d'une quelconque déprédation ; il s'agit des espèces appartenant au genre Macroraphis, Nezara, Piezodorus etc, associées au caféier de moyenne altitude et de la plupart des espèces de basse altitude pour lesquelles les caféiers ne constituent que des hôtes intermédiaires ou temporaires, leur hôtes habituels étant la végétation de la strate herbacée ou des plantes vivrières en culture associée dans la caféière. Dans cette catégorie, se rangent les *Nezara*, responsables de dégâts sur certaines légumineuses (soja).

Certaines variétés de caféiers comme *C. arabica* et C. congensis sont plus attractives vis à vis des différentes espèces d'Hémiptères ; elles sont habitées par plus de 60 % des espèces recensés et hébergent près de deux tiers des espèces de basse altitude. Parmi les Hémiptères de moyenne et de haute altitude quelques espèces : *A. lineaticollis ; C. corticina, N. viridula, S. annulus* etc ont fait l'objet d'études monographiques détaillées : Kirkpatrick (1947), Mbondji (1972), Mbondji (sous presse), Pavis (1986). Des recherches approfondies doivent être poursuivies concernant les autres espèces d'intérêt scientifique ou économique pour la culture caféière.

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RESUME

Cette étude indique que les Hémiptères sont parmi les ordres d'insectes, les plus abondants dans la caféière camerounaise ; 54 espèces au total réparties dans 43 genres appartenant à 12 familles ont été recensées dans quatre zones agro-écologiques de culture du caféier. La famille des Coreidae possède le plus grand nombre d'espèces (16 espèces) ; elle est suivie par celle des Pentatomidae ; cette dernière étant au plan économique, la plus importante par le nombre d'espèces nuisibles aux caféiers, notamment au *C. arabica*. L'analyse quantitative des insectes capturés montre que certains caféiers comme *C. arabica* et *C. congensis* sont plus attractifs pour les Hemiptères ; Ils hébergent le plus grand nombre d'individus et d'espèces : 29,87 % sur *C. arabica*, 23,37 % sur *C. congensis*. *C. robusta* et *C. arabusta* étant fréquentés à peu près par le même nombre d'espèces : 16,88 % et 15,58 % respectivement tandis que *C. libérica* serait le caféier le moins habité par les Hémiptères. Les différentes espèces ont été réparties en trois grands groupes comprenant les Hémiptères de basse altitude de 10 à 750 m ; les Hémiptères de moyenne et dehaute altitude : entre 1000 et 1800 m et les Hémiptères de basse et de moyenne altitude.

BIOMASS ACCUMULATION IN THE VARIOUS PLANT ORGANS OF *COFFEA ARABICA* L., CULTIVAR RUIRU 11, UNDER DRIP IRRIGATION IN KENYA

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INTRODUCTION

The growth and seed production of a plant is a result of the integrated processes of photosynthetic carbon dioxide assimilation and subsequent partitioning and utilization of the assimilated carbon (Yamagata et al., 1987). This requires efficient translocation of photoassimilates to the developing fruit. The need therefore to know how a crop plant such as coffee, partitions dry matter to the various plant organs cannot be overemphasized.

Harper and Ogden (1970) described a method of studying energy allocation in plants that requires seperating individual plants into component tissues according to their function and expressing energy allocation as a proportion of total biomass stored in each tissue type. There are several reasons why dry weight is used to measure biomass allocation patterns. Energy content and dry weight equally reflect energy allocation patterns (Hickman and Pitelka, 1975). Dry weight is reported to reflect the integration of all physiological processes throughout the growing season (Abrahamson and Caswell, 1982). In addition dry weight reflects the functional aspects of all assimilations (Chmielewaski and Ringius, 1987).

In many crop species, increased yields of improved cultivars has been related to changes in partitioning as opposed to an increase in total biomass (Egli, 1988). In the tall, mature field-grown Arabica coffee trees in Kenya, shoot growth is reported to be associated with seasonal changes in dry matter distribution within the trees (Cannell, 1971a). Work on glasshouse grown Arabica coffee cultivar Ruiru 11, has shown that dry matter partitioning to above - and below - ground plant parts varied according to progenies (Gathaara, 1990). Its dry matter partitioning under field conditions was however not studied. The aim of this study is to undertake the field investigation with a view to finding out the dry matter allocation to the various Ruiru 11 component plant parts. This information is necessary for field management of the cultivar.

MATERIALS AND METHODS

One year old seedlings of cultivar Ruiru 11 were transplanted in the field at a spacing of 2x2m. Field preparation and establishment procedures were as recommended by the Coffee Research Foundation (Mwangi, 1983). The training and pruning system of the trees was the uncapped multiple stem system (Fernie, 1966).

The experimental design was a 5x3 factorial in split plot. The main plots were three irrigation intervals namely 21, 28 and 42 days. The sub-plots were the amounts of water applied to offset the soil moisture deficit (SMD). These were 0, 25, 50, 75 and 100 mm of water applied through the dip irrigation system. The overall plot size consisted of 25 (5x5) trees but the recorded plot was 9 (3x3) trees. The recorded plots were therefore surrounded by two guard rows of coffee trees. In this way the treatments were four meters apart. The treatments were replicated four times.

The timing of the first experimental irrigation was when a SMD of 100 mm was recorded using an evaporation pan according to the method described by Pereira (1957). The soil moisture deficit of 100 mm is critical because at this SMD, Arabica coffee trees are reported to show signs of water stress (Kumar, 1979) and stomata start closing (Wormer, 1966). The periods of high SMD coincided with the hot/dry season between January and March and the cool/dry season between August and September. The drip irrigation system used was Netafim drip system from Israel. It consisted of drip line with a discharge of 2.3 litres per emitter per hour. The water was discharged at a pressure of 2.0 bars.

Nitrogen fertilizer was manually applied in April, May and June on the basis of leaf and soil analysis results as either calcium ammonium nitrate (CAN) or ammonium sulphate nitrate (ASN). In October a compound NPK fertilizer, 20-20-10 was applied. It was calculated so as to apply the same amount of nitrogen that would have been supplied if a straight N fertilizer had been applied

Three trees per plot were selected for destructive harvesting at the end of the five year growth and production cycle. The destructive harvesting was done during the hot-dry period between January and March 1996. The coffee tree was severed at the soil level with a pruning saw and separated into leaves (including petioles), twigs (primary branches and all lateral branches) and stem. These were brought to the laboratory and dried in an oven to constant weight at 85°C. Excavation of the root system was done according to the method described by Cannell (1971b). To estimate the extent of the root distribution within the soil profile the distance from the coffee tree stump at the soil surface level to the tip of the deepest root (vertical distribution) was measured. The root system was spread horizontally at right angles to the stump and the distance from tip to tip of the longest roots on opposite sides of the stump (horizontal distribution) measured. Vertical/horizontal ratio was calculated by dividing the vertical distribution by the horizontal distribution. The root system was then taken to the laboratory and its dry weight determined as described for the above-ground plant parts.

RESULTS

Effects of the irrigation intervals

The results of the effects of the drip irrigation intervals (Table 1) indicated that only the 21 day interval resulted in a significant

increase (P=0.05) of the leaf dry matter. In respect of the twig dry matter, the 28 day interval resulted in significantly more dry matter than the unirrigated treatment (Table 1). The 21 and the 42 day irrigation intervals were however not significantly different (P=0.05) from the unirrigated treatment. The effects of the irrigation intervals on the stem and root dry matter were not significant (Table 1).

Table 1. The distribution of dry matter (g) to various plant organs of cultivar Ruiru 11 as influenced by drip irrigation intervals (d).

Irrigation interval (days)	Plant Organs				Total plant biomass
	Leaves	Twigs	Stem	Roots	
0	626.09±120.29	1113.92±108.83	1242.25±52.55	1264.73±68.84	4246.73±329.58
21	820.98± 17.65	1274.86± 70.22	1494.75±69.57	1445.56±67.34	5136.15±214.60
28	678.64± 41.26	1307.64± 42.37	1410.00±44.76	1385.90±13.09	4782.18±114.70
42	567.76± 46.69	1172.29± 73.91	1357.60±55.12	1347.43±74.32	4445.08±235.00
Mean	673.37± 54.16	1242.18± 60.02	1376.15±52.82	1360.91±37.88	4652.61±195.41
LSD (5%)	122.65	174.13	NS	NS	526.08

In respect of the total plant biomass, the 42 day irrigation interval was not significantly different from the unirrigated treatment. However, the 21 day and the 28 day intervals resulted in significant increases of total plant biomass (Table 1).

Effects of the irrigation rates

Total plant biomass was significantly increased (P=0.05) over the unirrigated treatment by all the irrigation rates (Table 2). However, the rates were not significantly different in their effects on the leaf dry matter. With respect to the twigs, the 25mm rate, the 50mm rate and the 75mm rate significantly increased the twig dry matter (P = 0.05). The 100mm rate was however not significantly different from the unirrigated treatment (Table 2). All the irrigation rates resulted in significant (P=0.05) increases of stem dry matter, but there were no significant differences between them (Table 2).

In respect of the root dry matter, the 25mm rate and the 50mm rate did not differ significantly from the unirrigated treatment. However, the 75mm and the 100mm rates had significantly (P= 0.05) more root dry matter than the unirrigated treatment (Table 2).

Table 2: The dry matter (g) distribution to various plant organs of cultivar Ruiru 11 as influenced by drip irrigation rates (mm).

Irrigation rate (mm)	Plant Organs				Total plant biomass
	Leaves	Twigs	Stem	Roots	
0	626.09±120.29	1113.92±108.83	1242.25±52.55	1264.73±68.84	4246.99±329.58
25	738.91±68.71	1311.58±100.65	1433.17±43.95	1320.87±24.12	4804.53±230.62
50	696.20±72.83	1324.65±87.78	1462.92±92.45	1401.40±85.49	4885.17±330.69
75	744.57±71.90	1404.63±51.45	1537.33±31.24	1530.96±55.96	5217.49±173.14
100	639.86±72.81	1269.85±56.82	1428.25±56.04	1446.86±27.21	4784.82±193.07
Mean	689.13±24.46	1284.93±47.92	1420.78±48.62	1392.96±46.64	4787.80±155.75
LSD (5%)	NS	180.31	158.43	145.36	516.82

Intervals x Rate Interactions Effects

There were no significant (P=0.05) interval x rate interaction effects in respect of the leaf biomass at the 21 and 28 day intervals. However, at the 42 day interval all the rates except the 100mm resulted in significantly (P=0.05) more biomass than the unirrigated treatment.

In respect of twig, stem and root biomass at the 28 day interval, none of the rates resulted in significant biomass increase above the unirrigated treatment. At the 21 day interval however, the 50mm and 75 mm rates resulted in significant biomass increases of the three organs while at the 42 day interval 75 and 100mm rates resulted in significant biomass increases of the three organs.

Biomass Ratios

The results of the plant organ biomass ratios (i.e. the dry matter of a plant organ/whole plant biomass showed that the proportionate allocation of dry matter to the various organs of the Ruiru 11 cultivar, were not altered by the irrigation treatments (Table 3). The biomass ratios of the above - and below-ground parts in the unirrigated coffee trees were 0.70 and 0.30 respectively. In the irrigated trees it was 0.71 and 0.29 respectively (Table 3).

Table 3. The biomass ratios of the various plant organs of cultivar Ruiru 11 as influenced by drip irrigation.

Irrigation treatment	Plant organ					Total
	leaves	twigs	stem	above ground	root	
Unirrigated	0.15	0.26	0.29	0.70	0.30	1.00
Irrigated	0.14	0.27	0.30	0.71	0.29	1.00

Root Distribution

Results indicated no significant differences (P=0.05) between the intervals on their effects on the horizontal distribution of the root system. However, at the 42 day irrigation interval the vertical root distribution (116.25 ± 8.98cm) was significantly less (P= 0.05) than in the unirrigated treatment (129.50 ± 8.61cm). The 21 day and the 28 day intervals which resulted in vertical root distribution of 133.25 ± 2.21cm and 126.40 ± 5.65cm respectively were not significantly different from the unirrigated treatment.

The effects of the irrigation rates on both the horizontal and vertical root distribution were not significant (P=0.05). The ratio of the vertical to the horizontal root distribution showed that the vertical distribution was about half the horizontal distribution irrespective of the irrigation treatment (Table 4).

Table 4. The ratio of the vertical to the horizontal root distribution of Arabica coffee cultivar Ruiru 11 as influenced by drip irrigation intervals (days) and rates (mm water) in the sixth year of growth since field transplantation.

Irrigation interval (d)	Vertical horizontal ratio	Irrigation rate (mm)	Vertical horizontal ratio
0	0.56	0	0.54
21	0.57	25	0.53
28	0.53	50	0.51
42	0.51	75	0.55
		100	0.54
Mean	0.54	-	0.54
LSD (P=5%)	NS	-	NS

DISCUSSION

These data have shown that the dry matter accumulation in the various plant organs responded differently to the drip irrigation treatments. For instance, while the longest irrigation interval did not significantly influence the dry matter accumulation in any of the plant organs, and therefore total plant dry matter, the shortest interval resulted in significant increases of dry matter in both the leaves and twigs. This showed that for the cultivar Ruiru 11 to invest more dry matter in the productive wood, it requires short rather than long drip irrigation intervals at the plant density used in this study. However, the dry matter partition and accumulation to the leaves was more responsive to the frequency of moisture application than to the quantity. This probably indicated a need of sustained supply of moisture for the biochemical processes taking place in the leaf. This is important inasmuch as the leaf is the source of photoassimilates.

The drip irrigation rates resulted in significant increases of dry matter in all the plant organs with the exception of the leaves. The observed significant reduction of the twig dry matter when the entire soil moisture deficit was offset implied that offsetting the entire SMD by drip irrigation method may be inimical to the shoot growth of the cultivar Ruiru 11.

The dry matter allocation to the stem and root exhibited differences in their response to the irrigation treatments. All the rates resulted in significant dry matter allocation to the stem whereas only the higher rates resulted in significant increases of root dry matter. This may be indicative of a higher water requirement for root growth than for stem growth in the cultivar Ruiru 11. Huxley and Turk (1975) observed that the feeder roots of Arabica coffee took about 80% of total dry matter increment when the soil is well supplied with water as happens during the long rains.

The biomass allocation ratios of the various plant organs showed that the drip irrigation treatments did not alter the proportionate allocation of dry matter to the organs. A similar biomass allocation pattern has been observed in dryland cotton (Gossypium hirsutus L.) Mullins and Burmester (1990). The root biomass ratio reported here for the compact Ruiru 11 cultivar is far less than that observed in the tall Arabica coffee cultivar SL 28 (Cannell 1971b). The allocation of relatively little dry matter to the root system may be advantageous in that more dry matter may be diverted to the shoot system where it is available for fruit production. Huck et al., (1986) however, have reported that diversion of more dry matter to the root system has a survival value for drought-stressed plants as this results in an

extensive root system which has access to a larger reserve of stored soil moisture.

For the cultivar Ruiru 11 the problem may not be so much the root biomass ratio but the vertical to the horizontal ratio of the root distribution. This study has revealed that the vertical root distribution was about half the horizontal distribution. A large horizontal distribution of the root system has the advantage of access to more reserves of soil nutrients. However, restricted root depth may result in poor tree anchorage. The low vertical/horizontal root ratio may explain why some of the Ruiru 11 progenies lodge. On the basis of this finding, it is recommended that, in the coffee improvement programme a deep root system be considered as a selection criterion.

Lang and Thorpe (1986) have suggested that the mechanism regulating the carbon partition between plant parts is effected by the turgor pressure. A low turgor in the phloem, which enhances import, is achieved by either low internal solute concentration or a high concentration of solute in the apoplast (Williams et al., 1991). Translocation from source to sink has kinetic behaviour analogus to that of diffusion. It is a feature of such kinetics that a maximum posssible rate of mass movement falls off rapidly with increasing distance (Canny, 1973). In Arabica coffee, growing fruits draw assimilates from all but the terminal leaves on the same branch and from lateral branches (Cannell and Huxley, 1969; Cannell, 1970).

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ABSTRACT

In a field drip irrigation trial, Coffea arabica L. cultivar Ruiru 11 was used. Irrigation was applied when a soil moisture deficit of 100 mm was recorded to offset the deficit by 0,25,50,75 and 100%. Subsequent irrigation applications were at 21,28 and 42 day intervals. Using destructive harvesting method the trees were seperated into their component organs according to function, and their dry weights determined. The vertical and horizontal distribution of the root system was also determined. The vertical root distribution was half the horizontal distribution. The 21 day and 28 day intervals resulted in significant increases of leaf, twig and total plant biomass but the 42 day interval was not significantly different from the unirrigated treatment. All the drip irrigation rates resulted in significant increases in the stem biomass but with respect to the root biomass only the 75mm and 100mm rates resulted in significant increases. A higher water requirement for root than for stem growth was implied. The practical implications of the results are discussed.

LABORATORY EVALUATION OF THE RELATIVE PREFERENCES BY *EPICAMPOPTERA IVOIRENSIS* WATSON (LEPIDOPTERA : DREPANIDAE) ON FIVE COFFEE TYPES

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INTRODUCTION

Epicampoptera species (Lepidoptera: Drepanidae) have been reported as serious defoliators of coffee in Ghana and other West African countries (Wills, 1962; Forsyth, 1966; Hill, 1983; Padi, 1985, 1994). In Ghana, frequent outbreaks of *E. Strandi glauca* Hamps. and *E. ivoirensis* Watson occurred in many localities between 1979 and 1990 on *C. canephora*, the main coffee cultivar then grown in Ghana. Such outbreaks resulted in the serious defoliation of both young and old coffee and often required chemical treatment (Padi, 1994). In recent times serious sporadic outbreaks continue to occur mainly in the Ashanti and Brong-Ahafo regions of the country. Several species within the genus have also been reported on *Coffea arabica* in East Africa (Le Pelley, 1968).

To prevent crop losses resulting from outbreaks of *Epicampoptera* species and other important pests such as the coffee berry borer, *Hypothenemus hampei* Ferrarii and the stem borer *Bixadus sierricola* White, a study has been initiated at the Cocoa Research Institute of Ghana (CRIG) to screen the coffee germplasm collection and promising varieties, both in the laboratory and in the field (Padi & Kumah, in press) for insect preferences. The ultimate objective is to either select coffee types which emerge as the least preferred by insect pests, or to incorporate the genetic characters which render them unattractive into breeding varieties for cultivation.

The present study reports on a laboratory screening involving five coffee types, a local *Coffea canephora*, *C. arabica* and three introduced high-yielding *C. canephora* clones for their attractiveness to *Epicampoptera ivoirensis*, the commonest *Epicampoptera* species occurring in Ghana (Padi, 1984).

MATERIALS AND METHODS

Seventy 3rd instar caterpillars removed from laboratory cultures raised on seedlings of either the local *C. canephora* (LR1) or *C. arabica* (CA1), code-named "mother plants" were introduced onto fresh seedlings of the same coffee type. Each "mother plant" bearing the 70 caterpillars was placed at the centre of a metal tray and was surrounded by five seedlings, one local *C. canephora* (LR), one *C. arabica* (CA) and one each of introduced high-yielding clones code-named A129, B170 and E152 (Martinson *et al.*, 1986; Adu-Ampomah, 1995). To facilitate the free movement of caterpillars from seedling to seedling, the five seedlings in each tray were packed tightly together so that each seedling was in direct contact with the "mother plant" which was either *C. arabica* (Treatment 1) or local *C. canephora* (Treatment 2). The two treatments were each replicated three times with the five seedlings placed in different positions as illustrated in Fig. 1. Seedlings of approximately the same size and stage of maturity were used throughout the experiment.

The number of caterpillars on each seedling and the level of damage caused were observed and recorded daily for nine days.

Fig. 1

RESULTS:

Figure 2 shows that, for both treatments, the local *C. canephora* harboured the highest number of caterpillars and was, therefore, the best preferred coffee type, followed by *C. arabica*, A129, E152 and B170 in descending order of preference. This was the case even for Treatment 1 which involved caterpillars initially reared on *C. arabica* (CA1): most of the caterpillars had moved from the *C. arabica* "mother plant" onto the local *C. canephora* seedling within the first 24 hours. On the other hand, in Treatment 2, caterpillar migration from the local *C. canephora* "mother plant" onto *C. arabica* and the introduced *C. canephora* clones was minimal and much slower (Fig. 2). In both Treatments 1 and 2, the highest percentage of caterpillars continued to be recorded on the local Robusta seedlings up to day 7 and on day 9, when most of the caterpillars had pupated, the few remaining were found on the local Robusta seedlings only.

Fig. 2

It was further observed that feeding was most intensive on the local *C. canephora* seedlings and that from day 1 to day 8, the seedlings were completely defoliated and had to be replaced daily whereas some leaves remained on the four remaining coffee types. Moreover, feeding on the *C. canephora* seedlings often extended onto the bark of shoots when the leaves had all been eaten.

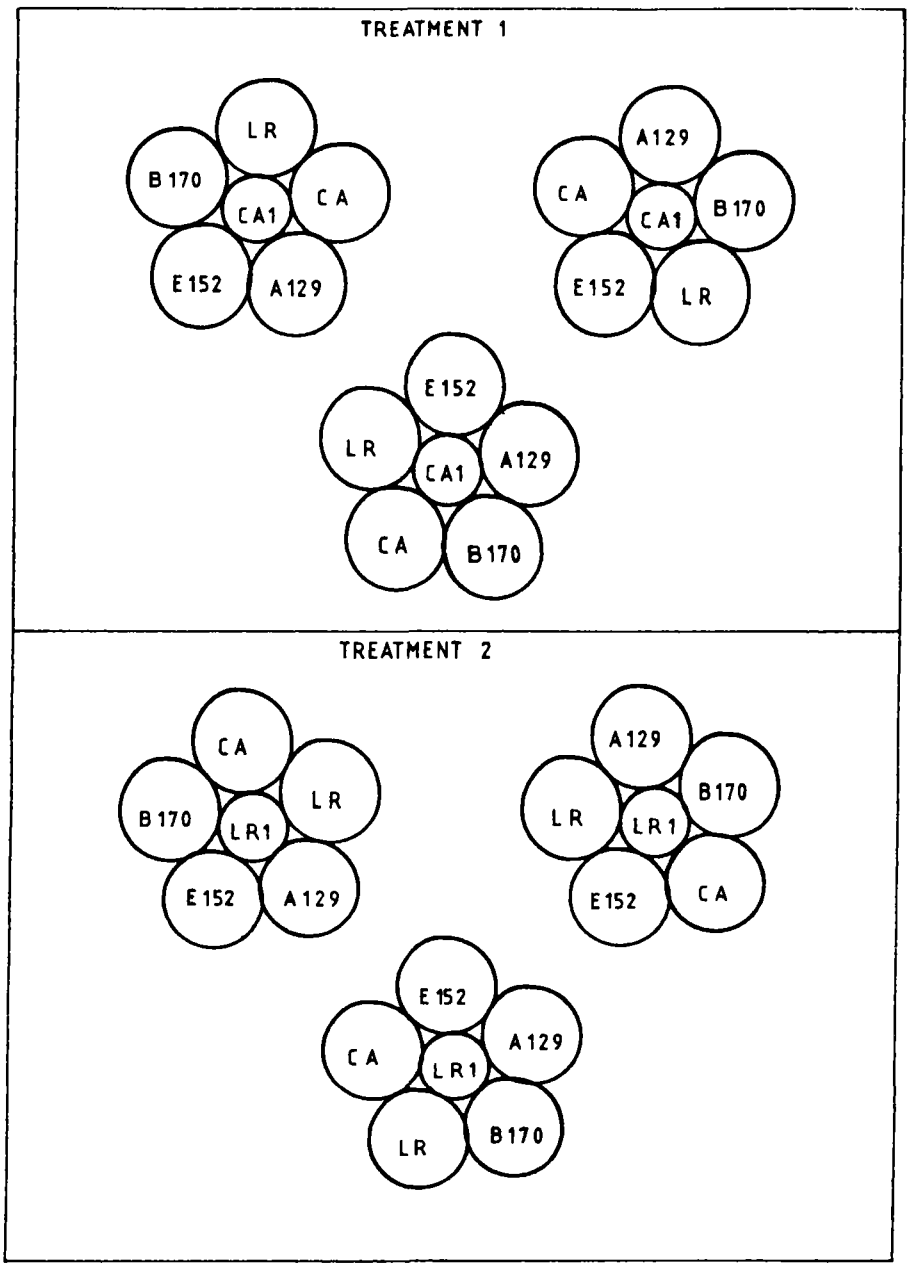


Fig. 1 Laboratory Host Preference Study

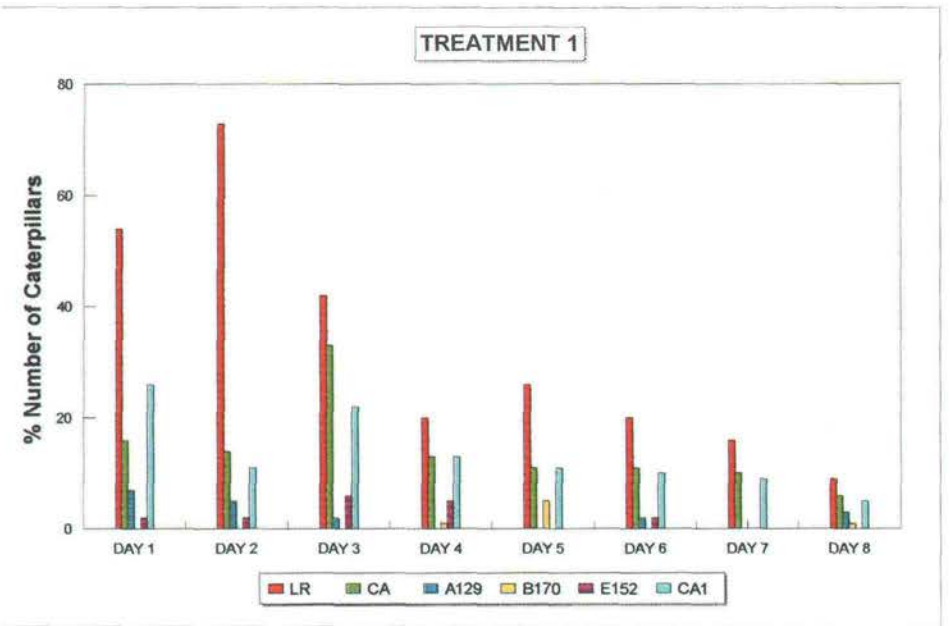
LR = Local Coffea Canephora (Robusta)

CA = Coffea arabica

LR1 = Local C. Canephora "mother plant"

CA1 = C. arabica "mother plant"

A129, B170 & E152 = Introduced C. Canephora clones



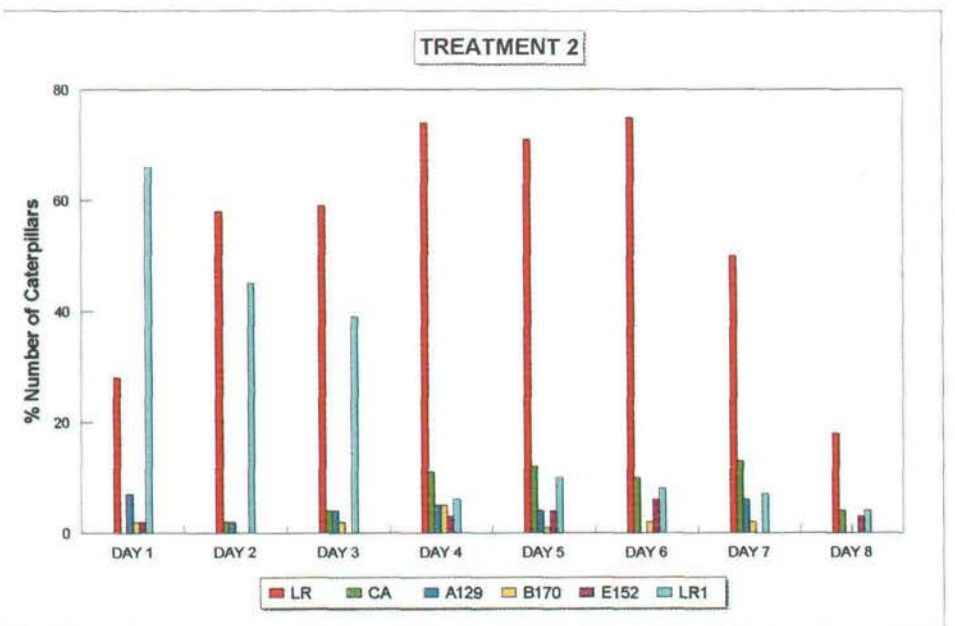


Figure 2. Relative abundance (% mean of three replicates) of caterpillars of Epicampoptera ivoirensis on five coffee types, local Coffea canephora (LR), C. arabica (CA) and introduced C. canephora clones A129, B170 and E152. Treatment 1 had C. arabica (CA1) as "mother plant"; Treatment 2 had local C. canephora (LR1) as "mother plant".

DISCUSSION:

Results from the present laboratory study have clearly shown that the defoliating caterpillars of *E. ivoirensis* preferred the local *C. canephora* to *C. arabica* and the three introduced Robusta clones, especially since this preference was evident even when the caterpillars were initially reared on *C. arabica*. There is the need to compared these results with results from field screening experiments to determine the level of correlation between the two. Should the two be positively correlated, the laboratory procedure which is a much simpler and quicker method could alone be used in the screening for *Epicampoptera* preferences on coffee.

SUMMARY

An insectary host-choice experiment involving five coffee types viz. a local *Coffea canephora, Coffea arabica,* and three promising high- yielding introduced *C. canephora,* code-named A129, B170 and E152, was conducted to determine the relative preference by the coffee defoliating caterpillars of *Epicampoptera ivoirensis* Watson. The experiment was replicated three times under two treatments in which the experimental insects were raised either on local *C. canephora* or on *C. arabica* seedlings. In each replicate experiment, the subsequent distribution of 70 caterpillars from the *C. canephora* or *C. arabica* seedling placed at the centre of a metal tray onto seedlings of the test coffee types packed closely around and touching the central seedling, was observed daily. The numbers of caterpillars and their feeding behaviour on each coffee type were recorded.

E. ivoirensis showed a clear preference for the local *C. canephora* followed by *C. arabica* and the introduced clones A129, E152 and B170 in descending order of preference. Feeding was also most intensive on the local *C. canephora*. Information from this and similar future experiments on promising coffee types could be incorporated into coffee breeding and selection programmes.

ACKNOWLEDGEMENT

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THE STATUS OF COFFEE WILT DISEASE (TRACHEOMYCOSIS) AND STRATEGIES FOR ITS CONTROL IN UGANDA

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INTRODUCTION

Coffee is the chief foreign exchange earner contributing over 50% of the country's entire foreign exchange earnings, valued between 300 - 400 million US dollars annually. Over 2.5 million people directly depend on coffee cultivation and trading for their livelihood. Both arabica (*Coffea arabica* L) and robusta (*C. canephora* Pierre) are cultivated. Robusta is cultivated at lower and warmer elevations (1000 - 1300m) on 240,000 ha, while arabica is grown from 1300 - 1800m, on 39,000ha. Total annual production is about 4 million bags (60 kg) of clean coffee (Anon.,1997). About 85 percent of the coffee-growing districts cultivate robusta. Robusta comprises 90% of total production and arabica makes up the other 10%.

Pests and diseases are among the major production constraints in coffee production. Diseases such as coffee leaf rust (*Hemileia vastatrix* Br.& Br.) and coffee berry disease (*Colletotrichum kahawae* Sp. Nov.) have well established control strategies. The appearance of coffee wilt disease in Uganda has caused grave concern due to limited available information on the disease and lack of effective control methods. This paper reviews the status of the disease in Uganda and discusses some of the control measures being advocated and research strategies being developed to combat the disease.

HISTORICAL ACCOUNT

Fusarium xylarioides Steyaert was first reported from the Central African Republic in 1946 on *C. excelsa* (Guillemat, 1946). It was later described in Zaire (now Democratic Republic of Congo) in 1948 on robusta (Steyaert, 1948). In 1958, the disease was reported from Ethiopia on arabica coffee (Lejuene, 1958).

Several outbreaks occurred in Zaire during the 1950's (Flood, 1996 - quoting Frasella, 1950). An effective breeding programme in Zaire is reported to have led to decrease in the disease (Flood, 1996). New outbreaks, however, occurred again during the 1980s (Mfwidi-Nitu, 1993) and 1991 (Mpinji, personal communications). Presently it is causing considerable losses on robusta coffee in north east of that country (Flood, 1996).

In 1990 coffee traders reported a devastating robusta coffee disease occurring in Zaire in the area bordering Uganda. In 1992 a similar report was received. The first report of the disease within Uganda's borders, in Bundibugyo and Rukungiri districts bordering Zaire, was received and confirmed in 1993. In 1994/95 coffee wilt disease was reported for the first time in Mukono district, east of Kampala (Hakiza, 1995).

CURRENT OUTLOOK

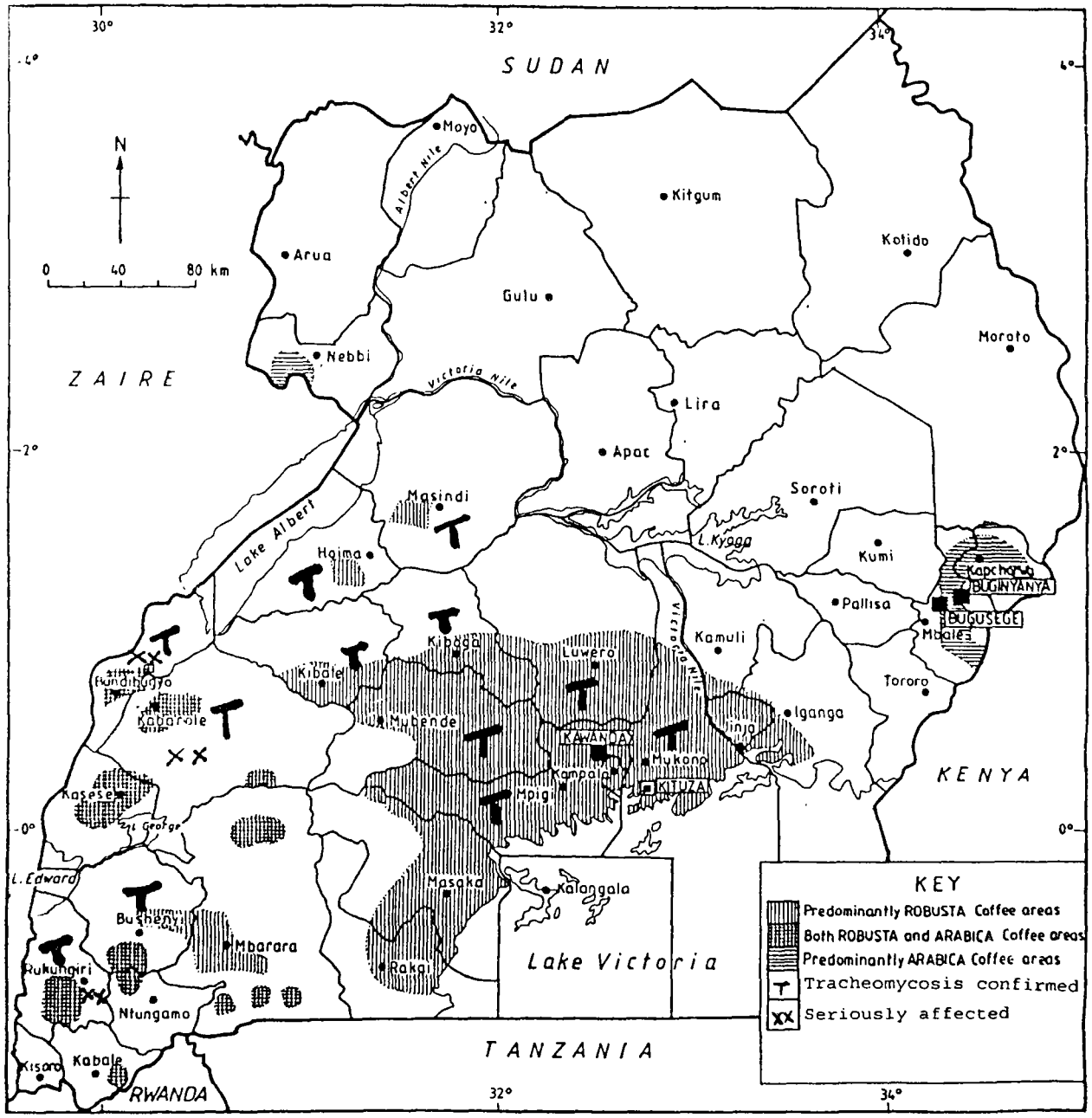
A Task Force was set up to keep track of the advance of the disease. Subsequently, a survey was conducted in April 1996 throughout most of the coffee growing areas to establish the distribution and severity of the disease. This led to identification of hot spot areas, and realisation that the disease was more widespread than previously believed. Over 10 coffee districts were found affected, the worst areas being Bundibugyo and Rukungiri ("hot spots"). Individual farm losses in many cases were as high as 100% even in areas with low incidences. Incidence and severity varied among districts and among farms in the same locality. Some farmers have abandoned coffee cultivation and are replanting with alternative crops. From two districts in 1993, wilt has spread to other areas and today (1997) its presence in 12 districts has been confirmed. The disease, however, has not yet been reported east of River Nile and so far it occurs only on robusta trees previously raised from seedlings. This does not imply that clonal robusta and arabica coffee are resistant/tolerant to wilt as this has not been established. Therefore, wilt continues to pause a serious threat to arabica coffee and the clonal robusta recommended for planting. Figure 1 shows the distribution of wilt on robusta coffee in Uganda. Reasons for the observed variations in disease incidence and severity can only be unravelled through research.

Symptoms

The symptoms exhibited by infected robusta coffee trees in Uganda are similar to those reported in literature (Vander Graaf and Pieters, 1978; Potchet, 1988; Coste, 1992).

- ♦On multiple stems, usually only a single stem is affected at a time, in both old and young coffee.
- ♦The first symptoms are seen as leaves curling inwards. The leaves may also wilt and feel dry to the touch. Yellowing may or may not occur.
- ♦Sudden leaf fall may occur within a few days of the first symptoms.
- ♦Primaries/bearing branches remain bare after leaf fall. A few leaves may sometimes remain at the tops of the main stems.
- ♦Black or brown to violet streaks/bands are observed on the wood when bark is peeled off the stem.
- ♦Sometimes cracks or cankers occur at the collar region. Black granular structures may sometimes be observed in between the cracks at the collar or just above the collar region. These are perithecia of the sexual stage (*Gibberella xylarioides* (Heim and Saccs)).
- ♦Affected trees remain firmly rooted to the ground and do not topple on pushing and may take a few months to die and dry up. Affected trees never recover. Even if they produce suckers, these later dry up and also die.

COFFEE GROWING AREAS AND TRACHEOMYCOSIS DISTRIBUTION IN UGANDA



CONTROL STRATEGIES IN OPERATION

The first step taken in this direction was training of both extension staff and farmers in disease recognition, followed by sensitisation of farmers and civic leaders.
Sanitary control measures were then implemented which include

- ♦Destruction of affected trees by cutting trees at ground level, chopping and burning *in situ*.
- ♦Restriction of movement of infected plants as firewood and coffee husks from infected areas to other areas.
- ♦Banning the use of coffee husks as mulch in coffee fields, as a precaution.
- ♦Milling coffee is done in the district of production.
- ♦Continous surveillancee of disease in all coffee growing areas to keep track of spread and ascertian the effectiveness of control measures.

The impact of these short term measures are yet to be ascertained.

RESEARCH STRATEGIES

Prospects for long term control measures depend on research activities, which have been implemented to generate information on:

- ♦Epidemiology and biology of the pathogen, to cover mode of spread and transmission, survival, presence of alternate hosts etc.
- ♦Host plant resistance/tolerance is being explored by inoculation of all available germplasm, breeders' materials and current recommended arabica and robusta varieties. Although the disease has not yet been reported on recommended robusta clones and arabica coffee, it still remains a threat and could be a matter of time before they are also affected.
- ♦The effects of production systems (intercropping, soil fertility management and cultivation of nonhost crops for 5 or more years followed by coffee) on wilt incidence and severity will be interesting to elucidate.
- ♦The role of weather factors e.g. rainfall, temperature, etc as well as soil types are also to be assessed and correlated to wilt incidence.

DISCUSSION AND CONCLUSION

The availability of epidemiological data on coffee wilt, particularly, the effects of rainfall, temperature, soil types, and crop management systems on the manifestation of this disease, would greatly facilitate coffee cultivation in Uganda.
Although wilt is widespread, replanting on fresh land not previously under coffee is advocated, to keep coffee cultivation going, while the search for resistance continues. All effort to devise a plan to prevent or delay the advance of wilt to other areas still free from the disease, especially arabica should be made. Coexistence of coffee with the disease will depend on cultivars with

some tolerance to the disease, and the implimentation of integrated control measures to reduce risk. It is imperative that the national research programme for wilt control in Uganda joins forces with other programmes in the region to successfully investigate this disease.

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SUMMARY

Coffee is an important and leading cash crop for the small scale farmers in Uganda. Pests and diseases are among the major production contraints which reduce both productivity and quality of coffee. Coffee wilt disease due to Fusarium xylarioides Steyaert on robusta coffee was confirmed in Uganda in 1993 in two districts bordering the Democratic Republic of Congo (Zaire). Currently, the wilt occurs in 12 districts, to the south and east of the original foci. Disease incidence varies from a few infected trees to over 50% tree mortality. The control measures being implemented are sensitisation of farmers and civic leaders about the disease, urging farmers to cut and burn affected trees in situ, restriction on movement of unhulled coffee, a ban on use of coffee husks as mulch in coffee, and replanting on new land. Little information is available on the biology and epidemiology of the disease. There is, therefore, urgent need to intensify research in these areas and to identify sources of resistance which are basic in formulating effective control strategies.

EFFECT OF STRIPPED MEALYBUG (*FERRISIA VIRGATA* COCKERREL) ON MARKETABLE QUALITY OF UGANDA ROBUSTA COFFEE (*COFFEA CANEPHORA* PIERRE)

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1. INTRODUCTION

Attack of coffee by pests may cause considerable yield losses, lower quality and increase costs of production since their control entails additional material, labour and time inputs. The damage to quality of the product by these pests may be a direct result of the degradation of the beans following attacks, or of some pesticides used in their control which impart undesirable flavours to the marketable product (Pochet, 1990).

Over 50 species of scales and mealybugs are reported to attack various parts of the coffee tree - roots, branches, leaves, flower clusters and berries where they suck sap and are of great economic importance on the crop (Wrigley, 1988; Coste, 1992). In Uganda, the stripped mealybug (*Ferrisia virgata* Cockerrel), previously a pest of sporadic occurrence (Butt *et al*, 1970; McNutt, 1970) is now considered the second most damaging pest of Robusta coffee after the berry borer (*Hypothenemus hampei* Ferr.). Attacks are concentrated on berry clusters, greenwood and leaves. Heavy infestation of berry clusters by the mealybugs results in berries ripening prematurely, and they either fall off the trees or remain on primaries and eventually dry out (Le Pelley, 1968). It has not been determined whether such prematurely rippened

berries have any effects on the final quality of the harvest. In this study, effort is made to establish effects of *F. virgata* attack, on marketable quality of Robusta coffee and to integrate information in quality management strategies for the crop.

2. MATERIAL AND METHODS

Assessment of effects of *F. virgata* attacks on marketable quality of 6 clonal varieties of Robusta coffee was carried out at Kawanda Agricultural Research Institute on a field planted in 1991 using the 1994/95 harvests, and at the quality analysis laboratory of the Uganda Coffee Development Authority (UCDA). Samples of berries were obtained from *F. virgata* infested and uninfested berry clusters during harvest of the 1994/95 crop. These were wet processed to give clean coffee beans which were sun dried to a uniform moisture content of about 12%, verified using SINAR moisture analyser. The berries were analysed for various quality parameters at the Uganda Coffee Development Authority (UCDA) quality analysis laboratories as below:

Effects on mean bean size and mean seed weight.

To determine effects of *F. virgata* infestation on bean size, one hundred grammes of beans from each sample were weighed out and screened, first using the larger screen guide 18, and the beans not retained again screened using a smaller screen guide 15. The percentage by weight of beans retained by both screen guides were obtained for infested and uninfested bean samples for each of the clones as estimate of bean size.
To determine effects of attack by *F. virgata* on mean seed weight of robusta coffee, and 100 beans counted out for each sample. Weight of the 100 beans was obtained using an electronic chemical balance. Three 100 bean samples, each for uninfested and infested beans samples per clone, were determined and these were used to derive 100-bean mean weight for all the samples.

Effects on roast appearance, centre cut and liquor taste.

To assess effects of attack by *F. virgata* on roast appearance, centre cut and liquor taste, samples of beans from infested and uninfested cherries for each clone were roasted in a PROBAT gas sample roaster to a medium level roast. Samples of roasted beans

ground to medium fine particles were brewed using freshly boiled tap water to prepare infusions for cup quality tests using the conventional sensory evaluation procedure.

3. RESULTS

Effect on bean size

Results of determination of effects of *F. virgata* infestation of berry clusters on beans size are summarised in Table 1.
Proportion of beans retained by screen 18 was significantly ($P \leq 0.05$) higher for uninfested than for infested cherries for clones 1s/3, 1s/6, 236s/23, 257s/56 and 258s/24(0). A reverse trend was however observed for clone 1s/2 with higher proportion retained for infested than for uninfested cherries.
The proportion of beans retained by the smaller screen 15 was significantly ($P \leq 0.05$) higher for beans obtained from infested cherries compared to those from uninfested clusters, and this applied to clones 1s/3, 1s/6, 236s/23, 257s/56 and 258s/24(0). Again, a negative trend occurred for clone 1s/2 with higher proportion retained for beans from uninfested cherries than for beans obtained from infested cherries.

Effects on mean seed weight

Table 2. shows mean seed weight and the relationship between infested and uninfested cherries derived beans for all the six clones. Differences in mean seed weight between beans derived from infested and uninfested cherries was significant ($P \leq 0.05$) among clones, with beans from uninfested cherries having a higher mean seed weight than for *F. virgata* attacked cherries. This difference was recorded for clones 1s/3, 1s/6, 236s/23, 257s/56 and 258s/24(0).
Interaction between *F. virgata* attack and clones for the same parameter was also significant ($P \leq 0.05$), with higher mean seed weight in clone 1s/2 obtained from infested cherries derived beans.

Table 1. Percentage of beans retained by screen guides 18 and 15 for infested and uninfested cherries derived beans

Clone	Screen 18		screen 15	
	Uninfested	Infested	Uninfested	Infested
1s/2	75.2	85.5	24.0	14.1
1s/3	86.7	82.7	10.9	12.0
1s/6	85.2	77.2	14.5	22.5
236s/23	91.2	72.1	8.4	27.7
257s/56	30.4	7.4	68.0	86.2
258s/24(0)	84.1	63.2	15.0	36.8
Sample means	75.4	64.7	23.7	33.0

Table 2. **Mean 100 seeds weight for *F. virgata* infested and uninfested cherries derived beans**

Clone	Uninfested	Infested
1s/2	22.2	22.8
1s/3	23.6	22.5
1s/6	22.6	21.2
236s/23	23.3	20.6
257s/56	19.6	16.1
258s/24(0)	21.3	19.4
Clone means	22.1	20.4

Effects of infestation on roast appearance, centre cut and liquor taste.

Results for roast appearance, centre cut and liquor taste analyses done on samples of beans from infested and uninfested berry clusters, roasted to a medium degree roast in a Probal gas sample roaster and ground to medium fine particles, are summarised in Table 11. Differences were observed between beans derived from *F. virgata* infested cherries and those for uninfested cherries for all the clones. Generally, fair roast colour and cup taste is represented by beans obtained from uninfested cherries, compared to beans obtained from infested cherries.

4. DISCUSSION

The results of bean size assessments show that infestation of berry clusters by *F. virgata* leads to decrease in bean size as indicated by a higher percentage of beans from uninfested cherries than those from infested ones retained by the larger screen 18, and the lower percentage of beans of beans from uninfested cherries than those from infested berry clusters retained by the smaller screen 15. The reduction in bean size

Table 3. Roast appearance, centre cut and liquor taste implications of *F. virgata* infestation of berry clusters of Uganda clonal robusta coffee.

	UNINFESTED			INFESTED		
CLONES	Roast	Centre cut	Liquor taste	Roast	Centre cut	Liquor taste
1S/2	Dull	Poor	Fair robusta	Irregular	Poor	Bitter, weak robusta
1S/3	Dull	Normal	Weak robusta	Irregular	Poor	Poor harsh robusta
1S/6	Dull	Normal	Weak robusta	Dull	Normal	Weak flat robusta
236S/26	Coated	Normal	Poor weak robusta	Coated	Normal	Poor bitter robusta
257S/56	Coated	Normal	Poor weak robusta	Dull	Normal	Poor weak bitter robusta
258S/24(0)	Coated	Normal	Fairly good robusta	Coated dull	Poor	Poor flattish flavour
Standard	*****	*****	*****	******	******	*******

following attack of berry clusters is possibly due to the sucking effects of the mealybugs, particularly on the berry navel which reduces the amount of assimilates reaching the developing berry and consequently interrupting full development of the beans. Incomplete development of infested berries is actually indicated by premature ripening of the beans which comes much earlier than normal ripening.

The results also showed a reverse trend for clone 1s/2 with a bigger bean size obtained for beans from infested cherries than for beans from uninfested cherries. This observation suggests presence of elements of resistance or tolerance to *F. virgata* attacks in clone 1s/2.

Infestation of cherries by *F. virgata* therefore lead to reduction in mean seed weight in clones 1s/3, 1s/6, 236s/23, 257s/56 and 258s/24(0), but again resulted into higher mean seed weight in clone 1s/2. Reduction in mean seed weight of the five clones following attack of this pest is probably due to reduced filling of the berries as a result of partial loss of assimilates partitioned to the berries to extraction by the mealybugs.

Again, increase in mean bean weight in clone 1s/2 following attack of *F. vargata* on berry clusters is yet another indication of presence of elements of resistance to the pest in this clone.

Infestation of berries by *F. virgata* also lowered quality of roast of the resultant beans, centre cut appearance and liquor taste. Beans derived from uninfested cherries exhibited ordinary roast which is superior to the dull roast exhibited by beans from infested berry clusters. Uninfested strains also exhibited good body and flavour, while the infested counterparts had reduced body, poor flavour, and generally bitter and harsh flat taste. These roast and cup taste effects are also possibly a result of reduced filling of the beans with loss of assimilates to the mealybugs, and the accompanying premature ripening of the berries.

5. CONCLUSION

Apart from loss in crop yields, heavy attack of Robusta coffee berry clusters by **F. virgata** interrupts normal bean development leading to premature rippening and drying of berries on primaries. Such berries yield beans of lower marketable quality characterised by smaller screen guide, lower mean bean weight and much poorer cup taste.

Farmers are therefore advised not to mix cherries from heavily attacked berry clusters to normal ones as these lower grade of coffee.

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ABSTRACT

Relationship of attack by Ferrisia virgata (Cockerell) to marketable quality of six Uganda Robusta coffee (Coffea canephora Pierre) clones was assessed at the Uganda Coffee Development Authority quality control laboratory, Uganda in 1995. Variations among beans derived from infested and uninfested berry clusters for bean size and mean seed weight were significant ($P < 0.05$). Infestation lowered 'screen guide 18' retention by 10.7%, while retention by the smaller 'screen guide 15' rose by 9.3%. Mean bean size was also reduced by 7.7%. Roast colour, centre-cut appearance and liqour quality were generally inferior for beans from infested berry clusters. Farmers should therefore not harvest heavily attacked berries which are prematurely ripen and dried out as these lower marketable quality of the coffee.

PLANT REGENERATION FROM SUSPENSION CULTURE PROTOPLASTS ESTABLISHED FROM HYPOCOTYL-DERIVED CALLUS OF TWO *COFFEA ARABICA* GENOTYPES

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INTRODUCTION

In coffee there have been reports of the attempted isolation of protoplasts from leaves (Orozco and Schieder 1984), leaf-derived callus (Söndahl *et al.* 1980), somatic embryos (Schöpke *et al.* 1987a), embryogenic cell suspensions induced from leaf-derived callus (Acuna and De Peña 1991; Spiral and Petiard 1991) and cell suspension cultures established from non-embryonic roots via callus cultures (Grézes *et al.* 1994). However, successful protoplast culture and plant regeneration through somatic embryogenesis has been achieved only recently in coffee (Acuna and De Peña 1991; Spiral and Petiard 1991; Schöpke *et al.* 1987b). These reports suggest that successful plant regeneration from protoplasts is highly genotype-dependent in *Coffea* spp. and that further studies are necessary to develop the potential of this technique for breeding purposes. The objective of this present study was to establish a method for isolation, culture and plant regeneration from protoplasts of cell suspension cultures which were established from hypocotyl-derived callus of two *C. arabica* genotypes N39 and Hybrido de Timor. N39 is a commercial cultivar in Tanzania and is highly susceptible to both coffee berry disease (CBD) (*Colletotrichum kahawae*) and leaf rust (*Hemileia vastatrix*), while Hybrido de Timor is used as an important progenitor of resistance to both diseases.

MATERIAL AND METHODS

Establishment of cell suspension cultures

Cell suspension cultures of genotypes N39 and Hybrido de Timor that served as sources of protoplasts were prepared by growing *c.* 3 g of calli in liquid callus maintenance medium (CMM, lacking agar) (Nyange *et al.* 1995a), but with 2.3 µM 2,4-D, 2.6 µM NAA, 2.3 µM/kinetin (KIN) and 2.2 µM 6-benzylaminopurine (BAP), contained in 250 ml flasks with screw caps or cotton wool plugs and sealed with Nescofilm. The cell suspension cultures were incubated at 26°C on an orbital shaker at 110 rpm in darkness and maintained by diluting three-fold into fresh medium weekly (Nyange *et al.* 1997).

Isolation and culture of protoplasts

Five-day old cell suspensions were used for the isolation of protoplasts, as described by Nyange *et al.* 1997.

Essentially, the protoplast culture medium was made of B5 salts (Gamborg *et al.* 1968) supplemented with vitamins, organic acids, sugars, MES and PVP. Cells (5 g wet weight) were pre-plasmolysed in liquid protoplast culture medium lacking phytohormones and vitamins for 60 min at 110 rpm in darkness at 26°C and 1 ml packed cell volume (PCV) was resuspended in 10 ml of enzyme solution. Several enzyme solutions of different compositions (Table 1) were evaluated for their ability to release protoplasts. They were dissolved in protoplast culture medium lacking phytohormones, vitamins and organic acids, but with Ca^{2+} raised to 6 mM. The Petri dishes containing cells were placed on an orbital shaker at 50 rpm in the dark at 28°C for 6 or 15 h. The protoplasts were collected by centrifugation at 70 g for 4–5 min and washed three times in protoplast culture medium, but with the Ca^{2+} concentration lowered to the original Gamborg's B5 salts. Protoplast yields were determined from two experiments, each with two replicates per enzyme solution per genotype. Protoplasts were cultured at 2 x 10^5 per ml in 30 x 15 mm single-vented Petri dishes (Sterilin) containing 2 ml of liquid protoplast culture medium supplemented with 2.3 μM 2,4-D, 2.6 μM NAA and 2.2 μM BAP. The cultures were incubated in the dark at 26°C and fresh culture medium added (1:3 v/v) 3–4 days after isolation to prevent aggregation of protoplasts. After 3 weeks, cultures were mixed with an equal volume of 0.8% agarose (Sigma type IV) in the same protoplast culture medium, but with glucose reduced to 0.3 M and cultured under the same conditions for 5 weeks to allow the formation of microcalli. The agarose plates were cut into blocks which were transferred to 60 mm Petri dishes containing protoplast culture medium with the concentration of glucose reduced to 0.2 M and incubated at 26°C under low light intensity (35 μmol s^{-1} m^{-2}) for 4 weeks. The osmotic pressure of the medium was further reduced by replacing the liquid medium around the agarose blocks with fresh protoplast medium containing glucose at 0.1 M and the plates were incubated under the same conditions for another 4 weeks. The plating efficiency, determined after 8 wk of protoplast culture, was based on the percentage of microcalli formed from the actual number of protoplasts cultured. The results were derived from two experiments, each with two replicates (Petri dishes) per genotype. The viability of freshly isolated protoplasts was assessed by staining them with fluorescein diacetate (FDA) [Widholm 1972].

Plant regeneration

After 16 weeks of culture, protoplast-derived microcalli (1–2 mm in diameter) were lifted by spatula and transferred to callus growing medium consisting of half-strength MS medium, B5 vitamins, sucrose (87.6 mM), Difco-Bacto agar (8 g l^{-1}) and BAP (4.4 μM) and grown for 8 weeks with subculturing every 4 weeks. The calli were then conditioned in liquid embryo induction medium (EIM) according to Neuenschwander and Baumann (1992) and incubated for 2 weeks on an orbital shaker at 50 rpm at 26°C in the dark. The conditioned calli were transferred to solid EIM in 30 mm Petri dishes under low light intensity (35 μM s^{-1} m^{-2}) at 26°C for another 6 weeks. Embryos which developed were cultured on to embryo germination medium (EGM) (Neuenschwander and Baumann 1992) under the same conditions for another 6 weeks. The plantlets were rooted on medium 4 (consisting of half-strength MS, sucrose (43.8 mM) and lacking phytohormones) contained in jars and cultured for more than 6 weeks at 26°C and a light intensity of 60 μmol s^{-1} m^{-2}, before final transfer to Jiffy-7 (peat pellets) in humid boxes in the greenhouse for weaning.

RESULTS AND DISCUSSION

Table 1 shows yields of intact protoplasts obtained with different enzyme solutions. The analysis of variance of the $\log_e$ transformed data showed that enzyme solution V was more efficient ($P<0.001$) than any of the other enzyme solutions in releasing protoplasts. Enzyme solution I gave a higher yield of protoplasts compared to II, III and IV after a 6 h incubation period. There were no significant differences observed in the isolation of protoplasts between the two genotypes tested. Therefore, enzyme solution V with a 15 h-incubation time was adopted as a standard method for the isolation of protoplasts in subsequent experiments. The viability of freshly isolated protoplasts ranged from 80–94%, as determined by FDA staining. The protoplasts were plated at an initial density of 2 x 10^5 protoplasts ml^{-1}. Within 10 days of culture, the protoplasts regenerated cell walls, attained an oval shape and underwent cell division. Colonies consisting of groups of more six cells were evident after 3 weeks of culture. Microcalli (1–2 mm in diameter) were formed after 16 weeks of culture. The protoplast-plating efficiencies, defined here as the percentage of the total number of protoplasts plated that developed into microcalli, were 3.16% and 4.17% for N39 and Hybrido de Timor respectively. Microcalli derived from protoplasts were transferred to callus growing medium (Nyange *et al.* 1995b) where they developed into

embryogenic calli of more than 8 mm diameter after 8 weeks culture. Callus proliferation was sustained after transfer of calli to EIM for 2 weeks and subsequently to solid EIM for another 6 weeks. On the latter medium, the calli became brown and a highly synchronous development of somatic embryos took place. About 70% of the brown calli became dark and necrotic, and no further growth occurred. Many of the somatic embryos were transferred to EGM. Some early-developed somatic embryos with cotyledonary leaves were grown further on to EGM until they were 6–10 mm. Although EGM is not normally used for rooting, some of the somatic embryos formed roots in this kinetin-containing medium. Extensive root development occurred after transfer of somatic embryos to half-strength MS lacking phytohormones. Plants with normal morphological features were weaned and transferred to a glasshouse.

The procedures described in this study have shown for the first time the applicability of embryogenic cell suspension cultures established from hypocotyl-derived callus for obtaining totipotent protoplasts and for the regeneration of plantlets. This is seen as an important advance since *Colletotrichum kahawae*, attacks the hypocotyls of *C. arabica*. The callus derived from hypocotyls, therefore, may offer a suitable source of material for *in vitro* studies of CBD resistance (Nyange *et al.* 1993, 1995a). Griffiths and Anderson (1987) suggested that protoplasts used for plant-pathogen studies should be isolated from cells which would be challenged by the pathogen under natural conditions. The fact that it has been possible to regenerate plants from the two genotypes N39 (a selection from the naturally occurring allotetraploid with 2n = 4x = 44, *C. arabica* germplasm (Fernie 1960) and Hybrido de Timor (a natural spontaneous tetraploid cross between *C. arabica* and *C. canephora* (Rodrigues *et al.* 1975)) may indicate the non-genotype specificity of this technique. The protoplast-to-plant system developed in this study offers the opportunity for improvement of *C. arabica* through techniques such as gene- and organelle-transfer, either by direct DNA or by protoplast fusion, and *in vitro* selection for resistance to diseases like CBD and the subsequent cloning of the variants obtained (Nyange *et al.* 1995b).

Table 1: The effect of enzyme solution on the release of protoplasts from cell suspensions of *C. arabica*

	Enzyme solutions[1] (%w/v)				
Composition	**I**	**II**	**III**	**IV**	**V**
Cellulase Onozuka R10[2]	2.25	2.25	2.25	2.0	1.5
Macerozyme R10[2]	1.0	1.0	1.0	–	1.0
Pectolyase Y-23[3]	0.05	0.2	–	0.2	–
Driselase[4]	0.2	–	0.5	0.2	0.5
	Log_e protoplast yield[5] ml^{-1} (PCV)				
	13.54	10.92	12.40	11.93	13.96
	($7.59x10^5$)	($5.50x10^4$)	($2.44x10^5$)	($1.50x10^5$)	($1.15x10^6$)
SED (df=9) 0.369					

[1]Incubation time: 6h (enzyme I-IV); 15 h (enzyme V); [2]Yakult Pharmaceutical Co., Japan; [3]Seishin Pharmaceutical Co., Japan; [4]Sigma UK; [5]Log_e transformation used for analysis, detransformed values are given below in parentheses.

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SUMMARY

Regeneration of plants of *Coffea arabica* from protoplast-derived calli was achieved through the induction of somatic embryogenesis. Protoplasts were enzymatically isolated from 5-day-old cell suspension cultures which were established from hypocotyl-derived callus. A high yield of protoplasts was released from the suspended cells (1.15×10^6 protoplasts ml^{-1} packed cell volume) when treated with an enzyme mixture containing 1.5% Cellulase Onozuka R10, 1.0% Macerozyme R10 and 0.5% Driselase. Culture of protoplasts in an agarose-solidified medium resulted in sustained proliferation of microcalli which were transferred to callus-growing medium and subsequently to embryo-induction medium. Embryoids which developed were grown on embryo-germination medium into plantlets. Plants with normal morphological features have been successfully transferred to the greenhouse. The establishment of this protoplast system should facilitate novel breeding approaches in *C. arabica.*

EVALUATION OF AGROCET 180, 360 EC AND ROUNDUP DRY 420 SOLID GRANULES, NEW ROUNDUP FORMULATIONS IN CONTROLLING WEEDS IN COFFEE

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INTRODUCTION

Pre- and Post-emergence herbicide trials has been carried out and recommendation made for coffee (Anon, 1988). Several soil acting and foliage acting herbicides have also been recommended for use in Kenya coffee farming (Anon, 1993). Glyphosate control a wide spectrum of annual weeds, broad leaved weeds and the deep-rooted perennials in which it is translocated easily from vegetative parts to underground roots, rhizomes or stolons and destroys them (Kessy, 1986; Njoroge, 1990). It interfere with the synthesis of amino acids. Leaf symptoms in most cases appear within 7 to 14 days after spraying glyphosate and complete desiccation usually occurs within 30 days after treatment application (Monsanto, 1982).

The purpose of this paper is to report on the current investigations of Roundup Dry 420 SG, Agrocet 180 EC and Agrocet 360 EC as compared with Roundup 360 EC carried at Lyamungu research station. The objectives of this study were (a) To monitor the efficacy of Roundup dry 420 SG, Agrosate 180 EC and 360 EC in controlling weeds in coffee. (b) To look at the effect of these herbicides on coffee yields when applied repeatedly for a period of three years on the same site.

MATERIALS AND METHODS

A herbicide trial was established at Lyamungu Research Station at two blocks of the farm. The trial was carried for three years (1994, 1995 and 1996). In all the three years, two applications were made and compared to two slashings per annum as a check. The experimental design was CRBD with three replications and a plot size of $15m^2$. Prior to treatment application, weed flora was assessed in species at each treatment plot using field guide to important arable weeds as identified by Vernon, 1983; Terry and Michieka, 1987. Herbicide treatments were applied using a hand "solo" knapsack sprayer with low-volume lurmark spray nozzle.

Treatment efficacy was based on % foliar weed-kill taken at 7, 14, 21, 28 and 35 days after treatment application (DAT). Also % Weed cover data from 8 to 14 WAT at an interval of two weeks averaged across sites and applications for two years was used to access treatment efficacy. The treatments are shown below:

		Formulation		Active ingredient	Water
Treatment	Commercial Product	(1)	kg or l/ha	kg/ha	l/ha
T1	Control	0	0	0	0
T2	Roundup dry 420 SG	6.6	0.867 kg	0.364	100
T3	" " "	13.3	1.733 kg	0.729	100
T4	" " "	20.0	2.600 kg	1.092	100
T5	Roundup 360 EC	-	4.000 l	1.440	100
T6	Agrosate 360 EC	-	2.000 l	0.920	100
T7	" "	-	4.000 l	1.440	100
T8	Agrosate 180EC	-	4.000 l	0.720	100
T9	" "	-	6.000 l	1.080	100
T10	" "	-	8.000 l	1.440	100
T11	Roundup dry 420 SG	26.0	3.466 kg	1.446	100

(1)No. of 130g sachets EC: emulsifiable concentrate, SG: solid granules

All four products have Glyphosate as active molecule. Roundup is manufactured by Monsanto, Agrosate by Transagro.

RESULTS AND DISCUSSIONS

Table I: Appearance and disappearance of weeds which were killed slowly

Year	1994		1995		1996	
Weed Species \Application	1st	2nd	1st	2nd	1st	2nd
Commelina spp.	+	+	+	+	+	+
C. Rotundus	+	+	+	+	+	+
Mucuna spp.	+	-	-	+	-	+
O. latifolia	+	-	+	+	+	+

- = absent + = present

(a) Weed-kill

In all the seasons and applications based at 35 DAT the *Commellina spp, Cyperus rotundus, Oxalis latifolia* and *Mucuna species* were killed slowly (Tables 3). However *Mucuna species* and *Oxalis latifolia* had a tendency of disappering and appearing in between years and applications as shown in table I.

(b) Appearance and disappearance of weed species by applications and across treatments

For three years the weeds shown are the ones which appeared and reappeared. *Tagetes minuta* did not show up after the 1st application in 1994. However *Galium sprium* and *Solanum nigrum* showed in 1996 indicating a change in weed flora (Table 2).

(c) % Weed cover:

The data is shown in Table 4 below for 8,10,12 and 14 weeks respectively. Weed regrowth was low in all roundup formulations but least in the higher rates ie. T4, T10 and T11. However the low %weed cover was not reflected in coffee yields.

(d) Coffee parchment yield (kg/ha)

There were no significant yield differences due to treatments. However significant year differences in yields were noted with 1996 having the highest yields (Table 5). The yearly differences in yield could be attributed to the highest total rainfall as shown in Table 6 and may be cumulative management of the trial.

From the above results it can be seen that *Commelina spp., O.latifolia* and *Macuna spp.* are killed slowly at 35 DAT. Some weed species appear and disappear with repeated applications. Roundup dry at 0.729 kg/ha appear to be economical. There was no yield advantage in screening the products at higher rates. All the herbicide products showed a superior % weed-cover compared to the control but this was not reflected in coffee yields.

Table 2: Appearance and disappearance of weed species by applications and across treatments

Year	1994		1995		1996	
Weed Species \ Application	1st	2nd	1st	2nd	1st	2nd
Oxalis latifolia	+	-	+	+	+	+
Mucuna spp.	+	-	-	+	-	+
Oxalis coniculata	+	-	-	+	+	-
Ipomoea spp.	+	-	+	+	+	+
Vernonia petersii	+	-	+	+	+	+
Tagetes minuta	+	-	-	-	-	-
Phyllanthus leucanthus	+	-	+	+	+	-
Ageratum spp.	+	+	-	+	+	-
Digitaria scalarum	-	-	-	+	+	+
Galium sprium	-	-	-	-	+	+
Solanum nigrum	-	-	-	-	-	+

- = absent + = present

Table 3: Mean % foliar weed-kill at 7 and 35 DAT at Lyamungu 1994 -1996. First and Second applications

1st Application of treatments

	7 DAT											35 DAT									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
1.	0	2	2	4	3	3	3	3	3	5	2	0	26	43	64	52	50	61	48	41	56	48
2.	0	10	15	14	16	14	19	15	14	14	15	0	100	100	100	100	100	100	100	100	100	100
3.	0	8	7	9	7	9	8	6	8	8	7	0	89	93	98	99	100	100	99	100	100	100
4.	0	13	19	17	21	16	18	16	16	18	18	0	100	98	100	100	100	100	100	100	100	100
5.	0	6	7	8	10	10	10	5	7	10	9	0	77	74	87	84	79	83	76	84	87	88
6.	0	5	13	10	10	10	7	8	10	13	7	0	95	99	95	100	97	100	99	56	100	100
7.	0	9	10	13	13	12	13	13	17	10	10	0	100	100	100	100	100	99	100	100	100	100
8.	0	15	8	10	5	10	10	5	7	8	5	0	62	100	100	100	100	100	100	98	100	100
9.	0	5	5	8	5	5	16	5	3	3	2	0	50	57	69	65	47	72	85	77	80	63
10.	0	10	15	16	20	16	29	15	19	20	17	0	100	100	100	100	98	100	100	100	100	100
11.	0	7	10	10	10	14	12	8	8	13	9	0	90	88	93	100	95	99	99	97	90	98
12.	0	0	1	3	0	2	2	5	2	3	2	0	5	13	87	60	23	43	73	48	53	65
13.	0	2	4	9	13	10	14	3	10	7	10	0	71	80	100	100	100	100	92	95	100	95

2nd Application of treatments

	7 DAT											35 DAT									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
1.	0	3	3	2	7	2	4	6	5	4	5	0	42	41	36	67	60	52	50	70	80	64
2.	0	7	9	10	9	8	12	9	9	8	10	0	91	98	100	100	82	99	100	100	99	100
3.	0	5	7	8	9	8	5	9	10	9	7	0	85	98	99	97	96	99	97	99	99	98
4.	0	8	9	11	9	10	12	9	10	11	13	0	85	91	100	99	96	100	97	99	100	98
5.	0	7	7	7	10	6	6	6	6	7	7	0	69	76	87	86	77	80	82	87	83	80
6.	0	5	8	9	9	8	14	7	13	11	8	0	82	95	88	98	85	97	82	97	95	95
7.	0	7	11	8	10	8	9	9	10	12	11	0	76	91	95	99	92	98	95	99	97	97
8.	0	7	9	10	9	10	12	10	12	9	10	0	83	92	97	99	95	99	98	99	99	98
9.	0	7	5	8	11	5	11	9	8	10	5	0	58	83	77	95	72	78	80	78	84	76
10.	0	8	11	12	7	19	15	14	9	11	15	0	98	100	100	100	100	99	98	99	98	100
11.	0	12	9	12	13	13	14	11	13	11	11	0	99	100	100	99	100	100	98	100	100	100
12.	0	6	5	11	20	10	15	12	18	8	7	0	55	67	92	67	69	98	52	74	90	81
13.	0	8	7	8	7	7	10	10	8	7	7	0	95	97	100	100	100	98	73	100	94	83

Weed species as they appear in the 1st and 2nd Applications:

1st Application	2nd Applications
1. Commelina spp	*1. Commelina spp*
2. Setaria homonyma	*2. Setaria homonyma*
3. Oxalis latifolia	*3. Oxalis latifolia*
4. Mucuna spp	*4. Mucuna spp*
5. Cyperus rotundus	

Table 4: %Weed cover from 8-14 weeks after treatment application (means of 1995 and 1996)

First Application (Early March)

	Weeks After Treatment Application			
Treatment	8	10	12	14
T1	100	100	100	100
T2	15	22	38	52
T3	14	25	40	58
T4	7	10	16	29
T5	13	26	47	67
T6	11	17	31	51
T7	11	18	36	57
T8	9	14	26	40
T9	12	20	30	46
T10	8	11	21	39
T11	10	13	25	40
Mean	18.52	24.56	36.98	52.56
CV(%)	34.68	44.61	52.28	48.69
LSD(0.05)	10.92	18.77	33.09	40.88

Second Application (Early July)

	Weeks After Treatment Application			
	8	10	12	14
	100	100	100	100
	6	11	22	37
	9	16	28	38
	4	7	13	21
	7	13	28	41
	8	17	26	38
	8	16	26	40
	6	11	22	34
	6	11	19	29
	6	11	21	31
	6	9	20	28
Mean	14.7	19.74	28.96	36.89
CV(%)	23.07	35.58	38.87	38.35
LSD	6.02	12.94	20.28	25.5

Table 5. Coffee parchment yields (kg/ha).

Treatment	1994	1995	1996	Mean
T1	449	486	1199	711
T2	340	596	1454	796
T3	739	538	1573	950
T4	630	406	1053	693
T5	312	518	1675	835
T6	486	467	1325	760
T7	370	467	1574	804
T8	563	651	1230	815
T9	398	492	1404	765
T10	599	383	1142	708
T11	391	590	1627	869
Mean	480	509	1386	791
CV %				42.4
LSD (P=0.05)	ns	ns	ns	ns

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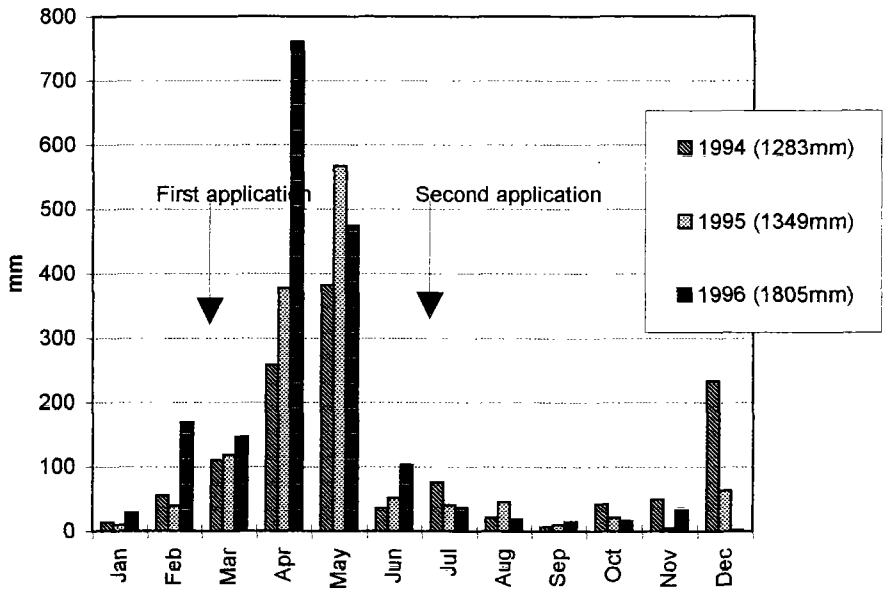
Figure 1: Monthly rainfall, Lyamungu, 1994 to 1996



SUMMARY

A Trial at Lyamungu Coffee Research Station for three years in two blocks of the farm is in discussion. Roundup dry 420 SG at rates of 0.364, 0.729, 1.092 and 1.446 kg/ha a.i., Agrocet 180 EC at 0.92 and 1.44 kg/ha a.i. and Agrocet 360 EC at 0.72, 1.08 and 1.44 kg/ha a.i. Comparisons are made with Roundup 360 EC at 1.44 kg/ha a.i. and a slashed check in each trial. All the rates were applied twice annually. All the formulations had equal efficacy in weed kill at 35 days after treatment application. Some weeds re-appeared while
others disappeared. There was no coffee yield advantage in applying the products at higher rates. At the rates screened, annual and perennial weeds were controlled. Hence with repeated applications of roundup dry the rate of 0.729 kg/ha ai. is recommended.

RESUME

Un essai d'herbicides a été mené pendant trois ans dans deux parcelles à Lyamungu. On y compare Roundup sec 420 à trois doses par hectare, Agrocet 180 CE à deux doses par hectare, et Agrocet 360 CE à trois doses par hectare. Deux témoins sont utilisés: Roundup 360 CE à 1,44 kg/ha et un traitement désherbé manuellement. Tous les traitments sont appliqués deux fois par an. Tous les traitements ont la même efficacité après 35 jours. L'application de doses élevées n'apporte aucun surplus de récolte. Tous les traitements permettent un contrôle efficace des mauvaises herbes annuelles et pérennes. On recommande donc l'application répétée de Roundup sec à 0,729 kg ma/ha.

SYNTHÈSE DES RÉSULTATS DE RECHERCHE SUR LES MÉTHODES CULTURALES DU CAFÉIER ARABICA (*COFFEA ARABICA*) AU RWANDA, AU 31 MARS 1994

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1 Introduction

Coffea arabica L. est la culture d'exportation la plus importante et la principale source de devises au Rwanda. La valeur du café représentait respectivement 75%, 73% et 56% de l'ensemble des exportations du pays pour les années 1987, 1988 et 1989. Le café est cultivé sur une superficie d'environ 53.000 ha par de petits planteurs dont le nombre s'élève à environ 700.000 soit environ 55% des exploitations agricoles familiales (Miniplan, 1988). La taille moyenne d'une caféière par exploitant est de 100-175 plants et la production moyenne varie de 300kg à 600kg de café parche/ha/an. La vente du café permet d'injecter en milieu rural une grande masse monétaire qui assure une intense activité économique à travers tout le pays.

2. Exigences écologiques du caféier arabica

Selon Coste (1955), l'espèce *C. arabica* prospère dans les milieux ayant des conditions écoclimatiques suivantes :
une altitude comprise entre 1300m et 1800m, une saison sèche de 2 à 3 mois au maximum, une pluviométrie de 1500 mm bien répartie, une humidité relative de 60%, peu de vents et une température de 20 à 22 °C avec des minima de 5 °C et des maxima de 30 °C.
Le caféier est une culture semi-héliophile qui peut cependant prospérer en plein ensoleillement s'il est en culture intensive avec paillage, bon entretien et fertilisation. Cette plante s'adapte à plusieurs types de sols mais profonds et de texture argileuse à argilo-sableuse. Le pH (eau) optimal du sol est de 5 à 7.

Ces conditions sont partiellement remplies au Rwanda. En effet, la région caféicole dans ce pays est située entre 1450m et 1900m d'altitude (De Vuyst et Brion, 1968) et au dessus de l'isohyète de 1000mm (van Minnebrugen, 1973). La température moyenne du mois le plus froid est supérieure à 18 °C. Le régime pluviométrique est bimodal (Djimde, 1988). Néanmoins, la durée de la grande saison sèche est variable et devient de plus en plus longue lorsqu'on se dirige de l'ouest (60-90jours) vers l'est (110-115 jours) du Rwanda. Les précipitations varient entre 950

et 1350 mm et sont inférieures à l'optimum (1500mm). Cette insuffisance d'eau défavorise le bon développement végétatif et la fructification normale et par conséquent la production des plantations de café. Les sols sont généralement des Oxisols, des Ultisols, des Inceptisols et parfois même des Entisols et dérivent des matériaux parentaux très divers (CPR, 1992). Leur teneur en matière organique et leur richesse chimique sont fort variables mais souvent faibles. L'acidification et l'érosion des sols sont un phénomène généralisé. Les plantations de café ont des productivités variables en fonction de l'altitude, la pluviométrie disponible, la nature des sols et du niveau de gestion des parcelles.

Sur base des rendements café parche enregistrés entre 1959 et 1961, De Vuyst et Brion (1968) ont distingué cinq différentes zones caféicoles, à savoir zone très propice (1kg/caféier/an), zone propice (0.75-1kg), zone assez propice (0.5-0.75kg), zone moyenne (0.5-0.6kg) et zone marginale (moins de 0.5kg/caféier/an). La délimitation de ces zones et les technologies pour obtenir un rendement plus élevé du café devraient être affinée avec le temps.

3 **Matériel et Méthodes**

De nombreuses études conduites en vue de lever ou atténuer les contraintes écologiques à la caféiculture rwandaise ont été réalisées soit sous forme d'enquêtes soit sous forme d'expériences en champs. Généralement, les essais en champs ont été conduits en blocks complétement aléatoires avec au moins tois répétitions; le nombre de caféiers réellement observés par parcelle élementaire variaient de 9 à 25. Les résultats étaient souvent collectés au cours de plusieurs saisons puis soumis à différentes méthodes d'analyse statistique ou évalués a l'aide du calcul de pourcentage d'augmentation par rapport au témoin. La présente synthèse a été réalisée en consultant les différentes publications et rapports annuells produits ainsi qu'en utilisant les données de recherche collectés par nous- mêmes.

4 **Résultats**

Les résultats saillants en matière de techniques culturales du caféier au Rwanda sont présentés ci-après.

4.1 **Techniques de plantation**

4.1.1 **Germoirs et pépinières**

Il a été prouvé que le café en parche sec devait être semé en germoir au mois de novembre/décembre à l'écartement de 10cm x 5cm, ce qui permet d'avoir 3000 à 4000 plantules/kg de graines. Un ombrage artificiel à partir de 2,25 m du sol est requis pour un bon germoir. Après 3 à 4 mois de séjour en germoir, les plantules au stade "cotylédon en parche" doivent être transplantées en pépinière établies à proximité des points d'eau et des champs à planter. Les jeunes plants doivent être repiqués dans les sachets de polyéthylène (18cm x 24cm) remplis d'un mélange de terre inerte (1/2) et de compost (1/2), ce qui garantit une meilleure reprise (De Vuyst, 1972). Les plants séjournent en pépinière ombragée 7 à 8 mois.

4.1.2 **Plantation**

Les plants de caféier doivent être installés en champ au début de la saison de pluie en octobre-novembre. D'après les essais conduits en tiges uniques à Rubona, l'écartement de 2m x 2m soit 2500 plants/ha ou 2.5m x 1.5 m est adéquat: de tels écartements assurent une production plus élevée et réduisent la prolifération des mauvaises herbes. La densité de 3333 caféiers/ha assure de meilleurs rendements/ha alors que la densité de 2500 caféiers/ha donne la meilleure production par caféier. Une courbe théorique ajustée de production a montré que la densité optimale de plantation se situe à 3880 caféiers/ha (ISAR, 1980).

La supériorité des densités élevées a été encore confirmée par les résultats obtenus à Rubona sur caféiers multicaules où les densités de 3784 plants/ha en carré ou rectangle et de 2890 plants/ha en triangle ont produit en fin du premier cycle de production (5 ans de récoltes), respectivement 17,3 et 11.4% de plus que la production de la densité de 2500 plants/ha actuellement utilisée (tableau 1).

Tableau 1. Production cumulée de café parche J2/1257 depuis 1989 à 1993 à Rubona

Ecartement (m) et dispositif	Densité caféiers/ha	Rdt kg /ha café parche	Rdt kg/arbre	% témoin (avec rdt de 2428kg/ha)
2.5 X 2.0 rectangle	2 000	2 397	1.399	98.7
2.0 X 2.0 carré	2 500	2 428	0.971	100.0
2.5 X 1.5 rectangle	2 666	2 370	0.889	97.6
2.0 X 2.0 triangle	2 890	2 848	0.985	117.3
2.0 X 1.5 rectangle	3 333	2 143	0.643	88.3
1.75X 1.75 triangle	3 484	2 705	0.715	111.4
PPDS 5%		416		
CV %		9		
ET		218		

4.2 **Couverture du sol dans les caféières**

Les modes de couverture à adopter dépendent des conditions écologiques des sites. Là où la sécheresse n'est pas un facteur limitant, le paillage du sol est facultatif et son effet dans l'augmentation de la production par rapport à celui du sarclage intégral n'est ni notoire ni économique. Par contre, une culture intercalaire de légumineuses fauchée plusieurs fois peut augmenter la production. La couverture vivante et la couverture sarclée peuvent causer la concurrence hydrique avec le caféier dans les zones à déficit hydrique (< 1500mm d'eau). Dans ces dernières conditions, le paillage permanent est le mode de couverture qui convient. Appliqué correctement en quantité suffisante sur sol encore frais, il assure une bonne économie en eau du sol, utilisable par le caféier pour bien mûrir ses fruits en saison sèche. Par contre, en cas de faible pluviométrie, le paillis peut intercepter la pluie et limiter son arrivée au sol.

L'état de pluviométrie en région caféicole du Rwanda fait du paillage permanent la meilleure technique culturale pour la production satisfaisante du caféier arabica. Cette constatation est appuyée par plusieurs résultats de recherche. Dans un essai avec huit cultivars de café à Rubona (tableau 2), la parcelle paillée a produit 2 à 6 fois plus par rapport aux parcelles non paillées.

Dans un autre essai, les caféiers Mibirizi plantés en 1943 à la densité de 1333 caféiers/ha et paillés en permanence ont donné un rendement supérieur à celui des caféiers sous couverture vivante (tableau 3).

De même le rendement cumulé de 1986 à 1991 d'une jeune caféière de J2 planté en 1984 à la densité de 2500 caféiers/ha et sous paillis permanent a été supérieur à celui des caféiers cultivés avec *Flemingia congesta* (0.354 kg contre 0.220kg /caféier) (ISAR, 1993). Les caféiers sous paillis permanent ont produit presque le double des caféiers soumis au sarclage intégral. Les légumineuses annuelles et celles vivaces plantées dans les caféières puis fauchées et utilisées comme paillis pendant la saison sèche, ont réduit le rendement du café. Le sarclage sélectif où les graminées sont enlevées laissant en place les dicotylées s'est également révélé inférieur au sarclage intégral.

Tableau 2. Effet du paillage sur la productivité de 8 cultivars à Rubona

Variétés	Production moyenne de cérises kg/arbre (moyenne de 8ans)	
	Sarclage à nu	Paillis permanents*
Amerelle Rubona	1.26	5.42
Keut du Kenya	1.55	4.77
Blue Mountain Kenya	1.15	5.53
Blue Mountain Nioleca	1.82	5.77
Jackson Hybrid Kenya	1.24	8.15
Mysore Kenya	1.53	5.05
Bourbon Kenya	2.03	4.88
Mokka Kenya	1.85	7.15

*: Quantité et types de paillis non précisés.

Tableau 3. Rendement moyen en café parche des caféiers Mibilizi sous différents types de couverture du sol (moyenne de 1982 à 1987).

Objets	kg/caféier	kg/ha	% témoin (par rapport à 786kg/ha)
Paillis	1.340	1 786	100
Desmodium	1.130	1 506	84
Stylosanthes	1.030	1 373	77
Mucuna	0.906	1 208	68
Soya fourrager	0.930	1 240	69

Haarer (1962) et Snoeck (1959) avaient montré avec les résultats des essais menés au Kenya et au Rwanda (tableau 4) l'effet bénéfique et supérieur du paillis permanent.

Tableau 4. Effet des différents types de couverture des caféières au Kenya et au Rwanda (respectivement 5 et 10 ans d'observation).

Mode de couverture	Production café en % du témoin	
	Kenya (moyenne de 5 ans)	Rwanda (moyenne de 10 ans)
Paillis permanent	100	100
Sarclage intégral	80	52
Plante de couverture	56	68

Les effets bénéfique du paillage tiennent au fait que le paillis protège le sol contre les effets destructeurs par la pluie (Tian, 1992) et contre l'érosion (Roose *et al.*, 1992), retarde l'évaporation de l'eau du sol (Mwakha,1987), enrichit le sol en humus et en éléments minéraux (Tian, 1992). L'humus produit contribue à l'amélioration du complexe adsorbant du sol. Pour cette raison, le paillis est un préalable indispensable à la bonne utilisation des engrais minéraux au Rwanda (De Vuyst, 1968). Le paillage peut ainsi favorisé le développement des racines de caféier dans les couches superficielles du sol (de l'ordre de 40% de poids des racines primaires et de l'ordre de 50% de poids des racines secondaires) est favorisé. Les caféiers paillés ont

généralement de plus gros grains et ont un rapport café marchand/cerises plus élévé (14,5% contre 13,2% en moyenne pour les caféiers non paillés). Les caféiers paillés ont produit 65% de refus au tamis de 5 mm contre 35% pour le sarclage intégral (Snoeck, 1959). La carence relative du Mg provoquée par l'apport élevé de potassium (par le paillis) a cependant un effet défavorable sur la qualité du café: la couleur cru devient brunâtre, la liqueur moins acide et l'aspect du café torréfié terne (Mitchell, 1968).

La couverture au moyen de Desmodium présente cependant quelques potentialités. Cette légumineuse couvre bien le sol, se régénère facilement après la coupe et produit une matière végétale importante (ISAR, 1986). Elle produit une matière verte évaluée à 40t/ha/an. Le *Flemingia* couvre aussi bien le sol et se régénère facilement après la coupe mais son installation par le semis présente des difficultés. Sa production moyenne annuelle en matière végétale est évaluée à 10- 15 t/ha. A défaut de paillis et dans les zones à déficit hydrique modéré, la couverture par *Desmodium entortum* donne des résultats acceptables.

Les matériaux les plus communs pour le paillage des caféières au Rwanda sont les feuilles et stipes de bananier, les produits de coupe de haies anti-érosives, le *Tripsacum*, le *Pennisetum*, les chaumes de maïs et de sorgho, les fanes de haricot, l'*Eragrostis*, les *Papyrus* des marais et *Themeda triandra*. *T. triandra* avec sa production moyenne annuelle (en 2 coupes) de 36 t/ha de matière verte peut aisément couvrir les exportation d'une tonne de café marchand/ha (Pasteels, 1979). L'analyse chimique de cette graminée a montré que 33t de sa matière verte apportent 53 kg N, 3 kg P et 78 kg K qui peuvent compenser les exportations par 1t de café marchand/ha. Ces exportations par le café sont de l'ordre de 39 kg N, 5 kg P et 37 kg K.

Le paillis est généralement un mélange de plusieurs matériaux et donc sa composition chimique est variable. Selon ISAR (1985), 15 à 20 t de paillis secs apportent 120 kg N, 8kg P, 107 kg K, 18 kg CaO et 30 kg MgO. En général, la teneur en matière sèche du paillis varie entre 10 et 15% mais peut atteindre 20 à 25% suivant l'âge des végétaux et la saison. L'azote varie entre 1 et 3%, le potassium entre 0.5 à 2% tandis que le phosphore et le calcium sont très faibles. Selon Mitchell (1968), l'apport en potassium peut être important avec certains types de paillis jusqu'à atteindre des valeurs de l'ordre de 580 kg K/ha, ce qui peut provoquer une déficience relative de magnésium et influencer la qualité du café. Toutes ces considérations montrent que la quantité de 20 kg de matière sèche/caféier/an soit 40 t/ha prescrite pour un paillage adéquat est bien suffisante.

L'époque idéale au Rwanda pour pailler le caféier en vue de lui permettre de bénéficier de l'économie d'eau du sol se situe aux mois de décembre et de mai précédents la petite et la grande saison sèche. Malheureusement, le paillage est fait tard en juillet et août. Ce retard est expliqué par le peu de disponibilité du matériel de paillage, les caféières éloignées de la source de paillis, la préférence des caféiculteurs à transporter le paillis sec, le souci des paysans d'éviter la cueillette précoce des cultures pour ne pas perdre des récoltes et la disponibilité du matériel de paillage seulement en saison sèche dans la plupart des régions à café (chaumes de sorgho).

Le caféiculteur rwandais est particulièrement confronté au problème d'insuffisance du paillage. Les raisons directes ou indirectes de cette situation sont:
- L'insuffisance des terres où installer les plantes à paillis;
- La diffusion inadéquate des plantes à paillis;
- La méconnaissance des ressources réelles du paillis (quantité, type de paillis, répartition des parcelles de production);
- L'utilisation variée des matériaux de paillage (fertilisation des cultures vivrières, matériel combustible, nourriture des animaux);
- L'effort considérable demandé au caféiculteur pour réaliser un paillage adéquat.

Quelques solutions alternatives au paillage pourraient être les suivantes:
- L'utilisation d'un film de plastic noir qui peut assurer une excellente conservation de l'eau dans le sol durant la saison sèche. Ce plastic coûte cependant relativement cher au paysan rwandais;
- L'utilisation des plantes intercalaires de couverture (*Desmodium, Flemingia, Leucaena* etc.) dont les produits de fauche avant la saison sèche sont utilisés comme paillis. Ces plantes de couverture ne remplacent cependant pas le paillis mais servent à en réduire la quantité nécessaire à apporter de l'extérieur.

4.3 **Ombrage dans les caféières**

Il a été rapporté que les arbres d'ombrage tels qu'*Albizzia stipulata, Leucaena leucocephala* et *Erythrina abyssinica* jouaient dans certains pays un rôle bénéfique dans la thermorégulation et l'enrichissement du sol en éléments nutritifs et n'exerce qu'une faible concurrence hydrique envers les caféiers. Cependant, les résultats d'un essai d'ombrage planté à Rubona en 1936 avec diverses plantes (bananiers, *Grevillea robusta, Albizzia stipulata, Cassia spectabilis* et *Gliricidia maculata* (Snoeck, 1959)) ont abouti aux recommandations que l'ombrage dans les caféières soit déconseillée au Rwanda à partir de 1943-1944 (Planard et Paquay, 1961). L'ombrage diminuait la production en général et seul l'ombrage d'*Albizzia* pouvait être toléré. Les bananiers diminuaient de 17% les rendements par rapport au non-ombrages. Même plantés comme source d'ombrage temporaire, ils épuisaient le sol et pompaient 2 fois autant d'eau et d'éléments minéraux que les caféiers. L'idéal était donc de planter sans plants d'ombrage pour autant qu'il s'agisse de variétés de café résistantes à la brûlure et que la protection du sol soit assurée par le paillis (Snoeck, 1959).

4.4 **Tailles du caféier**

4.4.1 **Taille de formation**

Les résultats d'essais effectués à Rubona ont montré la supériorité de la multicaulée par rapport à l'unicaulée (conduite en tige unique) au point de vue de la production et de la simplicité technique de la méthode. Cela a conduit à la généralisation du système de taille en tiges multiples chez les caféiculteurs. Il faut cependant noter que la supériorité de la multicaulée n'apparaissait pas au cours des premières années de production. D'après les résultats d'essais plantés à Rubona en 1930, ce n'est qu'à partir de la 12ème année que les rendements des caféiers en multicaulée ont commencé à dépasser ceux des caféiers à une tige. En effet, la taille de formation en tiges multiples retardait l'entrée en production. Ce retard persistait d'autant plus longtemps que le recepage était pratiqué de manière brutale (Snoeck 1959). Un recepage progressif qui maintenait la tire-sève pendant deux ans, permettait aux caféiers multicaules de rattraper le rendement des monocaules avant la 12ème année. Les résultats d'un essai planté à Rubona en 1936 montrent que la perte de production due à la taille multicaule est compensée entre la 10ème et 15ème année de production. Après 15 ans de récoltes, la production totale tend à s'équilibrer.

Coste (1955) a mentioné l'inconsistance des résultats obtenus dans deux pays (Kenya, Tanzanie) et a conclu qu'à égalité de rendements, les frais étaient sensiblement moins élevés pour la conduite en multicaulée. La conduite en tiges multiples est plus simple et exige un minimum de connaissances techniques de la part du planteur.

La conduite en tiges multiples exige une taille de formation précoce qui se fait de la manière suivante:
Dix mois après la plantation, les jeunes caféiers sont arqués afin de provoquer le départ des gourmands à la base du tronc. Quatre à cinq rejets sont seulement conservés. Lorsque les rejets atteignent 50cm de hauteur, 3 meilleurs sont conservés tandis que deux autres et la tige-mère sont supprimés.

La conduite en tiges multiples par l'étêtage en champ déprime le rendements du café et ne produit que 89 à 91% de la production moyenne de l'agobiada après 15 ans (Snoeck, 1959). L'agobiata semble la plus pratique pour la conduite en tiges multiples. Le retard à l'entrée en production par la taille de formation multicaule ont ces dernières années conduit certains caféiculteurs rwandais à revenir sur la conduite en tige unique. Cette situation a poussé la recherche à tester de nouveau les techniques de taille de formation. La taille de formation à l'arcure (fausse agobiada) a été comparée depuis 1982 à une plantation de 3 plants par trou et la plantation inclinée à 30° laissant croître 2 rejets et la tige-mère. Les premières observations (tableau 5) ont montré que le trois méthodes s'équivalent. Le désavantage d'utiliser plus de plants à l'installation était contrebalancé par le rejetonnage plus rapide et l'absence de perte de production dans le temps pour la méthode de plantation inclinée laissant 3 tiges.

Tableau 5. Production de café parche avec trois types de taille de formation à Rubona depuis 1982 à 1989

Méthodes de taille	Rendement moyen kg/ha) de 7 récoltes	% témoin J2	Caturra (Cat)
J2 Agobiada	1215	100	-
J2 incliné	1335	110	-
J2 3 plants/trou	1343	110	-
Cat 140 Agobiada	1641	135	100
Cat 140 incliné	1770	146	108
Cat 140 3 plants/trou	1723	142	103

4.4.2 **Taille de régénération**

Une période favorable au recépage se situe à la 4ème ou 5ème année après la récolte maximale. Cette récolte est en effet suivie par une très faible production à la 7ème année du cycle de production. La présence d'une bonne floraison sur des vieilles tiges tente le planteur pour différer la régénération de sa caféière. Pour cela, des caféières de 8 ans et même de 10 ans sans régénération se rencontrent à travers tout le pays.

4.5 **Fertilisation**

4.5.1 **Fertilisation du germoir et pépinière**

Une terre arglo-sabloneuse est la plus indiquée pour le germoir. L'incorporation du fumier a sur le pouvoir germinatif du café, un effet dépressif dû au développement des microorganismes parasites des semences (Snoeck, 1959). L'engrais minéral à dose unique de l'ordre de 5 g d'un mélange contenant 2/3 d'azote et 1/3 de potassium appliquées en pépinière au pied du caféier âgé de 9 mois a un effet bénéfique (ISAR, 1960).

4.5.2. **Fertilisation en champ des caféiers**

La fertilisation des cultures doit tenir compte du type de sol (Rutunga et Nsengimana, 1990). Pour les sols fertiles, la fumure n'augmente pas les rendements et l'azote peut être néfaste. Pour les sols moyennement fertiles, la fertilisation organique ou minérale, ou organo-minérale est indiquée et augmente sensiblement le rendement. En sols peu ou pas fertiles, la fumure minérale seule est peu indiquée: il est plutôt préférable de tester la fumure organique et organo minérale. Toutes ces observations sont à tenir en considération lors de l'examen des résultats des essais de fertilisation menés sur le caféier au Rwanda depuis 1955 jusqu'en 1993.

4.5.2.1 **Essais de fertilisation des caféiers en stations de recherche**

De 1957 à 1967, des observations sur la production de jeunes caféiers ont été effectuées à Rubona dans un essai factoriel N,P,K à 3 niveaux. Plantée à la densité de 1600 caféiers/ha et conduite sans apport de paillis, la variété Mokka a montré un effet hautement significatif et linéaire de l'azote, un effet bénéfique du potassium, et un effet non significatif du phosphore. La meilleure combinaison était constituée par une formule nitro-potassique 100kg N /ha et 120kg K/ha. Elle augmentait la production de 71% par rapport au témoin général de l'essai (0-0-0) qui a produit 585 kg. La fumure nitro-potassique et l'azote ont induit un effet bénéfique notoire. La production de café est d'autant plus élevée que la dose d'azote appliquée est plus forte (tableau 6).

Tableau 6. Rendement moyen de café parche pendant 9 ans dans un essai factoriel NPK à Rubona*

Traitements	kg/ha/an	kg/ha/an de café parche	% par rapport aux doses 0
NO	0	608	100
N1	50	732	120
N2	100	804	132
PO	0	704	100
P1	35	713	101
P2	70	726	103
KO	0	670	100
K1	60	696	103
K2	120	776	115
Témoin géneral	0:0:0	585	100
N + K	100 + 120	1005	171

Source: ISAR, Rapport Annuel 1967.

L'effet de N et de K fut ensuite étudié de façon approfondie, mais cette fois en présence de paillis permanent, sur la lignée élite Jackson 2/1257 plantée à la densité de 2000 caféiers/ha. Les deux éléments furent combinés dans un essai factoriel NK à 4 niveaux équidistants. L'azote était apporté sous forme de sulfate d'ammonium (0-126-252-378 kg N/ha/an) et le potassium sous forme de sulfate de potassium (0-96-192-288 kg K20/ha/an).

Les observations réalisées sur 7 années de récolte (1968 à 1974) ont montré un effet hautement significatif de l'azote, un effet significatif du potassium et une intéraction non significative entre N et K. L'effet de la fumure s'est réellement manifesté à partir de la 3ème année de production. Cette observation pourrait être expliquée par le fait que la fumure agit indirectement sur les rendements à travers une meilleure activité végétative. Cette hypothèse a été étayée par les corrélations positives hautement significatives qui ont été établies dans plusieurs essais à Rubona, entre le poids des récoltes et le poids des produits de taille (ISAR, 1961).

L'azote est donc l'élément fondamental dans la fertilisation du caféier arabica; le potassium produit un effet bénéfique lorsque la caféière n'est pas paillée et un effet nul en présence du paillis; le phosphore n'avait guère d'effet. Ces constatations sont à tenir avec réserve eu egard aux caractéristiques pédologiques rencontrées à Rubona où le sol sous essai est un Ferralsol humifère légèrement acide et bien saturé (pH H_2O: 5,7 à 6,3 et taux de saturation en base: 68%).

Des essais de vérification ont été ensuite conduits à Rubona. Quatre niveaux d'azote

uréique (0, 150, 300, 450 kg d'urée 46%/ha/an) appliqués sur une jeune plantation de J2 et de manière fractionnée (trois fractions:une en octobre, une autre en février et une 3ème en mai) à Rubona ont montré que les 3 niveaux d'N étaient équivalents mais différaient significativement du témoin sans engrais (ISAR, 1991). La dose de 150 kg/ha/an était ainsi la plus bénéfique.

Puisque les sols du Rwanda sont à majorité acides, l'azote sous forme d'urée et de sulfate d'ammonium (acidifiant) a été testé à Rubona à 2 niveaux (100 et 150 g urée, 210 et 315 g NH4S04 par arbre) et en 2 application (mars et novembre). Les résultats de 6 ans de récoltes ont mis en évidence que 150 g d'urée ont augmenté la production moyenne du café de 46% de plus par rapport aux autres niveaux. L'urée peut donc facilement être utilisée dans la fertilisation du caféier. Les résultats (7 années d'observation) d'un essai de dose fractionnée d'un engrais nitro-potassique à Rubona ont montré une différence significative et un avantage en faveur du fractionnement, ce qui justifie la recommandation de 2 application/an.

Récemment, l'ISAR a testé si la restitution à la caféière des pulpes de café sous forme de compost pouvait être bénéfique. Le résultat est que l'application de 2 kg/caféier/an d'un compost vieux de 2 ans bien décomposé était susceptible d'augmenter la production de l'ordre de 13% (ISAR, 1993).

4.5.2.3. **Essais de fertilisation en champs des paysans**

Depuis 1958, de nombreux essais ont été conduits en champs de caféiculteurs et étaient répartis en trois grandes phases: 1958-1961; 1962-1966; 1967-1993. Ces essais avaient le mérite d'étudier la réaction des caféiers à la fumure dans les conditions pédologiques et écologiques très diversifiées et souvent plus réalistes que les conditions optimales rencontrées en stations de recherche. Les paramètres observés étaient: développement des caféiers, vigueur de croissance de l'arbre et sa resistance à la défoliation en saison sèche, maturation des cérises, caractéristiques technologiques du café (rapports café parche/café cerise, café marchand/café parche, dimension des fruits, granulométrie des fèves) ainsi que les maladies. Les résultats obtenus ont fait l'objet de plusieurs publications dont les conclusions principales sont résumées ci-dessous.

La première phase (1958-1961) a permis de déterminer les zones caféicoles avec réponse économique à la fumure minérale et celles avec réponse non économique à la fumure minérale (De Vuyst et Paquay, 1964).

La deuxième phase (1961-1966) devait préciser les formules et les doses de fumures à appliquer et approfondir l'étude de la fumure minérale dans les zones où subsistaient des problèmes importants tels que carences minérales, doses économiques, réponses nulles ou insuffisantes à l'engrais minérale. Il est apparu que l'efficacité de la formule d'engrais minéral (NPKMg 10:10:20:5 ou 20:10:10:5 et/ou sans 1g de chaux/caféier tous les trois ans) à la dose de 500g/arbre/an était fonction des zones caféicoles et des types de sols. Les augmentations de rendement moyen en café parche/caféier/an dues à l'apport de la fumure variaient de 16 à 100% (De Vuyst, 1968).

La 3ème phase était constituée par des essais-ISAR en préfectures Butare, Gitarama et Kibungo.

Dans tous les terroirs où les essais furent installés, l'emploi de la fumure a favorisé le développement des caféiers qui était caractérisé par plus de feuillages, une coloration plus foncée, une faible défoliation en saison sèche et par une croissance plus vigoureuse de l'arbre. La maturation des cérises était légèrement retardée. Les caractéristiques technologiques du café n'étaient pas influencées. Aucun effet de l'engrais sur l'anthracnose (Colletotrichum coffeanum) n'a été observé. Par contre, l'application de l'engrais a réduit l'intensité de l'attaque de la rouille (Hemileia vastatrix) et du shedding des fruits.

Les sols dérivés des granites présentent la meilleure réaction à la fumure, suivent ensuite ceux dérivés des schistes et des quartzites et enfin ceux issus de roches basiques. Il a été également noté que ce sont les sols où les parcelles témoin des caféiers donnent des rendements relativement élevés qui répondent le mieux, en valeur absolue, à l'apport de l'engrais minéral.

L'effet du potassium s'est révélé beaucoup plus marqué et a dépassé même dans certains cas celui de l'azote. La carence magnésienne (visible aux mois d'avril-mai) affectait surtout les régions de Kibuye, Cyangugu et de la boutonnière granitique. Ces signes de déficience apparaissaient dès que la teneur en magnésium dans la couche 0 à 20 cm du sol était inférieure à 1,5 meq/100 g de sol (De Vuyst et Paquay, 1964). La carence magnésienne pouvait être corrigée par l'apport annuel de 100 g de kiesérite par arbre ou par la formule contenant du magnésium (De Vuyst et Brion, 1968). Pour cela, des formules de NPKMg 10:10:20:5 et NPKMg 20:10:10:5 ont été recommandées selon les zones caféicoles (De vuyst, 1968).

Des carences en manganèse ont été observées sur les sols dérivés de cendrées volcaniques récentes du Nord du Rwanda. Ces sols sont caractérisés par une forte teneur en allophanes, un pH légérement acide (6 à 6,5) et une forte capacité d'échange cationique. Ces conditions favorisent l'immobilisation du manganèse. Une pulvérisation de sulfate de manganèse en solution de 0,2% sur la surface foliaire faisait disparaître la carence manganique pour une période de deux à trois mois (Planard et Paquay, 1961).

L'application d'une fumure NPK 20:10:10 à raison de 400 g/arbre/an a provoqué, au cours de 7 ans, une augmentation de production significative de café de 61% et de 30% respectivement autour de Rubona et au Sud Mayaga, en préfecture Butare (ISAR,1982). Une telle fertilisation s'est révélée rentable.

Les résultats obtenus en zone Kibungo dans une plantation à densité de 2000 caféiers/ha (tableau 7) ont montré que l'engrais minéral à la dose de 39kg N, 8kg P et 17kg K accroît la production du caféier avec une rentabilité encore satisfaisante pour des prix de 33 FRw/kg d'engrais NPK ou urée et de 73.3 FRw/kg café parche respectivement.

Tableau 7. Production moyenne de café et bénéfice réalisé dans un essai de fertilisation à Kibungo (densité 2000 plants/ha)

Fumure/caféier	Fumure/ha	Rdt Café kg/arbre	%	Coût de l'engrais(FRw/ha)	Supplément de marge brut/ha	RV/C
Témoin	-	0.833	100	-	-	-
60g NPK 17-17-17 + 20g urée	160kg	0.913	109.6	5280	12320	3.3
120g NPK 17-17-17 + 40g urée	320kg	1.098	131.9	10560	47140	4.5

5 Conclusion générale

La caféiculture au Rwanda se pratique en conditions de pluviométrie insuffisante et sans ombrage, sur sols à fertilité fort variable. Les techniques d'installation et le régime de taille des caféiers ont été mis au point mais ne sont pas totalement appliqués par les caféiculteurs. Le paillage est la pratique la plus appropriée pour la bonne production de café arabica. Le paillis est malheuresement difficile à trouver en quantité adéquate au Rwanda. La couverture vivante de même que les arbres d'ombrage n'ont pas donnés des résultats satisfaisants. L'engrais minéral

et le compost de pulpes de café peuvent améliorer la production du café, particulièrement dans les caféières bien paillées. L'azote et le potassium parraissent de très loin les éléments les plus importants surtout si la caféière n'est pas paillée ou est paillée insuffisamment. Les fumures N.P.K. et N.P.K.Mg à dominance d'azote et de potassium sont donc recommendées, la dose requise étant fonction des types de sols.

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Summary

Arabica coffee plays an important role in the macro-economic performance of Rwanda because it is one between the most important cash crops. As a result, INEAC and ISAR have done agronomy research aimed at improving its productivity. The ecological conditions for coffee in Rwanda are characterized by low rainfall and high light intensity. The high light intensity increases coffee evapotranspiration and die-back spread. Soil parental material and chemical properties are variable amongst different small holders coffee producers.

The available results indicated that spacing in coffee planting of 2m x 2m or 2.5m x 1.5m are convenient. Higher density with 'multicaule' planting provided better production with some clones. Pruning regime must be correctly followed. Mulching is the best cultural technique to improve production. The mulching materials are variable and inadequate. They are applied late (June, July). Living cover with different species including the legumes cannot replace efficiently the mulch, although *Desmodium entortum* cover offers some advantages. Black plastic film can be used as a cover but it is too costly to be economic. Shade trees in coffee plantation have not been successful. High density planting and mulching reduce weed spread. Mulch also improves soil physical and chemical properties and water storage. It improved mineral fertilizer use efficiency. In Rwanda, N fertilizer followed by K (especially when mulching is not appropriate) improved coffee production. The effect of P was not clear while Magnesium deficiency was observed in some plantations. The formula of N.P.K. and N.P.K.Mg. can be recommended and their rates should vary according to the sites. Finally, a beneficial effect was observed in using coffee rinds compost in coffee.

GENETIC DIVERSITY AMONG ISOLATES OF *COLLETOTRICHUM KAHAWAE* CAUSING COFFEE BERRY DISEASE

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1.0 Introduction

Coffee berry disease (CBD) caused by *C. kahawae* is a serious anthracnose of green and ripening berries. The disease is so far confined to the African continent and is specific to *Coffea arabica*. A breeding programme aimed at developing *C. arabica* varieties combining resistance to major coffee diseases with improved yield and quality led to the release of cultivar Ruiru 11 in 1985. Pathogenicity tests using isolates from Ethiopian (van der Graaf, 1978) and Kenyan (Masaba and Van der Vossen, 1980) only revealed variation in aggressiveness (variety non-specific) but not in virulence (variety specific). Isolates of *C. kahawae* were also found to possess identical morphological characteristics (Masaba *et al.*, 1982 and Waller *et al.*, 1993).

In another comparative study with isolates obtained from different countries, Rodrigues Jr *et al.* (1991) found that the Kenyan strains had characteristics different from the Angolan and Malawian strains. These isolates were found to be highly aggressive on Catimor which is used in Kenya as a female parent in the production of the CBD resistant hybrid Ruiru 11. Rodrigues Jr *et al.* (1992) concluded on the basis of pathogenicity tests that physiological forms of the CBD pathogen might exist among the Angolan, Malawian and Kenyan isolates. Characterisation of *C. kahawae* isolates based substrate utilization has also led to different conclusions. Waller *et al.* (1993) observed homogeneity among *C. kahawae* isolates in their failure to utilize citrate or tartrate as a main carbon source. However, Beynon *et al.* (1995) distinguished at least five (5) Vegetative Compatibility Groups (VCGs) in the nitrogen assimilation pathway. Molecular analysis using RAPDs or Restriction Fragment Length Polymorphism (RFLP) (Sreenivasaprasad *et al.* 1993 and Beynon *et al.* 1995) detected no polymorphism among isolates of *C. kahawae*. In this study, an integrated analysis using, pathogenicity tests, protein, isozymes and RAPD markers were used to evaluate the level of genetic diversity among isolates of *C. kahawae*.

2.0 **Materials and Methods**

Green infected berries were obtained from 10 locations across the range of coffee growing Districts in Kenya. Nine berries were sampled from each location. Pure cultures from each berry were obtained by inoculating Potato Dextrose Agar (PDA) and subculturing from regions where culture characteristics corresponded with those documented for *C. kahawae*. Monoconidial isolates were derived from pure culture by serial dilution of conidial suspension and stored in replicates on PDA at 4°C until required.

Eleven coffee varieties consisting of Rume Sudan, Pretoria, Hibrido de Timor, K7, Padang, SL 28, SL 34, Caturra, Erecta, Mokka and Laurina were inoculated with the 90 isolates of *C. kahawae.* Based on pathogenicity, isolates with varying degrees of infection were selected for protein, isozymes and RAPD analysis.

Proteins were extracted from mats cultured in a nutrient broth described by Maas *et al* (1990). Electrophoresis was run with a constant current of 1000 v and 60 mA for two gels or 1000 v and 30 mA for a single gel using an LKB 2197 power supply. The protein bands were made visible by using the silver staining method of Blum *et al.* (1987).

A 10% polyacrylamide gel without SDS was used to assay 13 enzyme systems including esterase (EST, EC 3.1.1.2), Malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphoglucose dehydrogenase (6 PDH, EC 1.1.1.44), Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49), Isocitric dehydrogenase (IDH, EC 1.1.1.42), Lactate dehydrogenase (LDH, EC 1.1.1.27), Malic enzyme (ME, EC 1.1.1.40), Adenylate kinase (AK, EC 2.7.4.3), Hexokinase (HEX, EC 2.7.1.1) Phosphoglucose isomerase (PGI, EC 5.3.1.9), Mannose-6-phosphate isomerase (M6PI, E C 5.3.1.8), Fructose-1, 6-diphosphate (F1,6DP, EC 3.1.3.11) and Hexose phosphate isomerase (PHI, EC 5.3.1.9). Enzymes were extracted according to the method of Cole *et al.* (1991). Electrophoresis was run as described for proteins. Enzyme staining methodologies of Oudemans and Coffey (1991) were used.

DNA was extracted according to the procedure of Moller *et al.* (1992). The polymerase chain reaction (PCR) was conducted in a programmable thermocycler (MJ Research, Inc. Watertown MA, model PTC-100-96). The PCR products were separated on a 1.5% agarose gel in TAE, stained with ethidium bromide and photographed under UV (260 nm).

3.0 **Results**

Variation analysis revealed significant effect of isolates, varieties and isolate x variety interaction at P=0.01 (Table 1). Of the total variation, isolate effects accounted for 14.89%, varieties 49.83% and isolate x variety 14.60%. The highly significant interaction effect indicate that differential pathogenicity among the isolates might exist.

Based on protein electrophoresis, two bands of molecular weights 127 kDa and 57 kDa present in *C. acutatum* distinguished the species from *C. kahawae* isolates which lacked the bands. Although polymorphism was also revealed by a protein

band of kDa 111 among isolates of *C kahawae*,it was not suitable as a marker of pathogenicity.

Isolates of *C. kahawae* were distinct from *C. acutatum* and *C. gloeosporioides* in all the 13 enzyme systems assayed. Among the *C. kahawae* isolates, a total of 21 loci were resolved. M6PI and EST yielded 3 and 7 loci respectively. G6PDH, IDH, LDH, ME, AK, OGI, F1,6DP, 6PGD and PHI all stained for a single locus each. All enzyme systems were monomorphic with respect to *C. kahawae* isolates except EST. The EST 1 locus was present as a single or double band in all isolates obtained from the resistant host variety. Isolates from susceptible host varieties lacked the locus. Two Benomyl resistant strains obtained from susceptible hosts carried the EST 1 locus but were also found to initiate lesion development on resistant host varieties.

Out of the 48 oligonucleotide primers tested, 45 produced monomorphic DNA patterns while 3 detected polymorphism with respect to *C. kahawae* isolates. RAPD patterns for *C. acutatum* and *C. gloeosporioides* were not only distinct from each other but also from *C. kahawae*. Although genetic variation exists among the *C. kahawae* isolates, the primers tested did not detect any RAPD markers associated with pathogenicity.

Table 1: Analysis of variance for pathogenicity tests

Source	Df	Mean square	% Variation
Isolates	89	28.083***	14.89
Varieties	10	836.306***	49.83
Isol. x Var.	890	2.753***	14.60
Error	1980	1.753	20.68

4.0 **Discussion**

Variation in pathogenicity among isolates of *C. kahawae* is predominantly due to aggressiveness (large main effects). Differential pathogenicity (virulence), although small was highly significant (P=0.01) and therefore cannot be ignored. Variation in virulence is the major cause of resistance breakdown. Some of the mechanisms by which virulent strains develop are spontaneous mutations, sexual recombination and somatic hybridization (Burdon 1993). The clonal nature of reproduction of the CBD pathogen tend to propagate a very uniform pathogen population. The limited variation possibly arose from the rare mutations or somatic hybridization. This could partly be the reason why the proportion of pathogen variants have remained low and sometimes gone undetected.

The existing variation among strains of *C. kahawae* may also be underestimated because of the limited number of resistance genes present in the host. So far, there are only 3 genes known to condition resistance to CBD in *C. arabica* (Van der Vossen and Walyaro 1980). Theoretically, if the genes were operating independently, the maximum number of pathogen races which can be detected using the classical pathogenicity analysis are $2^n=2^3=8$, where n is the number of

loci. In *C.arabica* some varieties such as Rume Sudan and Pretoria carry the resistance genes in duplicate combinations limiting the number of detectable races further.

A powerful tool to detect variation in the pathogen population is by screening biochemical and molecular markers closely linked to pathogenicity. A serious drawback with the CBD pathogen is the inability to generate segregating populations such as F2 or backcrosses necessary for inheritance studies of the pathogenicity markers. However, contrary to the observation that the CBD isolates are largely uniform in culture and therefore could be genetically similar, large genetic differences among the isolates have been detected by protein, isozyme and DNA electrophoresis. These methods also distinguished the non-pathogenic *C. acutatum* and *C. gloeosporioides* from the pathogenic *C. kahawae*. Esterase enzyme produced a marker in the EST 1 locus which could be closely linked to pathogenicity. The failure of the other analyses especially RAPDs to detect pathogenicity markers may be attributed to the fact that the primers used may have amplified sections of the genome which do not control pathogenicity. Screening more primers that have hitherto not been tested may in future detect RAPD markers for pathogenicity.

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Abstract

Monoconidial isolates of *Colletotrichum kahawae* Waller and Bridge sp. nov (1993), the causal agent of Coffee Berry Disease (CBD) were subjected to genetic analysis using pathogenicity tests, protein, isozyme and Random Amplified Polymorphic DNA (RAPD) markers. Two non-pathogenic species *C. acutatum* and *C. gloeosporioides* commonly found colonising the coffee plant were included in the analysis for comparison. Pathogenicity analysis revealed significant variations (P=0.01) among isolates for aggressiveness (main effects) and virulence (interaction effects).

Buffer soluble proteins, separated on a 10% polyacrylamide gel and stained with silver nitrate not only revealed genetic differences between pathogenic and non-pathogenic species (for proteins with molecular weights of 57 KD and 127 KD) but also among the pathogenic isolates (for proteins of 111 KD). A total of 13 enzyme systems were also assayed and 21 loci were resolved. All enzymes systems distinguished *C. kahawae* from *C. acutatum* and *C. gloeosporioides* but were all monomorphic with respect to *C. kahawae* isolates except esterase. Of special interest was the observation that isolates obtained from resistant host variety Ruiru 11 had the EST 1 locus in a homo- or heterozygous state while isolates obtained from susceptible host varieties such as SL 28 and SL 34 lacked the locus. Benomyl resistant strains produced an overlapping presence of the EST 1 locus although they were obtained from susceptible hosts. Polymorphism was further detected between the pathogenic and non-pathogenic species by all the 48 decamer oligonucleotide primers screened for RAPD markers. However, among the pathogenic *C. kahawae* isolates, only 3 primers detected polymorphism

THE IMPORTANCE OF CHAMELEONS AND PRAYING MANTIDS AS BIOLOGICAL CONTROL AGENTS AGAINST SOME INSECT PESTS OF ECONOMIC SIGNIFICANCE ON COFFEE

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1. Introduction

It is only in the last few decades that the effect of pesticides to man and to the environment was realised. Before the publication of "The Silent Spring" in 1962, the application of chemicals was regarded as a solution to all pest problems in agriculture. Since then, the world became aware of the dangers that can occur to man and to the environment as a result of using these toxic chemicals when combating diseases and arthropods of economic importance in agriculture. So effort has been exerted all over the world in doing all types of research aimed at minimising the use of chemicals in agriculture, human and animal health.

2. The state and initial efforts to combat coffee insect pests in Tanzania

When coffee was introduced in Tanzania in 1890s, various species of insect pests were found to be of economic importance to the crop. Some 850 species of insects are recorded to attack coffee (Le Pelley, 1973), but in Tanzania, only a few of them are troublesome and are of economic importance.

In an effort to combat these harmful insects, various control measures were introduced: among these was the introduction to coffee of various groups and formulations of insecticides. As these chemicals were handled by farmers who were mostly poor and illiterate, in most cases, these toxic chemicals could not be used properly.
As a result, the problem of pesticide poisoning to people who were applying them, the problem of environmental pollution, the development of pest resistance to these chemicals, the upsurge of secondary pests and the destruction of non-target organisms became a major concern whenever these chemicals were applied.

3. Integrated Pest Management endeavours to reduce pest incidence on coffee

There are many species of parasites that have been reported in the literature to be of importance in reducing populations of insect pests of coffee. Crowe (1970), gave an annotated list of over 37 species of parasites of coffee leaf miner which have been found in East Africa.

Several families of parasites and parasitoids have also been recorded to be of importance in reducing long-horn beetles (*cerambycidae*), antestiopsis, coffee berry borer (*Stephanoderes hampei*), Scale insects (*Coccus spp.*) and mealybugs (*Planoccocus kenyae* (Le Pelley, 1968).

Some cultural practices have also been found to be of considerable importance in reducing insect pest incidence on coffee. It has been observed that the two species of *Leucoptera* in East Africa have different requirements: *Coffeina* thrives in cool - shaded conditions while *L. meyricki* in warm, dryer ones and occurs commonly on unshaded coffee (Kirkpatrick, 1935). In Bukoba (Tanzania, the infestation of coffee berry borer (*Hypothenemus hampei*) on robusta coffee was found to be heaviest under conditions of extreme humidity (Jervis, 1939).

Manipulation of the crop can also decrease pest incidence. *Antestiopsis* prefers dense tangled bushes, so pruning to open out the centre of the bush decreases populations (Bardner, 1985).

The condition that favour *H. hampei* are limited by altitude. This beetle is more common in low altitude coffee. In a survey carried out in Jamaica in 1979 (Rhodes and Mansing, 1986), infestation levels by the borer were generally low in plantations above an altitude of 1000 m a.s.l. The level of damage (national average) of 9.5% of perforated coffee berries was recorded. In Uganda (Hargreaves, 1935), it was found to be an important pest of arabica coffee up to 1250 m a.s.l., but at 1500 m a.s.l. it was rare.

The two species of green scale of coffee in East Africa (Haarar, 1962) appear to occur at different altitudes. From sea level to approximately 3000 ft, *Coccus viridis* (Green) is the one that is troublesome. Above 3000 ft, the species in Kenya is said to be *Coccus alpinus,* and *alpinus* is also the one that is common in Tanzania.

3.1 IPM approaches at Lyamungu Coffee Research Station

In an effort to minimise the use of chemicals while controlling insect pest in Tanzania, research efforts at Lyamungu Coffee Research Station were diverted at finding possible Integrated Pest Management methods that would be useful. Among these were supplementary investigations on beneficial cultural practices and further enquiry on the importance of the available biological control agents.

3.1.1 Some cultural practices

Investigations on the effect of some cultural practices in regulating insect populations on coffee was made. From trials that were conducted at Lyamungu recently, it was clearly shown that there were more parasites of coffee leaf miners from shaded plots than from the unshaded ones. The use of over-head irrigation was also found to minimise L/miner moth populations (Mcharo, 1995).

3.1.2 Parasite studies

Some studies were done to find leaf miner parasites that are found in Northern Tanzania coffee-growing areas. Some 18 species of parasites of coffee leaf miner were recorded from plantations at Arusha and Kilimanjaro (Mcharo, 1994 and 1995).

4.Investigations on the predators of importance in diminishing coffee insect pests

Apart from lady birds which have been reported to feed on scale insects mealybugs and aphids, the use of predators in the management of arthropods of economic importance on coffee has not been done anywhere. There is no information found in the literature where predacious organisms have been reported to be of importance in reducing populations of insect pests of the crop.

4.1 Observations with chameleons

Field observations at Lyamungu had shown that chameleons feed on antestia. So these reptiles were caged in the green house and several insect orders were introduced in the cages to find out what types of insects they preferred most.

Observations showed that chameleons eat many types of insects, but they seem not to feed on coleoptera. Although they feed on *antestiopsis* (a pentatomid of importance on coffee), they do not prefer them most. They generally favour softer insects like lepidoptera moths, diptera and grasshoppers. The prey has to be of the right size, as smaller chameleons can not manage to catch and feed on too large insects. Normally young ones subsist on fruit flies and other small insects.

4.2 Observations with praying mantids

Praying mantids were observed in the field feeding on antestia. Some observations were also done to monitor their feeding habit.

Initial observations showed that praying mantids eat all types of insects and the females seemed to eat more than the males. After copulation, the female devours the male and smaller mantids were victims of the larger ones.

Later, a male and female praying mantid were put in separate cages where antestia only and a mixture of antestia and grasshoppers were introduced in cages and the number of insects that were eaten were recorded at 24, 48 and 72 hours after they were introduced. Table 1 below shows the results that were obtained.

Table 1: Preliminary observation on the feeding habits and host preference of the praying mantid.

				Total insects consumed within			
Cage no.	**Mantid**	**Type of Food**	**No. of Insects**	**24 h**	**48h**	**72h**	**Total**
1	Female	Antestia	20	3	7	0	10
2	Female	Antestia	20	5	13	0	18
		Grasshoppers	5	0	5	0	5
3	Male	Antestia	20	2	8	1	11
4	Male	Antestia	20	0	3	3	6
		Grasshoppers	5	0	1	0	1
5	Female	Grasshoppers	15	11	1	1	13
6	Male	Grasshoppers	15	2	8	1	11

NB : One adult mantid/cage

The observation recorded on table 1 support earlier assumption that female mantids eat more than the males. In cage number 2 for example, a single female managed to eat 90% of all the antestia and 100% of grasshoppers while the male in cage No. 4 ate only 30% of antestia and 20% of grasshoppers. A similar trend is observed on cage No. 5 and No. 6.

5. The rational way of utilising chameleons and praying mantids in the management of coffee insect pests

5.1 Chameleons

Most Africans fear chameleon and some even find them more repugnant than snakes. This phobia is normally a result of stories and mythologies that the public receive from the tribal societies. When children see their parents running away from chameleons, they grow up believing that they are truly harmful; so it continues to be an obsession of the community, the tribe and the whole society. Chameleons are harmless reptiles, but the problem is that, due to this phobia that farmers have on them, they kill them without any good reason.

Another advantage of this reptile is its spectacular and unique camouflaging ability. Its amazing searching dexterity and its long tongue that can pick its prey from quite a long distances, is indeed one of the wonders of the world.

When a predator is so well blended to its environment, it can easily catch its prey without much effort because its victim can not easily notice its existence.
Since they feed on many types of insects, chameleons seem to be important in reducing many arthropods of economic importance on coffee. They can minimise in the coffee canopy antestiopsis, fruit flies, berry moths, moths of stinging caterpillars and of giant loopers.

5.2 Praying mantids

The capacity of praying mantids to control antestia and other arthropod species as explained and results shown on table 1, gives confidence that this insect is a useful biological control agent.

The population of *Antestiopsis* that can be tolerated is generally taken as two per tree. If in 3 days, a single female praying mantid can devour 18 antestia and 5 grasshoppers (Table 1), assuming they have nothing else on coffee to feed on except antestia and grasshoppers, the population of about 50 praying mantids per hectare would be enough to reduce antestia populations to levels below economic thresholds.

As reported earlier, praying mantids feed also on other insects; so they can reduce the population on coffee of berry moths, moths of stinging caterpillars and of giant loopers.

This bug has also other parasites that are reported to be useful biological control agents (Greathead & Waage, 1983). If these are augmented with praying mantids, it is most likely that coffee can be made free of *antestiopsis* without much reliance on insecticides.

6. Implications of using predacious organisms in Crop Protection.

For a long time, some farmers at Arusha plantations claim to use very little insecticide applications on coffee without having much insect problems. The reason for this phenomena has not been thoroughly investigated. It is quite likely that there may be many beneficial organisms in the crop environment that have not been detected. Perhaps chameleons and praying mantids are only a few of them!

When entomologists discuss the usefulness of a biological control agent, they normally think of how it can be multiplied and eventually distributed to the targeted areas. This is mainly because most of these organisms have been parasites and parasitoids which can easily be bread and multiplied.

I do not think that it is practically possible to breed, multiply and introduce predators to targeted areas. Predators kill their prey outright while parasites normally weaken their hosts first before they kill them. There can also be many parasites in a single host; but this is not the case with predacious organisms. So multiplication and release of predators might not work because one would soon eliminate them also as they would kill their victims faster than would be the case with parasites.
I feel therefore, that the only way of utilising predators in crop protection is by preservation. In this case, it would be by educating farmers that they are harmless and also by applying insecticides only when it is absolutely necessary. So it is important that the natural enemies of coffee pests should be conserved as much as possible. Conservation of natural enemies of pests is an indirect method in that, measures are taken to conserve natural enemies and enhance the numbers of species already present in the crop environment (Greathead and Waage, 1983).

Research in the management of arthropods of economic importance in agriculture is rapidly shifting direction all over the world. Total reliance on insecticides for combating insect pests is increasingly becoming outdated. Consumers of most agricultural commodities are progressively getting interested in organic farming i.e. agricultural products that are free from pesticide residues.

As the world population is increasingly becoming aware of the menace of pesticides to man and to the environment, it is eminent that all research endeavours should be exerted at finding ways that can manage insect pests in agriculture with minimum application of insecticides.

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MORPHOLOGICAL AND PHYSIOLOGICAL DIFFERENCES BETWEEN RESISTANT AND SUSCEPTIBLE STRAINS OF *HYPOTHENEMUS HAMPEI*

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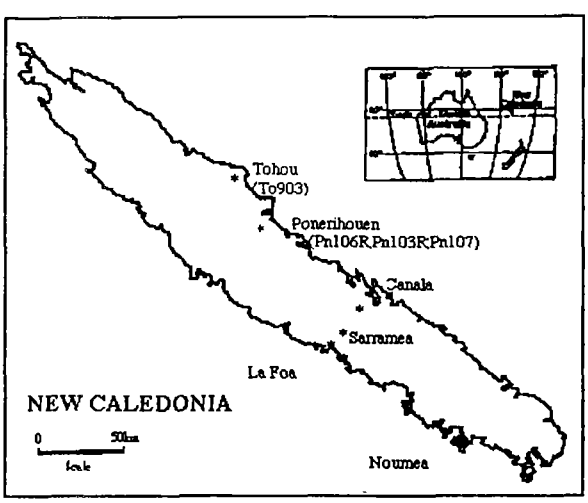
Introduction

The coffee pest *Hypothenemus hampei*, has expanded its geographical range along with the extension of coffee plantations worldwide during the last three centuries. This pest is mainly controlled with endosulfan, a cyclodiene insecticide, but resistance to this compound has recently been documented in New Caledonia (Brun et al. 1989). A similar mutation of independent origin would be a serious threat to the coffee industry.
Therefore, preliminary studies for fitness differences were conducted through morphological and physiological observations for possible significant variation between susceptible and recessive strains.



Materials and methods

Laboratory observations were conducted at ORSTOM, New Caledonia, South Pacific (Fig 1) and at the University of Bergen (Norway).

Origin of *H. hampei* strains :
Six strains were collected from New Caledonia (NC). Three resistant strains (R-To903, R-Pn106, R-Pn103) came from the East coast, as well as the susceptible strain S-Pn107. The other two susceptible strains (S-La Foa and S-Sarramea) were from the West coast (Fig 1). Other strains were collected from Ivory Coast, Mexico, Honduras, Jamaica, Philippines, Indonesia, and Thailand.

Figure 1: Collecting sites for CBB in New Caledonia

Rearing and crosses

All strains were maintained under standardized laboratory conditions (25±2°C). Mated females were placed individually in cells of microtitre plates filled with artificial diet (Brun et al. 1993). Cells were covered by glass microscope slides, and microtitre plates were kept in ziplock bags to avoid dessication. For crosses, female pupae were isolated. Then imagos were paired with males for a week for mating.

Observations

-Body size: A stereo dissecting microscope with measuring ocular was used. Comparisons were made through measurement of lenght and width of 30 females per strain. Five New Caledonian strains and two foreign strains were measured.

- Fecundity at three and six weeks: Progeny of 20-25 parents from the same strains were counted at three and six weeks by dissecting the media.

- Survival rate : As a measure of the capacity to survive during the inter-season period, survival was assessed without providing any food. Females were isolated in groups of four to six into holes made in perspex slides. Mortality check was made at 12h intervals. 12 strains were used, with a sample size of 38-40 females. This experiment was conducted under two different humidity levels: 60(±)5 and 90(±) 5 % R.H.

- Boring activity : Boring activity of the apical part of berries is part of the colonisation process of H. hampei. Thus, it is an important component its life history. Therefore, the ability of females to bore through the microtitre plates was recorded.

Results and discussion

- Body size : Table 1.

Mean values of width and length of resistant individuals were generally greater than for other strains. R-To903 was significantly longer and wider than the susceptible strains. The resistant strains R-Pn103 and R-Pn106 were larger (both in width and length) than the two susceptible strains from La Foa and Ivory Coast. R-Pn106 was also longer than S-Pn107, a susceptible strain collected from an adjacent field.

Table 1. Bodysize of Hypothenemus hampei. s.e.= Standard error. Sample size was 30 in all the experiments.

	Length	**Width**
	Mean (±s.e) in cm.	**Mean(±s.e) in cm.**
Pn103R	1.57 (±0.008)	0.69 (±0.003)
Pn106R	1.58 (±0.006)	0.69 (±0.003)
To903R	1.62 (±0.009)	0.70 (±0.003)
PN107	1.55 (±0.005)	0.68 (±0.003)
La Foa	1.53 (±0.008)	0.67 (±0.002)
Mexico	1.56 (±0.006)	0.68 (±0.003)
Ivory Coast	1.54 (±0.009)	0.66 (±0.004)

- Fecundity at 3 and 6 weeks: Table 2.

There were no clear trend comparing susceptible and resistant strains, even though mean progeny per female was significantly higher for R-To903 at three weeks than for the susceptible strain from Ivory Coast.

Table 2. Number of progeny after 3 and 6 weeks. s.e.= standard error. n=sample size.

	3 weeks		6 weeks	
	Mean (±s.e.) progeny	**n**	**Mean (±s.e.) progeny**	**n**
Pn103R	16.8 (±1.2)	20	16.7 (±0.7)	24
Pn106R	18.5 (±1.4)	20		
To903R	20.3 (±1.0)	20	21.0 (±0.8)	24
PN107	17.6 (±1.6)	9	19.5 (±1.3)	23
La Foa	19.3 (±1.4)	20	17.2 (±0.8)	23
Mexico	21.7 (±1.4)	20	27.0 (±1.2)	23
Ivory Coast	13.9 (±1.1)	20	18.9 (±1.0)	20

- Survival rate : Table 3.

Adult females from R-To903 show a higher survival capacity without food than any of the seven susceptible strains tested, at both relative humidities used (60 or 90%). When adults were fed, no significant differences among strains was found in survival capacity at 60% R.H.

Table 3. Length of survival for *Hypothenemus hampei* without food. s.e.=Standard error. R.H.= Relative humidity. Sample size was 38 in all experiments.

	R.H.=60 (±5)%	R.H.=90(±5)%
	Mean(±s.e.) in days	**Mean(±s.e) in days**
To903R	13.5 (±0.7)	16.0 (±0.8)
La Foa	9.7 (±0.6)	9.6 (±0.7)
Sarramea	6.8 (±0.6)	13.4 (±0.6)
Ivory Coast	6.7 (±0.6)	11.9 (±0.5)
Honduras4	5.1 (±0.3)	8.2 (±0.7)
Honduras5	3.7 (±0.2)	6.3 (±0.6)
Jamaica4	8.8 (±0.6)	11.0 (±0.6)
Jamaica5	6.6 (±0.5)	8.4 (±0.4)
Jamaica7	7.3 (±0.2)	10.2 (±0.4)
Jamaica8	10.3 (±0.4)	12.4 (±0.5)
Mexico	7.6 (±0.3)	9.4 (±0.6)
Philippines	5.9 (±0.4)	12.1 (±0.4)

-Boring activity: Tables 4 & 5.
The two resistant strains tested bored through their rearing containers much more often than any of the susceptible strains.

Table 4. Drilling activity for *Hypothenemus hampei.* n=sample size (number of cells). NC Susceptible strains (NC-S) from Sarramea, Canala and Koumac.

Strain	n	Number boring	% boring
To 903R	59	13	22
Pn103R	120	46	38
NC-S	384	2	<1
Philippines	55	0	0
Thailand	64	6	9
Honduras	127	4	3
Jamaica	447	12	3
Ivory Coast	43	3	7
Sumatra	256	2	<1

Table 5. Comparisons of drilling activity between resistant and susceptible strains. Differences are tested with a G-test (Zar 1996).

Lines tested	G-value	p-value
To903R vs. Pn103R	4.95	< 0.001
To903R vs. NC-S	43.81	<0.001
Pn103R vs. NC-S	132.22	<0.001
To903R vs. all susceptible	35.91	<0.001
Pn103R vs. all susceptible	154.13	<0.001

Conclusion

Since all strains had been maintained for several generations in laboratory conditions on a standard semi-artificial diet, it appears that differences observed were due to genetic variations among strains, both within and among regions.
Larger body size and greater survival under starvation for the resistant strains indicate that some fitness differences may be related to the resistance mutation to cyclodiene, or to strong linkage disequilibrium between the resistant allele and genes contributing to the quantitative traits we have measured. Strong linkage equilibrium arises rapidly in species with regular close inbreeding, such as for *H. hampei* (Gingerich et al. 1996).
Variation observed is an indication of possible fitness-related genetic differences among populations from different origins, presumably in quantitative traits. Due to the economic importance of the resistant mutation conferring resistance to endosulfan, additional studies should be conducted under field conditions. Those data will contribute to better management of resistance.

Statistics :

- Body size : ANOVA 1-way was used to see if there were any differences among strains ($\propto<0.01$), and Tukey-HSD ($\propto<0.05$) to find between which groups the difference were. (Normal distribution fits well with the data with homogeneity in variance for both length and width).
- Fecundity at 3 and 6 weeks: Kruskal Wallis one way ANOVA and Multiple Comparisons Between Treatments (Siegel 1988) were used.
- Survival rate: Two way ANOVA ($\propto=0.05$) was used to detect if there were any differences among groups. Tukey HSD was used to find between which groups the difference were.
- Boring activity : G-test for goodness of fit (Zar 1996) with significanse level $\propto=0.05$ was used.

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RAPPORT DE SYNTHÈSE / SUMMARY REPORT

Rapport de synthèse

Effets physiologiques

Les présentations concernant les aspects physiologiques du café étaient divisées principalement en trois sujets : 1) dépendance au café et à la caféine ; 2) effets anti-cancérigènes du café ; 3) contamination du café par l'ochratoxine, contrôle et analyses.

Dépendance à la caféine

Le Dr Astrid Nehlig de l'université de Strasbourg a passé en revue le statut actuel des connaissances relatives à la dépendance à la caféine. Elle a réussi à simplifier ce sujet complexe et à le rendre compréhensible pour les profanes. Elle a axé son exposé sur la polémique récente qui décrivait la caféine comme une substance d'abus potentielle. Cette polémique est due en grande partie aux modifications de la terminologie et des définitions utilisées dans le contexte de la toxicomanie. Le Dr Nehlig a comparé la caféine aux critères établis sur l'abus de drogues selon quatre critères cruciaux : sevrage, tolérance, renforcement et dépendance. On a estimé qu'il était essentiel de prendre en compte tous ces critères pour qu'une substance soit considérée comme une drogue.

Bien que la caféine produise des symptômes de sevrage chez certains sujets sensibles, ces symptômes sont très légers et passagers, comparés à ceux produits par la cocaïne et les amphétamines. De plus, la désaccoutumance à elle seule n'est pas un critère suffisant pour l'imputer à la dépendance. La caféine possède aussi des propriétés de renforcement, mais seulement à faibles doses, les hautes doses engendrent plutôt l'aversion. Le Dr Nehlig a montré que, contrairement aux drogues classiques, la caféine ne produit pratiquement pas de tolérance au niveau du système nerveux central. Par conséquent, l'abus de caféine présente un risque minime en comparaison des substances classiques induisant une dépendance. Les réponses à la caféine présentent d'importantes différences inter-individuelles et seulement un nombre restreint d'individus sensibles rencontrent des difficultés à arrêter l'ingestion de caféine. Cependant, les ambiguïtés et les inadéquations dans les données existantes, ainsi que les diverses possibilités d'interprétation par les experts vont probablement assurer la poursuite du débat sur la dépendance à la caféine.

Effets anti-cancérigènes

Il est de plus en plus évident que les composants du café peuvent avoir des effets bénéfiques sur la santé humaine. Le Dr Anthony Huggett du Centre de Recherche Nestlé à Lausanne a centré sa conférence sur deux constituants du café, le cafestol et le kahweol, qui ont précédemment fait la une à cause de leur association avec l'hypercholestérolémie. Il a démontré qu'à faible dose d'ingestion, ces constituants augmentent l'activité d'enzymes spécifiques qui sont impliquées dans la détoxication de certains carcinogènes dans le corps. Dans une série d'expériences *in vivo* et *in vitro* complémentaires, le Dr Huggett a démontré que le cafestol et le kahweol protègent l'ADN contre des dégâts causés par les carcinogènes alimentaires, benzo-[a]-pyrène et aflatoxine-B1. De plus, il fut mis en évidence que ces effets anti-cancérigènes, dus aux diterpènes du café, se produisent à des concentrations inférieures à celles provoquant l'augmentation du taux de cholestérol. Une consommation modérée de café instantané et espresso à faibles teneurs en diterpènes pourrait, par conséquent, induire d'importants effets bénéfiques sans produire les effets négatifs sur le métabolisme du cholestérol rapportés, suite à une consommation importante de café bouilli ou filtre.

Ochratoxine A

Les autres présentations de la session de physiologie fournirent une vaste revue de tous les aspects de la contamination du café par l'ochratoxine A (OTA). Comme souligné par le professeur Ron Walker de l'université du Surrey et président du comité WHO-JECFA d'évaluation des additifs, l'OTA est une mycotoxine produite par les moisissures *Aspergillus ochraceus* et *Penicillium verrucosum*. C'est un contaminant trouvé aussi bien dans le café torréfié et moulu que dans le café instantané à des taux bas de l'ordre du ng/g (ppb). Il n'y a qu'un faible pourcentage des cafés examinés qui contiennent plus de 5 ppb d'ochratoxine.

Le mécanisme de la toxicité de l'ochratoxine demeure un sujet très controversé. Il s'ensuit un débat sur la procédure à adopter pour déterminer la dose acceptable d'ingestion d'OTA. L'évaluation WHO-JECFA la plus récente propose une dose d'OTA hebdomadaire tolérable provisoire de 0-100 ng/kg de poids corporel. Les céréales sont la source alimentaire principale d'OTA, leur contribution à la dose hebdomadaire acceptable provisoire est d'environ 8-20 ng/kg de poids corporel, alors que celle du café est de seulement 2-6 %. C'est pourquoi, la JECFA considère que l'ingestion d'OTA par le café ne présente pas de danger véritable pour la santé.

La nécessité d'établir des limites d'OTA spécifiques pour le café est toutefois un sujet de discussion dans certaines agences réglementaires. Le professeur Walker a démontré que l'établissement de telles limites est une méthode coûteuse et inefficace de contrôle de l'OTA. La distribution hétérogène d'OTA dans le grain de café et la difficulté à établir des plans d'échantillonnage appropriés présentent d'importants problèmes. De surcroît, la validité scientifique des limites proposées est très contestable. Par conséquent, l'introduction des limites actuellement en discussion s'avérerait coûteuse et n'aurait qu'un impact limité sur l'ingestion d'OTA. Le professeur Walker conclut que la prévention de la contamination du café vert par l'OTA, et non pas une réglementation spécifique du

taux d'OTA dans le café, est le moyen le plus efficace pour contrôler le problème et assurer la sécurité humaine.

Cette opinion fut soutenue dans les deux présentations complémentaires qui suivirent celle du professeur Walker. L'impression générale fut qu'il serait préférable d'identifier des points de contrôle critiques au niveau des procédés de fabrication et de prendre des mesures pour réduire l'accès des moisissures aux fruits et grains de café pour prévenir la contamination par les mycotoxines plutôt que de contrôler l'OTA dans le produit. Le Dr Mick Frank de l'université du Surrey utilise une approche HACCP pour caractériser les étapes de la production de café qui peuvent être les cibles de contrôle des moisissures. Etant donné la grande diversité des pratiques de traitement du café, il est difficile de développer un modèle unique applicable à tous les procédés. C'est pourquoi, le Dr Frank a identifié cinq étapes clefs générales et fonctionnelles : (1) nouaison et maturation des fruits ; (2) récolte et tri ; (3) séchage et séparation des tissus ; (4) élimination des défauts ; (5) stockage et transport. Il est actuellement en train de caractériser chacune de ces étapes en fonction de leur potentiel de contamination par la moisissure et l'influence des caractéristiques critiques de contrôle.

Le Dr Naidu de l'institut central de recherche sur le café de l'Inde, partisan de cette approche, décrit les efforts pratiques entrepris en Inde pour gérer les problèmes causés par la mycotoxine au niveau de la production du café. La première étape consista à identifier certains paramètres conduisant à une contamination du café par la moisissure et à suggérer des stratégies de contrôle de ces paramètres. Ceci inclut par exemple la mise en place de dispositifs d'ombrage adéquats pour éviter le dessèchement par le soleil, le contrôle intégré des parasites pour réduire les dommages causés aux baies et la séparation des fruits endommagés lors de la cueillette ainsi que bien d'autres précautions qui doivent être prises pendant les étapes de séchage et de stockage. La clef du succès de l'approche du Dr Naidu réside dans la formation des cultivateurs et commerçants ainsi que dans l'identification des chefs de village et des personnes influentes dans les zones de plantations de caféiers. L'efficacité de ces procédures est actuellement étudiée en collaboration avec les Drs Frank et Illy et l'extension de l'approche pratique à d'autres pays d'Afrique, d'Amérique du Sud et d'Asie a été proposée. L'objectif scientifique et l'approche pratique ébauchés par le Dr Naidu sont les clefs du futur pour la gestion efficace du problème que constitue l'OTA.

A. Huggett

Atelier consacré à l'amélioration de la qualité du café par la réduction des moisissures

A la suite d'une réunion spéciale regroupant trente-trois scientifiques issus de neuf pays producteurs et de huit pays consommateurs, en présence de M. Arega Worku de l'OIAC et de M. E. Boutrif de la FAO, deux importants documents ont pu être édités :

1. *Vue d'ensemble de la situation relative à l'ochratoxine A – Actions à entreprendre pour élaborer les marches à suivre GAP (bonnes pratiques agricoles) et GMP (bonnes pratiques de fabrication) dans le but d'améliorer la qualité du café par la prévention des moisissures.*

On a pu compter sur l'appui de tous les participants durant la réunion, ainsi que sur celui du Dr Celsius Lodder, directeur administratif de l'OIC, en particulier.

2. *Version préliminaire des instructions pour de bonnes pratiques agricoles et de fabrication.*

Une version préliminaire de toutes les recommandations pratiques déjà existantes a été rédigée. Les informations qu'elle contient vont du champ à la production de café, en passant par le transport et la fabrication du café soluble et en grain. Cette liste sera régulièrement mise à jour sur la base des résultats du programme en cours.

R. Viani

Chimie

Dans le premier exposé magistral, M. N. Clifford présente une revue exhaustive de la diversité des acides chlorogéniques et de leurs composés. Les effets antioxydants des métabolites de l'acide chlorogénique amènent l'auteur à la conclusion que les acides chlorogéniques sont des composants très utiles du café.

A. Wilson *et al.* ont étudié, à l'aide de techniques utilisant la microscopie optique et la microscopie électronique, le devenir de l'huile contenue dans les fruits du caféier jusqu'à la torréfaction.

L'art de la préparation d'un espresso est, d'après M. Petracco *et al.*, basé sur la sélection optimale de caféiers d'origine botanique Arabica, de cafés présentant une flaveur sans défaut, et sur une sélection très sérieuse de tous les paramètres qui pourraient affecter le goût final. 810 plantes ont été soumises à une évaluation.

G. R. Waller *et al.* ont résumé les résultats de récentes études menées en Grande-Bretagne, en Suisse, au Japon et aux Etats-Unis sur le métabolisme et le catabolisme de la caféine chez le caféier (et le théier).

S. Homma *et al.* ont présenté une méthode fiable pour détecter les fractions de mélanoïdine dans le café soluble en combinant des méthodes chromatographiques HPLC à trois dimensions, LC-MS et GC-MS. Après complexation avec une solution de zinc (II), les auteurs purent caractériser une fraction brune Ap-V, de poids moléculaire 48 000, qui présentait une intéressante activité anti-oxydante.

L'importance des glucides dans les produits finis du café a été soulignée par V. Leloup *et al.*, qui ont mis au point une méthode fiable de séparation des oligosaccharides par chromatographie d'échange d'ions. Des données quantitatives ont été présentées pour les oligomannanes, les oligogalactanes et les oligosaccharides, dont le degré de polymérisation allait de DP1 à DP6, à trois niveaux de torréfaction différents.

A. Bradbury et E. Atkins ont étudié la structure des sédiments qui apparaissent pendant la fabrication du café soluble. La β(1-4) mannane, dans sa forme cristalline insoluble, était le principal composant. Sa teneur augmente avec le temps et la température.

La technique moderne associant la désorption par laser et la spectrométrie de masse a été appliquée par J. Zapp et R. Khun pour déterminer le poids moléculaire des oligosaccharides présents dans les extraits de café. Par cette méthode de spectrométrie de masse, également appelée MALDI-TOF, le poids moléculaire des oligosaccharides, jusqu'à un degré de polymérisation de 20, a pu être directement déterminé. Une application similaire aux protéines du café a été démontrée.

F. Kurzrock et K. Speer traitèrent de la distribution de quelque treize esters d'acide gras du cafestol dans différents cafés Robusta verts. 0,5 % seulement de ces esters se retrouvent dans le café boisson.

I. Kölling-Speer et K. Speer confirmèrent que les diterpènes déterminés dans les fèves de café sont également

présents dans les feuilles de caféier. Cependant, le 16-O-méthylcafestol, composant spécifique du café Robusta, n'a pas été retrouvé dans les feuilles de caféier Robusta analysées.

H. Steinhart et K. Luger ont mis au point une méthode analytique pour l'étude des cafés dits « traités à la vapeur » (cafés dont les propriétés physiologiques auraient été améliorées, qui ne sont commercialisés que dans quelques pays européens). Les auteurs proposent l'analyse de glucides à poids moléculaire élevé dans la fraction mélanoïdine et donnent des résultats pour des échantillons du commerce.

La caractérisation de la protéine la plus abondante dans l'endosperme du grain de *Coffea arabica* à maturité a été faite par W. I. Rogers *et al.* Sa ressemblance avec des protéines de réserve d'autres plantes laisse supposer qu'elle joue un rôle de réserve. L'ADN qui code cette protéine a été isolé.

W. Holscher *et al.* montrèrent comment a été élucidée l'origine des fèves puantes présentes dans le café vert trop fermenté. L'examen des effluents résultant de la chromatographie en phase gazeuse de cafés Arabica et Robusta avariés, associée à la spectrométrie de masse, conduit à l'identification de l'éthylester de l'acide 2-3-méthylbutyrique et de l'acide cyclohexanoïque, en tant que composés majeurs clés. Bien que ces composés soient présents dans presque tous les cafés en quantité de l'ordre des ppt, ils ne provoquent l'apparition de fèves puantes que lorsqu'ils atteignent des quantités de l'ordre des ppb.

J. Gretch *et al.* ont mis à l'épreuve deux types de capteurs de gaz électroniques destinés au contrôle de la qualité de l'arôme de café soluble conservé en flacon. Des améliorations doivent être apportées à ces « nez électroniques », bien que les deux types de capteurs, les polymères conducteurs et les oxydes métalliques, soient sensibles à de petites quantités d'huile aromatisée. La possible influence de l'humidité, du gaz carbonique, de la pression totale et de la teneur en oxygène dans l'espace de tête a été discutée.

Afin d'obtenir davantage de résultats quantitatifs, à partir de la chromatographie en phase gazeuse-olfactométrie, sur l'influence des composants de l'arôme du café, A. Chaintreau *et al.* ont utilisé, pour standardiser, une nouvelle cellule d'espace de tête. Les échantillons de gaz qui en provenaient ont été évalués par plusieurs juges, à l'orifice du chromatographe, en appuyant sur un bouton qui déterminait la durée de l'émission de chaque odeur. Une bonne reproductibilité et une bonne répétabilité ont été obtenues avec un panel de six à dix juges, après avoir établi sur ordinateur une moyenne des « fréquences de l'impact nasal » (NIF) et la « surface de la fréquence de l'impact nasal » (SNIF).

Génie alimentaire

H. K. Cammenga *et al.* ont étudié le processus de préparation du café boisson à l'aide d'un appareil ménager conventionnel à filtration. Plusieurs étapes ont été examinées : remplissage et forme de l'extracteur, débit d'eau, immersion et extraction par percolation. Des profils de concentration ont été déterminés.

F. Suggi Liverani *et al.* présentèrent un nouveau modèle informatisé, destiné à simuler les phénomènes de percolation, basé sur une machine à réaction-diffusion (R-DM). L'application à la percolation du café, compte tenu de la distribution de la taille des particules et du rapport liquide/solide, permet de prévoir la vitesse d'écoulement et d'extraction des substances solides. Les résultats préliminaires sont comparables à ceux obtenus expérimentalement.

D. Jaganyi et P. S. Madlala ont étudié la cinétique de l'extraction de la caféine et des ions minéraux en fonction de différentes origines géographiques des cafés. La diffusion retardée et plus lente dans les fèves de café, par comparaison avec des coefficients de diffusion connus, obtenus dans l'eau, est expliquée.

W. Göpel a fait une revue des principes des capteurs de gaz disponibles à l'heure actuelle dans le commerce, sous l'appellation de « nez électroniques ». Chaque modèle présentant des inconvénients, la combinaison de plusieurs capteurs est recommandée. Un aperçu de futurs nez artificiels est présenté, ceux-ci devant donner plus directement une perception analogue à celle du nez humain.

W. Heilmann fit un tour d'horizon des procédés de décaféination, y compris ceux de récupération de la caféine, en insistant sur la récupération de la caféine à partir de charbon activé, procédé qui est appliqué dans la décaféination à l'aide de CO_2.

V. D. Nataraju et K. Ramalaxmi explorèrent une nouvelle technique de torréfaction rapide en lit fluidisé. Les échantillons provenant d'un torréfacteur de laboratoire ont été analysés en prenant en compte leurs propriétés biochimiques et organoleptiques.

La formation d'un complexe caféine/acide caféique dans le rapport molaire 1 : 1, après l'addition d'acide caféique à des solutions de café, a été rapportée par B. L. Zeller et F. Saleeb. La possible application de ce phénomène à la décaféination de la caféine aqueuse et de solutions d'extraits de café a été démontrée en laboratoire.

J. A. Vaessen, dans un exposé qui contrastait avec les exposés techniques, a souligné qu'il fallait toujours avoir à l'esprit l'opinion du consommateur. La qualité est un concept à multiples facettes. Il faut en tenir compte, tant dans le cadre du commerce international, que de la législation nationale et internationale ainsi que dans ses implications dans le « marketing » et les perceptions du consommateur. En outre, et ce qui n'est pas le moindre, la qualité doit répondre aux attentes subjectives du consommateur. Ce dernier, outre le goût du produit, tient compte d'autres facteurs tels que le caractère sain des aliments, les aspects sociaux et environnementaux.

P. Justus décrivit les avantages d'une trieuse à café vert disponible sur le marché. L'élimination des grains indésirables est possible par sélection monochromatique, bichromatique et par fluorescence. Les derniers progrès dans ce domaine ont permis de mettre au point une trieuse trichromatique (Elexso) qui couvre le spectre des ultraviolets aux infra-rouges.

F. Suggi Liverani a illustré la diffusion des connaissances sur le café au moyen de deux expériences, l'une à l'aide d'un CD-ROM, l'autre en utilisant Internet. L'emploi des nouvelles techniques de diffusion de l'information, qui peuvent véhiculer des hypertextes, des illustrations, des films, des sons, permet de divulguer de façon efficace des connaissances et des faits sélectionnés dans les différents aspects de la science et de la technique du café.

Je n'ai plus qu'à remercier les auteurs pour le niveau élevé des exposés qu'ils ont présentés au cours des sessions de Chimie et de Génie alimentaire.

O. G. Vitzthum
(traduction)

Transfert de technologie en milieu rural

A l'occasion de ce 17e colloque, l'ASIC s'est ouverte à un nouveau champ de réflexion, celui du transfert de technologie en milieu rural, afin que les résultats de la recherche profitent au plus grand nombre.

Quatre communications principales étaient prévues sur ce thème :

- Dans un « Message d'un chercheur pour l'amélioration de la caféiculture par une meilleure utilisation des données de la recherche », le Dr R. A. Muller (ancien Directeur Scientifique de l'IRCC/CIRAD, France) a recommandé que ces données soient saisies et transmises dans leur globalité, car formant un tout cohérent indissociable, et sans les modifier, car établies en tenant compte de réalités bioécologiques incontournables ; un effort de formation des développeurs et des paysans est par conséquent nécessaire ; l'environnement socio-économique des paysans doit être adapté pour qu'ils puissent obtenir, en particulier par leur groupement en coopératives ou autres formes d'associations, les moyens indispensables à la mise en pratique des connaissances accumulées par les chercheurs.
- Dans une seconde intervention principale, le Dr B. Goud (CIRAD/SAR, France) a expliqué les travaux conduits en collaboration avec le Dr B. Sallée (CIRAD/CP, France) dans le bassin caféier de Coatepec au Mexique dans le cadre d'un projet de coopération franco-mexicaine pour la modernisation de la caféiculture. Ce projet de dix ans s'est attaché à comprendre le milieu physique et économique, à mettre en place des actions concrètes de modernisation en partenariat avec les paysans qui étaient les acteurs de ces transformations, après avoir été organisés en groupements solidaires. L'amélioration de la qualité, premier souhait des paysans, a conduit à la création d'ateliers de traitement des récoltes avec un système de dépollution des eaux résiduaires ; l'utilisation rationnelle des engrais a constitué un second volet important de ce projet qui a compris en outre des efforts en vue de la diversification des productions paysannes et a été caractérisé par un travail interdisciplinaire et une démarche participative.
- Le pofesseur B. P. Louant (Université de Louvain, Belgique), à son tour, dans une troisième communication principale, insista sur la nécessité de professionnaliser les paysans, en les formant techniquement pour qu'ils soient en mesure d'atteindre un niveau d'intensification raisonnable et respectueux de l'environnement. Des efforts importants doivent être faits pour que le message scientifique soit transmis et saisi : l'audiovisuel, avec les techniques modernes de l'informatique, peut être à la fois attrayant et convaincant pour ce transfert. Deux exemples des possibilités de l'audiovisuel, portant en particulier sur la régénération des caféières, ont été présentés pour illustrer le propos.
- La quatrième communication principale n'a pu être présentée, son auteur, le Dr J.-C. Chartier (Bureau pour le Développement de l'Agriculture, France) en ayant été empêché. Sous le titre « Les difficultés du transfert de technologie en milieu rural », cette communication sera néanmoins reprise dans les Actes du Colloque, compte tenu de l'importance du sujet traité

Le Dr A. M. Karanja (Coffee Research Foundation of Kenya) analysa ensuite les conséquences du mouvement de libéralisation et de privatisation sur la productivité des plantations de caféiers, conduisant à une nécessaire introduction des nouvelles technologies et à la participation des organisations d'agriculteurs dans les actions de transfert de technologie.

C'est encore cette nécessaire participation des paysans qui est soulignée par le Dr B. Nyambo (Coffee Research Foundation of Kenya) en ce qui concerne la recherche et la mise en place des opérations de lutte intégrée contre les parasites. Un projet en cours depuis 1996 comprend un volet important de formation des encadreurs et des paysans, pour les aider à la prise de décision dans le cadre d'une approche globale en vue de l'adoption de systèmes de production durables.

Traitant des interactions entre entreprises et disponibilité des ressources dans le secteur des petits producteurs de café au Kenya, le Dr M. T. K. Onsongo (Coffee Research Foundation of Kenya) plaide pour que des technologies bien adaptées aux possibilités des petits producteurs soient recherchées, tenant compte de leurs contraintes : terres disponibles, force de travail et ressources financières.

Ce propos rejoint celui du Dr E. M. Njoka (Université Egerton, Nairobi, Kenya), qui explique ce que devrait être une caféiculture durable, responsable de l'environnement par la protection du sol et la lutte antiérosive, la mise en œuvre de méthodes de lutte non polluantes contre les ennemis du caféier, en soulignant la nécessité d'une intégration des paysans à la mise au point et à la diffusion des méthodes et des techniques adaptées à cet objectif.

Soulignant une fois de plus la difficulté et l'insuffisance du passage des données de la recherche au niveau des paysans, le Dr J. O. E. Rakotomalala (Madagascar) explique que, dans ce pays, on a en quelque sorte renoncé à la diffusion de clones sélectionnés de *Coffea canephora*, qui exigent plus de soins pour leur culture, au profit d'un retour à la distribution de semenceaux, moins productifs, mais plus faciles à conduire par les paysans.

Pour terminer cette demi-journée consacrée au transfert de technologie en milieu rural, le Dr P. Ngategize (Coffee Research Center, Kituza, Ouganda) présenta le Réseau de Recherche Caféière en Afrique (RECA), groupant différents pays africains avec la coopération de différents pays européens et l'appui de l'Union européenne : ce réseau devrait être un élément performant pour la conduite des recherches dans le futur.

Un poster présenté par le Dr E. M. K. Koinange (ARTI, Lyamungu, Tanzanie) et le Dr P. Charmetant (CIRAD/CP, France) donne les grandes lignes des recherches conduites en Tanzanie, et leurs prolongements par les mesures prises pour leur transfert en milieu rural, y compris la distribution de clones de caféiers obtenus par multiplication végétative, la lutte contre le CBD et la rouille orangée.

Pour conclure, on peut dire que cette demi-journée a permis de constater que, si les problèmes posés par le transfert de technologie en milieu rural apparaissent à tous, de la même manière la nécessité de la formation des développeurs et des paysans, la nécessité de l'organisation des paysans en groupements solidaires, et de leur participation active à la mise au point des techniques et à leur application, semblent également faire l'unanimité, ce qui montre qu'il devrait être possible, par la concertation, par la confrontation des expériences, de faire progresser les méthodes les plus aptes à amener les paysans à profiter enfin au mieux des recherches conduites en vue de l'amélioration de leur situation.

R. A. Muller

Agronomie

La session d'agronomie, avec quatre exposés de synthèse, trente-sept communications orales et dix-neuf

affiches, a donné une vue d'ensemble des avancées de la recherche.

Amélioration génétique

Dans son exposé introductif sur **l'amélioration génétique** de *C. arabica*, D. J. Walyaro (Kenya) a présenté un rapport sur l'impact des principales maladies et des ravageurs du caféier, et a analysé les stratégies couramment utilisées pour améliorer la résistance à ces maladies de façon durable. Il a aussi fait le point sur les caractères de qualité à prendre en considération pour l'amélioration du café Arabica.

Bellachev Bayetta a fait une revue du programme d'amélioration du café en Ethiopie. Le matériel végétal local de *C. arabica* comprenant près de 2 000 origines est conservé dans une collection en champ. L'hérédité de la résistance au « Coffee berry disease » (CBD) et la vigueur hybride ont été étudiées. Dix-huit variétés améliorées résistantes au CBD sont en cours d'évaluation avant leur diffusion.

A. Charrier a résumé le programme original d'amélioration génétique de *C. arabica* réalisé en Amérique centrale par le CATIE, le PROMECAFE et la coopération française. Ce programme a pour objectif la sélection de variétés hybrides F1 multipliées par embryogenèse somatique. Ainsi, le temps nécessaire à la sélection s'en trouve réduit et l'on valorise le phénomène de vigueur hybride.

C. Agwanda a analysé l'influence de l'interaction génotype × environnement sur les caractères de qualité du grain et la qualité de la boisson de *C. arabica* au Kenya. Les différences génotypiques pour les caractères du café boisson étaient mieux révélées en conditions de déficit hydrique intervenant pendant la phase de remplissage de la fève de café.

H.A.M. van der Vossen a présenté une analyse critique des programmes d'amélioration génétique de *C. arabica* de cinq pays africains producteurs en se fondant sur leurs stratégies d'amélioration, les créations variétales et les techniques de diffusion. Il a aussi recommandé le recours aux techniques modernes de l'amélioration des plantes, ainsi que la collaboration des équipes aux niveaux régional et industriel.

L'amélioration génétique de *C. canephora* pour les zones tropicales de basse altitude a aussi fait l'objet de plusieurs communications :

1 – C. Montagnon a évalué l'efficacité de la sélection récurrente réciproque (SRR) conduite en Côte d'Ivoire en comparant, pour la sélection clonale, les performances pied-mères *vs* clones et, pour la sélection d'hybrides, les performances géniteurs *vs* hybrides.

2 – N. E. Nyange a réalisé une collecte de caféiers cultivés par les agriculteurs de la région de Kagera (Tanzanie) : 141 numéros de *C. canephora* ont été introduits en collection pour évaluation.

3 – Des recherches sur les hybrides Arabusta tétraploïdes sont poursuivies pour améliorer la qualité du café produit en zone Robusta. E. Anim-Kwapong a présenté les résultats des populations F2 d'Arabusta *stricto sensu* évaluées au Ghana. A. Rabemiafara a décrit les caféiers F2 sélectionnés dans une population hybride trois voies, tétraploïde, à Madagascar, dénommée Ratelo ; des caractères originaux des trois espèces parentales – *C. canephora*, *C. eugenioïdes* et *C. arabica* – sont observés chez quelques caféiers manifestant des anomalies limitées de fructification.

4 – Dans un croisement entre l'espèce spontanée sans caféine *C. pseudozanguebariae* originaire du Kenya et l'espèce *C. liberica dewevrei*, P. Barre a évalué l'introgression des caractères de qualité. Le transfert pourrait être facilité par le recours aux nouvelles techniques, comme l'hybridation génomique *in situ*, la cytométrie en flux et les marqueurs moléculaires. L'hérédité de l'absence de caféine serait due à un gène majeur à l'état récessif.

Biotechnologies végétales

Les **biotechnologies applicables à l'amélioration des caféiers** sont en cours de développement dans plusieurs directions :

1 – T. Leroy a rapporté les résultats des recherches sur le génie génétique obtenus par le CIRAD et Nestlé-France : des embryons somatiques ont été transformés avec succès par coculture avec une souche d'*Agrobacterium tumefaciens*. Ainsi, de jeunes plantes transformées avec un gène de *Bacillus thuringiensis* ont été régénérées et expriment une endotoxine dont l'efficacité vis-à-vis de la mineuse des feuilles est en cours d'étude.

2 – M. Sondhal et M. Petracco ont porté leur attention sur la sélection de variétés *arabica* améliorées pour la qualité du café à la tasse à partir de variants somaclonaux provenant des populations d'origine interspécifique Icatu avec *C. canephora* et Aramosa avec *C. racemosa* développés au Brésil.

3 – M. Berthouly a décrit les progrès réalisés dans la maîtrise des différentes phases de la multiplication végétative des caféiers par embryogenèse somatique. Le procédé est en cours d'application au CATIE sur les hybrides F1 de *C. arabica* et un laboratoire est opérationnel en Ouganda pour *C. canephora*.

4 – Un protocole de cryoconservation des graines de caféiers a été développé pour la première fois par S. Dussert. Son application à la conservation de longue durée des ressources génétiques de *C. arabica* est envisageable par déshydratation des graines et immersion directe dans l'azote liquide, puis reprise par culture d'embryons avec un taux élevé de germination.

5 – L'intérêt potentiel de la sélection assistée par marqueurs génétiques (SAM) de *C. arabica* a été analysé par P. Lashermes. Elle peut être intégrée aux programmes classiques d'amélioration génétique, en particulier pour conduire l'introgression de gènes d'intérêt agronomique. Les résultats préliminaires obtenus pour la résistance de *C. arabica* au CBD ont illustré cette démarche.

Sous forme d'affiche, V. M. Carneiro a montré comment déterminer le niveau de ploïdie de jeunes caféiers régénérés par culture d'anthères grâce au comptage des chloroplastes des cellules stomatiques. De même, N. E. Nyange a rapporté la régénération de deux génotypes de *C. arabica* par culture de protoplastes issus de cals d'hypocotyles, au SCRI (Dundee).

Méthodes agronomiques

Plusieurs communications ont concerné les **systèmes agronomiques et les techniques utilisées dans différents pays producteurs de café**. Dans son exposé introductif, J. M. Njoroge a fait une revue des pratiques agronomiques, de la culture des caféiers à la préparation du café, dans l'objectif de promouvoir une caféiculture durable pour *C. arabica*. M. Sondhal et M. Petracco ont évalué l'efficacité des applications d'Ethrel chez *C. arabica*, soit sur de jeunes fruits pour provoquer la chute des dernières nouaisons, soit sur des fruits complètement développés pour provoquer une maturation groupée. La pulvérisation précoce de l'Ethrel a un effet favorable sur la qualité à la tasse, en réduisant la proportion de fruits immatures.

F. Amoah a présenté les performances des cultivars de *C. canephora* au Ghana cultivés avec ou sans ombrage, à différents écartements et selon la taille pratiquée.

J. W. J. Msaky a analysé les productions de trois cultivars de *C. arabica*, sur trois cycles de recépage, à différentes densités en vue d'établir des normes techniques pour une caféiculture rentable pour les planteurs.

M. Kanua a déterminé l'effet de la culture associée de patate douce sur la croissance et la production d'une plantation de *C. arabica* var. *typica ;* il a évalué l'intérêt économique de cette pratique en Papouasie-Nouvelle-Guinée.

M. Koudjega a observé l'action bénéfique des arbres d'ombrage *Albizzia* spp. sur la production des caféiers *C. canephora* au Togo.

Des informations agronomiques complémentaires ont été rapportées dans les affiches exposées :

– Ph. Vaast a présenté les effets du pH, de la température et des formes de l'azote sur la capacité d'absorption de jeunes plantes de la variété Catuai.

– P. Dubale a évalué les effets de la taille, de la fertilisation et du désherbage sur la production dans différents lieux en Ethiopie.

– M. P. H. Gathaara a étudié la biomasse accumulée dans les différents organes des caféiers de la variété Ruiru 11.

– V. Rutunga a rappelé les résultats agronomiques obtenus pour la culture de *C. arabica* au Rwanda.

Le Dr J. K. Kimemia (Coffee Research Foundation of Kenya) fit un exposé sur la possibilité de produire un café biologique au Kenya. Un tel café de qualité pourrait être offert à une clientèle particulière, et être obtenu par utilisation de caféiers résistants aux maladies évitant l'emploi de pesticides, le remplacement des engrais chimiques par une fumure organique sous forme de fumier de ferme ou d'engrais verts produits entre les lignes de caféiers.

Le Dr Ph. Vaast (CIRAD/CP, France) montre, dans des essais en serre, qu'une endomycorhization précoce du caféier Arabica lui confère une forte tolérance vis-à-vis des deux principaux nématodes, *Pratylenchus* et *Meloidogyne,* en même temps qu'une vigueur accrue.

Les Drs K. Ngoran (IDEFOR/DCC, Côte d'Ivoire) et P. Jadin (CIRAD/CP, France) ont étudié l'influence des engrais azotés sur le stock azoté du sol sous caféiers Robusta en Côte d'Ivoire et au Cameroun. Il apparaît que l'apport d'urée permet de maintenir la réserve potentiellement minéralisable à un niveau plus élevé que dans le cas des apports de sulfate d'ammoniaque.

Les Drs S. Abdoellah et A. M. Nur (Indonesian Coffee and Cocoa Research Institute) ont présenté des résultats sur les effets de l'apport de matière organique, de plantes de couverture (*Calopogonium* et Vetiver), et d'engrais azotés, sur la croissance du caféier Robusta en Indonésie. Les apports de matière organique sont trop récents encore (deux ans) pour avoir eu un effet significatif. La fertilité du sol est cependant améliorée. En conséquence, on conseille encore l'apport d'engrais azoté pour assurer une bonne croissance aux caféiers.

Un poster du Dr P. R. Matowo (Agricultural Research Station, Lyamungu, Tanzanie), donne en outre une méthode d'évaluation des herbicides pour le contrôle des adventices dans les plantations de caféiers.

Préparation du café vert

Au chapitre des procédés de première transformation des récoltes, le Dr J. K. Mburu (Coffee Research Foundation of Kenya) montre que, pour servir de tampon entre les apports de récolte et leur traitement, il est possible de stocker les grains en parche dans de l'eau renouvelée chaque jour pendant un temps assez long, soit sept jours sans altérer les cafés de bonne qualité, et même en améliorant la qualité des cafés de classe inférieure.

Deux communications s'intéressent à la protection de l'environnement par le traitement des pulpes et des eaux résiduaires des stations de dépulpage :

- c'est le Dr J. R. Ramirez-Martinez (Universidad Nacional del Tachira, Venezuela) qui propose l'ensilage des pulpes aboutissant à l'obtention d'un produit utilisable pour l'alimentation des porcs et des poissons ;
- c'est le Dr J. Noble (University of Leeds) qui, étudiant différents systèmes de dépollution des eaux, propose une méthode raisonnée pour orienter le choix des techniques les plus appropriées à chaque cas, en tenant compte de tous les critères d'appréciation des coûts, de la masse à traiter, du degré de pollution acceptable, etc.

Enfin, un système de séchage solaire, couplé avec un brûleur à bois est proposé par le Dr S. Mulato (Indonesian Coffee and Cocoa Research Institute) pour le séchage du café des petites exploitations, afin d'améliorer la qualité du produit marchand auquel il est reproché couramment de porter des moisissures.

Un poster présenté par les Drs L. Lendaro (Agromatica, Italie) et A. Balci (Illycafé, Italie), fait état d'un nouvel outil pour faciliter et rendre plus rapide la récolte du café.

Phytopathologie

Lors d'une présentation générale, le Dr D. Nandris (ORSTOM, France, Nouvelle-Calédonie) a exposé les règles de la lutte intégrée et la complexité que couvre ce concept, prenant en compte tous les éléments biotiques et abiotiques intervenant dans un pathosystème. En application de ces principes, une étude a été conduite en Nouvelle-Calédonie, intégrant trois maladies du caféier, l'anthracnose des rameaux, la rouille orangée et la cercosporiose. Cette étude faite en de nombreux points de l'île et intégrant la diversité des pathogènes, du matériel végétal et de l'environnement, a conduit, grâce à une analyse mettant en œuvre de puissants moyens informatiques, de caractériser les risques et d'expliquer la dynamique des différentes maladies étudiées. Il s'agit là d'un outil méthodologique qui devrait être mis en œuvre chaque fois que l'on est confronté à une affection parasitaire ou autre.

Dans la même orientation, le Dr J. Avelino (CIRAD/CP, IHCAFE/Honduras) a fait part d'une enquête-diagnostic portant sur la rouille orangée, et intégrant les niveaux d'attaque, les caractéristiques de l'environnement naturel (composantes du sol et du climat) et cultural ; cette enquête conduit à élaborer un modèle prédictif des risques reposant sur l'analyse factorielle et la segmentation, et devant permettre de moduler les recommandations de la lutte chimique en fonction des réalités bioécologiques locales, alors qu'elles sont actuellement trop générales pour satisfaire chacun des agriculteurs.

Deux présentations ont ensuite traité de l'identification précise d'un pathogène grave du caféier, le *Colletotrichum kahawae,* agent de l'anthracnose des baies de l'Arabica (Coffee berry disease, ou CBD des auteurs de langue anglaise) :

– le professeur H. Hindorf (Institut für Pflanzenkrankheiten, Université de Bonn, Allemagne) a d'abord fait l'historique de la dénomination de plus en plus précise de cet organisme, aboutissant à la nomenclature actuelle qui a le mérite d'éviter les confusions d'autrefois avec les autres espèces de *Colletotrichum* rencontrées sur caféier ; puis il a donné un certain nombre de règles permettant cette identification précise : caractéristiques morphoculturales, modalités de croissance en culture, mise en œuvre des méthodes de biologie moléculaire, et tests de pathogénicité ;

– pour le Dr Bella Manga (IRA, Cameroun) travaillant dans le cadre du Projet RECA, en coopération avec le Kenya, la France et le Portugal, il est en plus nécessaire, au sein de l'espèce *Colletotrichum kahawae,* de bien identifier les souches diverses pouvant exister dans les différents pays concernés, afin de cibler au plus juste l'agent pathogène à combattre dans les programmes d'amélioration en vue de la résistance ; fondée sur la technique des groupes de compatibilité végétative et l'étude du pouvoir pathogène, ce travail a conduit à identifier deux sous-populations (Cameroun et Afrique de l'Est), les souches camerounaises étant particulièrement agressives. Le fait qu'aucune interaction hôte-parasite n'a été mise en évidence et la grande variabilité dans l'agressivité des isolats du Cameroun montrent que l'on se trouve dans un système de résistance générale ; des génotypes de caféiers d'origine éthiopienne, hautement résistants pourront être introduits dans les programmes d'amélioration.

Enfin, le Dr Julie Flood (International Mycological Institute, Royaume-Uni) a montré que la trachéomycose du caféier, grave maladie d'autrefois en Afrique de l'Ouest et du Centre sur Excelsa, Liberica et Robusta, et qui avait été perdue de vue, revient en force depuis dix ans, en République démocratique du Congo et en Ouganda sur Robusta. Il est urgent de trouver une solution à ce problème et, dans un premier temps, il serait très utile de faire une enquête pour identifier les causes de la résurgence de cette affection : mutation du pathogène ou modifications de l'environnement naturel et cultural ? Un atelier de travail va se tenir à Kampala du 28 au 30 juillet 1997 pour tenter d'établir les grandes lignes des actions à entreprendre.

Deux posters complètent les travaux de phytopathologie : l'un, du Dr G. O. Omondi (Coffee Research Foundation of Kenya) sur la diversité génétique des isolats de *Colletotrichum kahawae,* l'autre, du Dr J. B. Birikunzira (Coffee Research Center, Kituza, Ouganda) sur la trachéomycose en Ouganda et les stratégies possibles de lutte.

Entomologie et lutte intégrée

Revenant sur le concept de lutte intégrée, le Dr B. T. Nyambo (Coffee Research Foundation of Kenya) fit un tableau très complet des ravageurs, maladies et nématodes qui menacent le caféier, en montrant la nécessité d'une approche globale de ces différents aléas, sous peine, en luttant contre les uns, de favoriser les autres. Elle a souligné la nécessité d'intégrer les moyens de lutte dans une agriculture durable, respectant au mieux l'environnement, et la nécessité d'associer les paysans eux-mêmes à la mise au point et à la mise en œuvre des recommandations ; une réflexion sur les limites et les besoins d'une telle approche a été faite pour terminer.

Le Dr L. O. Brun (ORSTOM/France, Nouvelle-Calédonie) a fait une étude sur la résistance à l'endosulfan du scolyte du grain, *Hypothenemus hampei,* après dix ans d'utilisation de cet insecticide. Les populations résistantes ont été identifiées dans cinq régions sur quinze ; il est constaté que leur niveau de résistance décroît après l'arrêt des traitements. L'étude génétique de cette résistance a été faite, mettant en évidence un gène récepteur, avec une semi-dominance de la résistance que l'on peut qualifier de haplo-diploïde fonctionnelle, du fait que la moitié du matériel chromosomique paternel dégénère alors que celui de la lignée maternelle est totalement transmis.

Le Dr H. Breilid (Université de Bergen, Norvège) a ensuite présenté une étude sur la phylogéographie des populations du scolyte des grains. Cette étude, qui repose sur le séquençage de l'ADN mitochondrial, dans des populations provenant du monde entier, a conduit à identifier une population centre et sudaméricaine, une population incluant des individus asiatiques, ivoiriens, jamaïcains et néo-calédoniens, ce qui doit permettre de savoir quelle a été l'histoire de la dispersion de ce ravageur. Des populations de Nouvelle-Calédonie étant résistantes à l'endosulfan et aux autres insecticides de type cyclodiène, il est important de tenir compte de cette résistance pour en éviter la dispersion.

Enfin, le Dr Ch. Cilas (CIRAD/CP, France) a présenté une étude sur la définition des pratiques d'échantillonnage pour estimer les dégâts dus au scolyte des grains. Cette étude critique comparative des différentes méthodes d'échantillonnage au champ aboutit à renoncer à l'échantillonnage au hasard et à recommander des procédés d'échantillonnage systématiques, beaucoup plus précis. Ces méthodes, un peu difficiles à mettre en œuvre pour l'étude de l'évaluation de la nécessité d'effectuer des traitements, sont au contraire bien adaptées à l'expérimentation.

Dans le cadre de l'entomologie, il faut pour finir faire état de l'intervention du Dr S. Sithanantham (International Center of Insects Physiology and Ecology, Nairobi, Kenya) qui, proposant des études pour la mise en place de solutions alternatives à la lutte chimique contre le scolyte des grains, lance un appel pour une coopération internationale.

Cinq posters ont été présentés dans le cadre de cette session :

- celui du Dr P. Kucel (Coffee Research Center, Kituza, Ouganda) concerne les cochenilles en Ouganda ;
- celui du Dr G. O. Omondi (Coffee Research Foundation of Kenya) se rapporte au contrôle biologique des insectes ravageurs du caféier au Kenya ;
- celui du Dr B. Padi (Cocoa Research Institute of Ghana) traite de la préférence relative des Epicampoptères sur cinq types de caféier ;
- celui du Dr E. Y. Mcharo explique que les mantes religieuses et les caméléons pourraient jouer un rôle dans la lutte contre les insectes ravageurs ;
- celui du Dr P. Mbondji Mbondji, qui présente l'abondance, la diversité et la répartition géographique des Hémiptères vivant sur les caféiers au Cameroun.

A. Charrier et R. A. Muller

Summary report

Physiology

The presentations concerning the physiological aspects of coffee were essentially divided into three subjects : 1) coffee and caffeine dependence ; 2) anti-cancer effects of coffee ; 3) ochratoxin contamination of coffee, its control and analysis.

Caffeine dependence

Dr. Astrid Nehlig from the University of Strasbourg reviewed the current status of knowledge concerning caffeine dependence. She managed to make a very complex subject understandable to the lay-person. She highlighted the recent controversy that has arisen with regard to the status of caffeine as a potential substance of abuse. This controversy is largely due to changes in terminologies and definitions used in drug dependence. In a comparison of caffeine with established drugs of abuse Dr. Nehlig examined four critical criteria : withdrawal, tolerance, reinforcement and dependence. All of these criteria should be exhibited if a substance is to be considered a drug of abuse.

Although caffeine can induce withdrawal symptoms in certain sensitive individuals, the symptoms are moderate and transient compared to cocaine and amphetamine. Moreover withdrawal alone is not a sufficient criterion for ascribing dependence. Caffeine also possesses reinforcing properties, but this only occurs at low doses and high doses tend to produce aversion. In contrast to classical drugs of abuse, Dr. Nehlig showed that there is almost no tolerance to caffeine at the level of the central nervous system. Therefore compared to classical addictive compounds caffeine abuse can be considered a very minimal risk. There are large inter-individual differences in response to caffeine and only a very small proportion of specifically sensitive people appears to have difficulties in stopping caffeine intake. Nevertheless, ambiguities and inadequacies in the existing data, and the potential for large differences in the way data are interpreted by experts, are likely to ensure that the debate of caffeine dependency will continue.

Anti-cancer effects

There is increasing evidence that coffee components can have beneficial effects on human health. Dr. Anthony Huggett from Nestlé Research Centre, Lausanne focused on two constituents of coffee, cafestol and kahweol, which have previously been in the news due to their association with hypercholesterolemia. He demonstrated that at low intake levels these constituents increase the activity of specific enzymes that are involved in the detoxification of certain carcinogens in the body. In a series of complementary *in vivo* and *in vitro* experiments, Dr. Huggett showed that cafestol and kahweol protected against DNA damage caused by the dietary carcinogens benzo[a]pyrene and aflatoxin B1. Furthermore, evidence was presented that these anti-cancer effects of the coffee diterpenes occur at lower concentrations than their cholesterol-raising effects. Thus moderate consumption of coffee brews such as instant coffee and espresso which have low diterpene contents may produce important benefits without producing any of the negative effects on cholesterol metabolism that have been reported following heavy consumption of boiled or cafetière coffee brews.

Ochratoxin A

The remaining presentations in the physiology session provided a comprehensive review of all aspects concerning the contamination of coffee by ochratoxin A (OTA). As highlighted by Professor Ron Walker of the University of Surrey and Chairman of the WHO-JECFA additive evaluation committee, OTA is a mycotoxin produced by the moulds *Aspergillus ochraceus* and *Penicillium verrucosum*. It has been found as a contaminant of both roast and ground as well as instant coffee at low ng/g (ppb) levels. However, only a very small percentage of coffees that have been examined contain more than 5 ppb ochratoxin.

The mechanism by which ochratoxin causes toxicity remains a highly controversial issue and as a result there is a debate over the risk assessment procedure that should be used to assess a tolerable level of intake of OTA. The most recent WHO-JECFA evaluation suggests a provisional tolerable weekly intake of OTA of 0-100 ng/kg bw. Cereals are the major dietary source of OTA contributing about 8-20 ng/kg bw/week, while coffee contributes only between 2-6 % of the provisional tolerable weekly intake. Therefore, JECFA would consider that OTA intake from coffee does not present an appreciable health risk.

Nevertheless, there is discussion within certain regulatory agencies of the need for setting up specific limits for OTA in coffee. As demonstrated by Professor Walker, the establishment of such limits represents a costly and inefficient method of OTA control. The heterogeneous distribution of OTA in the coffee bean and the difficulty of establishing an appropriate sampling plan present important problems and furthermore the scientific validity of the limits that have been proposed has been strongly questioned. Consequently, the implementation of limits currently under discussion would be costly and would have little impact on OTA intake. Professor Walker concluded that the prevention of green coffee bean contamination with OTA and not a specific regulation on OTA levels in coffee was the most effective way of controlling this issue and ensuring human safety.

This view was strongly endorsed in the two complementary presentations which followed that of Professor Walker. Rather than OTA control at the product level it was felt that critical control points in the production process should be identified and steps taken to reduce the access of moulds to coffee fruit and beans, thus preventing mycotoxin contamination. Dr. Mick Frank of the University of Surrey is using a HACCP approach for characterising the steps in coffee production that can be the targets for fungal control. Since coffee-processing practices can be highly variable, it is difficult to develop a single model applicable to all processes. Therefore Dr. Frank has identified five key general functional steps : (1) setting and development of fruit ; (2) harvesting and sorting ; (3) drying and separation of tissues ; (4) grading out defects ; (5) storage and transport. He is currently characterising each of these steps with regard to their potential for fungal contamination and the influence of critical control features.

As a supporter of such an approach, Dr. Naidu of the Central Coffee Research Institute, India described the practical efforts that have been taken in India with regard to mycotoxin management in coffee production. In the first stage, certain parameters leading to fungal contamination of coffee were identified and management strategies to control these parameters were suggested. For example, these include the provision of adequate shade to avoid sun-scorching, integrated pest management to reduce berry damage, and the separation of damaged fruit at harvesting, as well as numerous other precautions to be taken during the drying and storage steps. A key to the success of the approach being used by Dr. Naidu is the education of farmers, traders and curers and the identification of village leaders and trend-setters in coffee growing areas. The effectiveness of the procedures is now being studied in collaboration with Dr. Frank and Dr. Illy and the extension of this practical approach to other countries in Africa, South America and Asia has been proposed. The scientific objective and practical approach outlined by Dr. Naidu hold the key to the future for effectively dealing with the OTA issue.

A. Huggett

Special workshop on the enhancement of coffee quality by reduction of mould growth

A special meeting of thirty-three scientists from nine producing and eight consuming countries in the presence of Mr Arega Worku of IACO and Mr E. Boutrif of FAO has produced two important documents :

1. *Current overview of ochratoxin A – further suggested action to develop GAP (good agricultural practices) and GMP (good manufacturing practices) procedures to enhance coffee quality by prevention of mould growth.*

The commitment of all the participants at the meeting, and of Dr Celsius Lodder, executive director of ICO was obtained.

2. *Draft guidelines for good agricultural practices and good manufacturing practices.*

A preliminary list of all practical recommendations already available from the field through coffee production, transport, roast and instant coffee manufacture was drafted. This list shall be regularly updated based on the results of the ongoing program.

R. Viani

Chemistry

In the first plenary paper, presented by M. N. Clifford, a comprehensive overview on the variety of chlorogenic acids and related compounds was given. The antioxidative effects of chlorogenic acid metabolites lead the author to the conclusion, that the chlorogenic acids are useful ingredients of coffee.

A. J. Wilson *et al.* investigated by light and electron microscopic techniques the fate of the oil from the coffee fruits to the roasted beans.

The « art » of espresso making, according to M. Petracco *et al.*, is based on optimal selection of the botanical origin of Arabica coffee with no flavour defects and on a careful selection of all variables that could affect the sensory balance. 810 plants had been evaluated.

G. R. Waller *et al.* summarized the results on caffeine metabolism and catabolism in *Coffea,* based on recent studies in the UK, Switzerland, Japan and the US.

S. Homma *et al.* demonstrated a successful way of elucidating melanoidin fractions from instant coffee, combining 3-dimensional HPLC, LC-MS and GC-MS techniques. After complexation with Zinc-II-solution he could characterize a brown fraction Ap-V of m.w. 48,000 that showed an interesting antioxidative activity.

The importance of carbohydrates in coffee products was stressed by V. Leloup *et al.*, having developed a reliable anion exchange chromatography method for oligosaccharides. Quantitative data were presented for oligomannans, oligogalactans and oligosaccharides of degree of polymerisation from DP1 to DP6 at three different roasts.

A. Bradbury and E. Atkins investigated the chemical structure of the sediments that occur during instant coffee processing. β(1-4) mannan in its insoluble crystalline form was the major component. Its content is increasing with time and temperature.

The modern technique of Laser desorption-mass spectrometry was applied by J. Zapp and R. Kuhn for the molecular weight determination of oligosaccharides in coffee extracts. By this also called MALDI-TOF ms the molecular weights of oligosaccharides up to a degree of polymerisation of 20 could be directly determined. Similar applicability to proteins in coffee was demonstrated.

F. Kurzrock and K. Speer reported on the distribution of some 13 cafestol fatty acid esters in various green Robusta coffees. It was found only 0,5 % of the esters will pass into the beverage.

I. Kölling-Speer and K. Speer confirmed that the diterpenes known from coffee beans, also will be present in the leaves of the coffee tree.

However the « Robusta indicator compound » 16-O-methylcafestol was not found in the coffee leaves.

H. Steinhart and A. Luger developed an analytical method for the analysis of so called « steam-treated » coffees (these are coffees claiming improved physiological properties ; they are only in some European countries on the market). The authors propose the analysis of high molecular weight carbohydrates in the melanoidin fraction and give results for commercial samples.

The characterisation of the most abundant protein in mature *Coffea arabica* grain endosperm was described by W. J. Rogers *et al.* Similarity with other plant storage proteins supports the assumption of its storage function.

The corresponding DNA, coding for this protein, has been isolated.

W. Holscher *et al.* reported the elucidation of the « stinker » off flavour in overfermented green beans. Gaschromatographic effluent sniffing of spoiled Arabicas and Robustas – in combination with GC-MS – lead to the identification of 2- and 3-methylbutyric acid ethylester and cyclohexanoic acid ethylester as being the key impact compounds. Though these compounds being present in nearly all coffees in the ppt range, they cause the perceptible off flavors of stinker beans only in the ppb level.

J. Gretsch *et al.* tested two types of electronic gas sensors for the quality control of instant coffee in-jar aroma. These « electronic noses » will need further improvement, though both types, the conducting polymers and the metal oxide sensors, could distinguish between samples with and without aroma oil. The problematic influence of moisture, carbon dioxide, total pressure and oxygen content in the headspace has been discussed.

In order to get more quantitative results out of the subjective olfactometric characterisation of coffee aroma impact compounds, A. Chaintreau *et al.* used a newly developed head space cell for standardization. Gas samples from there were evaluated by several panelists at

the GC sniffing port, pressing a button for the duration of each odor. Good reproducibility and repeatability was achieved with six panelists after computerized averaging of the « nasal impact frequencies » (NIF) and the « surface of nasal impact frequency » (SNIF).

Food engineering

H. K. Cammenga *et al.* have studied the coffee brewing process in a conventional filter household machine. Several stages were investigated : the filling and form of the extractor, the flowthrough of water, immersion and percolation extraction. Concentration profiles were determined.

F. Suggi Liverani *et al.* presented an innovative computer based modelling to simulate diffusion phenomena based on a Reaction Diffusion Machine. The application to coffee percolation, given particle size distribution and liquid/solid ratio, allows to predict flow rate and soluble substances' extraction. Preliminary results are comparable with experimental ones.

D. Jaganyi and P. S. Madlala studied the kinetics of extraction of caffeine and mineral ions from coffee beans in relevance to different geographical regions. Explanations were given for the hindered slower diffusion within the coffee beans comparing with known diffusion coefficients obtained in water.

W. Göpel gave a survey on the principles of gas sensors, also commercially available to-day under the name of « electronic noses ». Due to different disadvantages of each sensory type a combination of various sensors is recommendable. An outlook for future artificial nose systems, representing more directly the recognition that is perceived in the human nose, was given.

W. Heilmann gave an overview on decaffeination processes including ways of caffeine recovery. Special emphasis was given to the caffeine recovery from activated carbon, which process is applied in CO_2 decaffeination.

V. D. Nagaraju and K. Ramalaxmi explored a new method of fluid bed technique for quick roasting of coffee. Samples from a lab scale roaster were analysed, referring to their biochemical and organoleptical properties.

The formation of 1:1 molar caffeine/caffeic acid complexes after adding caffeic acid to coffee solutions was reported by B. L. Zeller and F. Saleeb. Applicability for decaffeination of aqueous caffeine and coffee extract solutions was demonstrated on a lab scale base.

J. A. Vaessen in contrast to the technical programme here, stressed the importance of the consumer opinion that we always have to keep in mind.

Quality is a multidimensional concept. It has to be considered under its relation to the international trade as well as national and international legislation, the implications given by marketing and consumer perception and last but not least – quality must meet the expectations of the « emotional » consumer. This one, besides taste of the product, is including also other factors like wholesomeness, environmental and social aspects.

P. Justus described the advantages of a commercial available sorting machine for green beans. Besides monochromatic also bichromatic and fluorescence selection of unwanted coffee beans is possible. Latest developments comprise a trichromatic color sorting machine (Elexso), that covers the spectrum from UV to infrared.

Two experiments in spreading coffee knowledge, the first based on a CD ROM and the second on Internet were illustrated by F. Suggi Liverani. The usage of new multimedia technology, that incorporates hypertext, pictures, films and sound, easily allows to efficiently transfer knowledge and facts, selected out of various aspects of coffee science and technology.

Finally I have to thank the authors in Chemistry and Food engineering for their overall high standard of presentations in this symposium.

O. G. Vitzthum

Technology transfer

As a new field of discussion for ASIC, problems of technology transfer in rural circle, took place at the 17th colloquium.

Four keynotes were presented :

- In a « Message of a researcher to improve coffee production through a better use of research datas », Dr. R. A. Muller (ex-Scientific Director of IRCC/CIRAD, France) gave some recommendations : research results must be transmitted in total because they constitute a non-separable coherent package, and without any modification because they are obtained taking into account all bioecological constraints ; for the author a great effort of training of all extension agents and farmers is therefore needed ; the socio-economical environment of the farmers needs to be adapted (cooperatives or other types of farmers' associations) to give them more possibilities to use the knowledge given by the researchers.
- In a second keynote, Dr. B. Goud (CIRAD/SAR, France) gave a complete overview of the works carried out with Dr. B. Sallée (CIRAD/CP) in the coffee producing area of Coatepec (Mexico, Vera Cruz), through a French-Mexican cooperative project aiming at the modernization of coffee growing. During ten years, after having studied the physical and economical environment, the two authors worked in cooperation with farmers, who were the true actors of all the activities : farmers were organized in associations ; their first wishes being to improve the quality of their product, pulping and processing stations were set up with a used waters cleaning system ; rational use of fertilizers was also an important part of the project ; trials to diversify the production of the farms were done. All this work was conducted following an interdisciplinary and participative way.
- Pr. B. P. Louant (University of Louvain, Belgium) presented the third keynote speech which emphasized the need for farmers to be technically trained and professionalized to give a good level of intensification in coffee growing in the respect of natural environment. Important efforts must be done to bring them scientific messages : modern audio-visual methods with the help of computers seem to be the best way for transferring technology to farmers because they are very attractive. Two projections were given to illustrate these possibilities (one about the regeneration of coffee trees).
- The fourth keynote (Dr. J.-C. Chartier, BDPA, France) was not presented, the author being absent ; under the title « The difficulties of technology transfer in rural circle », this paper will nevertheless be found in the final Acts of the Colloquium, due to the importance of the subject.

Then, Dr. A. M. Karanja (Coffee Research Foundation of Kenya) proposed an analysis of the consequences of the

liberalization and privatization tendencies on coffee plantations productivity : the need of new techniques and the necessary organization of the farmers in cooperative structures are underlined.

For Dr. B. Nyambo (Coffee Research Foundation of Kenya), the search and the application of integrated pest management measures must be conducted with a strong participation from farmers. Since 1996, a project includes an important part on training of extensionists and farmers, to help them to reach a global approach and adopt sustainable production systems.

For Dr. M. T. K. Onsongo (Coffee Research Foundation of Kenya) speaking about the interactions between enterprises and resource disponibilities, technologies well adapted to small farmers must be found, taking into account their specific constraints : disponibility of land, work capacity, and financial resources.

The same theme was discussed by Dr. E. M. Njoka (Egerton University, Nairobi, Kenya) who explained what might be a sustainable coffee growing, respecting the environment, with soil protection and antierosion measures, use of a non-polluting pest management techniques; the author emphasized the need for a true integration of farmers to find and apply the methods and techniques adapted to those objectives.

Showing once more the difficulties and the lack of transmission of research data to farmers, Dr. J. O. E. Rakotomalala (Madagascar) said that, in his country, the diffusion of selected *Coffea canephora* clones was abandoned due to their requirements, too difficult to meet for small farmers ; on the contrary, seedlings, although less productive, but easier to be grown, are distributed.

Finally, Dr. P. Ngategize (Coffee Research Center, Kituza, Uganda) presented the African Coffee Research Network, which gathers various African countries with the cooperation of some European ones, and a financial aid from the European Union : this network might be a very good tool to lead fruitful researches in the future.

A poster, presented by Dr. E. M. K. Koinange (ARTI, Lyamungu, Tanzania), and Dr. P. Charmetant (CIRAD/CP, France/Tanzania), gave the main objectives of coffee research in Tanzania, and the measures applied for transfering the results to farmers, including the diffusion of selected clones produced through vegetative multiplication, and the control of CBD and leaf rust.

In conclusion, we can say that the problems inherent in technology transfer to farmers, the need for extensionists and farmers to be well trained, the necessary organization of farmers' associations, and the participation of farmers in the search and application of cultural techniques seem to be clear to everybody. Therefore it seems possible, through discussions and comparisons of experiences, to elaborate efficient methods for transferring to farmers the results obtained by researchers and, by that way, to improve their economical situation.

R. A. Muller

Agronomy

Four keynotes, thirty-seven oral communications and nineteen posters during the agronomy session provided an overview of advances in research in the different coffee growing countries in the world.

Genetic improvement

In his introductory speech on the breeding of *C. arabica,* D. J. Walyaro (Kenya) presented a report on the impact of major diseases and pests of coffee and analysed strategies currently employed in breeding for stable and durable resistance to the main coffee diseases. He also reviewed the important quality traits in Arabica coffee breeding.

Bellachew Bayetta made a review of coffee breeding programme in Ethiopia. A local germplasm collection of *C. arabica* comprising 2 000 accessions is maintained. Heredity of CBD resistance and hybrid vigor have been studied. Eighteen improved varieties, with resistance to CBD are being evaluated prior to release to the farmers.

A. Charrier summarized an original programme of genetic improvement of Arabica in Central America supported by CATIE, PROMECAFE and the French cooperation. The programme is based on the selection of F_1 hybrids varieties multiplied by somatic embryogenesis. This programme allows the shortening of the breeding time and the exploitation of hybrid vigour phenomenon.

C. Agwanda analysed the genotype × environment interaction for bean and liquor quality in Arabica coffee in Kenya. Differences between genotypes for liquor traits were best detected under conditions of water stress prevailing during the bean filling stage of berry development.

H. A. M. van der Vossen presented a critical analysis of Arabica coffee breeding programmes in five African countries based on their strengths, in respect to breeding strategies, new cultivars and technology transfer. He also emphasized the need to adopt improved breeding techniques, as well as regional and international collaboration.

Attempts have also been made to improve Robusta coffee production in tropical lowlands :

1. C. Montagnon has assessed the efficiency of the reciprocal recurrent selection (RRS) conducted in Côte d'Ivoire. For clonal selection he compared orthets *vs* clones and for hybrid selection the performances of progenitors *vs* hybrids.

2. N. E. Nyange has undertaken a collecting mission in Kagera region (Tanzania) on farmers plots. 141 new accessions of *C. canephora* have been introduced in the living collections for evaluation.

3. Studies of tetraploid Arabusta hybrids for improving the cup quality continued. E. Anim-Kwapong from Ghana presented the evaluation results of F_2 populations of true Arabusta. A. Rabemiafara described F_2 plants named Ratelo selected from a three-way hybrid, at the tetraploid level, in Madagascar. Original traits of the three progenitors species *C. eugenioides, C. canephora* and *C. arabica* were observed in few trees with limited abnormal fructification.

4. Introgression of quality traits from wild coffee species has been evaluated by P. Barre through a cross between a wild caffeine-free species from Kenya-Tanzania and a cultivated one, *C. dewevrei.* This transfer could be facilitated by the use of new techniques such as the *in situ* genomic hybridization, flow cytometry and molecular markers. The absence of caffeine could be determined by a major gene at the recessive status.

Plant biotechnologies

Biotechnologies applied to coffee breeding are developing in several directions :

1. T. Leroy presented the progress of the CIRAD-Nestlé France research on coffee genetic engineering. Successful transformation of somatic embryos of *C. canephora* with *Agrobacterium tumefaciens* and regeneration of transformed plants has been realized. The use of a synthetic gene of *B. t.* coding for five endotoxins against leaf miner is being investigated.

2. M. Sondahl and M. Petracco have paid attention to bred varieties with enhanced cup quality from somaclones

developed from interspecific populations of Icatu and Aramosa in Brazil.

3. M. Berthouly reported progress made at the different phases of the somatic embryogenesis in coffee. This is currently being tested in CATIE in *C. arabica* F_1 hybrids and in a laboratory set up in Uganda for Robusta.

4. A cryopreservation protocol for seeds has been developed for the first time by S. Dussert. Long term conservation of *C. arabica* genetic resources through dehydration, direct immersion in liquid nitrogen and embryoculture with a high rate of germination can now be envisaged.

5. The potential usefulness of molecular marker-assisted selection (MAS) for *C. arabica* was stressed by P. Lashermes. Its use in monitoring gene introgression and its integration in conventional breeding programmes were suggested. Preliminary results obtained for CBD resistance in Arabica coffee were given as illustration.

In the poster session, V. M. Carneiro showed the determination of ploidy level of coffee plants regenerated through anthers culture by chloroplast counting in stomatal cells.

N. E. Nyange obtained at SCRI plant regeneration from suspension culture of protoplasts established from hypocotyl-derived callus of two *C. arabica* genotypes.

Agronomic practices

Several examples were given of the agronomic systems and practices employed in different coffee producing countries.

J. M. Njoroge gave an overview of agronomic practices, from nursery to processing with particular reference to *C. arabica,* to promote sustainable coffee management.

M. Sondahl and M. Petracco have evaluated the efficiency of early Ethrel applications as a « running agent » of late flowers, and late Ethrel applications as ripening regulation on cultivars of Arabica coffee in Brazil. Early Ethrel application had a positive effect in cup quality, reducing the presence of immature fruits.

F. Amoah reported the performances of Robusta cultivars in Ghana under different conditions of shade, spacing and pruning.

J. W. J. Msaky analysed coffee Arabica yields in Tanzania after three cycles for three cultivars, different densities and their interactions, in order to make recommendations on the most economical practice to farmers.

M. Kanua determined the effect of intercropping sweet potato on the growth, and the yield of a typical variety of Arabica and assessed the economic benefits of permanent food crops *vs* coffee intercropping in Papua-New-Guinea.

M. Koudjega evaluated the positive effect of shade trees *Albizzia* species on yield of Robusta in Togo.

Some complementary information were reported in poster sessions :

– Ph. Vaast studied effects of pH, temperature, forms of nitrogen on uptake capacity of seedlings of Catuai.

– P. Dubale assessed effect of pruning, weeding and fertilization on yield at different locations in Ethiopia.

– M. H. P. Gathaara evaluated biomass accumulation in the various plant organs of Ruiru 11.

– V. Rutunga gave agronomic results on *C. arabica* in Rwanda.

The possibility of producing an organic coffee in Kenya is studied by Dr. J. K. Kimemia (Coffee Research Foundation of Kenya), who explained that there is a demand in European and American markets for this kind of coffee. According to some experimental results this coffee could be produced using disease-resistant Arabica hybrids avoiding use of pesticides, and replacing chemical fertilizers by organic ones, such as farm manure or green manure grown between the rows of coffee trees.

According to Dr. Ph. Vaast (CIRAD/CP, France), a good tolerance to the two main nematode species, *Pratylenchus* and *Meloidogyne,* is conferred to Arabica plants by early endomycorrhization in green house experiences. At the same time the plants become more vigorous than the others.

Drs. K. Ngoran (IDEFOR/DCC, Côte d'Ivoire) and P. Jadin (CIRAD/CP, France) studied the influence of several kinds of nitrogen fertilizers on the nitrogen storage of soils under Robusta coffee in Côte d'Ivoire and Cameroon. Urea maintained the content of nitrogen which can be mineralized at a higher level than ammonium sulphate.

Robusta coffee growth is studied in Indonesia by Drs. S. Abdoellah and A. M. Nur (Indonesian Coffee and Cocoa Research Institute), comparing the effects of organic material supplies, cover crop (*Calopogonium* and Vetiver), and nitrogen fertilizers. Organic material supplies are too recent (two years only) to have significant effects. But soil fertility is improved. In conclusion it is advised to add nitrogen fertilizers for a good growth of the trees.

Through a poster, Dr. P. R. Matowo (Agricultural Research Station, Lyamungu, Tanzania) gave a method to evaluate the efficiency of chemical herbicides in the control of weeds in coffee plantations.

Green coffee processing

In the chapter of crop first processing, Dr. J. K. Mburu (Coffee Research Foundation of Kenya) showed that it is possible to store in water the seeds after pulping. Water being renewed every day, the storage can last seven days without altering the quality of the good coffee beans, and with an improvement of the lower quality beans.

Two papers considered the protection of environment :

- For Dr. J. Ramirez-Martinez (Universidad Nacional del Tachira, Venezuela), pulps after silage give a usable product for feeding pigs and fishes.
- Dr. J. Noble (University of Leeds) explained a rational method to make the best choice between the different water clearing techniques, according to the different quantitative and qualitative parameters to be considered.

Finally, a sun-drying system completed with a wood burner is proposed to small farmers, by Dr. S. Mulato (Indonesian Coffee and Cocoa Research Institute), to improve the quality of their final product which is often moulds contaminated.

Through a poster, Drs. L. Lendaro (Agromatica, Italy) and A. Balci (Illycafe', Italy) present a new tool making coffee harvest quicker and easier.

Plant protection, plant pathology

In a first keynote, Dr. D. Nandris (ORSTOM, France/New-Caledonia) explained the rules of integrated pest management and the great complexity of that concept which covers all the biotic and abiotic factors involved in a pathosystem. According to these principles, a study was carried out in New-Caledonia, on three coffee diseases, orange leaf rust, cercosporiosis and die-back. This study carried out at various locations in the island took into account the plant and pathogen diversity, and the various environmental conditions. Thanks to powerful tools of analysis, this study gives the possibility of evaluating the risks and explains the dynamics of the diseases. The methodology is proposed as a tool for studying all cases of parasitic or non parasitic diseases.

In the same way, Dr. J. Avelino (CIRAD/CP, France/IHCAFE, Honduras) showed the results of a survey on coffee leaf rust, taking into account the levels of attacks, the various natural (soil and climate) and cultural environmental factors. This survey makes possible the prediction of the risks through a factorial and sequential analysis. Thanks to this method, it could be possible to adapt the chemical control recommendations according to local bioecological realities, while these recommendations are, up to now, too general to be adapted to each case.

The precise identification of a very serious parasite of Arabica coffee, *Colletotrichum kahawae* Waller, the causal agent of CBD, has been discussed by two authors :

• Pr. H. Hindorf (Institut für Pflanzenkrankheiten, University of Bonn, Germany) drew the history of the more and more precise denomination of this microorganism, leading to the actual name which clarifies the situation and avoids the confusions of the past, when all the *Colletotrichum* found on coffee tissues were considered as *C. coffeanum* ; the best criteria for a precise identification are given : morphocultural aspects, growth conditions in sterile media, molecular biological technics, and pathogenicity tests.

• For Dr. Bella Manga (IRA, Cameroon), working in the cooperative project ACRN which associates Kenya, France and Portugal for a CBD in-depth study, it is also necessary to recognize the various strains belonging to the *C. kahawae* species and existing in the different countries to identify for each place which regional pathogenic strain is to be fought through breeding programs for resistance. Based on the vegetative compatibility groups technique, and on pathogenicity studies, the work leads to identify two subpopulations (Cameroon and East Africa), the Cameroonian one being particularly virulent. It has been demonstrated 1) that there is no host-parasite interaction ; 2) that there is a great variability in the aggressiveness of the different Cameroonian strains : these two points suggest that the resistance to CBD is a non-specific one. Some Ethiopian Arabica genotypes appearing strongly resistant will be introduced in the breeding programs.

Finally, Dr. J. Flood (International Mycological Institute, U.K.) gave a picture of the *Fusarium* wilt of coffee : after having been very serious in the past in West and Central Africa, affecting Excelsa, Canephora Kouilou and Canephora Robusta, and having desappeared during almost 25 years, the disease came back ten years ago in the Democratic Republic of Congo and in Uganda, causing great damages to Robusta plantations. It is very urgent to find a solution : it is proposed to make a survey to identify the true causes of the resurgence of the disease (mutation of the pathogen, or changes in the natural or cultural environmental conditions ?). A workshop will be held in Kampala, Uganda, from July 28 to July 30, 1997, to draw the main lines of action to be adopted.

Two posters :

• Dr. G. O. Omondi (Coffee Research Foundation of Kenya) gave some data on the genetic diversity of *C. kahawae.*

• Dr. J. B. Birikunzira (Coffee Research Center, Kituza, Uganda) drew the lines of possible control strategies against the *Fusarium* wilt.

Entomology and integrated pest management

Coming back to the pest management concept, Dr. B. T. Nyambo (Coffee Research Foundation of Kenya) drew a very complete picture of pests, diseases and nematodes which can attack coffee trees, and showed the need for a global approach of these various ennemies : if we fight one of them, there is a risk of favouring the others. She underlined the necessary integration of the control measures in a sustainable agriculture, which respects the environment ; she thinks it is necessary to associate farmers themselves in the search and application of the recommendations ; the limits and the needs of such an approach were also discussed.

Dr. L. O. Brun (ORSTOM, France/New-Caledonia) studied the Coffee Berry Borer (*Hypothenemus hampei*) resistance to endosulfan, after ten years using this insecticide in New-Caledonia. Resistant populations of the insect have been identified in five of the fifteen regions of the island, but the level of resistance decreases when treatments are stopped. A genetic study of the resistance has been made : a reception gene was found, with a partial dominance of the resistance, which can be said « haplodiploïd functional », due to the degenerescence of half of the father chromosome material, the maternal one being completely transmittted.

Dr. H. Breilid (University of Bergen, Norway) presented a study on Coffee Berry Borer populations phylogeography. This study, based on the mitochondrial DNA analysis in populations coming from all over the world, leads to identify a Center and South American population, and a population involving individuals from Asia, Côte d'Ivoire, Jamaica and New-Caledonia ; through such a study it might be possible to know what was the spreading history of this pest. New-Caledonian populations being resistant to endosulfan and other cycladiene-type insecticides, it is necessary to consider that resistance for avoiding its dispersion.

Dr. Ch. Cilas (CIRAD/CP, France) gave the results of a study comparing field sampling methods to estimate Coffee Berry Borer damages. According to that critical comparative study, the preference might be given to systematic sampling methods and not to randomized ones. These methods seem a little difficult to be used when evaluating levels of attacks to decide chemical treatments, but they are well adapted for experimentation.

Finally, Dr. S. Sithanantham (International Center of Insects Physiology and Ecology, Nairobi, Kenya), emphasized the need of an international cooperation to carry out studies for an alternative solution to Coffee Berry Borer chemical control.

Five posters gave information about mealy bugs in Uganda (Dr. P. Kucel, Coffee Research Center, Kituza, Uganda), biological control of coffee pests in Kenya (Dr. G. O. Omondi, Coffee Research Foundation of Kenya), the relative attractivity of five types of coffee trees for Epicampoptera (Dr. B. Padi, Cocoa Research Institute of Ghana), the possible use of Mantas and Chameleons for the control of insects (Dr. E. Y. Mcharo), and abundance, diversity and geographical distribution of the Hemiptera living on coffee trees in Cameroon (Dr. P. Mbondji Mbondji).

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