Association Scientifique Internationale du Café

QUATORZIÈME COLLOQUE SCIENTIFIQUE INTERNATIONAL SUR LE CAFÉ
San Francisco, 14-19 juillet 1991

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R. COSTE

Secrétaire administratif permanent de l'ASIC

Nous voici, nombreux réunis à San Francisco pour le XIVème Colloque de notre Association.

Notre présence dans cette grande et belle ville, nous la devons à l'invitation de la "National Coffee Association" (NCA) des Etats-Unis en la personne de son Président, M. George Boecklin. Nous la devons aussi au Comité d'Organisation que préside le Dr. James Coughlin, assisté de M. M. Scott Mac Mullin et de M. James MacCarthy.

Je n'aurai garde d'oublier le concours diligent et efficace apporté à la préparation de ce Colloque par l'Agence "Meeting Planning Associates", dirigée par M. Gary Bertolucci avec qui, malgré notre éloignement, nous avons collaboré dans d'excellentes conditions.

A tous, et en particulier au Dr. George Boecklin, Président de la National Coffee Association" j'exprimerai en votre nom, nos très vifs sentiments de reconnaissance.

A cette adresse de remerciements je voudrais associer ceux qui, appartenant à la grande famille du Café dans le monde, n'ont pu se joindre à nous, mais conscients de l'intérêt des travaux de l'ASIC, partagent les sentiments, qu'en votre nom, je viens d'exprimer.

Quel chemin parcouru depuis la première réunion tenue près de Paris, à Nogent/Marne, en 1963, à l'initiative de l'Institut Français du Café et du Cacao (IFCC), réunissant une quinzaine de participants, puis la suivante, encore à Paris, en 1965, dans les locaux mis à notre disposition par le Centre National de la Recherche Scientifique (CNRS), avec alors près de cent participants. C'est d'ailleurs à l'issue de celle-ci, qu'à la requête pressante des congressistes, fut décidée la création d'une Association qui assurerait la continuité de ces réunions.

L'Association Scientifique Internationale du Café fut donc légalement constituée en 1966 et son premier Colloque, succédant aux deux réunions informelles précédentes, eut lieu en Italie, à Trieste, à la diligence du Dr E. Illy, notre premier président.

Au cours des années suivantes les réunions se sont déroulées tous les deux ans un peu partout dans le monde, alternant Pays producteur et pays consommateur, enregistrant chaque fois un franc succès. Ce furent successivement, après Trieste : Amsterdam, Lisbonne, Bogota, Hambourg, Abidjan, Londres, Salvador, Lomé, Montreux et, il y a deux ans, Paipa en Colombie avec, chaque fois 250 à 300 participants.

Aujourd'hui San Francisco, confirmerait, s'il en était besoin, le succès de l'ASIC dans le monde : un nouveau maillon, et quel maillon ! s'ajoute donc à cette succession de manifestations scientifiques internationales en faveur des caféiers et des cafés.

La documentation rassemblée par ces colloques, avec un millier de communications, dont de nombreuses prestigieuses par la haute qualification de leurs auteurs, publiées dans les volumes de comptes-rendus, constitue une source originale d'information d'une exceptionnelle valeur technique et scientifique sur les caféiers et les cafés, indispensable aux spécialistes comme aux professionnels.

Et demain ?

Les travaux de recherche dans le monde connaissent de nos jours une remarquable extension dans tous les domaines; les caféiers et les cafés y participent. On connaît la prodigieuse avancée des problèmes de multiplication caféière avec les cultures "in vitro" ... . Il faut s'attendre aussi à un approfondissement de nos connaissances en biologie cellulaire et plus particulièrement dans le domaine de l'équipement chromosomique de la cellule et de la localisation de ses fonctions avec, comme corollaire, les possibilités offertes par les manipulations et leurs conséquences pour l'amélioration des caféiers. La lutte contre les ennemis des cultures tend de plus en plus à faire appel aux ressources de la génétique, afin de mieux associer à la lutte chimique les moyens naturels de défense du végétal contre les adversités. Bien d'autres exemples pourraient être cités avec, entre autres, l'étude des composants aromatiques de l'arôme du café, dont le nombre dépasse aujourd'hui "le millier", celle de son action physiologique sur l'homme, etc.

L'ASIC est là pour rassembler, aux côtés des chercheurs, agronomes et techniciens, les professionnels des cafés, au cours des rencontres, amicales et fécondes, qu'elle organise périodiquement, à l'exemple de cette semaine à San Francisco. Elle donne ainsi la possibilité aux hommes de science d'échanger le fruit de leur expérience et de leurs travaux et leur en assure la diffusion dans le monde.

J'espère donc, chers amis, que nous nous retrouverons, encore plus nombreux, dans deux ans probablement, en France, à Montpellier, pour le XVème Colloque de notre Association.

A. F. BELTRAO

Directeur exécutif de l'Organisation Internationale du Café

Il y a vingt ans, j'ai participé, pour la première fois, à un colloque organisé par l'ASIC à Lisbonne. Ce colloque était, en fait, le cinquième d'une série qui avait débuté à Paris en 1963. Dès le commencement, René Coste, qui à l'époque était le Directeur général de l'Institut français du café et du cacao, a été une des forces motrices de cette entreprise et j'aimerais saisir cette occasion --étant donné que M. Coste est des nôtres aujourd'hui-- afin de rendre hommage à ses efforts inlassables, pendant plus d'un quart de siècle, en faveur de la recherche scientifique sur le café.

En juin 1980, le Colloque s'est tenu à Londres, au siège de l'Organisation Internationale du Café. A ce moment-là, l'économie mondiale du café était atteinte par des prix anormalement élevés du fait des gels qui avaient eu lieu au Brésil en 1975. C'est pourquoi j'ai dit, dans mon discours d'ouverture, que :

> "Pour obtenir une économie caféière saine dans laquelle des profits équitables sont assurés à tous les secteurs de la profession, il faut établir un lien étroit entre la recherche pure et appliquée, la gestion sociale et économique des ressources agraires et les politiques de production et d'approvisionnement. Sinon, nous continuerons à connaître dans l'avenir les mêmes cycles de prix élevés et de prix bas que dans le passé et les hommes de science et chercheurs exerceront les fonctions d'un service d'urgence au lieu de constituer un chaînon permanent du processus global de production et de commercialisation".

Aujourd'hui, nous nous trouvons dans une phase différente de ce même cycle : à la suite de la suspension des contingents en juillet 1989, les prix, en valeur réelle, sont plus bas qu'ils ne l'ont jamais été depuis les années trente. Ceci étant le cas, il n'est guère surprenant que je doive vous annoncer la triste nouvelle de la suspension des activités du Fonds de propagande de l'Organisation Internationale du Café et, par voie de conséquence, qu'il est mis un terme à l'appui fourni par l'Organisation, pendant de nombreuses années, en faveur de la recherche scientifique dans des domaines tels que le café et la santé. Néanmoins, en qualité de membre d'organismes de recherche établis en coopération avec le Fonds, tels que l'Institut Scientifique d'Information sur le Café (ISIC), piloté par le Dr. Illy, je continuerai à manifester un intérêt des plus constructifs à cet égard. En outre, les difficultés que connaissent actuellement les pays producteurs réduisent à la fois les programmes de recherche et les investissements dans de nouvelles techniques qui sont les fruits de la recherche.
Je suis, par contre, très encouragé de constater qu'il y a un volume important de nouvelles recherches sur le café actuellement en cours. Il me semble que la tendance clé, à cet égard, est que la recherche sur le café s'oriente vers un environnement beaucoup plus holistique.

En effet, par le truchement de la psychologie expérimentale, des techniques d'analyses sensorielles se trouvent maintenant liées à la recherche sur les mécanismes de perception et les fonctions cérébrales en passant par la composition chimique. Le vif intérêt que suscite la recherche visant à renforcer la résistance aux maladies, aux fléaux et autres agents nuisibles est également lié à l'enthousiasme croissant des consommateurs en faveur de la qualité du café. De même que les producteurs, les consommateurs se soucient davantage de la conservation de l'environnement. A ce sujet, je voudrais noter l'intérêt croissant, en matière de production, que suscite l'emploi de faibles niveaux de pesticides ou d'engrais (café organique) et je voudrais faire observer que, par comparaison à certaines autres cultures ou autres activités, la culture du café a tendance à respecter l'environnement naturel existant et à créer des communautés humaines dynamiques et stables.

Pour vous donner un aperçu sur l'avenir, j'aimerais rappeler l'occasion en 1980, lorsque j'ai demandé à M. Coste son avis sur les travaux accomplis par l'ASIC en matière d'amélioration de la qualité du café. M. Coste a bien voulu préparer un rapport qui a été diffusé sous forme de document de l'Organisation internationale du Café. Dans ce rapport il déclarait que "la question de déterminer, à des fins commerciales, l'arôme d'un café sur la base d'une analyse qualitative de ses éléments constitutifs peut paraître une entreprise utopique aujourd'hui". Il ajoutait qu'il espérait voir venir le jour où "le jugement subjectif des dégustateurs céderait le pas à des méthodes scientifiques afin d'établir les qualités organoleptiques du café". Il me semble que cet enjeu est toujours valable aujourd'hui. A sa façon modeste, l'Unité technique de l'Organisation, dirigée par M. Feria-Morales, qui est parmi nous aujourd'hui, a consacré ces dernières années à un effort en vue d'atteindre les objectifs de M. Coste. La nécessité d'une coordination internationale de la recherche, non seulement par le maintien de liens entre les hommes de science, mais en reliant cette recherche aux besoins économiques de la profession dans son ensemble, est un secteur d'intérêt vital auquel il me semble que l'Organisation internationale du Café peut contribuer de façon concrète en s'associant au thème de ce Colloque : "les aspects scientifiques du café dans un contexte global".

La coopération internationale, qui est reflétée de la façon la plus complète par la gamme de secteurs couverts par les Accords internationaux sur le Café, a, comme je l'ai déjà indiqué, traversé un certain nombre de cycles. Lorsque nous nous retrouverons dans un cycle plus positif, nous allons conseiller aux Membres, dans le cadre de l'Organisation internationale du Café, de considérer que la recherche scientifique, qu'elle soit liée à des préoccupation sur la santé ou l'amélioration de la quantité et de la qualité de la production, doit être traitée sérieusement et faire partie de tout nouvel Accord. Notre tâche aujourd'hui est de croîre fermement aux avantages de la coopération internationale sur le Café, de maintenir notre réseau exceptionnel de recherche et d'échanges de données et d'être prêts à promouvoir toute proposition qui permettra de donner à notre profession le meilleur avenir possible.

A.F. BELTRAO

Executive Director of the International Coffee Organization

Twenty years ago I took part in my first ASIC Conference, in Lisbon. That Conference was in fact the fifth in a series started in Paris in 1963. From the beginning, René Coste, who at the times was the Director General of the French Coffee and Cocoa Institute, has been a moving spirit behind this endeavour and I should like to take the opportunity --since Mr. Coste remains very much with us today-- to pay tribute to his unstinting efforts over a quarter of a century in the cause of scientific research on coffee.
In June 1980 the Conference was held in London at the headquarters of the International Coffee Organization. On that occasion the global coffee economy was affected by the situation of abnormally high prices arising from the Brazilian frosts in 1975. In those circumstances I said, in adressing the Conference, that :

> "We will obtain a healthy coffee economy with fair profit for all sides of the industry only if there is a close link between pure and applied research and sound economic and social management of land resources, production and supply policies. If this is not done we will continue to see in the future a repetition of the past cycles of high and low prices, with scientists and researchers being used as a sort of emergency service and not as a permanent part of the process of coordinated production and marketing".

Today we are in a different phase of just such a cycle : following the suspension of quotas in July 1989 prices in real terms are lower than at any time since the 1930s. In this circumstances it is hardly surprising that I have to convey the sad news that, as a consequence of the winding down of the Promotion Fund of the International Coffee Organization, the backing for scientific research provided by the Organization for many years in areas such as coffee and health can no longer continue, although I will still, as a member of research bodies established in cooperation with the Fund, such as the Institute for Scientific Information on Coffee (ISIC) under the leadership of Dr. Illy, take the keenest possible constructive interest in this area. To add to this, the difficulties currently being experienced by producing countries are curtailing both research programmes and investment in the new techniques which are the fruits of research.
On a positive note, I am very encouraged to note the enormous amount of new research in coffee currently under way. I believe that the key trend in this area is that research in coffee is moving into an altogether more holistic environment. Sensory analysis techniques are being linked through experimental psychology into research on the mechanisms of perception and brain functions, with a further link into the area of chemical composition. The intense interest in research into breeding for resistance to diseases and pests and other attributes also links up with increasing consumer enthusiam for quality coffee and a concern, amongst producers as well as consumers, for the preservation of the environment. In this connection I would like to note the increasing interest in production using low levels of pesticides or fertilisers (organic coffee) and to point out that, certainly compared with some other crops or activities, cultivation of coffee tends to respect the existing natural environment, as well as create dynamic and stable human communities.

If I were asked to look into the future I should like to recall the occasion in 1980 when I asked Mr. Coste to comment on the work done by ASIC on improving the quality of coffee. In a report volunteered by Mr. Coste and circulated as a document of the International Coffee Organization he stated that "the determination for commercial purposes of the aroma of a coffee on the basis of a qualitative and quantitative analysis of its constituents may still seen Utopian today". He added that he looked forward to the time when the "subjective judgment of tasters will give way to scientific methods of establishing the organoleptic qualities of a coffee.

I would suggest that this challenge remains valid today. In a modest way the Technical Unit of the Organization, headed by Mr. Feria-Morales, who is with us today, has devoted the last few years to fostering Mr. Coste's goals. The need for a coordination of research internationally, not just through maintaining links between scientists but by relating these matters to the economic needs of the global industry, is an area of vital concern and one where I believe the International Coffee Organization can contribute in concrete terms of the theme of this conference : "Coffee Science in the Globale Age".

As I have hinted already, the process of international cooperation, which, in its fullest form, is shown by the range of areas covered by the International Coffee Agreements, has been through a number of cycles. Within the framework of the International Coffee Organization, as and when we see a more positive cycle returning, we will be advising Members that scientific research, whether connected to concerns about health or improving the conditions and quality of production, must be treated seriously and become a part of any new Agreement. Our task today is to keep faith in the benefits of international coffee cooperation, to maintain our unique network of research and exchange of information, and be ready to promote all proposals leading to the goal of securing the best future for our industry.

A REVIEW OF THE EFFECTS OF COFFEE DRINKING ON MENTAL PERFORMANCE

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Research on behavioral effects of coffee and caffeine started with the pioneering work of Kraepelin and Hollingworth at the end of the last century and in the early part of this century. Before I go through the most recent work, it may help to give a brief overview of the functions that have been assessed as well as the particular methodological frameworks.

Mood is usually assessed by presenting the subjects scales that range from "very" to "not at all" for a given adjective such as tired, or from one mood adjective to an opposite one such as from calm to tense.

Attention and vigilance are mostly assessed in long-lasting and monotonous tasks. The subjects have to detect infrequent target stimuli against more frequent nontarget stimuli.

Sleepiness measurements have become highly standardized in recent years. Subjects are put to bed, the EEG is continuously monitored, and sleep latencies are objectively defined by the onset of sleep spindles and characteristic slow activity. As soon as this happens, the subjects are awakened.

Speed is measured in a multitude of more simple or more complex reaction tasks.

Motor performance measurements are mostly designed so as to assess speed, motor coordination or endurance.

Cognitive functions which involve learning, memory, and problem solving capacities are relatively easy to assess as long as one relies on standardized tasks. However, the extrapolation from such tasks to the complex overall functioning of cognitive abilities remains of course a very difficult goal.

Considering the reward value of coffee or caffeine, the strength of self-administration, its relation to tolerance, possible effects of withdrawal and correlations to personality have only recently become the objects of scientific discussion.

Neurological functions, which also have been approached only more recently, involve mainly the analysis of the EEG and of cerebral blood flow.

As a preface to the more recent results obtained with such methods I should like to refer you to a few of the most extensive reviews covering the earlier work.

Weiss and Laties [9] concluded early, in 1962, that a very wide range of behaviors (with the notable exception of intellectual tasks) can be enhanced by caffeine beyond a simple restoration of fatigue. But, also, that in this respect the superiority of the amphetamines over caffeine in unquestionable.

Gilbert [5, p. 76] used a quote from Prosper Montagné to characterize his impression: "Coffee soothes, eliminates the feeling of tiredness and exhaustion, makes mental work

easier, dispels drowsiness. A larger dose can bring on nervous excitation, trembling, insomnia."

Estler [4] remarked that the small number of well-controlled studies is in sharp contrast to the importance of caffeine as a commonly used psychostimulatory drug. The results are by no means unequivocal or clear-cut. Nevertheless, they substantiate the stimulatory effect on certain psychomotor functions, which becomes especially apparent when these functions are impaired by fatigue.

Dews [3], finally, pointed out that the effects of caffeine are modest and even subtle. It is clear that caffeine is not a simple stimulant. The evidence at present is compatible with the view that the selective effects of caffeine are not determined by the nature of the behavior on which it is imposed, but by the condition of freshness or fatigue of the subject.

My own review [1], which is in press, concentrates on studies of the last few years and also serves as the basis of this lecture. For my presentation I have also included a few results from our laboratory and tried to structure this complex material thematically.

Multitask Experiments

constitute the first topic. They have a long tradition going back to the turn of the century. The technique remains popular in human psychopharmacology with manifold drugs but only a few have been carried out over the last years using caffeine only as the active substance. The method has the advantage of serving as a screening for the profile of action across different abilities. It has also the disadvantage that testing lasts too long to be carried out both before and after ingesting a substance. Therefore predrug level of freshness or boredom and mood escape control, and only robust effects of a substance can be detected. The task which was significantly facilitated by caffeine in the two studies by Lieberman et al. (1987) [cited in 1] as well as in the study by Fagan et al. (1988) [cited in 1] was an auditory vigilance task. It is a long-lasting task of listening to monotonous peep signals and responding only to those few which are a bit louder or different in pitch or otherwise. This test, which lasts nearly an hour, was facilitated in the first study by Lieberman et al. by all doses of caffeine from 256 mg all the way down to 32 mg, and in their second study, by a low dose with and without the addition of 800 mg aspirin. In Fagan's experiment it was seen that the overall improvement was mainly due to the second part of the test, and a natural explanation would be that caffeine prevented fatigue. The effects on mood were remarkably inconsistent across the three studies and suggest that mood may also be affected by the tasks per se, as well as by the duration of the testing sessions.

A couple of additional multitask experiments have been done with caffeine and benzodiazepines, alone and in combination. The benzodiazepines, which are clinically used as anxiolytics, are also well known for their depressive effects on performance and for inducing sleepiness. The many derivatives that are used today differ in part considerably in their pharmacokinetics and thereby also in their sedative action. Roache and Griffiths (1987), Loke et al. (1985), and Ghoneim et al. (1986) [cited in 1] have used caffeine and diazepam at relatively similar dose levels. All studies measured several standardized reaction time, memory, and cognitive tasks and assessed several mood dimensions. Diazepam generally impaired performance and induced fatigue. Caffeine given alone improved only some of the performance measures such as reaction times or letter cancellation but also increased tension and restlessness at the higher dose levels. Caffeine mostly failed to antagonize the diazepam performance impairments but tended to antagonize diazepam-induced sedation of mood, whereas vice versa, diazepam antagonized high-dose caffeine restlessness.

The Ghoneim et al. study is particularly interesting, as it followed the plasma levels of the two substances across 3 hours. For both substances, these levels peaked between 90 and 120 minutes after administration and decreased thereafter by about 70 percent until the end of the third hour. The effects of the two substances on mood and performance also peaked simultaneously with the plasma peak levels but returned to near baseline levels at the end of the 3 hours, suggesting that for both substances acute tolerance develops rather rapidly.

Tasks requiring more specifically continuous

Attention and Short-term Memory

are the subjects of my second section. A series of tasks are based on the model used first by Bakan, and it requires not only prolonged attention but also short-term memory. The subjects watch randomized single digits presented in rapid succession on a screen. For each digit it has to be decided whether it is even or odd, and the same distinction has to be remembered for the two previous digits. Responses for an even digit are required when the two previous ones were also even, and the same has to be done for the odd numbers. The studies done with different versions of the task in the last years include the experiments by Bättig and Buzzi [2], Hasenfratz et al. [6], Smith et al. [8], Lieberman et al. (1987), and Fagan (1988) [cited in 1]. Among these, those experiments failed to detect improvements after caffeine which were easier than the original Bakan task. In the Lieberman experiment, a response was required whenever a figure 4 appeared that was preceded by the figure 7. In the experiment by Fagan, geometrical figures rather than digits were used, and responses were required for repetitions of the same figure. The three studies by Bättig and Buzzi [2], Hasenfratz et al. [6], and Smith et al. [8], which detected improvements after caffeine, differed in several additional aspects from the other two studies. They were not only more difficult as a result of adhering to the high difficulty level of the original version. In addition, the tasks lasted longer and were presented not only after but also before caffeine-containing coffee was drunk. In two of these studies, the level of difficulty was further raised with subject-paced presentation of the digits: more rapid after correct responses and slower after errors. These two studies were carried out in our laboratory, and I should like to comment on them in more detail.

For the Bättig and Buzzi [2] experiment we selected 10 female subjects for extreme extraversion and 10 others for the extreme of introversion. This personality aspect did not affect performance either with or without caffeine, and I will come back later on to the problem of possible interactions between personality and caffeine. The task was presented for the rather long period of 30 minutes both before and after drinking the test beverages. Before and after, and with or without caffeine in between, average performance, evaluated by the number of digits processed per minute, declined moderately from the first to the last 10 minutes by around 10 percent. The same was true for the maxima and minima of performance. Caffeine, regardless whether given at the dose level of 150 or 450 mg or whether mixed in decaffeinated coffee or fruit juice, elevated average, maximal, and minimal posttreatment performance similarly for all three successive 10-minute periods of the task. Although significance was reached for these effects, they remained modest in magnitude, amounting to improvements of about 5 to 10 percent .

The second experiment by Hasenfratz et al. [6] tested whether this effect might be modified by smoking a cigarette or having a lunch together with caffeine. The task remained the same as before. The pre- and posttreatment runs lasted 30 minutes in the lunch experiment with 8 female subjects, but only 20 minutes in the smoking experiment with 12 male subjects. A single dose of 250 mg caffeine was used in both experiments. Nearly no pre- to posttreatment differences were observed for the control condition without caffeine and without smoking. Similar performance increments of around 15 digits per minute, roughly 10 percent, were seen when the subjects got caffeine only, were allowed to smoke a cigarette only, or when both interventions were combined, and this was similarly the case for the first and second 10 minutes of the test. This raises the question, of course, why the beneficial effects of coffee and smoking on performance did not add to each other. One possible, although untested, speculation would be that the limits for improvement are small with this task and were already reached with caffeine alone or with smoking alone.

In the lunch experiment, we observed also minimal and nonsignificant improvements from the pre- to the posttreatment runs when neither a lunch nor coffee was given in between, and we observed a rather remarkable improvement for both the first and the second 15 minutes when only the coffee with 250 mg caffeine was given. The lunch alone failed to produce a postlunch performance dip as compared to the control condition, and caffeine given with the lunch did not affect in any way the missing lunch effect. There are two facts that may explain this outcome. The lunch that we provided may not have been sufficient to produce postlunch fatigue, as its size was not manipulated but fixed to a single pizza. On the other hand, the cardiovascular data which were also monitored suggested that the absorption of the pizza may have considerably slowed the absorption of caffeine.

Considering the nature of performance required by the Bakan test, it is clear that both sustained attention and concentration as well as short-term memory are needed. It is more difficult, however, to guess which of these functions was improved by caffeine. Short-term memory has in the past been investigated in several studies in a more specific

fashion, using the task of recalling previously shown word lists without time pressure. All these studies by Loke (1988), Foreman et al. (1989), Terry and Phifer (1986), and Erikson et al. (1985) [cited in 1] observed either no effects of caffeine or slight impairments for recalling the last words of the lists. This was discussed as an indication that caffeine might improve sustained memory, but also interfere negatively with short-term or immediate memory. Several explanations could be offered for the fact that improvements with the Bakan test, which also requires short-term memory, were seen rather consistently. One might be that eventual lapses of memory encoding in the Bakan test were offset by increases of attention and improved stimulus processing. Another possibility would be that the memory load with the Bakan test is smaller than with word lists and thereby not affected by caffeine.

Thus, for prolonged auditory vigilance as well as for prolonged attention requiring short-term memory, both other investigators and we ourselves obtained good arguments that caffeine might not only facilitate endurance but also accelerate some neuromental processes. In order to pursue this question I will relate my next section to

Reaction Times and Response Times.

A multitude of experiments has been done toward this question in the past. Jacobson and Edgley (1987) [cited in 1] most recently investigated this question in a very strictly controlled experiment. All the subjects had to do was to lift their arm as promptly as possible and as rapidly as possible; 300 mg caffeine shortened both the reaction time to the command signal and the speed of the arm movement. However, such a task leaves open the question as to whether the increment of speed was due to direct effects of caffeine on the muscular system or to an indirect effect on attention, concentration, and response readiness. The latter hypothesis seems more likely in view of the findings of Jacobson and Edwards (1990) [cited in 1]. They investigated caffeine effects on the speed of the monosynaptic arc of the knee-stretch reflex and were unable to see an acceleration with a medium dose of caffeine, but found an impairment with the highest dose of 6 mg/kg. Measuring reaction time to auditory stimuli, Roache and Griffiths (1987) [cited in 1] obtained improvements with caffeine, as did also many earlier studies.

Estler [4] in his review on reaction and response time studies commented that improvements are frequently found but their appearance and robustness depend to a considerable extent on different factors such as the habituation to caffeine, the initial response level, the rigidity of the experimental design, and several predrug conditions.

A recent example are the coffee, lunch, smoking experiments by Hasenfratz et al. [6], for which I already presented in the previous section the positive effects on the rate of processing single digits. In this task the subjects are not required to press the response button for the target stimulus as rapidly as possible. In fact, the computerized program accepts responses for a given digit until the disappearance of the next stimulus. In addition to the scoring of the responses for correctness, the response times to the target stimuli were also measured. These were shortened by caffeine both in the caffeine-smoking interaction experiment and in the lunch-caffeine interaction experiment. However, this improvement appeared for the second 10 or 15 minutes of the test runs only, whereas correctness of the responses was improved throughout the entire runs, as reported in the previous section.

In a recent experiment, we investigated the effects of caffeine on speed using the rather complex Stroop task paradigm. The Stroop task compares response speed between straightforward and conflicting stimuli. As a straightforward stimulus, the subject may see the word RED printed in red letters and is required to answer with "red." If, however, the word RED is printed in green letters, the stimulus becomes conflicting, and in general more time is required for a correct response. We used a numerical version of the task. Symbols are presented as nonconflicting stimuli, and the subjects' task is simply to count them. Digits are used as the conflicting stimuli because they can be both read and counted, and the task is not to read them but to count them. The subjects had to perform the computerized task before and after the treatments, each time for 15 minutes. Twenty female subjects took part in four experimental sessions with different treatments. Once no treatment was given, once smoking was allowed, once coffee with 250 mg caffeine was offered, and once caffeinated coffee and a cigarette were combined. In addition, the difficulty level was varied in all runs by presenting half the stimuli at 0-second intervals and, as the easier condition, the other half of the stimuli at 1-second intervals.

The obtained data revealed that the reaction times were affected by the difficulty level as well as by the caffeine and smoking treatments. As was to be expected, the longest reaction times were obtained when numbers as conflicting stimuli were presented in immediate succession at 0-second intervals. When the same conflicting stimuli were presented at 1-second intervals the reaction times became slightly, although consistently, shorter. They became still shorter when the nonconflicting symbol stimuli were presented at 0-second intervals, and the shortest reaction times were obtained when these nonconflicting stimuli were presented at 1-second intervals. With all four conditions, the response times changed only minimally from the pre- to the posttreatment run in the control condition when no treatment was given. For all treatments, however, smoking alone, caffeine alone, and the combination of the two, improvements of similar magnitude were obtained.

Most Stroop task experiments, however, have concentrated on the difference between conflicting and nonconflicting stimuli as an index of the speed of mental processing rather than on the separate reaction times to either one of the two stimulus categories. We also analyzed this difference, which in the literature is commonly designated as the Stroop effect.

With the control condition, the Stroop effect at 0-second intervals was greater after than before treatment. This prolongation might presumably have been a consequence of fatigue. With smoking or caffeine, however, the posttreatment Stroop effect became smaller than the pretreatment effect, but this was not the case with the combination of the two treatments. On the other hand, with the 1-second interval between the stimuli the effect was smaller, did not increase from pre- to posttreatment in the control condition, and changed only slightly with the treatments to the extent that significance was missed in contrast to the 0-second interval condition. This result fits well with the already mentioned impression of Estler [4] that improvements in reaction with caffeine depend very much on how much room there is for improvement. It is of course smaller, the more prompt and rapid the baseline performance is without the drug. Nevertheless, such results can be taken as an indication that caffeine may accelerate some yet undefined mental processes.

This hypothesis was recently explored at our laboratory by Mr. Berz, a doctoral student, who analyzed the effects of caffeine in rats on a DRL task, which could be considered as a test for time estimation. For this test, the animals are first trained to press a lever to get single food pellets. Then they are trained to wait with lever pressing for progressively longer periods after each food pellet. In this way the animals were trained to wait 18 seconds after each reward. Earlier responses remained not only without reward, they were even punished by postponing the reward availability by another 18 seconds. A rather large group of 18 animals was trained in this task until they reached an efficiency level of 60 percent rewarded lever presses. All animals then underwent the task six times, each time with a different dose level of caffeine. The great number of data resulting from this experiment allowed a detailed analysis of the distribution of the responses of the animals across successive interresponse intervals. Without caffeine, the peak of responses occurred 19 seconds after the last reward, and for these responses and all responses that occurred even later the animals got their rewards. With 12 mg/kg caffeine this peak frequency decreased to 17 seconds, and with the high dose of 24 mg/kg this peak decreased further to 13 seconds. The result might suggest therefore that subjectively for these animals time runs progressively faster the more caffeine they were given. Assuming such a speculation, an analysis of brain activity would be tempting.

The most classical approach to assess brain activity consists in the analysis of the electroencephalogram (EEG). For this reason

Brain Waves and the EEG

will represent the next topic. The traces of the electroencephalogram are highly irregular, as the activity is composed of different frequencies. The usual procedure to analyze such patterns consists in the spectral analysis. Such analysis reveals that power increases for the low delta (1-3 cps) and theta (4-7 cps) frequencies in deep sleep. Rather regular patterns also appear in the alpha frequency band from 8-12 cps when a subject in the awake state closes his eyes and tries to relax. These EEG states are also called the synchronous stage, and they are typical for the inactive states of deep sleep and drowsiness. Higher, more irregular and rather low amplitudes become typical in states of alertness. The EEG is then dominated by frequencies from 13 all the way up to 30 or even 50 cps. This is called the asynchronous state or beta band of the EEG.

However, performance efficiency is by no means a direct function of EEG asynchrony. With increasing EEG asynchrony, performance efficiency increases first up to a maximum. With further increases in asynchrony, the behavioral state changes to overarousal and even anxiety with decreases of performance efficiency.

Caffeine and the arousal state of the brain have so far been considered in several contexts and situations.

A first one might be seen in the effect of caffeine on the EEG in the awake but relaxed state. Only a few studies have been done so far. Knott [7], who has recently reviewed the five studies available so far, had to remark that these studies differ considerably both in their experimental design and in their procedures for data analysis. Mainly on the basis of the study which can be considered to be the strongest from a methodological point of view he proposes that caffeine might be expected to decrease power in the theta and alpha frequency range and to increase the peak frequency within the alpha range and eventually also the beta range.

A first attempt by our laboratory to look for such changes was done in connection with the experiment involving the Stroop task, for which I have already presented the performance data. In the rest periods before and after the treatments the EEG was recorded using three different electrodes.

The main findings with caffeine were a marginal increase of the peak frequency in the alpha band, a decrease of power in the low frequency theta range, and an increase of power in the beta range. These changes fit rather well the expectations formulated by Knott. A perhaps even more important finding can be seen in a comparison of the changes induced by smoking. These differed from those of caffeine in several respects. The smoking effects were clearly more robust than the effects of caffeine, but they were also shorter lasting. Furthermore, it appears that the two substances affected the EEG in a differential and distinct fashion, as no interactions were obtained for any of the multiple variables.

Another approach to relate effects of caffeine to cortical arousal state is a rather hypothetical one based on the assumption that some personality patterns are related to cortical arousal states.

The work on the

Personality-Arousal-Caffeine

interaction is based mainly on the personality dimensions elaborated by Eysenck. According to this hypothetical concept, introverts are characterized by high arousal states of the brain, whereas extraversion would go along with a low arousal state of the brain. In recent years, it was proposed that impulsivity, which is a subscale of extraversion, or sensation seeking, which is closely related to extraversion, might better fit the postulated relation than the original dimension of extraversion. Several studies have followed this proposal in the last years with caffeine as an instrument to manipulate arousal. The prediction of such studies is that caffeine, as an enhancer of arousal, would facilitate performance in extraverts, because their arousal is too low, and that it would impair performance in introverts, because they become overaroused. However, the reported results are rather inconsistent. Anderson et al. (1989) [cited in 1] tested memory scanning as the dependent performance and impulsivity as the personality dimension, but they obtained no caffeine-personality interaction. The same negative finding was reported by Arnold et al. (1987) [cited in 1], who tested memory for word lists. A tendency toward the working hypothesis was obtained by Gupta and Gupta (1990) [cited in 1], who found dose dependent improvements of performance in high impulsives. However, they did not obtain the expected impairments in the low impulsives in a dose dependent fashion. Finally, Davidson and Smith (1989) [cited in 1], who measured skin conductance changes elicited by digit span tasks as an objective measure of vegetative arousal, also failed to see any significant interactions between sensation seeking and caffeine effects.

Taken together, although the scientific theoretical background of these studies is highly interesting, the results in general give only weak support to the idea of a robust interaction between personality and the effects of caffeine. In addition, it also seems that direct and objective EEG-based measures of arousal would be needed to further pursue this hypothesis.

More robust findings with caffeine were obtained in

EEG-Based Sleep Latency Studies.

The onset of sleep goes along with different characteristic changes of the EEG pattern. These changes are now analyzed in a highly standardized fashion. Subjects are fitted with EEG electrodes. Then they are put to bed, the light is turned off, and the subjects are told to relax and try to sleep. The time until the onset of the sleep patterns is considered as sleep latency. As soon as such patterns appear, the subject is waked up, taken out of bed, and the procedure is repeated, usually every 2 hours. Lumley et al. (1987) [cited in 1] allowed subjects to sleep 11, 8, or only 5 hours before either caffeine or alcohol were given in the morning. The shorter the night rest, the shorter the sleep latencies were throughout the next day. This effect was clearly enhanced by alcohol and attenuated by caffeine. Walsh et al. (1990) [cited in 1] monitored sleep latency throughout the night. As expected, the latencies became gradually shorter throughout the night. Caffeine given before bedtime prolonged the sleep latencies similarly throughout the night. Therefore the effect of caffeine did not fade off, although it can be assumed that the plasma levels of caffeine had decreased substantially. Rosenthal et al. (1991) [cited in 1] reported a similar finding. They let the subjects sleep either 5 or 8 hours and observed shorter sleep latencies on the following day after the shorter duration of sleep. However, the lengthening of sleep latencies by caffeine was independent of how much the subjects had slept the previous night. Johnson et al. (1990) [cited in 1] manipulated sleepiness by giving subjects different doses of benzodiazepines on the previous evening. With benzodiazepines, the subjects were sleepier the following day, as assessed by the sleep latencies, and caffeine significantly antagonized this sleepiness, although it failed to antagonize the benzodiazepine-induced performance drop. The only study which failed to observe caffeine antagonism of benzodiazepine sleepiness was that by Roehrs et al. (1988) [cited in 1].

Taken together, these studies on sleepiness at night or during the day as a consequence of either previous sleep deficits or benzodiazepine medication reveal the quite interesting picture that caffeine appears to be more efficient in maintaining wakefulness than in improving performance when under the influence of sedative drugs.

A final aspect, which has received scientific attention only in the last year, is the subject of my last section.

The Pleasure and Reward

of coffee drinking have recently been proposed by several researchers as a factor in drug dependence which might even lead to considering coffee as a drug of abuse. This argumentation borrows heavily from the arguments that were put forward a few years ago by the Surgeon General of the U.S. for seeing cigarette smoking as drug abuse, similar to heroin and cocaine. Holtzman (1990) [cited in 1] argued recently on the basis of these ideas that caffeine could indeed be considered a drug of dependence and potential abuse. He further remarked that, as negative effects on physical, psychological, or social health are not documented, caffeine would be an ideal substance for investigating the development and underlying factors of drug dependence. Looking more closely at the available facts, however, the arguments are in general weak.

A first prerequisite for a substance to produce dependence would be that it can be recognized. In quite many studies comparing decaffeinated and caffeinated coffee, the subjects were asked afterward to guess which one they had received. The answers were quite consistently at chance level. A first systematic experiment using a design which is common in animal experiments was done by Chait and Johanson (1988) [cited in 1]. This careful experiment revealed only poor discrimination and for the high dose only. In contrast, Griffiths reported an experiment in which he and the six coauthors of the study (1990) [cited in 1] were able to detect doses of caffeine all the way down to between 5 and 30 mg. However, the conditions for detecting subjectively the effect of the substance were optimized to a degree that may call for caution. At short intervals, these six subjects swallowed two capsules, and they knew one of them would contain caffeine. The possibility that the placebo capsule was not an ideal placebo can therefore not be excluded.

Self-administration in a dose dependent manner would be another, more direct prerequisite for drug dependence. It is extremely poor in animals, as reviewed by Griffiths and Woodson (1988) [cited in 1] and limited in humans to small doses when given in capsules, as done by Griffiths and Woodson (1988) [cited in 1] and by Stern et al. (1989) [cited in 1]. Furthermore, Griffiths et al. (1986) [cited in 1] also observed that extremely high coffee consumers did not change the number of daily coffee cups when given decaffeinated coffee or preloads with caffeine. In another experiment, Griffiths et al.

(1989) [cited in 1] tested how much physical work would be done on an ergometer bicycle to obtain a cup of coffee or a capsule with 100 mg caffeine. Indeed, some subjects worked up to half an hour for that goal. However, as the subjects worked similarly for decaffeinated coffee, it remains open to what extent they worked for the taste of coffee or the effects of caffeine.

A further aspect of dependence would be seen with possible withdrawal effects. Griffiths and Woodson (1988) [cited in 1] have reviewed all reports on this matter up to 1988. These considered mostly single cases and were extremely inhomogenous. Probably the first experimental study was done by Griffiths et al. (1986) [cited in 1] in a small group of extremely heavy consumers drinking on the average about 15 cups of coffee per day. Replacing under blind conditions caffeinated with decaffeinated coffee was accompanied by slightly increased fatigue for about 1 day and slight headache for about 3 days. Rizzo et al. (1988) [cited in 1] tested reaction time before 1 week of abstinence and again after this period in both regular and infrequent users and observed an improvement of performance in the infrequent users but not in the users.

An interesting paradigm which also might help to explain dependence is that of state-dependent learning, which is frequently used in animal psychopharmacology. With this paradigm, learning is done under the influence of a given drug, and retention, as measured later on, is usually better when it is tested under the influence of the same drug rather than another one or placebo. Lowe (1988) [cited in 1] observed this effect with alcohol, caffeine, smoking, and placebo.

Drug use is also frequently related to distinct personality patterns. Andrucci et al. (1989) [cited in 1] found personality patterns to be related to alcoholism. However, the relations to coffee use were negligible with the exception of a slight correlation to sensation seeking, which could hardly be seen as specific.

Relations between personality and coffee consumption were also analyzed by our lab in a sample of about 340 females aged 20 to 40 years. Overall the relations were very modest. The strongest one was seen for age. Coffee consumption increased in this sample significantly with age, but this did not explain more than about 5 percent of the variance and is consistent with many other reports.

All relations to personality factors which reached significance were even more modest but consistently in a favorable direction. The suggested tendencies are that frequent consumers are less anxious, less nervous, less depressed, less restrained, and more stable, self-confident, and extraverted than infrequent users. This is an overall picture which is quite different from that seen for alcohol abuse or chronic use of any illegal drug.

A further question to be considered is whether coffee consumption leads to chronic tolerance, which could explain the increased consumption. Several studies in the last few years have compared heavy vs. moderate or infrequent users. Lieberman et al. (1987) [cited in 1] found no difference between such groups in the effects of caffeine in a multitask experiment, and a similar negative result was obtained by Landrum et al. (1988) [cited in 1] for caffeine effects on writing, reading, and learning and by Zahn and Rapoport (1987) [cited in 1] for effects of caffeine on skin conductance. Several other studies found differences under placebo conditions which place frequent users in an even better light than infrequent users. In Erikson et al.'s study (1985) [cited in 1] the females, with a generally higher self-reported coffee consumption than the males, outperformed the males on memory performance without caffeine, and a similar observation was made across all subjects by Kuznicki and Turner (1986) [cited in 1]. Finally, Zahn and Rapoport (1987) [cited in 1] found nonusers to be more reactive in skin conductance responses than users.

Summarizing

the results of these numerous studies of the last five years might be easiest by going through the different sections of my lecture. Multitask experiments as a tool to characterize the action profile suggest that caffeine quite specifically helps to fight the consequences of fatigue in long-lasting and monotonous tasks. Usually such tasks require continuous discriminations but not memory. Continuous prolonged attention and short-term memory are involved in the Bakan type tasks, and in these caffeine improved performance, although studies specifically for memory obtained no effects or, in some cases, even slight impairments with caffeine. Reaction times and response times are quite consistently accelerated by caffeine under the condition that rigid experimental designs are used. Only a few studies have been done so far on the effects of caffeine on the EEG in the awake state, and they suggest that caffeine modestly arouses cortical activity. On the other

hand, theoretical concepts on arousal differences between personality types and corresponding differential effects of caffeine are only weakly supported by facts. The ability of caffeine to antagonize sleepiness has been supported quite unequivocally with objective EEG-based methods. It even appears that this may perhaps be the most robust one among the many effects of caffeine.

However, the question as to how and why caffeine acts as a reward requires a closer consideration of the important problem of tolerance. This aspect has recently been clarified to a remarkable extent for the cardiovascular effects of coffee and caffeine [see 1]. The findings are quite straightforward: In nonusers or in users who have been cleared of caffeine by a sufficient period of abstinence, doses in the order of 200 mg and upward produce modest but significant increases of blood pressure. However, under daily-life conditions when such an amount is distributed in several smaller doses over the day, the effects on blood pressure become minimal or absent, a finding which has been obtained in several ambulatory field studies over periods of up to several weeks. This type of experimentation, however, is to date nearly absent in the behavioral investigation of coffee. By far most of the results reported so far are based on effects seen in subjects treated after previous abstinence with doses exceeding those of a normal coffee serving.

What results would have to be expected if behavioral functions were assessed under ambulatory conditions? Although an answer cannot be given, one would expect that the effects of the substance would be even more subtle than in the laboratory experiments that I have summarized in this lecture.

This makes it quite unlikely that the postabsorption psychopharmacologic effects would be the main or only reason for drinking coffee. Most subjects report that drinking a cup of coffee constitutes a reward per se in terms of the gustatory and olfactory experience. To what extent such qualities constitute a primary reward or a sensation conditioned to the positively experienced postingestive effects is a question that is still wide open.

The arguments for seeing caffeine or coffee consumption as a behavior of dependence are weak in comparison to what could be said in this respect for cocaine and amphetamine. Dose titration is very weak or missing completely. The same holds for chronic tolerance and for withdrawal symptoms. In addition, the effects of the substance are mostly in the positive direction, and the use of single high doses to induce toxicomanic states is unknown for caffeine. Therefore I would expect that future research will see reward and dependence in more relative terms and eventually rediscover the category "Genussmittel" or "substance of enjoyment."

Summary

A review of the effects of coffee drinking on mental performance

Research on caffeine effects on performance in the last years can be seen under four different headings. The classical approach, going back to the early work of Kraepelin and Hollingworth, consists in measuring behavioral performance (attention, speed and endurance in stimulus processing, memory, learning, etc.) in subjects given relatively high doses after previous abstinence. A second approach consists in using the same methodology in subjects presumed to differ in their baseline cortical arousal level (introverts vs. extraverts, etc.). A more recent approach consists in the application of objective EEG-based measurements of sleepiness during the daytime. The most recent area of research concerns the rewarding mechanisms underlying the popularity of coffee beverages. Surprisingly, however, there remain also very interesting questions which so far have not been addressed. Almost no research has been done to delineate pharmacological and behavioral tolerance. The role of gustatory and social pleasure with coffee drinking has also escaped so far the interest of modern science.

Résumé

Compte-rendu des effets de la consommation de café sur les performances mentales

Les recherches effectuées ces dernières années, concernant les effets de la caféine sur les performances mentales peuvent être examinées de quatre manières différentes. La méthode classique, se référant aux premiers travaux de Kraepelin et Hollingworth, consiste à mesurer la performance comportementale (attention, vitesse et endurance dans le traitement de stimulis, mémoire, apprentissage, etc.) sur des sujets à qui l'on a donné des doses relativement fortes après abstinence préalable. Une deuxième méthode consiste à utiliser la

même méthodologie sur des sujets présumés différents quant à leur niveau d'éveil cortical de base (introvertis et extravertis, etc.). Une méthode plus récente consiste en l'application de mesures objectives, basées sur l'EEG, de la somnolence pendant la journée. Le domaine le plus récent de la recherche concerne les mécanismes de satisfaction qui sont à la base de la popularité des boissons caféinées. Etonamment, il reste des questions très interessantes qui n'ont pas encore été abordées. Presqu'aucune recherche n'a été entreprise en vue de décrire la tolérance pharmacologique et comportementale. Le rôle du goût et du plaisir social liés à la consommation de café a échappé jusqu'à présent à l'intérêt de la science moderne.

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AN EVALUATION OF EPIDEMIOLOGY STUDIES OF COFFEE CONSUMPTION AND CANCER

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Introduction - The epidemiological study of the possible association between coffee consumption and cancer in humans has been pursued for several decades. In attempting to identify the possible causes of cancer in humans, the examination of coffee is a natural consequence of it being the beverage of highest consumption in the world. Although many organ sites have been studies over the past years, the majority of the data relate to five, bladder, breast, colon, ovary and pancreas. By far the organ site that has received the most attention is the bladder, and that will be examined in detail in the following presentation. This evaluation will address the other four organ sites where substantial data are available examining the association between coffee consumption and cancer.

Methods - The procedures used to depict the data that will be presented are those described by Peto (1). In no sense is the presentation meant to be a formal meta-analysis with rigorous analytic methodology or statistical treatment. Rather, the intent is to present the total body of relevant data as reported by the authors. The model for the data presentation is shown in Figure 1. The data elements are the point estimates of relative risk for cancer at each of the organ sites, the 95% confidence limits, where they have been reported, and a measure of the size of the case population of each study as depicted by the size of the box at the point estimate of relative risk. While a formal meta-analysis was not carried out, these data elements do provide a measure of the uncertainties and power of the studies used in this evaluation. In addition, an overall relative risk was calculated based on a weighting of the individual study relative risks by the size of the case population in each study.

Results and Discussion - The evaluation of the studies dealing with breast cancer provide strong support for the view that coffee consumption is not associated with breast cancer occurrence in women (Figure 2). The relative risk values cluster closely around 1.0, with 2 major studies totaling over 3000 cases demonstrating no increased risk with coffee consumption. Lubin and Ron have recently reviewed this subject (2).

For colon cancer, there is increasing evidence the coffee consumption may, in fact, exert a protective effect (Figure 3). The consistency of findings of relative risks for colon cancer significantly below 1.0 are quite persuasive in supporting this conclusion. Except for several studies with very small case populations, the reported values in the majority of publications for relative risk of colon cancer range between 0.5 and 0.85. The possible mechanisms for this finding are unknown at the present time, but the strength of the evidence suggests that one or more components of coffee may exert an inhibiting effect on tumor formation. Such materials have been identified in experimental animal carcinogenesis studies (3). An overview of colon cancer and coffee consumption has been reported by Rosenberg (4).

The analysis of the data for cancer of the pancreas is made difficult by the uniform small sizes of the case populations throughout the entire range of studies considered. Pancreas cancer is a disease of rare occurrence, thus the case sizes will be small, accompanied by general large confidence limits in the statistical analysis of the relative risk values. The study of the relationship between coffee consumption and pancreas cancer had its impetus from the report by McMahon et al. 1981 (3), showing a significantly increased risk for cancer at this site associated with coffee consumption among a set of hospital patients. A later more complete analysis with a larger study population reported a much lower relative risk (1.3), with the lower boundary for the 95% confidence interval below 1.0 (4). The relative risks in individual studies ranged from 0.6 to 1.6, with the combined relative risk of 1.22. (Figure 4). These findings with the wide statistical boundries do not indicate an association between coffee consumption and cancer of the pancreas. The issue of confounding factors, especially smoking, requires careful attention, since several investigators have shown a strong association (Relative Risks - 3.2 - 5.15) of cigarette smoking with pancreas cancer. As Morrison has shown for bladder cancer, the inclusion of a relatively small proportion of misidentified smokers in a study population can substantially influence the relative risk values yielding an inappropriate association (5). A discussion of the general issues of pancreas cancer and coffee consumption has been presented by Gordis (6).

There were 7 studies on ovarian cancer considered, and they exhibit a wide range of relative risks from 1.0 to 2.2, with a combined relative risk of 1.4 (Figure 5). Those studies reporting 95% confidence limits show them to be very large. In contrast to the studies of cancers at other organ sites, there appears to be a dichotomy of results, with 4 showing relative risk values at or near 1.0, 2 studies showing relative risk values of about 2.0, and one small study with an intermediate value. The reasons for the wide disparity among the results of these studies is not apparent, however, there is a possibility that some factors not associated with coffee drinking may be responsible for the findings in the studies reporting relative risks of 2.0 or higher. Leviton has recently reviewed the epidemiology literature related to ovarian cancer and coffee consumption (7).

Though epidemiological studies often center on a single exposure and single outcome, the evaluation of an exposure pattern, especially such a highly integrated one as a dietary factor, raises questions about whether the outcome is related in a causal way to the exposure, or rather to some associated behavior that may be the true causal influence. The extremely high quantitative correlation of coffee drinking with cigarette smoking demonstrates the need to consider associated behaviors when making causal inferences relating to exposure and outcome (Figure 6). Beyond this sort of direct association, there have been identified other behavioral patterns that associate with coffee consumption, such as a diet with high fat, low fiber and low vitamin intake and a high stress score (Table 1)(7). Moreover, the extremely complex chemical nature of the foods in the diet of most humans strongly indicates caution in ascribing an elevated risk for cancer attributable to any single dietary intake.

When the epidemiological data on coffee consumption and cancer occurrence in human breast, colon, pancreas and ovary are examined and compared, the evidence strongly suggests that coffee consumption is not associated with increased cancer risk at any of these organ sites.

Summary - The published data for epidemiology studies relating to coffee consumption and human cancer are reviewed for four organ sites, breast, colon, ovary and pancreas. For each organ site, the data were aggregated using the descriptive procedure of Peto. For breast cancer 10 studies were evaluated and the combined relative risk was 1.08, with a range from 0.6 to 1.26. For colon cancer
(15 studies), the combined relative risk for cancer was 0.72, with a range from 0.5 to 2.0. For pancreas (16 studies), the combined relative risk was 1.22, with a range from 0.6 to 1.6. For cancer of the ovary in 7 studies, the combined relative risk was 1.4, with a range of 1.0 to 2.2. Thus, for breast and colon the data clearly show no increased cancer risk associated with coffee consumption, and for colon there is evidence for a protective effect. For ovary and pancreas, the small increase in the calculated relative risk, taken with the large ranges, including relative risks of 1.0 or lower in some studies, strongly suggests that coffee consumption is not linked causally to cancer occurrence at these sites.

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FIGURE 1

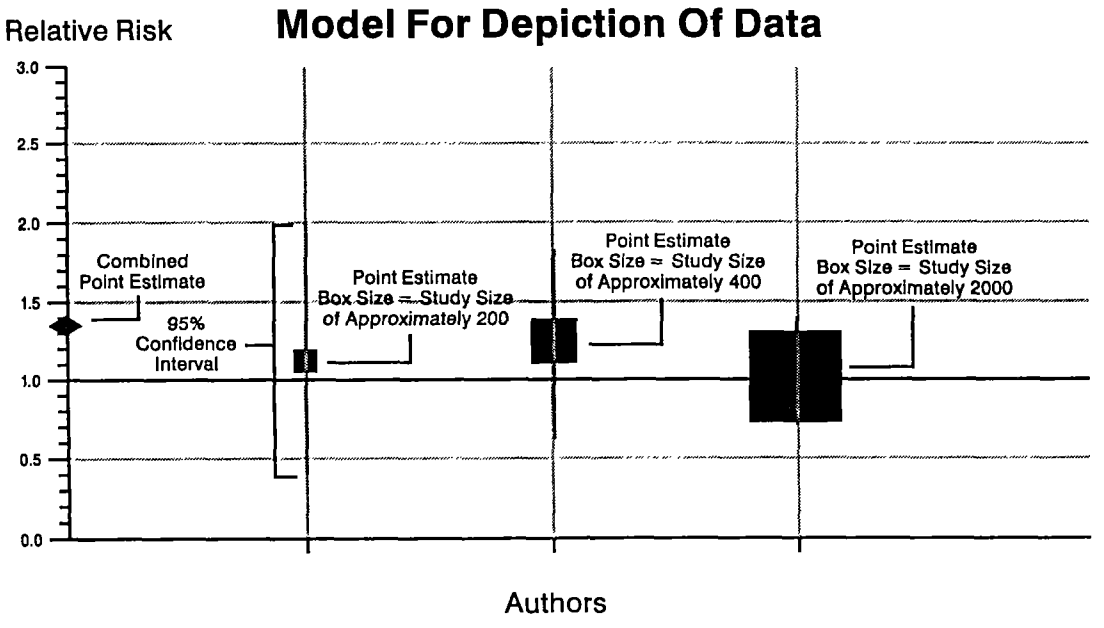


FIGURE 2 - EPIDEMIOLOGY STUDIES ON BREAST CANCER AND COFFEE CONSUMPTION

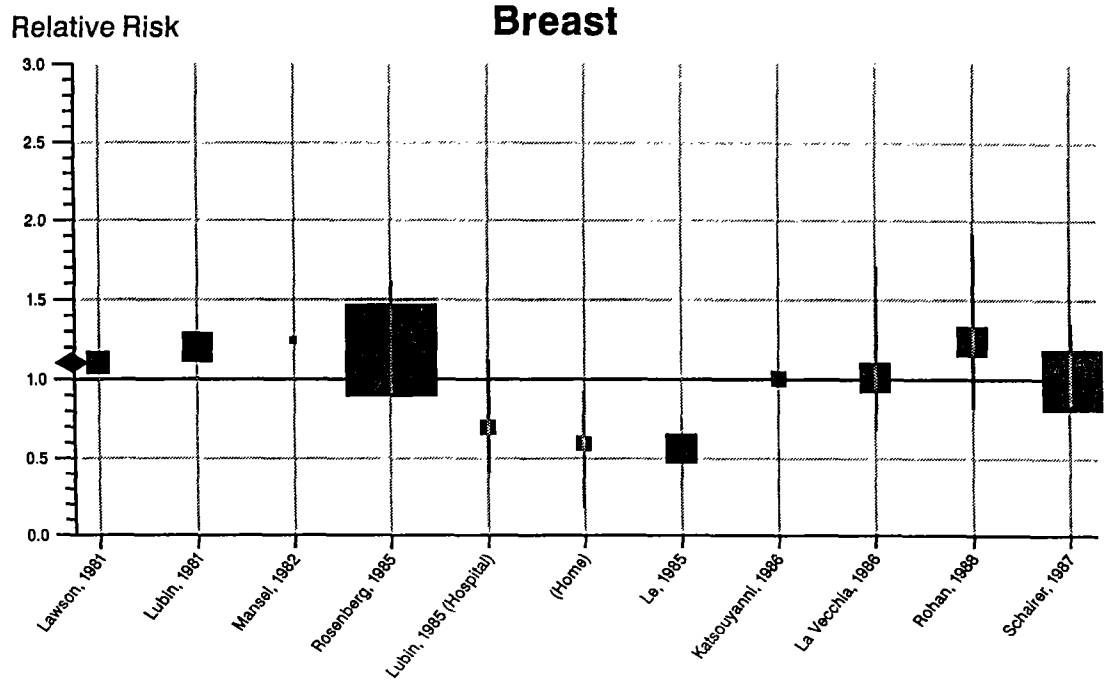


FIGURE 3 - EPIDEMIOLOGY STUDIES ON COLON CANCER AND COFFEE CONSUMPTION

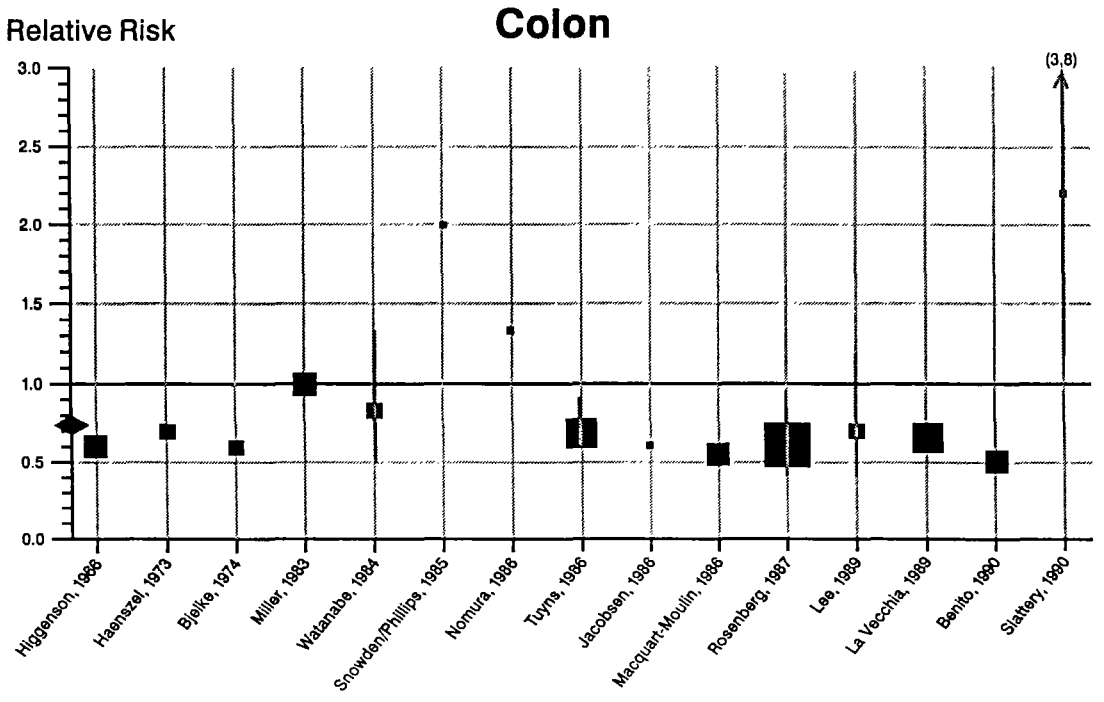


FIGURE 4 - EPIDEMIOLOGY STUDIES ON PANCREATIC CANCER AND COFFEE CONSUMPTION

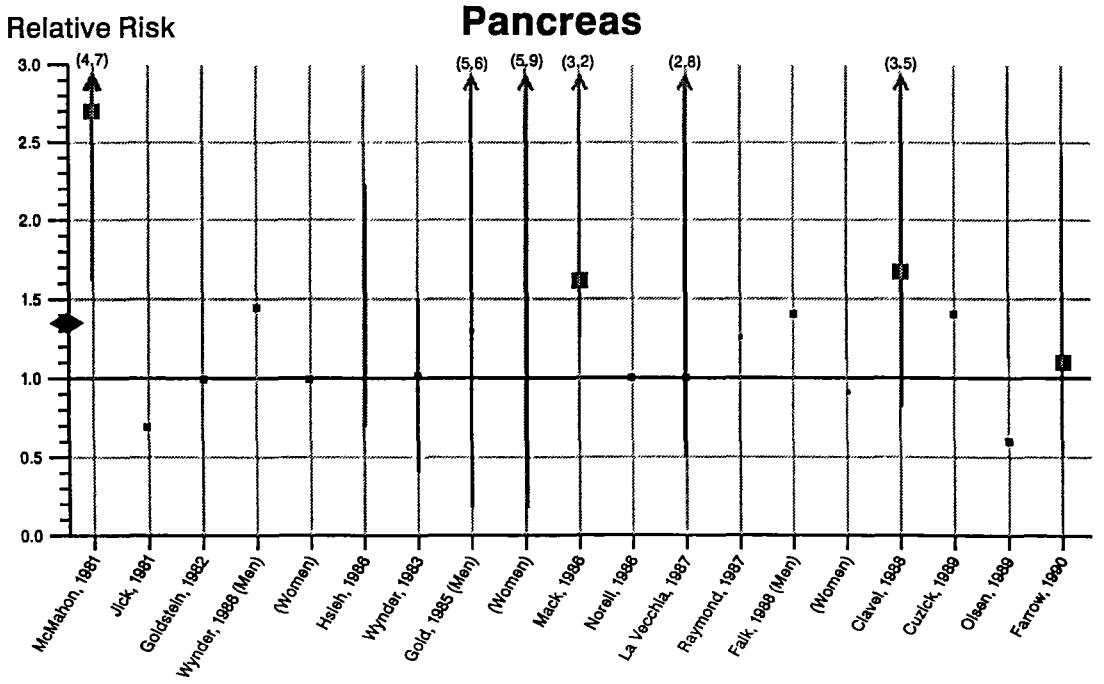


FIGURE 5 - EPIDEMIOLOGY STUDIES ON OVARIAN CANCER AND COFFEE CONSUMPTION

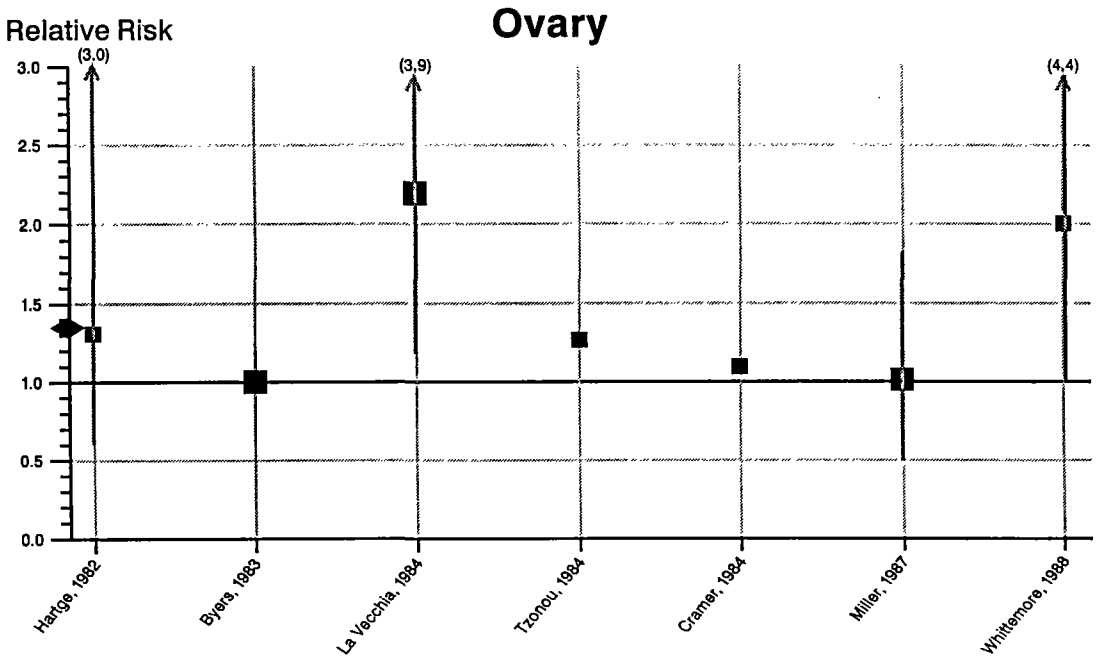
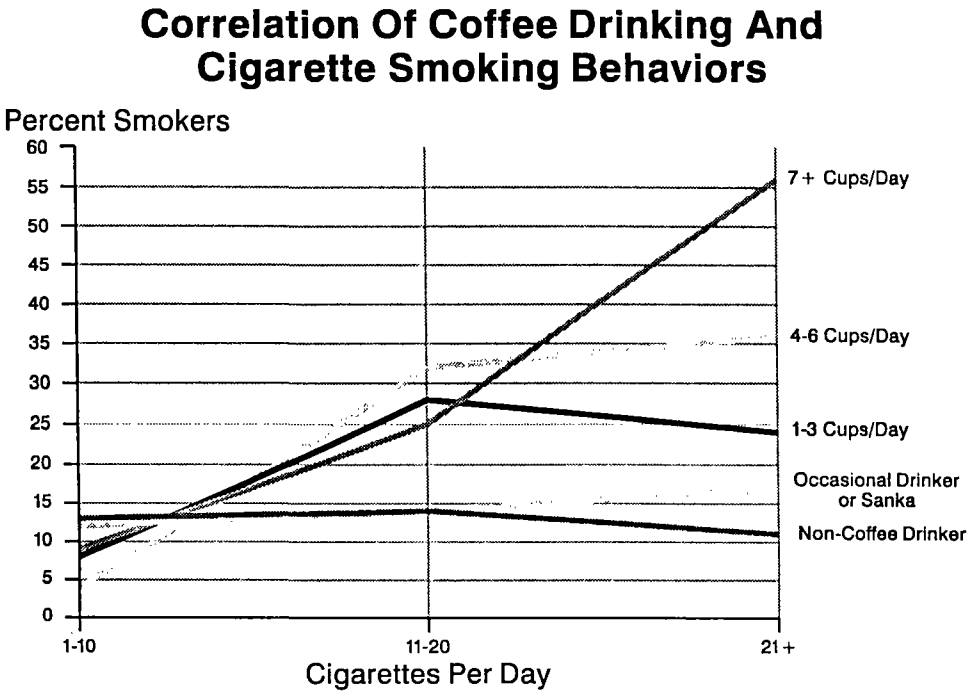


FIGURE 6



Adapted from Wynder et al. (1977)

TABLE 1

Behavior Correlates Of Coffee Consumption

Variable	Level Associated with Top Quartile	P Value	r
Cigarettes	Current Smoker	0.0000	0.23
Diet	High Fat/Low Vitamin Low Fiber	0.0000	0.08
Health Perception	Good	0.0002	0.06
Stress Score	High	0.0186	0.03
Vitamin Supplement	None	0.0385	0.03

Leviton et al. (1987) (8)

A META-ANALYSIS OF CASE-CONTROL STUDIES OF BLADDER CANCER IN COFFEE DRINKERS

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In 1971, Cole reported the results of a case control study originally undertaken to investigate the risk of bladder cancer in cigarette smokers. A significant association was discovered, a finding was subsequently replicated in several studies that followed. An unanticipated byproduct of that research was an additional association that was the subject of a report appearing in the Lancet in July of 1971: responding yes to the query "do you drink coffee?" was also found to be associated with cancer of the lower urinary tract.

In Cole's study, 468 patients with lower urinary tract cancer were matched to 498 controls. Among the health, lifestyle, and occupation questions was included the query: Do you drink coffee? Those who responded affirmatively were also asked about the daily amount of coffee consumed. In an analysis that controlled for age, smoking, and high risk occupations, women who consumed one or more cups of coffee per day had a relative risk for lower urinary tract cancer of 2.58 with 95% confidence ranging from 1.3 to 5.1 when compared to women who did not drink coffee. Male coffee drinkers had a statistically non-significant relative risk of 1.24. Noting the lack of a dose response relationship and the disparate risks in men and women, Cole concluded simply that "the relationship between coffee drinking and bladder cancer warranted further investigation."

Investigated it has been. Over 20 years and some 30 case control studies later, the association between coffee drinking and bladder cancer is still under investigation without suitable resolution. Relative risks have ranged from 0.6 to over twelve at the highest levels of coffee exposure in an Argentinean study. The question then arises, why such variability in the relative risks of studies investigating this association?

Meta-analysis is a technique whereby the results of many sufficiently

homogenous studies are pooled. Uses of meta-analysis include the detection of effects that would not achieve statistical significance in smaller studies as well as the resolution of conflicting studies. The purpose of the current research then, was first to review existing case control studies of coffee drinking and bladder cancer in an attempt to understand the reason for discordant findings, and to develop a methodologic framework for a meta-analysis.

Studies were identified through a medline search of all English publications in which "coffee" and "bladder" cancer appeared in the same article. The references of these articles were also reviewed to insure no studies were overlooked. All case control studies were eligible for inclusion. When results were reported more than once, we selected the most recent publication. Prospective cohort studies were also identified and noted as part of this process, but they were not considered in the formal analysis. Ultimately, 32 studies were identified for analysis in this manner, and a standardized coding form was used to extract information on methods including: case and control selection (e.g. anatomic site of cancer, actual histologies, degree of tissue invasion), matching criteria, coffee exposure (type, amount duration, and sequence), adjustment for potential confounders (e.g. cigarette smoking), and dose response relationship.

These publications spanned the years 1968 to 1990 and assembled case and control groups from secular periods 1958 to 1983. The number of cases ranged from 47 to 2892, and the number of controls from 94 to nearly 5782. The exposure prevalences in the cases ranged from 62 to 98% and in the controls from 48-97%, although for both cases and controls the majority of exposure prevalences clustered in the 85 to 95% range. The lower exposure prevalences generally reflect unique study populations such as mormons.

In order to more formally assess these reports, we undertook a consensus process in which three investigators met regularly to determine the requirements for similarity of methods, and to develop criteria to evaluate the quality of each study. Studies that met these methodologic requirements were considered to constitute the "core" of reports that were considered for further evaluation. We also retained other studies that were not part of the core group for later analyses. The three core criteria for inclusion were (1) appropriate selection of cases and controls, (2) suitable specification of the exposure, and (3) adjustment for the effects of smoking.

To meet the definition of a case, we required that studies include only histologically confirmed lower urinary tract cancer, over 90% of which had to be bladder. We also required that incident cases only be included. The natural history of bladder cancer is such that patients with one cancer are at increased risk for recurrence at the same and other sites, and patients with an index lesions are typically kept under close cystoscopic surveillance at regular intervals. Finally, we required that the core studies include cases only from population based sources, although hospital based studies that met other core criteria were noted and retained for analyses.

When we assessed case selection in the 32 identified studies, we found that 1 study included cancers that were not histologically confirmed, 2 studies included prevalent cases, and that 2 additional studies did both. For 10 studies, we could not determine from the methods section of the paper whether these criteria had been adhered to and these were therefore categorized them as uncertain. This left 17 studies that satisfied all core criteria for case selection.

For the selection of controls, we stipulated that the studies avoid constraints on controls that were not equally imposed on the cases. Examples

of these included: tobacco related conditions, any previous cancer, conditions that would modify the diet, and absence of other urinary tract problems. As for cases, we excluded studies which selected controls from non-population based sources although they were considered in a subsequent analysis. Applying these criteria to control selection, we found that six studies were hospital based, that ten studies were hospital based and had constraints on controls, and that for one study we could not determine the source of the controls. This left 15 of the 32 studies as satisfying the control selection criteria.

For exposure specification criteria, we had a single requirement; that the investigators ascertain exposure in the period before the development of lower urinary tract cancer; simply to inquire about current use was unacceptable. Here we were insisting on the appropriate exposure-disease sequence so that the exposure precede the development of the cancer. When we evaluated exposure specification, we found that 5 studies asked about current consumption only. In 10 studies, we could not determine the exposure disease sequence from the description of methods, and these studies were classified as uncertain. Ultimately, 17 studies satisfied our exposure specification criteria.

As our last core methodologic criteria, we required an adjustment for smoking. There is both epidemiologic and laboratory evidence that cigarettes are bladder carcinogens. Most studies have shown smoking and coffee drinking to be correlated to varying degrees. While we did not stipulate a statistical method for adjustment, we did require that some method of be employed to adjust for the effects of smoking. Only five of 32 studies failed to make any attempt to adjust for smoking in their analysis of coffee drinking and bladder cancer, leaving 27 as satisfactory on this criterion.

After applying methodologic core criteria to all 32 studies we found that eight satisfied all the criteria. Table 1 shows these studies, their authors, the year of publication, the number of cases and controls, and the smoking adjusted odds ratios for males and females. These estimates of risk are combined over exposure strata using a weighting strategy based on the inverse of the variance. This was calculated to get a smoking adjusted odds ratio for any regular use of coffee, which we defined as greater than or equal to 1 cup of coffee per day. This was necessary because many studies only reported smoking adjusted odds ratios for individual exposure strata, and not for all levels of consumption. The Boston component the Morrison study satisfied the core criteria, but did not provide confidence intervals around the smoking adjusted odds ratios so that an odds ratio combined over strata could not be calculated.

There are several notable features to table 1. The lowest odds ratios in these studies was 0.69 in women from a study by Ohno, while the highest was 1.85 in Connecticut study by Marrett. Curiously, studies showing the highest odds ratios in men tended to show less impressive odds ratios in women, and studies with higher odds ratios in women, showed less impressive odds ratios in men. We then proceeded to pool odds ratios for the core studies both adjusted and unadjusted for smoking. In men coffee drinking was associated with an odds ratio of 1.27 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 1.14-1.42. In women coffee drinking was associated with an odds ratio of 1.05 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 0.87 to 1.26.

We then pooled the odds ratios for the core studies after adjusting for smoking. In men coffee drinking was associated with a smoking adjusted odds

ratio of 1.07 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 1.00-1.14. In women coffee drinking was associated with a smoking adjusted odds ratio of 0.91 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 0.81 to 1.03.

We next looked at the association at the highest exposure strata, since if a true cause and effect relationship existed one would postulate it to be most pronounced at the highest levels of consumption. These odds ratios are also smoking adjusted. Males drinking more than 4-5 cups of coffee per day had an odds ratio of 1.16 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 1.05 to 1.28. Females drinking more than 4-5 cups of coffee per day had an odds ratio of 0.94 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 0.79 to 1.13.

In the next series of analyses, we added studies that we originally deemed to be uncertain in fulfilling the methodologic requirements for a core study to see what effect they exerted on the analysis. These are exposures of greater than 1 cup per day combined over exposure strata and adjusted for smoking. In men coffee drinking was associated with an odds ratio of 1.10 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 1.03-1.17. In women coffee drinking was associated with an odds ratio of 0.95 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 0.84 to 1.06.

Finally we repeated the analysis for all studies - core, uncertain, and unacceptable. In men coffee drinking was associated with an odds ratio of 1.14 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 1.08-1.21. In women coffee drinking was associated with an odds ratio of 0.95 for the development of lower urinary tract cancer with 95% confidence intervals ranging from 0.89 to 1.08.

On the basis of these preliminary analyses, we can make several conclusions. First, case-control studies designed to investigate the association between coffee drinking and lower urinary tract cancer exhibit substantial methodologic heterogeneity. Second, a meta-analysis of quality "core" studies based on pre-determined criteria suggests that coffee drinkers are not at substantially increased risk of lower urinary tract cancer. Furthermore, this lack of a substantial association persisted after (1) including studies with an incomplete description of methods that precluded us from interpreting them as "core", and (2) performing the same meta-analysis for core studies at the highest levels of coffee exposure.

Summary

A preliminary meta-analysis of case-control studies investigating the risk of bladder cancer in coffee drinkers was performed. Three investigators developed core criteria for studies to be included in the meta-analysis that included appropriate selection of cases and controls, ascertainment of exposure disease sequence, and adjustment for the confounding effects of smoking. Significant methodologic heterogeneity was noted, and eight of 32 identified studies adhered to all these criteria and were included in the meta-analysis. Coffee drinkers were not found to be at substantially increased risk of lower urinary tract cancer, and this finding persisted at the highest levels of coffee exposure.

TABLE 1
STUDIES MEETING CORE CRITERIA

Author	Year	Cases	Ctls	Smoking Adjusted OR for ≥ 1cup/day Males	Females
Howe	1980	632	632	1.46 (1.06,2.02)	1.03 (0.60,1.78)
Morrison (Boston)	1982	741	741	---	---
Marrett	1983	412	881	1.85 (1.25,2.73)	1.18 (0.69,2.01)
Hartge	1983	2982	5782	1.05 (0.96,1.14)	0.84 (0.73,0.97)
Ohno	1985	293	589	0.88 (0.68,1.13)	0.69 (0.40,1.21)
Jensen	1986	371	771	0.90 (0.63,1.27)	1.70 (0.91,3.15)
Risch	1988	835	792	1.05 (0.86,1.29)	1.22 (0.85,1.76)
Slattery	1988	332	686	1.11 (0.85,1.45)	NA

EVIDENCE FOR THE PRESENCE OF MULTIPLE CANCER CHEMOPREVENTIVE AGENTS IN GREEN COFFEE BEANS

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INTRODUCTION

The research that I would like to present is an outgrowth of previous work in Dr. Lee Wattenberg's laboratory at the University of Minnesota. Those studies showed that the addition of powdered green coffee beans to the diet of experimental animals could inhibit or suppress the formation of carcinogen-induced mammary neoplasia (1,2). Thus far, two chemicals with cancer chemopreventive activity have been isolated from green coffee beans (2,3). Research with these chemicals, kahweol and cafestol, suggest that they are blocking agents that inhibit chemical carcinogenesis by preventing carcinogens from reaching or reacting with the DNA. Blocking agents may have one of several mechanisms of action; however, the evidence now suggests that kahweol and cafestol enhance the activity of enzymes known to detoxify mutagens and carcinogens (3,4). One of these enzymes is glutathione S-transferase.

MATERIALS AND METHODS

Our laboratory has continued this research on the cancer chemopreventive activity of green coffee beans (5,6). In each of the three separate experiments, I will be reviewing, the green coffee beans, the green coffee bean fractions, and the isolated chemicals, kahweol and cafestol, were incorporated into the diets of the experimental animals. The basic diet in each of these experiments was a powdered Purina Lab Chow specifically formulated for small rodents. The animals remained on their respective diets throughout the course of the experiment. In each experiment, the hamster cheek pouch model for oral carcinogenesis was used.

The procedure used to produce these oral tumors was fairly simple. One of the buccal pouches was inverted and painted 3 x weekly with a dilute

solution of the carcinogen, 7,12-dimethylbenz[a]anthracene (DMBA). The DMBA was dissolved in heavy mineral oil at a concentration of 0.5% (w/v). In each group, the pouches of the control animals were treated 3 x weekly with heavy mineral oil. All of the solutions were applied topically and each painting was done with a small camel hair brush. Each application placed approximately 0.05 ml of the solution on the surface of the pouch.

After approximately 12-13 weeks, the treatment with either the heavy mineral oil (control animals) or the 0.5% solution of DMBA (experimental animals) was discontinued. One week later the hamsters were sacrificed and the pouches were removed. Tumors, when present grossly, were counted and measured (length, width, and height). Since the tumors were exophytic and well defined, these measurements were easily obtained. The excised pouches were fixed in 10% formalin, sectioned in paraffin, and stained with hematoxylin and eosin.

There are a number of reasons why we like this tumor model. One, we feel that it tends to mimic the human situation. Unlike most tumor models, in which the animals are treated with one or two massive doses of the carcinogen, this model uses a procedure in which the carcinogen is repeatedly applied (35-40 treatments) to the tissue over an extended period of time. Two, the hamster cheek pouch model is an established technique developed in 1954 that has been used routinely to study cancer chemopreventive agents. Three, the latent period is relatively long and the changes in the tissues are readily visible. Four, the cancer chemopreventive agent can be added to the diet or applied topically to the pouches. Five, multiple tumors are common in this model. This permits the use of fewer animals. Six, the carcinomas that are produced are slow-growing tumors. This minimizes any pain or discomfort that the animals might have to endure.

As might be expected, there are also a number of negative features for this tumor model. One, the technique is labor intensive. Repeated applications of the carcinogen mean that the investigator must work with the animals over an extended period of time. Two, there is very little similarity between these DMBA-induced epidermoid carcinomas and oral tumors in humans. As a matter of fact these papillary carcinomas resemble quite closely some forms of colon cancer.

RESULTS AND DISCUSSION

The data for the first experiment is given in Tables I and II. Two groups of hamster were used. The animals in group 1 received a normal diet, while the animals in group 2 received the same diet supplemented with 20% powdered green coffee beans (Colombian). After the hamsters adjusted to their diets, treatment with the carcinogen, DMBA, was initiated. After 40 treatments with DMBA, the experimental animals were sacrificed, the pouches were removed, and the tumors were counted and measured.

TABLE I.
TUMOR INCIDENCE (EXPERIMENT #1)

Group	No. of Animals	No. of Tumor Bearing Animals	No. of Tumors
1	15	15 (100%)	76
2	12	3 (25%)	3

As indicated in Table I, all of the pouches from the hamsters in group 1 contained tumors. In 13 of these animals, multiple tumors were found, ranging from 2 to 11 tumors per pouch. By comparison, only three tumors in three different animals were found in the hamsters that were fed the diet containing whole green coffee beans.

TABLE II.
TUMOR NUMBER, BURDEN AND MASS (EXPERIMENT #1)

Group	Avg. No.* of Tumors	Avg. Tumor* Burden, mm^3	Avg. Mass of Tumors, mm^3
1	5.1 ± 0.9	170 ± 68	33.3
2	0.3 ± 0.1**	0.9 ± 0.6**	3.5

*values are means ± S.E. **$p<0.05$ when group 1 is compared to group 2.

The data for tumor number, tumor burden and tumor mass are given in Table II. The treatment with green coffee beans reduced tumor number by more than 90% and tumor mass by 90%. Tumor burden takes into account both tumor number and tumor mass. The figures in this column show a 95% reduction in tumor burden for the animals in group 2.

As indicated, two cancer chemopreventive agents have been isolated from green coffee beans. Experiment #2 examined the effects of kahweol and cafestol on the development of DMBA-induced buccal pouch carcinomas. With the help of Dr. Liardon and Mr. Bertholet at the Nestlé Research Centre, we were able to obtain large quantities of a 50:50 mixture of the two chemicals. Calculations taking into account the molecular weights of kahweol, cafestol, kahweol palmitate, and cafestol palmitate, as well as the percent of each of these compounds in green coffee beans (Colombian), allowed us to construct a diet that approximated quite closely the kahweol and cafestol content of a diet containing 20% green coffee beans (Colombian). The diet contained 2 g of the kahweol and cafestol mixture per kg of food. The modified diet was given to the hamsters in group 2. An unmodified diet was given to the animals in group 1. The data for this second experiment are given in Tables III and IV.

TABLE III.
TUMOR INCIDENCE (EXPERIMENT #2)

Group	No. of Animals	No. of Tumor Bearing Animals	No. of Tumors
1	14	14 (100%)	91
2	15	14 (93%)	61

As can be seen in Table III, all of the pouches from the hamsters in group 1 had visible tumors. In group 2, the group receiving kahweol and cafestol in the diet, 14 of the 15 animals had visible tumors. Multiple tumors were found in most of the animals (14/14 in group 1 and 11/15 in group 2). Ninety-one tumors were found in the animals in group 1 and sixty-one tumors were found in the animals in group 2.

TABLE IV.
TUMOR NUMBER, BURDEN AND MASS (EXPERIMENT #2)

Group	Avg. No.* of Tumors	Avg. Tumor* Burden, mm^3	Avg. Mass of Tumors, mm^3
1	6.5 ± 0.8	167 ± 16	25.7
2	4.1 ± 0.8**	110 ± 31	26.9

*values are means ± S.E. **$p<0.05$ when group 1 is compared to group 2.

Table IV shows that the treatment with kahweol and cafestol reduced tumor number by 35 to 40%. No effect on average tumor mass was seen, while the figures for average tumor burden show a reduction of approximately 35%.

Taken together, the results from experiments one and two suggest that green coffee beans may contain other cancer chemopreventive agents. To test this possibility, we went back to the green coffee bean and to two fractions of the green coffee bean, green coffee bean oil and defatted green coffee beans. For experiment three, there were four groups of animals. The hamsters in group 1 received a normal diet. The animals in groups 2, 3, and 4 were given the same diet supplemented with 15% green coffee beans (group 2), 12.75% defatted green coffee beans (group 3), or 2.25% green coffee bean oil (group 4). In the Colombian green coffee bean, the green coffee bean oil accounts for approximately 15% (w/w) of the whole bean. The oil contains kahweol and cafestol, as well as, other plant oils and lipids. After the green coffee bean oil is removed, the other fraction accounting for the remainder of the coffee bean (85%) is the defatted green coffee bean. This fraction is essentially devoid of kahweol and cafestol. After 36 treatments with DMBA, the hamsters were sacrificed and the tumor data were collected. The data are given in Tables V and VI.

TABLE V.
TUMOR INCIDENCE (EXPERIMENT #3)

Group	No. of Animals	No. of Tumor Bearing Animals	No. of Tumors
1	16	16 (100%)	75
2	15	12 (80%)	39
3	15	13 (87%)	39
4	16	16 (100%)	56

Table V shows that all of the hamsters in groups 1 and 4, 12 of the 15 hamsters in group 2, and 13 of the 15 hamsters in group 3 had visible tumors. Most of the animals, 15 of 16 in group 1, 10 of 15 in group 2, 13 of 15 in group 3, and 14 of 16 in group 4 had multiple tumors. The total number of tumors ranged from a low of 39 for groups 2 and 3 to a high of 75 for group 1.

TABLE VI.
TUMOR NUMBER, BURDEN AND MASS (EXPERIMENT #3)

Group	Avg. No.* of Tumors	Avg. Tumor* Burden, mm^3	Avg. Mass of Tumors, mm^3
1	4.7 ± 0.5	145 ± 41	30.9
2	2.6 ± 0.6**	43 ± 15**	16.5
3	2.6 ± 0.4**	62 ± 34	23.8
4	3.5 ± 0.6	57 ± 15**	16.3

*values are means ± S.E. **$p<0.05$ when the means for these groups are compared to the corresponding mean for group 1.

In Table VI, it can be seen that the treatment with green coffee beans produced a 45% reduction in average tumor number and a 55% reduction in average tumor mass. The decrease in average tumor burden was 70%. A similar comparison between groups 1 and 3 (defatted green coffee bean diet) gave a 45% reduction in average tumor number and only a 25% reduction in average tumor mass. Overall there was a 55% decrease in average tumor burden. The same comparison for groups 1 and 4 (green coffee bean oil diet) yielded a 25% reduction in average tumor number, a 55% reduction in average tumor mass and a 60% reduction in average tumor burden.

The data on the extraction of the green coffee bean oil showed that 98% of the oil was removed. The residual material, the defatted green coffee beans, contained the remaining 2%. This data when coupled with the data on tumorigenesis indicates that the cancer chemopreventive activity in defatted green coffee beans cannot be entirely due to kahweol and cafestol. For this reason, we feel that these results support our earlier tentative conclusion from experiment #2 that green coffee beans contain additional cancer chemopreventive agents. These results also indicate that one or more of these additional cancer chemopreventive agents are in the defatted green coffee bean.

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SUMMARY

In conclusion, we feel the data from these experiments showed the following:

1. Diets containing 15-20% powdered whole green coffee beans significantly inhibited the formation of DMBA-induced buccal pouch carcinomas in the hamster cheek pouch model for oral carcinogenesis.

2. Part of this inhibition in DMBA-induced neoplasia was due to two diterpenes in green coffee beans, kahweol and cafestol.

3. Besides kahweol and cafestol, the results indicated that there were other cancer chemopreventive agents in green coffee beans. One or more of these as yet unidentified compounds appears to be in the defatted portion of the bean.

RESUMEN

En conclusión, creemos que los datos de estos esperimentos demuestran lo siguiente.

1. Las dietas que contienen de 15% a 20% de granos verdes completos pulverizados de café inhiben la formación de los carcinomas bucales inducidos por el DMBA en el modelo de carcinogénesis oral en el abazón de hámster.

2. Parte de esta inhibición en la neoplasia inducida por el DMBA se debe a dos biterpenos en los granos verdes de café, kahweol y cafestol.

3. Los resultados indicaron que, además del kahweol y el cafestol, habia otros agentes quimiopreventivos de cáncer en los granos verdes de café. Uno o más de estos compuestos aún no identificados parecen estar en la porción desgrasada del grano.

THE EFFECT OF COFFEE ON *N*-NITROSAMINE FORMATION IN HUMANS AND *IN VITRO*

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Evidence has accumulated over the past 25 years linking exposure to *N*-nitroso compounds and an increased risk of cancer (1-3). Humans are exposed to preformed *N*-nitroso compounds in food, water and air (4,5), and also to *N*-nitroso compounds that form endogenously in the acidic stomach (6,7) and at other sites (8). It was once suggested that readily oxidized phenols, including the coffee component chlorogenic acid, could catalyzed nitrosation (9). This hypothesis, however, was based on artefactual experimental results and subsequent work clearly demonstrated that chlorogenic acid inhibited nitrosation *in vitro* (10). Nitrosation is effectively inhibited by chemicals that react rapidly with the nitrosating agent including ascorbic acid, α-tocopherol, and other compounds that occur naturally in foods (11-14).

Using a variety of endpoints, researchers have shown that coffee and coffee constituents inhibit nitrosation. Mutagenicity in *Salmonella typhimurium* resulting from the nitrosation of methylurea, for instance, was reduced when chlorogenic acid or instant coffee was added to the nitrite and methylurea test mixture (15). Coffee also decreased hepatoxicity in rats treated with the precursors of *N*-nitrosodimethylamine, nitrite and aminopyrine (16). Furthermore, mice given nitrite and methylurea by gavage had decreased DNA damage in bone marrow erythrocytes and colon epithelial cells when coffee or coffee constituents were also administered (17).

In order to examine the effect of coffee on nitrosation in humans we used *N*-nitrosoproline (NPRO) as a biomarker. NPRO is widely used for demonstrating gastric acid-catalyzed nitrosation in humans because it is not mutagenic, carcinogenic, or metabolizable and is excreted quantitatively in urine (7,18,19). In a typical example, a human subject consuming a low nitrate diet is given a large, oral dose of nitrate followed by a similar dose of L-proline one hour later. The nitrate is rapidly absorbed in the small intestine, circulated, secreted in saliva, and reduced by oral bacteria to nitrite, which is re-swallowed. Nitrosating agents are produced from nitrite in the stomach's acidic environment and react readily with proline (Figure 1), and the resulting NPRO is excreted within 12-24 hours. If a large dose (1 gram) of ascorbic acid is given with the proline, an increase from baseline urinary NPRO levels is not seen.

In this study, nitrate and proline were administered to volunteers on five days, and instant decaffeinated coffee was given on three of these days. The results of the human study are compared to those obtained from a simple reaction system *in vitro*, where morpholine was used as the amine substrate.

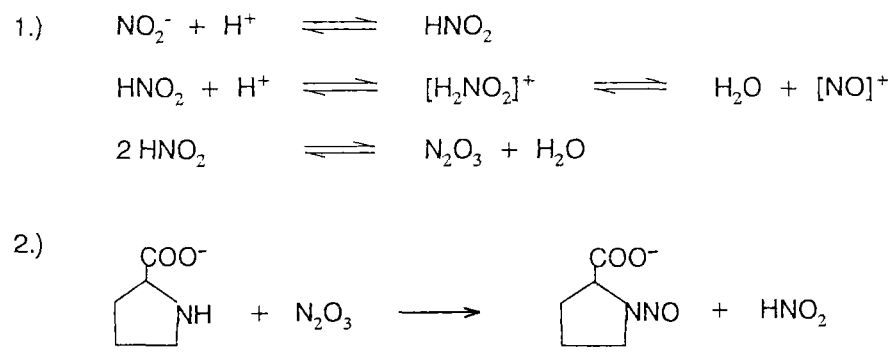

FIGURE 1
1.) Generation of nitrosating agents ($[H_2NO_2]^+$, $[NO]^+$, and N_2O_3) from nitrite in the presence of acid. 2.) Nitrosation of proline.

MATERIALS AND METHODS

Nitrosation *in vitro*

Morpholine (50 μmol) and instant decaffeinated coffee (0-0.5 g) were added to citrate/phosphate buffer (9.9 mL) in a glass vial with a teflon sealed cap. The solution was adjusted to pH 3, and sodium nitrite solution (12.5 μmol in 100 μl citrate/phosphate buffer) was added. The mixture was incubated for 1 hour in a 37°C shaking water bath and 5N NaOH (2 ml) was added to stop the reaction. *N*-nitrosodipropylamine (NDPA) was added as an internal standard, NDPA and *N*-nitrosomorpholine (NMOR), were extracted with dichloromethane from the reaction mixture and quantitated with a gas chromatograph-Thermal Energy Analyzer (GC-TEA).

Nitrosation in humans

Subjects, diet, and urine collection. The protocol was approved by the Nestlé Research Center Ethical Committee and subjects signed consent forms after the study was explained to them. There were no health risks associated with the study; L-proline is a naturally occurring amino acid and nitrate is found in water and in vegetables. The size of the nitrate dose in this study was approximately equal to the amount in a large serving of spinach or in 250 ml of beet juice. Daily nitrate intake may actually have been lower for some subjects during the study than during their regular diet.

Healthy males (25-50 years old) who did not smoke or take any medications or vitamin supplements participated in the study. Volunteers kept a daily diary in which any deviations from the protocol or diet and any changes in health were recorded. A diet was designed that was low in nitrate and did not contain any fruits or vegetables or caffeine. The volunteers consumed only the defined diet (<150 μmol nitrate/24 hr) and drank low nitrate bottled water (70 μmol nitrate/L) during the course of the study. The bottled water was also used for nitrate, proline, and coffee doses.

Urine was excreted directly into bottles that contained 5N NaOH to inhibit microbial growth and the formation of artefactual nitrosamines. Urine samples were pooled for 24 hour periods that began at the time of the nitrate dose.

Protocol. After two days on the diet, which allowed for clearance of nitrate and NPRO that were consumed before the subjects began the controlled diet (data not shown) and a baseline urine collection, subjects were given an oral dose of sodium nitrate (3.7 mmol in 15 ml water) immediately followed by 100 ml water. The nitrate was administered in mid-afternoon about 2 hours after lunch. One hour after the nitrate dose, the subjects were given an oral dose of L-proline (4.3 mmol in 15 ml water) that was followed by 150 ml water, or on subsequent days, by instant decaffeinated coffee (1, 2, or 4 g) in 100 ml water and an additional 50 ml water.

N-Nitrosoproline and nitrate analyses. NPRO was extracted from urine after *N*-nitrosopipecolic acid was added as an internal standard. The methyl-ester derivatives were formed as described in Ladd *et al.* (20) and quantitated using a GC-TEA. Nitrate was extracted from a sample of the blended diet with warm water and quantitated by an automated procedure (21).

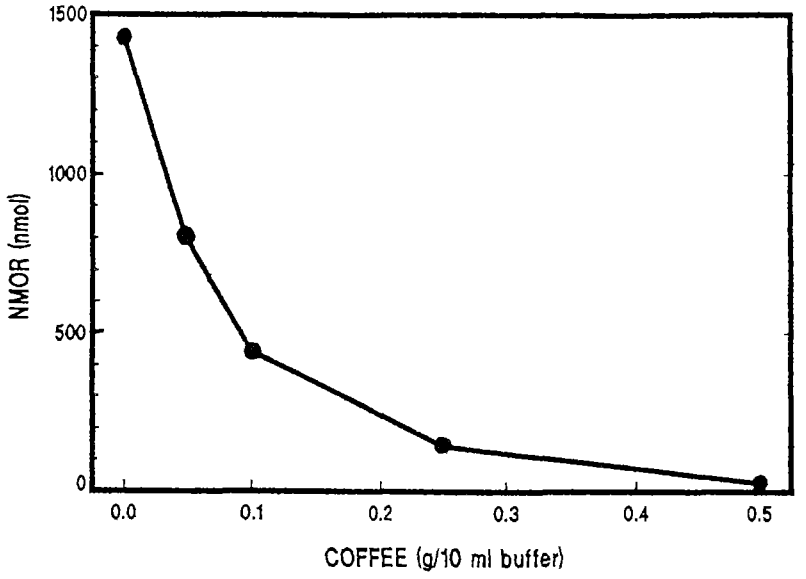

FIGURE 2
Nitrosation of morpholine *in vitro*. Reaction conditions: morpholine (50 μmol) and coffee (0.05-5 g) were added to citrate/phosphate buffer (9.9 ml) and adjusted to pH 3.0. Sodium nitrite (12.5 μmol) was added, the mixture was incubated for 1 hour at 37°C, and the reaction was stopped by adding 5N NaOH (2 ml). Trials were done in duplicate.

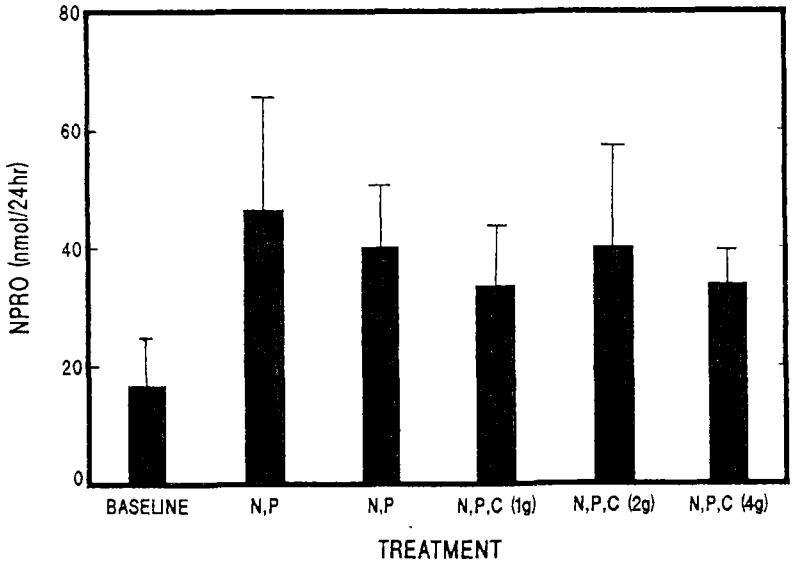

FIGURE 3
Urinary *N*-nitrosoproline levels in humans treated with nitrate, proline and coffee. The average (bar) and standard deviation (line) of six subject's urinary NPRO/24 hr are shown. Treatments: Baseline = no doses, N = 3.7 mmol $NaNO_3$, P = 4.3 mmol L-proline, C = instant decaffeinated coffee (amount). There was no significant effect ($p = 0.28$) from the coffee treatment.

RESULTS AND DISCUSSION

Morpholine was chosen as the substrate for the model system because it is rapidly nitrosated and because the product, *N*-nitrosomorpholine (NMOR), is volatile and therefore, easily analyzed. The range of coffee concentrations used in the assay was similar to what is typically consumed, i.e. it was as if the reaction were performed in a cup of coffee. Coffee inhibited the nitrosation of morpholine *in vitro* in a concentration dependent manner (Figure 2). As little as 50 mg of coffee reduced NMOR formation by 50%, but even ten times more coffee did not completely suppress NMOR formation. Many coffee components may be responsible for the inhibition including, chlorogenic acid, Maillard reaction products and melanoidins (15,17,22).

NMOR is carcinogenic (23) and therefore, cannot be used for human studies. Although the rate constant for proline nitrosation is about ten times smaller than for morpholine (11), proline is a satisfactory and proven substrate for examining gastric nitrosation in humans (7,18-20). Dosing with nitrate and proline increased urinary NPRO about 2.5-fold from 15 nmol/24 hr to over 40 nmol/24 hr ($p = 0.046$), in accord with results from similar experiments (18,19). There was no significant effect ($p = 0.28$) on urinary NPRO levels, however, when instant decaffeinated coffee was given with the proline. Furthermore, a dose-response relationship was not observed between the coffee dose and urinary NPRO levels; doses of 1, 2, and 4 grams of coffee resulted in urinary NPRO levels of 33.6, 40.2, 33.8 nmol/24 hr, respectively.

Nitrosation of secondary amines by aqueous nitrous acid is catalyzed by nucleophilic anions, including two which occur *in vivo*, chloride and thiocyanate (14). The presence of these catalytic ions may have been one reason why the results from the reaction *in vitro* were not repeated in humans. The situation is similar with ascorbic acid; a 2:1 molar ratio of ascorbic acid:nitrite is sufficient to prevent NPRO formation *in vitro* (12,24), yet, ascorbic acid doses 20-fold larger than the estimated gastric nitrite (assuming 5% of the nitrate dose was reduced to nitrite (25)) are necessary to completely inhibit proline nitrosation in the stomach (18,19). In a study where chlorogenic acid was given by stomach tube to rats simultaneously with nitrite and proline, a 5:1 ratio of chlorogenic acid:nitrite decreased NPRO formation by 50% (22). Assuming instant decaffeinated coffee contains about 2-5% chlorogenic acid by weight, as much as 10 g of coffee would have been necessary to reproduce this effect in humans.

A larger coffee dose may have been more effective in reducing nitrosation in humans as was observed in animal (16,17) and *in vitro* mutagenicity studies (15). More concentrated coffee, however, is not palatable and is not usually consumed, and the alternative, a larger volume of coffee, would have diluted the reactants. Although a significant decrease in urinary NPRO levels was not observed at the coffee doses tested, when results from experiments in humans, in animals, and *in vitro* are taken together, we conclude that coffee clearly does not increase, and at higher concentrations, could potentially decrease endogenous gastric nitrosation in humans.

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MYCOTOXINS IN COFFEE

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Introduction

Under certain conditions of humidity and temperature mycotoxins that are produced by several fungal species (e.g. Aspergillus or Penicillium) can occur in coffee beans. The occurrence of aflatoxin B1, sterigmatocystin, and ochratoxin A has been described in the literature. Various groups have carried out studies that can be taken into account when estimating the possible risk for humans imposed by these mycotoxins. The results of these experiments are, however, partly contradictory; investigations concerning the influence of roasting on the mycotoxin content in coffee beans e.g. led to inconsistent results. Furthermore it remains unclear whether mycotoxins in roasted coffee beans can be transferred into the brew. In this paper the data available are summarized and, in spite of the mentioned open questions, a risk assessment is attempted.

Occurrence of mycotoxins in coffee

The most recent comprehensive review on the occurrence of mycotoxins in coffee is by R. Strobel, 1988 (see Table 1): almost 3000 normal commercial as well as obviously contaminated green coffee samples have been considered by various authors. Few samples were actually contaminated with ochratoxin A or aflatoxin B1 (about 2% each in total); contamination was mostly found in mouldy samples and in quite low amounts (ochratoxin A 10-100 µg/kg, aflatoxin <3 up to 12 µg/kg. Sterigmatocystin was found in 2 out of 700 samples, both of which were very mouldy, one was two years old; they contained 1 and 12 mg/kg, respectively). Strobel

does not state exactly how samples were chosen; the reason for this is that the details are usually lacking in the original papers. Most of the original papers were rechecked by us.

Additional data have been published by Micco et al. in 1989; they found that 58% of commercial green coffee samples were contaminated with 0.2-15 µg ochratoxin A per kg.

Table 1: Summary of the occurrence of mycotoxins in green coffee samples, from Strobel 1988

Mould toxins determined in green coffee beans[a]

Coffee samples analysed	*Aflatoxin B_1*	*Ochratoxin A*	*Sterigmatocystin*	*Reference*
640 commercial	0	NA	NA	1964–69 Procter & Gamble (Heusinkveld *et al.*[197])
58 very mouldy	0	NA	NA	1964–68 (Levi and Borker[17])
2 commercial	0	NA	NA	1968 (Scott[176])
18 commercial	0	NA	NA	1969 (Boutibonnes and Jacquet[177])
2 very mouldy	0	NA	(1) 1100	1973 (Purchase and Pretorius[194])
267 hand cleaned from mouldy lots	NA	(1)–360 (2)–20+ (16)–20	NA	1974 General Foods (Levi *et al.*[186])
68 commercial	NA	(3) 20, 20, 80	NA	1974 (Levi *et al.*[186])
15 (5 no mould, 10 mouldy)	NA	NA	0	1975 (Levi *et al.*[197])
502 (2 years old, one sample mouldy)	0	0	(1)–12000	Illicaffe (via Levi[22])
201 commercial	(2)[b] 3; 12	(2) 96; 24	0	FDA USA, Stoloff, L. and Francis, O., via Levi[22]
65 commercial	(28) < 3	NA	NA	Tsuboi *et al.*[198]
22 commercial	0	(4) 9·9–46	NA	Tsubouchi *et al.*[187]
1 commercial	0	NA	NA	Kiermeyer[180]

[a] All quantities in $\mu g\,kg^{-1}$ or $ng\,g^{-1}$.
[b] Numbers in parentheses indicate the number of samples infested with mycotoxin.
NA = Not analysed.

Inconsistencies in the literature were found on the effect of roasting on the ochratoxin A content: Tsubouchi and coworkers (1987) reported a maximum reduction of 12% in the mycotoxin content of artificially contaminated beans, while Levi et al. (1974) found much higher reductions (80-90%). Tsubouchi et al. hypothesize that their way of contaminating the coffee beans (inoculating the beans with Aspergillus ochraceus) is closer to the natural situation than the method of Levi et al. ("mycotoxins were added"). Micco and coworkers (1989), on the other hand, found a more efficient mycotoxin destruction in naturally contaminated versus artificially contaminated ("added") samples. Their ochratoxin A concentrations were in a realistic range, between 5 and 40 µg/kg, while Tsubouchi's samples had concentrations 10 to 10'000 times higher. This might be a reason for the different findings. Gallaz and Stalder (1976), who inoculated green coffee beans with Aspergillus ochraceus and obtained coffee contaminated with 20-40 µg/kg, also found a 80-90% mycotoxin destruction by roasting. This again supports the postulated concentration-related reason for the different findings. Data are summarized in Table 2. Still, the effect of roasting remains somewhat unclear. In order to clarify this point, more research should be done.

Table 2: Experimental studies on the destruction of ochratoxin A during roasting of coffee beans

Method of contamination	Mycotoxin content (µg/kg)	Roasting conditions	Destruction	Reference
mycotoxin added	210-350	198-210°C 5-20 min	80-90%	Levi et al. 1974
A.ochraceus inoculation	17-43	not specified	80-90%	Gallaz and Stalder 1976
mycotoxin added	80	not specified	90%	
naturally contaminated	4-9	not specified	90-100%	Micco et al. 1989
mycotoxin added	40	not specified	50-87%	
A.ochraceus inoculation	200-140,000	200°C 10-20 min	0-12%	Tsubouchi et al. 1987

Tsubouchi and coworkers (1988) investigated mycotoxins found in commercially roasted coffee samples. They found 3-17 µg ochratoxin A per kg, 5 out of 68 samples were contaminated. To our knowledge there are no other publications on this point. In addition, Tsubouchi and coworkers (1987) are so far the only authors to describe a transferral of mycotoxins into the brew: ochratoxin A in contaminated ground roasted samples was almost quantitatively transferred into the brew. Micco et al. (1989), who also examined the mycotoxin content in beverages prepared from artificially contaminated coffee, found no residues of ochratoxin A; this is, however, not so surprising since in their experiments the ochratoxin A concentration was low in the green beans and 90-100% was destroyed by the roasting process.

Toxicological considerations and risk assessment

In experimental animals both aflatoxin B1 and ochratoxin A induce tumours (see below). This has to be taken into consideration when making a risk assessment. The intake of carcinogens should be as low as possible: a life time risk of $\leq 1:10^6$ is generally regarded as acceptable (virtually safe dose, VSD).

Aflatoxin B1 is a classic genotoxic (mutagenic) carcinogen. In experimental animals it induces tumours: 1.34 µg/kg body weight given orally to rats daily over their lifetime led to an incidence of liver carcinoma of 50% (Gold et al. 1984). Extrapolating linearly to a carcinogenic risk of $1:10^6$ gives 2.7 pg/kg bw daily for life. Aflatoxin is also suspected to play a role in the induction of human liver carcinoma in tropical areas. For humans a risk of $1:10^6$ may be achieved by a daily intake of about 20 pg/kg body weight or 1.5 ng per adult (based on the linear regression $y = 0.106x + 2.2$, where y is the liver cancer incidence per 10^5 cases per

year and *x* is the daily aflatoxin intake in ng/kg bw, as published by Peers and Linsell in 1977).

Ochratoxin A does not induce point mutations, but there is some evidence for DNA single strand breaks. It possibly exerts indirect genotoxicity by increasing the formation of oxygen radicals. There are also some indications for a promoting activity of ochratoxin A (DFG 1990). In experimental animals an induction of tumours was found: rats fed 70 µg/kg bw daily for life developed kidney carcinoma (males 31%, females 2%); 210 µg/kg led to an incidence of 60% in males and 6% in females (NTP 1989). Extrapolation to a carcinogenic risk of $1:10^6$ would amount to a daily intake of about 0.5 ng/kg bw for life in rats. In humans ochratoxin A is possibly a cause of the "Balkan endemic nephropathy" (DFG 1990), data are incomplete, however. Calculating the dose responsible for a risk of $1:10^6$ is therefore very hypothetical; based on Chernozemsky et al. 1977, Petkova and Castegnaro 1985, as well as on our own calculations it may lie at about 0.05-0.2 ng per adult or 1-3 pg/kg body weight.

As for coffee, daily consumption of 30 g coffee with 2% or less of the samples being contaminated with an average of 3 ppb aflatoxin B1 amounts to ≤1.5 ng aflatoxin B1 per person per day. The same 30 g coffee with 2 to 3% of the samples being contaminated with 20 ppb ochratoxin A on average amounts to 12-18 ng ochratoxin A per person per day (in both cases degradation during roasting and possibly limited transfer into the brew not considered!). Since these data therefore represent figures that might be (far) too high, we do not consider them alarming in relation to the virtually safe dose mentioned above. Still, these calculations show the importance of determining the extent of degradation of ochratoxin A during the roasting procedure in order to have more exact data on a possible intake via coffee consumption.

For sterigmatocystin similar estimations cannot be made as no reliable data are available. If one considers, however, that both the coffee samples in which sterigmatocystin was found were very mouldy, it can be concluded that sterigmatocystin contamination does not represent a priority item since mouldy samples usually are excluded from consumption.

In order to put the possible health risk imposed by mycotoxins in coffee in relation to other foods possibly containing mycotoxins some comparisons have been made.

Aflatoxin B1: 20 g mais (corn) per day, 5-10% contaminated with 5 ppb or less (Tutelyan et al. 1989) amounts to 5-10 ng aflatoxin B1 daily per person. 15 g peanuts or peanut products, 10% contaminated with less than 10 to 100 ppb (Fritz 1983) amount to 15-150 ng aflatoxin B1 per person per day .

Ochratoxin A: consuming 200 g wheat per day with 2-8% of the samples being contaminated with 20-50 ppb on average (Rati Rao et al. 1979), (Fritz et al. 1979), amounts to 12-800 ng ochratoxin A per person daily. Mais (corn), 20 g per day, with 10-20% contaminated with 50-200 ppb (Jarvis 1982) contributes 100-400 ng ochratoxin A per person and day.

These data are summarized in Table 3.

Table 3: Contribution of various food items to the intake of mycotoxins

	Average contamination	Daily intake of food item	Resulting average human intake of mycotoxin
Aflatoxin B1			
coffee	≤ 2% with 3 ppb	30 g	≤ 1.5 ng
corn	5-10% with ≤5 ppb	20 g	5-10 ng
peanuts or p. products	10% with ≤10-100 ppb	15 g	15-150 ng
Ochratoxin A			
coffee	2-3% with 20 ppb	30 g	12-18 ng
wheat	2-8% with 20-50 ppb	200 g	12-800 ng
corn	10-20% with 50-200 ppb	20 g	100-400 ng

Conclusions

Aflatoxin B1 and sterigmatocystin do not seem to represent a considerable health risk for coffee consumers. With the data available at present ochratoxin A intake via coffee consumption may be higher than the calculated virtually safe dose; a somewhat increased carcinogenic risk can therefore not be excluded. If one considers, however, the possible mycotoxin intake from other food items, it is apparent that coffee consumption contributes only 5-10% of the total mycotoxin burden. If roasting the coffee beans in fact decreases the mycotoxin concentration by 90 percent or even more, the contribution of coffee to the intake of mycotoxins would become even smaller, and, the intake could then lie below the virtually safe dose even for ochratoxin A. It is therefore essential to clarify both the influence of roasting on mycotoxin destruction and the extent of transferral of mycotoxins from ground roasted beans to the coffee brew.

Summary

Aflatoxin B1, sterigmatocystin, and ochratoxin A are known to occur sporadically in green coffee beans. While aflatoxin B1 and sterigmatocystin do not seem to represent a considerable health risk for coffee consumers, there remain some open questions about ochratoxin A: data available at present indicate that the intake via coffee consumption might be higher than the virtually safe dose if one makes a worst case calculation. However,the influence of roasting is not yet clear, data on the decrease of ochratoxin A concentration during the roasting procedure vary between <10% and 90%. Furthermore it remains unclear whether ochratoxin A in roasted coffee beans can be transferred into the brew. Still, the contribution of coffee to the daily intake of ochratoxin A most probably lies below 10-20%.

Zusammenfassung

Aflatoxin B1, Sterigmatocystin und Ochratoxin A können sporadisch in grünen Kaffeebohnen vorkommen. Während Aflatoxin B1 und Sterigmatocystin kein wesentliches gesundheitliches Risiko darstellen für Kaffeetrinker, existieren bezüglich Ochratoxin einige noch ungeklärte Fragen. Wenn vom schlechtesten Fall ausgegangen wird, weisen die heute vorhandenen Literaturdaten darauf hin, dass die Ochratoxinaufnahme mit dem Kaffee höher sein könnte als die "virtuell sichere Dosis". Wie stark der Röstprozess jedoch den Ochratoxingehalt beeinflusst, ist umstritten: in der Literatur varieren die Angaben über die Zerstörung von Ochratoxin während des Röstens zwischen <10% und 90%. Zudem ist unklar, ob in gerösteten Kaffeebohnen enthaltenes Ochratoxin beim Brauen in den Kaffee übergeht. Trotz dieser noch offenen Fragen kann davon ausgegangen werden, dass der Beitrag des Kaffees zur täglichen Ochratoxinaufnahme wahrscheinlich unterhalb 10-20% liegt.

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DOES COFFEE/CAFFEINE CONSUMPTION BY WOMEN INFLUENCE THEIR RISK OF REPRODUCTIVE HAZARDS ?

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Although only about 30 studies have addressed the issue of coffee/caffeine consumption and the risk of reproductive adversities, the literature is so confusing that a total of 16 reviews have been published (1-16). Why should the confusion and controversy exist in the first place, and why should it continue? In what follows, I emphasize two phenomena that have not been adequately addressed.

Before I deal with these two phenomena, however, let me emphasize the limitations of epidemiology in general. Epidemiology is an inexact science, relying on observational studies. Attempts at making groups of humans truly comparable can never achieve what is possible with rodents in the laboratory. A relatively high proportion of the 30 studies had one or more methodologic limitations that limit the inferences that should be drawn. These limitations include problems with exposure ascertainment, sample selection, sample size, and attention to potential confounders.

Sometimes efforts at controlling for differences may be legitimate, but results faulty because the data are substandard. This is illustrated by what I call the Morrison phenomenon, named after Alan Morrison, Professor of Epidemiology at Brown University in Providence, Rhode Island (17).

People who consume relatively large amounts of coffee are more likely than others to consume alcohol and tobacco (18-23). Some people tend not to fully acknowledge their alcohol and tobacco consumption, apparently because they think others view consumption of large amounts of these as socially undesirable (24). This may be particularly true for pregnant women. If people do indeed underreport their tobacco and

alcohol consumption, but fully acknowledge their coffee consumption, then the coffee consumption variable conveys some information about the amount of alcohol and tobacco consumed.

Morrison has shown how underreporting tobacco consumption can account for an association between coffee and a disease, when, indeed, coffee and the disease are not associated, but tobacco and the disease are. Let me illustrate this.

The numbers in the following table are simplified for illustration. To make them appear more reasonable, add a zero to each.

TABLE 1: Ignoring smoking status

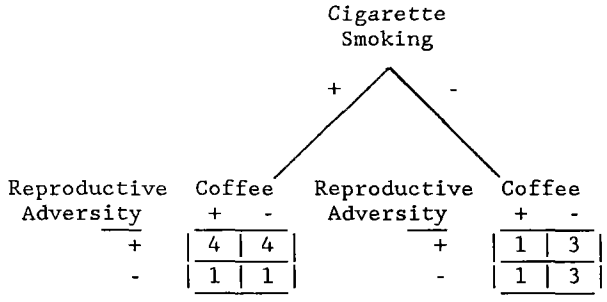
Reproductive Adversity	Coffee Consumption +	Coffee Consumption -
+	5	7
-	2	4

Odds Ratio = (5x4)/(7x2) = 1.4

If no attention is paid to tobacco, then coffee drinkers appear to be 1.4 times more likely than women who do not drink coffee to have experienced a reproductive adversity (Table 1).

Epidemiologists use the term confounder to identify a variable that is associated with the putative exposure and with the outcome of interest. Confounding occurs when our perception of the relationship between the exposure and the outcome is distorted. One technique to explore if confounding is occurring is to stratify by the confounder. Bacause cigarette smoking, if not adequately considered, might distort our perception of the relationship between coffee drinking and reproductive adversities, most careful investigators will at some point divide their sample into those who smoke cigarettes and those who do not.

TABLE 2: Stratifying by smoking status



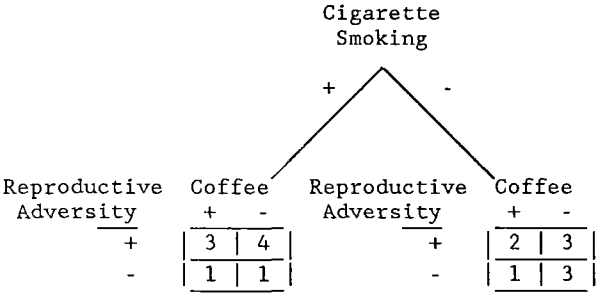
Odds Ratio	1.0	1.0

When we stratify by smoking status, coffee drinkers are not at increased risk of reproductive adversity. Notice that if you combine these two tables and ignore smoking status, the result will be Table 1.

One of the best ways to eliminate confounding is the exclusion strategy. This entails excluding all subjects who possess the putative confounder. In our example, it means excluding all women who acknowledge smoking cigarettes during the pregnancy (i.e., the left component of Table 2), and focusing our attention on those who reported they did not smoke at all since their last menstrual period (i.e., the right component of Table 2).

Consider the possibility that 20% of coffee drinkers who did smoke cigarettes denied tobacco exposure. Therefore, one of the five coffee drinkers in the left table (of Table 2) would not be in that table, but rather in the table on the right.

TABLE 3: If 20% of women who smoke and drink coffee deny smoking, then Table 2 becomes:



As a consequence, the estimate of the risk of adversity is twice as high in coffee drinkers as in women who did not drink coffee.

This is the Morrison phenomenon. In essence, truth has not changed, only the appearance of truth.

Now let's go on to another explanation for some of the claims that coffee consumption is not good for pregnant women. A number of reproductive hazards are associated with placental difficulties (25,26). Zena Stein and Mervyn Susser of Columbia University in New York City recently raised the possibility that some of these placental difficulties may influence a woman's consumption of coffee (27). I have attached their names to this concept mainly because I could not find a simple descriptive phrase. In addition, however, they have developed their concept with such elegance, and their concept is so important, that they deserve this recognition.

Their concept has a number of components.

1. Nausea is associated with a good reproductive outcome.

Studies have shown that nausea is less common in women who experience fetal wastage (i.e., miscarriage and stillbirth) than in women who give birth to an apparently healthy baby (28-30).

2. Nausea is a reflection of desirably-increased levels of hormones.

The higher the level of human chorionic gonadotropin (hCG), the greater the likelihood of nausea (31). Nausea does not persist throughout pregnancy. The diminution in nausea is temporally associated with a decrease in hCG.

3. In some instances, the absence or minimal presence of nausea reflects impaired placental synthesis of hormones.

Suboptimal increase in hormonal levels is associated with small placenta size and elevated risk of fetal wastage (32) Therefore, as might be expected, the absence of nausea conveys information about the heightened risk of reproductive adversities (27).

4. Nausea is associated with reduced coffee/caffeine consumption

In the study of Fenster and her colleagues that prompted the Stein-Susser commentary, 6.5% (81/1244) of women who were nauseated consumed three or more cups of coffee per day, whereas among women who were not nauseated, 9% drank an equivalent amount of coffee (58/642) (30). In that study, 16% of women who miscarried in the first trimester and 16% of women who carried to term reduced their caffeine but were not nauseated. In contrast, women who gave birth to a liveborn at term were considerably more likely than women who had a miscarriage to reduce caffeine consumption in the setting of nausea.

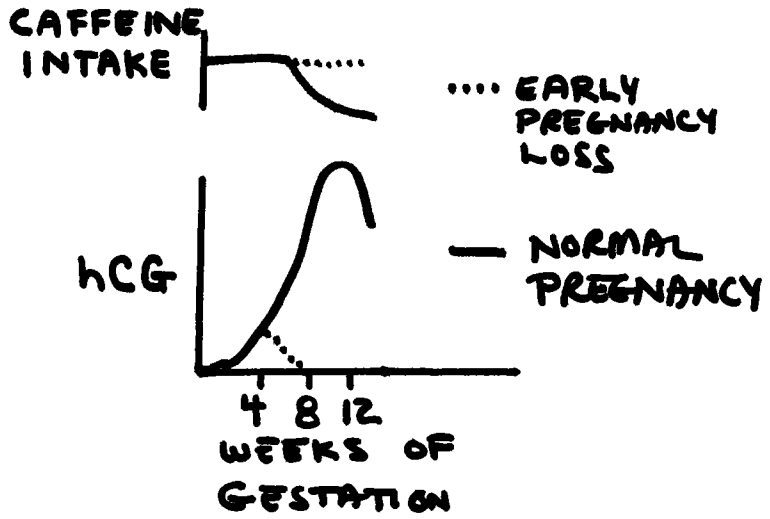
TABLE 4: Nausea and caffeine reduction in the spontaneous abortion case-control study of Fenster et al. (29). Percents are of column totals.

Nausea	Caffeine Reduction	Miscarriage	Livebirth
-	+	16%	16%
+	+	24	43
Total	+	40	59
Total	-	60	31

Caffeine metabolism is slowed in women near the end of their pregnancy (33-35). Evidence is not yet at hand, however, to demonstrate that caffeine metabolism is indeed slowed early in pregnancy. Thus, it is not yet known how much of the early reduction in caffeine consumption reflects delayed degradation. Between 16 and 40% of women reduce their coffee consumption in response to doubts about the wisdom of exposing their fetuses to caffeine, rather than because of nausea.

Stein and Susser, concerned about the sequence of events, offer the hypothesis that the viability of the pregnancy influences caffeine/coffee consumption, rather than the more frequently-made claim that caffeine/coffee consumption influences pregnancy viability. They offer something approaching the sequence in Figure 1. I have modified their own figure because I do not have evidence that women who miscarry reduce their coffee consumption because of nausea.

FIGURE 1: As chorionic gonadotropin (hCG) levels rise in normal pregnancy, caffeine consumption falls. Early pregnancy loss, however is associated with a limited hCG rise and with a minimal reduction in caffeine consumption.



The major point here is that a well-implanted, well-functioning placenta will secrete large amounts of human chorionic gonadotropin, whereas the placenta of a conceptus likely to be expelled in the next four to six weeks will not secrete very much HCG at any time. In this model, both nausea and coffee/caffeine reduction are manifestations that the placenta is functioning well. Thus, unchanged coffee consumption is a consequence, and not a cause, of the phenomenon leading to pregnancy loss.

If the Stein-Susser phenomenon is indeed true, then many of the allegations of an association between coffee consumption and reproduction hazards reflect epiphenomena (i.e., occurrences not in the causal chain). I have presented two explanations for an apparent link between coffee consumption and pregnancy adversities. One was identified a decade ago, and the other only two months ago. These phenomena delight epidemiologists because they promote and reinforce the skepticism that characterizes our profession. We admonish each other to "explain away our findings." Morrison, Stein and Susser have risen to the challenge, and we are all the better for it.

Let me conclude by trying to answer the question that is the title of this presentation. No matter how I look at the data from all the studies, I cannot find convincing evidence that coffee consumption adversely affects a woman's reproductive capacity or the well-being of her fetus. Some of the problems attributed to coffee and caffeine consumption may well reflect the Morrison and Stein-Susser phenomena.

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SUMMARY

Inferences about the relationship between coffee consumption and reproductive adversities might be wrong because of two concepts that, all too often, are not adequately addressed. One is the concept that coffee consumption is a marker of other exposures not fully adjusted for even with stratified and multivariate analyses. The second is that pregnant women who do not develop nausea, and therefore are less likely to reduce their coffee consumption, are at increased risk of reproductive hazards, but not because of coffee consumption.

THE METABOLISM AND ROLE OF PARAXANTHINE IN MEDIATING THE PHYSIOLOGICAL EFFECTS OF CAFFEINE

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INTRODUCTION

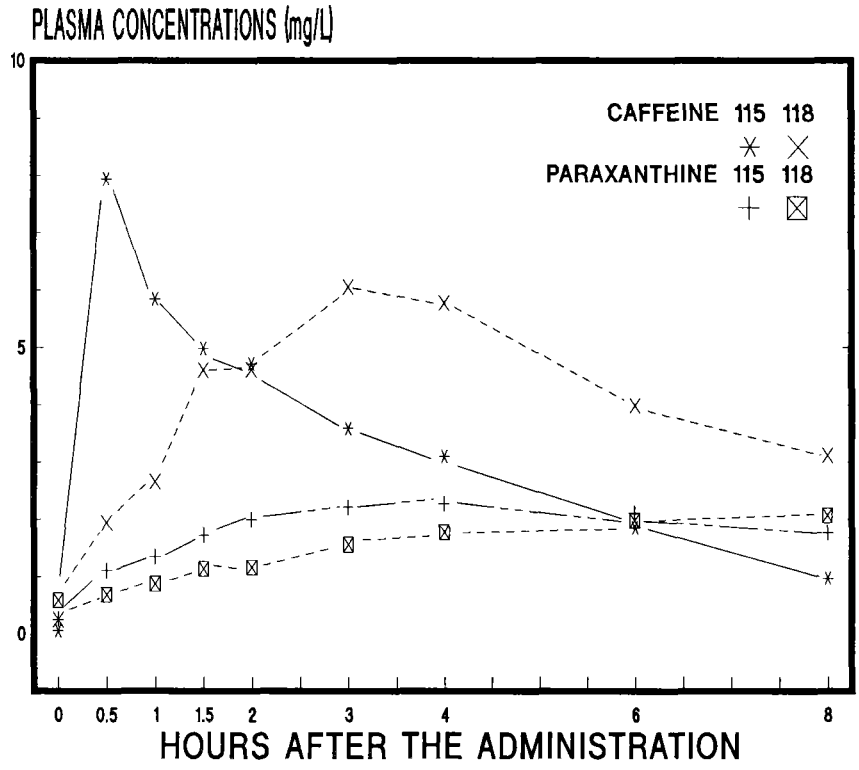
While the presence of caffeine (1,3,7-trimethylxanthine) was discovered in green coffee beans in 1820, theobromine (3,7-dimethylxanthine) in cocoa beans in 1842 and theophylline (1,3-dimethylxanthine) from tea leaves in 1888, it was only in 1980 that paraxanthine (1,7-dimethylxanthine) was identified as a constituent of coffea Arabica (Chou and Waller). This biosynthesis in plants leads to the accumulation of caffeine, theobromine and theophylline but only trace amounts of paraxanthine. Paraxanthine is, however, one of the most important caffeine metabolites in both animals and man, for that reason paraxanthine was first isolated from human urine in 1883 (Salomon). Important quantitative differences in the metabolism of caffeine through paraxanthine have been reported between animals and man. Since the physiological and pharmacological properties of caffeine correspond to the cumulative effects of the parent compound and its metabolites, the specific effects produced by paraxanthine have to be evaluated when results obtained in animals are extrapolated to man.
All of the biological effects observed in response to caffeine are presently attributed to caffeine itself and, in contrast with the other dimethylxanthines, paraxanthine is even not mentioned in the chapter on Xanthines in Pharmacology Textbook (Rall, 1990). Discrimination of the specific effects of paraxanthine and in consequence of caffeine will perhaps help us in the understanding of largely unexplained individual variations in caffeine sensitivity.
When caffeine formation and accumulation was discovered from theophylline administered for the management of apnea in the newborn infant, it was proposed that both theophylline and caffeine plasma concentrations should be monitored to evaluate the treatment better. We can hypothesize that in the case of caffeine ingestion in man both caffeine and paraxanthine plasma concentrations must be monitored to understand the physiogical effects observed.

DISTRIBUTION AND ELIMINATION OF PARAXANTHINE

Presence of paraxanthine in plasma, saliva, bile and urine

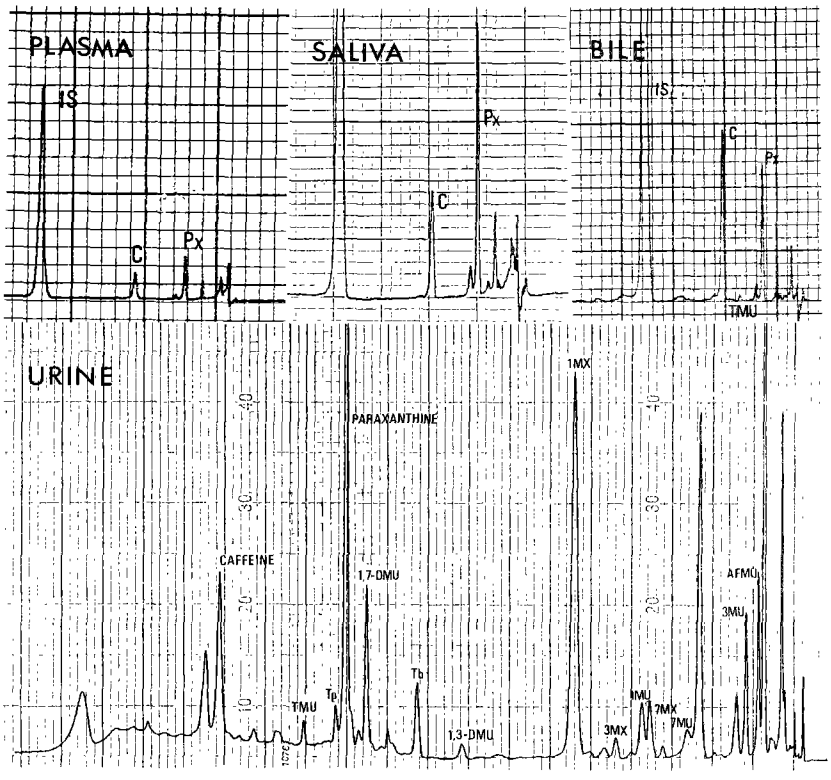
After coffee ingestion, the maximum value of caffeine plasma concentration as well as the time of the peak depends on the rate of gastric emptying and thus on the co-ingested foods. The concentration of caffeine then declines according to half-lives ranging from 2 to 6 hours, while on the contrary, the paraxanthine plasma concentrations, metabolized from caffeine, increase continuously to attain a plateau (Fig. 1). Paraxanthine has been reported to be formed at about seven times the rate of theophylline and theobromine (Bonati et al., 1982).

Fig.1
CAFFEINE AND PARAXANTHINE PLASMA CONCENTRATIONS
Variations observed between two volunteers taking coffee simultaneously with a standardized breakfast.



Following coffee administration caffeine plasma values become lower than those of paraxanthine after 5 to 8 hours. This relationship remains constant and fasting plasma levels of paraxanthine are always higher. It has been reported in man that caffeine, theophylline and theobromine are easily transferred from plasma to saliva so that a constant saliva/plasma concentration ratio can be calculated. We measured a mean ratio of 0.77 ± 0.12 (n=30) for caffeine, a value close to the fraction of unbound caffeine in plasma (0.75) (Suzuki Y. et al., 1989). In the literature, this saliva/plasma ratio varies from 0.61 to 1.02. Although micro assays have been developed for paraxanthine measurement in saliva (Badcock N.R., 1990), no saliva/plasma ratio has yet been published. We found a ratio of 0.87 ± 0.14 (n=32). This value is in agreement with the percentage of unbound paraxanthine to plasma protein reported in the rat. These in vitro measurement performed in the animal over a range of 1-100 mg/l varied from 83 ± 5 % to a maximum of 91 ± 3 % (Bortolotti et al., 1985).
In patients submitted to gall-bladder resection, bile was collected continuously after the ingestion of a cup of coffee containing 150 mg caffeine. Paraxanthine was present in each bile sample as was caffeine, theophylline and theobromine. The pattern of these metabolites closely followed those found in saliva and plasma (Arnaud and Grimaldi, unpublished results).
In the urine, paraxanthine is excreted in amounts 6-fold higher than caffeine (Arnaud and Welsch, 1980; Callahan et al., 1982). Fig. 2 shows the HPLC chromatograms of plasma, saliva, bile and urine collected 8 hours after the ingestion of coffee or caffeine.

Fig.2
PARAXANTHINE IN PLASMA, SALIVA, BILE AND URINE
Samples collected 8 hours after the administration of coffee.



Pharmacokininetics and distribution of paraxanthine
The distribution of paraxanthine has been evaluated from pharmacokinetics studies in rats (Bonati and Garattini, 1984; Bortolotti et al., 1985) and in humans (Lelo et al., 1989). In the rat close values were obtained for the volume of distribution (Vd) of caffeine and paraxanthine. In man, the Vd of 0.60 l/kg found for paraxanthine is also similar to that of caffeine. After oral administration of [8-^{14}C] paraxanthine in the rat, autoradiography of sections obtained 30 minutes later showed a distribution of radioactivity throughout the body: liver, heart, muscle, thymus, lungs and the gastrointestinal membranes. Higher concentrations of radioactivity were observed in the stomach, the kidney and the bladder showing that a fraction of the dose administered had still not emptied from the stomach and that another fraction was already actively excreted in the urine. Two hours after administration, the distribution pattern was similar except that lower concentrations of radioactivity were present in the stomach. Feces in the colon were radioactive but to a considerably lower level than the urine in the bladder. At that time, the concentration of radioactivity in the blood and the liver was the same indicating a complete equilibrium between blood and the tissues except for the brain. The concentration of radioactivity was lower in the brain which is protected by a blood-brain barrier. The brain to blood concentrations ratio was 0.30 for paraxanthine while this ratio is 1 for caffeine.
After 10 hours, only traces of radioactivity were detected in the body. Low concentrations were still present in the liver and the intestinal membranes while some feces in the colon exhibited the highest levels. However, radioactivity was not detectable in the blood.
In the rat, the kinetic parameters of caffeine and paraxanthine are similar with half-lives between 1 and 1.5 hour. The area under the plasma concentrations curve (AUC) was shown not to increase proportionally with doses higher than 15 mg/kgm, indicating that in the rat, both caffeine and paraxanthine are eliminated by a saturable process.
Pharmacokinetic parameters of caffeine and paraxanthine have been studied by Dorrbecker et al. (1987) in the New Zealand White rabbits which showed a polymorphism

with rapid (1.1 hour half-life) and slow (4.5 hours) caffeine metabolizers. At the highest dose of paraxanthine administered (8.5 mg/kg), the half-life was similar to that found in the rat. For these doses, paraxanthine elimination was not saturable in either slow or fast caffeine metabolizer. Interestingly, an additional experiment showed that the administration of paraxanthine simultaneously with caffeine inhibits caffeine metabolism and contributes to the saturation process observed in rapid metabolizer.

In man receiving a single dose of 3-4 mg/kg paraxanthine, a mean half-life of 3.9 ± 0.7 hours has been reported. This value is not significantly different from that of caffeine (Lelo et al., 1989). In that study, Cimetidine pretreatment resulted in a 40 % prolongation in paraxanthine elimination, an effect similar in magnitude to the effect of Cimetidine on caffeine (May et al., 1982). Saturation mechanisms for paraxanthine have been described in man at higher caffeine concentrations (Tang-Liu et al., 1983). It has been accepted for more than 10 years that the elimination of caffeine is independent on the dose (Newton et al., 1981; Bonati et al., 1982; Blanchard and Sawers, 1983) for doses ranging from 2 to 10 mg/kg. However, Kotake et al. (1982) and Tang-Liu et al., (1983) using breath tests and pharmacokinetic model respectively suggested saturation of caffeine metabolism. The results have been confirmed recently by Cheng et al. (1990) who demonstrated a higher caffeine clearance at 1 mg/kg dose and dose-dependent pharmacokinetics for higher doses up to 4 mg/kg. This disagreement observed in the dose range 1-4 mg/kg needs further investigations to confirm and evaluate the physiological significance of a saturation process in the elimination of caffeine and paraxanthine in man.

SPECIES DIFFERENCES IN THE METABOLISM OF CAFFEINE AND PARAXANTHINE

When different animal species such as rats, mice, Chinese hamsters (Arnaud, 1985) and monkeys (Bonati and Garattini, 1984) are compared, humans are characterized by a major demethylation of caffeine into paraxanthine. This pathway corresponds to 75-80 % of the first step of caffeine metabolism in man (Arnaud and Welsch, 1980) while it represents a maximum of 40 % in rats, 65 % in mice, 55 % in Chinese hamsters and less than 10 % in monkeys. Although incomplete, the results obtained with rabbits indicate that this animal seems the closest model to man with paraxanthine as the primary caffeine metabolite in the blood (Bonati and Garattini, 1984). However, unlike man, the rabbit seems relatively inefficient at further metabolizing paraxanthine (Dorrbecker et al., 1987).

In 1980 the urinary metabolic profile observed after the administration of paraxanthine to volunteers at a dose of 4 mg/kg (Arnaud and Welsch, 1980) was published. The recovery in urine collected on the two following days was only 61 % of the dose and reached 76 % when AAMU (Callahan et al., 1982) or its labile precursor

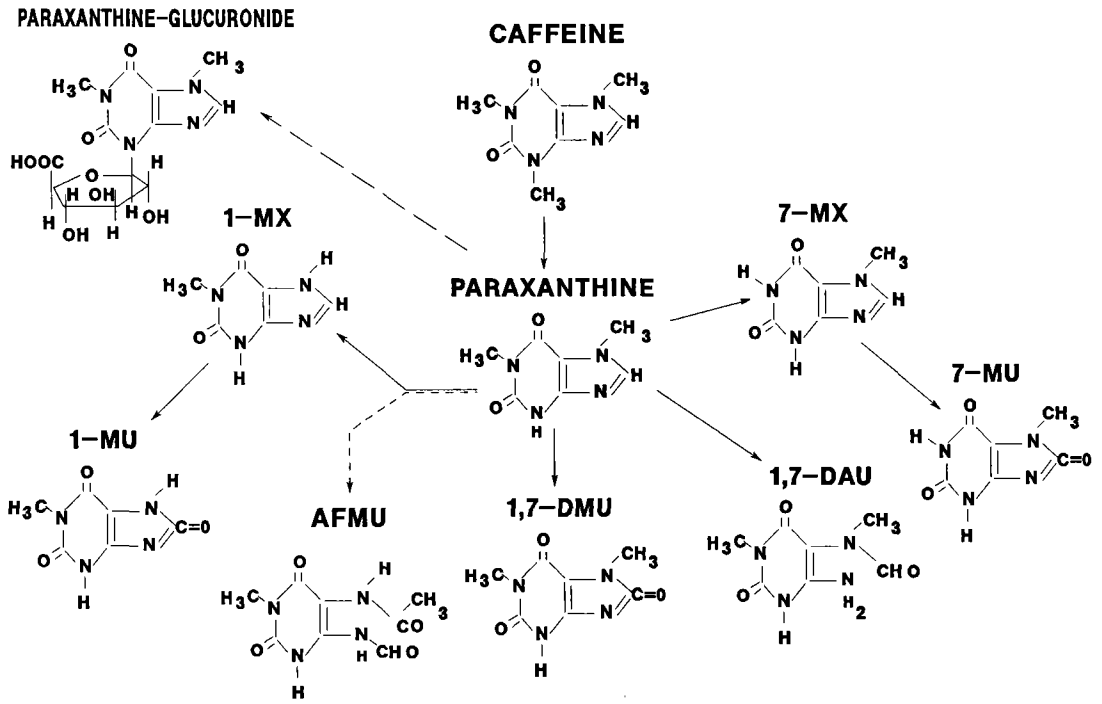
TABLE I: URINARY EXCRETION OF PARAXANTHINE METABOLITES 24-48 HOURS AFTER PARAXANTHINE ADMINISTRATION #

Metabolites	Human Studies		Rat Study
	Arnaud & Welsch (1980)	Lelo & al. (1989)	Arnaud & Welsch (1979)
Paraxanthine	10 ± 4	9 ± 3	44.2 ± 3
7-MX/7MU	0.3 ± 1*	5.2 ± 3*	2.3 ± 0.2
1-MX	15 ± 4	17.8 ± 4	9.3 ± 1
1-MU	17 ± 2	34.8 ± 10	17.8 ± 3
1,7-DMU	15 ± 3	7.7 ± 1	12.7 ± 2
1,7-DAU	4 ± 2	NA	$
AFMU	ND	14.2 ± 5	ND
Total	61 ± 3	88.7 ± 2	85 ± 3

Results are expressed as percentage (mean ± SEM) of the dose administered
* Result obtained for 7-MX. $ Result for 1,7-DAU included with 1,7-DMU
ND: not detected; NA: not analyzed

AFMU (Tang et al., 1983) was included. More recent results have been published (Lelo et al., 1989) with a recovery of the administered dose close to 90 %. The comparison of these studies is shown in Table 1. The recovery of 32 % of the dose as [1-methylxanthine+1-methyluric acid] found also by Tang-Liu et al. (1983) is certainly underestimated due to the difficulties encountered in quantitatively analyzing 1-methyluric acid.
The recovery of approximately 50 % found by Lelo et al. (1989) is in agreement with the 56 % reported after caffeine administration by Bonati et al. (1982).
The urinary metabolites of [1-Me-^{14}C]paraxanthine given orally to the rat have been analyzed (Arnaud and Welsch, 1979) and revealed that most of it is excreted unchanged in the rat (44 % of the dose) suggesting a limited capacity to metabolize paraxanthine (Table 1). This increased excretion of paraxanthine was compensated for by lower recoveries of 1-methylxanthine and 1-methyluric acid and the absence of AFMU.
The metabolic pathway of paraxanthine is presented in Fig. 3 for man, rats and mice (Arnaud and Welsch, 1980; Grant et al., 1984). The acetylated derivatives have not yet been identified in animal while a glucuroconjugate of paraxanthine was identified only in mice (Arnaud et al., 1986). Quantitative variations in this metabolic pathway were observed in man (Grant et al., 1983a) and it has been proposed (Grant et al., 1983b) that the molar ratio of AFMU to 1-methylxanthine or even better AFMU to [1-methylxanthine+1-methyluric acid] (Tang et al., 1987) be used to determine acetylator phenotype. Others ratios: 1-methyluric acid to 1-methylxanthine, 1,7-dimethyluric acid to paraxanthine, [AFMU+1-methylxanthine+1-methyluric acid] to paraxanthine (Kalow, 1984) and more recently [AFMU+1-methyl-xanthine+1-methyluric acid] to 1,7-dimethyluric acid (Campbell et al., 1987) represent indices of the activities of xanthine oxidase, microsomal 8-hydroxylation and 7-demethylation and the caffeine metabolic ratio (CMR) which has been shown to be correlated with the caffeine demethylation rate.

Fig.3
METABOLIC PATHWAYS OF PARAXANTHINE IN ANIMALS AND HUMANS



In ten women receiving two cups of coffee in the morning, one at noon and one in the afternoon, no correlation was found between paraxanthine saliva concentrations and acetylator phenotype (M. Arnaud, unpublished results). These results suggest that paraxanthine saliva or plasma concentrations are not controlled by metabolic clearance of paraxanthine. An inverse significant correlation ($p < 0.05$) was found between caffeine and paraxanthine saliva concentrations showing that paraxanthine concentrations are probably dependent on the 3-methyl demethylation of caffeine. This hypothesis was confirmed when caffeine and paraxanthine were shown to be inversely and directly correlated respectively to the metabolic clearance ratio (CMR) proposed by Campbell et al. (1987). This CMR index is based on the molar recovery of paraxanthine 7-demethylation products relative to a paraxanthine 8-hydroxylation product: CMR = (1-methylxanthine + 1-methyluric acid + AFMU/1,7-dimethyluric acid). Campbell et al. (1987) showed that CMR is correlated with all N-demethylation of caffeine and dimethylxanthines, these pathways being mediated in large part by polycyclic aromatic hydrocarbon-inducible P-450.

PHYSIOLOGICAL EFFECTS OF PARAXANTHINE

Adenosine receptors and spontaneous locomotor activity

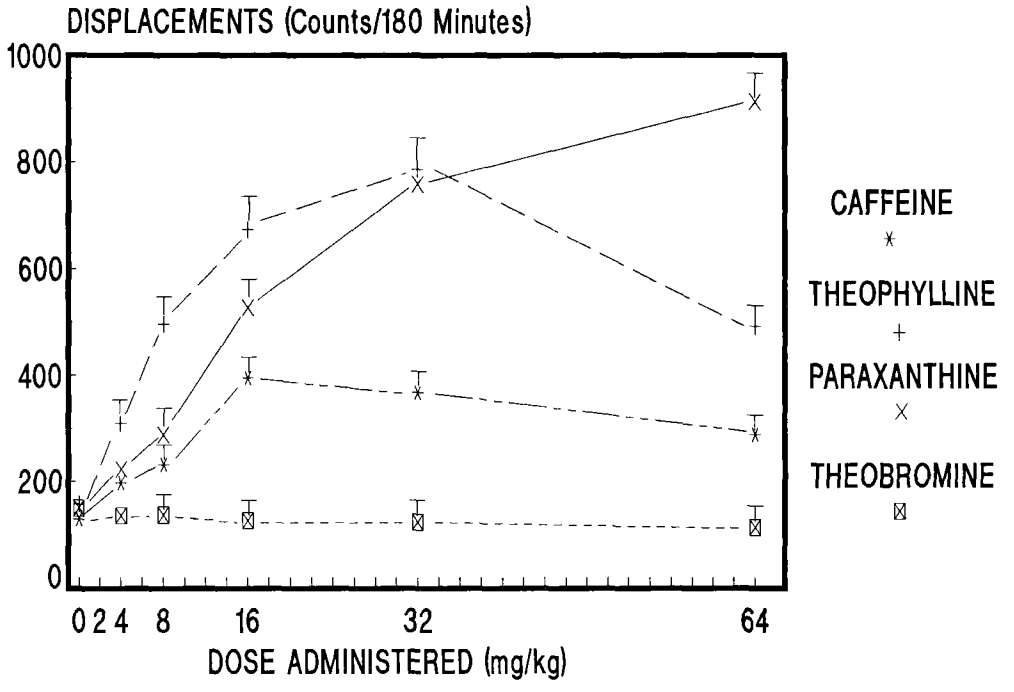
A review of methylxanthine toxicity to man (Stavric, 1988) mentioned without any references, that paraxanthine and its two metabolites 7-methylxanthine and 7-methyluric acid seem not to be pharmacologically active. This statement was supported by experiments performed in vitro on the isolated guinea-pig trachea (Persson et al., 1982). Paraxanthine exhibits a tracheal relaxant potency similar to theobromine but very low when compared to caffeine and theophylline. However, they reported that paraxanthine was more active as a adenosine-antagonist than caffeine but was less active than theophylline. They concluded that only methylxanthines with a methyl in a 1-position consistently antagonized the relaxant effect of adenosine. These results confirmed those of Snyder et al. (1981) showing that the order of potency on adenosine receptor binding was theophylline > paraxanthine > caffeine. A recent report suggests that paraxanthine could be a D-1 dopaminergic receptor antagonist and thus could be involved in the behavioral stimulation of caffeine (Ferré et al., 1990). The inhibition of the binding of adenosine receptor ligands has also been proposed as the mechanism. Snyder et al. (1981) showed in mice a biphasic effect of caffeine and paraxanthine 0.5-1 hour after their intraperitoneal injection. Locomotor depression was observed with doses lower than 1.8 mg/kg and stimulation at higher doses from 5 to 18 mg/kg. With subcutaneous injection of caffeine and a record of spontaneous motor activity for 3 hours stimulant effects of caffeine were already observed with a dose as small as 1 mg/kg (Natsuno and Inada, 1972). There was a progressive increase in spontaneous motor activity with increasing caffeine up to 16 mg/kg. This increase declined at 32 mg/kg.

We confirmed these results on male Sprague-Dawley rats aged 8-10 months with oral administration of caffeine (Fig.4). The locomotor activity of the rats, recorded for 3 hours, was also studied after the administration of dimethylxanthines: theobromine exhibited no stimulant effect while theophylline and paraxanthine were more potent than caffeine at all doses from 2 to 16 mg/kg. The stimulant effects still increased for doses of 24 and 32 mg/kg and for this last dose, the potency was similar for paraxanthine and theophylline. While paraxanthine stimulation was even higher at the 64 mg/kg dose, the number of displacements decreased for theophylline. This biphasic curve for theophylline, with a peak stimulation occuring at 30 mg/kg has already been reported in rats (Finn and Holtzman, 1987). We can hypothesize that a decreased stimulation of spontaneous motor activity may appear when toxic effects are produced. If this is the case, theophylline and caffeine would be more toxic than paraxanthine and these results would support the statement that the toxicological effects of paraxanthine are very low when compared with those of caffeine and theophylline (Stavric, 1988). The teratogenicity of paraxanthine has been studied in mice and high doses (300 mg/kg/day) were shown to be slightly less toxic to the embryo than caffeine (York et al., 1986).

Effects on lipolysis

The effects of caffeine on fat mobilization *in vitro* and *in vivo* has been known for a long time (Bellet et al., 1968) and the mechanism of action has also been

Fig. 4
EFECTS OF DIFFERENT ORAL DOSES OF CAFFEINE, PARAXANTHINE, THEOPHYLLINE AND THEOBROMINE ON SPONTANEOUS LOCOMOTOR ACTIVITY OF RATS



suggested to be the blockage of adenosine receptors (Fredholm, 1985). Hetzler et al. (1990) determined the relationship between the appearance of paraxanthine and free fatty acid (FFA) mobilization after intravenous caffeine administration (4 mg/kg lean body mass) in 10 healthy adult men. From venous blood samples, a high negative correlation was seen between decreases in caffeine and increases in FFA and a high positive correlation between the appearance of paraxanthine and FFA. Their conclusion that paraxanthine mediates the increased lipolytic affect after caffeine administration in humans needs confirmation by carrying out similar experiments using direct paraxanthine administration. It is quite possible that the negative correlation observed for caffeine can be explained by a time delay between the peak of plasma concentration and the lipolytic effect finally observed.

CONCLUSIONS

This review of the literature as well as the new results presented on paraxanthine metabolism and physiological properties demonstrate that paraxanthine metabolism has been actively investigated during the last decade and several reliable tests of hepatic enzyme activities have been established. Among these tests, the determination of acetylation phenotype is the most widely used as a research tool for pharmacologists and epidemiologists. Dose-dependent pharmacokinetics of paraxanthine elimination needs to be clearly established and its physiological significance must be evaluated. The mechanism of AFMU formation must also be known. Paraxanthine has been shown to be more potent than caffeine for physiological effects such as the stimulation of locomotor activity and at the cellular level as an antagonist of adenosine receptors. More research will be necessary in the coming years to discriminate the physiological effects of caffeine which are mediated by paraxanthine.

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SUMMARY

THE METABOLISM AND ROLE OF PARAXANTHINE IN MEDIATING THE PHYSIOLOGICAL EFFECTS OF CAFFEINE

Paraxanthine is not found in plants and appears only in man and animals as a metabolite of caffeine. While the formation of paraxanthine from caffeine is not the main metabolic pathway in animals, in man 80 % of caffeine is demethylated to form paraxanthine. Thus, when extrapolating results obtained in animals to man we have to take into account the specific effects of paraxanthine. This presentation reviews all data reported on paraxanthine.

Animal experiments have shown that paraxanthine at levels lower than 8 mg/kg is as potent as caffeine to stimulate the Central Nervous System but its potency is lower than that of theophylline. With higher doses (20 to 100 mg/kg), a dose-dependent increase in CNS activity was observed with paraxanthine while a decrease occurs with both caffeine and theophylline. These effects could be explained by a lower toxicity of paraxanthine. The increase of plasma free fatty acids attributed to the lipolytic effect of caffeine has been shown rather to be correlated to paraxanthine than caffeine plasma concentrations. In contrast with the few studies on the physiology of paraxanthine, its metabolism has been extensively investigated. Paraxanthine metabolism is used as a multifunctional liver test to evaluate the enzymatic activities of xanthine oxidase, microsomal 7-methyl demethylation and C-8 hydroxylation. Paraxanthine has been shown to be excreted as an acetylated metabolite and a test for the determination of human acetylation phenotype has been established. However, acetylator phenotype did not explain interindividual differences in the elimination of caffeine. A significant inverse relationship between paraxanthine and caffeine plasma concentrations demonstrated the importance of the rate of 3-methyl demethylation in the individual variations observed.

RESUME

METABOLISME ET ROLE SPECIFIQUE DE LA PARAXANTHINE DANS LES EFFETS PHYSIOLOGIQUES ATTRIBUES A LA CAFEINE

La paraxanthine n'est pas un constituant des plantes, elle apparaît seulement chez l'homme et l'animal comme un métabolite de la caféine. Alors que la formation de paraxanthine à partir de la caféine ne constitue pas une voie de transformation importante chez l'animal, 80 % de la caféine est déméthylée chez l'homme pour former la paraxanthine. Ainsi, lorsque l'on extrapole les résultats obtenus chez l'animal à l'homme, il est nécessaire de tenir compte et de connaître les effets spécifiques dus à la paraxanthine. Cette communication résume nos connaissances acquises sur la paraxanthine.

Des études chez l'animal démontrent que la paraxanthine à des doses inférieures à 8 mg/kg stimule autant le Système Nerveux Central que la caféine mais beaucoup moins que la théophylline. Toutefois, pour des doses plus élevées (20 à 100 mg/kg), une augmentation de l'effet stimulant dépendant de la dose est observée pour la paraxanthine tandis que cet effet diminue pour la caféine et la théophylline. Ces effets pourraient être expliqués par une plus faible toxicité de la paraxanthine. La lipolyse attribuée à la caféine, provoquant une élévation des concentrations plasmatiques d'acides gras libres, est mieux corrélée avec les concentrations de paraxanthine que celles de caféine. S'il y a eu encore peu d'études sur les effets physiologiques de la paraxanthine, son métabolisme a été plus fréquemment étudié. Le métabolisme de la paraxanthine est utilisé comme test multifonctionnel hépatique pour évaluer les activités enzymatiques de la xanthine oxydase et des activités microsomiales de déméthylation du groupe 7-Méthyle et d'hydroxylation en C-8. La paraxanthine est aussi éliminée sous la forme d'un métabolite acétylé et un test a été établi permettant la détermination du phénotype d'acétylation chez l'homme. Toutefois, ce phénotype d'acétylation n'explique pas les variations interindividuelles dans l'élimination de la caféine. Par contre, une corrélation inverse significative entre les concentrations plasmatiques de la paraxanthine et de la caféine démontre l'importance de la cinétique de la déméthylation du groupe 3-Méthyle dans les variations individuelles observées.

COFFEE - AN UNEXPECTED CHOLESTEROL-RAISING FACTOR

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Background

Coffee has like all nutritional items been examined for health hazards. The experiment on coffee and cholesterol was in 1970 when Egede-Nissen reported on the effect of abstaining from coffee on total cholesterol in hypercholesterolaemic subjects. The author claimed that refraining from coffee reduced the total cholesterol level by 17 % (1).

A report from northern Norway in 1983 on the cross-sectional association between total cholesterol and coffee intake brought coffee back on the cardiovascular agenda and again as a possible cholesterol-increasing factor (2). In this Norwegian study those drinking 5-8 cups per day had 0.51 mmol higher total cholesterol levels than those consuming less than one cup of coffee per day.

Epidemiological evidence

A review of twenty-four cross-sectional studies of the effect of coffee on serum cholesterol illustrates the difficulties in assessing this possible relationship. Ten studies, involving nearly 110,000 subjects, demonstrated a significant positive relationship between coffee consumption and serum cholesterol for both sexes (2,4-12).

In the remaining 14 studies, the evidence for a link between coffee and cholesterol is less clear-cut. In five studies, involving a total of 13,000 people, no relationship at all was demonstrated (13-17) and in a further three, a link between coffee consumption and serum cholesterol was apparent only in women (18-20). Six studies all of which dealt only with men, demonstrated a firm association in four cases (3,21-23) results from the last two studies being inconclusive (24,25). A recent study in Belgium observed a weak but consistent association between coffee and cholesterol in male soldiers, but not in females (26). The discrepancies between the studies would suggest that confounding factors such as brewing methods were distorting the relationship between coffee and cholesterol.
An extensive meta-analysis including 24 cross-sectional studies demonstrated that the beta-coefficient or increase in total cholesterol per cup of coffee was 0.008 mmol/l in populations consuming filtered brews, whereas the corresponding figure was 0.038 mmol/l for those drinking unfiltered (boiled) coffee (27).

Brewing methods

This observation has been confirmed by experiments (28-30) where it was shown that brewing methods involving filtering resulted only in a very slight increase in cholesterol levels compared to that of the unfiltered brew.

The idea that coffee might contain a lipid-raising factor was further taken up by Zock et al. in Wageningen (31). They heated 1350 kg of water to boiling in 150 kg batches with 15 kg of coarsely ground coffee per batch. After centrifugation a supernatant was visible. This supernatant was lipid rich, and the enrichment was ten times that of boiled coffee. It was given to ten volunteers mixed in their daily meals for 42 days, correspondingly to drinking approximately 6-7 cups of unfiltered or boiled coffee per day. During the trial the LDL-cholesterol increased by 0.85 mmol/l, which again is consistent with what has been observed in experiments exposing people to boiled non-filtered coffee.

Thus it is highly possible that brewing methods really do play a role, and explain why there are national and regional differences in the cholesterol raising effect of coffee.

The exact nature of the lipid-raising in coffee factor is still unknown, as well as the mechanism by which this naturally occurring substance is exerting its effect. In the Nordic countries the cholesterol levels have long been recognized as remarkably much higher than what is observed in other parts of Europe (32). Part of this increased level can be explained by the particular coffee-brewing methods which are still in use in certain areas (33). Salonen et al. actually ascribed a substantial proportion of the decline in total cholesterol levels which has occurred in Finland the last fifteen years to the change in coffeebrewing methods (34).

From a public health point of view coffee is virtually harmless as long as one prepares it by methods including filtering. Thus one may continue enjoying this popular beverage which, even if it is without any known nutritional value, remains one of our most stimulating drinks (35).

Summary

The first experiment on coffee and cholesterol is mentioned in 1970. The author claimed that refraining from coffee reduced the total cholesterol level by 17 %. Ten out of 24 studies, involving nearly 110,000 subjects, demonstrated a significant positive relationship between coffee consumption and serum cholesterol for both sexes. In the remaining 14 studies, the evidence for a link between coffee and cholesterol is less clear-cut. An extensive meta-analysis including the 24 cross-sectional studies showed that the beta-coefficient or increase in total cholesterol per cup of coffee was 0.008 mmol/l in populations consuming filtered brews, whereas the corresponding figure was 0.038 mmol/l for those drinking unfiltered (boiled) coffee. This observation has been confirmed by experiments conducted in the Nordic countries and the Netherlands. In these experiments it was shown that brewing methods involving filtering resulted in only a very slight increase in cholesterol levels compared to that of the unfiltered brew. Zock et al. in Wageningen concluded that there is a lipid-raising factor in coffee which must be a powerful naturally occurring substance. It is also reasonable to conclude that filtering might retain this substance as the brewing methods seem to be of such importance. This finding, that brewing methods really do play a role may explain why there are national and regional differences in the cholesterol raising effect of coffee.

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COFFEE AND CARDIOVASCULAR DISEASE : NO GROUNDS FOR FEAR

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Introduction

An association between coffee and cardiovascular mortality and morbidity has been debated for several decades. In 1963, Paul and coworkers observed a link between coffee use and cardiovascular disease in a cohort study (1). This report was followed by many others, providing both confirmation and refutation of the hypothesis that coffee consumption increases the risk of a cardiovascular event (2-26). Tables 1 and summarizes the main characteristics and results of the studies on coffee and fatal and non-fatal cardiovascular disease, including 17 studies with a cohort design and 10 case-control studies. The discrepancy between the findings may result from issues such as study design, choice of control group in case-control studies, recall bias and time interval between coffee intake assessment and cardiovascular event. Moreover, coffee consumption has been related to a number of cardiovascular risk indicators, such as smoking, dietary fat intake and low physical activity (27). Inadequate or incomplete adjustment for these confounding variables may affect the results of a study on coffee and cardiovascular disease. In the following paragraphs the available studies will be discussed, with special emphasis on methodological aspects.

Design and bias

The relationship between coffee and cardiovascular disease has been investigated in both cohort studies and case-control studies with inconsistent results. Most case-control studies show an increased risk of myocardial infarction in heavy coffee consumers (3,4,10,12,21-23). By contrast, the results of most cohort studies suggest the absence of a link between coffee and cardiovascular disease (6,7,9,10,11,13,14,16,19,24,26).

Recall bias Generally, prospective follow-up studies offer the best basis to obtain valid conclusions regarding the relation of any host or environmental factor to disease development (7). In particular, the cohort design reduces the possibility of

biased reporting of coffee use or other characteristics. This so called "recall bias" may act differently for coffee as for smoking. In a case-control study, cases may systematically overreport coffee-consumption because they are aware of a possible link between coffee and myocardial infarction and as a result the effect will be overestimated. Alternatively, cases may underreport smoking relative to controls, possibly caused by feelings of guilt, and again overestimation of the effect will result, since part of the apparent effect of coffee is confounded by heavier smoking in the cases. Similarly, proposed that the psychological impact of the experience of a myocardial infarction may influence the patients dietary reporting (10). The possibility that questions about coffee use in the period prior to hospitalization are subject to recall bias is difficult to exclude. For this reason, findings in case-control studies always need careful interpretation. In a case-control study of Hennekens et al. the cases had died of coronary heart disease. Wives of cases and controls were asked about coffee consumption habits of their husbands. If one considers coffee drinking unhealthy, wives of patients might have overestimated their husbands' coffee consumption, whereas wives of controls might have underestimated, resulting in overestimation of a fatal effect of coffee use. The investigators, however, reported no increased risk of coffee use on fatal myocardial infarction (8).

Hospital controls Two early case-control studies performed in Boston showed increased risk of non-fatal myocardial infarction with increased coffee consumption (3,4). The results, however, are often criticised to be distorted by the inclusion of hospitalized patients with chronic conditions among the controls, artificially inducing an effect. Rosenberg and co-workers expressed their concern of inclusion of an excessive number of subjects with gastro-intestinal or other diseases in which coffee drinking has been either abandoned or medically proscribed. Among hospitalized women, those admitted for chronic conditions reported significantly less coffee consumption than those admitted for acute conditions (28). In another study, coffee use in hospitalized subjects was compared with coffee consumption in the general population. Those hospitalized for gastro-intestinal disorders and conditions such as diabetes mellitus, rheumatic heart disease, chronic lung disorders, cardiovascular disease and chronic nephritis, were consuming less coffee than population controls (29). The results indicate that the referent group in hospital based case-control studies on coffee and cardiovascular disease should be restricted to patients hospitalized for conditions not affecting dietary habits. If not, an association observed in a case-control study might be due to decreased coffee consumption among controls rather than to an excessive consumption among the cases. The group of Rosenberg performed several case-control studies, in which controls were hospital admitted for conditions unrelated to coffee consumption such as traumatic injury and non-respiratory infections (12,21,22). Conditions of recent and rapid onset are unlikely to have influenced coffee consumption in the period before admission. By contrast, Jick reanalyzed the Boston results and concluded that coffee drinking habits were not different between acute and chronic patients and thus the results could not have been affected by the inclusion of patients with chronic conditions in the control series (30). The choice of hospital controls is an important methodological issue, potentially leading to biased results. The principle issue, however, is the comparability of cases and controls with respect to the accuracy of information of the determinant under study (31).

Follow-up time

In a study of Schreiber and co-workers coffee drinking habits were found to vary significantly over time. A random sample of 2,714 US citizens were asked to compare present caffeinated coffee drinking with consumption in the 10 preceding years. 55 % had changed, with 38 % currently drinking less and 17 % drinking more

Table 1. A summary of studies on coffee and fatal and nonfatal cardiovascular disease.* The last column reflects the opinion of the authors on the association between coffee and cardiovascular risk.

Ref.	*Year publ.*	*First Author*	*Design*	*Size n or c/c*	*Years of follow-up*	*Relative risk estimate*		*Statistical significance*	*Endpoints*	*Authors conclusion*
1	63	Paul	cohort	1162	4	1.3	5 c/d vs.0	< 0.025	all chd	present
2	65	Little	cc	86/84	-	1.0	?	?	nonfatal mi	absent
3	72	BCDSP	cc	276/1104	-	1.3	1-5 c/d vs.0		nonfatal mi	present
						2.1	6+ c/d vs.0	< 0.003 trend		
4	73	Jick	cc	440/123190	-	1.6	1-5 c/d vs.0	< 0.001	nonfatal mi	present
5	73	Klatsky	cc	464/928	-	1.0	6+ vs. < 6	?	nonfatal mi	absent
6	73	Hrubec	cohort	10744	?	1.3	1-5 c/d vs.0		angina	dubious
						1.5	6+ c/d vs.0	0.06 trend		
7	74	Dawber	cohort	4492	12	1.0	any level	?	mortality	absent
						1.0	any level	?	all chd	absent
8	76	Hennekens	cc	649/649	-	1.2	1-5 c/d vs.0	ns	fatal mi	absent
						1.0	6+ c/d vs.0	?		absent
9	77	Yano	cohort	7705	6	?	5+ c/d vs.0	ns	all chd	absent
10	77	Wilhelmsen	cohort	846	12	?		?	all chd	absent
			cc	220/846	-	?		"significant"	nonfatal mi	present?
11	78	Heyden	cohort	2530	4.5	1.0	5+ vs. < 5	?	mortality	absent
						1.0	5+ vs. < 5	?	fatal chd	absent
						1.0	5+ vs. < 5	?	fatal cva	absent
12	80	Rosenberg	cc	487/980	-	1.4	5+ c/d vs.0	0.05	nonfatal mi	present?
13	81	Murray	cohort	16911	11.5	1.0	any level	?	fatal chd	absent
						0.9	7+ c/d vs.0	0.01 trend	non-chd death	artificial
14	84	Welin	cohort	855	17	?	any level	ns	all chd	absent
			cohort	6500	3-7	?	any level	ns	all chd	absent
						?	any level	ns	mortality	absent

continued...

Table 1 (cont.).

Ref.	*Year publ.*	*First Author*	*Design*	*Size n or c/c*	*Years of follow-up*	*Relative risk estimate*		*Statistical significance*	*Endpoints*	*Authors conclusion*
15	84	Kahn	cohort	27,530	21	1.2	3+ vs. < 1	ns	mortality	absent
16	86	Jacobsen	cohort	16,555	11.5	0.9	7+ vs. < 2	ns	all chd	absent
17	86	LaCroix	cohort	1,130	19-35	1.8	5+ c/d vs. 0	ns	all chd	absent
					< 5	2.5	5+ c/d vs.0	< 0.05	all chd	present
18	86	Vandenbroucke	cohort	1,583 (m)	25	1.4	5+ c/d vs.0	± 0.10	mortality	present
				1,508 (f)		0.8	5+ c/d vs.0	ns		absent
19	87	Yano	cohort	7,194	15	1.2	coffee yes/no	ns	all chd	absent
20	87	LeGrady	cohort	1,910	19	1.3	6+ vs. < 6	< 0.05	mortality	dubious
						1.7	6+ vs. < 6	< 0.05	fatal chd	present
21	87	Rosenberg	cc	491/1,119	-	0.8	1-4 c/d vs.0	ns	non-fatal mi	
						2.1	5+ c/d vs.0	0.05		present
						1.0	1-4 c/d (caf)	?		
						1.6	5-9 c/d (caf)	0.05		
						2.1	10+ c/d (caf)	< 0.05		
						1.3	1-4 c/d (decaf)	ns		
						1.2	5+ c/d (decaf)	ns		
22	88	Rosenberg	cc	1,873/1,161	-	1.4	1-2 c/d vs.0	< 0.001 trend	nonfatal mi	present
						1.6	3-4 c/d vs.0			
						1.8	5-9 c/d vs.0			
						2.9	10+ c/d vs.0			
23	89	LaVecchia	cc	262/519	-	1.7	4+ c/d vs.0	0.02 trend	nonfatal mi	present
24	89	Wilson	cohort	5,209	10-20	1.0	per cup	ns	all cvd	absent
25	89	Tverdal	cohort	38,564	6.4	2.2	9+ vs.< 1 (m)	< 0.05	fatal chd	present
						5.1	9+ vs.< 1	ns		present
26	90	Grobbee	cohort	45,589	2	1.0	4+ c/d vs. 0		all cvd	absent

chd = coronary heart disease, mi = myocardial infarction, cva = cerebrovascular accident, cvd = cardiovascular disease, cc = case control, c/d = cups/day

* As the presentation of data varies markedly across data, some of the information needed to be inferred from tables or figures rather than taken directly from the published work.

than they had 10 years earlier (32). These results suggest that past coffee and caffeine consumption habits may be poor surrogates for current intake and if it is recent or cumulative use of coffee that affects the risk, a single measure in the distant past is unsatisfactory and could result in underestimation of an effect. This is supported by the relatively consistent absence of coffee effects on cardiovascular disease in studies where the intake was assessed long before the coronary events. The positive finding of LeGrady et al. in a study with a follow-up time of 19 years is an exception (20). The limitation of inadequate consideration of changes in coffee consumption over time was overcome by LaCroix et al. by measuring coffee drinking and smoking repeatedly during a long-term cohort study of medical students (17). The positive association observed, was strongest when the time between reported coffee intake and the coronary event was shortest. The relative risk of coronary heart disease for consumers of 5 cups or more per day compared to non-consumers was observed to be 1.8 (n.s.) with coffee intake assessed 19 to 34 years before the coronary event and 2.5 (95 % CL 1.1, 5.8) with coffee consumption assessed less than 5 years ago (17). By contrast, several authors emphasized the need in follow-up studies on coffee and mortality for exclusion of deaths occurring shortly after the coffee consumption is reported (13,16). In two cohort studies mortality in the first years of follow-up related strongly to coffee drinking habits; those with low coffee consumption having higher than expected mortality (13,16). Apparently, patients with chronic diseases and a higher risk of dying tend to have lower coffee consumption than the general population.

Confounding

In a cross-sectional study of 14,582 men and women, coffee drinking was negatively related to the use of low-fat milk, use of table fat high in poly-unsaturated fatty acids, use of fruits and vegetables, and positively associated with bread consumption. Three persons out of four with high coffee consumption (> 8 cups per day) were daily smokers, in contrast to about a quarter of those with low coffee consumption (<1 cup per day). In women and young men, high coffee consumption was associated with low physical activity at leisure. These data suggest that high coffee consumption may be an indicator of a lifestyle with high risk of coronary heart disease (27). Clustering of risk factors was also found by others (33,34). Hennekens proposed that the Boston investigators reporting a positive association between coffee use and nonfatal myocardial infarction did not control for enough variables, leaving room for residual confounding. In their own case-control study they demonstrated a nearly two-fold increase in risk of death from ischemic heart disease in heavy coffee drinkers when controlling only for those variables used in the Boston study, but when they controlled for additional variables, such as physical activity and coffee additives, no increase in risk was found (8). Rosenberg et al. emphasized the need for studies with detailed information on dietary factors that are possibly related to coffee consumption and cholesterol levels (22). An atherogenic diet was indeed shown to be more common among men who drink a great deal of coffee than among other men (35). Other investigators suggested the possibility that coffee consumption is a marker of stress (17,36). Highly stressed subjects may drink

considerable amounts of coffee during the day. In a study of 2,714 white US adults, however, of 32 risk factors analyzed by linear and logistic regression, only sex and cigarette smoking were found to be important potential confounders of the relationship between coffee intake and disease (37). Of all potential confounders of the coffee-cardiovascular disease association, smoking is without doubt the most important one. The significant correlation between coffee consumption and the later development of ischemic heart disease observed by Paul et al. in 1968 was later demonstrated to be entirely accounted for by tobacco use (38). This observation is shared by many other investigators (7,9,11,15,18,22). In the study of LeGrady, however, an association between coffee and fatal coronary heart disease was present in both smokers and non-smokers (20). It seems important to consider changes over time in smoking (17). In populations with growing numbers of subjects who have quit smoking, the measure of smoking at baseline may greatly overestimate actual exposure to cigarettes as follow-up time increases (39). Probably the best way to exclude the confounding effects of smoking is to study non-smoking subjects only. This was closely achieved in the recently published Health Professionals Follow Study, the cohort of which comprised only 10% smokers.

According to LaCroix et al., some investigators did not find an association, because the comparison group in their studies comprised a substantial number of coffee drinkers, e.g. persons consuming less than 5 cups per day (11), rather than the completely unexposed segment of the cohort of non-drinkers alone (17). By contrast, Rosenberg et al. argued that the health related behaviour of men who never drink coffee may differ in important ways, that are difficult to measure, from that of men who drink coffee (22). Thus, a comparison of coffee drinkers with never drinkers may well overestimate an adverse effect of coffee on the risk of myocardial infarction because of the inability to control completely for such behaviour. Therefore, it is more convincing if a statistically significant trend is observed over the entire range of coffee consumption categories. On the other hand, the possibility of a threshold phenomenon, where no effect is present until a certain level of the exposure is reached, cannot be ruled out. According to the threshold, a very high coffee intake may increase an individual's risk (3,4,21,25).

Biological plausibility, indirect and direct effects

Only a few papers on coffee and cardiovascular disease discuss the mechanism of the potential connection (16,17,23,26,40). The most plausible biological explanation is a positive link between coffee intake and serum cholesterol level or blood pressure. Alternatively, coffee may induce cardiac arrhythmias (41,42). These variables, potentially in the causal pathway from coffee to cardiovascular disease are frequently included in the multivariate analyses (5,8,10,12,14,17,18,20,21,23-25). This approach may be questioned when the crude effect of coffee use is of interest. Adjustment for intermediate factors may then lead to underestimation of the risk (22,43,44). Yet, when the direct effect of coffee is at issue, adjustment for intermediate risk factors may be appropriate. Preferably, results of both analyses

should be provided. From both non-experimental studies and randomized trials, boiled coffee was shown to raise serum cholesterol levels (45-48,49). The cholesterol increasing effect of coffee is probably mediated by brewing method and seems to be confined to methods where no filter is used, such as boiled coffee, Turkish coffee, espresso and percolated coffee. The positive association between coffee and non-fatal myocardial infarction in an Italian study may for example have resulted in part from the potential serum cholesterol increasing effect of espresso coffee (23). Likewise, the positive results of some American studies might be explained by the use of percolated coffee (3,4,12,17,20-22). Given the strong association observed between coffee consumption and serum cholesterol level in the Norwegian population, it is remarkable that Jacobsen et al. did not find an association between coffee consumption and ischemic heart disease in a large cohort study (16). By contrast, a positive association between heavy coffee use and cardiovascular death was reported by Tverdal and colleagues (25). The relative risk estimate reached statistical significance in men, but not in women. The evidence for a blood pressure raising effect of coffee is inconsistent and, if present, only small (46,50-52). The study of Martin et al. comprised 10,064 diagnosed hypertensive individuals. During 4 years of follow-up, all-cause mortality and cardiovascular mortality did not change by level of caffeine consumption (40). In a double-blind, placebo-controlled study, a large dose of caffeine (300 mg) did not cause ventricular arrhythmias in 70 patients with a recent myocardial infarction (53). A recent review of available data did similarly conclude that caffeine is not arrhythmogenic (54).

Conclusions

The study of coffee and cardiovascular disease is complicated by many potential sources of bias. Early case-control studies linking coffee intake to myocardial infarction have not been consistently supported by several relevant case-control and prospective follow-up studies with diverse design features.
The main problem in case-control studies is recall bias. Cases just recovering from a myocardial infarction, or partners of cases who just died of a coronary event, may overestimate coffee consumption and consequently, the risk estimate of coffee use may be too high. In addition, a careful choice of control subjects in case-control studies is of paramount importance (26). If the study is hospital-based, subjects with conditions leading to decreased coffee consumption should preferably be excluded from the control group. If not, the overall risk estimate for coffee use may be determined by diminished coffee consumption among controls rather than increased consumption among cases.
In a prospective cohort study, the interval between the measurement of coffee intake and the manifestations of coronary disease may be too long for an association to be observed. Alternatively, the interval may be too short, resulting in high event rates among low coffee consumers. This can be explained by the observation that patients suffering from chronic diseases and therefore at a higher risk of dying, tend

to drink less coffee than healthy subjects. Since coronary atherosclerosis is thought to develop gradually over a period of several years before it produces clinical symptoms it seems most accurate to evaluate the cumulative effect of coffee consumption over a short period up to 5 years (22,53). This, however, would not apply to more acute effects of coffee, e.g. arrhythmias and hemostatic changes. Coffee consumers may be at increased risk of a number of diseases, perhaps not because of coffee intake per se, but because of other aspects of their lives and lifestyle. Coffee consumption appears to be strongly related to smoking habits. Moreover, high dietary fat intake, low physical activity, psychological stress and other risk factors for coronary heart disease are reported to be associated with heavy coffee use. These variables should be accounted for in the analysis of studies dealing with coffee and cardiovascular disease. Adjustment for potential intermediate factors such as serum cholesterol and blood pressure should be avoided or reported separately.

Only four out of seventeen follow-up studies however have shown a positive association between coffee use and cardiovascular disease (1,17,20,25). The report of Paul et al. was followed several years later by a paper in which the positive findings were contributed to smoking habits among coffee drinkers (1,38). The prospective study of LaCroix et al. included only 47 cases of coronary heart disease and consequently, the confidence interval around the risk estimate was wide (RR 2.5, 95% CL 1.1, 5.8) (17). In the study of LeGrady et al, the relative risk of a fatal coronary event was 1.33 (1.07, 1.65) for men drinking 6 or more cups of coffee per day compared with those drinking less (20). The time interval between coffee intake assessment and coronary event was 19 years. Recently, a risk associated with coffee was reported from a large Norwegian study (25). For heavy coffee consumers (more than 9 cups a day) relative to subjects drinking less than 1 cup per day, the relative risk was 2.2 (1.1, 4.5) for men and 5.1 (0.4, 60.3) for women.

Findings in the large Health Professionals Follow Up Study, however, virtually exclude any increased risk for major cardiovascular events from moderate coffee consumption in healthy subjects (26). The finding, in this study, of an increased risk with increasing use of decaffeinated coffee warrants further attention and at present its basis and significance remains unexplained. On balance, the available data justify the conclusion that moderate coffee consumption is not likely to be a major risk factor for cardiovascular disease.

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BREWING MAKES THE DIFFERENCE

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1. Introduction.

This afternoon we have heard a number of lectures on the physiological effects of drinking coffee. The consumption of the drink is the final stage of the long route from growing the coffee tree to preparing the drink. Use of green coffee beans from different origins in blending, variations in roasting, grinding and packaging are all factors influencing the final result: a cup of coffee. But also from this point on with the choice of brewing conditions, and the coffee to go with it, a wide range of variations can be introduced. The properties of a cup of coffee are determined by either the operator in the out-of-home field or the consumer at home. In the latter case in particular much is left to the choice of the consumer. Then very often brewing makes the difference.

In this lecture I want to give an impression of how much variation can exist between coffee brews. This also means a wide variation in the actual intake of the various compounds present in the coffee.
At the previous ASIC-meeting an extensive study of the properties of espresso coffee brews was presentented by Petracco (1). For our research we selected a number of different brews: some popular home-use brewing methods from various countries. It is not meant to be a complete review of all brewing methods, merely an illustration of the point we want to make: when looking at the effects of coffee the properties of the brew will have to be taken into account since not all cups of coffee are the same.

2. Methods & materials.

2.1. Brewing methods.

For the comparison seven brews from various European countries were chosen some of which much used, others by a smaller group of consumers. With it a coffee (in this case always a roast&ground) of a major brand, suitable for the method, was chosen. An average amount of coffee was used. It is obvious that with the same method variations in the amount of coffee will also have effect on the properties of the brew. This effect was not included in this study.

Included in the study were:

- Two types of dripfilter coffee from different countries. Brews prepared with a regular electric dripfilter apparatus (960 W) capable of dosing 1 l hot water in 6 minutes. A standard paper filter was used. Since it is a widely used brewing method two different coffees were selected for the test.

- The Mocha and Napolitana system. Water in a closed reservoir to be heated by an external source, is pushed through a coffee bed into another reservoir by build-up of steam pressure. Of this particular system two examples were chosen, one from Italy (called Mocha) and one from Spain (called Napolitana). In these countries it is a very frequently used method. The principle of the two is exactly the same. However the size of reservoirs for coffee and water were quite different, as was the coffee used.

- The cafetiere. In this system hot water is poured onto coffee in a glass reservoir, and left standing for a while. Brew and spent grounds are separated by pushing down a perforated plunger.

- The percolator. An electric percolator has a point of heating at the bottom thus pushing up hot water through a pipe and on top of the coffee reservoir. The coffee is extracted and the brew drips back in the water reservoir.

- Boiled coffee. Boiling water is poured onto roast and ground coffee and left boiling for some minutes. The brew is obtained by decanting.

Fig.1 shows some of these brewing methods.

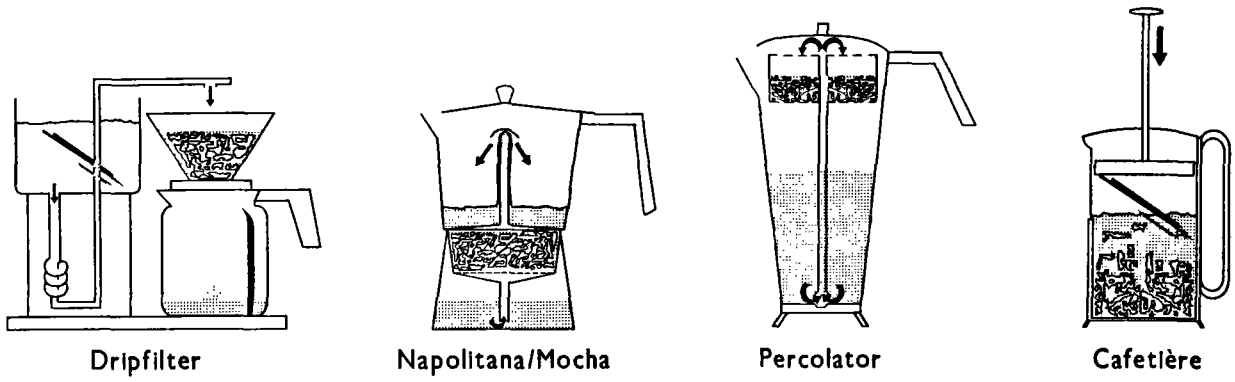

Figure 1 - Brewing methods

Some of the process parameters of the brewing methods are given in table 1. It is obvious from these data how differences between brews are created. The initial water temperature as given in the first column is usually close to 100 C. For most systems the pressure created by boiling is the transportation force for the water. Somewhat different is the temperature in the cafetiere: it decreases steadily and the starting temperature is more or less free to choose. The temperature of the brew at the end of the process (second column) ranges from 80 to 100 C.

Much larger are the differences in time of extraction (from 1 to 10 minutes) and the coffee/water ratio (with the same amount of coffee the amount of water can be almost three times as high).

Table 1 - Brewing parameters of various brewing systems

method	T-water (°C)	T-brew (°C) *1	brewing-time	ratio coffee/water	total brew (gr)
Dripfilter I	98	80	6'30"	40 gr/1000 ml = 1:25	905
Dripfilter II	98	81	6'24"	40 gr/1000 ml = 1:25	905
Mocha	100	83	1'00"	18 gr/ 160 ml = 1: 9	138
Napolitana	100	98	1'58"	23 gr/ 300 ml = 1:13	251
Cafetiere	98->82 *2	82	4'00"	49 gr/ 875 ml = 1:18	793
Percolator	100	92	7'30"	40 gr/1000 ml = 1:25	901
Boiled coffee	100	100	10'00"	40 gr/1000 ml = 1:25	800 *3

*1 temperature of the brew in the brewing device
*2 temperature going down during the process due to heat loss to the environment; start and finish temperature are given
*3 amount very much depending on how much is decanted; at 800 gr the first solid particles came into the brew

During the boiling an average of 40 gr evaporated, from the percolator about 30 gr; from the cafetiere little weight was lost. In all calculations the amount of brew that is in fact obtained for consumption has been used for calculation (column 'total brew'). For the first four this will contain practically all material that is extracted. In the last three the water that is left in the coffee bed contains the same material as the rest of the brew. Separate figures will be included in the tables in which this material, which is in fact extracted but is not separated from the grounds, is taken into account.

2.2. Coffee analyses.

For the two dripfilter brews, the Mocha and the Napolitana a major brand coffee was chosen from the country in which the system is most used. Since such a coffee was not available for the other three methods another suitable coffee was chosen. Table 2 contains a summary of the physical and chemical properties of the coffees involved in the test.

The coffee data indicate that in some cases the coffee is also a major source of variation. The amount of moisture in the coffees varies considerably, from very little in the Napolitana coffee to relatively very much in the second dripfilter coffee. However for the brewing methods involved here there will be little influence of the amount of water in the coffee.

The average particle size has an influence on various properties and the behaviour in a brewing system. A smaller particle can be extracted faster and to a higher degree. The resistance of the coffee bed will increase which can lead to longer brewtimes.
The results in table 2 for the Mocha coffee show the combination of small particles and

high extractability. Large particles of the coffee for cafetiere and percolator lead to less extractable matter.

Table 2 - Some properties of R&G coffee

coffee	moist (%ww)	average particle size (mm)	extract ability (%ds)	caf-feine (%ds)	chloro-genic acids (g/kg)	non-arom. acids (g/kg)
Dripfilter I	3.28	0.423	31.2	1.57	23.85	27.0
Dripfilter II	4.28	0.460	33.2	2.31	16.96	26.6
Mocha	1.09	0.261	35.3	1.99	21.37	25.5
Napolitana	0.73	0.461	30.1	1.51	12.23	24.0
Cafetiere	3.79	0.635	29.6	1.59	24.07	26.9
Percolator	3.79	0.635	29.6	1.59	24.07	26.9
Boiled coffee	3.28	0.423	31.2	1.57	23.85	27.0

The caffeine content of three of the coffees is between 1.5 and 1.6%. Higher in content are the Mocha coffee (1.99%) and in particular the dripfilter II (2.31%). The content of chlorogenic acids varies considerably. The content depends on the amount present in the green coffee and the degree of roast. The overall content of non-aromatic acids and their anions (like citrate and acetate) is very much the same for all coffees and will not be the cause of large differences in the brews.

2.3. Analytical methods.

The avarage particle size of the R&G coffees was determined by means of sieving. To establish the maximum extraction yield coffee was extracted in plenty of water (1:100) for 25 min. at 85 C. The moist content was defined as the weight loss after heating for 17 hrs at 103 C. For caffeine, chlorogenic acids and the non-aromatic organic acids/anions were determined using the HPLC-methods presented a.o. at previous ASIC-colloquia (2,3,4). The total content of material of the various brews was calculated from the density measured. For the pH and acidity measurements a standard pH electrode was used. The fines (larger than 3μ) were filtered off. The yield of the brewing process was calculated from the content of solids and the amount of brew obtained.

For the highly volatile aroma compounds a sample was taken from the closed container in which the brew was put immediately after preparation and immediately injected on a GC/MS column. For the aroma compounds with a higher boiling point an extractive steam destillate was prepared from which the aroma material was extracted and measured using GC/MS.

3. Water soluble compounds in coffee brews.

It is obvious that the material being extracted from the coffee consists for a very large part of water soluble material. Of this material a number of compounds has been chosen to be analized. Of these compounds the concentration in the brews was measured. In the tables and figures to come the overall content is given to demonstrate the difference between the brews. The percentage the particular compound makes up of the total amount extracted is also given. The amount of a compound in a brew is determined by a combination of factors. In the first place there is the amount of extractable matter present in the roast and ground coffee (given in table 2). The second factor is the water/coffee ratio: the more coffee used the more concentrated the brew, but also the more incomplete extraction can become. Finally there is the influence of time and temperature of extraction. In the last column of the tables the effect of the brewing method itself is illustrated with the percentage of the available amount that is extracted.

3.1. Total dry matter.

Table 3 and clearly demonstrate that there can be a large difference between the total content of solubles of the brews. Very high was the content of the Mocha brew, up to three times as much as most of the others (4.11% vs 1.30 to 1.40%). The Napolitana contained 2.76% on average, also quite much compared to the others.

From the third column with the figures for the efficiency of the extraction it is clear that with a number of methods a high degree of extraction is reached (90% or more). The cafetiere and the percolator extract slightly less, but still about 80%. This is caused by two factors. One is that the extraction is probably less complete because of the lack of 'fresh' water being added during the process. Compounds will divide between brew and coffee in an equilibrium. The other factor is that in these systems a part of the brew being of the same concentration as the rest -unlike for instance the dripfilter system where the water remaining in the bed contains virtually no coffee material- is not separated from the grounds. Therefore values are added to the last column (in brackets) taking this amount into account and showing what is actually extracted. In the latter case the value for boiled coffee becomes higher than 100%. The boiling apparently releases more material than the method of analysis (which uses a lower temperature).

Results in the tables are given as concentrations in the brew, leaving all effects of cupsize out of this report. As a final remark it must be added that an average amount of coffee has been used for the dripfilter system. Here the amounts used by consumers vary a great deal. The possible maximum amount of coffee for the Moka and Napolitana is more limited by the size of the coffee reservoir than in case of dripfilter brewing.

Table 3 - Details on overall extraction of solids, pH and acidity

brew	dry solids (%ww)	yield (% of dry)	% solubles extracted	pH	acidity (meq/l)
Dripfilter I	1.30	30.4	94	5.15	8.35
Dripfilter II	1.37	32.3	93	5.60	6.00
Mocha	4.11	31.9	89	5.45	20.55
Napolitana	2.69	29.6	97	5.40	12.75
Cafetiere	1.42	23.9 (26.4)	81 (89)	5.30	8.90
Percolator	1.09	25.5 (27.5)	86 (93)	5.30	6.30
Boiled coffee	1.30	26.9 (32.3)	91 (109)	5.25	7.35

The pH of all brews was between 5.1 and 5.6. There is no clear relation between these figures and any of the brewing parameters. Apart from a stronger distinction between the two dripfilter brews, due to different coffees, the acidity -the overall amount of titratable acid equivalents- more or less follows the total amount of solids extracted from the coffee.

3.2. Caffeine.

Table 4 shows the amount of caffeine present in each of the brews. Differences are substantial, due to both a variation in caffeine content of the coffee and different brewing conditions. The dripfilter brews contain less than 1 g/l, as do boiled coffee, cafetiere coffee and percolator coffee. The Napolitana coffee takes a middle position with 1.3 to 1.4 g/l. Once again the Mocha has the highest content: 2.4 g/l.
The dripfilter system is very efficient in extracting caffeine, as are the Napolitana and to a lesser extent the Mocha (all close to 100%). In this case the caffeine content is determined by the content of the coffee and how much of the coffee is used.
In systems in which no fresh water is added during the process (boiled coffee, cafetiere, percolator) the extraction becomes less. This even when including the brew left in the coffee bed. (Value in brackets in third column)

Table 4 - Caffeine extraction

brew	caffeine in brew (g/l)	%-age of d.s. in brew	%-age extracted
Dripfilter I	0.67	5.2	100
Dripfilter II	0.95	6.9	97
Mocha	2.36	5.7	92
Napolitana	1.35	5.0	98
Cafetiere	0.69	4.9	73 (81)
Percolator	0.58	5.3	85 (95)
Boiled coffee	0.57	4.4	75 (89)

3.3. Chlorogenic acids.

About 5 to 10% of the solids in the cup consist of chlorogenic acids (second column in table 5). Like with the caffeine content the Mocha is by far the highest in chlorogenic acid content, but the Napolitana is only slightly higher than the others. From table 5 it is clear that this is due to a low content in the coffee not to incomplete extraction. (In fact values higher than 100% are calculated when looking at average values; the variance in the analysis could explain these figures). This means that the amount of chlorogenic acid is determined by the content of the coffee.

As with caffeine in boiled coffee, cafetiere and percolator coffee not all available chlorogenic acids were extracted. Once again partly because of incomplete extraction (%age in brackets), partly because incomplete separation of brew and grounds.

Table 5 - Chlorogenic acids in coffee brews

brew	chlorogen. acids (g/l)	%-age of d.s. in brew	%-age extracted	
Dripfilter I	1.08	8.3	106	
Dripfilter II	0.76	5.5	106	
Mocha	2.76	6.7	100	
Napolitana	1.18	4.4	106	
Cafetiere	1.19	8.4	83	(92)
Percolator	0.87	8.0	85	(92)
Boiled coffee	0.90	6.9	78	(94)

3.5. Non-aromatic organic acids.

The remaining group of compounds of which the content was examined is that of the non-aromatic organic acids ('acid' stands for both acid and its anion). Coffee brews contain a number of these acids. Most prominent of these are citric, malic, quinic, succinic, formic, lactic and acetic acid/-anions. An extensive study into the acids in coffee was presented by Maier o.a. in a previous ASIC-colloquium (5). In table 6 the total amount of these acids is given for each of the brews.

Table 6 - Non-aromatic organic acids in coffee brews

brew	organic acids in brew		%-age of d.s. in brew	%-age extracted (%ww)	
	meq/l	g/l			
Dripfilter I	16	1.2	9.2	100	
Dripfilter II	12	0.9	6.6	80	
Mocha	45	3.6	8.8	109	
Napolitana	28	2.2	8.2	101	
Cafetiere	24	1.9	13.4	118	(131)
Percolator	17	1.3	11.9	113	(122)
Boiled coffee	12	1.0	7.7	77	(93)

The non-aromatic organic acids follow the pattern of the overall acidity when looking at equivalents of acid present. The total content of non-aromatic acids in the coffees from which the brews are prepared is very much the same. Brewing method and coffee/water ratio determine the total amount of acids.

In the column of percentages extracted in some cases values of more than 100% appear. This would suggest hydrolysis of esters during brewing, thus releasing more acids.
In cases of more than 100% yield in particular the contribution of glycolic acid has increased (both Moka and cafetiere and percolator coffee) and to some extent the amount of succinic acid (in cafetiere and percolator). The content of boiled coffee is very low. The prolonged boiling apparently causes evaporation of the acids.

4. Other compounds in coffee brews.

Apart from the water soluble material there is also a large amount of insoluble material present in roast coffee. Two other groups of compounds can be characterized: the insoluble polysaccharides (cell wall material, up to 50% of the total weight) and lipids: coffee contains 10 to 15% lipid material.

Although the water soluble compounds make up (as can be expected) the major part of the material in the brew, some of the insoluble material is also released into the brew. The amount of these insolubles in the brew has been determined in a number of ways. Small particles can be separated by filtering over a very fine filter. The amount of volatile compounds (some of which are soluble in water, many are not) was determined using GC/MS, and all lipids by means of hexane extraction.

4.1. Coffee fines.

It is not only by dissolving into water that coffee material is transferred from the coffee bed to the brew. Small particles can be washed out when the separation between brew and coffee particles is insufficient. Though subject to quite some fluctuation between individual measurements the average figures give an indication of how efficient the barrier is between brew and coffee (table 7).

Table 7 - Fines in coffee brews

coffee brew	fines mg/100g
Dripfilter I	7
Dripfilter II	6
Mocha	110
Napolitana	171
Cafetiere	106
Percolator	22
Boiled coffee	204

It is clear that use of a paper filter leaves only a small amount of particles. The water, forced with pressure through the bed in the Mocha and Napolitana, takes along a fairly large amount of fines. The coarser grind and the absence of force in the percolator keeps the figure low for this system. The amount of particles in boiled and cafetiere coffee very much depend on how much of the brew is decanted. Though present in relatively small amounts the difference between the brews in content of these particles is much larger than that of any of the water soluble material.

4.2. Oil.

Roast coffee contains, depending on origin, 10 to 15% oil. Though not soluble in water some of this oil is extracted from the coffee grounds. The amount of oil in coffee brews was determined by means of extraction of the cold brew with hexane.

It has turned out that the oil content, like the amount of fines, is subject to strong fluctuation in some cases. When looking at the oil content the brews can be divided into three groups. In the first place the dripfilter brews, which are very low in oil. Only

0.3% of the total amount of oil is extracted. The paper filter apparently is -like for the fines- an efficient barrier for oil leaving 10 to 20 mg in the brew.

The second group consists of the brewing methods in which brew and spent grounds are less efficiently separated: the Mocha, the Napolitana and the percolator brew. In this case about 2 to 3 % of the oil passed through the barrier (200-400 mg/l). Finally in the third group with boiled and cafetiere coffee up to 15% of the oil was decanted with the brew. In this group, in which exact duplication of the brewing process is difficult, the results fluctuate strongest (600-1100 mg/l).
These results match with figures recently published by V.Dusseldorp et al (6). She mentions contents of 0.01 g/l for filter coffee and 1.0 g/l for boiled coffee.

4.3. Aroma compounds.

Very important for the quality of the the coffee are the volatile aroma compounds. We have determined the total amount present, divided into two groups: the more volatile ones (boiling point to 100 C) and the less volatile (b.p. 100 to 250 C). It is an overall figure, not an exact measure for intensity or quality of organoleptical properties.

Table 8 - Aroma compounds in coffee brews

brew	total content aroma compounds (mg/l)		
	b.p. <100°C	bp 100-250°C	total
Dripfilter I	39	118	157
Dripfilter II	70	200	270
Mocha	374	526	900
Napolitana	165	538	703
.....	.....	.....	.....
Cafetiere	98	280	378
Percolator	16	158	174
Boiled coffee	3	125	128

As can be seen in table 8 there is a considerable variation in aroma present in the brew. Boiled coffee is very low in high-volatile compounds since most of these will have evaporated during brewing. Less volatile compounds are present in about the same amount as in the dripfilter coffee. The same is true, to a lesser extent, for percolator coffee.

5. Conclusions.

Summarizing the results of the chemical analyses of various European brews it can be said that there is a large difference in total concentration of solids dissolved in the brew between the various methods. Brews prepared with a Mocha or Napolitana system are both very high in solids (up to three times as much as the others).

The individual water soluble chemical compounds chosen for this research only make up about some 30% of the total amount of soluble material. The remaining part consists largely of minerals, carbohydrates and brown pigments. Most of the brewing methods in this study are fairly efficient in extracting the available material from the coffee (usually between 90 and 100%). Main factors in creating differences are therefore the water/coffee ratio used and the availability of extractable material in the coffee. Individual water soluble compounds like caffeine, chlorogenic acids and non-aromatic organic acids follow the same pattern.

A relatively much larger difference between brews exists for insoluble material like coffee fines and volatile compounds. A paper filter has turned out to be the optimal barrier for this material. When brew and spent grounds are less efficiently separated the content of insoluble material becomes much higher. A separation by decantation of the brew leaves even more insoluble material in the brew.

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Summary

Brewing makes the difference
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A selection of various European coffee brews, prepared using different methods, was chemically analized to illustrate the wide range of variations that exist in coffee brews. The results show a large difference in total concentration of solids dissolved in the brew. With it the amounts of individual water soluble compounds like caffeine and chlorogenic acids vary. The extraction of water soluble material is usually quite effective.
Properties of the brews are determined by the composition of the roast and ground coffee and the coffee/water ratio. The amount of insoluble material like coffee fines is very much depending on the type of separation of brew and spent grounds.

Des préparations de café, préparer selon des méthodes différentes, ont été analisée pour illustrer la grande variation qui existe entre les boissons. La concentration totale des solubles, et celles des solubles individuels comme la cafeine et les acides chlorogéniques, étaient tres différentes. L'extraction des solubles était en general très efficace la plupart des méthodes.
La composition du café torrefié et le dosage du café déterminent la composition de l'infusion. La teneur en matières insolubles dans le boisson est surtout déterminée par la méthode de séparer l'infusion et le marc de café.

AN OVERVIEW OF COFFEE AROMA AND FLAVOR CHEMISTRY

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Coffee is one of the most popular beverages among people in the western countries. Its unique flavor has been studied intensively and constantly since the beginning of this century. The analysis of coffee volatiles became active in the early 1960s after the invention of gas chromatography/mass spectrometry, which advanced flavor chemistry dramatically. Since then the number of volatile chemicals identified in coffee has increased steadily to a current number of 800. This value is expected to be over 1,000 by the end of this century. The volatiles found in coffee vary from nonpolar compounds such as hydrocarbons to highly polar compounds such as alcohols and acids. The components which have received the most attention from flavor chemists are heterocyclic compounds, in particular pyrazines and thiazoles, because of their characteristic odor and low odor threshold. The major pathway involved in formation of volatile heterocyclic compounds is the Maillard reaction which was proposed in 1912. These heterocyclic compounds are known to form from the reaction between amines (amino acids or proteins) and carbonyl compounds (carbohydrates or sugars). Therefore, any materials containing amines and carbonyls produce tremendous numbers of volatile compounds. However, determination of the flavor impact by an individual component in heated foods or beverages is very difficult due to the large number of compounds produced and their different flavor threshold values. The analysis of flavor chemicals formed in coffee during heat treatment is one avenue to understanding the nature of complex mechanisms of volatile formation in coffee.

INTRODUCTION

Coffee, which possesses a unique and characteristic aroma, is one of the most popular beverages in the world. Some twenty million people currently enjoy its aroma and taste daily. Even though the beverage was introduced to the Western world around the year 1600, it was less than a century ago that the aroma chemicals in coffee began to receive attention. This may have been due to a lack of proper analytical techniques for volatile chemicals. The pioneer workers found a limited number of volatiles, including acetic acid, acetone, and furfural, at the beginning of this century. However, obtaining in-depth knowledge of the aroma constituents in coffee had to wait until the development of gas chromatography. As Figure 1 shows, the number of volatile constituents found in coffee was increased drastically by the invention of gas chromatography.

Since Nobel Prize winners James and Martin introduced gas-liquid chromatography in 1952, isolation and identification of aroma chemicals in foods and beverages have advanced greatly. The maximum number of volatiles identified in coffee before the invention of gas chromatography was approximately 20. However, this number reached 100 shortly after a

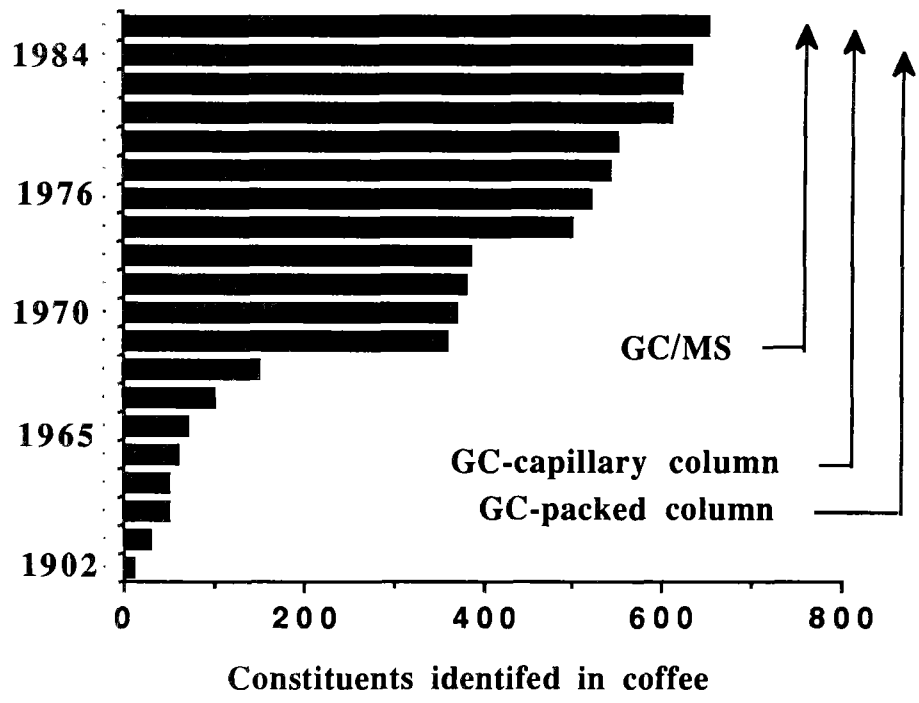

Figure 1. Progress of the number of volatiles found in coffee and the development of analytical techniques (Flament and Chevalier, 1988).

commercial GC became available. Stoll et al. (1967) had done impressive analyses of coffee volatiles just before the introduction of gas chromatography/mass spectrometry. They used many conventional isolation techniques, such as fractional distillation and column chromatography, to prepare the samples for instruments including UV, IR, MS, and NMR. Because of their work the number of volatiles found in coffee was doubled and reached 350 at the end of the 1960s. The MS data published in their paper were used as a bible of mass spectra of volatiles by flavor chemists for many years.

The column plays the most important role in gas chromatography because it performs separation. The number of volatiles found in coffee closely paralleled the development of columns. Figure 2 shows typical gas chromatograms of coffee volatiles obtained by different columns. In 1957, M.J.E. Golay devised a stainless steel open tubular column which increased column resolution significantly. Around the same time, the technique in which a gas chromatograph was interfaced to a mass spectrometer (GC/MS) was developed. The GC/MS has advanced the analysis of volatile chemicals not only in coffee but also in many other foods and beverages. However, because of the active metal surface of a conventional stainless steel column, satisfactory analysis of certain volatiles such as sulfur-containing compounds had to wait until the introduction of the glass capillary column in the 1970s. In the late 1970s, the more inert and flexible fused silica capillary column, which allowed separation of sulfur and nitrogen containing compounds, was invented. Therefore, many sulfur- and nitrogen-containing compounds were isolated and identified in foods and beverages in the 1980s (Tressl and Silwar, 1981; Tressl et al., 1981). These compounds are particularly important to the flavor of coffee because of their characteristic aroma and low odor threshold (Holscher et al., 1990).

Because of the development of analytical techniques, research in coffee aroma became quite diverse. For example, it is now possible to monitor the GC profile of a coffee aroma immediately after brewing using a direct headspace sampling technique (Shimoda and Shibamoto, 1990). However, determination of the flavor impact by an individual component in heated foods or beverages is very difficult due to the large number of compounds produced and their different flavor threshold values. The number of combinations between volatiles' odor threshold and quantity is almost infinite and those combinations make up the unique aroma of each food and beverage. It should be noted, however, that the nearly 800

compounds found in coffee are only one portion of the over 4,000 constituents found in various foods and beverages (Ishii, 1987).

Precursors of Coffee Volatiles

It is well known that many aroma or flavor chemicals are produced by heat-treatment in foods. Thermally generated flavors vary considerably between foods. For example, even though there are no significant differences in constituents between raw coffee beans and Hazel nuts (Table I), the thermally generated flavors are significantly different. It is not well understood yet how these specific flavors are formed by heat in each food. There must be specific precursors which produce these aroma chemicals by heating. Table II shows the percentage of each non-volatile constituent in green and roasted coffee beans (Feldman et al., 1969). The major compositional difference between green and roasted coffee beans is the amount of proteins (including amino acids) and sugars, suggesting that the Maillard reaction is the major mechanism in forming volatiles. Table III shows the composition of amino acids in the hydrolyzed green and roasted coffee beans (Feldman et al., 1969). Arginine, cysteine, lysine, serine, and threonine are significantly reduced by heat treatment. These amino acids might react with sugars (sucrose and its monosaccharides) to produce aroma chemicals in roasted coffee.

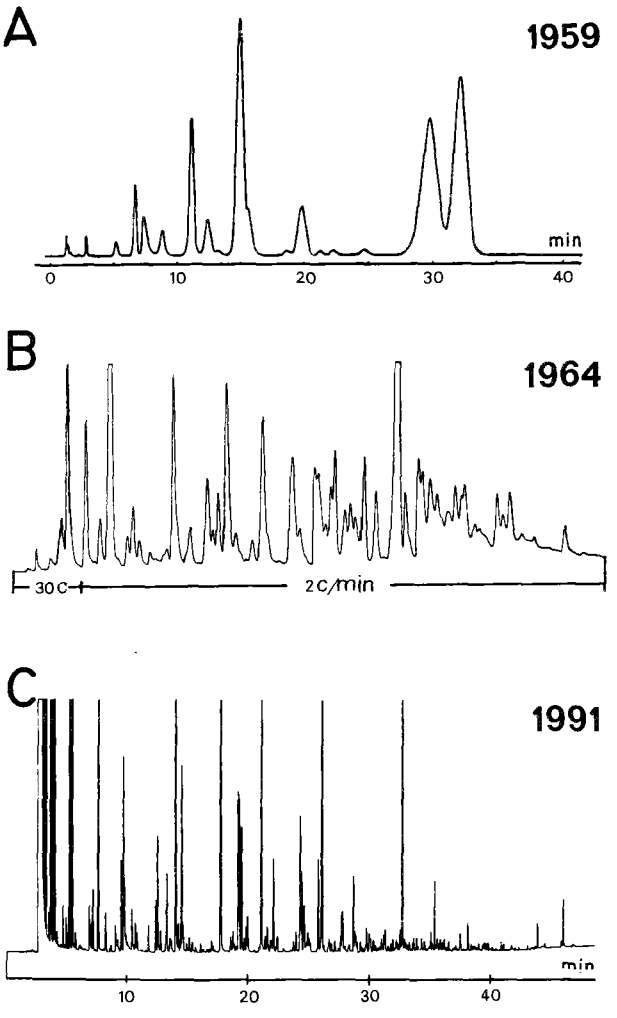

Figure 2. Gas chromatograms of coffee volatiles obtained by different columns.

The interaction between amino acids and sugars is the major phenomenon which produces aroma chemicals. This is the so-called browning reaction, also known as the Maillard reaction (Maillard, 1912). Therefore, the Maillard reaction must play an important role in the formation of aroma chemicals in coffee as well as in other foods and beverages.

In addition to amino acids and sugars, lipids have begun to receive much attention as precursors of volatile compounds. Lipids produce numerous carbonyl compounds which react with amines, such as amino acids and proteins, to produce heterocyclic aroma compounds (Ohnishi and Shibamoto, 1984).

Aroma Compounds Found in Coffee

Figure 3 shows the number of derivatives in different chemical groups found in coffee (Flament and Chevallier, 1988).

Table I. **Composition of major non-volatiles in raw coffee beans and Hazel nuts.**

Component	Coffee beans	Hazel nuts
Minerals	4.2	6.0
Proteins (including amino acids)	15.0	12.7
Lipids	18.0	60.9
Sugars	55.0	4.8

Extracted from Woodnoof (1982) and Smith (1985).

Table III. **Typical composition of amino acids (%) in green and roasted colombian coffee.**

Amino acid	Green	Roasted
Asparagine	10.63	7.13
Leucine	8.77	10.34
Lysine	6.81	2.76
Proline	6.60	7.01
Serine	5.88	0.80
Threonine	3.82	1.38
Arginine	3.61	0.00
Cysteine	2.89	0.69

Extracted from Feldman et al. (1969).

Table II. **Average composition of chemicals (%) in green and roasted coffee.**

Constituent	Green	Roasted
Hemicelluloses	23.0	24.0
Cellulose	12.7	13.2
Proteins (including amino acids)	11.6	3.1
Lipid	11.4	11.9
Chlorogenic acid	7.6	3.5
Sucrose	7.3	0.3
Lignin	5.6	5.8
Caffeine	1.2	1.3

Extracted from Feldman et al. (1969).

Carbonyl Compounds

A total of nearly 100 aldehydes and ketones have been reported in the aroma of coffee. Some volatile carbonyl compounds are important aroma chemicals. For example, diacetyl possesses a butter-like aroma and its odor threshold is 4 ppb in water. *trans*-2-Nonenal is used to enhance a fresh woody note in instant coffee (Parliament et al., 1973). Carbonyl compounds have been known to produce heterocyclic aroma compounds upon secondary reactions in foods and beverages. Therefore, they are more important as a precursor of aroma chemicals rather than as aroma chemicals themselves.

The highly volatile aldehydes, such as formaldehyde and acrolein, were reported in various coffee products (Hayashi and Shibamoto, 1986) and lipid-rich foods (Umano and Shibamoto, 1987). Table IV shows the amount of formaldehyde found in coffees and Table V shows the amount of acrolein determined in headspace of heated lipids. These compounds posses a somewhat pungent odor and stimulate the taste and olfactory organs, suggesting that they play an important role in physiological effects of coffee.

Furans

Furans are most abundant volatile chemicals in roasted coffee. Among numerous furans found in coffee volatiles, furfural has been known to form from a monosaccharide by the action of heat or acid. Furfural, 5-methyl-furfural, and furfuryl alcohol are present in coffee at levels of 100 ppm (van Straten et al., 1983).

Furans have been known as sugar caramelization or degradation products and posses a caramel-like aroma. Some sugar degradation products, such as 4-hydroxy-5-methyl-3(2H)-

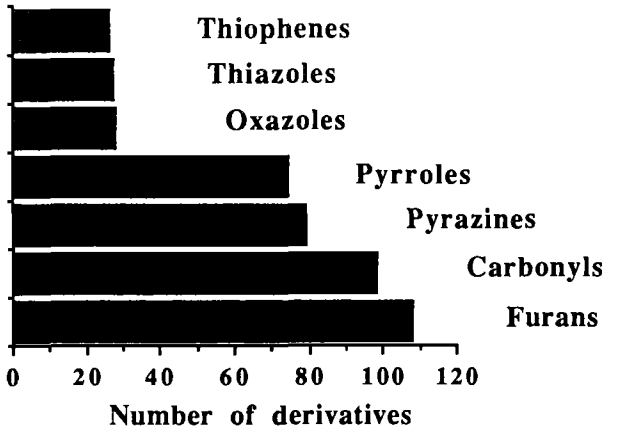

Figure 3. Number of derivatives in different chemical groups found in coffee (reformed Flament and Chevallier, 1988)

Table IV. Amounts of formaldehyde found in coffees

Type	Source	Amount (ppm)
Regular	A	4.5
Decaffeinated	B	3.4
Instant	A	16.3
Instant	B	10

Hayashi et al. (1986).

Table V. Amounts of acrolein recovered from the headspace of lipids heated at 300 °C

Lipid	Amount (ppm)
Corn oil	0.68
Soybean oil	0.63
Sunflower oil	0.48
Olive oil	0.86
Sesame oil	0.71
Beef fat	0.63

Umano and Shibamoto (1987).

furanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and 2-hydroxy-3-methyl-2-cyclopentene-1-one (cyclotene), have been known as chemicals giving a predominant caramel-like or burnt sugar aroma. Furans may, therefore, form only from sugars, with amino acids acting just as a catalyst. Figure 4 shows the role of amino acid in furan formation from sugar (Feather and Huang, 1985).

The major role of furans in coffee aroma is that they produce the key-note aroma chemicals of roasted coffee upon secondary reactions with sulfur containing compounds. Many sulfur containing furan derivatives are formed from the reaction between furfural and hydrogen sulfide (Shibamoto, 1977). Furfurylmercaptan was reported in coffee in 1926 (Reichstein and Staudinger, 1926) and has been known as one of the most important key-note chemicals in roasted coffee. Its odor threshold in water is 5 ppt (Flament and Chevallier, 1988). Typical sulfur containing furans found in coffee are shown in Figure 5.

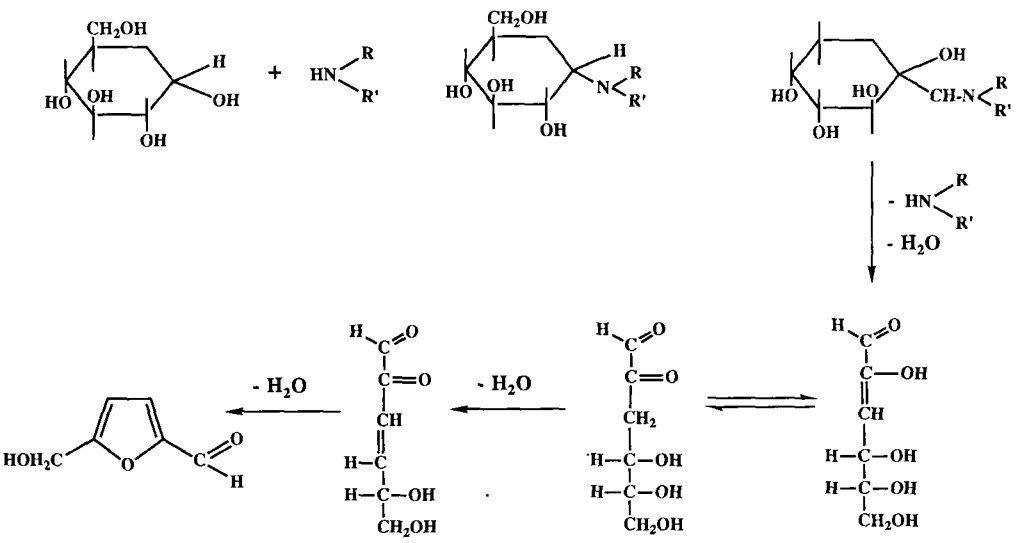

Figure 4. Proposed formation mechanisms of furans from a sugar with an amino acid (Feather and Huang, 1986).

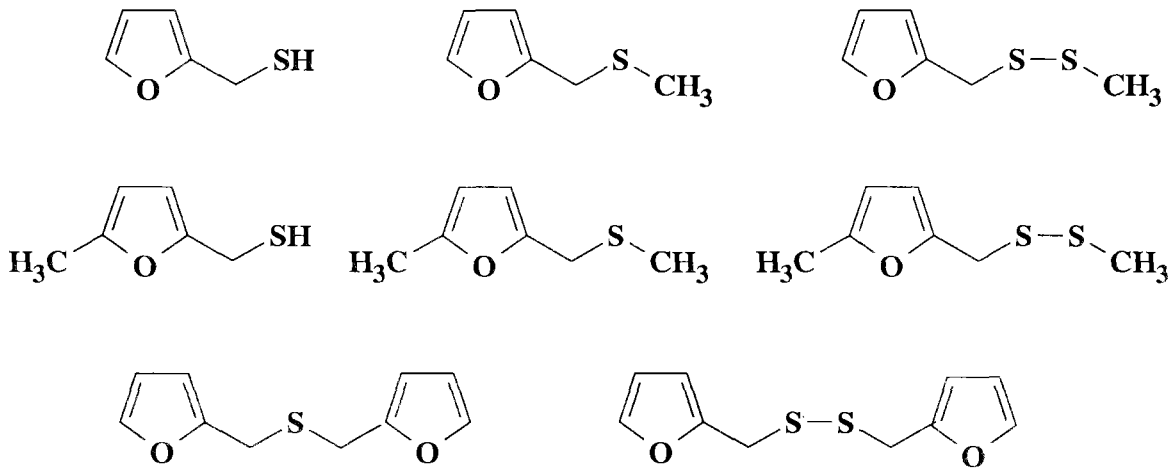

Figure 5. Major sulfur containing furans found in coffee (extracted from Tressl et al., 1981).

Pyrazines

Pyrazines are the second most abundant heterocyclic compounds found in coffee after furans. Over 80 pyrazines have been reported in coffee aroma up to today (Vizthum, 1976). Pyrazines have been known by organic chemists since the last century (Stoehr, 1896) but their importance in flavor was discovered in relatively recent time. Once pyrazines were found in some processed foods, including coffee, they received much attention as chemicals which play an important role in cooked-food flavors. Pyrazines are well characterized as the compounds which directly contribute to roasted or toasted flavors and some of them posses an extremely low odor threshold (Table VI).

Table VI. Odor threshold of selected pyrazines in water

Pyrazine	Threshold (ppm)
Methylpyrazine	60.0
2,3-Dimethylpyrazine	25.0
2,5-Dimethylpyrazine	18.0
2,6-Dimethylpyrazine	15.0
5-Isopentyl-2,3-dimethylpyrazine	6.00
5-Pentyl-2,3-dimethylpyrazine	0.09
Methoxypyrazine	0.035
2-Hexyl-3-methoxypyrazine	0.000001

From Guadagni et al. (1972) and Shibamoto (1986).

Even though the formation of pyrazines in a reaction of a free sugar with ammonia was reported before Maillard developed his hypothesis of browning reactions, their formation mechanisms in foods have not been well established yet. The most common mechanisms proposed in many articles are that α-amino carbonyls, such as 3-amino butane-2-one, form a dihydropyrazine which is subsequently oxidized to pyrazine. The oxidation step from dihydropyrazine to pyrazine is the most difficult step to prove because it occurs with or without oxygen. The observation of conversion of dihydropyrazine to pyrazine in a gas chromatograph suggested the involvement of aa disproportionation reaction (Shibamoto, 1975). The hydroxy dihydropyrazines were proposed to be an intermediate in pyrazine formation (Shibamoto and Bernhard, 1977) but their presence has never been proved. The presence of a dialkylpyrazine radical as a possible intermediate in the pyrazine formation was also reported (Hayashi and Namiki, 1986).

It should be noted that this simple step from dihydropyrazine to pyrazine is not yet thoroughly understood.

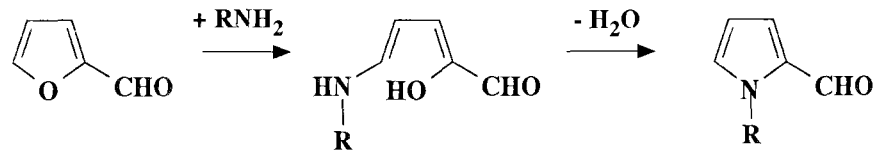

Figure 6. Proposed formation mechanisms of pyrroles from a furan and an amino acid (Baltes and Bochmann, 1987).

Pyrroles

Pyrroles are a widely distributed heterocyclic compound in processed foods, including coffee. Eleven pyrroles were reported in roasted coffee in the late 1960s (Stoll et al., 1967). Among them, 2-acetylpyrrole, 2-formylpyrrole, and 2-formyl-1-methylpyrrole are present in roasted coffee at the highest levels (apx. 10 ppm). Large numbers of pyrroles have been also found in Maillard reaction systems (Shibamoto, 1983). For example, a simple model system consisting of rhamnose and ammonia produced eight pyrroles that were proposed to form from a direct reaction between sugar and ammonia (Shibamoto and Bernhard, 1978). Baltes and Bochmann (1987) proposed that N-alkylpyrroles can form from serine and threonine via a pyrolytic degradation (Figure 6), whereas the formation of N-furfurylpyrroles requires the presence of sugar. Tressl et al. (1981) found 20 N-substituted pyrroles including 15 furfurylpyrroles in roasted coffee.

Pyrroles have not received as much attention as flavor components as other heterocyclic compounds such as pyrazines and thiazoles even though the number of derivatives found in foods and beverages is almost the same as that of pyrazines (Figure 3). 2-Formylpyrrole possesses a sweet and corn-like aroma and 2-acetylpyrrole has caramel-like flavor. 1-Furfuryl-pyrrole and 1-(5-methylfurfuryl)pyrrole, which have odor thresholds of 10 ppb in water (Tressl et al., 1981), have a green mushroom-like aroma.

Thiophenes

Thiophenes have been known to form from vegetables of the cruciferae family, such as cabbage and turnip, by heat treatment. Many thiophenes have been isolated from onion and garlic and play an important role in the cooked flavor of those vegetables (Boelens et al., 1971).

Over 30 thiophenes have been reported in coffee aroma. The presence of thiophenes in coffee may be unique among beverages because there is virtually no report in other beverages such as cocoa and tea. Maillard model systems consisting of a sugar and a sulfur-containing amino acid produce a large number of thiophenes. For example, glucose produced many thiophenes by reaction with hydrogen sulfide (Shibamoto and Russell, 1977; Sakaguchi and Shibamoto, 1978). Thiophenes, as well as pyrroles, are known to form at the early stage of browning reactions and their formation does not require a high degree of sugar fragmentation.

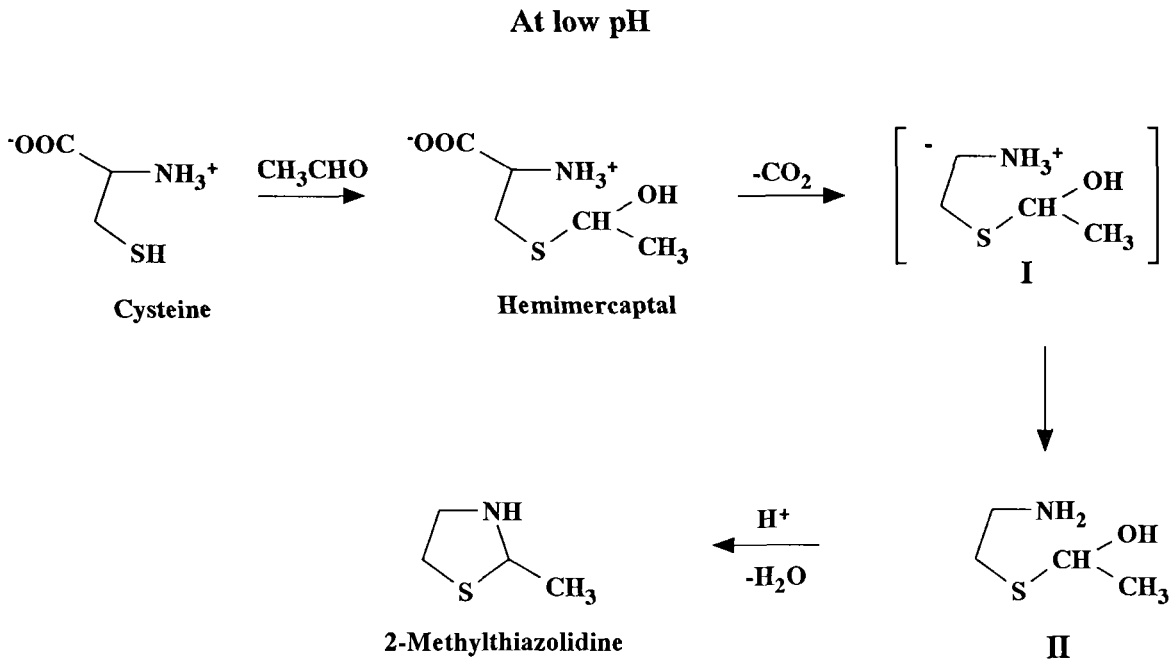

Figure 7. Formation mechanisms of thiazolidines from cysteamine at different pH (Yeo and Shibamoto, 1991).

Some thiophenes posses a sulfurous aroma which contributes to the characteristic flavor of cooked meat (Shibamoto, 1980). They give a sulfurous aroma to coffee along with the other sulfur-containing compounds including hydrogen sulfide, alkyl thiols, and alkyl disulfides.

Thiazoles, Thiazolines, and Thiazolidines

Thiazoles are known as chemicals possessing a cooked meat-like flavor and have been widely used in formulation of imitation meat flavors (Katz et al, 1969; Pittet et al., 1969). Following a report of 2-acetyl-2-thiazoline in beef broth (Tonsbeek and Copier, 1971), many thiazoles and thiazolines have been found in cooked foods, in particular in cooked meat (Shibamoto, 1984). Stoll et al. (1967) found two thiazoles for the first time in coffee. Later, Vizthum and Werkhoff (1974) reported 24 additional thiazoles in roasted coffee.

Formation of thiazoles requires sugar degradation to first form a smaller carbon unit. Pyrolysis of cysteine reportedly produced 2-methylthiazolidine (Fujimaki et al., 1969). Cysteamine, which is a decarboxylated cysteine, produced many thiazolidines upon reaction with aldehydes (Sakaguchi and Shibamoto, 1978).

Thiazolidines have never been reported in foods, whereas many thiazoles and thiazolines have been found in various foods. Recently, pH dependence of 2-methylthiazolidine was observed in a microwaved model system consisting cysteine and D-glucose (Yeo and Shibamoto, 1991). The proposed formation mechanisms of 2-methylthiazolidine are shown in Figure 7. At lower pH (2, 5, and 7), cysteine exists predominantly in the zwitterionic form. The amino group (pKa = 10.7) is protonated, and therefore it cannot undergo nucleophilic attack. On the other hand, the thiol group (pKa = 8.3) on cysteine can act as a nucleophile and can attack the carbonyl group on acetaldehyde to form a hemimercaptal. This is followed by decarboxylation to form an intermediate, I, which readily isomerizes to form II via intramolecular hydrogen transfer, freeing the amino group. A water molecule is subsequently eliminated to form 2-methylthiazolidine.

Under basic conditions (pH 9), the thiol goup on cysteine is deprotonated, giving a nucleophilic thiolate anion. However, the hemimercaptal intermediate mentioned above is not formed because the hydroxyl group would react with acetaldehyde more readily than with the thiolate anion. This explains the absence of 2-methylthiazolidine in the pH 9 sample. The formation of thiazolidine is proved to be pH dependent but its actual formation mechanism in foods is still unknown.

Oxazoles

5-Acetyl-2-methyloxazole was found for the first time in coffee in the late 1960s (Stoffelsma et al., 1968). Later, a series of alkyloxazoles was identified in roasted coffee (Vitzthum and Werkhoff, 1974). Two possible formation mechanisms of oxazoles were proposed. One is the formation from α-amino ketone formed from an amino acid via Strecker degradation. The other is the direct formation from serine or threonine through decarboxyration (Figure 8). The former is an analogue to the pyrazine formation and the latter is an analogue to the thiazole formation.

Even though the presence of oxazole in foods was recognized in the 1950s, their role in flavors has not received much attention due to the lack of a characteristic aroma compared with other heterocyclic compunds.

Concluding Remarks

Coffee will remain as one of the most popular beverages in the future. Enjoying the taste and aroma generated from freshly brewed coffee is one of the most pleasant practices in our daily life. Numerous volatile chemicals make up its unique flavor. Even though we have found over 4,000 aroma chemicals in foods and beverages, we are aware of the presence of more undiscovered aroma chemicals, because our olfactory organ has a much higher sensitivity toward

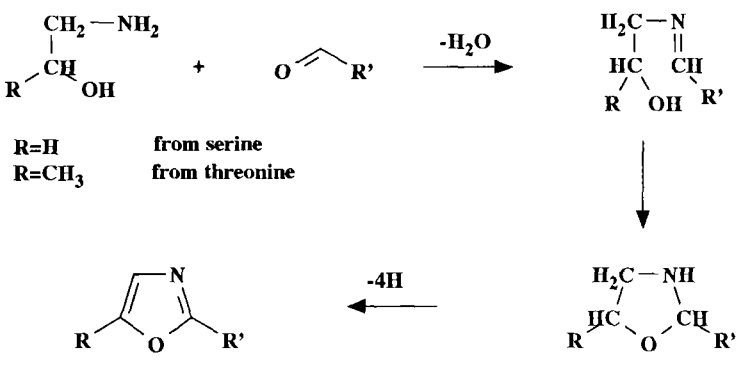

Figure 8. Proposed formation mechanisms of oxazoles (Vitzthum and Werkhoff, 1974).

certain chemicals than do any mechanical detectors. Isolation and identification of aroma chemicals are the first steps in understanding the myth of flavor.

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Zusammenfassung

In den westlichen Ländern, Kaffee ist eines der beliebtesten Getränke. Seit Beginn dieses Jahrhunderts worde sein einzigartiger Geschmack gründlich und beständig untersucht. Die Analytik der flüchtigen Kaffeeverbindungen begann nach der Establierung von Gaschromatographie/Massenspektrometrie, die seit den frühen Sechzigerjahren dramatische Fortschritte in die Aroma-Chemie gebracht haben. Seit damals ist die Anzahl der identifizierten flüchtigen Chemikalien im Kaffee laufend auf 800 bis heute angestiegen. Bis zum Ende dieses Jahrhunderts werden über 1.000 erwartet. Die im Kaffee gefundenen flüchtigen Bestandteile variieren von nichtpolaren Verbindungen wie Kohlenwasserstoffe bis zu hochpolaren Komponenten wie Alkohole und Säuren. Die meiste Beachtung von den Aroma-Chemikern erhielten die heterozyklischen Bestandteile, besonders Pyrazine und Thiazole, wegen ihres charakteristichen Geruchs und der niedrigen Geruchsschwelle. Der Hauptsyntheseweg, der am Aufbau flüchtiger heterozyklischer Komponenten beteiligst ist, ist die Maillard-Reaktion, die 1912 vorgeschlagen wurde. Diese heterozyklischen Verbindungen sind dafür bekannt die Reaktion zwischen Aminen (Aminosäuren oder Proteine) und Carbonylgruppen (Kohlenhydrate oder Zucker). Deshalb entsteht in jedem Material mit Aminen und Carbonylen eine riesige Anzahl von flüchtigen Bestandteilen. Jedoch ist, aufgrund der grossen Anzahl produzierter Verbindungen und ihrer unterschiedlichen Geruchsschwellenwerte, die Bestimmung der Geschmackseinwirkung einer einzelnen Komponente in erhitzten Nahrungsmitteln oder Getränken sehr schwierig. Die analyse der Aroma-Chemikalien, die im Kaffee während der Hitzebehandlung entstehen, ist ein Weg, um die Natur komplexer Mechanismen der Entstehung flüchtiger Bestandteile im Kaffee zu verstehen.

AROMA IMPACT COMPOUNDS OF ARABICA AND ROBUSTA COFFEE. QUALITATIVE AND QUANTITATIVE INVESTIGATIONS

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Introduction

The volatile fraction of roasted coffee is extremely complex, consisting of more than 700 compounds [1] with a wide variety of functional groups.

During the last decade, efforts have been undertaken to evaluate those volatile compounds which contribute significantly to the aroma of roasted coffee. On the basis of the odor unit concept [2], *Tressl* [3] has suggested that 2-furfurylthiol is the most important odorant. In addition, he reported that the other compounds listed in Table 1 are of significance for the coffee flavor. Recently, *Holscher et al.* [4, 5], using gas chromatography/olfactometry (GC/O) of serial dilutions of the volatile fraction (aroma extract dilution analysis, AEDA [6]), confirmed that some of the odorants suggested by *Tressl* [3] are indeed intensely involved in the coffee flavor (Table 1). In addition, these authors identified further character impact compounds which are summarized in Table 1.

It is well-known [review in 7] that the two varieties of coffee, Arabica and Robusta, differ in their aromas. Several authors have compared the volatile fractions of the two varieties, but they have not evaluated the contribution of the identified compounds to the flavor differences of the two coffees. *Vitzthum et al.* [8] have recently reported, that 2-methylisoborneol resembles the typical earthy aroma impression of the Robusta coffee.

Table 1: Important odorants of the coffee flavor according to *Tressl* [3] and *Holscher et al.* [4, 5]

Compound	*Tressl* [3]	*Holscher et al.* [4, 5]
2-Furfurylthiol	+	+
2-Methyl-3-furanthiol	-	+
5-Methyl-2-furfurylthiol	+	-
3-Methyl-2-buten-1-thiol	-	+
Furfurylmethyldisulphide	+	-
3-Mercapto-3-methylbutylformate	-	+
3-Mercapto-3-methyl-1-butanol	-	+
Methional	-	+
Kahweofuran	+	-
Ethyldimethylpyrazine	+	+
Acetylpyrazine	+	-
Trimethylpyrazine	-	+
2-Methoxy-3-isobutylpyrazine	+	+
2-Methoxy-3-isopropylpyrazine	-	+
Linalool	-	+
Guaiacol	+	+
4-Vinylguaiacol	+	+
ß-Damascenone	-	+
Furaneol	+	+
2,3-Pentandione	+	+
2,3-Butandione (diacetyl)	-	+
(E)-2-Nonenal	+	-
2-/3-Methylbutanal	+	+
2-/3-Methylbutanoic acid	-	+
Acetic acid	-	+

The aim of the following study was to identify the potent odorants of Arabica and Robusta coffee (powder and brew) and to show the differences of these two varieties. The identification experiments were focussed on those compounds which were evaluated by AEDA as important odorants. As AEDA is only a screening method [9], some odorants, contributing to important notes within the odor profile of the two varieties, were quantified and the odor units were calculated on the basis of odor/taste threshold values in water.

Methods

The methods used for the analysis of the coffee aroma are summarized in Table 2. The volatiles were isolated by solvent extraction from both, the roasted ground powder and the brew. The aroma extract was separated from the non-volatile compounds by high vacuum transfer and the volatile fraction obtained was analysed by GC/O. The odorants were characterized by their retention index, odor quality and relative odor activity (FD-factor). The odorants with high FD-factors were identified and their odor threshold values were determined. Important odorants were quantified using stable isotope dilution methods and then compared on the basis of odor activity values calculated by dividing the concentration levels in coffee (powder and brew) through the flavor thresholds in water.

Table 2: Methods used for the analysis of coffee aroma

Method	Result
Solvent extraction, high vacuum transfer [10]	Aroma extract
Gas chromatography/olfactometry (GC/O [11])	Retention index and odor of the volatiles
Aroma extract dilution analysis (AEDA [6])	FD-Chromatogram (FD-factors: ranking of the volatiles on the basis of their odor units determined by GC/O)
Enrichment procedures (column chromatography, HPLC, preparative GC)	Pure odorants
Identification experiments (Capillary GC, MS, NMR)	Chemical structure
Sensory characterization [11]	Threshold values (in air, water)
Synthesis	Reference compounds, labeled compounds
Stable isotope dilution assay (SIDA [12]); calculation of odor units [2]	Quantitative data of some important odorants; their flavor significance expressed as odor units

Results and Discussion

Identification of important odorants

About 50-60 odorants were found in the GC-effluent of the aroma extracts of Arabica and Robusta coffee. As examplified for the Arabica coffee (roasted powder) the FD-chromatogram (Fig. 1) indicated 38 odorants with FD-factors ≥ 16. Most of the 38 odorants were identified on the basis of GC and MS data (footnote "d" in Table 3). Only the amounts of the compounds nos. 5, 21 and 37 were so low in the volatile fraction that no clear MS signals were obtained. The identification of these compounds as 2-methyl-3-furanthiol (no. 5), 2,3-diethyl-5-methylpyrazine (no. 21) and bis(2-methyl-3-furyl)disulphide (no. 37) was based only on comparison of GC and sensory data with that of the corresponding reference substances. Their sensory importance can be explained by the low threshold values (0.001-0.01 ng/l air) [13].

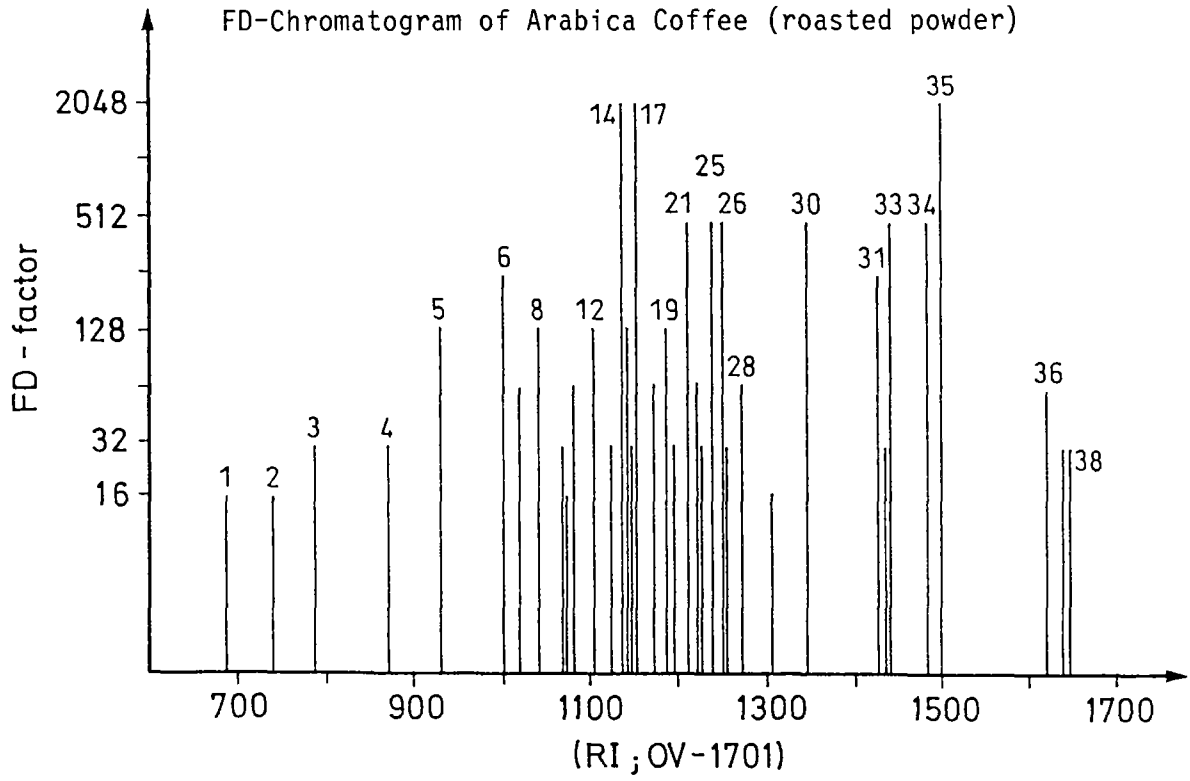

Figure 1. Flavor dilution chromatogram of the volatiles isolated from the roasted powder of Arabica coffee. Numbering of the flavor compounds as in Table 3; FD-factor: flavor dilution factor; RI: retention index.

Three odorants (nos. 14, 17 and 35) appeared with the highest FD-factor (FD = 2048) in the FD-chromatogram (Fig. 1). As shown in Table 3 they were identified as 3-mercapto-3-methylbutylformate (no. 14), 3,5-dimethyl-2-ethylpyrazine (no. 17) and (E)-ß-damascenone (no. 35). Their odor qualities were described as "catty", "earthy-roasty" and "honey-like", respectively.

Further odorants summarized in Table 3 were sotolon (no. 30), abhexon (no. 33), 4-methoxybenzaldehyde (no. 32) and bis(2-methyl-3-furyl)disulphide (no. 37). These compounds were identified for the first time in the coffee aroma. Sotolon and abhexon smelled "seasoning-like" and, in higher dilution, "caramel-like". Other volatiles contributing with "caramel-like, sweet" odor qualities to the aroma of coffee were furaneol (no. 24), 3,4-dimethyl-2-cyclopentenol-1-one (no. 22) and an unknown compound (no. 29).

The identification of such polar enoloxo compounds in low concentrations is difficult, since they are more or less adsorbed at the capillary during the GC-procedure [14]. It was observed that the lower the amount injected on the capillary the higher the proportion which was adsorbed. These effects were the smallest on the FFAP stationary phase which was, therefore, used for the AEDA of the enoloxo compounds.

Odorants with the impression "sweet" in combination with an additional odor quality were diacetyl (no. 1, buttery), 2,3-pentandione (no. 3, buttery), methional (no. 8, potato-like), 5H-5-methyl-6,7-dihydrocyclopentapyrazine (no. 27, nutty), p-anisaldehyde (no. 32, minty), bis(2-methyl-3-furyl)disulphide (no. 37, meaty) and vanillin (no. 38, vanilla-like).

About one third of the potent odorants were described as "roasty" in combination with an additional odor quality. 2-Furfurylthiol (no. 6, coffee-like), 3-mercapto-3-methylbutylformate (no. 14, catty), 2-methoxy-3-isopropylpyrazine (no. 15, earthy), 3,5-dimethyl-2-ethylpyrazine (no. 17, earthy), 2,3-diethyl-5-methylpyrazine (no. 21, earthy) and two unknown compounds (nos. 19 and 26, earthy) belonged to this group.

Important odorants with "honey-like" or "spicy" aroma qualities were (E)-ß-damascenone (no. 35), phenylacetaldehyde (no. 18) and guaiacol (no. 23), 4-ethyl- and 4-vinylguaiacol (nos. 31 and 34), respectively.

The compounds found in this study as important for the flavor of roasted coffee powder are in good agreement with those reported by *Holscher et al.* [4, 5] (Table 1). The presence of 2-furfurylthiol, ethyldimethylpyrazine, 2-methoxy-3-isobutylpyrazine, 4-vinylguaiacol, furaneol and 2,3-pentandione

Table 3: Important odorants of the roasted powder of Arabica coffee (FD ≥ 16)[a]

No.	Compound	Retention index[b]			Aroma quality[c]
		OV-1701	SE-54	FFAP	
1	2,3-Butandione (Diacetyl)[d]	686	580	990	buttery
2	3-Methylbutanal[d]	739	650	950	malty
3	2,3-Pentandione[d]	791	695	1060	buttery
4	3-Methyl-2-buten-1-thiol	874	821		amine-like
5	2-Methyl-3-furanthiol[e]	930	870		meaty
6	2-Furfurylthiol[d]	1004	913	1440	roasty (coffee)
7	2-/3-Methylbutanoic acid[d]	1022	860		sweaty
8	Methional[d]	1040	909	1455	potato-like, sweet
9	Unknown	1073		1365	fruity
10	2,4,5-Trimethylthiazole[d]	1074	997	1370	roasty, earthy
11	2,3,5-Trimethylpyrazine[d]	1078	1000	1395	roasty, earthy
12	Unknown	1107	1055		roasty, sulfury
13	3-Methyl-3-mercapto-1-butanol[d]	1127	972	1655	meaty (broth)
14	3-Methyl-3-mercapto-butylformate[d]	1138	1023	1517	catty, roasty
15	2-Methoxy-3-isopropylpyrazine[d]	1146	1097	1428	earthy, roasty
16	2,4-Dimethyl-5-ethylthiazole[d]	1149	1078	1435	earthy, roasty
17	3,5-Dimethyl-2-ethylpyrazine[d]	1154	1083	1453	earthy, roasty
18	Phenylacetaldehyde[d]	1178	1053	1635	honey-like
19	Unknown	1185	1103		roasty, earthy
20	Linalool[d]	1193	1102		flowery
21	2,3-Diethyl-5-methylpyrazine[e]	1218	1155	1485	earthy, roasty
22	3,4-Dimethyl-2-cyclopentenol-1-one[d]	1226	1075	1840	caramel-like
23	Guaiacol[d]	1228		1990	phenolic, spicy
24	2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Furaneol)[d]	1235	1065	2035	caramel-like
25	2-Methoxy-3-isobutylpyrazine[d]	1237	1186	1520	earthy
26	Unknown	1254	1184		roasty, earthy
27	5H-5-Methyl-6,7-Dihydrocyclopentapyrazine[d]	1260	1145		roasty, sweet
28	(E)-2-Nonenal[d]	1275	1160		fatty
29	Unknown	1305		2090	caramel-like
30	4,5-Dimethyl-3-hydroxy-2(5H)-furanone (Sotolon)[d]	1347	1107	2200	seasoning-like
31	4-Ethylguaiacol[d]	1424	1287	2032	spicy
32	p-Anisaldehyde[d]	1431	1263	2030	sweet, minty
33	4-Methyl-5-ethyl-3-hydroxy-2(5H)-furanone (Abhexon)[d]	1433	1193	2270	seasoning-like
34	4-Vinylguaiacol[d]	1482	1323	2205	spicy
35	(E)-ß-Damascenone[d]	1502	1395	1815	honey-like, fruity
36	Unknown	1620		2355	amine-like
37	Bis(2-methyl-3-furyl)disulphide[e]	1640	1540	2150	meaty, sweet
38	Vanillin[d]	1645	1410	>2500	sweet (vanilla)

Footnotes of Table 3

a Numbering as in Fig. 1.

b RI: Retention index on the capillary [10].

c Odor quality perceived at the sniffing-port.

d The compound was identified by comparing it with the reference substance on the basis of the following criteria: MS/EI, MS/CI, RI data (on OV-1701, SE-54 and FFAP) as well as of the odor quality and threshold, which was perceived at the sniffing-port.

e The MS signals of the substance were too weak for an interpretation; the compound was only identified by comparing it with the reference substance on the basis of the resting criteria reported in footnote d.

also agrees with the suggestion of Tressl [3], that these odorants belong to the key compounds of the coffee flavor. In the capillary gas chromatograms of the coffee volatiles, *Holscher et al.* [5] have localized two additional odorants of unknown structure with high FD-factors. In the present study, they were identified as sotolon and abhexon.

Differences between the powders of roasted Arabica and Robusta coffee

The odorants of roasted Arabica and Robusta coffee powders were compared on the basis of their FD-factors. All of the odorants identified with FD-factors ≥ 16 contribute to the aroma of both coffee varieties. Linalool was an exception occurring only in Arabica coffee. According to the data summarized in Table 4 3,5-dimethyl-2-ethylpyrazine appeared with the highest FD-factor in both coffee varieties. However, differences were found in the concentration levels of some important coffee odorants.

2,3-Diethyl-5-methylpyrazine and 4-ethylguaiacol were predominant in the Robusta coffee and 3-mercapto-3-methylbutylformate, sotolon and abhexon in the Arabica coffee. Further significant differences were found for 2-methyl-3-furanthiol, phenylacetaldehyde, 3,4-dimethyl-2-cyclopentenol-1-one, 2-/3-methylbutanoic acid and linalool, all predominating in the Arabica coffee, and for 3-methyl-2-buten-1-thiol, which prevailed in the Robusta coffee.

The comparison of the coffee varieties indicated in addition that compounds causing "caramel-like, sweet-roasty" odor qualities were high in Arabica coffee, while those having "spicy" and "earthy-roasty" qualities contributed more significantly to those of the Robusta species. These

Table 4: Important odorants of roasted Arabica and Robusta coffee (powder and brew)

No.[a]	Compound	FD-factor$_{rel}$[b]			
		Powder[c]		Brew[d]	
		Arabica	Robusta	Arabica	Robusta
17	3,5-Dimethyl-2-ethylpyrazine	100	100	50	100
35	(E)-ß-Damascenone	100	50	3	6
14	3-Mercapto-3-methylbutylformate	100	25	13	3
21	2,3-Diethyl-5-methylpyrazine	25	100	6	50
34	4-Vinylguaiacol	25	50	25	50
25	2-Methoxy-3-isobutylpyrazine	25	25	6	3
19	Unknown	25	25	6	25
30	Sotolon	25[e]	2[e]	100[e]	6[e]
33	Abhexon	25[e]	3[e]	50[e]	3[e]
31	4-Ethylguaiacol	13	50	25	50
26	Unknown	13	25	2	6
6	2-Furfurylthiol	13	13	3	3
8	Methional	6	3	25	13
15	2-Methoxy-3-isopropylpyrazine	6	3	2	3
5	2-Methyl-3-furanthiol	6[f]	2[f]	<1[f]	<1[f]
11	2,3,5-Trimethylpyrazine	3	2	2	3
28	(E)-2-Nonenal	3	2	<1	<1
36	Unknown	3	2	3	6
18	Phenylacetaldehyde	3	1	2	1
22	3,4-Dimethyl-2-cyclopentenol-1-one	3[e]	<1[e]	6[e]	2[e]
7	2-/3-Methylbutanoic acid	3[e]	<1[e]	3[e]	<1[e]
20	Linalool	3[e]	-	<1	-
5	3-Methyl-2-buten-1-thiol	2	6	<1	<1
27	5H-5-Methyl-6,7-dihydrocyclopenta-pyrazine	2	3	<1	<1
23	Guaiacol	2[e]	3[e]	1[e]	3[e]
13	3-Mercapto-3-methyl-1-butanol	2[e]	<1[e]	3[e]	2[e]
38	Vanillin	2	2	25[e]	13[e]
37	Bis(2-methyl-3-furyl)disulphide	2	2	6	3
24	Furaneol	1[e]	<1[e]	13[e]	3[e]

[a] Numbering as in Fig. 1 and Table 3.

[b] The FD-factor (OV-1701) of each compound (Table 3) was related to the compound with highest FD-factor which was set to 100.

[c] Both coffee varieties were roasted 3 min using a jetzone roaster, particle size of the coffee powder: 300-500 µm.

[d] The brew was obtained by extracting 54 g of the powder with 1 L of hot water (80-100°C).

[e] The FD-factor was determined on FFAP.

[f] The FD-factor was determined on SE-54.

differences in the composition of the important odorants corresponded to the differences in the overall aromas of the two varieties.

Differences between the brews of Arabica and Robusta coffee

A comparison of the odorants isolated from the brews of Arabica and Robusta coffee (Table 4) revealed a shift in the predominating flavor compounds. Sotolon, abhexon, furaneol and 3,4-dimethyl-2-cyclopentenol-1-one showed significant higher FD-factors in the Arabica than in the Robusta coffee. This difference suggested that these odorants were mainly responsible for the "sweet, mild" aroma of the Arabica coffee.

During the extraction with hot water the water-soluble enoloxo compounds were enriched in the brew and, thus, enhanced the caramel-like flavor notes, in particular of the Arabica coffee. *Hodge* [15] has reported that a planar enoloxo structural element in a volatile compound is responsible for the caramel-like odor impression.

The aroma of the Robusta coffee brew was mainly influenced by compounds having "roasty-earthy" and "spicy" odor qualities like 2,3-diethyl-5-methylpyrazine, 3,5-dimethyl-2-ethylpyrazine, 4-ethylguaiacol, 4-vinylguaiacol and the odorant no. 19. These odorants, in combination with the lower amounts of compounds having caramel-like aromas, were responsible for the "harsh, earthy, less pleasant" flavor notes of Robusta coffee.

Compared to the powders, vanillin, methional, furaneol and sotolon increased in the brews, in particular of the Arabica coffee. On the other hand (E)-ß-damascenone, (E)-2-nonenal, the temperature labile thiols 3-mercapto-3-methylbutylformate, 2-furfurylthiol, 2-methyl-3-furanthiol, 3-methyl-2-buten-1-thiol, and also linalool decreased strongly. This effect was also observed for 2,3-diethyl-5-methylpyrazine and 2-methoxy-3-isobutylpyrazine, which also decreased especially in the Arabica coffee.

Quantitative data

The quantitative analysis of the compounds were performed by means of a stable isotope dilution analysis (SIDA) in order to compensate for losses during the isolation procedure [12]. In the SIDA the odorant labeled with a stable isotope is used as internal standard. Until now a SIDA was developed for the quantification of furaneol, diacetyl [16], 2,3-pentandione, 4-

Table 5: Concentrations of some important odorants in the brews of Arabica and Robusta coffees

Compound[a]	Concentration[b] Arabica	Robusta
Diacetyl ($^{13}C_2$)	1.7	1.3
2,3-Pentandione (d_3)	1.3	0.7
Furaneol ($^{13}C_2$)	6.6	1.5
Sotolon[c]	1.0	0.2
Abhexon (d_3)	0.1	<0.03
4-Ethylguaiacol (d_2)	0.06	0.4
4-Vinylguaiacol[d]	1.0	n.d.
3-Mercapto-3-methylbutylformate (d_6)	0.006	0.002
(E)-ß-Damascenone (d_6)	n.d.	0.003

[a] The quantification was performed as stable isotope dilution assay. The labeling of the internal standard with the stable isotope is given in bracketts (^{13}C: carbon-13, d: deuterium).

[b] Concentration: mg/l brew prepared from 54 g roasted coffee powder.

[c] Sotolon was determined using d_3-abhexon as internal standard.

[d] 4-Vinylguaiacol was determined using d_2-4-ethylguaiacol as internal standard.

n.d.: not determined.

Tabel 6. Odor activity values (OAV) of important flavor compounds in the brews of Arabica and Robusta coffees

Compound	Threshold[a]	Arabica OAV	Robusta OAV
Diacetyl	15[b]	113	87
2,3-Pentandione	30[b]	43	24
Furaneol	100[b]	66	15
	30[c]	220	50

[a] µg/kg water.

[b] Odor threshold (nasal perception).

[c] Flavor threshold (retronasal perception).

ethylguaiacol, abhexon, (E)-ß-damascenone and 3-mercapto-3-methylbutylformate in the brews of both, Arabica and Robusta coffees.

As shown in Table 5, the amounts of diacetyl and 2,3-pentandione were higher in Arabica than in Robusta coffee brew indicating the importance of the buttery top-notes for the mild aroma of Arabica coffee. This becomes more clear when comparing the odor activity values (OAV, Table 6). The higher OAV of diacetyl indicated that this dione contributed more significantly to the aroma of the brews than 2,3-pentandione.

Quantitative measurements of furaneol which was used as indicator substance for the caramel-like odorants revealed one reason for the odor difference between Arabica and Robusta. The concentration of furaneol was 4.5 fold higher in the brew of Arabica coffee than in the corresponding sample of the Robusta species (Table 5). Calculation of OAV (Table 6) confirmed the stronger effect of furaneol on the flavor of Arabica coffee compared to the Robusta variety. The concentrations of sotolon and abhexon were lower than those of furaneol, but also these enoloxo compounds prevailed in the Arabica coffee (Table 5).

The OAV of diacetyl in Arabica coffee brew was 2-fold higher than the OAV of furaneol. In contrast, the FD-factor of furaneol was 8-fold higher. This difference indicates the great losses of diacetyl during the isolation procedure compared to the higher boiling compounds like furaneol. Thus, the importance of high volatile compounds was underestimated by the AEDA.

The results in Table 5 show, furthermore, the significantly higher concentration of 4-ethylguaiacol in the Robusta coffee compared to the Arabica coffee. This agreed with the sensory data obtained by the AEDA indicating the importance of phenol-derivatives for the aroma of Robusta coffee.

The predominance of 3-mercapto-3-methylbutylformate in the Arabica coffee was also in agreement with the results of the AEDA.

Acknowledgements. The work was supported by the Forschungskreis der Ernährungsindustrie (Hannover) and the AIF (Köln). We are grateful to Miss Kustermann, Miss Foschum and Miss Reinhard for skilful technical assistance.

Summary

The volatile components of roasted Arabica and Robusta coffees (powder and brew) were analysed by gas chromatography-olfactometry (GC/O) which revealed the odorants having the highest odor-activity values (ratio of concentration to odor threshold). This procedure resulted in 38 odorants of which 32 were identified. The powders of the two coffee varieties differed in the concentration levels of these compounds.

The results indicate that the flavor difference between Arabica and Robusta coffee (powder and brew) are mainly due to the predominance of enoloxo compounds (sotolon, abhexon, furaneol, 3,4-dimethylcyclopentenol-1-one) in the former and of 3,5-dimethyl-2-ethylpyrazine, 2,3-diethyl-5-methylpyrazine, 4-ethylguaiacol and 4-vinylguaiacol in the latter. Preparation of brews enhanced the flavor difference, as the concentration levels of water-soluble odorants (furaneol, sotolon, abhexon) responsible for the "sweet-caramel" flavour note increased more in the Arabica than in the Robusta coffee. On the other hand, the alkylpyrazines and guaiacols were responsible for the "spicy, harsh-earthy" aroma of the Robusta coffee.

Quantification of selected odorants using a stable isotope dilution assay confirmed the differences between the Arabica and Robusta coffees (brew) found by GC/O.

Zusammenfassung

Die flüchtige Fraktion von Arabica- und Robusta-Röstkaffee (Pulver und Getränk) wurde durch Gaschromatographie-Olfaktometrie (GC/O) untersucht. Die Analyse ergab 38 aromaaktive Verbindungen, von denen 32 identifiziert wurden. Die beiden Kaffeesorten zeigten Unterschiede in der Konzentration dieser Aromastoffe.

Das Aroma des Arabica-Kaffees wird hauptsächlich durch Enoloxo-Verbindungen (Sotolon, Abhexon, Furaneol und 3,4-Dimethyl-2-cyclopentenol-1-on) geprägt, während im Robusta-Kaffee 3,5-Dimethyl-2-ethylpyrazin, 2,3-Diethyl-5-methylpyrazin, 4-Ethyl- und 4-Vinylguajacol überwiegen. Die Bedeutung der Enoloxo-Verbindungen nimmt im Arabica-Kaffeegetränk wegen der guten Wasserlöslichkeit zu, so daß sie für das süßlich-karamelartige Aroma verantwortlich sind. Die stechend-erdige Aromanote von Robusta-Kaffee wird dagegen von Alkylpyrazinen und Guajacol-Derivaten geprägt.

Quantitative Daten bestätigten die durch GC/O erhaltenen Ergebnisse in bezug auf die Unterschiede von Arabica- und Robusta-Kaffee (Getränk).

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NEW SULFUR-CONTAINING AROMA-IMPACT-COMPOUNDS IN ROASTED COFFEE

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INTRODUCTION

The literature about volatiles in roast coffee is undoubtedly numerous. Some 800 compounds are described and a structural elucidation has been made for about 1000 compounds. The improvement of analytical chemistry with regard to detector sensitivity and column separation efficiency facilitated the discovery of compounds down to the ppt-range. Among roasted coffee volatiles, many different kinds of chemical classes are present. Nevertheless, little is known about the individual contribution of these hundreds of components to the sensory impression of roasted coffee flavor. Recent work in our group indicated, that only a relative small number of volatiles is of greater importance for the overall perceptible coffee aroma, and that the most aroma-intensive volatiles are mainly found in the group of the sulfur-containing compounds and the N-heterocycles (Holscher et al., 1990). Further systematic studies of all the peaks obtained by gaschromatographic separations of roasted coffee volatiles would yield several 100 substances more but this only will lead to a prolongation of a never-ending list of volatiles including artifacts formed during the cleaning-up procedure. More and more it became clear that the chances of discovering a single key component responsible for the overall perceptible aroma of roasted coffee has become less and less likely. Current aroma research uses another methodical approach. The new target therefore, is the elucidation of the character-impact-compounds, which means those compounds which contribute most to the coffee aroma. This paper describes the identification of some aroma-intensive sulfur-containing compounds in roasted coffee. As the identified components have a common precursor, a possible formation pathway is proposed.

METHODOLOGICAL APPROACH

Fig. 1 gives an idea about the methodology currently applied in our laboratory for the identification of some aroma-intensive odorants in roasted coffee. For the isolation of roasted coffee volatiles, one of the two methods was used: either the high vaccuum distillation according to fig. 1 or the simultaneous distillation/extraction (SDE). The first one delivered fewer artifacts and a rather naturally

smelling aroma concentrate that represented the properties of authentic coffee flavor to a great extent. On the other hand, SDE often is advantageous due to better recoveries, simple handling and less time consumption and was used for enrichment of trace compounds. The next steps were the concentration and the fractionation of the total aroma extracts. Prefractionation, either by column chromatography, prep. HPLC or prep. GC, was an obligate step in order to avoid peak overlapping and coelution. The identification of the single peaks was carried out via retention data, GC-MS and GC-FTIR. NMR was used mainly for the structural confirmation of synthetic reference substances.

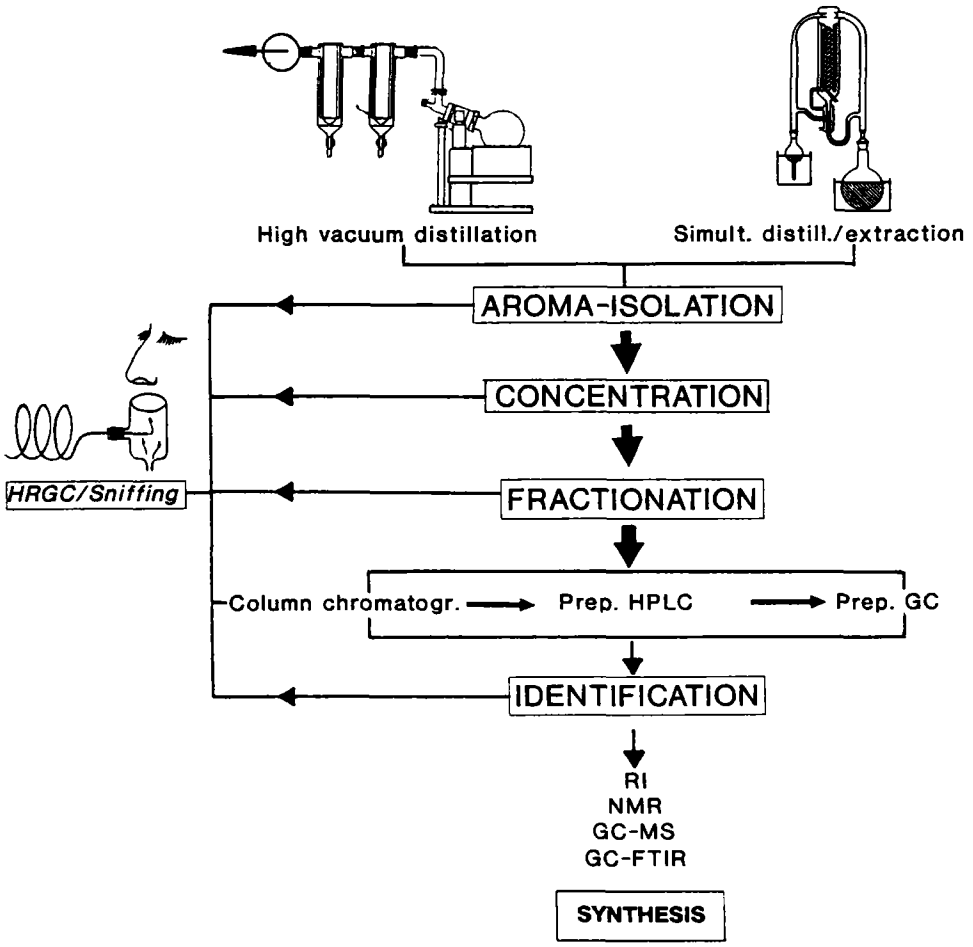

***Fig. 1:** Diagram of the experimental procedure*

GC-EFFLUENT SNIFFING

As visualized in fig. 1, GC/effluent sniffing was applied at each step of the procedure and was the most important tool for the differentiation between aroma-potent and less or non-important volatiles. Therefore the capillary column was splitted into a stream for flame ionization detection as well as for sensory evaluation by means of a sniffing port. Sometimes only sniffing allowed the detection of ultra trace compounds in certain subfractions of column chromatography or prep. HPLC. Simultaneous GC/sniffing evaluation was done even with total aroma extracts. Many kind of different aroma notes, e.g. buttery, roasty or honey-like notes, were perceivable. Some of these odor perceptions, for instance some "foxy", "animal-like" or "soup-like" notes, were of special interest because of their intensive and characteristic odor impressions. One aroma note however, attracted a particular interest because of a strong "sweaty" and "catty" smell at the sniffing port.

IDENTIFICATION

As an example, the cleaning-up and enrichment procedure prior to structural elucidation of the "catty" smelling compound is illustrated in fig. 2. Roasted coffee volatiles were obtained by SDE as already mentioned. After concentration, prefractionation by means of column chromatography on silica gel was carried out (Silwar et al., 1987). The volatiles were separated according to their polarity with a pentane/dichloromethane/diethylether gradient. The subfraction I to III out of VI were combined and submitted to preparative HPLC on a silica gel column. The compound of interest was perceivable in the eluate and the corresponding fractions were cut out and collected. GC of this subfraction indicated a large number of components despite of a narrow cut but the compound of interest was enriched to a remarkable discrete peak. On the basis of mass spectral and GC-FTIR-data the strong "catty"-smelling component was proposed as 3-mercapto-3-methyl-butylformate.

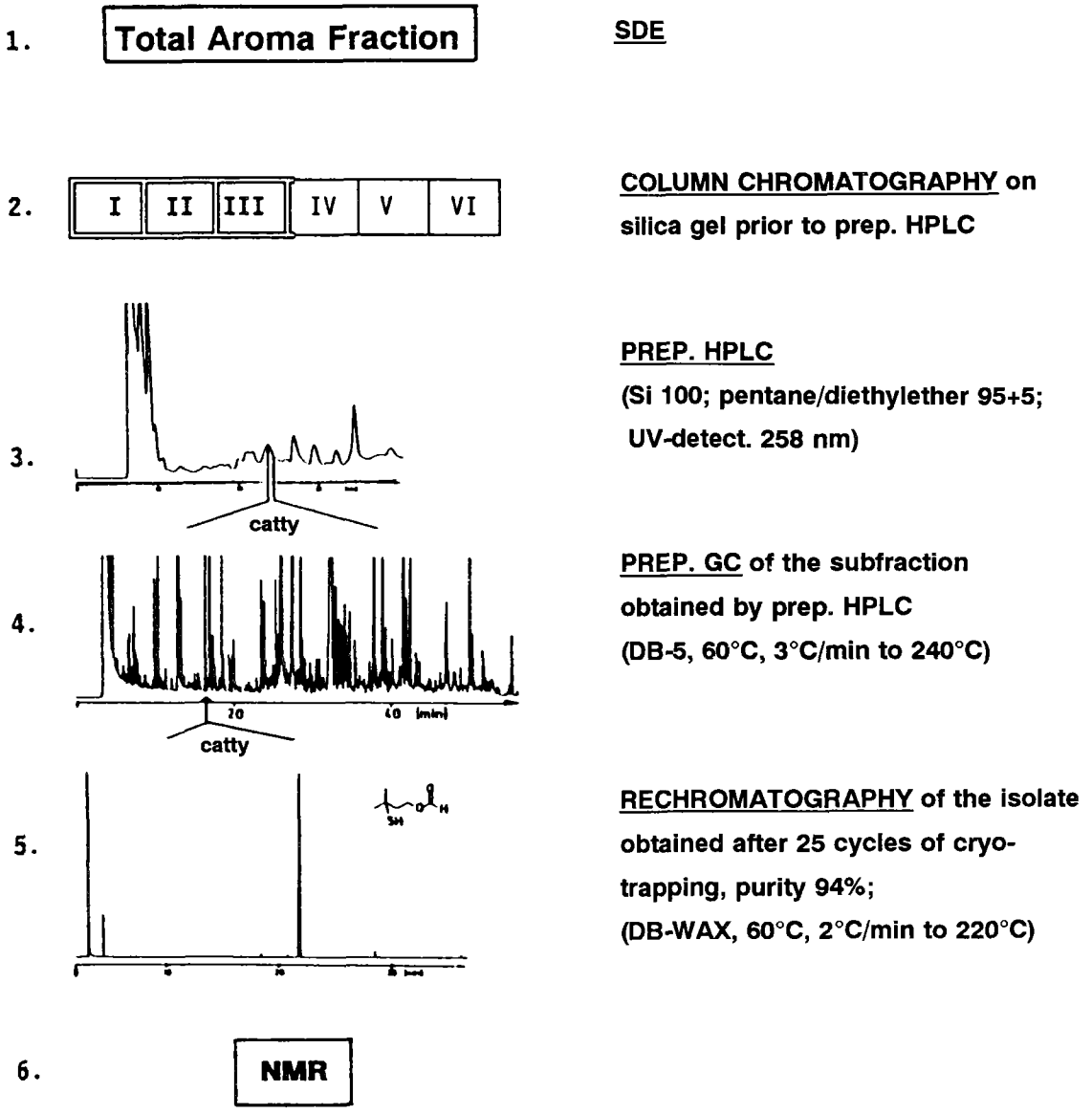

Fig. 2: Enrichment and identification of 3-mercapto-3-methylbutylformate

In order to facilitate the synthesis of this compound, confirmation of the chemical structure was carried out by off-line NMR-analysis. Therefore, a total amount of about 20 kg of roasted coffee was cleaned-up according to fig. 2 and the compound of interest was isolated by means of preparative GC. For this purpose, the peak of interest was cryo-trapped during 25 GC-runs by means of a dry-ice-cooled glas tube thus obtaining enough material, about 20 μg, for subsequent NMR-analysis, which confirmed the chemical structure (fig. 3). The re-chromatography of the isolate, eluted with several microliters of tetrachloromethane, proved a purity of the cryo-trapped mercapto-compound of over 90 %. In a similar manner, but with different cleaning-up expense, another two sulfur-containing compounds of specific interest were identified: 3-methyl-2-buten-1-thiol and 3-mercapto-3-methyl-butanol.

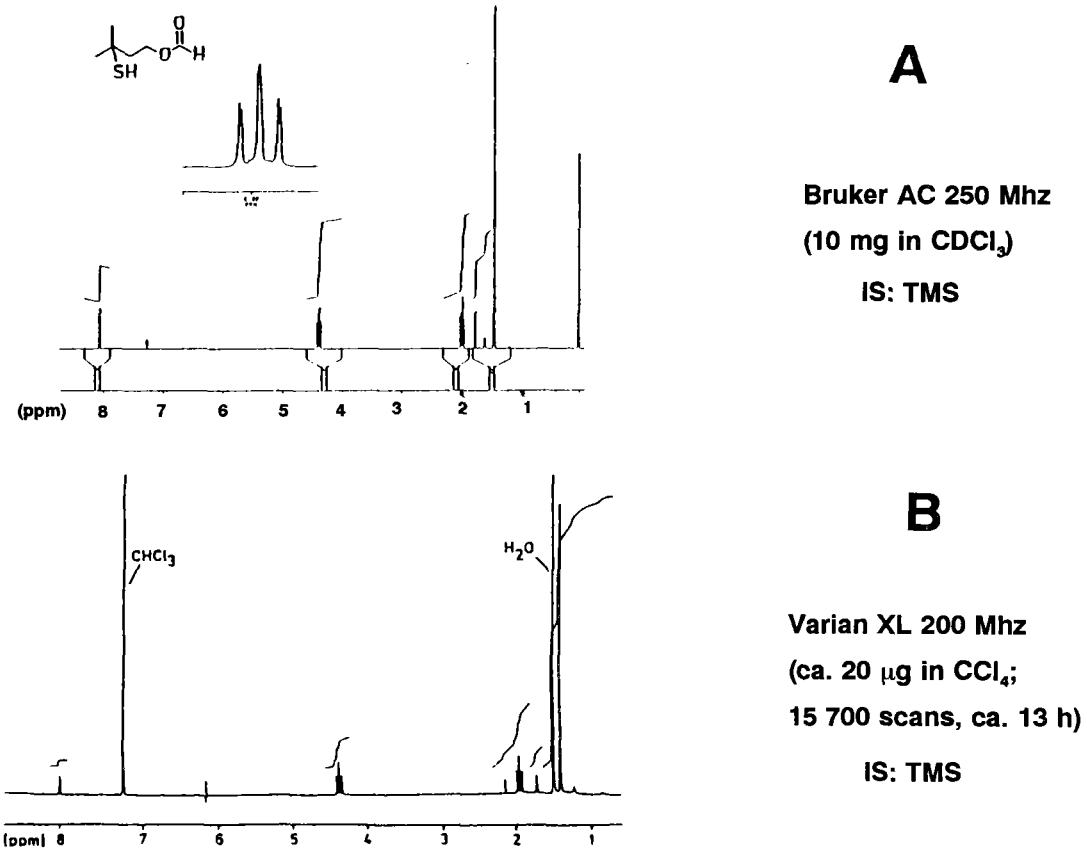

Fig. 3: *Comparison of ^{1}H-NMR-spectra of synthetic 3-mercapto-3-methyl-butylformate (A) and isolated from roasted coffee (B); ($CHCl_3$: signal from impurities of the solvent)*

SENSORY PROPERTIES

In order to confirm their chemical structures and to investigate the sensory properties, syntheses were carried out according to Kofod (1955) for the preparation of 3-methyl-2-buten-1-thiol and according to Stoffelsma and Pijpker (1973) for 3-mercapto-3-methylbutanol and 3-mercapto-3-methyl-butylformate, respectively. The thiol exhibited a "pungent, leek-like" aroma note in high concentrations, whereas in high dilution a "foxy, animal-like" or "skunky" odor dominated. Its odor threshold goes down to the ppq-level. 3-Mercapto-3-methylbutanol possessed a "sweet, soup-like" note in high concentrations, whereas in higher dilution and at the sniffing-port a "spicy, cooked-meal-like" odor impression was perceivable. The odor threshold was in the low ppb-level and this

was comparatively high with regard to the corresponding formic-acid-ester. This latter compound exhibited a strong "sweaty" aroma note, also described as "blackcurrant-like" or "catty" because of a certain similarity to the excrements of cats (Pearce, 1967). "Catty"-smelling compounds play an important role in aroma research. For the exhibition of the "catty" aroma note, the structure element of the tert.-amylmercaptan group is sufficient (Polak et al., 1988) and can be identified in all chemical structures in fig. 4.

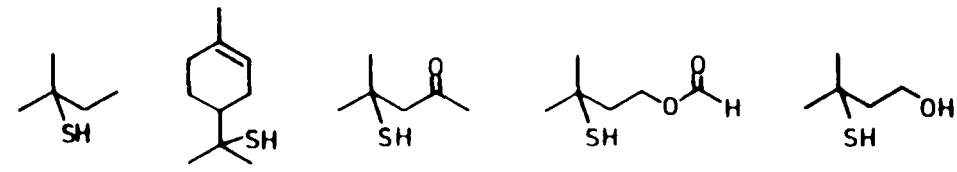

Fig. 4: Structure-odor relationships of the "catty" aroma perception (modified according to Polak et al., 1988)

The polarity of the oxygen-containing functional group influences the aroma quality. 1-p-Menthene-8-thiol, the character-impact-compound of grapefruits, and 4-mercapto-4-methyl-2-pentanone, known as an off-flavor causing odorant in meat cans, exhibit the "catty" note whereas in 3-mercapto-3-methyl-butylformate a slightly fruity odor impression is already recognizable. In the corresponding alcohol the catty note is totally lost and converted to a soup-like note.

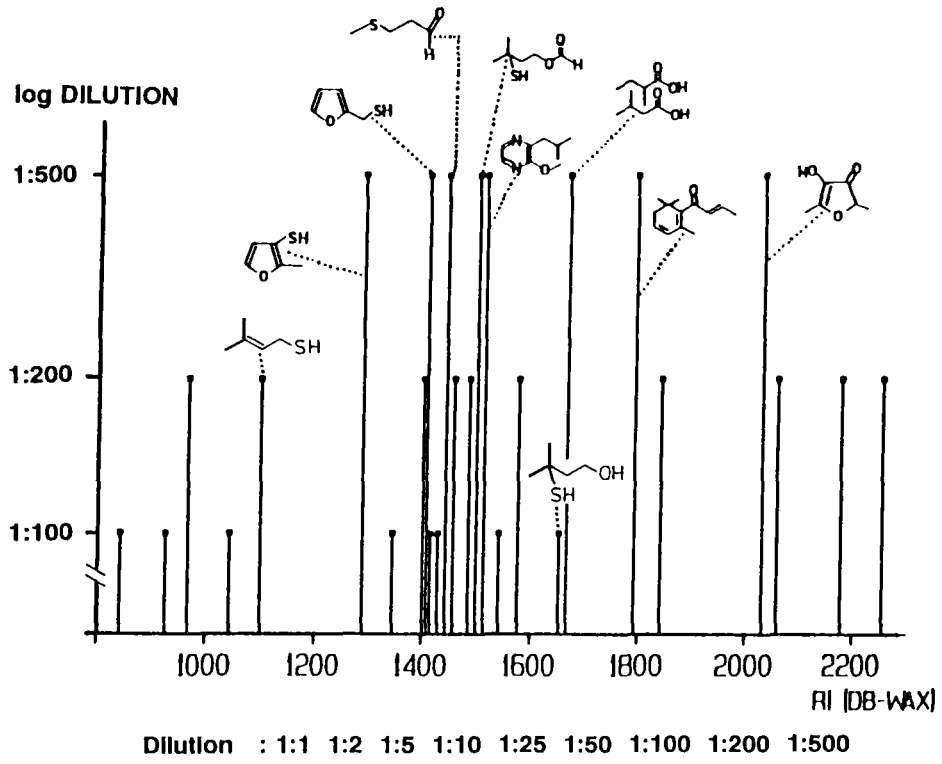

Fig. 5: Aromagram of a total aroma extract obtained from roasted Colombian coffee

A further objective was to estimate the contribution of the newly identified compounds to the overall perceptible flavor of roasted coffee. For this task, a very promising technique has been introduced, the so-called aroma dilution analysis (Ullrich and Grosch, 1987). In practice, gas chromatographic separation of a total aroma extract is done as usual, including simultaneous sniffing. The original aroma concentrate will be evaluated at the sniffing port and the aroma perceptions are noted. This extract will be diluted stepwise after each GC-run. The higher the dilution the more aroma impressions disappear at the sniffing port. The procedure has to be continued until no or fewer odor perceptions are still recognizable. If the aroma dilution analysis is carried out by well experienced and trained testers, very reliable results can be obtained. These results can be visualized by plotting the retention index of a certain aroma compound versus the dilution step as a characteristic aromagram. Fig. 5. shows a cut from such an aromagram we obtained by our investigations (Holscher et al., 1990) and illustrates the approximate contribution of the three sulfur compounds mentioned before to the overall perceptible flavor of roasted coffee. At the dilution step 1:500 the most intensive aroma notes are recognized at the sniffing port. Among other and partly better known compounds, e.g. 2-furfurylmercaptan, 3-mercato-3-methyl-butylformate still was perceivable even at this high dilution. Slightly lower intensity showed the thiol which could not be recognized at this dilution step but in a previous step. And even the contribution of the butanol is still comparatively high with respect to a total amount of 9 dilution steps (in fig. 5 only the 3 highest dilution steps are given).

HYPOTHETICAL FORMATION PATHWAY

Although not obvious at once, the three sulfur compounds are related to each other by a common precursor. Tressl et al. (1983) proposed a hypothetical pathway for the formation of several sulfur containing volatiles in roasted coffee but, most of them were not identified yet. The key compound of this pathway is 3-methyl-2-buten-1-ol, also called prenylalcohol. The diphosphate of this alcohol is widely spread as a tautomer of the "active isopren,"which is well known as a key compound during biosynthesis of isoprenoids. The prenylalcohol occurs in roasted coffee in considerably large amounts (Holscher, 1991). During roasting of the green beans, many kind of chemical reaction can take place under elevated pressures and temperatures. Sulfur containing amino acids may form hydrogen sulfide which reacts with the double-bonding of prenylalcohol according to the rule of Markownikow to form 3-mercapto-3-methylbutanol. In addition to the mechanism proposed by Tressl et al. (1983) the "catty"-smelling 3-mercapto-3-methyl-butylformate was identified as an aroma-potent constituent of roasted coffee flavor and arises from esterification with formic acid. On the other hand the hydroxy-group of prenylalcohol may be substituted to form the "foxy"-smelling 3-methyl-2-buten-1-thiol.

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SUMMARY. *Investigation of the character-impact-compounds of roasted Colombian coffee by means of simultaneous GC-MS / sniffing resulted in the identification of several aliphatic sulfur containing components: 3-methyl-2-buten-1-thiol, 3-mercapto-3-methylbutanol and 3-mercapto-3-methyl-butylformate. Simultaneous distillation / extraction was used for their isolation. Preseparation and enrichment prior to spectroscopy was carried out by means of column chromatography as well as by HPLC and preparative GC. Said compounds possess low odor thresholds and show certain structure-odor relationships. Results of semi-quantitative sniffing assessment, carried out as aroma dilution analysis, indicated a remarkable contribution to roasted coffee flavor. These compounds are presumably related to each other by the common precursor prenylalcohol (3-methyl-2-buten-1-ol).*

RÉSUMÉ. *La recherche sur les liaisons arômatiques sur café torréfié de Colombie par la combination GC-MS / olfactométrie, a permis l'identification de plusieurs combinaisons sulfuriques: 3-méthyle-2-butène-1-thiole, 3-mercapto-3-méthyle-butyle-alcool et 3-mercapto-3-méthyle-butyle-formiate. La distillation / extraction simultanée a été appliquée pour l'isolation de ces combinaisons. La séparation et l'enrichissement précédent la spectroscopie ont été réalisés au moyen de la chromatographie préparative liquide et en phase gazeuse. Ces substances arômatiques sont caractérisées par un seuil d'odeur très bas et donnent certaines relations de structures chimiques / d'odeurs. Les résultats d'olfactométrie semi-quantitative donnent une intéressante contribution sur l'arôme du café torrefié. Toutes ces liaisons sont vraisemblablement reliées ensemble par un précurseur (3-méthyle-2-butène-1-ol).*

DISCRIMINATION OF VARIETIES AND ROASTING LEVELS IN COFFEE BEANS BY PATTERN RECOGNITION ANALYSIS OF RESPONSES FROM A SEMICONDUCTOR GAS SENSOR ARRAY

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1 INTRODUCTION

Aroma analysis of coffee has intensively been conducted by many researchers as reviewed in a recent article [1]. Until now nearly 700 aroma compounds have been identified, however, no single compounds responsible for its peculiar aroma have not been reported. From the view point of coffee business, objective discrimination of coffee varieties based on their components has been important subjects. In coffee manufacturing, on the other hand, many efforts have been concentrated to establish the objective assessment of roasting levels of coffee beans to supplement or substitute color lightness.

Chemometric pattern recognition has widely been applied in the flavor research to objective sample classification, discrimination and identification on the basis of their chromatographic profiles of constituents [2]. Especially recent progress and propagation of personal computers and software is accelerating the utilization of such techniques [3]. Concerning coffee aromas, several groups have already reported their successful results for variety discrimination by applying pattern recognition for gas chromatographic data [4, 5]. The purpose of pattern recognition of spectra or chromatographic data can be expressed as the extraction of useful information from complicated data matrix by using statistical methods.

Today, several different kinds of gas sensors are available for gas detection. So far, semiconductor gas sensors and quarts-resonator sensors have been applied to discriminating gases or gas mixtures due to their durabilities and high sensitivity [6-8]. Although sensor properties can be somewhat controlled by doping noble metals into metal oxides or adjusting their amounts, every gas sensor is non-specific in nature [9]. It means that a gas sensor responds to any gases when it contacts. From the view point of mimicking olfaction system, however, we do not need highly specific sensors because receptors at our olfactory epithelium are also non-specific in nature. The human brain discriminates different aromas by conducting a kind of pattern recognition to decode the signals sent from the olfactory neurons [10]. This is the rational premise for constructing so-called an artificial nose by integrating multi-gas sensors carrying somewhat different properties (Fig. 1) [11].

Six different semiconductor gas sensors made of SnO_2 were selected to construct the aroma sensing system in this study. By applying pattern recognition to the resulting responses, this system has already succeeded in discriminating four different coffee samples: freeze dried and spray dried instant coffees, and two species of ground coffee, i.e., Coffea arabica and C. robusta [12]. In this study, at first, discrimination of aromas in different varieties of coffee beans was attempted based on their response patterns. Next, discrimination of aromas in coffee beans at different roasting levels was tried by using the same system.

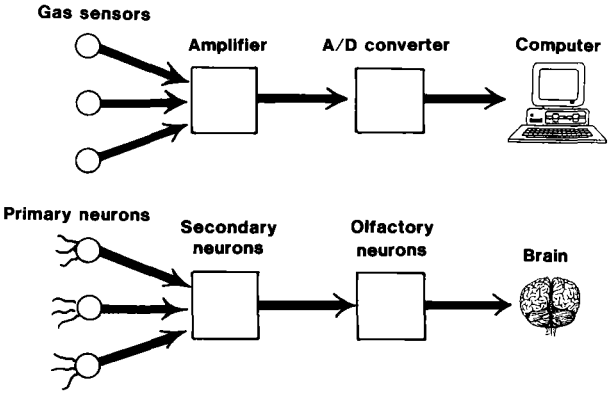

Fig.1 Comparison of olfaction system and "artificial nose" using gas sensors

2 MATERIALS AND METHODS

2.1 Coffee samples

All coffee beans were courtesy of Tokyo Allied Coffee Roasters Co., Ltd. (Tokyo, Japan). Coffee beans were roasted with hot combustion gases based on the predetermined level. Six varieties of C. arabica and two varieties of C. robusta shown below were selected for variety discrimination because of their popularity in the Japanese coffee market. Abbreviations for the subsequent tables and figures are indicated in parentheses.

C. arabica: Mandheling (A), Blue Mountain (B), Guatemala (G), Mocha (M), Santos (S), and Tanzania (T)
C. robusta: AP-1 (P), and WIB (W)

Columbian coffee beans were roasted at five predetermined levels with hot combustion gases. The degrees of roasting levels were checked by colorimetry and expressed at L values as shown below. Abbreviations for the subsequent tables and figures are shown in parentheses.

L27.2 (1), L24.2 (2), L22.3 (3), L19.6 (4) and L18.4 (5)

All coffee beans were ground by a Unic T80 coffee grinder.

2.2 Sample treatment for sensing

A Tekmar LSC 2000 semiautomatic headspace concentrator was used for the pretreatment of sample aroma. The conditions for sample treatment were fixed for all samples. Sample aroma was purged with N_2 gas for 30 min at 40 mL/min at 50°C. The volatiles trapped on Tenax TA (ca. 150 mg) were dry-purged with N_2 gas for 5 min at 40 mL/min. Aroma desorption from the trap was performed at 180°C for 4 min.

2.3 Gas sensing system

The whole scheme of the gas sensing system is shown in Fig. 2 [8]. Six semiconductor gas sensors used to construct the gas sensor array were courtesy of Figaro Sensor Inc. (Minoo, Osaka, Japan) (Table 1).

One sensor array was installed in the three-necked 5 L sample flask, and another array was used as reference sensors. The circuit voltage for all sensors was kept ca. 2 V. Every heater temperature was kept around 350°C during the sensing period. The resistance decreased when reducing gas, i.e., aroma compounds, contacted at the surface of gas sensors. Amplified differences between sample sensors and reference sensors were recorded by recorders. After each measurement was finished, the flask was ventilated with air purified through charcoal and silica gel columns until responses returned to the blank levels.

The distances (mm) from the blank levels were measured as the sensor responses. To collect enough number of data for the subsequent statistical analysis and to confirm repeatability of this methodology, the measurement repeated 15 times for the same variety or roasting level by renewing coffee particles in the 25 mL purge sampler.

The sensor responses were used as variables for the subsequent pattern recognition analysis.

Table 1 Properties and operating conditions of semiconductor gas sensors [13]

Sensor	Circuit voltage (V)	Heater voltage (V)	Analyte gases
TGS812	1.7	4.4	Alcohols, organic solvents
TGS813	1.8	4.4	General combustive gases
E71N	1.8	4.8	Sulfur compounds
TGS800	1.5	4.4	General gases
TGS815D	1.8	4.4	General combustive gases
E71M	1.8	4.8	Carbon monoxide, hydrogen, alcohols

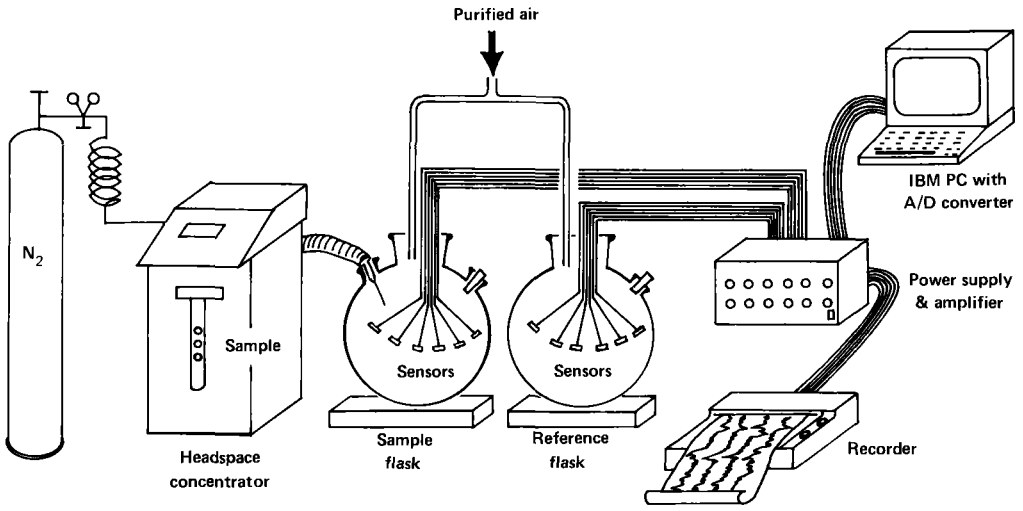

Fig. 2 Gas sensing system using gas sensor array

2.4 Gas chromatographic analysis

Headspace analysis of coffee aroma was performed by coupling a Tekmar LSC 2000 with a Shimadzu GC-9A gas chromatograph equipped with an FID detector through a cryofocusing interface. A fuzed silica capillary column (30m x 0.25 mm i.d.) coated with DW-WAX (0.25μ m)(J&W Scientific, Folsom, CA) was used. The oven temperature was kept at 50°C for 4 min and then elevated to 200°C at 3°C/min.

2.5 Pattern recognition

Two different pattern recognition techniques were applied to the data matrix of sensor responses by using SPSS PC+ V3.0 programs [14] on an IBM PS/55 5551T system, as follows.

Hierarchical cluster analysis can sort sample relationships in the form of a tree based on their multidimensional distances or similarities. This method is widely used to examine the relationships of samples in unsupervised pattern recognition analysis. Various methods are available for measuring distances among samples and clusters but Euclidean distance and Ward method were used.

Linear discriminant analysis (LDA) is used to classify and then assign samples into specific known groups based on multi-dimensional distances. In this study stepwise LDA, in which the most effective combination of variables can be selected based on the contribution of each variables for discrimination, was applied to obtain the most discriminative results using the minimum number of sensor responses. Variables which could make Wilks'Λ minimum were selected in this stepwise LDA. LDA is a supervised pattern recognition technique because it uses group names as criterion variables for calculating discriminant functions. In the LDA, if the number of variables increases, then correct ratios can automatically improve. Thus the ratio of sample number to variable number should not exceed 3.0 to avoid chance separation.

3 RESULTS AND DISCUSSION

3.1 Sensor responses and GC profiles

The six sensors responded immediately when sample aroma was introduced into the flask as shown in Fig. 3. The recorder attenuation was adjusted so as to make all the responses within the full scale. The responses from the six sensors were similar to each other. The initial sharp peaks typically shown by TGS813 in Fig. 3 were not included in the data because these peaks changed among measurements even in the same coffee sample. Correlation coefficients were calculated for every combination of six sensors on the basis of responses for 120 samples of the eight coffee varieties (Table 2). Except for E71N, highly significant correlations were observed among the responses of other five sensors.

The aromas concentrated by Tekmar LSC 2000 under the same operational conditions were injected into GC to compare the patterns of volatile components among coffee samples. More than 100 peaks appeared in every chromatogram as shown in Fig. 4.

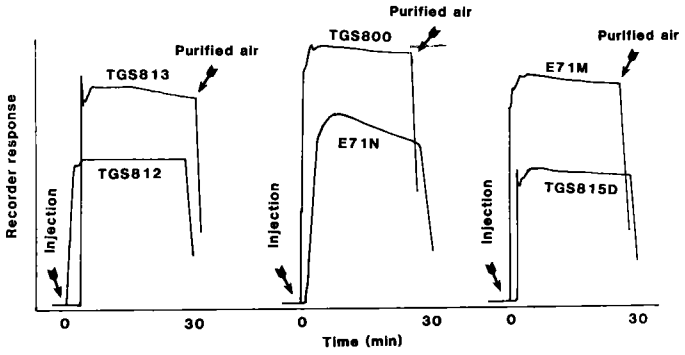

Fig. 3 Sensor responses to coffee aroma

Table 2. Correlation coefficients of responses in six gas sensors (n=120)

	Sensors					
Sensor	TGS812	TGS813	E71N	TGS880	TGS815D	E71M
TGS812	1.000	0.779*	0.186	0.706**	0.900**	0.895**
TGS813		1.000	-0.127	0.942**	0.760**	0.810**
E71N			1.000	0.004	0.339**	0.242*
TGS880				1.000	0.712**	0.769**
TGS815D					1.000	0.891**
E71M						1.000

*P<0.01, **P<0.001

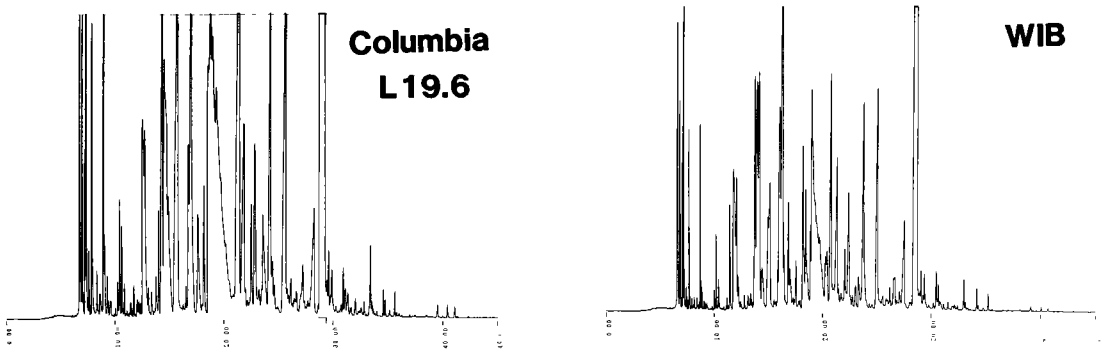

Fig. 4 Headspace gas chromatograms of coffee samples

3.2 Coffee varieties

Mean heights (averages of three heights measured at 8, 16 and 24 min), standard deviations (SD) and relative standard deviations (RSD=100xSD/mean) observed for different varieties are shown in Table 3. Differences in sensor responses for varieties are not so apparent due both to similarity among sample aromas and nonselectivity of gas sensors. This is the rational basis for applying pattern recognition analysis for the resulting sensor responses.

Table 3 Basic statistics of sensor responses for eight varieties

	Sensor					
Variety	TGS812	TGS813	E71N	TGS880	TGS815D	E71M
Mandheling (n=15)						
Mean height (mm)	202.4	134.5	285.1	144.5	177.3	302.7
SD	26.8	15.2	17.4	9.9	19.1	31.3
RSD (%)	13.2	11.3	6.1	6.9	10.8	10.3
Blue mountain (n=15)						
Mean height (mm)	210.1	179.2	235.0	196.5	186.7	330.8
SD	17.6	10.8	14.5	10.9	15.4	22.7
RSD (%)	8.4	6.0	6.2	5.5	8.2	6.9
Guatemala (n=15)						
Mean height (mm)	206.5	151.3	267.7	169.1	187.3	315.9
SD	16.6	11.6	11.3	10.5	14.1	25.7
RSD (%)	8.0	7.7	4.2	6.2	7.5	8.1
Mocha (n=15)						
Mean height (mm)	241.8	197.7	221.6	212.1	207.7	344.3
SD	19.2	14.8	12.0	9.1	19.4	21.3
RSD (%)	7.9	7.5	5.4	4.3	9.3	6.2
Santos (n=15)						
Mean height (mm)	226.8	173.0	287.9	195.0	207.7	342.5
SD	18.4	11.2	13.8	12.4	12.6	23.2
RSD (%)	8.1	6.5	4.8	6.4	6.1	6.8
Tanzania (n=15)						
Mean height (mm)	251.9	192.7	260.9	205.3	224.3	356.5
SD	19.2	12.3	12.5	7.7	16.9	26.4
RSD	7.6	6.4	4.8	3.8	7.5	7.4
Indonesia AP1 (n=15)						
Mean height (mm)	203.8	165.9	154.7	173.7	172.7	304.4
SD	18.4	17.8	32.6	20.4	18.2	29.2
RSD	9.0	10.7	21.1	11.7	10.5	9.6
Indonesia WIB (n=15)						
Mean height (mm)	200.3	167.3	212.8	177.1	188.1	308.4
SD	25.5	11.3	8.8	10.3	24.3	22.6
RSD	12.7	6.8	4.1	5.8	12.9	7.3

Cluster analysis suggested the existence of two major groups, C. arabica and C. robusta. in the dendrogram (Fig. 5). In the two major clusters, minor clusters corresponding to each of eight varieties were observed. Three clusters were formed in the C. arabica cluster, i.e., [Mocha and Tanzania], [Blue mountain and Santos], and [Mandheling and Guatemala]. Combinations of these three clusters may not fully coincide with our olfactory perception, however, in near future these contradictory results will be overcome by increasing number of sensors or finding more appropriate sensor combinations.

LDA stopped at step 5 and the correct discrimination was 95.8% with 0.007 of Wilks' Λ (Table 4). At the first step E71N was selected as the most discriminative sensor but the correct discrimination was only 42.5%. This low correct discrimination ratio is understandable because information from single sensor implies only absolute amounts of volatiles in samples. At step 2, TGS880 was selected in order to combine with E71N and the correct ratio improved up to 76.7% and then at steps 3 and 4, TGS 812 and E71M were entered into the discriminant functions, respectively. The correct ratios became 85% and then 90% at the two steps.

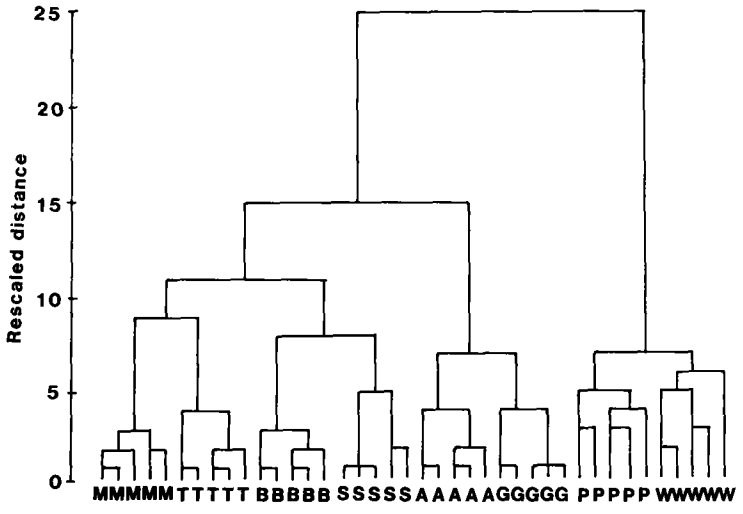

Fig. 5 Clustering of samples in eight coffee varieties

Table 4 Discrimination results for eight varieties at step 5

Actual variety	Assigned variety A	B	G	M	S	T	P	W	Correct %
Mandheling:A	15	0	0	0	0	0	0	0	100
Blue mountain:B	0	15	0	0	0	0	0	0	100
Guatemala:G	0	0	13	0	2	0	0	0	86.7
Mocha:M	0	0	0	15	0	0	0	0	100
Santos:S	0	1	2	0	12	0	0	0	80.0
Tanzania:S	0	0	0	0	0	15	0	0	100
Indonesia AP1:P	0	0	0	0	0	0	15	0	100
Indonesia WIB:W	0	0	0	0	0	0	0	15	100

3.3 Roasting levels

Basic statistics of sensor responses for the five roasting levels were shown in Table 5. Apparent differences among responses of five roasting levels were not observed. This data set was applied for the subsequent pattern recognition.

First, LDA was applied to discriminating aromas of five roasting levels. At step 1, E71N was selected but correct discrimination ratio was only 34.7%. At steps 2, 3, 4 and 5, TGS880, E71M, TGS813, and TGS815D were selected, respectively. The correct ratios improved 57.3%, 72%, 82.7% and 90.7% as the LDA step progressed. However, some samples belonging to L24.2 and L19.6 were falsely assigned even at step 5 as shown in Table 6. This result suggested the difficulty in discriminating whole five roasting levels.

Next, LDA was applied to three roasting levels by eliminating samples belonging to L24.2 and L19.6 from the data base. As clearly shown in Fig. 6, three roasting levels were unambiguously discriminated. In this analysis, E71N was also selected at step 1 with 77.9% of correct ratio. At step 2, TGS813 was entered into the discriminant functions and the correct ratio improved up to 88.9%. At step 3, E71M was selected as the third sensor by showing perfectly correct sample assignment. Comparing actual roasting levels, closer location of L27.2 to L18.4 than to L23.3 does not seem reasonable. This contradictory result may be

Table 5 Basic statistics of sensor responses for five roasting levels

Roasting levels	Sensor					
	TGS812	TGS813	E71N	TGS880	TGS815D	E71M
L27.2 (n=15)						
Mean height (mm)	185.3	169.5	155.5	162.3	140.3	299.2
SD	25.5	18.6	19.4	20.6	16.4	27.8
RSD (%)	13.8	11.0	12.5	12.7	11.7	9.3
L24.2 (n=15)						
Mean height (mm)	200.2	182.8	149.4	171.2	160.9	300.7
SD	16.1	12.0	24.7	16.0	20.6	14.3
RSD (%)	8.0	6.6	16.5	9.3	12.8	4.8
L23.3 (n=15)						
Mean height (mm)	176.1	173.1	119.3	152.4	124.3	279.3
SD	14.8	11.4	11.2	8.9	5.4	13.7
RSD	8.4	6.6	9.4	5.8	4.3	4.9
L19.6 (n=15)						
Mean height (mm)	196.9	183.9	131.3	171.5	144.9	308.5
SD	17.3	14.4	9.4	10.7	11.5	19.4
RSD (%)	8.7	7.8	7.2	6.2	7.9	6.3
L18.4 (n=15)						
Mean height (mm)	200.6	191.1	149.4	177.7	145.7	306.1
SD	17.3	21.4	10.6	18.4	16.6	27.8
RSD (%)	8.6	11.2	7.1	10.4	11.4	9.1

Table 6 Discrimination results for five roasting levels

Actual roasting level	Assigned roasting level					Correct %
	L27.2	L24.2	L23.3	L19.6	L18.4	
L27.2	15	0	0	0	0	100
L24.2	0	11	1	3	0	73.3
L23.3	0	0	15	0	0	100
L19.6	0	0	0	12	3	80
L18.4	0	0	0	0	15	100

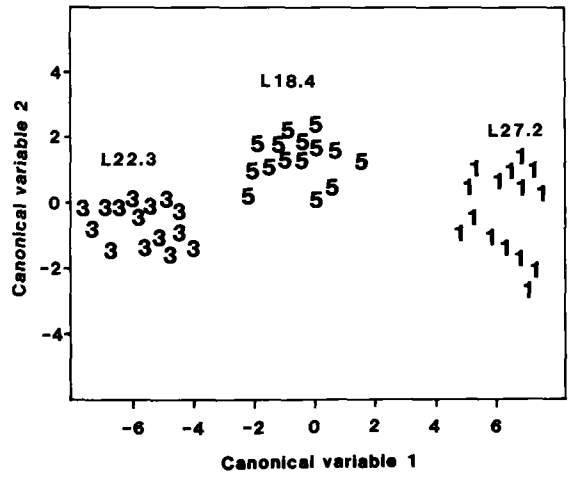

Fig. 6 Canonical plot of samples in three roasting levels

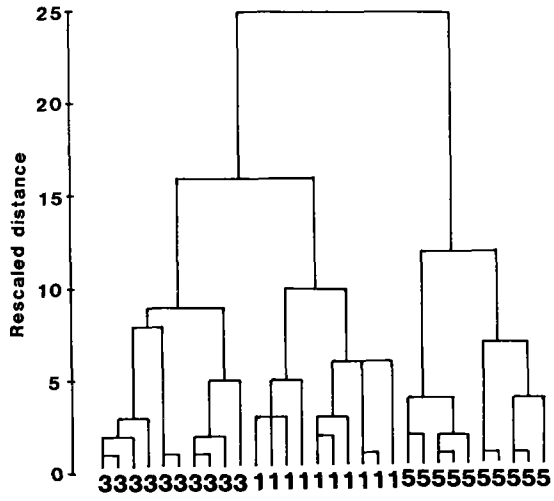

Fig. 7 Clustering of samples in three roasting levels

derived from sensor property itself because the aroma detection mechanism of gas sensors is not same with our olfactory system.

Cluster analysis applied to the same data matrix to confirm the aroma difference among roasting levels. As shown in Fig. 7, all samples were clearly classified into three clusters according to the roasting levels.

Summary

An array composed of six semiconductor gas sensors was applied to discriminating coffee aromas in eight varieties and in five roasting levels. A semiautomatic headspace concentrator was utilized to standardize aroma introduction to the sensor array. Every sensor immediately responded to the injected aroma but apparent difference in response patterns was not found among different coffee beans due to the similarity in their aromas. Sample classification was conducted by applying pattern recognition analysis for the response patterns. In the hierarchical cluster analysis, two major clusters each corresponding to C. robusta and C. arabica were observed. These two clusters separated into smaller ones each corresponding to the eight varieties. Most samples also clustered according to the roasting levels. More than 90% of samples were correctly classified into the derived varieties and the roasting levels by linear discriminant analysis. Although some results contradicted our olfactory perceptions, pattern recognition showed highly accurate sample assignments.

Résumé

Un groupe de six semiconducteurs detecteurs de gaz était utilisé pour discriminer les arômes du café dans huit variétés et dans cinq niveau de rotissage. Un concentrateur semiautomatique était utilisé pour standardiser l'introduction des arômes au niveau des detecteurs. Chaque detecteur a immediatement reconnu l'arôme injectée mais les différentes apparentes dans la séquence de responses n'étaient pas identifiées parmi les differents cafés à cause de la similarité entre leurs arômes. La classification d'échantillons était faite (realisée) en appliquant l'analyse de reconnaissance des sequences de reponses. Dans l'analyse hierarchique des groupes, deux groupes majeurs correspondant l'un au café arabica et l'autre au café robusta étaient identifiés. Les deux groupes étaient subdivisés en petits groupes correspondant aux huit variétés. La plupart d'échantillons étaient groupés suivant le niveau de rotissage. L'analyse discriminante linéaire a permis de classifier plus de 90% d'échantillons dans les differentes variétés et suivant le niveau de rotissage. Bien que certains résultats soient en contradiction avec les perceptions olfactoires, la séquence de reconnaissance a bien assuré la classification des échantillons.

Acknowledgement

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VOLATILE ANTIOXIDANTS ISOLATED FROM BREWED COFFEE

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INTRODUCTION

There has always been an interest in searching for possible food antioxidants isolated from natural sources, especially from plant material (Pratt and Hudson, 1990). The motivation behind this endeavor is to replace artificial antioxidants for natural compounds. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are utilized as food additives. However, there is a concern about their chronic and side effects (Branen, 1975).

Recently, Kroyer and co-workers (1989) reported on the antioxidative activity of extracts isolated from green and roasted coffee beans. These authors attributed the antioxidative properties to compounds such as chlorogenic acid, quinic acid, and caffeic acid.

The antioxidative activity of plant extracts are usually caused by the combined effect of several compounds (Kroyer et al., 1989; Pratt and Hudson, 1990). An investigation has been undertaken to determine the antioxidative activity of coffee volatiles. In this study, the volatile compounds of freshly brewed coffee were isolated by dynamic headspace and analyzed for antioxidative activity by using a newly developed antioxidation test.

EXPERIMENTAL PROCEDURES

Materials

Ground coffee was purchased from a local market. Pentyl aldehyde, hexyl aldehyde, and 2,5-dimethylhexane (25DMH) were purchased from Aldrich Chemical Co. (Milwaukee, WI). All authentic samples were obtained from reliable commercial sources.

Sample Preparation

Twenty-five grams of ground coffee were placed in a stove-top coffee brewer, containing 300 mL of tap water. The freshly brewed coffee was mixed with 17.5 g of NaCl (final concentration 1 M). The solution was placed in a 500 mL, two-neck, round-bottom flask interfaced to a simultaneous purging and solvent extraction apparatus (SPE) deviced by Umano and Shibamoto (1987). The mixture was stirred and kept at 60 °C for 3 hrs.

The headspace was purged into the SPE with a purified nitrogen stream (flow rate, 10 mL/min). The procedure was repeated four times (four batches). The extracts were combined and concentrated to a final volume (1 mL) by fractional distillation.

Column Chromatography (CC)

The final headspace extract was transferred to a glass column (15 cm x 1 cm i.d.), packed with silica gel (Kieselgel 60, E. Merck, Darmstadt, Germany). The extract was developed with pentane and ethyl acetate (30 mL aliquots) into five fractions. Each fraction was concentrated to a final volume (0.5 mL) by fractional distillation and stored at -5 °C for subsequent experiments.

Antioxidation Test

A newly developed aldehyde/carboxylic acid antioxidation test (Macku and Shibamoto, 1991a) was used to assess the antioxidative activity of coffee volatiles. CC fractions were mixed with pentyl aldehyde and hexyl aldehyde to final concentrations of 1 and 3 mg/mL, respectively. 25DMH was added as a gas chromatographic internal standard (0.1 mg/mL). The mixtures were diluted with dichlroromethane to a final volume of 1 mL and stored in 5 mL vials. A control solution was made, which contained the C_5 and C_6 aldehydes and 25DMH. All vials were analyzed by gas chromatography (GC) for a period of 10 days.

A Hewlett-Packard (HP) model 5790 GC, equipped with a DB-5 (60 m x 0.25 mm i.d.) bonded-phase fused-silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID), was used to monitor the relative amounts of pentyl aldehyde and hexyl aldehyde present in the vials. The GC peak areas were integrated with an HP 5880A series GC terminal. The injector temperature was 250 °C and the detector temperature was 300 °C. The oven temperature was programmed from 50 °C to 200 °C at 6 °C/min. The relative peak areas (RPA) were calculated by dividing the GC peak area of pentyl aldehyde or hexyl aldehyde by the GC peak area of 25DMH.

Qualitative and Quantitative Analyses

The coffee headspace extract and the CC fractions were analyzed by GC and gas chromatography/mass spectrometry (GC/MS). The GC retention index (Kovats, 1965) and the MS fragmentation pattern of each component were compared to those of the authentic compounds.

A HP model 5890 GC, equipped with a DB-WAX (30 m x 0.25 mm i.d.) bonded-phase fused-silica capillary column (J&W Scientific) and a FID, was used for routine analysis of the samples. The GC peak areas were integrated with a SP4290 integrator (Spectra-Physics, San Jose, CA). The injector temperature was 200 °C and the detector temperature was 250 °C. The oven temperature was held at 40 °C for 10 min and then programmed to 180 °C at 3 °C/min. Another HP model 5890 GC, interfaced to a VG Trio II mass spectrometer with a VG 11-250 computer data system, was used for MS identification of the GC components at MS ionization voltage of 70 eV. The column and oven conditions for GC/MS were as described for the GC/FID.

A HP model 5880 GC, equipped with a DB-1701 (30 m x 0.25 mm i.d.) bonded phase fused-silica capillary column (J&W Scientific) and a FID, was used for quantitative analysis of 1-methylpyrrole (a coffee volatile). The GC peak areas were integrated with a HP 5880A series GC terminal. The injector temperature was 200 °C and the detector temperature was 250 °C. The oven temperature was held at 30 °C for 5 min and then programmed to 220 °C at 3 °C/min.

RESULTS AND DISCUSSION

A typical DB-WAX gas chromatogram (FID) of the headspace volatiles obtained from freshly brewed coffee is shown in Figure 1. Thirty-three compounds were positively identified out of approximately 80 peaks. Seven other compounds were tentatively identified. Table I shows a list of the coffee volatiles identified in the present work, along with their Kovats values on DB-WAX column, their percentage peak areas, and the eluting fraction number during CC. These compounds and their relative amounts are comparable with the results of Shimoda and Shibamoto (1990), who analyzed coffee headspace volatiles by on-column injection technique.

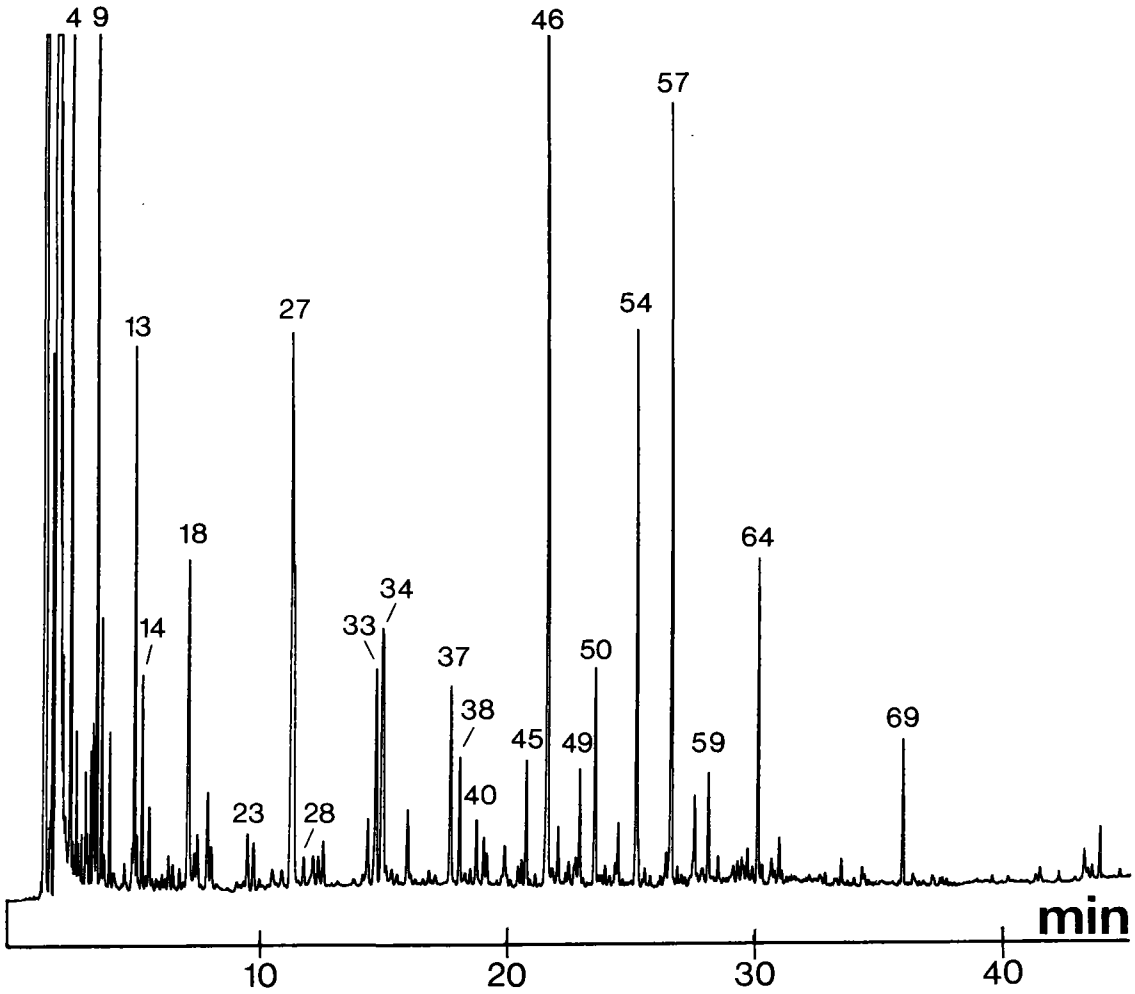

Figure 1. Typical gas chromatogram of the headspace coffee volatiles. Chromatogram was performed on a DB-WAX capillary column. Temperature program: 3 °C/min, starting at 40 °C (hold-up time = 10 min) up to 180 °C.

The aldehyde/carboxylic acid test is a fast method to assess the antioxidative properties of coffee volatiles. Simple and affordable materials such as straight chain aldehydes (pentyl aldehyde and hexyl aldehyde), and a hydrocarbon (2,5-dimethylhexane, an inert compound to monitor variations in GC injections, solvent volatilization, and day-to-day GC response) are mixed with possible antioxidants. If the coffee volatiles do not have any antioxidative property, the aldehydes readily oxidize to their corresponding carboxylic acids in the oxygen-rich dichloromethane solution through a radical type of reaction (Nonhebel et al., 1979).

Table I. Headspace Coffee Volatiles.

Peak # [a]	KI [b]	Fraction [c]	% Peak Area	Identity [d]
4	<1000	3	1.42	2,3-butanedione
5	1014	3	0.12	thiophene
8	1061	-	0.12	dimethyl disulfide
9	1070	3	3.03	2,3-pentanedione
10	1080	3	0.21	hexanal
11*	1095	3	0.14	a furan
13	1133	3	0.61	1-methylpyrrole
16	1175	3	0.03	2-heptanone
17*	1181	4	0.03	a furan
18	1186	-	0.59	pyridine
20	1195	-	0.08	isoamyl alcohol
23	1234	3	0.11	2-(methoxymethyl)furan
27	1264	4 & 5	1.08	2-methylpyrazine
28	1271	4	0.04	4-methylthiazole
29*	1276	3	0.07	a cyclic ketone
30*	1279	-	0.05	an alcohol
33	1312	-	0.38	2-ethylpyrazine
34	1318	5	0.66	2,6-dimethylpyrazine
35	1327	-	0.02	dimethyl trisulfide
36	1339	5	0.13	2,3-dimethylpyrazine
37	1371	5	0.29	2-ethyl-6-methylpyrazine
38	1377	5	0.18	2-ethyl-5-methylpyrazine
39*	1384	-	<0.01	a cyclic alcohol
40	1388	5	0.09	2-ethyl-3-methylpyrazine
41	1393	5	0.07	2,3,5-trimethylpyrazine
42*	1395	-	0.05	a furan
43*	1423	4	0.03	a furan
44	1426	-	0.03	acetic acid
46	1451	4	2.06	furfural
48	1469	5	0.03	2-methyl-6-vinylpyrazine
50	1491	4	0.32	2-furyl methyl ketone
54	1532	3	0.69	furfuryl acetate
56	1562	-	0.03	2,2'-bifuran
57	1566	4	1.02	5-methyl-2-furfural
58	1588	-	0.19	2,2'-methylenebis-furan
59	1600	4	0.14	1-methyl-2-formylpyrrole
62	1639	-	0.06	isopentanoic acid
63	1646	4	0.14	1-methyl-2-acetylpyrrole
64	1657	5	0.44	2-furanmethanol
69	1822	3	0.18	1-furfurylpyrrole

a Peak numbers in chromatogram Figure 1.
b Kovats index values on DB-WAX capillary column.
c Column chromatography fraction number: Fraction 3 (95% pentane, 5% ethyl acetate); fraction 4 (90% pentane, 10% ethyl acetate); fraction 5 (75% pentane, 25% ethyl acetate).
d Identification based on Kovats index values and mass spectral data.
* Tentative identification.

Figure 2 shows a typical DB-5 chromatogram of the aldehydes present in the control vial (C_5 and C_6 aldehydes) and their corresponding carboxylic acids after a storage period of 2 days. Figure 3 shows the RPA of pentanal for all CC fractions and for the control throughout a storage period of 10 days. Fraction 3 (95% pentane and 5% ethyl acetate) was found to inhibit the aldehyde/carboxylic acid turnover for more than 10 days, while most of the other fractions showed poor inhibitory effects. Figure 4 shows a typical DB-WAX gas chromatogram of the volatiles found in fraction 3. It is interesting to notice that peak #13 was present in fraction 3 (Table I). 1-Methylpyrrole, along with other 1,2-dialkylpyrroles, have been reported to show strong antioxidative activities (Macku and Shibamoto, 1991a). 1-Methyl-2-alkylpyrroles interrupt peroxidative processes by scavenging radicals (Gritter and Chriss, 1964; Gordon, 1990).

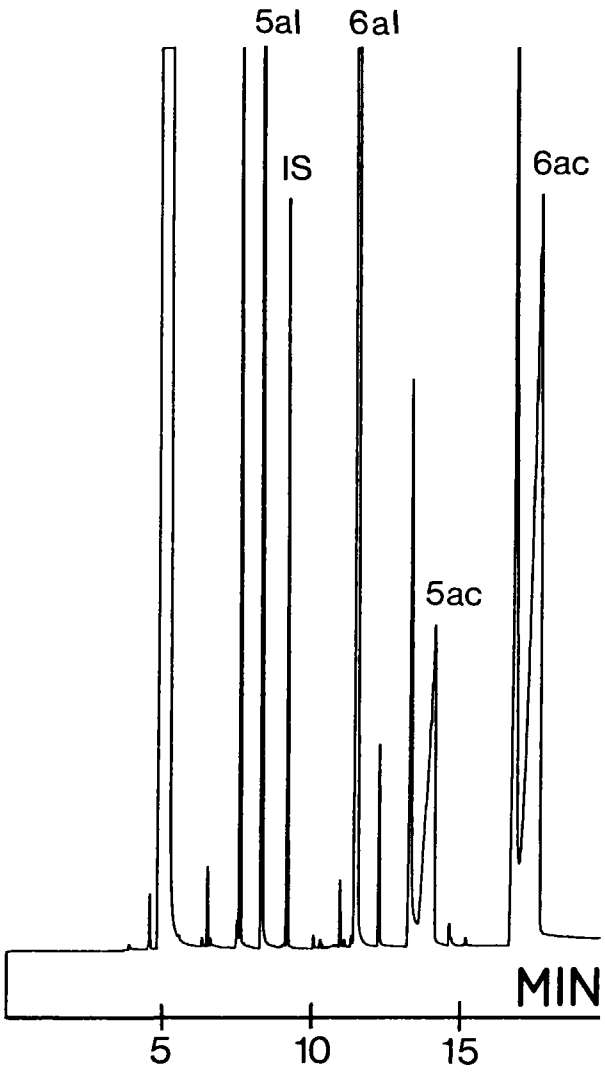

Figure 2. Typical gas chromatogram of pentyl and hexyl aldehydes (5al and 6 al, respectively), and corresponding carboxylic acids (5ac and 6ac, respectively) present in control vial after two days of storage. Internal standard (IS) is 2,5-dimethylhexane. Chromatogram was performed on a DB-5 capillary column. Temperature program: 50 to 200 °C at 6 °C/min.

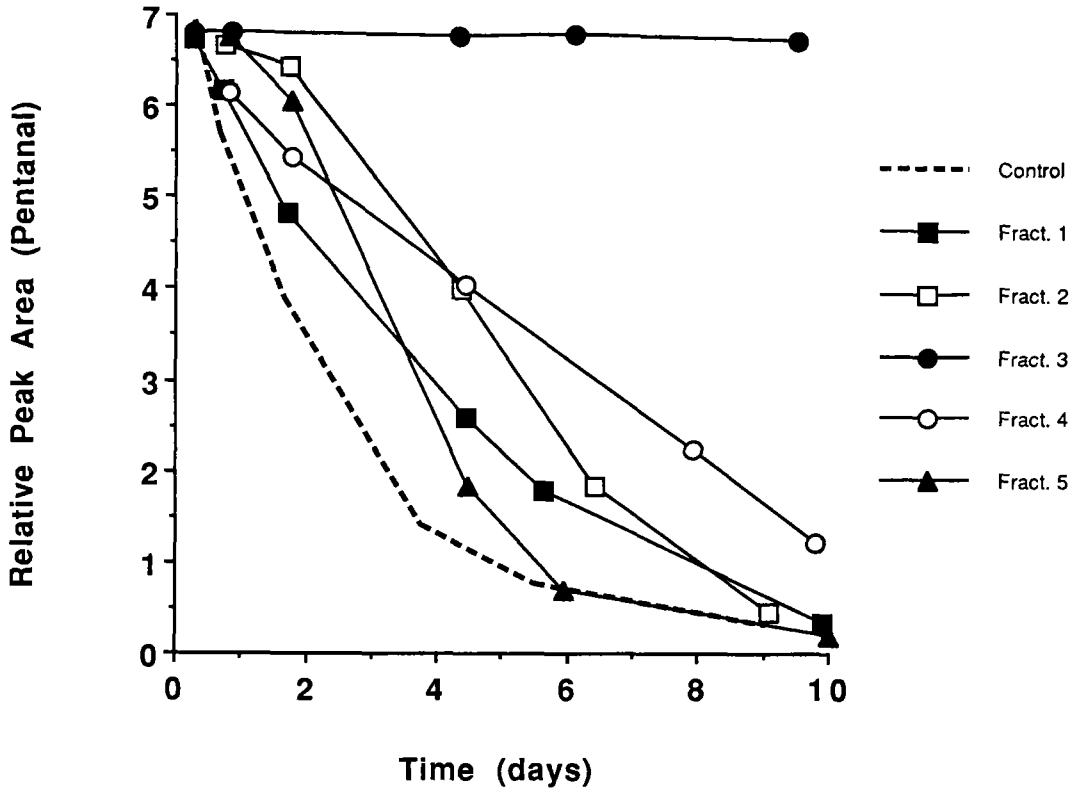

Figure 3. Remaining pentanal, expressed as relative peak area (RPA), present in vials containing column chromatography fractions throughout a storage period of 10 days.

1-Alkylpyrroles and their 2-alkyl homologs are thermally generated compounds. They have been reported in many foodstuffs such as in cooked meats (chicken, schrimp, and squid), roasted beans and nuts (coffee, cocoa, peanuts, and filberts), fermented products (beer, sherry, and tea) and in baked goods such as bread (van Straten and Maarse, 1983). These compounds are particularly important in roasted coffee (Flament and Chevalier, 1988), where fifteen different 1,2-dialkylatedpyrroles have been reported (Vitzthum and Werkhoff, 1976; Tressl et al., 1981a, 1981b). Figure 5 shows a typical DB-1701 gas chromatogram of the headspace coffee volatiles. Quantitation of 1-methylpyrrole was carried out with this intermediate polarity column due to the co-elution of this compound with another coffee volatile on DB-WAX column. In the present study, 1-methylpyrrole is found in ground-roasted coffee at a concentration of 200 ppb, a value 10 times smaller than the value reported by Tressl and co-workers (1981b). 1-Methylpyrrole has a DB-1701 Kovats index value of 833.

1,2-Dialkylpyrroles are also known to be formed from the thermal interaction between carbohydrates, such as sucrose, and amino acids, such as serine and threonine (Baltes and Bochmann, 1987). These nitrogen-containing compounds are found in headspace extracts of heated corn oil/amino acid mixtures (Macku and Shibamoto, 1991b). Extracts made out of these precursors could be added to processed foods in order to increase their shelf lifes. Extracts made out of glycine and coffee oil (similar fatty acid composition as corn oil) could be added as natural additives to coffee products such as coffee flavored chocolate, frozen coffee deserts, and ready-to-drink coffee products.

Kroyer et al. (1990) attributed the antioxidative activity of coffee extracts to the presence of hydroxylated cinnamic acid derivatives, such as chlorogenic and caffeic acids. However, these authors found that the extracts from roasted coffee beans showed a stronger antioxidative activity than that from the extracts of green coffee beans. During coffee roasting, up to 90% of the above mentioned acids are destroyed (Feldman et al., 1969; Clifford, 1979). The antioxidative activity of the coffee extracts is caused by other compounds which are produced during roasting. This activity is probably caused by the remaining hydroxylated cinnamic acid derivatives, thermally generated volatile compounds, and polymeric Maillard reaction products (MRP) (Lingnert and Eriksson, 1981; Yamaguchi, 1986; Bailey, 1988). Future studies are necessary to understand the formation pathways of these natural compounds, maximize their production, and discern their function within the food matrix.

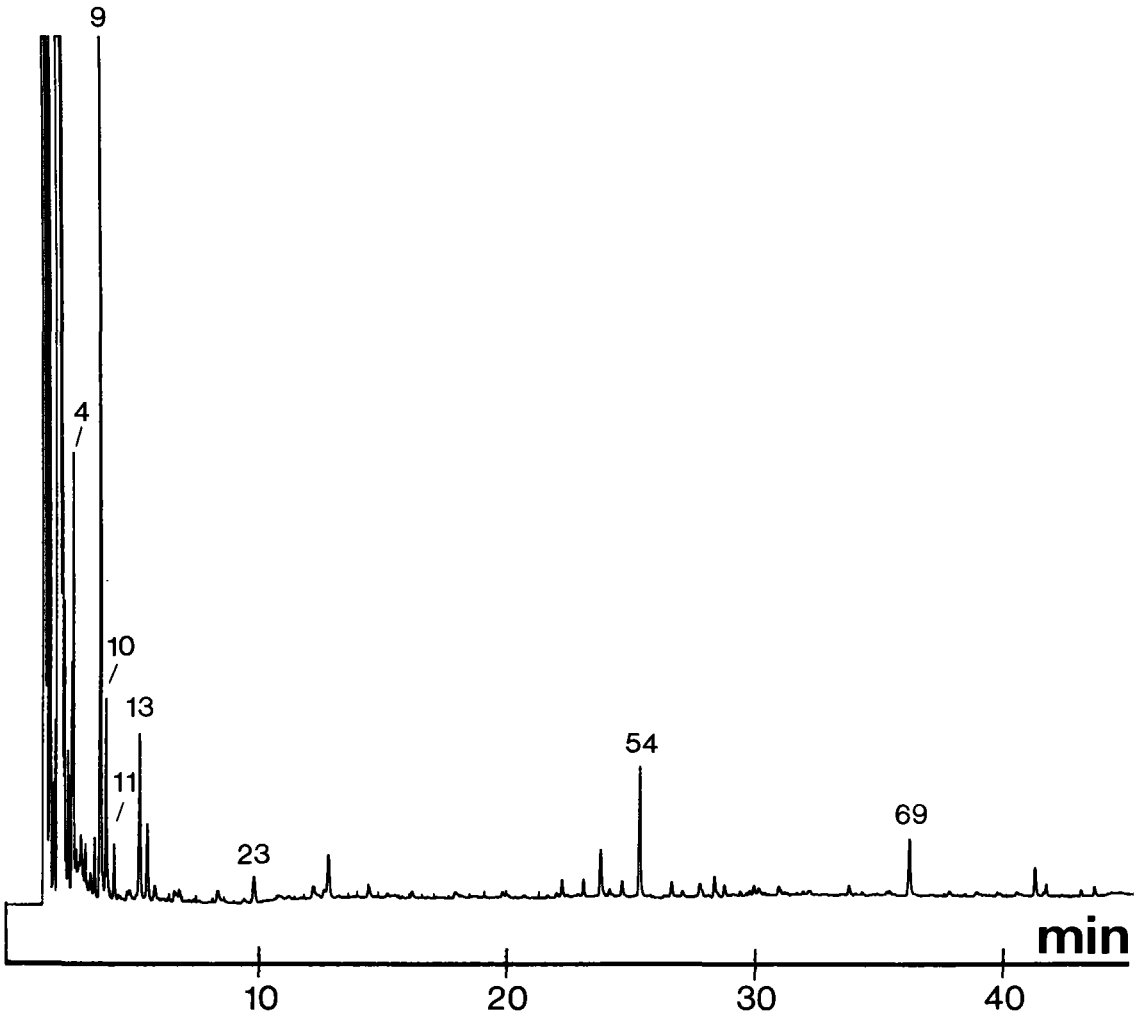

Figure 4. Typical gas chromatogram of the headspace coffee volatiles found in CC fraction 3. Same column and conditions as in Figure 1.

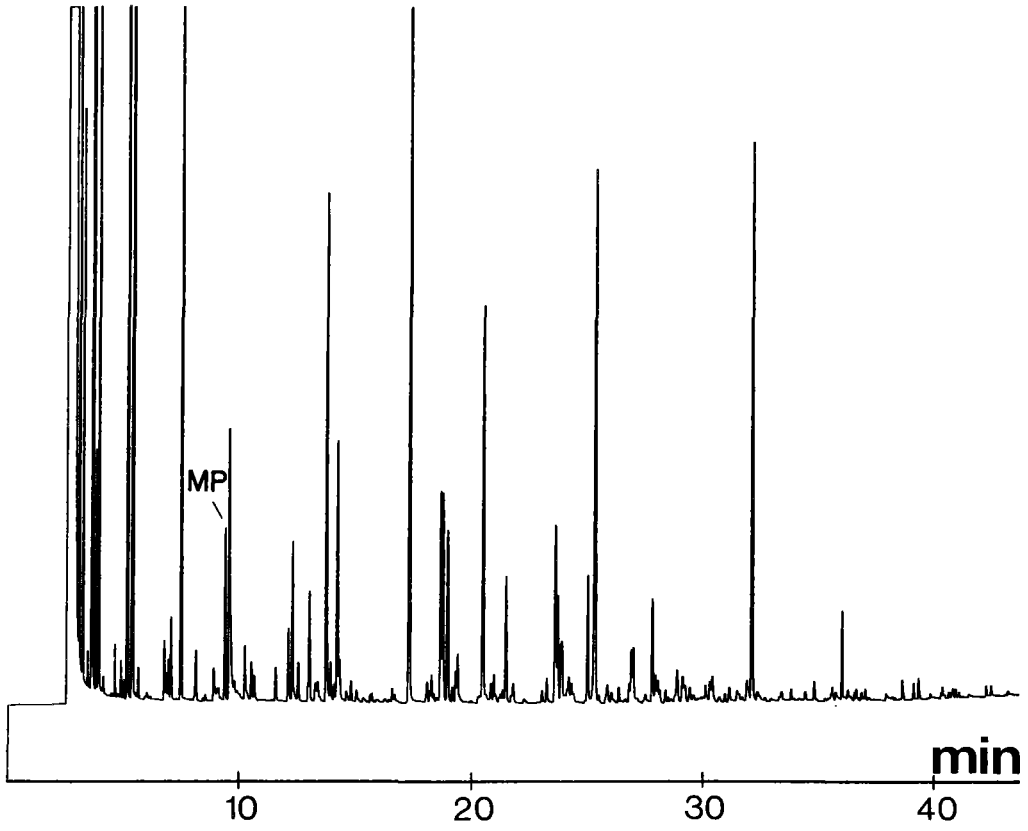

Figure 5. Typical gas chromatogram of the headspace coffee volatiles. Chromatogram was performed on a DB-1701 capillary column. Temperature program: 3 °C/min, starting at 30 °C (hold-up time = 5 min) up to 220 °C. 1-Methylpyrrole (MP) with a Kovats value of 833.

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SUMMARY

The headspace volatiles collected from freshly brewed coffee were analyzed by gas chromatography and gas chromatography/mass spectrometry. Column chromatographic fractions of the volatiles were tested for antioxidative activity. The fraction containing 1-methylpyrrole (a common roasted coffee volatile) was found to inhibit the oxidation of pentyl and hexyl aldehydes to the corresponding acids. Volatiles, such as 1,2-dialkylated pyrroles, along with other constituents are responsible for the antioxidative effect of roasted coffee extracts. This effect can be used to increase the shelf life of foods.

COMPUESTOS VOLATILES CON PROPIEDADES ANTIOXIDATIVAS AISLADOS A PARTIR DEL CAFE

RESUMEN

Compuestos aromáticos del café, aislados a partir de extracciones "headspace", se analizaron por cromatografía de gases y por espectrometría de masas. La técnica de cromatografía de columna se implementó para fraccionar los volátiles del café tostado. La fracción que contenía 1-metilpirrol (compuesto aromático, típico del café tostado) inhibió la oxidación de pentanal y hexanal a los correspondientes ácidos carboxílicos. Compuestos volátiles como los 1,2-dialquilpirroles, en conjunción con otros constituyentes, son los responsables del efecto antioxidativo del café. Este resultado podría ser usado para incrementar el tiempo de almacenaje de productos alimentícios.

STORAGE-RELATED CHANGES OF LOW-BOILING VOLATILES IN WHOLE COFFEE BEANS

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1. INTRODUCTION

Besides origin, one of the most important quality issues of roasted coffee is its aroma freshness. In this context aroma freshness means the fine and pleasant smell arising from freshly roasted coffee beans. In German-speaking countries the market share of whole beans sold in non-air-tight packs is substantial. This kind of packaging is, however, only suited for short distribution periods. Storage related changes of the coffee aroma, generally described as staling, as well as the preservation of aroma freshness have been a wide field of investigation in the past (Shuman and Elder, 1943; Feldman et al., 1971; Borchers and Maier, 1988; Spadone and Liardon, 1990). According to Arackal and Lehmann (1979), three stages of staling of whole coffee beans can be characterized:

- **After 10 days : certain loss of freshness**
- **After 6 to 8 weeks : significantly perceptible staleness**
- **After 4 to 5 months : rancidity**

The knowledge on the chemical causes of staling is incomplete. We suggest two main reasons for staling. Short time after roasting loss of low-boiling components is predominant, a mainly physical process, which is then more and more overlayed by oxidative events. A field of practical interest is the question whether it is possible to characterize the term "aroma freshness" by means of objective analytical methods. Many attempts had been done in the past which have resulted in some indicator systems (Vitzthum and Werkhoff, 1978 and 1979; Noomen, 1979; Radtke-Granzer and Piringer, 1981; Kallio et al., 1990). However, the applicability of these methods to unspecified coffee samples without knowledge of origin, degree of roast or age is limited. Therefore one explanation may be that too little is known about the individual contribution of single volatiles to the typical aroma of freshly roasted coffee. The major objective of this work was to investigate the loss of aroma freshness several days to weeks after roasting during storage of whole roasted beans in air. For this purpose, three main features have been looked at:

- **Improvement of an analytical method for the objective evaluation of roasted coffee freshness**
- **Quantitation of storage-related changes of low-boiling volatiles in whole beans**
- **Application to practical samples purchased from trade**

2. EXPERIMENTAL

A scheme of the experimental procedure is given in fig. 1.

2.1 Sample material and storage

All green coffee samples were roasted to a medium degree with a Probat laboratory roaster. Commercially available whole coffee beans, sold in non-air-tight packs with special claims of aroma freshness, were purchased at different times during October 1989 to May 1990 mainly in the northern part of Germany. Those samples which could not be analyzed immediately were stored at -40°C. Controlled storage tests were carried out mainly with Colombian coffee. Each 500 g were stored in glass vials at 20°C under light protection, but without any oxygen protection. Reference samples were stored under nitrogen at -40°C. For comparison with respect to head-space-profiles coffee samples from Colombia, Burundi, Mexico, Ethiopia, Kenya, Costa Rica, Tansania, El Salvador, Brazil, Guatemala and New Guinea represented the Arabicas whereas the Robusta-type was represented by coffees from Ivory coast, Uganda, Cental Africa, Zaire, Vietnam, Indonesia and Madagascar.

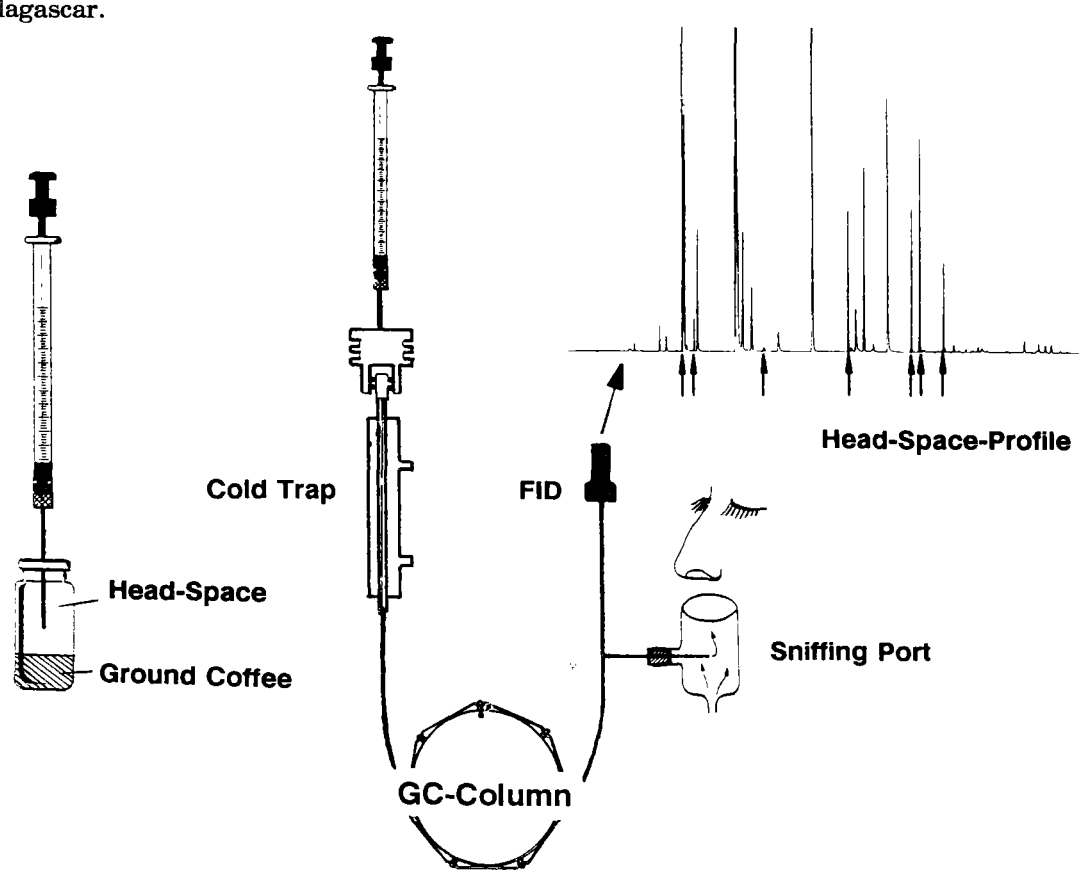

Fig. 1: *Sensory and analytical evaluation of low-boiling volatiles in whole coffee beans: Scheme of the experimental approach.*

2.2 Sample preparation

Whole coffee beans were frozen to -20°C (trade samples were mixed previously) and ground to standardized particle size (average 300-500 μm). 15 g of ground coffee were transferred into a 100 ml sample vial together with 10 small glass balls. The vial was tightly closed and lightly shaken for 2 h at room temperature (20°C). Thereafter 500 μl from the head-space were taken from the vial by means of a gas-tight syringe and injected for gas chromatographic separation.

2.3 Gas chromatography (HRGC)

The gas samples were injected by means of a Chrompack purge and trap-system, modified to a static head-space-injector, in connection with a Carlo Erba 4100 gas chromatograph. The volatiles were cryo-focused by cooling the cold trap to -120°C with liquid nitrogen. Injection of the trapped volatiles was achieved by resistance-heating of the trap to 180°C within a few seconds. Gas chromatographic separations were performed on a DB-5 capillary column (60 m x 0.32 mm; 1 μm film thickness; temperature program: 20°C for 10 min, then 10°C/ min to 160°C, hold for 2 min) using helium as carrier gas at a flow rate of 2-3 ml/min.

2.4 Gas chromatography/mass spectrometry

GC-equipment was connected to an ion trap detector (ITD 800 - Finnigan MAT). Mass spectra were generated at 70 eV in the electron impact modus.

2.5 Data evaluation

The peak areas of 13 selected peaks of the head-space-profile obtained by a flame ionization detector were calculated as percentages relative to the total peak area (tab. 1). The data of each sample were submitted to a computer-aided discriminant analysis (SAS/STAF, 1988; Backhaus et al., 1989) in order to maximize the differentiations between certain classes. The results were plotted in a coordinate system (centre set to zero by definition) where each sample was localized by their canonical variables.

2.6 Sensory testing

HRGC/sniffing. GC-effluent sniffing was carried out by splitting the capillary column for flame ionization detection as well as for sensory evaluation by means of a sniffing port.
Olfactory evaluation of odor intensity. 15 g of ground coffee were loaded into a 100 ml glass vial, covered and allowed to equilibrate for 10 minutes at room temperature. Evaluation of odor intensity was carried out by 13 well trained testers by sniffing the glass vials. The samples were presented as triangular-testing according to DIN 10951.

3. RESULTS AND DISCUSSION

For a long time, coffee chemists have been looking for an objective analytical approach to determine the freshness of a roasted coffee in order to replace or facilitate sensory testings which are subjective. For this purpose head-space techniques have been widely applied because of their simple handling and good reproducibilty and were chosen for these investigations. In order to analyze the aroma arising from a ground coffee, as it can be smelled by the human nose, the coffee samples were

prepared at room temperature. As shown in fig. 1, the head-space samples were introduced into the GC-system by means of automatic cryo-focusing. In this way, considerably larger gas volumes can be injected even on a medium-bore capillary column without band broadening. Connection of the capillary column to a sniffing port made possible simultaneous flame ionization detection as well as sensory evaluation by the human nose thus helping to optimize the analytical procedure. The use of a non-polar thickfilm capillary column allowed the separation of nearly all components of sensory interest and was advantageous with respect to column longevity.

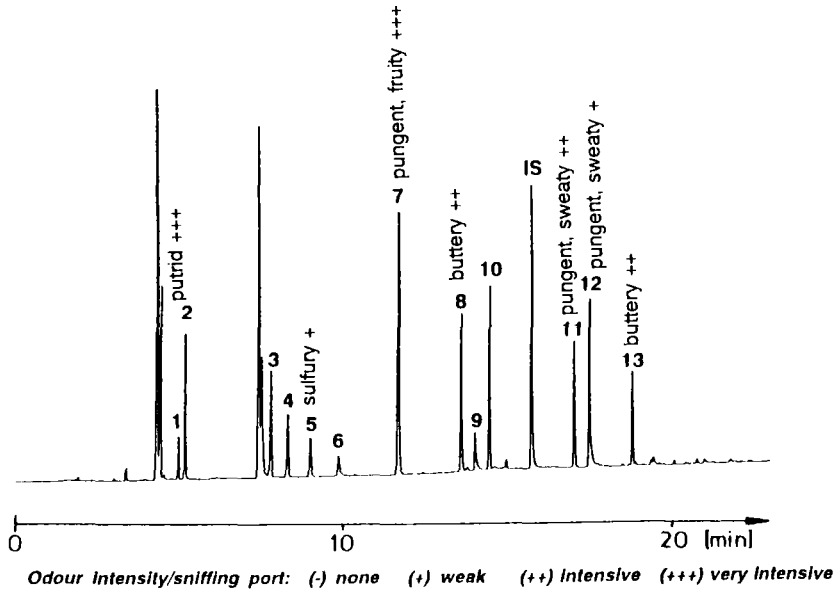

Fig. 2: *Typical head-space-profile of a freshly roasted coffee with sniffing evaluation (numbers refer to tab. 1, IS = int. stand. tetrahydrofuran)*

Fig. 2 shows a typical head-space profile obtained under these conditions. Identification of 13 selected peaks was achieved by comparison of retention- and mass spectral data as well as sensory properties with those of authentic reference substances. During effluent sniffing low-molecular sulfur compounds (1,5), Strecker-aldehydes (7,11,12) and α-dicarbonyls (8,13) gave aroma notes. Results indicated that the "putrid, sulfury" note of methanethiol as well as the "pungent, fruity" aroma note of 2-methylpropanal were the most aroma-intensive ones in addition to the "buttery" notes of 2,3-butanedione and 2,3-pentanedione and the "pungent, sweaty" notes of the methylbutanal-isomers possessing lower intensity. Several further weaker aroma notes were perceivable at the sniffing port but gave no detector response and seem to be of lower sensory importance.

Shelf-life studies were carried out storing roasted whole beans in non-air-tight packs at room temperature and applying the optimized head-space technique for kinetic studies of single volatiles. The major results are visualized in fig. 3. For each component, residual percentages relative to the start were calculated. It is obvious that the biggest changes of light volatiles occur within 3 weeks. The gas chromatographically obtained total peak area decreased to less than half of the starting value. Nearly the same change was obtained for the aroma-intensive 2-methylpropanal, whereas, 3-methylbutanal and 2,3-pentanedione apparently show a smaller decrease than the average. On the other hand, a larger decrease was measured in case of dimethylsulfide. This may be due to the fact that sulfur-containing compounds are known to be much more oxygen-sensitive. One of the most important findings was the kinetics of methanethiol. This compound having a strong impact on the aroma freshness showed the significantly largest decrease which was already recognizable one day after roasting. After 8-days storage, the peak area of methanethiol went to a level of about 30 % and

decreased further to 10-20% relative to the starting value after 3-weeks storage. The dotted lines show the values for the peak area of methanethiol and the total peak area of the preserved reference samples (Ref. in fig. 3) which nearly remained unchanged during the time period of hundred days.

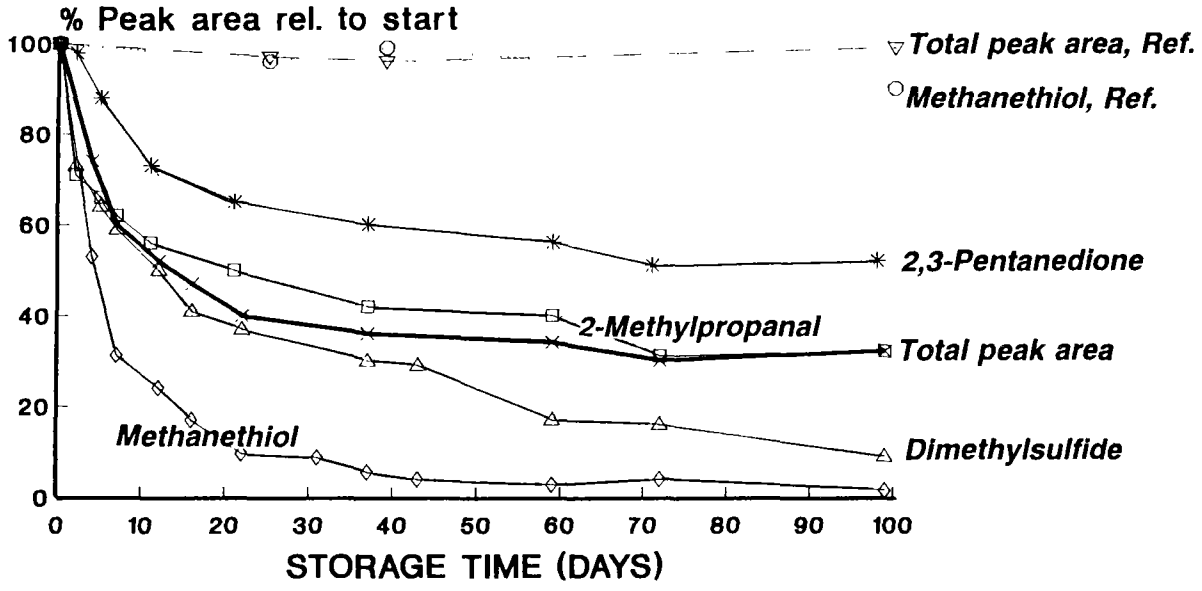

Fig. 3: *Kinetics of selected low-boiling odorants during storage in non-air-tight packs.*

Results of olfactory evaluation indicated an excellent correlation with those obtained by objective head-space analysis. The coffee samples were evaluated by sniffing 8, 12 and 22 days after roasting and storage in air. A sensory deviation compared to a freeze-stored reference sample was significant at least after 8-days storage (fig. 4). The one-week-old samples were described as "distinctly less odor-intensive" and showed "less aroma freshness". To our knowledge the important role of methanethiol with respect to the loss of aroma freshness was not yet reported.

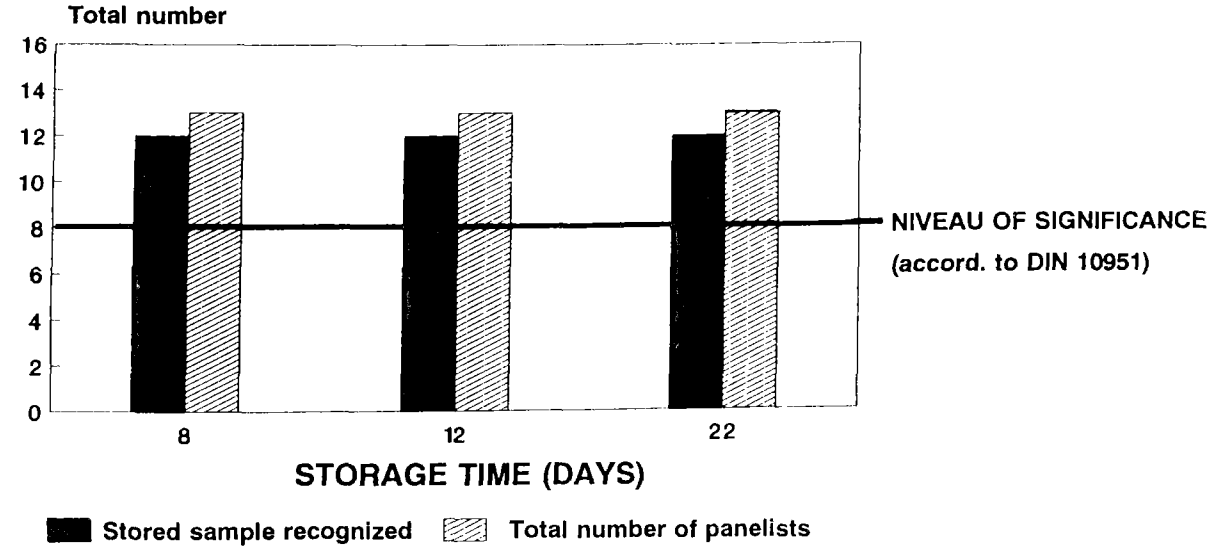

Fig. 4: *Results of olfactory sensory testing: stored whole beans versus reference samples.*

As the head-space-profile analysis delivered good results during kinetic studies, the question was posed whether this technique could be applied in combination with a computer-aided discriminant analysis to get tools for an objective analytical characterization of whole bean freshness. For this purpose 13 peaks from the head-space-profile were chosen (fig. 2 and tab. 1). For canonical discriminant analysis the values "% COMPONENT" were calculated for each component according to tab.1 thus obtaining more reliable relative results by minimizing the influence of different sample amounts or oscillating detector response.

1	Methanethiol	
2	Methylformate	
3	Furan	
4	Isoprene	
5	Dimethylsulfide	
6	Methylacetate	$\%\ \text{COMPONENT} = \frac{\text{Peak area component} \times 100}{\text{Total peak area}}$
7	2-Methylpropanal	
8	2,3-Butanedione	
9	2-Butanone	
10	2-Methylfuran	
11	3-Methylbutanal	
12	2-Methylbutanal	
13	2,3-Pentanedione	

Tab. 1: *Head-space components chosen for canonical discriminant analysis*

In order to check the influence of coffee origin to the results of head-space analysis a wide range of single strains of the variety Arabica as well as Robusta were investigated. The head-space-profile of each sample was analyzed immediately after roasting and after 10 days storage under non-air-tight conditions as whole beans. In fig. 5 the results of the computer-aided data evaluation are plotted.

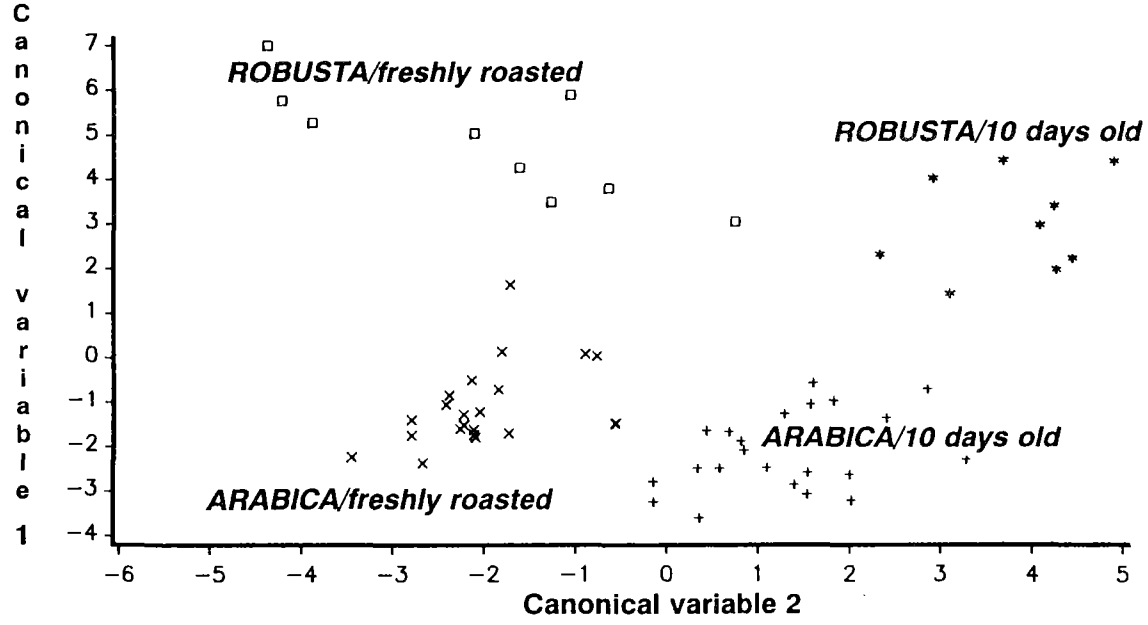

Fig. 5: *Canonical discriminant analysis: discrimination of roast coffee beans with respect to botanical variety and freshness based on head-space-profile analysis.*

Approximately the canonical variable 1 (Y-axis) corresponds to the botanical variety whereas the canonical variable 2 (X-axis) corresponds to the freshness. Despite of the fact that only 13 peaks were taken into account, canonical discriminant analysis allowed an exact classification of all samples into four distinguished classes: *ARABICA/freshly roasted* and *ARABICA/10 days old*, as well as *ROBUSTA/freshly roasted* and *ROBUSTA/10 days old*.

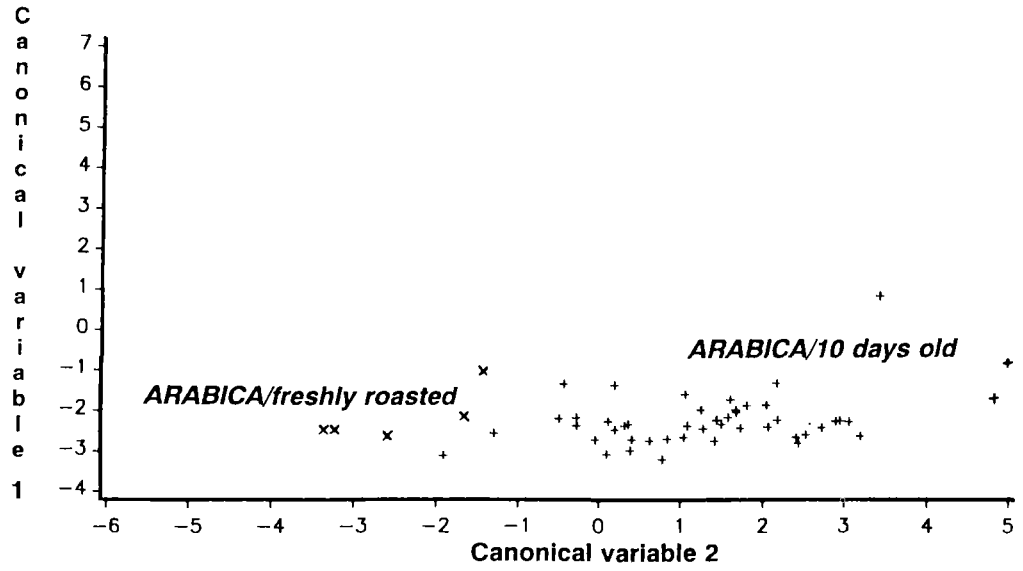

Fig. 6: *Canonical discriminant analysis: discrimination of roast coffee beans purchased from trade with respect to botanical variety and freshness.*

These data, obtained under standardized conditions, were taken for calibration with respect to the head-space-profile analysis of over 60 coffee samples purchased from trade as whole beans in non-air-tight packs. Data handling was carried out in the same manner. The results are plotted in fig. 6 and 7.

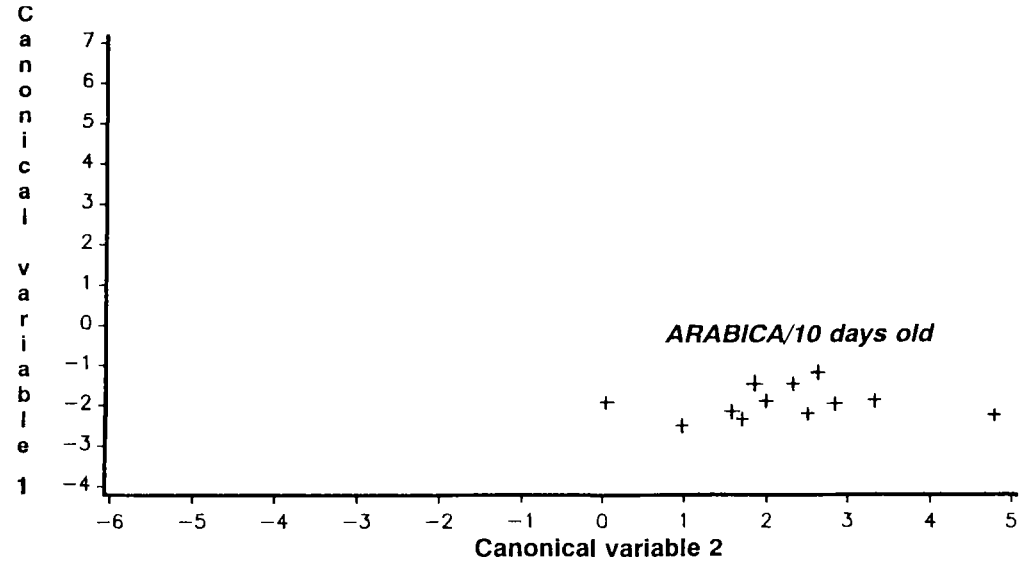

Fig. 7: *Canonical discriminant analysis: Discrimination of roast coffee beans purchased from trade with respect to botanical variety and freshness (steam-treated and decaffeinated coffees).*

Under these conditions the majority of the samples was classified as *ARABICA / 10 days old*, which seems to be true according to experience. Nevertheless, among those were some samples discriminant analysis indicated a considerable staleness. These objective analytical results could be verified by sensory testing. Five samples (marked with x) out of a total of 7, claimed as being roasted the same day when they were bought, were correctly classified as *ARABICA / freshly roasted* but on the other hand this declaration could not be verified for the two residual samples, because head-space data in combination with discriminant analysis indicated several days storage in air.

The samples purchased from trade included several decaffeinated and steam-treated coffees as whole beans. As fig. 7 shows, these coffees were classified alike the untreated coffees in fig. 6 as *ARABICA / 10 days old.*

4. CONCLUSIONS

- Overall results indicated that the aroma freshness of whole roasted coffee beans is mainly formed by certain low-boiling components, namely low-molecular sulfur compounds, Strecker-aldehydes and α-dicarbonyls.

- Loss of aroma freshness during several weeks storage of whole beans in non-air-tight packs mainly is due to the loss of certain aroma-potent volatiles. Therefore, the decrease of methanethiol is the most important indicator.

- Head-space-profile analysis, optimized on the basis of GC-effluent sniffing, in combination with a computer-aided discriminant analysis allowed an objective evaluation of the aroma freshness of whole coffee beans already after a few days storage and showed a wide applicability even without exact knowledge of coffee origin, degree of roast or age.

Acknowledgement

We have to thank Dr. W. Wosniok, University of Bremen, for computer-aided discriminant analysis.

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SUMMARY. *The volatiles forming the pleasant odour perception arising from freshly roasted coffee beans were investigated by means of head-space gas chromatography with intermediate cryo-focusing and simultaneous sniffing analysis. Shelf life tests in non-air-tight packs indicate, that the loss of aroma freshness several days to weeks after roasting corresponds to the diminution of certain low boiling components, mainly methanethiol. Head-space-profile analysis in combination with computer-aided discriminant analysis allowed an objective evaluation of the aroma freshness of whole coffee beans after only a few days storage and showed a wide applicability even without exact knowledge of coffee origin, degree of roast or age.*

RÉSUMÉ. *La fraction volatile formant l'odeur agréable libéré du café en grains torréfiés récessement a été investigé par la chromatographie en phase gazeuse et simultanément étudié au moyen de l'olfactométrie. Les tests après entreposage dans des sachets non hermétiques ont prouvé que la perte d'arôma en odeur fraîche du café torréfié, diminue avec la disparition d'éléments arômatiques très volatiles, surtout en particuliers le Méthyle-mercaptan. La chromatographie en phase gazeuse combiné avec l'analyse sur ordinateur permettent l'analyse discriminante et par là l'évaluation objective de la perte en arômas fraîs déjà après quelques jours d'entreposage. Ces valeurs trouvées permettent sans connaître l'origine du café, le taux de torréfaction ou son âge, de déterminer le taux de fraîcheur du café en échantillon.*

RATES OF OXIDATION OF ROAST AND GROUND COFFEE AND THE EFFECT ON SHELF-LIFE

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INTRODUCTION

The staling of roast and ground coffee is related to the consumption of oxygen. Radtke (1) showed that the effect of the reaction with oxygen was perceived only after several months. Clinton's study (2) gave essentially the same results. Much of the work done to date on the effect of oxygen on shelf-life has focused on the exposure of the packaged coffee to limited amounts of oxygen sealed with it in the package. Relatively little data exists on the actual rates of reaction of roast and ground coffee with oxygen. The longest-term such study was that of Punnett (3), who conducted a steady-state determination of the uptake of oxygen in coffee over a period of six years. This study defined several regimes of reaction. The earliest period was characterized by rapid reaction over a period of 20 days, followed by a slow reaction for a period of six months, then a rapid reaction over another 8 - 10 months, and at last a gradually slackening rate persisting for years. The initial reaction was accompanied by a period of about 2-4 days in which there was a significant exchange of gas from the outside of the ground coffee particles to the inside. Most other studies of the reaction rate, including Radtke's and this work, are non-steady state, and are thus limited to the initial stages of reaction. The importance of this reaction rate lies in understanding the degree to which the staling or quality degradation of the coffee is potentiated by reactions taking place before the coffee is sealed into the package. In the two studies cited above (1,2), most of the reaction with oxygen took place after the roasted and ground coffee was sealed in the can. Even so, the reaction was substantially complete long before an effect on quality was perceived. Although the consumption of oxygen by coffee has been described as slow (4), we will show that there can be in some circumstances an appreciable reaction with oxygen under ordinary in-process storage conditions, and that this reaction might affect the ensuing degradation of quality attributes. Further, we will show that the change in roasting conditions which has been part of the change in the standard pack size in coffee for the domestic US market from the 16-oz size prevalent up to the early 1980's to the current

13-, 12-, and 11.5-oz sizes can result in more rapid oxygen consumption.

METHODS

Reaction rates were measured by monitoring the partial pressure of oxygen in the headspace of flasks. The headspace volume was calculated from the measured flask volume and the absolute density of the coffee as determined by gas pycnometry. First-order rate constants were calculated from the best-fit slopes, K, of plots of $\ln P(O_2)$ against time. The values of $P(O_2)$ used were those due to reaction only, with physical changes in partial pressure subtracted. Thus if

1. $$K = \Delta \ln P(O_2)(rxn)/\Delta t$$

then

2. $$k' = K(1/m)[V - (m/\rho)]$$

where k' is the first-order rate constant in cc(STP)/g.hr, m is the mass of coffee, V is the empty volume of the flask, and ρ is the absolute density of the coffee.

The Colombian arabica coffee used in these experiments was roasted using two small experimental roasters capable of variations in heat and airflow. These conditions were varied to produce both a low- and a high-density whole bean at the same medium degree of roast. Most of the experiments were conducted using coffee which had been roasted in Roaster B, which produced sufficient sample for these studies. The roasting conditions and densities are shown in Table 1.

Table 1

Roaster	Temp °C	ρ(whole bean) g/cc	$\%H_2O$
Roaster A	246	0.385	2.5
Roaster A	213	0.412	2.2
Roaster B	240	0.367	1.7
Roaster B	210	0.444	1.8

To begin each rate measurement, a 50.0g sample of freshly-ground coffee was put into a 125mL Erlenmeyer flask with a 24/40 ground joint. A mating septum adapter with stopcock was used to seal the flask. The flask was then pumped for one minute without the septum in place. A mixture of oxygen and nitrogen was then delivered to the flask to a total pressure of about 0.80atm to allow for degassing of the coffee. The stopcock was then closed and the septum affixed. The small volume between the septum and the stopcock was then evacuated through a needle, the needle withdrawn, and the stopcock opened, leaving the flask sealed by the septum. Sampling started one day later to avoid gross error in the early stages of gas mixing.

RESULTS

Effect of Pressure

The reactions were judged to be overall first-order on the basis of the regular pattern of linearity in the plot of the logarithm of $P(O_2)$ against time. It would be expected that the reaction rate would be directly proportional to the partial pressure of oxygen in the samples. This was not found to be the case, as seen in Figure 1. The persistent pattern of these

measurements was that the slope of the plot of $\ln P(O_2)$ against time would increase somewhat as the pressure decreased. The effect persisted despite changes in the oxygen/nitrogen ratio, total pressure and weight to volume ratio in the flask. This behavior might be an artifact due to inadequate compensation for the physical behavior of the oxygen in the system, or it might be mechanistically based. In what follows, all of the determinations used for the calculations were at matched oxygen concentrations, since this effect was observed equally for all roasting types.

Effect of Density The values of the first-order rate constants measured at $P(O_2)$ = 0.20atm for the samples produced on both roasters at 30°C are shown in Table 2. Each of these production methods results in an increase in the rate of reaction as the density decreases. The densities used for comparison are the whole bean free-fall densities, which are the most reliable indicator of expansion in the bean during roasting. The increase is slightly greater than in direct proportion to the density, and the effect is somewhat greater in the samples produced in Roaster A than in Roaster B.

Table 2

Roaster	ρ(WB), g/cc	Ratio (ρ)	10^3k'(30°C), cc(STP)/g.hr	1/Ratio (k')
A	0.385	0.934	3.03	0.878
A	0.412		2.66	
B	0.367	0.827	2.98	0.802
B	0.444		2.39	

The Roaster B samples were used to measure the dependence of the reaction rate over a range of temperature, moisture, and particle size. As nearly as the samples could be brought to the same conditions, the results showed this density effect quite consistently.

Effect of Temperature and Moisture Figure 2 shows the values of the rate constants for the reaction of the two roast types plotted in an Arrhenius plot. The slopes of these plots are not significantly different. The values of the Arrhenius activation energies are in the range of 8000K, or about 16kcal/mole. Although the data are very sketchy, they seem to show that the activation energy is constant up to at least 4.0%H_2O. Figure 3 shows the effect of the moisture content on the reaction rate, measured at 46.5°C. The data show a larger effect of moisture on the lower-density product.

Effect of Particle Size Figure 4 shows the effect of particle size on the rate of reaction, at two different moisture levels over a range of temperatures. Although the reaction rate is more strongly affected by temperature, the particle size effect seems to be greater at lower than at higher temperature, with no apparent effect at the highest temperature, 46.5°C. Reaction rates were measured for whole-bean coffee at 37°C, with k' values in the range 1.6×10^{-3}.

DISCUSSION

Model of Staling The data presented by Clinton (2) can be seen as showing that the loss in organoleptic rating is related to the total oxygen consumption and to time. No simple function described these data adequately. The principal difficulty was that the degradation in organoleptic rating seemed to take place in two stages, a rapid initial

stage during the first 1 - 2 months, and a second stage during the remainder of the 2-year storage life studied for these products. In addition to this difficulty, the functions fit will be critically dependent on the rating scale. Though no mechanistic information can be inferred directly from the data, they do support the notion that the organoleptic rating is made up of disparate components. It must be emphasized that many functions will fit these data, and that data from other types and scales of evaluation will require different functions. We have fitted Clinton's data to a simple linear model in which the parameters are time and oxygen exposure, V', which is reckoned in cc(STP) of oxygen per gram of coffee. The vacuum levels are translated into oxygen exposure through the absolute density and the can volume, assuming the gas remaining at packing is air. Consideration of the details of the initial loss of freshness is avoided by including data only at and after one month's storage, and fitting the results to the function

3. $$F/S = A - B\sqrt{t}$$

where F/S is the organoleptic Freshness/Staleness used by Clinton. The A parameter of this function is the effective initial value of F/S after the initial decay, which is 6.82 for these data, and the B parameter is the rate of loss, which depends on the oxygen exposure, e.g.,

4. $$B = 0.172 + 8.07V'$$

in the units of cc(STP)/g and months. The data of Clinton are shown with the best-fit curves in Figure 5. Thirteen ounces of roast and ground coffee packed in a US standard 401 can at 27 inches.Hg vacuum would have an oxygen exposure of about 0.30 at a typical value of the absolute density.

These results indicate that the course of events is dictated by the amount of oxygen with which the coffee reacts early in its storage. A curve useful for scaling the degradation of quality with time as a function of product weight and can volume in the form of oxygen exposure can be derived from the functions above. If a criterion for the end of shelf-life is inserted, a calculation of shelf-life can be made. The function below represents the shelf-life in months for Clinton's criterion of F/S = 5 for the termination of product shelf-life.

5. $$t_{SL}(mo) = [1.82/(0.172 + 8.07V')]^2$$

This function is plotted in Figure 6. Using this function and the oxygen exposure, one can calculate the effect of different processing and packaging options on the expected shelf-life of the product. Whatever criterion for evaluating shelf-life might be used, it is to be expected that at very least an effective proportionality would obtain between the realized shelf-lives of products packed at different weight and volume.

Effect of In-Process Oxygen Exposure As noted above, the flavor degradation measured in the studies of Radtke and Clinton was due primarily to the oxygen remaining in the package after sealing. In these studies, the reaction with oxygen took place with a limited amount of reactant in a period of time more than sufficient for its practical completion. The rate of reaction is in this case not very important. Most coffee production is accompanied by holdup of the roasted whole bean and ground coffee for varying periods of time, as a production buffer, and in some cases for degassing. In this case, the amount of oxygen exposure is limited only by the design of the equipment, and takes place over a very limited period of time. The rate of reaction is then very important during in-process holdup. Using the measured values of the reaction rates, we can calculate the effect of this holdup time on the expected shelf-life of the coffee. If we have an

O_2 partial pressure $P(O_2)$, we can combine Equations 1 and 2, and get a rate of

6. $$\Delta \ln P/\Delta t = \Delta P/P\Delta t = mk'/[V - (m/\rho)] = mk'/V_{hs}$$

or

7. $$\Delta P/\Delta t = (m/V_{hs})k'P(O_2)$$

If the container is open, the oxygen partial pressure and thus the rate is constant, and the amount of oxygen taken up in an hour is given by

8. $$P\Delta V]_{open} = V\Delta P]_{closed} = V_{hs}(m/V_{hs})k'P(O_2)\Delta t$$
$$= mk'P(O_2)$$

To give the conventional units of cc(STP), this result must be multiplied by $(273/T_{reaction})$. This calculation is performed for coffee under various conditions using the rate data above. If we assume a 13-oz product packed in a 401 can at 27in.Hg vacuum, we can calculate the fractional reduction in shelf-life F(holdup) using the model described above. The reduction in shelf-life shown here would be at the extreme that the atmosphere in an unflushed bin were air. The results are shown in Table 3.

Table 3

Reaction of R&G Coffee in Storage
Air, 1 hr, 4.5% Moisture, MPS = 700μ
Packed at V' = 0.03cc/g

ρ(RWB), cc/g	Temperature, °F	10^3k', cc/g.hr	ΔV', cc/g.hr	F(holdup) 1 hr	F(holdup) 8 hr
0.444	116	8.5	0.0015	0.94	0.66
0.444	88	2.8	0.0005	0.98	0.86
0.367	116	11.5	0.0021	0.92	0.57
0.367	88	3.2	0.0006	0.98	0.84

We can see from this crude calculation that exposure to air in holding up roast and ground coffee for one hour can result in shelf-life reductions up to eight percent. Longer holdup times, which are sometimes used for degassing vacuum bag products, would result in correspondingly larger reductions in shelf-life, as shown in the last column. The effect of coffee density on the reduction in shelf-life is far greater at the higher temperatures.

Effect of Permeable Package

Modern coffee packaging is characterized by the use of many alternatives to the steel 401 can. Paper/polymer composites and mono- and multi-layer polymers are used in several forms. The measured rates of reaction of oxygen with roasted and ground coffee presented here can also be used to calculate the additional oxygen exposure resulting from permeation. As an example, we will use a coffee package made of PET of 20-mil thickness. If we base our package geometry on the 401 can, a PET package would have a permeability of about 0.07cc(STP)/pkg.day. Then for a permeability Π in units of cc(STP)/pkg.day, the rate of permeation in cc(STP)/hr is given by

9. $$dV/dt = (\Pi/24)(298/273)[(0.2098 - P(O_2))/0.2098]$$

and in units of atm,

10. $dP/dt = (1/V_{hs})(dV/dt)$

But for reaction,

11. $dP/dt = (m/V_{hs})k'P(O_2)$

The amount of reaction and permeation after packaging can be calculated numerically by using small time increments and calculating $P(O_2)$ at the end of each increment. This calculation is shown for both the model permeable container and an impermeable container in Figure 7. This figure shows that the amount of reaction six months after packaging is more than double in the permeable container. The oxygen exposure of this coffee increases over the entire storage period in the permeable container. Although this cannot be described by any simple model, further reductions in shelf-life will ensue.

CONCLUSIONS

Although the reaction of roasted and ground coffee with oxygen is fairly slow, there can be an appreciable effect of this reaction during processing. The empirical model presented here gives the means to relate the amount of reaction during processing to the shelf-life. The coffee density has an influence on the rate of reaction, but this effect is much smaller than that of temperature or moisture. The effect of lower density is more important in changing the ratio of headspace volume to weight of coffee in the package. This change, which results from changes in grinding as well as roasting techniques, increases the oxygen exposure after packaging for a constant can vacuum. A higher density, higher weight product will have a significantly lower oxygen exposure than a lower density, lower weight product. Shelf-life changes in permeable packages are beyond the scope of this simple empirical model. However, the early incremental reaction in a permeable package will ensure a reduction in evaluated quality within the time scope of ordinary shelf retention.

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SUMMARY

The uptake of oxygen in roast and ground coffee has been examined as a first-order reaction. Rate constants of the order 10^{-3} cc/g.hr at 30°C were measured, with an Arrhenius activation energy of about 8000K. Effects of roasting speed, particle size, and moisture content were determined. A model of the decay of sensory freshness using the amount of reaction with oxygen as a parameter was applied to literature data. Using this model, the significance of reaction before packaging can be examined. On the basis of this model, reductions in shelf-life from two to eight percent for an exposure of one hour can be expected. The effect of packaging in permeable materials is examined as well.

Zusammenfassung

Die Sauerstoffaufnahm in gemahlenen Röstkaffee wurde als Reaktion 1. Ordnung untersucht. Reaktionsgeschwindigkeitskonstante in Bereich von 10^{-3} cc/g.hr bei 30°C wurden gemessen mit einer Arrhenius Aktivierungsenergie von ca. 8000K. Die Einflüsse von Röstzeit, Partikelgrosse und Wassergehalt wurde bestimmt. Ein Modell über die Abnahme der sensorischen Frische unter Verwendung der in der Reaktion verbrauchte Sauerstoffmenge wurde auf Literaturdata angewendet. Unter Verwendung dieses Modells kann die Bedeutung einer Reaktion vor der Verpackung untersucht werden. Bei Zugrundelegung eines 1-stundiges Luftkontakt kann die Reduzierung der Haltbarkeit von 2-8% erwartet werden. Die Einfluss einer Verpackung von durchlässiger Materialen wurde ebenfalls untersucht.

Figure 1

Oxygen Uptake in R&G Coffee, 30°C

Density = 0.444

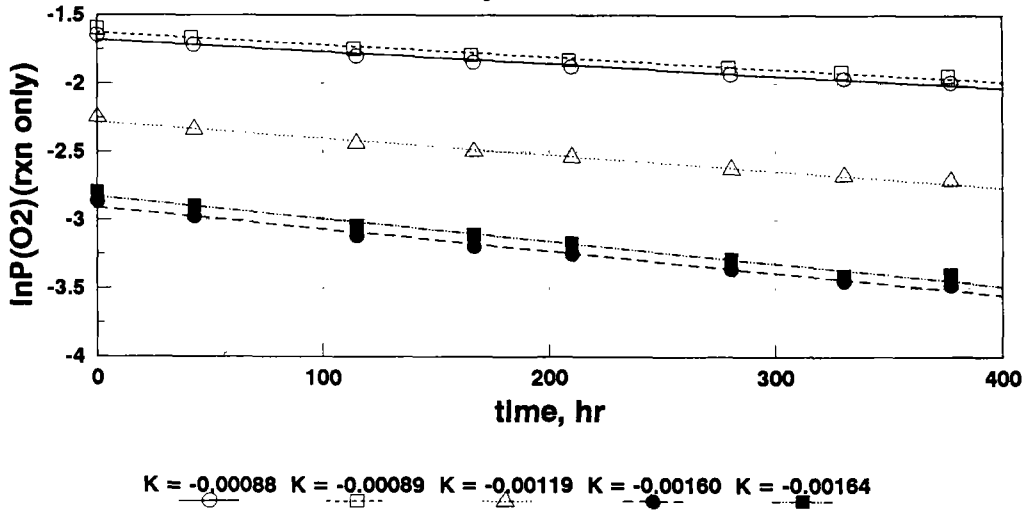


Figure 2

Oxygen Uptake In R&G Coffee
Effect of Temperature

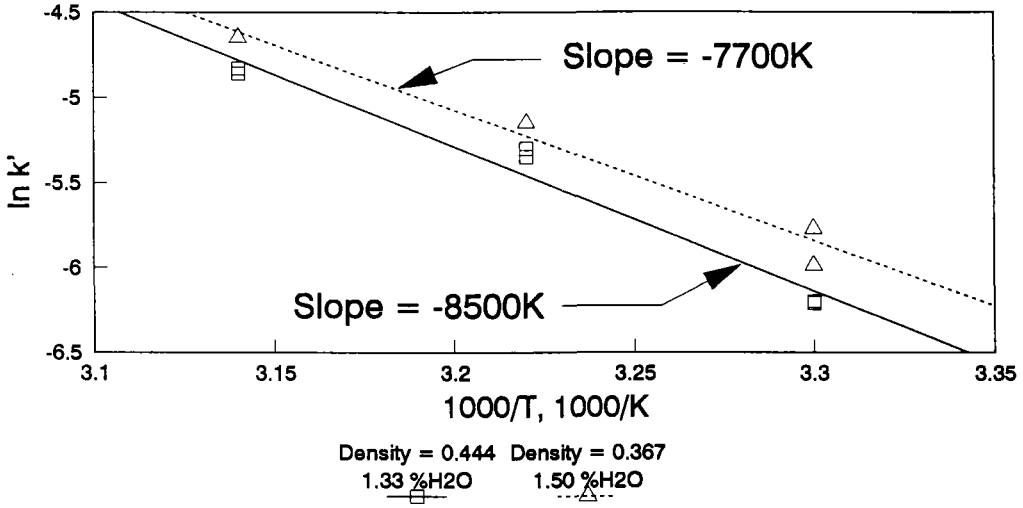


Figure 3

Oxygen Uptake In R&G Coffee
Effect of Moisture

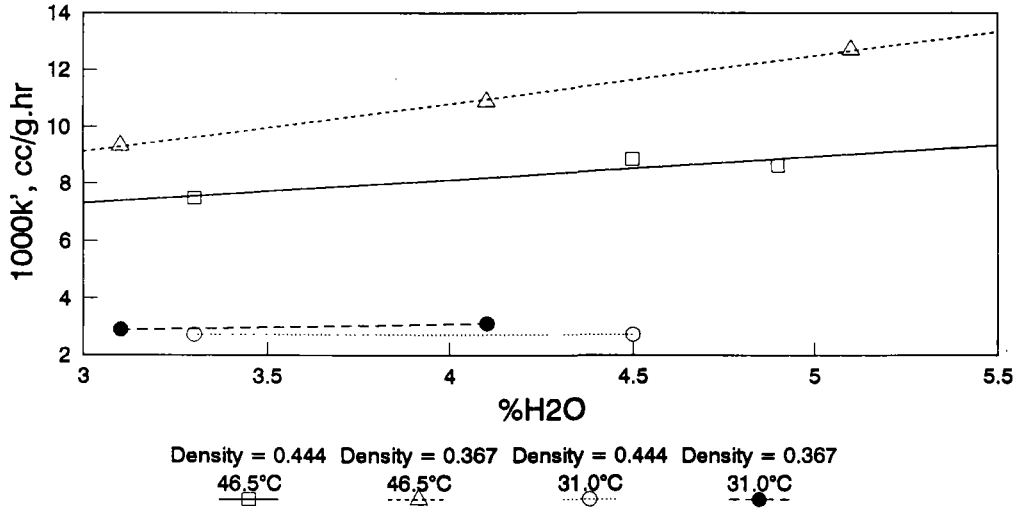


Figure 4

Oxygen Uptake in R&G Coffee
Effect of Particle Size

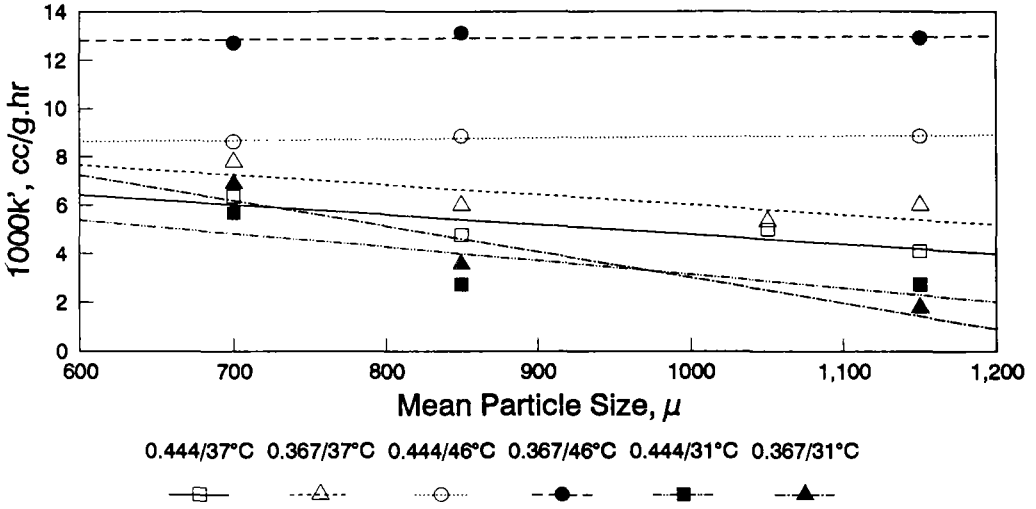


Figure 5

Measured and Modeled F/S
16-oz Product, 401 Can

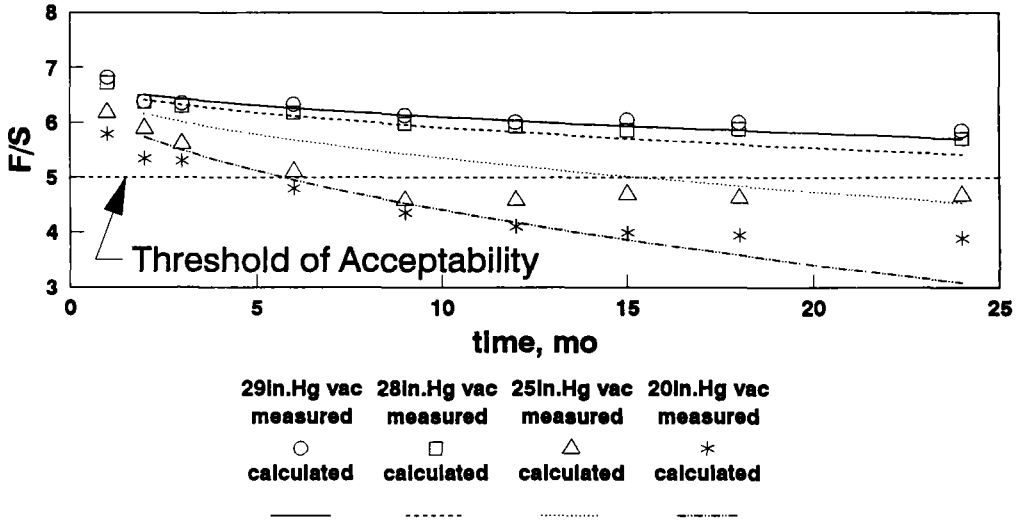


Figure 6

Shelf-life of Roasted and Ground Coffee
Calculated from Equation 5

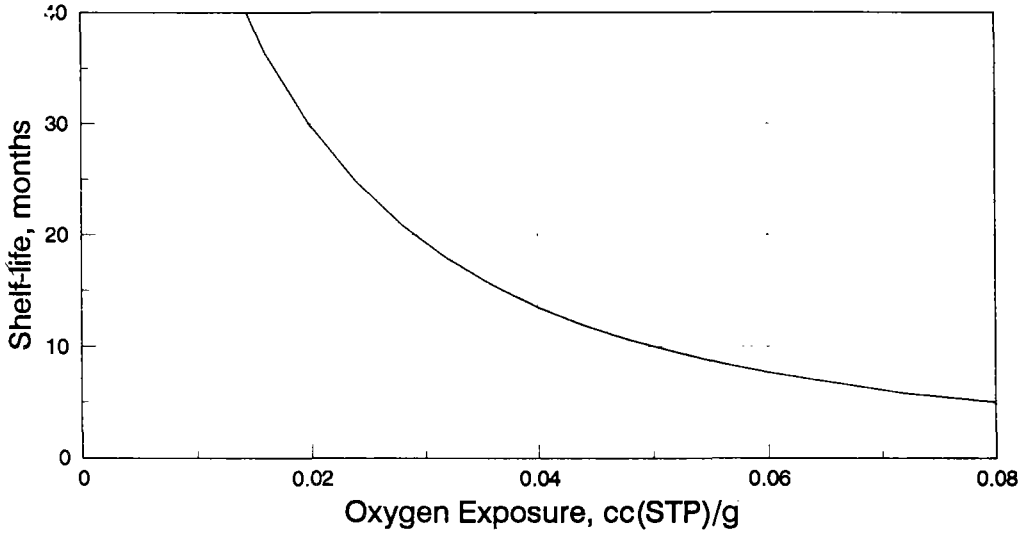
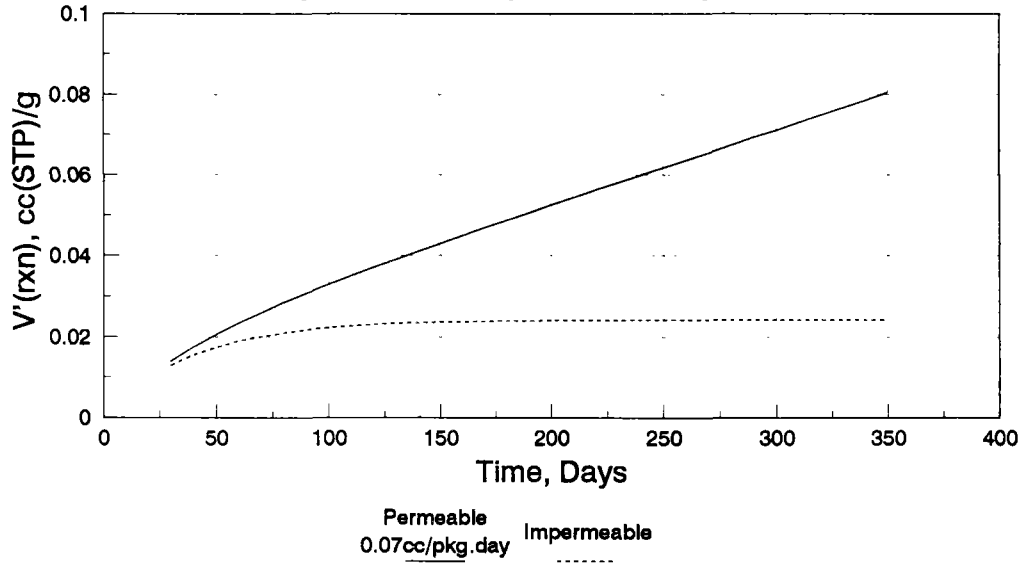


Figure 7

Oxygen Uptake by Container Type



NCA SURVEY OF PESTICIDE RESIDUES IN BREWED COFFEES

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Introduction

The National Coffee Association of USA is the oldest and largest of the coffee trade associations in the US. One of the socially responsible activities of the NCA is conducting scientific research on coffee in areas of public concern.

Pesticides in food and beverage products available to the consumer has been an area of growing concern in the public arena. While some work has been reported in the literature on the level and incidence of pesticide residues in green coffee, very little work exists on the residues present in roasted coffee and even less information exists on possible residues transferred to coffee brews.

Green coffee and tea imports into the United States are routinely monitored for pesticide residues and reported on an annual basis as a part of the USFDA's pesticide monitoring program. Figure 1 shows the incidence of combined coffee and tea samples collected as a part of this program in the period from 1978 - 1989 which showed no pesticide residues (1-5). It is clear from the graph that the percent of samples with no residues has generally increased over that time period, indicating decreasing incidence of green coffee with pesticide residues entering the United States. Additionally, when pesticide residues were found, their level was determined to be quite low.

Figure 1. FDA Pesticide Monitoring Program combined coffee/tea import results in the period 1978-1989.

The results of Centinkaya et al (6) and Blumenthal et al (7) are consistent with those findings of the FDA Pesticide Monitoring Program. Both of these studies show very low levels of organochlorine pesticide residues on green coffee when such residues were detected at all. Blumenthal et al further hypothesize that such low levels present in the green coffee would not be transferred to the brew based on their findings on tea infusions. Centinkaya et al noted an 85-100% reduction in residue level on roasting. They also did not pursue further work in the brew due to the resultant low residue levels. Further, USFDA Total Diet Studies, carried out annually, have not indicated any incidence of positive results for coffee.

As can be seen here, limited work has been done to study the fate of pesticide residues upon roasting and even less on the occurrence of residues in coffee brews. The lack of work in this area leaves no definitive data for the levels of pesticide residues in brewed coffee. Accordingly, the NCA board approved this study to extend the Association's overall data base and address these areas.

This study incorporates residue screening for organophospate and organochlorine pesticides and PCB's. These pesticides are tested for in standard screens and represent pesticides which have been in global use for a number of years. Some of the compounds in these screens are known to persist in the environment and are thus frequently detected. Many of these pesticides are currently banned. This work reports on the loss of these pesticides on storage, roasting and brewing and also surveys brews prepared from fifty commercial US coffees for residues of these compounds.

Experimental

This study is divided into two phases. The first phase was designed to investigate the loss of pesticides which occurs during coffee roasting and brewing. Green coffees received in 1988 which showed detectable pesticide residues and green coffees which were intentionally spiked with selected pesticides were tested for the pesticide residues mentioned above. Additionally, these green coffees were roasted, ground, and brewed. The R&G coffee and brews were also tested for pesticide residues.

The second phase was designed to evaluate the levels of pesticide residues in coffee brews prepared from commercial 100% coffee products on the U.S. market. Brews were prepared using the procedure described below and were subsequently tested for PCB's, and chlorinated and organophosphate pesticide residues.

Samples: Two samples which were received and screened for the pesticides of interest in this study in 1988 and which contained detectable residues were roasted, ground, and brewed. One sample was a wet processed Arabica originating from Peru and the other was a dry processed Arabica originating from India. The green, roasted and brewed coffees were screened for pesticide residues. These samples were stored at ambient conditions (i.e. room temperature in a cloth bag) in the period from 1988-1990.

A sample of new crop Colombian coffee was obtained. A portion of this coffee was spiked with five pesticides (technical grade BHC, DDT, dieldrin, malathion and pirimiphos methyl) which have been detected in previous studies. These compounds are representative of the types of pesticides screened in this study. Spike levels were on the order of 0.2-0.25ppm on the green coffee. These levels are 2-4 times the highest levels of pesticide residues typically detected in green coffee samples which have been received with detectable residues. The initial green coffee, spiked green coffee, roasted coffee and coffee brew were analyzed via the aforementioned pesticide screens.

Fifty samples of commercial coffees were obtained in September - October 1990. These samples were chosen to be typical regular R&G, decaffeinated R&G, regular soluble and decaffeinated soluble products. Both retail and foodservice products were represented. Sample distribution by geographic region and brand was done according to market share. Retail samples were obtained in the areas of San Francisco, CA, Cincinnati, OH, and New York, NY. All foodservice products were obtained in the Madison, WI area. A summary of commercial sample distribution by geographic region is given in Table 1 and by product type in Table 2.

Table 1
Retail Commercial Product Distribution

	East	Central	West
Area Purchased:	White Plains, New York	Cincinnati, Ohio	San Francisco, California
R&G Samples (%)	31	39	30
Soluble Samples (%)	50	23	27

All foodservice samples were purchased in the Madison, WI area.

Table 2
Commercial Coffee Products Used for Brew Preparation

Retail Products

Product Type	Number of Samples
Regular Roast and Ground	22
Decaffeinated Roast and Ground	5
Regular Soluble	9
Decaffeinated Soluble	4

Foodservice Products

Product Type	Number of Samples
Regular Roast and Ground	6
Decaffeinated Roast and Ground	1
Regular Soluble	2
Decaffeinated Soluble	1

Sample Preparation: Roasting was done in a gas fired laboratory type roaster. All roasted coffees in the first phase of the study were roasted to light medium roast (Agtron color 62) and dark medium roast (Agtron color 56) color visually matched to NCA supplied roasted whole bean targets. Grinding was done in a laboratory mill to an approximate commercial grind for filter brewing.

R&G products were brewed using 64 fluid ounces of water per 2 ounces of R&G coffee or fast roast process equivalent (30g regular roast coffee per liter of water) using a home style, single pass, filter coffee maker (ADC brewer). Soluble products were prepared by dissolving 1.8g product per 8 fluid ounces of water at 180°F (7.6g per liter of water).

Pesticide Screening: All sample preparation and analyses for PCB's, and chlorinated and organophosphate residues were carried out by the laboratories of Hazleton Wisconsin, Inc., Madison, Wisconsin. The standard methods utilized are based on AOAC methods and those in the FDA Pesticide Analytical Manual (8-11). Lower limits of detection for both brew and ground coffee matrices are given in Table 3. An indication of less than the lower limit of detection (LOD) does not imply that any of the pesticide was present.

The applicability of the test methods to the coffee matrices in this study was verified by Hazleton Wisconsin by spiking the coffee and brew matrices with known amounts of pesticides and determining the amount of pesticides recovered by the methods. Recovery samples were run in a 10% ratio to total samples in all matrices as part of Hazleton's analytical protocol. All recovery results were within the 70-120% recovery criteria used by Hazleton's laboratories.

Table 3
Lower Limits of Detection (LOD) for Organophosphate, ChlorinatedPesticides and PCB Screens

Compound	LOD (ppm) Ground Coffee	LOD (ppb) Coffee Brew
Vapona	<0.02	<0.50
Thimet	<0.02	<0.50
Diazinon	<0.02	<0.50
Methyl Parathion	<0.02	<0.50
Ronnel	<0.02	<0.50
Malathion	<0.02	<0.50
Parathion	<0.02	<0.50
Pirimiphos-Methyl	<0.02	<0.50
DDE	<0.005	<0.10
DDD	<0.005	<0.10
DDT	<0.005	<0.10
PCB	<0.01	<1.00
Dieldrin	<0.005	<0.10
Technical BHC	<0.005	<0.10
HCB	<0.005	<0.10
Endrin	<0.005	<0.10
Heptachlor Epoxide	<0.005	<0.10
Mirex	<0.01	<0.50
Methoxychlor	<0.01	<0.50
Toxaphene	<0.10	<2.00
Technical Chlordane	<0.01	<0.50

Results and Discussion

As mentioned previously, little work has been done on pesticide reduction during coffee roasting and little work, if any, has been reported on pesticide reduction with coffee drip style filter brewing (ADC), the most common form of brewing in the US. The initial results in this work address both of these areas.

Residue levels of 1988 green coffees received with detectable residues were significantly reduced during the two years storage from 1988 to 1990 (Table 4). These coffees were stored at ambient conditions. The storage loss ranged from 43% to 67% (DDT >58%). DDD showed no reduction, however the DDD levels are at the LOD where relative imprecision of the measurement can be as high as 50% making those results inconclusive. The low levels of pesticides and the pesticides detected on these samples are typical of coffee samples entering the U.S. in which pesticide residues are found.

The degree of loss due to roasting for the pesticides present in the 1988 coffees received with residues was observed to be in the range of 72% to >88% (Table 4) relative to the residue levels determined in 1990. Reductions indicated with "greater than" values are expressed in this manner since no pesticides were detected in the roasted product. Thus, the LOD's for the pesticides were used in the calculation since these represent the maximum amount which could possibly be present.

Table 4
Pesticide Loss Due to Roasting

1988 Green Coffee Samples with Residues as Received

	Green Coffee(ppm)			Roast Coffee(ppm)		Residue Loss (%)	
Compound	1988	1990	Storage Loss(%)	Light Medium	Dark Medium	Light Medium	Dark Medium
Peruvian Wet Process Arabica							
DDD	<0.005	0.005	0	0.006	0.006	*	*
DDT	0.049	0.028	43	<0.005	<0.005	>82	>82
Technical BHC	0.123	0.040	67	0.011	<0.005	72	>88
Indian Dry Process Arabica							
DDT	0.012	<0.005	>58	<0.005	<0.005	-	-
Technical BHC	0.107	0.043	60	<0.005	<0.005	>88	>88

* Negative percentage caused by residue levels very near the LOD where imprecision is large (result inconclusive)
\- No residue detected in either the green or roasted sample

Where residue levels are sufficient to monitor the effect of increasing degree of roast (Technical BHC - Peruvian sample), increased loss is observed with increased degree of roast.

The trend for reduction of pesticides on roasting and the degree of this reduction are similar for coffee samples with indigenous pesticide residues and for those samples which were spiked in this study. The degree of pesticide reduction with roasting was most accurately assessed using the spiked coffee samples in this study. The higher degree of accuracy is possible since the initial levels are sufficiently high for detectable residues to be present in some of the roast coffees. It should be reiterated that the green coffee spike levels in this study are significantly higher (2-4 times) than those typically encountered in green coffee samples with detectable residues.

DDD was not intentionally spiked in these samples but was present as a minor impurity in one of the spiked components, probably DDT. As can be seen in Table 5, DDD is present at an extremely low level and thus these results will not be considered here.

Reduction of the spiked components ranged from 83% to 98% (malathion and pirimophos methyl >92%) for the roasted samples (Table 5). These samples also show progressive reduction with degree of roast. The substantial losses of pesticides during roasting are in agreement with the previously cited German work (6).

No residues were detected in any brews prepared from coffees in this portion of the study; neither those samples received with detectable residues nor the spiked coffees (Tables 6,7). Due to this situation, LOD's in the brews were used to calculate the minimum pesticide losses during brewing as with the roasting portion of the study. The minimum pesticide loss values span a large range due to the low pesticide levels in the roast coffees.

Pesticide losses on brewing over all samples range from >44% to >88% based on levels in the roast coffees (where possible to calculate). Relative to the original green coffees, pesticide losses range from >33% to >92% for the samples received with residues and from >93% to >99% for the spiked samples.

Table 5
Pesticide Loss Due to Roasting

Spiked Colombian Wet Process Arabica

Compound	Green Coffee (ppm)	Spiked Green Coffee (ppm)	Roasted Coffee(ppm) Light Medium	Roasted Coffee(ppm) Dark Medium	Residue Loss(%) Light Medium	Residue Loss(%) Dark Medium
Malathion	<0.02	0.24	0.03	<0.02	88	>92
Pirimiphos-Methyl	<0.02	0.24	0.04	<0.02	83	>92
DDT	<0.005	0.242	0.019	0.013	92	95
Dieldrin	<0.005	0.233	0.028	0.018	88	92
Technical BHC	<0.005	0.204	0.006	<0.005	97	98
(DDD*	<0.005	0.008	<0.005	<0.005	>38	>38)

* Impurity in a spiked pesticide, probably DDT

One of the major driving forces for residue level reduction on brewing is the water insolubility of many pesticides. The water solubilities of the spiked compounds are given in Table 8 and are consistent with this hypothesis.

For all pesticides screened, no residues were detected in any of the brews prepared from any of the commercial products analyzed in this survey as shown in Table 9. As previously mentioned, the brew LOD's are in the part per billion range as shown in Table 3.

These results are consistent with the decreasing incidence and low levels of pesticide residues detected in green coffee as observed in the FDA pesticide monitoring program (1-5) and with the residue reduction on processing shown in this work.

The previously mentioned German work (6) indicates loss of many pesticides occurs in roasting. The Swiss experience with tea infusions (7) and the limited water solubility of many pesticides is consistent with further pesticide reduction in brewing. These works further corroborate the findings in this study.

Table 6
Pesticide Loss Due to Brewing

1988 Green Coffee Samples with Pesticide Residues as Received

Compound	Coffee Brew(ppb) Lt. Medium Roast	Coffee Brew(ppb) Dk. Medium Roast	% Loss on Roast Coffee Basis Lt. Medium Roast	% Loss on Roast Coffee Basis Dk. Medium Roast	% Loss on Green Coffee Basis (1990)*
Peruvian Wet Process Arabica					
DDD	<0.10	<0.10	>44	>44	>33
DDT	<0.10	<0.10	**	**	>88
Technical BHC	<0.10	<0.10	>70	**	>92
Indian Dry Process Arabica					
DDT	<0.10	<0.10	**	**	+
Technical BHC	<0.10	<0.10	**	**	>92

* Light and Dark Medium Roast brew values are identical in this column since all brews are <LOD and relative to the same green coffee.
** No pesticide residues detected in the roast coffee.
+ No pesticide residue detected in the green coffee.

Table 7
Pesticide Loss Due to Brewing

Spiked Colombian Wet Process Arabica

Compound	Coffee Brew(ppb) Lt. Medium Roast	Coffee Brew(ppb) Dk. Medium Roast	% Loss on Roast Coffee Basis Lt. Medium Roast	% Loss on Roast Coffee Basis Dk. Medium Roast	% Loss on Green Coffee Basis(Spiked)*
Malathion	<0.50	<0.50	>44	+	>93
Pirimiphos-Methyl	<0.50	<0.50	>58	+	>93
DDT	<0.10	<0.10	>82	>74	>99
Dieldrin	<0.10	<0.10	>88	>81	>99
Technical BHC	<0.10	<0.10	>44	+	>98
(DDD**	<0.10	<0.10	+	+	>58)

* Light and Dark Medium Roast brew values are identical in this column since all brews are <LOD and relative to the same spiked green coffee.
** Impurity in a spiked pesticide, probably DDT.
+ No pesticide residue detected in the roast coffee.

Table 8
Water Solubilities of Spiked Pesticides (12)

Compound	Water Solubility
Malathion	145 ppm
Pirimiphos-Methyl	5 ppm (30C)
DDT	Insoluble
Dieldrin	Insoluble
Technical BHC (represented by Lindane)	Insoluble

Table 9
Brews Prepared From Commercial Coffees

Product Type	# Samples	Total Residues
Regular Roast & Ground	28	None Detected
Decaffeinated Roast & Ground	6	None Detected
Regular Soluble	11	None Detected
Decaffeinated Soluble	5	None Detected

Conclusions.

No detectable residues were found in any of the brews analyzed throughout this study, including roast/brew study and commercial samples. Brews were tested to the sub-part per billion level. This work represents one of the first comprehensive surveys on the measurement of pesticide residues in coffee brews.

Substantial reduction in pesticide levels during storage at ambient conditions was observed for the pesticides found in green coffee received in 1988 and stored until 1990. Over a two year period, this loss can amount to 67% for Technical BHC.

Loss of pesticides on roasting in this work is consistent with that reported previously in the literature. The same degree of pesticide reduction and the trend of reduced pesticide levels with increased roasting were exhibited for both coffees received with detectable pesticide residues and spiked samples. Roast losses ranged from 72% to 98% (>88%).

Losses on brewing ranged from >33% to >99% relative to the levels in the green coffees tested. These results are consistent with the low water solubilities of these pesticides.

The results from the cited literature and the FDA's pesticide monitoring program show very low levels of pesticides are present in green coffee imports into the United States when such residues are even detected. These results along with the substantial reduction of pesticide residues during storage, roasting and brewing found in this work show consumer exposure to the tested pesticides is negligible, if any, due to typical coffee consumption.

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SUMMARY

NCA Survey of Pesticide Residues in Brewed Coffees

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The loss of pesticide residues on green coffee storage, roasting and paper filter brewing has been assessed. Additionally, fifty coffee brews prepared from US retail and foodservice commercial coffees both roast and ground and soluble (regular and decaffeinated) were tested for pesticide residues. Pesticide residue screens included organochlorine and organophosphate pesticides and PCB's. No residues were detected in any of the coffee brews in this study.

Substantial pesticide reduction was observed over storage, roasting and brewing: 0-67% loss on storage; 72-98% (>88%) on roasting; >33->99% on brewing relative to initial green coffee levels. These results are consistent with those reported in the literature where such data exist.

The results from this study coupled with the low incidence and levels of pesticide occurence in imported green coffee show consumer exposure to the tested pesticides is negligible, if any, due to typical coffee consumption.

RESUME

Etude de la NCA sur les residus de pesticides dans les cafes infuses

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La reduction des residus de pesticides lors de l'entreposage du cafe vert, de la torrefaction et de l'infusion avec filtres en papier a ete evaluee. De plus, cinquante cafes infuses prepares en utilisant des cafes commerciaux des Etats-Unis vendus normalement au detail ou par des services de restauration, et comprenant a la fois des cafes torrefies, moulus et solubles (ordinaires ou decafeines), ont ete testes pour determiner la presence de residus de pesticides. Les residus de pesticides recherches comprenaient l'organochlore, l'organophosphate et le PCB. Aucun residu n'a ete detecte dans les infusions de cafes ayant fait l'objet de cette etude.

Une reduction substantielle des pesticides a ete observee lors de l'entreposage, de la torrefaction et de l'infusion: perte de 0-67 % sur l'entreposage; 72-98 % (>88 %) sur la torrefaction; >33->99 % sur l'infusion par sapport aux niveaux initiaux dans le cafe vert. Ces resultats sont similaires a ceux qui ont ete mentionnes dans la litterature quand de telles donnees existent.

Les resultats de cette etude, conjointement avec la faible incidence et les faibles niveaux de presence de pesticides dans les cafes verts importes, demontrent que l'exposition des consommateurs aux pesticides testes est negligeable, ou meme peut-etre nulle, dans le cas d-une consommation ordinaire de cafe.

THE EFFECT OF ROASTING ON THE FATE OF AFLATOXIN B1 IN ARTIFICIALLY CONTAMINATED GREEN COFFEE BEANS

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INTRODUCTION

The occurrence of mycotoxin contamination in green coffee beans has, so far, never reached levels critical for human health. This is probably due to the proven inhibitory effects of caffeine on both the growth of toxigenic Aspergillus and Penicillium fungi and their production of mycotoxins such as aflatoxins, ochratoxins, sterigmatocystine, citrinin and patulin (Buchanan and Flechter, 1978; Nartowicz et al. 1979, Buchanan et al. 1981, and 1983 a, b).

Nevertheless the climactic conditions in the areas of coffee production prompt a continuous control of the incidence of mycotoxin contamination. Most of the papers relating to this problem have focused attention on commercial coffee samples, but no information is available on the contamination level in large lots of coffee beans obtained by a statistically based sampling plan.

The obtaining a laboratory sample representative of the lot from which it is drawn is in fact crucial to the analysis of mycotoxins in food (Whitaker, 1977), in order to obtain a better evaluation of the actual toxin content and more accurate information on the safety of the commodities for import.

Representative sampling is necessary to better estimated levels of toxin content in coffee beans and to provide more accurate information on the safety of food commodities for import.

The aim of this study, was to investigate the status of aflatoxin B1 (AFB1) contamination in several lots of imported green coffee beans from the major producing countries.

The samples were collected on the basis of a statistical sampling plan as in our previous paper on ochratoxin A contamination of green coffee beans (Micco et al., 1989).

In addition the percentage of destruction of aflatoxin B1 after the roasting process and the potential migration of the toxin into the beverage were also evaluated.

MATERIAL AND METHODS

Samples

Forty one green coffee samples were collected by sampling single lots ranging

in size from 50 to 4000 bags (average weight of each bag: 70 kg).

Green coffee was not flown directly from the producing countries, but was sampled during the storage period at two ports of entry into Italy (Trieste and Genova).

Sampling was carried out following EEC procedures (Official Journal of the Community, 1976) for aflatoxins in feed as a guideline, considering the lack of a specific sampling plan for the commodity under examination. The adopted sampling plan is reported in Table 1. Depending on lot size, a variable number of samples (4 kg each) were drawn. Each sample was then properly mixed and 1 kg subsamples were obtained by "quartering" technique.

The collected subsample (1 kg) was finely ground and thoroughly blended, and analytical samples (50 g) were taken by further quartering subdivision.

For two samples (Columbia) a preliminary freezing was necessary before grinding, probably because of the higher moisture content of the beans under examination.

Roasting procedure

The green coffee beans were artificially contaminated as follows: the beans were soaked in a benzene - acetonitrile solution (98:2) of AFB1 and after a properly blending the solvent was removed in a rotary evaporator under vacuum.

The samples used in the spiking experiments has been previously analyzed for the presence of AFB1. No toxin was found in any of the samples tested.

Three samples of artificially contaminated samples were roasted by using two types of laboratory roasters, i.e. electrical and by gas, in order to reproduce as closely as possible the industrial roasting condition.

Coffee beverage

Beverages were prepared after roasting from an artificially contaminated sample (0.8 ug/kg) employing three different kind of coffee preparation commonly used in Italy : bar, mocha and by infusion.

Bar method: nineteen grams of contaminated coffee powder were extracted with water (150 ml). The yield of coffee beverage was 96.6%.

Mocha method: sixteen grams of the contaminated sample were extracted with water (165 ml). The yield of coffee beverage was 43.6%.

By infusion: seven grams of the contaminated powder were extracted with water (200 ml). The yield of beverage was 84%.

Analytical procedure

AFB1 level was determined by employing Paulsch et al. (1988) method in feed with the following modifications:

- The size of each analytical sample was reduced to 20 grams for powdered samples and 50 ml for beverage.

- For all kind of coffee samples the C18 Sep-pak cartridge cleanup step was omitted since the HPLC chromatogram of the eluate from Florisil cartridge showed no interference in the elution zone of AFB1.

The HPLC apparatus used was a liquid chromatograph Perkin Elmer series 10LC (Pump A:water/acetonitrile/methanol 130/70/40; Pump B: saturated iodine aqueous solution) equipped with a Lichrospher 100 RP 18 column (Merck) fitted up

with a precolumn C18 Lichrospher (Merck).

Detection was performed by a Perkin Elmer LS4 Fluorescence spectrometer (wavelength of excitation 365 nm - emission 425 nm), and registration of data was obtained by a Varian 4290 electronic integrator.

The confirmation of positive results was performed by adding known amounts of AFB1 standards to the positive samples. In addition the contaminated samples were reinjected omitting the derivatization step with iodine: the absence of AFB1 peak resulted as a confirmatory test.

The detection limit attainable for AFB1 was 0.1 ug/kg for coffee beans and 0.01 ug/kg for the beverage.

In order to evaluate the accuracy and precision of the method employed, five recoveries were carried out both on green and on roasted coffee. The average recovery value calculated for five determinations at spiking levels of 1 and 5 ug/kg was 95% and 98% respectively, with a standard deviation of 4.5 and 3 and a coefficient of variation of 4.7% and 3%. These values were found satisfactory for the reliability of the modified method for the matrix under examination.

RESULTS AND DISCUSSION

The results of the analysis of coffee samples are summarized in Table 1. None of the forty one samples resulted contaminated by AFB1 showing good quality coffee lots strictly relating to the lot under analysis.

The presence of caffeine probably act in this commodity as a protective agent against AFB1 production, although coffee plants grow in climactic conditions favorable for the sprouting of Aspergillus moulds.

In order to evaluate the fate of AFB1 following the industrial process three samples of green coffee were artificially contaminated at levels of 8, 9 and 11 ug/kg respectively.

After evaporation of the solvent, coffee samples were roasted at 200°C for 8-10 minutes in the electrical treatment and at 210°C for 15-20 minutes in the procedure performed by gas .

By these procedures, two degrees of roasting were achieved, i.e. light and dark.

The latter is more commonly employed in Italy.

The results showed a marked destruction of AFB1 in all of the three samples at levels ranging from 90% to approximately 100%. Nevertheless a slight difference was noted in electrical as opposed to the gas procedure.

The destruction of AFB1 was more consistent in the dark roasting than in the light roasting procedure as the similar results obtained by Levi (1980).

A study was also performed to evaluate the potential migration of the toxin into the beverage. One sample of roasted coffee (0.8 ug/kg) was used to prepare three kinds of coffee beverages: bar, mocha and infusion.

All obtained results were calculated relating to powdered coffee.

A very low level of AFB1 (0.01 - 0.2 ug/kg) was detected in all three beverages but a higher level of the toxin was detected in the sample obtained by the mocha procedure.

This particular finding might be caused by a higher extraction power of the mocha method.

In conclusion, the whole process from raw coffee to the beverage showed a marked destruction of AFB1 ranging from 97 to 100% depending on the extraction technique employed.

This might presumably assure the safety of coffee from aflatoxin contamination, taking also in account that this particular commodity is naturally

quite resistant to the production of AFB1 because of the presence of caffeine in the matrix. Nevertheless in our opinion a control of the incidence of mycotoxin contamination in coffee should be performed, considering both of the climate characteristic of the areas where coffee grows, and the shipping conditions of bags particularly favourable to a propagation of toxigenic moulds.

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ABSTRACT

A study was undertaken to evaluate aflatoxin B1 contamination in coffee beans.

Forty-one samples of green coffee were collected from large lots of material by representative sampling. The raw samples were analyzed and showed

no detectable levels of aflatoxin B1. In order to establish the heat stability of the toxin, three artificially contaminated samples (average level 10 ug/kg) were roasted at ca. 200°C for different operation time periods so as to reproduce light and dark roasting procedures. Each sample was roasted both electrically and by gas.

The percentage of toxin destruction was up to 93% for light roasted and 99% for dark roasted coffee with a slightly higher rate up to 100% for the electrically roasted coffee for light and dark roasting. In order to evaluate the potential migration of the aflatoxin B1 into the coffee beverage, one sample found contaminated after roasting treatment (0.8 ug/kg) was extracted using each of the three most common types of coffee makers. Additional destruction of the toxin was observed (up to 99%) in two cases while only 75% of fate was obtained in the third.

The process from raw coffee beans to beverage showed a meaningful destruction of aflatoxin B1, ranging from 97 to 100% depending on the extraction technique adopted in the preparation of the beverages.

SOMMARIO

E'stato eseguito uno studio per valutare lo stato di contaminazione da aflatossina B1 nel caffè.

Quarantuno campioni di caffè verde sono stati prelevati da grosse partite di caffè, eseguendo un campionamento su basi statistiche. Il prodotto grezzo è stato analizzato e non ha evidenziato presenza di aflatossina B1 (limite di rivelazione del metodo 0.01 ug/kg). Al fine di stabilire la resistenza alla tostatura della eventuale tossina presente nel caffè, tre campioni di caffè verde sono stati contaminati artificialmente (contaminazione media 10 ug/kg), e successivamente sottoposti a tostatura a circa 200 °C per tempi differenti, in modo da riprodurre la tostatura chiara e scura. Ciascuno dei due tipi di tostatura è stata eseguita con tostino sia elettrico che a gas.

I risultati ottenuti dall'analisi dei campioni tostati hanno evidenziato una percentuale massima di distruzione della tossina del 93% per la tostatura chiara a gas e del 99% per la tostatura scura a gas; per la tostatura elettrica è stato evidenziato un lieve incremento nella percentuale di distruzione sia per la tostatura chiara che per la scura (100% max).

Inoltre, per poter valutare la possibilità di passaggio dell'aflatossina B1 nella bevanda, un campione risultato contaminato (0.8 ug/kg) dopo la tostatura, è stato estratto utilizzando i metodi più diffusi di preparazione della bevanda (bar moka ed infusione). L'analisi delle bevande ottenute ha evidenziato una ulteriore distruzione della tossina (99% max per il procedimento bar e infusione, e 75% per il procedimento moka).

Pertanto l'intero ciclo di lavorazione dal caffè grezzo alla bevanda ha mostrato una capacità di distruzione sull'aflatossina B1 variabile dal 97% al 100% a seconda del tipo di tostatura e delle modalità di preparazione della bevanda.

TABLE 1. EVALUATION OF AFB1 CONTAMINATION IN IMPORTED GREEN COFFEE BEANS

VARIETY	COUNTRY	LOT SIZE (BAGS)	SAMPLES (4 KG)	AFB1 (UG/KG)*
ROBUSTA	INDONESIA	270	3	ND**
	IVORY COAST	460	3	ND
	IVORY COAST	987	4	ND
	IVORY COAST	252	3	ND
	ZAIRE	160	2	ND
	CAMEROON	50	2	ND
ARABICA	SANTOS	380	3	ND
	SANTOS	255	3	ND
	SANTOS	2620	4	ND
	SANTOS	1225	4	ND
WASHED	KENYA	150	2	ND
	COSTARICA	1000	4	ND
	COLUMBIA	4000	4	ND

* Values of triplicate analysis

** Less than detection limit

TABLE 2. EFFECT OF ROASTING PROCEDURE ON AFLATOXIN B1 (UG/KG) IN ARTIFICIALLY GREEN COFFEE SAMPLES

GREEN COFFEE	ROASTING			
	LIGHT		DARK	
	GAS	ELECTRICAL	GAS	ELECTRICAL
SAMPLE 1 (IVORY COAST-ROBUSTA) (8 ug/kg)	0.8 (90%) [a, b]	ND (100%) [c]	0.1 (99%)	ND
SAMPLE 2 (SANTOS-ARABICA) (11 ug/kg)	0.8 (93%)	0.1 (99%)	0.2 (98%)	ND
SAMPLE 3 (COLUMBIA-WASHED) (9 ug/kg)	0.8 (91%)	0.3 (97%)	0.2 (98%)	0.1 (99%)
BAR		0.04 (95%) [d]	(99.6%) [e]	
MOCHA		0.20 (75%)	(97.7%)	
INFUSION		0.01 (99%)	(99.9%)	

a) Mean of triplicate analysis
b) AFB1 destruction (%)
c) Less than detection limit
d) Calculated on powdered coffee
e) Total destruction (%)

LA BIOLOGÍA ATÓMICA, EL CAFETO Y LA ROYA

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1. Introducción

Probablemente todas las reacciones catalizadas por enzimas son influenciadas por iones inorgánicos que interactúan bien sea con la enzima, el sustrato, los productos o las combinaciones de unos y otros, Mahler (1961).

El reconocimiento del papel de los elementos traza en biología, del cual son un buen ejemplo los cuatro metales de transición, claves para las plantas, hierro, manganeso, cobre y zinc, (Kabata-Pendias, Pendias, 1984), ha resultado en el desarrollo extraordinario de un segmento de la biología que es algo así como una contraparte de la biología molecular. Frieden (1984) lo denomina "biología atómica".

Diversos compuestos de cobre, manganeso y zinc siguen siendo populares para el control de enfermedades causadas por hongos (Becker - Raterink, 1991).

El control químico mediante compuestos de metales es una aplicación de la biología atómica.

Otro aspecto práctico de la biología atómica es el del empleo de la nutrición de las plantas como una componente importante del control de enfermedades. Cada vez se acrecenta más la toma de conciencia sobre el rol vital de los nutrientes minerales en el control de infecciones (Huber y Wilhelm 1988).

En nuestro laboratorio se hacen investigaciones en estas dos direcciones. Se busca con ellas contribuir, por ejemplo, al perfeccionamiento de la lucha química, para mejorar la eficiencia de fungicidas y disminuir su impacto ambiental.

2. Algunos rasgos químicos de las esporas de la roya del cafeto.

Nuestro objeto preferido de investigación, por su sensibilidad a la acción de iones metálicos, son las esporas de la roya del cafeto, Hemileia vastatrix Berk y Br.

En otra parte presentamos el contenido de 16 elementos, en uredosporas de 6 razas de H. vastatrix, (Quijano-Rico, 1991).

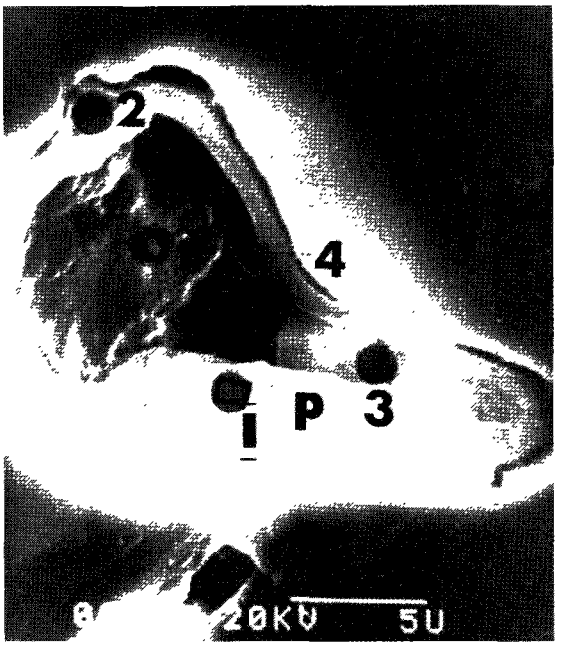


FIGURA 1. CORTE LONGITUDINAL DE UNA ESPORA DE ROYA DEL CAFETO. MICROSCOPIA ELECTRONICA DE BARRIDO. EL CORTE SE REALIZO POR MEDIO DE INCLUSION DE LAS ESPORAS EN RESINA Y PULIMENTO CON POLVO DE DIAMANTE. LA PARED, P., PARECE BASTANTE ROBUSTA. EN 1, 2, 3 Y 4 SE MIDIO EL ESPECTRO DE FLUORESCENCIA DE RAYOS X. HUTH, J. KUENSTLER F. Y QUIJANO-RICO, M. 1986.

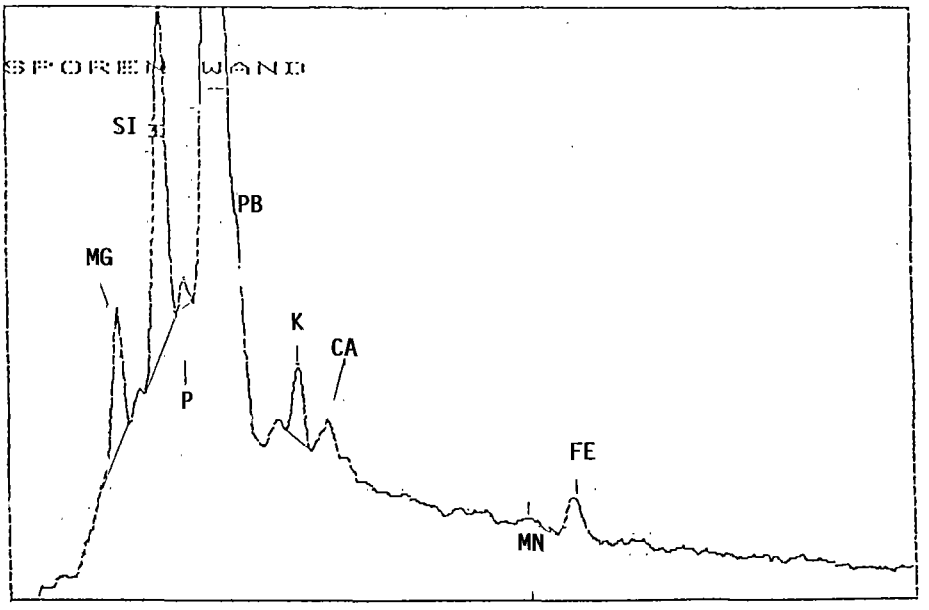


FIGURA 1-1. ESPECTRO DE EMISION POR FLUORESCENCIA DE RAYOS X DEL PUNTO 1 DE LA FIGURA 1, PARED DE LA ESPORA. OBSERVESE LA PRESENCIA DEL CALCIO, HIERRO Y MANGANESO Y LA GRAN RIQUEZA EN SILICIO. HUTH, J.; KUENSTLER, F. Y QUIJANO-RICO, M. 1986.

En la figura 1 se observa un corte longitudinal, de una de las muchas esporas de roya del cafeto que analizamos por microscopía electrónica de barrido y fluorescencia de rayos X. La técnica utilizada para la preparación de los cortes no permite preservar el material citoplasmático. La figura 1-1 corresponde al punto 1 en el centro de la pared de la espora. Se detectaron varios elementos químicos. Entre ellos llaman la atención la intensidad del pico del silicio y la presencia de manganeso y hierro.

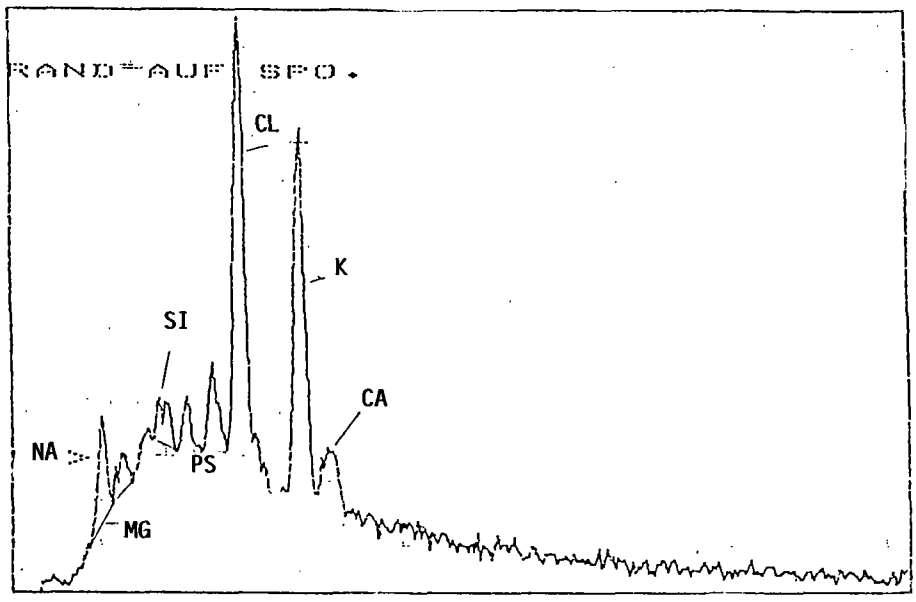


FIGURA 1-2. ESPECTRO DE EMISION POR FLUORESCENCIA DE RAYOS X DEL PUNTO 2 DE LA FIGURA 1, PARED DE LA ESPORA. OBSERVESE LA PRESENCIA DE SILICIO, FOSFORO, AZUFRE Y CALCIO ENTRE OTROS. HUTH, J.; KUENSTLER, F. Y QUIJANO-RICO, M. 1986.

Figura 1-2 corresponde también a la pared, punto 2, de nuevo aparece el silicio aunque en menor proporción.

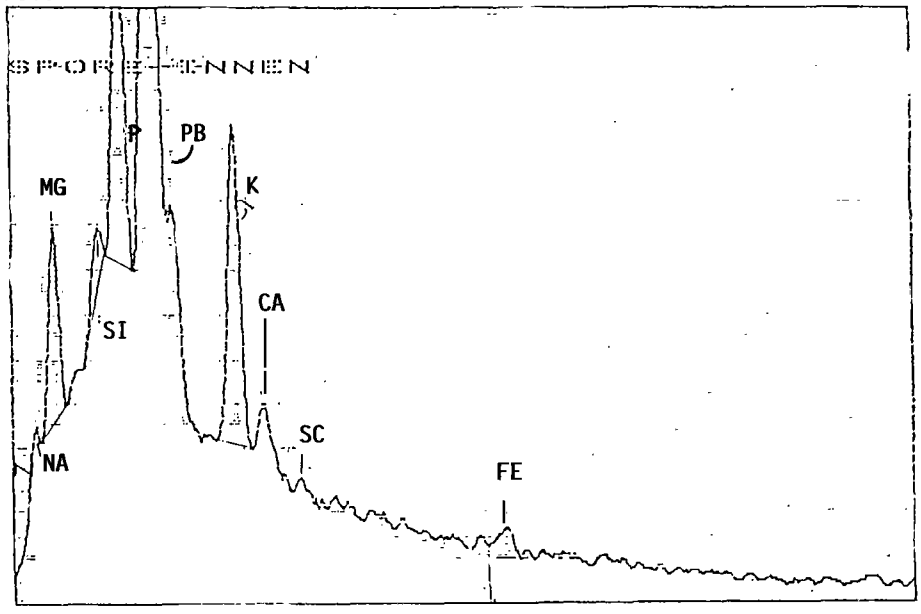


FIGURA 1-3. ESPECTRO DE EMISION DE FLUORESCENCIA DE RAYOS X EN EL PUNTO 3 DE LA FIGURA 1, INTERIOR DE LA ESPORA. SE OBSERVA LA PRESENCIA DE SILICIO, FOSFORO, CALCIO Y HIERRO ENTRE OTROS. HUTH, J.; KUENSTLER, F. Y QUIJANO-RICO, M. 1986.

Figura 1-3, punto 3, próximo a la pared, en el interior de la espora, aquí se alcanza a detectar la presencia de silicio.

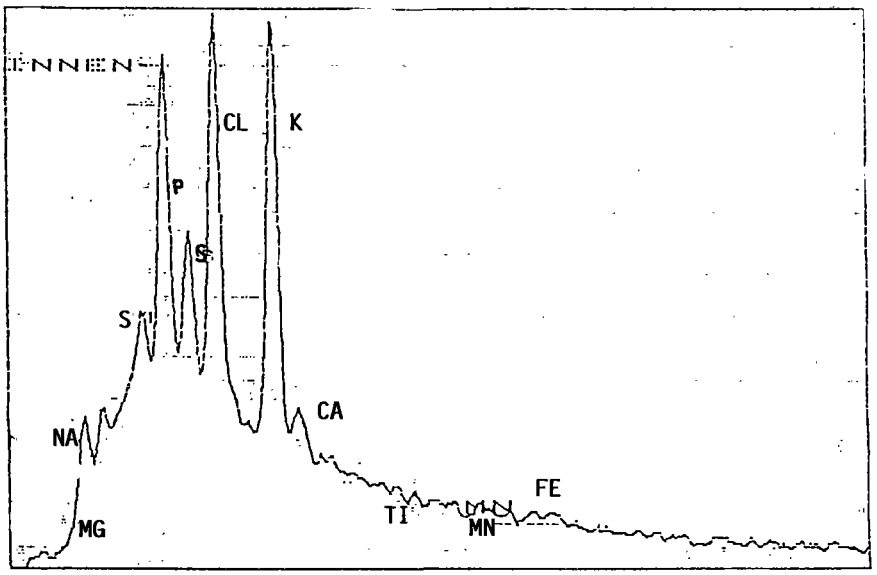


FIGURA 1-4. ESPECTRO DE EMISION POR FLUORESCENCIA DE RAYOS X DEL PUNTO 4 DE LA FIGURA 1, INTERIOR DE LA ESPORA. OBSERVESE LA PRESENCIA DE SILICIO, CALCIO, HIERRO Y MANGANESO, ENTRE OTROS Y LA RIQUEZA EN FOSFORO Y AZUFRE. HUTH, J.; KUENSTLER, F. Y QUIJANO-RICO, M. 1986.

Figura 1-4, punto 4, en el centro del interior de la espora. El silicio está siempre presente, son notables los picos del fósforo y del azufre.

Se ha descrito la presencia de silicio en la pared de esporas de Puccinia graminis, (Harder et al., 1986), pero no se conocen aún implicaciones del silicio en la germinación (Kunoh, 1990). También pudimos detectar silicio en las hifas de H. vastatrix.

3. Efectos de iones de metales de transición sobre los pigmentos de esporas de roya del cafeto.

El superoxido y sus derivados, se involucran en la degradación de pigmentos vegetales (Oswald y Eltsner, 1986).

Las esporas de roya del cafeto tienen un atractivo color naranja, debido a una provisión importante en carotenoides. Es sabido que los carotenoides son los agentes naturales más eficaces para la protección de varios sistemas celulares contra los efectos nocivos de oxidantes y la oxidación inducida fotoquímicamente (Krinsky, 1979). La decoloración de pigmentos ha sido asociada con daños importantes de las membranas y de la integridad celular (Knox y Dodge, 1985). Se ha supuesto que la inhibición de la germinación de esporas de la roya por el cobre es debida sobre todo a dos fenómenos: a) la inactivación de enzimas, principalmente del ciclo de Krebs, inactivación reversible por el manganeso. b) a una inactivación no reversible por el manganeso debida a la destrucción, localizada, por oxidación de moléculas de enzimas en los sitios en que coexisten aniones superóxido y sus derivados y iones metálicos que catalizan el daño (Quijano-Rico, 1987). Se sabe que el daño biológico causado por el superóxido es localizado e incrementado dramáticamente por iones de cobre (Samuni et al., 1981). Aunque el cobre según los autores anteriores es un potente inductor de daños por el superóxido, el mejor candidato para promoverlos, a través de la formación de radicales hidroxilo, es para otros autores, el hierro (Hallivell y Gutteridge, 1986).

Al contrario del cobre y del hierro, no se ha podido demostrar la producción de superóxido

por el manganeso, Mn^{++}, (Halliwell y Gutteridge, 1984). En cambio el manganeso, Mn^{++} puede destruir los radicales superóxido en presencia de fosfato (Benon, 1984).

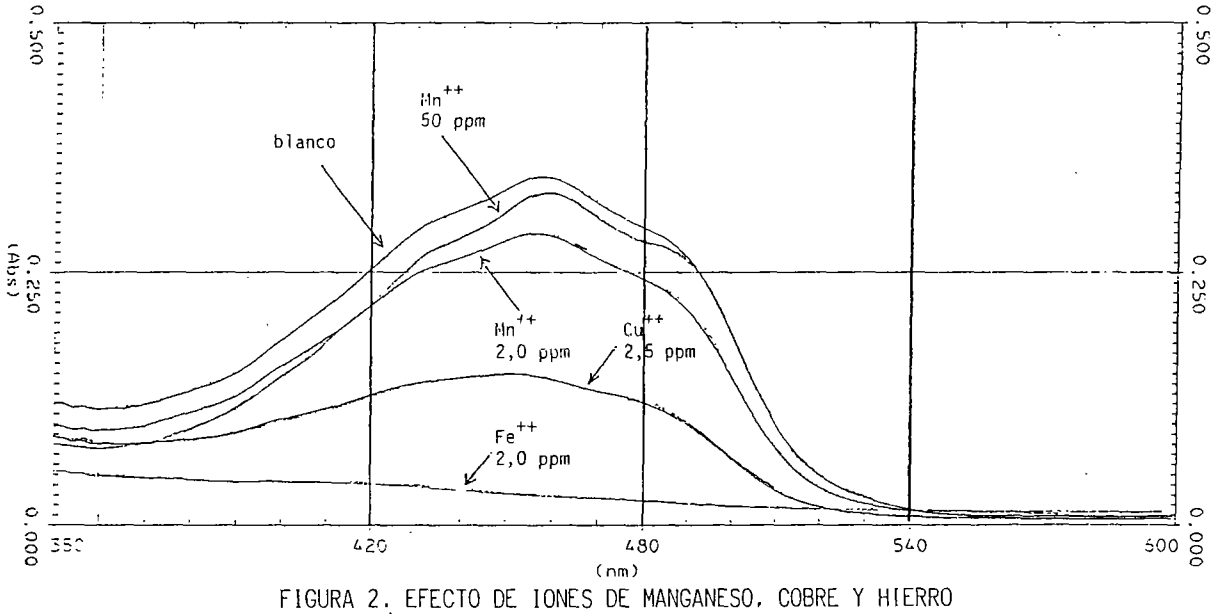


FIGURA 2. EFECTO DE IONES DE MANGANESO, COBRE Y HIERRO SOBRE LOS ESPECTROS DE ABSORCION DE PIGMENTOS DE ESPORAS DE LA ROYA DEL CAFETO. (ESPORAS DOPADAS).

Los pigmentos de esporas de la roya del cafeto deberían ser buenos monitores de daños por oxidación. La figura 2 muestra los cambios que sufren los pigmentos de esporas de roya del cafeto tratadas con dosis similares de Cu II, Fe II y Mn II. Para el manganeso se usó además una dosis elevada de 50 ppm (Ospina y Quintero, 1991).

Se observa cómo el efecto del manganeso es relativamente pequeño y disminuye cuando se aumenta la dosis, mientras que el del cobre es mucho mayor y el del hierro es francamente impresionante. Estos resultados están de acuerdo con las observaciones de los autores que hemos citado y muestran que los fenómenos de oxidación pueden jugar un papel importante en los mecanismos de inhibición de la germinación de esporas de roya por iones metálicos.

Es interesante anotar que en un trabajo reciente se encontró que las esporas de H. vastatrix poseen únicamente una superóxido dismutasa de manganeso, mientras que las hojas de cafetos arábica y de la variedad Colombia disponen de superóxido dismutasas de manganeso, cobre-zinc y hierro (Daza, 1990).

4. Ihbibición de la germinación de esporas de roya del cafeto por iones de cobre y hierro y su interacción con iones de manganeso.

La potente inducción de la oxidación de los pigmentos de la roya del cafeto por iones de hierro II permite suponer que deben inhibir la germinación de esporas de manera similar al cobre.

En el cuadro 1 se comparan las toxicidades de diversos metales en orden decreciente obtenidas en nuestro laboratorio, (DI_{50}), para esporas de la roya del cafeto, con datos de la literatura. Las esporas de la roya del cafeto tienen un comportamiento excepcional:

a) son tanto ó más sensibles al hierro que al cobre
b) son mas bien estimuladas que inhibidas por el níquel incluso a concentraciones de 100 ppm

Cuadro 1. Toxicidad para hongos en orden decreciente de diversos iones.

(1)	Hg › Ag › Fe › Cu › Mn › Zn » Ni
(2)	Ag › Hg › Cu › Cd › Cr › Ni › Pb › Co › Zn › Fe › Ca
(3)	Hg › Ag › Cu › Ni › Mn › Zn

(1) Este trabajo.

(2) Horsfall(1956), Lukens(1971) Citados por Nieboer & Richardson (1980).

(3) Sommers(1959).

En cuanto al manganeso se refiere, su acción sobre la inhibición por el cobre, figura 3 la hemos discutido en otras oportunidades (Quijano-Rico, 1988). Se caracteriza esencialmente por una reversión parcial (de la inhibición). En el caso de la inhibición por el hierro se observa un fenómeno de reversión similar, figura 4.

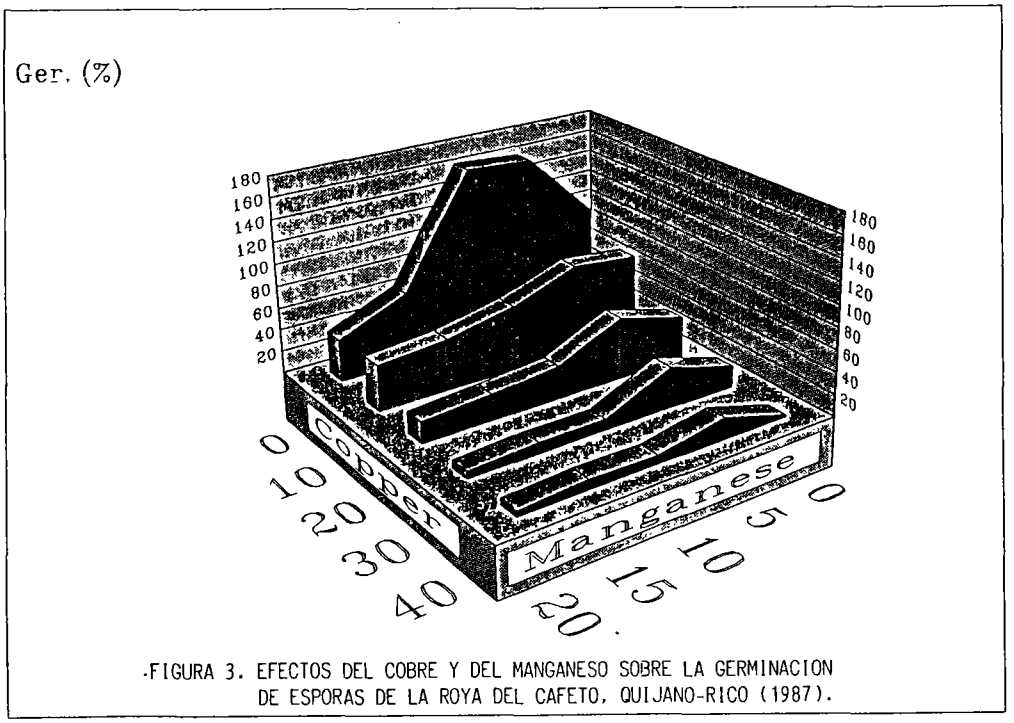


FIGURA 3. EFECTOS DEL COBRE Y DEL MANGANESO SOBRE LA GERMINACION DE ESPORAS DE LA ROYA DEL CAFETO, QUIJANO-RICO (1987).

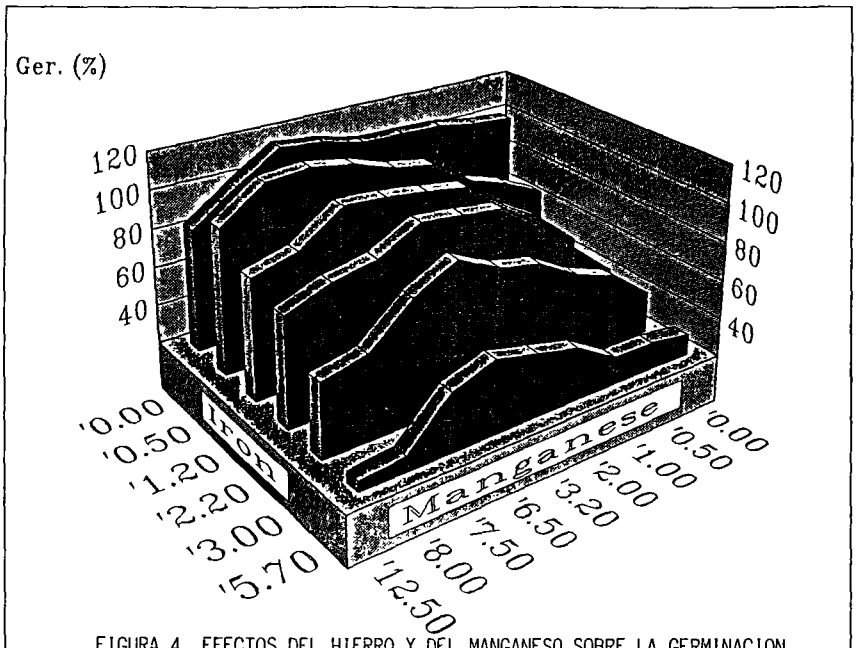


FIGURA 4. EFECTOS DEL HIERRO Y DEL MANGANESO SOBRE LA GERMINACION DE ESPORAS DE LA ROYA DEL CAFETO, ESTE TRABAJO.

La similitud de estos fenómenos permite suponer que el cobre y el hierro actúan por medio de mecanismos y en sitios bastante parecidos.

5. Influencia de iones de cobre y de manganeso sobre las relaciones cafeto-roya.

Los resultados que vamos a describir fueron obtenidos variando las concentraciones de los elementos mencionados en la solución nutritiva de cafetos, de dos meses, cultivados en medio hidropónico, sin soportes sólidos, en condiciones de invernadero.

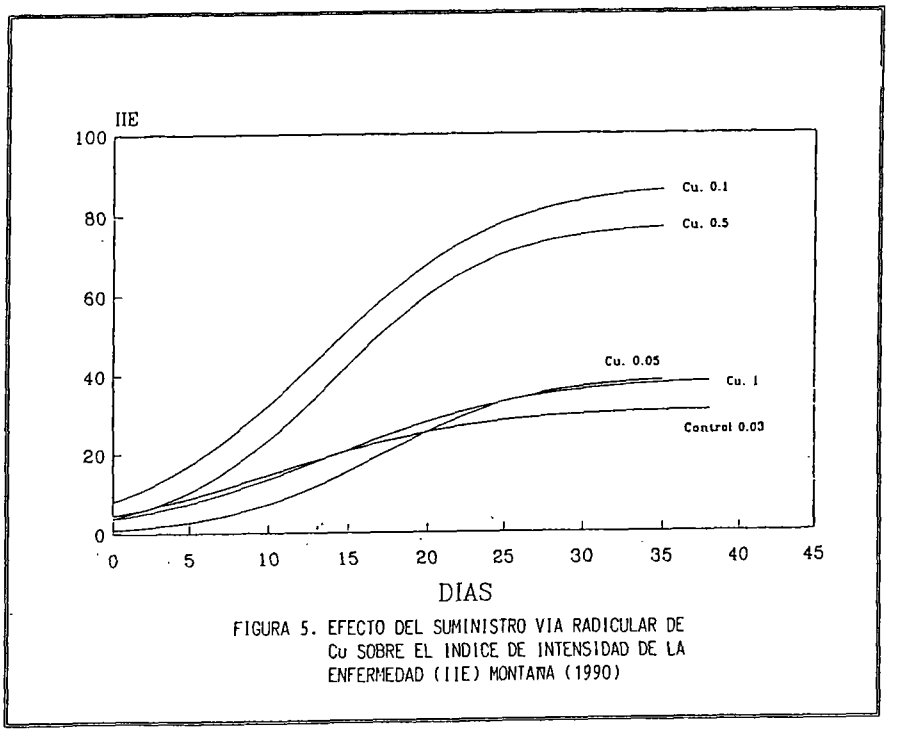


FIGURA 5. EFECTO DEL SUMINISTRO VIA RADICULAR DE Cu SOBRE EL INDICE DE INTENSIDAD DE LA ENFERMEDAD (IIE) MONTAÑA (1990)

En la figura 5 se aprecia el efecto en función del tiempo de diversas concentraciones de Cu II sobre el índice de intensidad de la enfermedad definido por Leguizamón (1985). Las biodisponibilidades naturales hacen al suministro de cobre dificilmente manipulable para disminuir el índice de intensidad de la infección, excepto a concentraciones relativamente elevadas próximas al nivel de fitotoxicidad (Montaña, 1990).

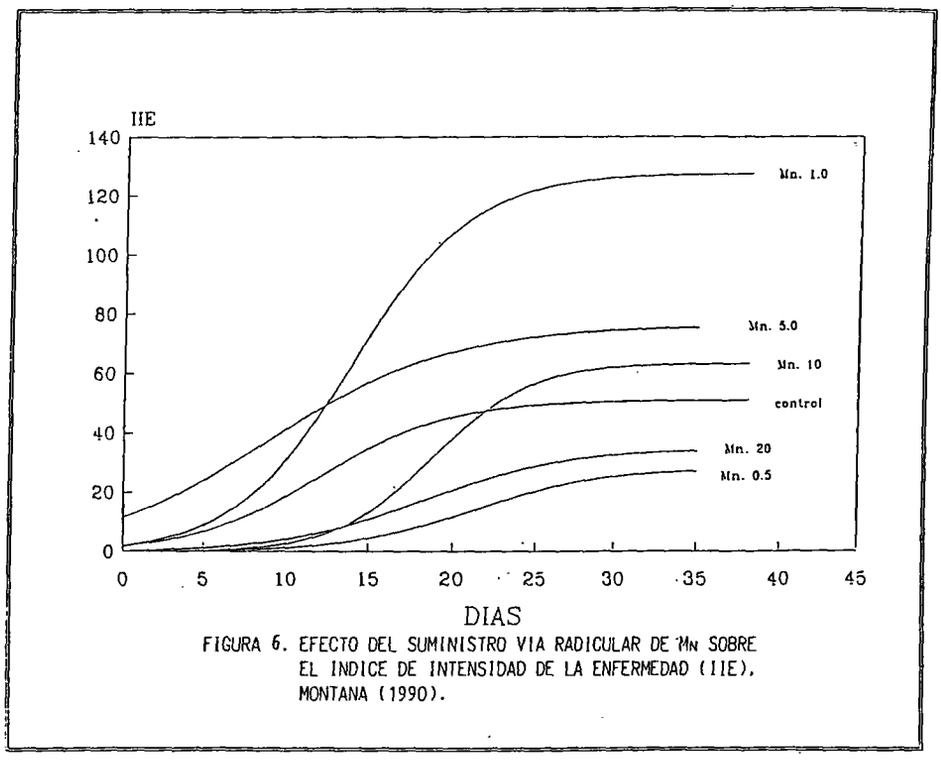


FIGURA 6. EFECTO DEL SUMINISTRO VIA RADICULAR DE Mn SOBRE EL INDICE DE INTENSIDAD DE LA ENFERMEDAD (IIE). MONTANA (1990).

En la figura 6, equivalente a la anterior pero siendo el ión utilizado Mn II, se observa que dosis relativamente altas de manganeso (20 ppm) permiten reducir el índice de intensidad de la enfermedad a valores más bajos que el del control. Desde este punto de vista el manganeso sería un mejor candidato que el cobre para reducir el impacto de la roya del cafeto, por medio de la manipulación nutricional.

En las figuras 7 y 8 se observan fenómenos similares para el caso del rendimiento de germinación. Mientras que para lograr una reducción poco notable con el cobre, las dosis son próximas a las fitotoxicas, con el manganeso lejos de las dosis fitotoxicas se obtienen reducciones apreciables del rendimiento de germinación, de las esporas producidas en plantas dopadas (Montaña, 1990). Estas dosis se pueden incrementar considerablemente aún, debido a la baja toxicidad del manganeso para C. arabica.

FIGURA 7. CONCENTRACION DE Cu II (EN SOLUCION HIDROPONICA) Y RENDIMIENTO DE GERMINACION DE ESPORAS.

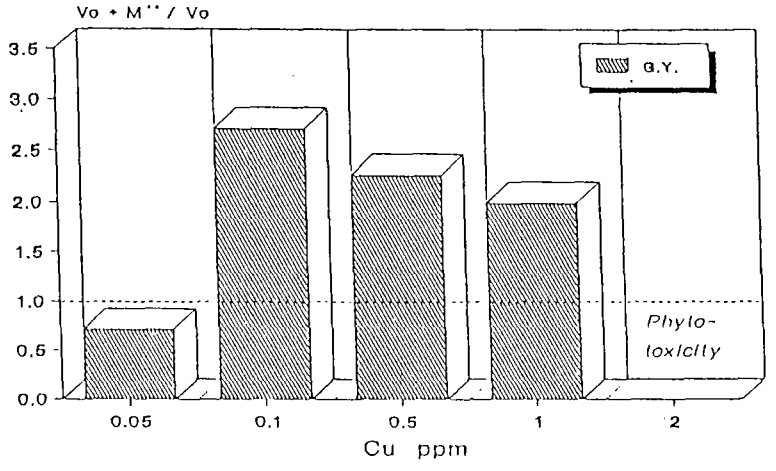
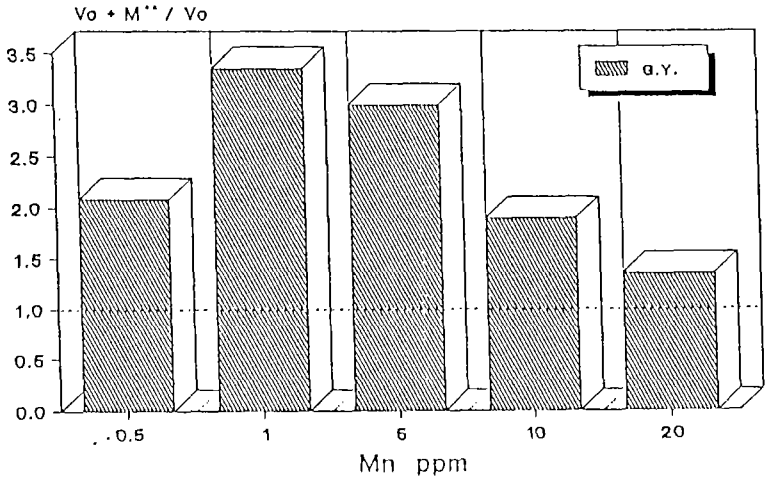


FIGURA 8. CONCENTRACION DE Mn II (EN SOLUCION HIDROPONICA) Y RENDIMIENTO DE GERMINACION DE ESPORAS.



6. Conclusiones

Los resultados que hemos descritos permiten asumir:

a) Las reacciones de oxidación pueden jugar un rol importante en la inhibición de la germinación de esporas de roya del cafeto.

b) Derivados del hierro con menos impacto para el medio ambiente y de costo bastante más bajo que los del cobre, pueden servir de base para un control perfeccionado de la roya del cafeto.

c) Los nutrientes minerales y especialmente el manganeso pueden abrir nuevas perspectivas para el control de la roya del cafeto. Muchos ejemplos del potencial del manganeso con estos fines han sido descritos recientemente en la literatura.

d) La función del silicio en las esporas, en la germinación y en la interacción planta-roya merece más atención por parte de los investigadores.

RESUMEN

En pocas décadas la atención de las actividades de investigación en biología pasó de las células a las moléculas. Vivimos la edad de la biología molecular y ya aparece la biología atómica como una nueva etapa en la comprensión de la vida y de los fenómenos con ella asociados. La biología atómica es sobre todo el campo de trazas de elementos en el interior de sistemas biológicos. Allí controlan fenómenos vitales como la fotosíntesis y otros procesos bioquímicos. Los elementos claves de las plantas son Fe, Mn, Cu y Zn. La función de muchas biomoléculas, por ejemplo metaloenzimas, depende de las propiedades intrínsecas de los iones metálicos con ellas asociados. Algunos elementos trazas se pueden emplear como sondas para conocer sus mecanismos de acción. Usamos en calidad de sistemas de estudio cafetos, esporas de royas y el conjunto cafeto-roya. Se describen nuevas posibilidades para el control de la roya del cafeto.

ABSTRACT

In few decades the attention of research in biology moved from the cell to the molecule. We are living in the age of molecular biology and already atomic biology appears as a new step for the understanding of life and related phenomena.

Atomic biology is mostly the domaine of traces of elements in the interior of biological systems. They control vital phenomena like photosyntesis and other biochemical processes. Key elements of plants are Fe, Mn, Cu and Zn. The function of many biomolecules, metalloenzymes by example, depends of the intrinsic chemical properties of the ions associated with them. Trace elements can be used as probes to know about their action mechanisms. We are doing some works in this direction by doping infected and unifected coffee plants, with coffee rust and coffee rust spores. New possibilities for perfecting the control of coffee rust are described.

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THE VARIATION OF XYLOSE, MANNITOL, AND FREE SUGAR LEVELS IN INSTANT COFFEE

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INTRODUCTION

The transformation of green coffee into roasted coffee into spray dried coffee powder involves a multitude of steps. During those steps, numerous physical and chemical changes take place. Of particular interest in this work are the changes which occur that affect the level of xylose, mannitol, and free sugars in the instant coffee. This has been an area of recent interest to buyers of soluble coffee. It is important to understand the chemical composition of the coffee that is purchased and how the composition is affected by raw materials and processing conditions.

It is well established that the predominant free sugar in green coffee is sucrose. Further, the roasting process destroys more than 90% of the free sugars in the green coffee. It has also been reported that the monosaccharides of arabinose, mannose, and galactose are mainly formed during industrial extraction while glucose and fructose are mainly formed during roasting (1).

Other research has shown the presence of total xylose in instant coffee and has suggested a direct correlation of the xylose level to the level of coffee husks coextracted with the roasted coffee bean (2).

More recently, mannitol, a polyhydric alcohol, has been detected in both sound green coffee up to 0.05% and in dried coffee husks in amounts of 1.61-2.03%. It has also been suggested that the level of mannitol in an instant coffee sample directly correlates to the level of coffee husks coextracted with the roasted coffee bean (3).

CURRENT WORK

Realizing that the coffee cherry is an agricultural commodity and that there is considerable variation in the chemical profile between types of beans, this work was aimed at studying factors which could influence the observed level of xylose, mannitol, and free sugars in an instant coffee product. Specifically, this work focused on the variation that could be accounted for by the level of coffee husks, the type of coffee husks, the green coffee blend, roasting, and extraction conditions. The specific compounds that were studied in this work were total xylose, mannitol, and the combined total of the six free sugars of sucrose, fructose, glucose, galactose, arabinose, and mannose.

ANALYTICAL METHODS

Xylose occurs in coffee as a free sugar only in a trace amount (4). Xylans, though, are present in green coffee and coffee husks and these can be hydrolyzed to form xylose. The following analytical procedure was used. A sample of the green coffee bean, roasted coffee bean, or instant coffee powder is hydrolyzed under reflux conditions with hydrochloric acid. The hydrolyzed solution is cleaned up and injected into an HPLC system equipped with a post column reactor and a UV detector operating at 410 nanometers. The quantity of total xylose is determined directly from the chromatogram against known external standards. This methodology is similar in principle to that proposed by Blanc et al (2).

The determination of mannitol and the free sugars was accomplished by extracting a sample with dimethyl sulfoxide to solubilize the sugars and then silating the liquor with tri-sil concentrate. This causes the free sugars to form a volatile complex which is then analyzed by direct injection into a gas chromatograph. Quantitation is achieved against a known internal standard.

The reproducibility of these methods is shown in Table 1 and Table 2. For the xylose method, both a short term and long term reproducibility study was carried out. For the mannitol/free sugars method, only a long term study was conducted.

Table 1

VARIATION IN XYLOSE METHODOLOGY

Short-Term And Long Term Reproducibility
Study On The Same Coffee Sample

Short-Term (Same Day) Reproducibility		Long-Term (Multiple Days) Reproducibility	
Run Number	Xylose	Date	Xylose
1	0 818%	5/23	0 563%
2	0 763	6/4	0 624
3	0 719	6/4	0 731
4	0 725	6/5	0 802
5	0.725	6/6	0.714
Average = 0 750		Average = 0.687	
Standard Deviation = 0.0418		Standard Deviation = 0 0939	
% Relative Standard Deviation = 5 57%		% Relative Standard Deviation = 13 66%	

Table 2

VARIATION IN MANNITOL AND TOTAL FREE SUGARS METHODOLOGY

Reproducibility On The Same Instant Coffee Sample Over Ten Days

Day Number	Mannitol	Total Free Sugars
1	.3818%	3 2157%
2	.3611	3.2554
3	.3644	3.1619
4	.3564	3.2200
5	.3587	3.1501
6	.3707	3 2179
7	.3772	3.2629
8	.3564	3.1460
9	.3566	3 2492
10	.3759	3 2933
Average =	.3659	3.2172
Standard Deviation =	.0092	.0480
% Relative Standard Deviation =	2.52%	1.49%

EXPERIMENTAL DESIGN

For the laboratory study, a series of extraction runs were made using a laboratory autoclave (Parr Instrument Company, Model Number 4522M, 2000 ml). This autoclave was equipped with a Parr 4842 temperature and speed controller. For each extraction run, 35 grams of coffee were extracted using 700 ml of water for 20-25 minutes. The extraction was carried out in two stages. The first stage extraction, which was intended to simulate fresh end extraction in an industrial system, was accomplished at either 90°C or 135°C. The second stage extraction, which simulated the hydrolysis end of an industrial system, was accomplished at 170°C or 190°C. The resulting liquors from the first stage and second stage were mixed together, freeze dried, and the dried solids were analyzed.

The specific variables that were studied in the laboratory autoclave experiments were: 1) the total level of husks in the blend, 2) the type of husks - arabica or robusta, 3) whether the husks were roasted or not, 4) the green coffee blend, 5) the degree of roast for the green blend, 6) the extraction conditions.

A 2^4 statistical factorial design was used to quantify the contribution of each of the six variables to the total observed variation of the carbohydrates studied. For each variable, a "high" and a "low" value was assigned as shown in Table 3. The statistical design then dictated the appropriate high and low values for each variable for each run that was made.

Table 3

AUTOCLAVE EXPERIMENTAL DESIGN

Variable	High Value	Low Value
Total Level of Husks	75%	25%
Type of Husks	100% Arabica; 0% Robusta	0% Arabica; 100% Robusta
Husks: Raw or Roasted	100% Roasted	0% Roasted
Green Coffee Blend	80% Arabicas; 20% Robustas	20% Arabicas; 80% Robustas
Roast Color	80 Photovolts (Light)	60 Photovolts (Dark)
Extraction Conditions (Hydro Yield)	1st Stage 90°C; 2nd Stage 190°C	1st Stage 135°C, 2nd Stage 170°C

Total level of husks refers to the combined total of arabica husks and robusta husks, raw or roasted, that were mixed with roasted coffee. High levels of coffee husks (much higher than used commercially) were used in order to best quantify the variation. The variable labeled type of husks refer to the percentage of total husks that are either arabicas or robusta. For example, the high value for "type of husks" combined with the low value for "total level of husks" means that only arabica husks were used at 25% of the total blend.

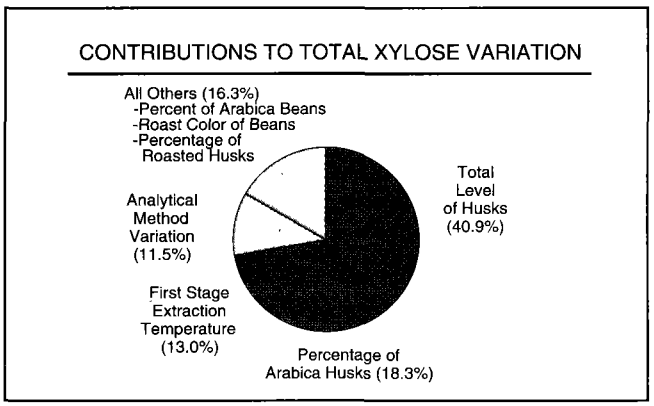
RAW MATERIALS

Sixteen autoclave extractions were carried out using the 2^4 factorial design. For all extraction runs, the same raw materials were used. For the green coffee, a Brazilian natural arabica and a Brazilian Conilon robusta were used. Each coffee was roasted to the desired color in a laboratory roaster. For the coffee husks, a sample of arabica husks and Conilon robusta husks were obtained from Brazil. Each type of husk was also separately roasted in a laboratory roaster.

RESULTS

Total Xylose: Of the observed variation in total xylose, 72.2% can be accounted for by three variables: total level of husks (40.9%), the percentage of arabica husks (18.3%), and the first stage extraction temperature (13.0%). Analytical method variation accounted for 11.5% of the total variation while 16.3% was due to the percent of arabica beans, the roast color of the beans, the percentage of roasted husks, and second order interactions. This is shown in Chart 1.

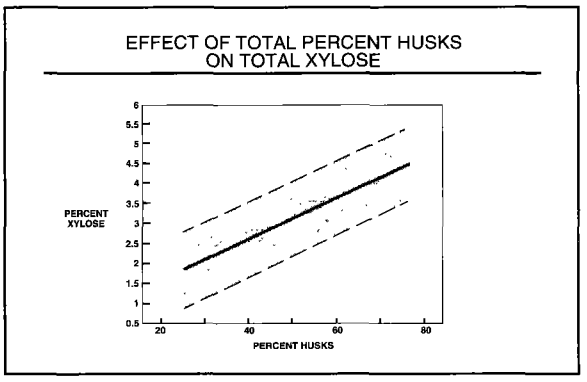
Chart 1



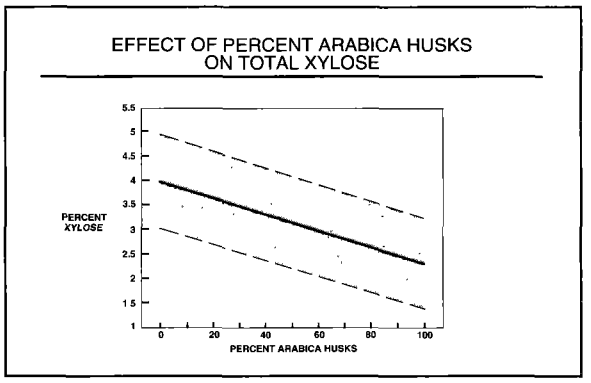
As shown in Graph 1, the total level of husks accounted for 40.9% of the variation and a positive correlation is seen between the level of husks and the level of total xylose. But at any specific level of husks, considerable variation in the level of total xylose was observed which is shown by the dotted lines. These lines represent the 2 sigma confidence intervals for the means. That is, 95% of the average values for total xylose at a constant husks level will fall within the dotted lines.

Graph 2 shows that the level of total xylose is inversely proportional to the percentage of arabica husks which accounted for 18.3% of the variation. Increasing the arabica husks resulted in a decrease in total xylose.

Graph 1

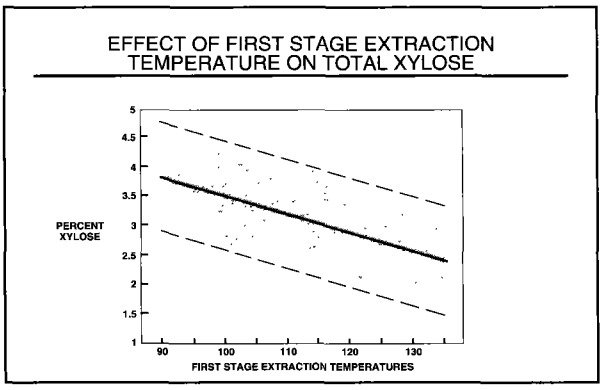


Graph 2



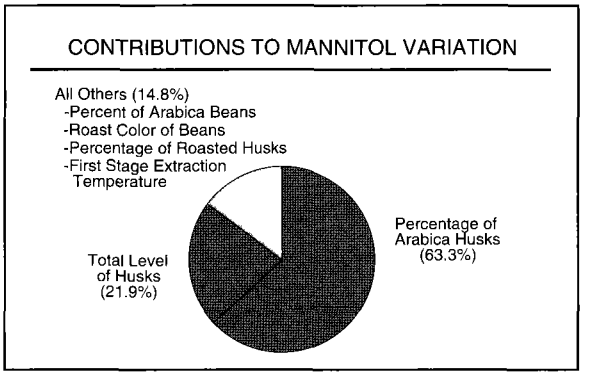
Graph 3 shows that the level of total xylose is inversely proportional to the first stage extraction temperature which accounted for 13.0% of the variation. Increasing the first stage extraction temperature resulted in a decrease in total xylose.

Graph 3



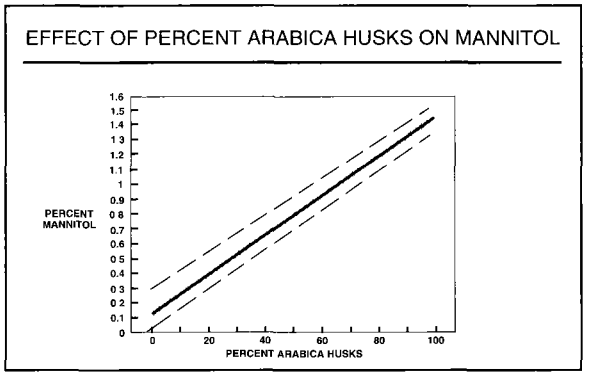
Mannitol: Of the observed variation on mannitol, 85.2% can be attributed to two variables: the percentage of arabica husks (63.3%) and the total level of husks (21.9%). The remaining 14.8% of the variation was attributable to the percentage of arabica beans, the roast color of the beans, the percentage of roasted husks, the first stage extraction temperature, and second order interactions. Analytical method variation was negligible. This is shown in Chart 2.

Chart 2

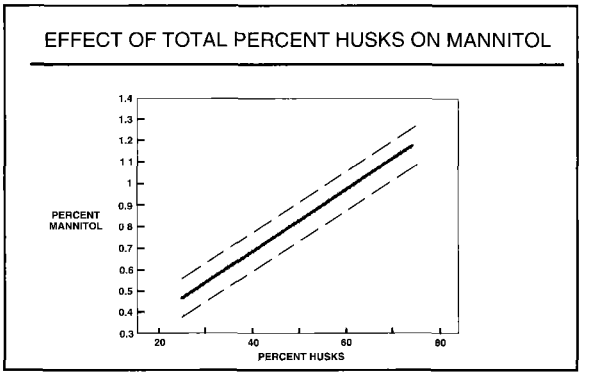


As shown in Graph 4, there is a positive correlation to the percentage of arabica husks and the percent mannitol. Graph 5 shows a positive correlation of total percent husks and percent mannitol.

Graph 4

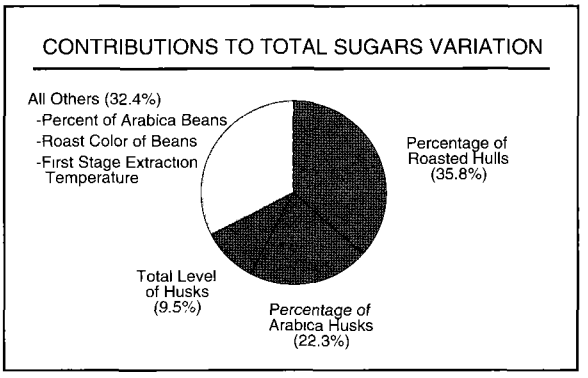


Graph 5



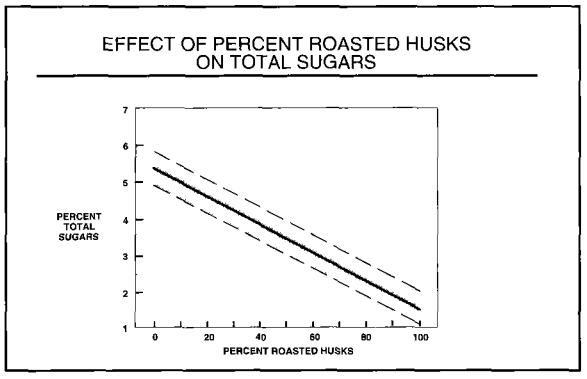
Total Free Sugars: Of the observed variation in total free sugars, 67.6% can be attributed to three variables: the percentage of roasted hulls (35.8%), the percentage of arabica husks (22.3%), and the total level of husks (9.5%). The remaining 32.4% of the variation is due to the percentage of arabica beans, the roast color of the beans, the first stage extraction temperature, and second order effects. Analytical method variation was negligible. This is shown in Chart 3.

Chart 3

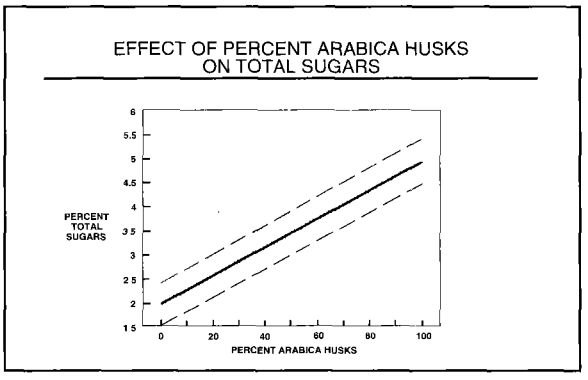


As shown on Graph 6, an inverse relationship exists between the percentage of roasted husks and the total sugars. Roasting of the husks destroys free sugars. Graph 7 shows that increasing the percentage of arabica husks increases total sugars. Graph 8 shows that increasing the total percentage of husks also increases total sugars.

Graph 6



Graph 7



Graph 8

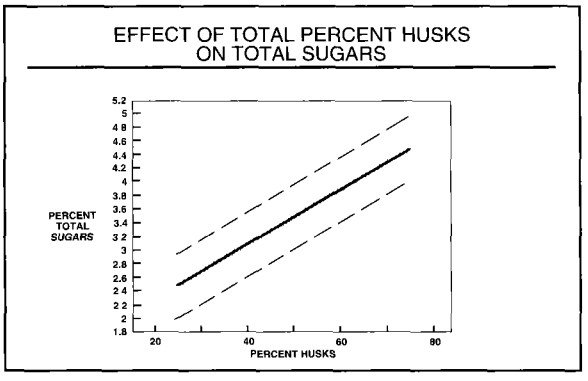


Table 4 summarizes the key learnings obtained from the autoclave study. Note that the effects seen on total free sugars were the same as seen on free fructose and free glucose.

Table 4

SUMMARY OF AUTOCLAVE STUDY

	Increasing Total Level of Husks	Increasing Percentage of Roasted Husks	Increasing Percentage of Arabica Husks	Increasing Percentage of Arabica Beans	Roasting Darker	Increasing First Stage Extraction Temperature
Xylose	Increases	Same	Decreases	Same	Same	Decreases
Mannitol	Increases	Same	Increases	Same	Same	Same
Total Free Sugars	Increases	Decreases	Increases	Same	Same	Same
Fructose	Increases	Decreases	Increases	Same	Same	Same
Glucose	Increases	Decreases	Increases	Same	Same	Same

OTHER COFFEE HUSKS

While all of the autoclave studies were carried out using the same coffee husks, analyses were made on husks from different sources to examine the range that may be observed in total xylose, mannitol, glucose, and fructose. This is summarized in Table 5.

Table 5

RAW COFFEE HUSKS

	Arabicas			
Source	Xylose	Mannitol	Glucose	Fructose
Guatemala	6.755	1.132	1.180	2.889
Brazil - Esp. Santo	3.573	0.198	0.235	0.553
Brazil - Sao'Paulo	3.600	1.199	1.705	2.886
Brazil - Parana'	4.263	1.348	1.508	2.866
Brazil - Mina Gerais Zona Da Mata	3.002	0.673	1.219	2.172
Kenya Mbuni	8.376	0.255	0.707	1.840
Brazil -Mina Gerais Varghinia	2.584	0.953	1.234	2.505
Brazil	3.014	1.502	1.723	3.997
Average	4.3959	0.9075	1.1890	2.4635
Range	2.6-8.4	0.2-1.5	0.2-1.7	0.5-4.0
	Robustas			
Source	Xylose	Mannitol	Glucose	Fructose
Brazil - Rondonia	5.052	0.068	0.060	0.068
Brazil - Esp. Santo	2.799	0.141	0.131	0.392
Brazil	5.187	0.214	0.326	0.914
Average	4.3460	0.1409	0.1723	0.4580
Range	2.8-5.2	0.07-0.21	0.06-0.33	0.07-0.91

COMPARISON OF AUTOCLAVE EXTRACTION TO INDUSTRIAL EXTRACTION

In order to validate the autoclave extraction technique, a comparison was made of the carbohydrate level of coffee extracted with the autoclave to coffee extracted on an industrial scale. Four different coffee samples which had been processed through an industrial extractor were extracted in the autoclave. The autoclave extracted solids were then compared to the industrial spray dried powder. As shown in Table 6, the autoclave resulted in 48% of the xylose, 73% of the mannitol, and 80% of the total free sugars as compared to the industrial extractor.

Table 6

AUTOCLAVE EXTRACTION VERSUS INDUSTRIAL EXTRACTION

Four Parallel Extractions

	Xylose		Mannitol		Total Sugars	
	Mean	Range	Mean	Range	Mean	Range
Autoclave	0.2535	0.11-0.41	0.0294	0.02-0.04	1.5261	1.3-1.6
Industrial	0.5270	0.43-0.56	0.0406	0.03-0.05	1.9098	1.87-1.93
Ratio (Autoclave/ Industrial)	0.48		0.73		0.80	

INDUSTRIAL SAMPLES

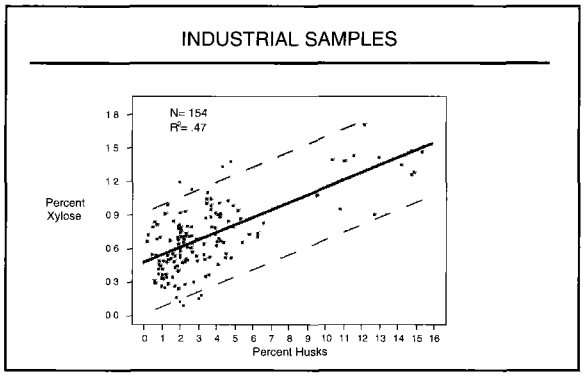
Spray dried instant coffee made on industrial extractors has been analyzed routinely over the past three years. A rather large data base has been generated correlating the percent of total xylose to the total percent husks as well as the percent mannitol to the percent husks.

Graph 9 shows that a positive relationship exists between the total percent husks and the percent total xylose. The number of individual samples in this data base is 154. As indicated by the value for R^2, 47% of the observed variation is accounted for by a variation in the percentage of husks. The dotted lines indicate the 95% confidence interval for any single sample.

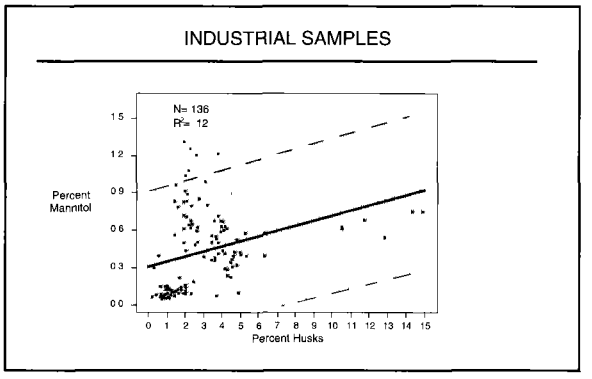
Graph 10 shows that a positive correlation exists between the percent mannitol and the percent husks. The data base includes 136 samples. The R^2 value is 0.12. Only 12% of the observed variation is due to variation in the total level of husks. The dotted lines are the 95% confidence intervals for a single sample.

This level of variation is significant. For example, at a 1% husks level, xylose was observed to vary from 0.1 to 1.0% while mannitol varied from 0.1 to 0.9%. This high degree of variation implies that an intimate knowledge of raw materials and processing conditions is needed prior to establishing any product limits.

Graph 9



Graph 10



SUMMARY

A laboratory autoclave was used to extract a mixture of roast & ground coffee and coffee husks while varying the percentage of husks, the type of husks, the roasting of the husks, the roast color of the coffee, the green coffee blend, and the extraction conditions. The effect that these variables had on the observed levels of total xylose, mannitol, and free sugars was studied.

Increasing the total percentage of husks will increase the level of total xylose, mannitol, and free sugars. Increasing the level of roasted husks, or substituting arabica husks for conilon husks, can have a mixed effect on these carbohydrates. Varying the first stage extraction temperature affected only the total xylose level. The green coffee blend and the degree of roast of the green blend had no significant effect on these carbohydrates.

Using an autoclave extractor to simulate an industrial system requires the use of a correction factor to properly predict the industrial values for total xylose, mannitol, and free sugars. In this work, the autoclave yielded 48% of the total xylose, 73% of the mannitol, and 80% of the free sugars as compared to the industrial system.

For industrial samples, the correlation of total percent coffee husks to percent xylose accounts for 47% of the total observed variation in xylose. The correlation of total percent coffee husks to percent mannitol accounts for 12% of the total variation seen in mannitol. The remainder of the variation is due to seasonal variations, coffee types, and processing conditions.

RÉSUMÉ

On s'est employé un marmite à laboratoire pour extraire une combination du café torréfié et mélangé et les cosses du café en variant le pourcentage, le type, la torréfaction des cosses, le couleur de la torréfaction du café, le mélange du cafe vert et les conditions de l'extraction. On a étudié l'effet que ces variables ont eu sur les niveaux observés du xylose, du mannitol et des sucres libres totaux.

En augmentant le pourcentage total de cosses augmentera le niveau de xylose, du mannitol et des sucres libres totaux. En augmentant le niveau des cosses torréfiees, ou en remplacant les cosses arabicas pour les cosses conilons peut avoir un effet mixte dans ces carbohydrates. La variatin du temperature du premier niveau de l'extraction ne s'est effectué que le niveau total du xylose. Le mélange du café vert et le degré de la torréfaction du mélange vert n'a eu aucun effet important sur les carbohydrates.

En employant un extracteur d'un marmite pour simuler un sistème d'extrait, on doit employer un facteur de correction pour prédire proprement les valeurs industrielles du xylose total, du mannitol et des sucres libres. Dans cette épreuve, le marmite a rendu 48 pour cent du xylose total, 73 pour cent du mannitol, et 80 pour cent des sucres libres en comparaison du sistème industrielle.

Pour les échantillons industrielles, la correlation du pourcentage total des cosses du café au pourcentage total du xylose explique la variation totale observée de 47 pour cent du xylose. La correlation du pourcentage total des cosses du café compare au pourcentage du mannitol explique le 12 pour cent de la variation totale vu dans le mannitol. Le reste de la variation est par suite des variations des saisons, des types du café et des conditions du procés.

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DETERMINATION OF CARBOHYDRATES IN SOLUBLE COFFEE BY HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION

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INTRODUCTION

It has been recognised that differences in processing conditions as well as fraudulent addition of coffee substitutes greatly influence the free and total carbohydrate profile of soluble coffee (1-4). The HPLC (1-3), GC (3) and enzymatic (4) methods used so far for carbohydrate determination in soluble coffee are limited either by insufficient resolution, by complex sample preparation or by specific enzyme availability. The present study describes a simple HPLC procedure in which all relevant carbohydrates are separated in one single run on a special anion-exchange resin and detected by pulsed amperometric detection. Results on representative samples are compared with those obtained by the previously published methods.

EXPERIMENTAL

Materials

Samples were commercial products purchased in different countries. In addition, two samples were pure instant coffees deliberately adulterated with 3 % chicory and 3 % cereals respectively. The method for the preparation of these adulterated coffees is described elsewhere (4).

Sample preparation

Free carbohydrates. Instant coffee (300 mg) was dissolved in 100 mL of demineralised water. About 10 mL of the solution were filtered through a Sep-Pak C18 cartridge (Waters). The filtrate was passed through a 0.2 µm filter (Sartorius) prior to injection.

Total carbohydrates - strong hydrolysis. Instant coffee (300 mg) was allowed to react with 50 mL 1.00 N hydrochloric acid in a boiling water bath for 150 minutes. The solution was cooled to room temperature, diluted to 100 mL with demineralised water and filtered through folded filter paper. The filtrate (3 mL) was filtered through a OnGuard-Ag cartridge (Dionex). The neutralised solution was filtered through a 0.2 µm filter prior injection.

Total carbohydrates - mild hydrolysis. The same procedure as above was used but hydrolysis was performed at 60°C for 60 minutes.

Apparatus

Liquid chromatograph. BioLC system (Dionex) consisting of a Model GPM-II quarternary gradient pump (Dionex); Model SP 8875 autosampler (Spectra-Physics) fitted with a 20 µL loop; Model EDM-II eluant degas module (Dionex); reagent reservoir for NaOH postcolumn addition (Dionex); Model PAD-II pulsed amperometric detector (Dionex); Model AutoIon AI-450 chromatography data station.

HPLC column. CarboPac PA1 (10 µm, 250 x 4 mm) analytical column (Dionex); CarboPac PA precolumn (Dionex).

Chromatographic conditions. Degassed demineralised water was pumped at 1.0 mL/min for 50 min to elute the carbohydrates, 300 mM NaOH pumped at 1.0 mL/min for 15 min to clean the column and demineralised water pumped at 1.0 mL/min for 15 min to reequilibrate the column. Total cycle time per sample was 80 min. Mobile phase and column were maintained at room temperature. A solution of 300 mM NaOH was added postcolumn at 0.6 mL/min to the PAD electrochemical cell. The same solvent was used to fill up the reference electrode. The potentials and pulse durations applied to the gold working electrode were E_1 = + 0.05 V (300 ms), E_2 = + 0.60 V (120 ms) and E_3 = - 0.80 V (300 ms).

RESULTS AND DISCUSSION

Evaluation of the method

In the last few years, high-performance anion-exchange (HPAE) chromatography with pulsed amperometric detection (PAD) has been applied for the determination of carbohydrates in a wide range of foods. The technique has been reviewed (5-6). Briefly, the unique resolution capacity of a pellicular anion-exchange polystyrene-divinylbenzene resin is combined with the sensitivity, specificity and reliability of an amperometric detector in which a repeating sequence of three applied potentials is used to constantly regenerate the surface of a gold working electrode.

Carbohydrate analysis by HPAE-PAD is usually performed in an alkaline medium using sodium hydroxide as mobile phase. Under these conditions all important monosaccharides and some sugar alcohols can be completely separated. However, sucrose elutes between xylose and mannose when very dilute NaOH is used. At higher pH, sucrose is well resolved but the two monosaccharides coelute. These elution patterns are obviously not favourable to establish the carbohydrate profile of instant coffee, in which all three sugars are present simultaneously.

We found (Figure 1) that the most important simple carbohydrates present in instant coffee can be completely separated in less than 50 min by elution with pure water. Detector response of each individual carbohydrate was linear over concentrations ranging from 0,5 (mannitol) - 4,0 (sucrose, fructose) to 2000 µg/mL. The detection limit measured on a standard solution and defined arbitrarily as the amount of product on the column that produces a signal/noise ratio of 5, lay around 10 ng for mannitol, 20 ng for glucose and xylose, and 40 ng for sucrose and fructose. Finally, the precision of the method was assessed by the repeatability coefficients of variation (CV_r) for six replicate injections of the same mixed standard solution. CV_r varied from 0.08 % (arabinose) to 0.70 % (fructose).

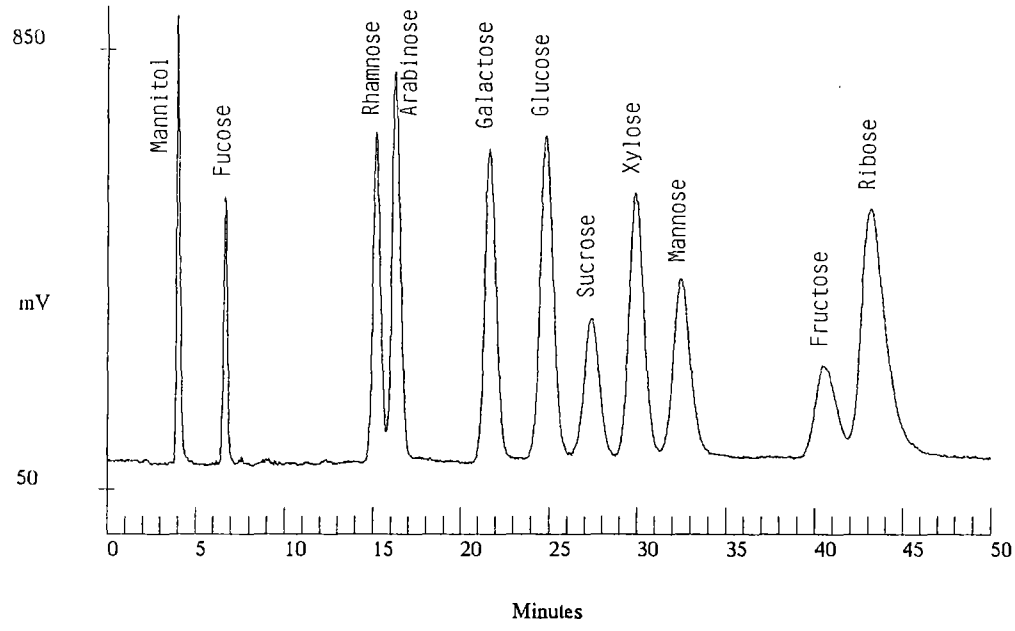

Figure 1. HPAE-PAD chromatogram of a mixed standard solution. Mannitol (15 ppm), fucose (15 ppm), rhamnose (35 ppm), arabinose (40 ppm), galactose (50 ppm), glucose (55 ppm), sucrose (45 ppm), xylose (55 ppm), mannose (45 ppm), fructose (90 ppm), ribose (90 ppm).

Recovery experiments

Recoveries were assessed by adding known amounts of each carbohydrate to a pure soluble coffee and by submitting the spiked sample to the same treatment as ordinary samples (either simple dissolution or strong hydrolysis). Recoveries of carbohydrates varied from 97 to 103 % without hydrolysis. After hydrolysis, recoveries ranged from 90 to 98 %, except in the case of fructose for which it was very poor (about 30 %), indicating an intense degradation of the monosaccharide under strong acidic conditions. When pure carbohydrates were hydrolysed separately, recoveries of monosaccharides varied from 82 to 89 %. Recoveries of fructose and mannitol were respectively 25 % and 98 %. This indicates a relatively strong matrix effect by the soluble coffee on the hydrolysis.

Analysis of instant coffee

Carbohydrates were determined according to the described method (HPAE-PAD) in 18 commercial instant coffees from different origins and in two deliberately adulterated samples. The free carbohydrate profile was obtained after a simple dissolution of the product in water and clean-up with a C18 disposable cartridge. The total carbohydrate profile was measured after a strong hydrolysis of the product with 1N HCl. Subsequent neutralisation was achieved by filtration through an anion-exchange disposable cartridge. Table 1 compares the results obtained on six representative samples with those determined by an enzymatic procedure (4) and by a different HPLC method (1) in which carbohydrates are separated on an amino-bonded phase and spectrophotometrically detected after post-column derivatization with tetrazolium blue (HPLC-TTB).

Table 1. Determination of carbohydrates in instant coffee[a].

Carbohydrate	Method	Coffee A		Coffee B		Coffee C		Coffee D		Coffee E		Coffee F	
		Free	Total	Free	Total	Free	Total	Free	Total[b]	Free	Total	Free	Total
Mannitol	HPAE-PAD	0.03	0.17	0.02	0.18	1.17	1.13	ND[c]	0.03	0.02	0.16	0.07	0.11
Fucose	HPAE-PAD	ND	ND	ND	ND	0.02	0.04	ND	ND	ND	ND	ND	ND
Rhamnose	HPAE-PAD	ND	0.35	0.05	0.32	0.08	0.64	ND	ND	0.03	0.34	0.03	0.16
Arabinose	HPAE-PAD	1.07	3.05	0.93	2.32	0.77	3.49	1.07	2.25	1.11	2.97	0.10	1.50
	HPLC-TTB	0.94	3.75	0.73	2.70	0.62	3.90	0.91	2.76	0.91	3.55	0.10	1.49
Galactose	HPAE-PAD	0.34	18.3	0.55	19.3	0.46	10.3	0.33	0.69	0.33	17.7	0.09	8.82
	HPLC-TTB	0.31	17.6	0.44	16.0	0.37	8.75	0.30	0.68	0.31	14.8	0.12	7.52
Glucose	HPAE-PAD	0.06	0.85	0.09	0.76	0.73	3.10	0.15	0.43	0.04	3.41	0.68	42.5
	HPLC-TTB	0.07	0.77	0.15	0.59	0.59	3.02	0.15	0.69	0.07	3.30	0.77	40.4
	ENZYM.	0.03	0.74	0.09	0.67	0.82	2.86	0.14	0.45	0.03	3.17	0.76	41.4
Sucrose	HPAE-PAD	0.07	ND	ND	ND	0.18	ND	0.24	ND	0.06	ND	3.58	ND
	ENZYM.	0.06	ND	ND	ND	0.03	ND	0.31	ND	0.11	ND	4.01	ND
Xylose	HPAE-PAD	ND	0.14	ND	0.08	0.10	3.15	ND	0.03	ND	0.16	ND	0.17
	HPLC-TTB	ND	0.12	ND	0.09	ND	2.21	ND	0.06	ND	0.13	ND	0.15
Mannose	HPAE-PAD	0.16	15.3	0.56	18.0	0.26	4.34	0.19	0.30	0.18	14.8	0.15	4.29
	HPLC-TTB	0.20	14.2	0.45	16.2	0.25	3.59	0.21	0.71	0.20	13.2	0.15	3.55
	ENZYM.	0.15	14.2	0.52	17.7	0.30	3.82	0.16	0.75	0.17	13.6	0.06	3.87
Fructose	HPAE-PAD	ND	ND	0.14	0.11	0.87	0.35	0.47	2.19	ND	0.06	0.19	0.88
	HPLC-TTB	0.05	0.14	0.14	0.11	0.91	0.24	0.52	1.99	0.06	0.14	0.36	0.70
	ENZYM.	0.08	0.01	0.15	0.07	1.11	0.20	0.63	2.29	0.07	0.03	0.36	0.64
Ribose	HPAE-PAD	0.02	0.09	0.15	0.06	ND	0.16	ND	0.11	0.04	0.07	ND	ND

[a] Results are expressed in % on the product as is and are averages of duplicate determinations
[b] Mild hydrolysis
[c] Not detected

As only reducing sugars are derivatized with tetrazolium blue, the determination of sucrose and mannitol is precluded by HPLC-TTB. Furthermore, the method does not allow a precise analysis of fucose, rhamnose and ribose. On the other hand, the availability of pure specific enzymes limits enzymatic determination to glucose, fructose, sucrose and mannose only. However, a complete separation and the detection of all these carbohydrates was achieved by HPAE-PAD.

For those carbohydrates where a comparison was possible, the results obtained by all three methods were in close agreement even if different hydrolysis conditions were used in HPLC-TTB (1N H_2SO_4 / 100°C / 4h).

The precision of HPAE-PAD was superior to that of the other two methods. The mean repeatability (r) of the measurements, calculated from the differences between the duplicates, were 0,03 % for free carbohydrates and 0,11 % for total carboyhdrates, whereas the mean repeatability coefficients of variation (CV_r) were 2,23 and 1,70 % respectively. HPLC-TTB and enzymatic determinations were respectively three and two times less precise than HPAE-PAD.

Five different types of instant coffee could be clearly distinguished from the figures. Samples A and B were characterised by low free carbohydrate levels and high amounts of total galactose and mannose. According to Blanc *and Coll.* this profile is typical for pure soluble coffee (1). The chromatograms for sample B are shown in Figures 2 and 3.

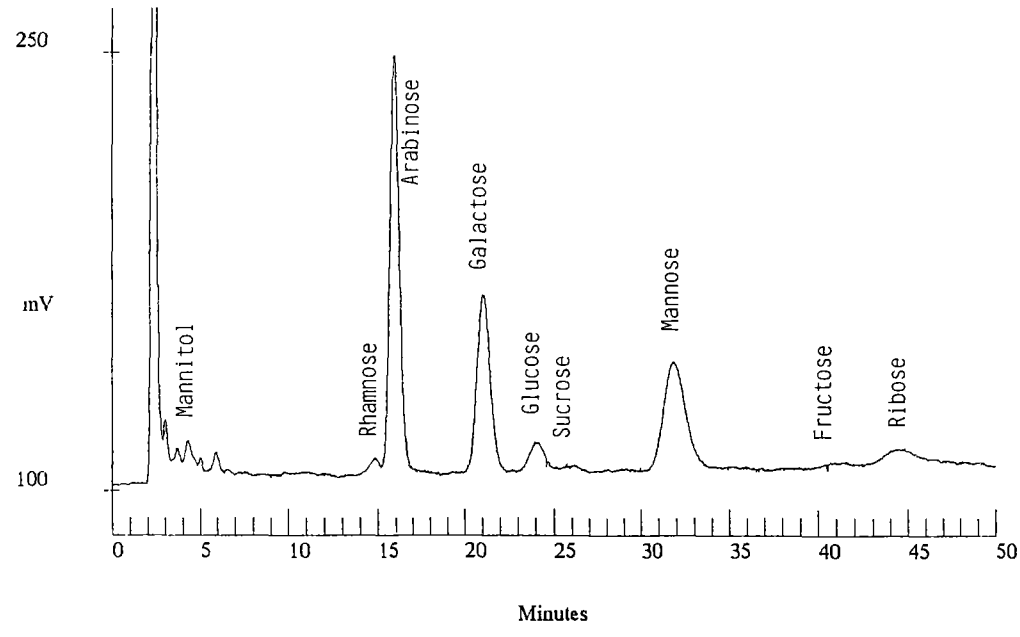

Figure 2. HPAE-PAD free carbohydrate profile of a pure soluble coffee (sample B).

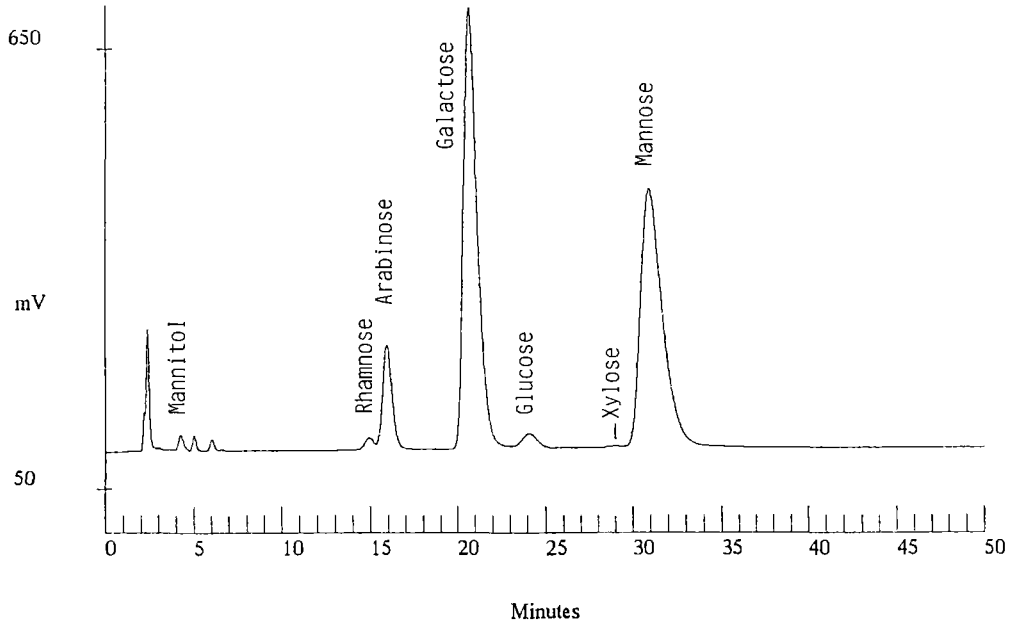

Figure 3. HPAE-PAD total carbohydrate profile of a pure soluble coffee (sample B) after strong hydrolysis.

Sample C presented high levels of free mannitol, free glucose, free fructose, total glucose and total xylose. This profile is typical for coextraction of coffee with up to 25 % unroasted coffee husks and/or parchments (1,2). Figure 4 shows the chromatogram obtained after strong hydrolysis of sample C.

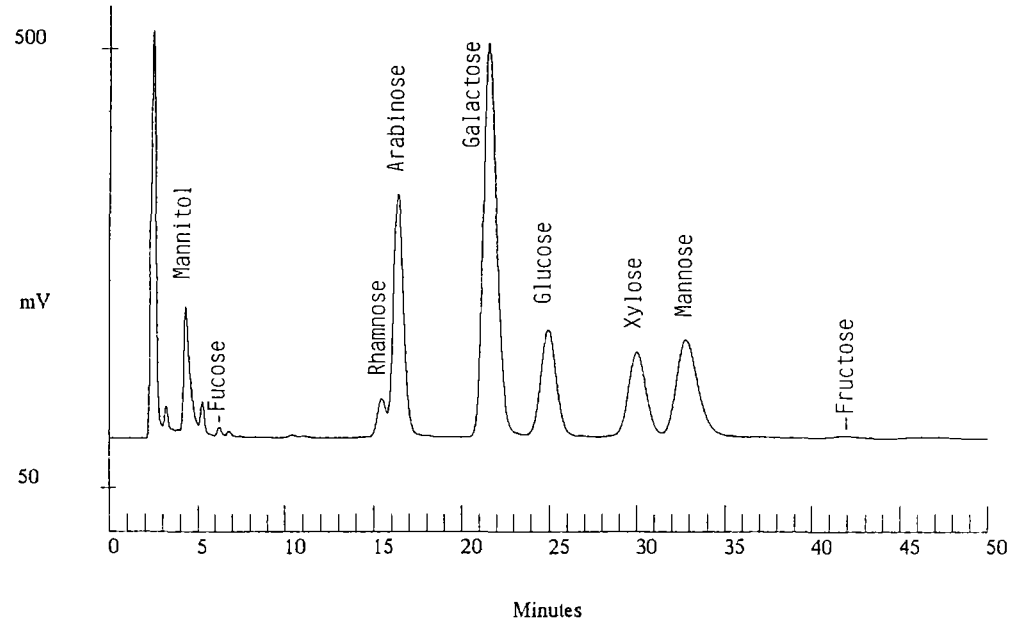

Figure 4. HPAE-PAD total carbohydrate profile of a soluble coffee containing coffee husks/parchments (sample C) after strong hydrolysis.

When pure coffee A was deliberately adulterated with 3 % chicory and 3 % cereals, the resulting mixtures (samples D and E respectively) presented a free carbohydrate profile very similar to the original coffee. However, submitting sample D to a mild hydrolysis provoked a complete depolymerisation of the chicory inulin (4) and generated high amounts of total fructose (Figure 5). On the other hand, strong hydrolysis of sample E formed high levels of total glucose due to the degradation of cereal starch (Figure 6).

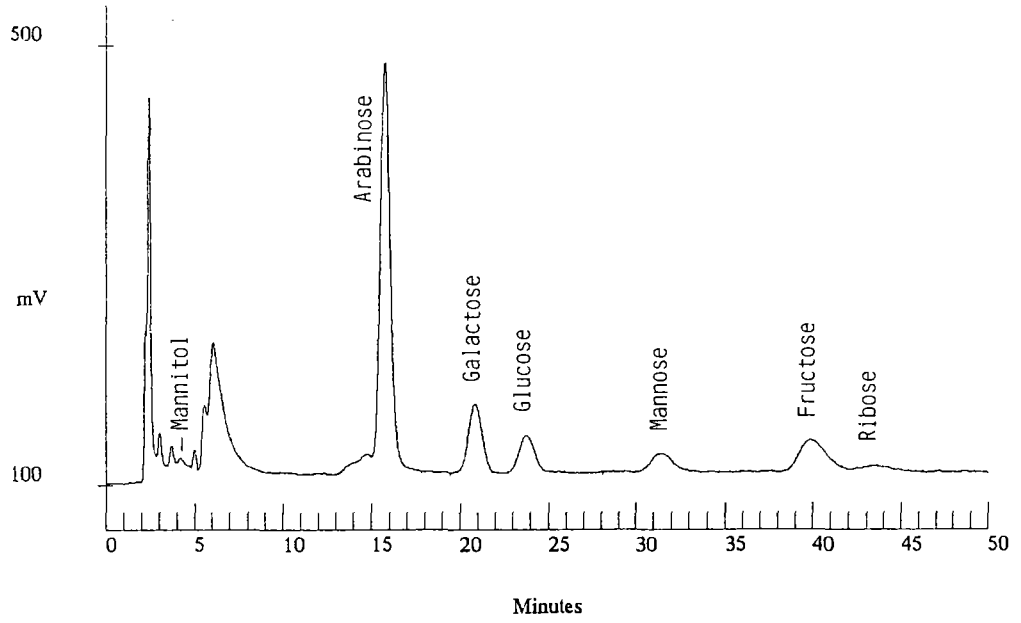

Figure 5. HPAE-PAD total carbohydrate profile of a soluble coffee containing 3 % chicory (sample D) after mild hydrolysis.

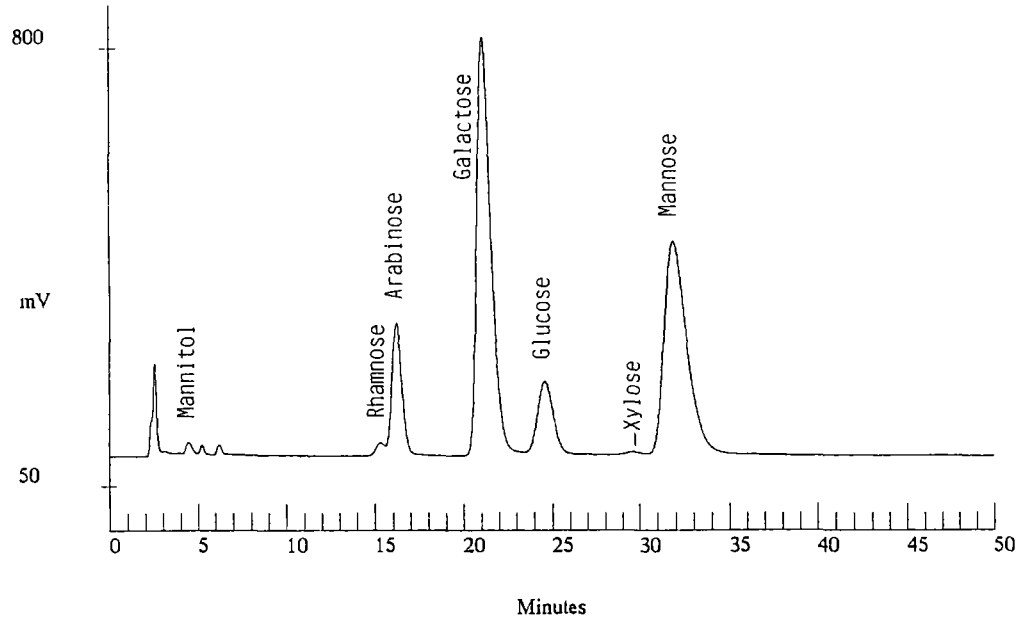

Figure 6. HPAE-PAD total carbohydrate profile of a soluble coffee containing 3 % cereals (sample E) after strong hydrolysis.

Finally, products containing added maltodextrins were also recognised. Their total carbohydrate profile (Figure 7) exhibited very large glucose peaks formed by the maltooligomer hydrolysis. Sample F was a typical example of such adulteration.

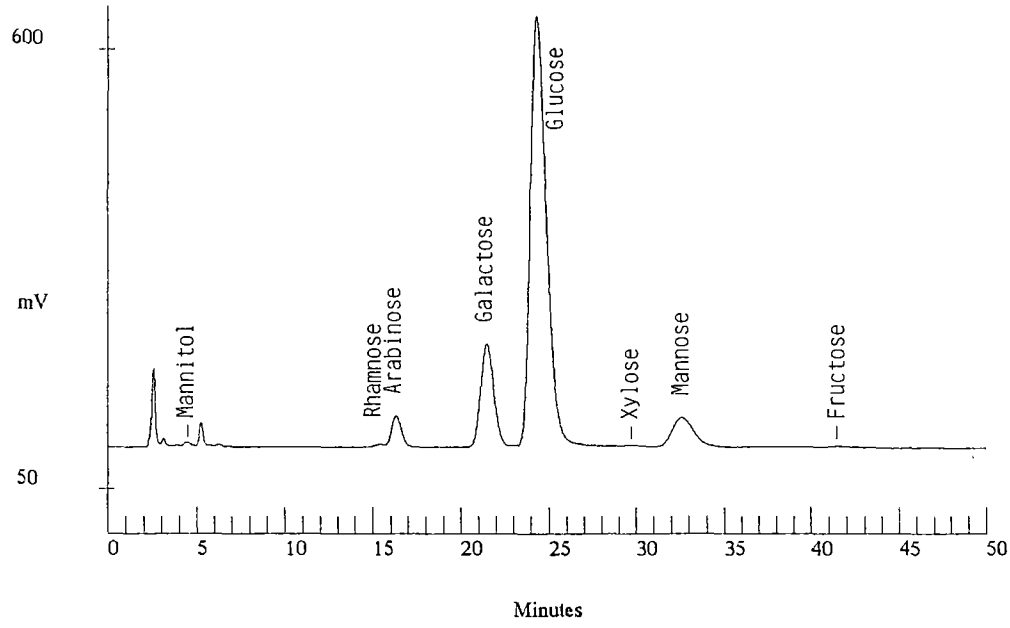

Figure 7. HPAE-PAD total carbohydrate profile of a soluble coffee containing maltodextrins (sample F) after strong hydrolysis.

CONCLUSIONS

The HPAE-PAD method, as described in this study, allows the determination of all relevant carbohydrates present in soluble coffee in one single run. The technique is simple, precise and sensitive. It is therefore a very powerful tool for routine analysis and for purity assessment of soluble coffee.

ACKNOWLEDGMENT

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SUMMARY

Simple carbohydrates present in soluble coffee can be easily separated on a pellicular anion-exchange resin and detected by pulsed amperometry. Results on representative samples compared very well with those obtained by an enzymatic or a different HPLC procedure. The technique allows the simple, precise and sensitive quantification of all relevant carbohydrates in one single run. It is therefore a very powerful tool for purity assessment of soluble coffee.

RESUME

Les glucides simples présents dans le café soluble peuvent aisément être séparés sur une résine échangeuse d'anions pelliculée et détectés par amperométrie pulsée. Les résultats sur des échantillons représentatifs sont tout à fait comparables à ceux obtenus par une analyse enzymatique ou par une méthode HPLC différente. La technique permet une quantification simple, précise et sensible de tous les glucides simples importants en une seule injection. C'est donc un outil très puissant pour le contrôle de la pureté du café soluble.

ISOMERS OF QUINIC ACID AND QUINIDES IN ROASTED COFFEE : INDICATORS FOR THE DEGREE OF ROAST ?

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Speaking about quinic acid in coffee, we think of (-)-quinic acid, because it is known to occur free or bound, mainly as chlorogenic acids. The sum of free and bound quinic acid in green coffee makes up around 4% on dmb.

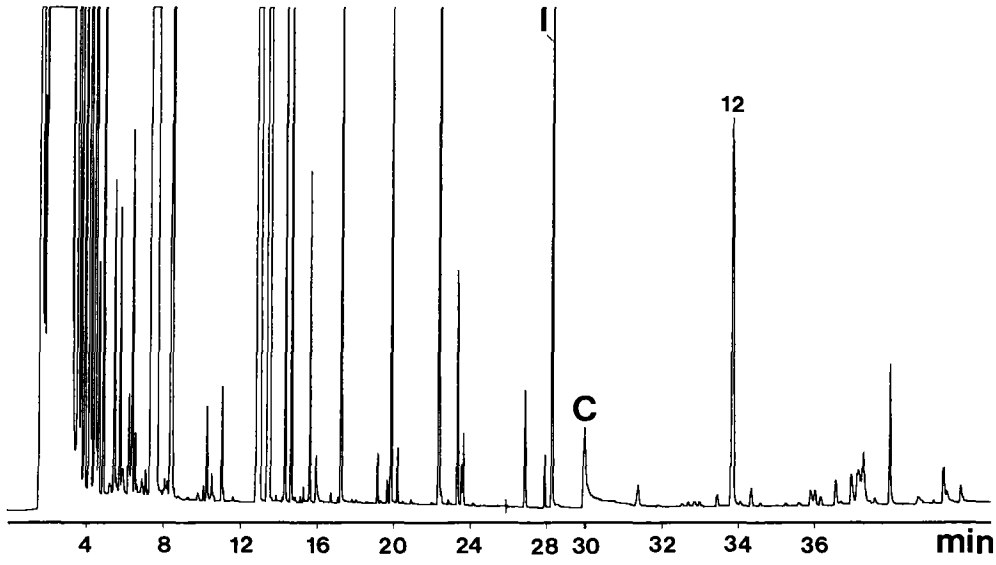
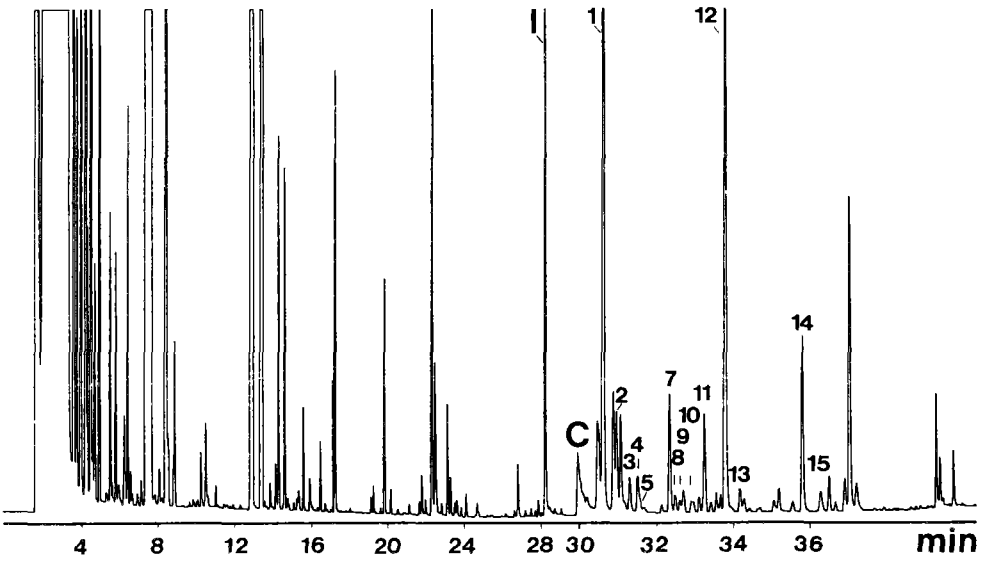


Fig.1. Gas chromatogram of a trimethylsilylated ethanolic extract of green coffee (SALVADOR ARABICA). Symbols below; method cf. fig.2

Figure 1 shows the gas chromatogram of a trimethylsilylated ethanolic extract of green coffee. In the area of interest only the internal standard (heptadecane, I), caffeine (C) and (±)-quinic acid (12) are visible.



1. (±)-γ-quinide	7. (±)-*epi*-γ-quinide	12. (±)-quinic acid
2. *scyllo*-δ-quinide	8. *neo*-quinic acid	13. (±)-*epi*-quinic acid
3. quinide No.1	9. *neo*-δ-quinide	14. *scyllo*-quinic acid
4. (±)-*epi*-δ-quinide	10. (±)-*neo*-γ-quinide	15. *meso*-quinic acid II
5. protocatecharic acid	11. *meso*-quinic acid I	

I = internal standard, heptadecane; C = caffeine; temperature program: 65°C/2min - 270°C/4°C/min, isotherm ; column: FS-OV-1-DF-0.25, 25 m x 0.25 mm id, GC/FID

Fig.2. Gas chromatogram of a trimethylsilylated ethanolic extract of roasted coffee (SALVADOR ARABICA)

During the roasting process, more peaks are arising in this area (fig.2). Using GC/MS-coupling, 5 further quinic acids and 7 quinides can be identified, including (±)-γ-quinide, which had been already described in roasted coffee.

From its structure quinic acid is very similar to pyranoide hexoses, except that it is an acid and that no oxygen shares the ring. From hexoses we know a great variety of stereoisomers, but none of the stereoisomers of quinic acid, which are theoretical possible, has been described in natural products.

Figure 3 shows the 8 stereoisomeric quinic acids. There are two pairs of enantiomers ((±)- and (±)-epi-quinic acid) and four meso forms (scyllo-, neo-, muco- and cis-quinic acid). In the following a nomenclature derived from inositols is used, because the correct IUPAC names turned out to be tongue twisters for an oral presentation.

From the 8 quinic acids 12 quinides could be generated (fig.4): 4 pairs of enantiomer γ-quinides and 4 δ-quinides including one enantiomer pair, too.

On the apolar, nonchiral methylsicon phase used for the gas chromatographic separation, pairs of enantiomers coelute, therefore the detectable number of quinic acids and quinides is reduced to six resp. eight and correspond to the number found in roasted coffee.

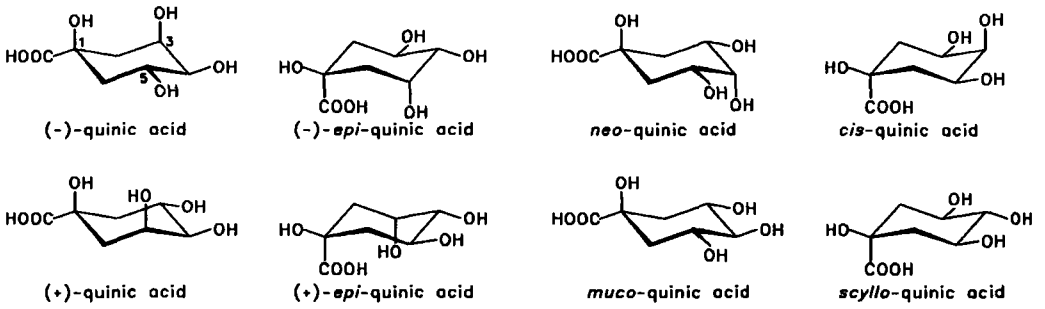


Fig.3. Stereoisomers of quinic acid

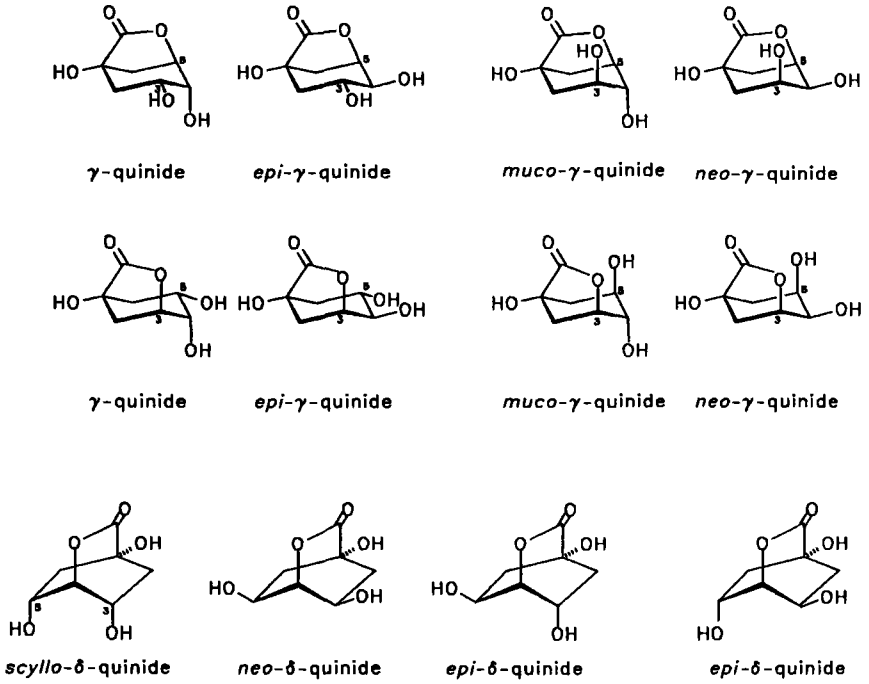


Fig.4. Quinic acid lactones

Basing on configuration inversion experiments with (-)-quinic acid in an acetic acid/mineral acid mixture carried out by Gorin (1963) as well as by Corse and Lundin (1970), reference material was synthesized. Besides (±)-epi-γ-quinide, (±)-epi- and scyllo-quinic acid described by the authors, all other quinic acids and quinides were found in the mixture - some of them only in traces, which could not be isolated.

The next figures are showing the schemes of the isolation, characterization and identification steps using NMR and GC/MS techniques.

First the acetate mixture was isolated and fractionated into an acid and a lactone fraction by several fluid extraction steps (fig.5). These fractions were worked up separately.

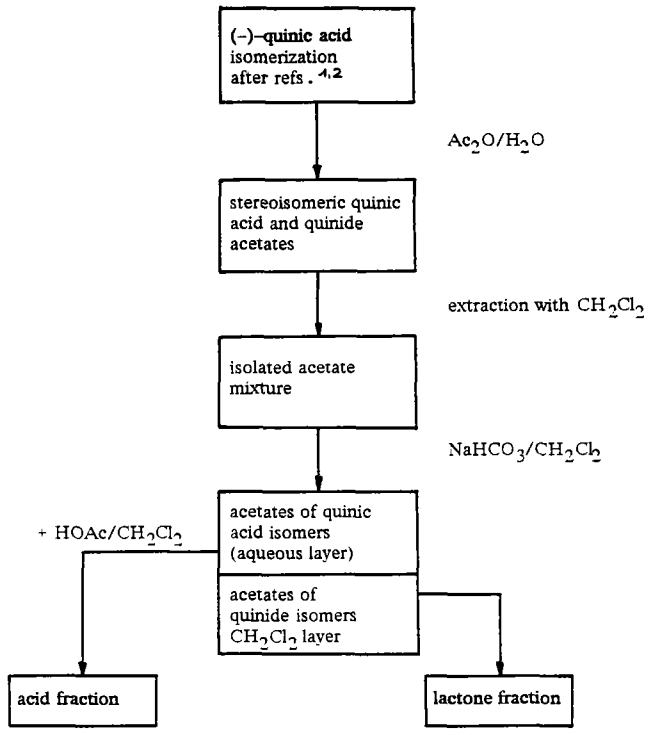


Fig.5. Isolation of stereoisomeric quinic acids and lactones I

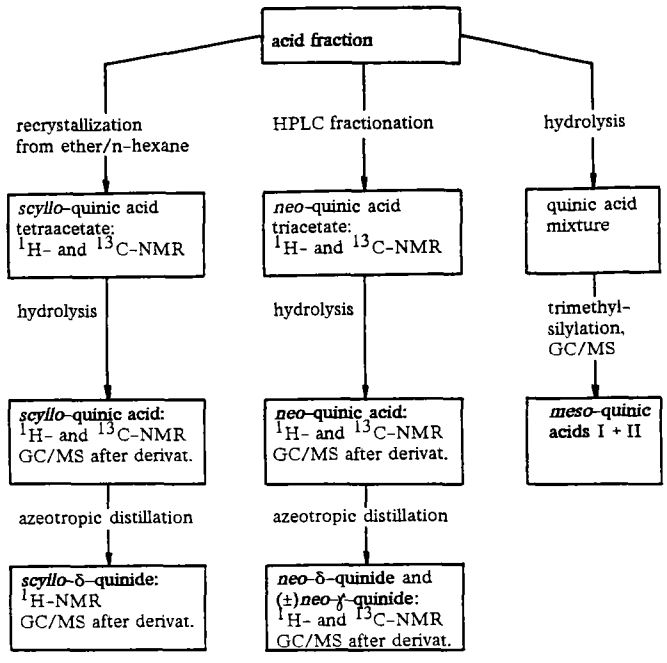


Fig.6. Isolation of stereoisomeric quinic acids and lactones II

Out of the acid fraction the acetates of scyllo- and neo-quinic acid could be isolated (fig.6). The free acids were prepared using alkaline hydrolysis. Their quinides were generated via azeotropic distillations. The existence of neo-quinic acid and its quinides has been established for the first time. The two other quinic acids, meso I and II (muco- resp. cis-quinic acid, but not necessarily corresponding to the order mentioned before), which had been found in roasted coffee, were also generated during the isomerisation process. They could neither be isolated nor accumulated out of the isomer mixture to an extent which would have been sufficient for establishing their configuration. Only their mass spectra characterize them as quinic acids.

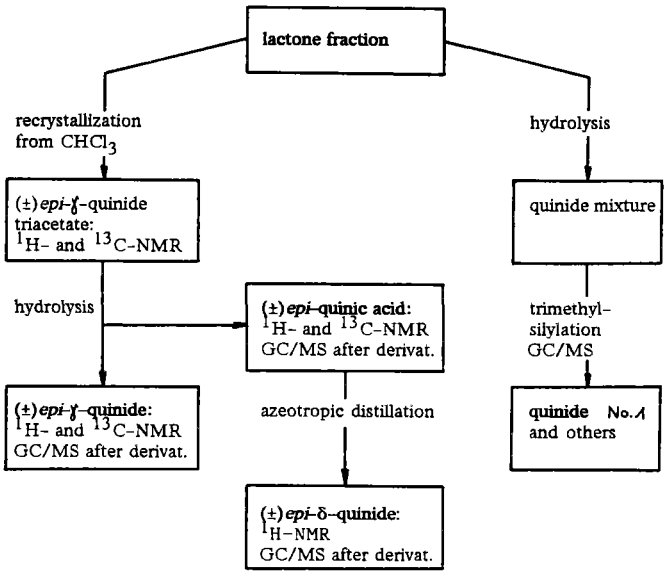


Fig.7. Isolation of stereoisomeric quinic acids III

From the lactone fraction (±)-epi-γ-quinide acetate was isolated (fig.7). After hydrolysis (±)-epi-γ-quinide as well as (±)-epi-quinic acid were received. After the azeotropic distillation of (±)-epi-quinic acid, (±)-epi-δ-quinide could be generated for the first time.
The lactone fraction includes a further quinide which might be the γ-lactone of muco-quinic acid. It could only be characterized via its mass spectrum and is called quinide No. 1.

For the detailed identification steps including NMR- and MS-data see Scholz-Böttcher, Ernst and Maier (1991).

From the five new quinic acids and six new quinides found in roasted coffee, three acids and five quinides could be completely identified. All quinic acids and quinides were analyzed in roasted coffee.

Figure 8 shows the extraction of quinic acid and quinides from coffee. The trimethylsilylated extract was analyzed via GC/MS or GC/FID. Postulating a detector signal which is independent of the conformation, all quinic acids were quantified as quinic acid. For the quantification of all quinides, except epi-γ-quinide, (±)-γ-quinide was used as a standard.

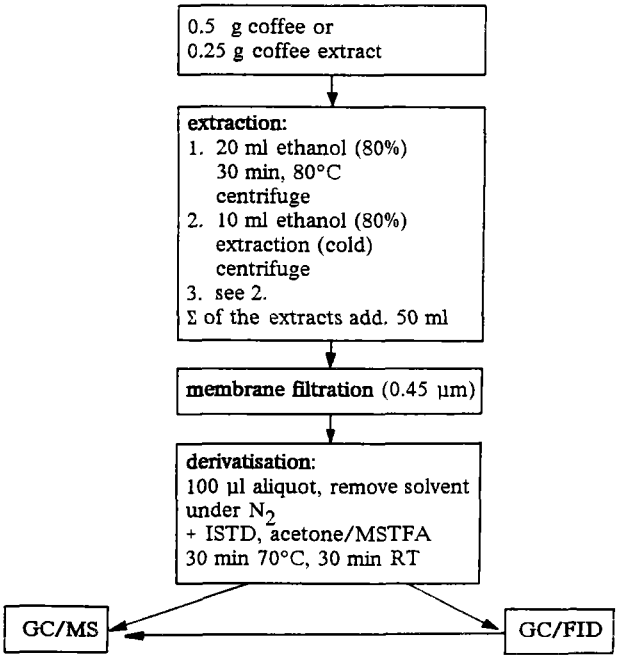


Fig.8 Determination of quinic acids and quinides in coffee

The changes in the quinic acids and quinides contents during the roasting process of a SALVADOR ARABICA roast series will be shown using three representative examples.

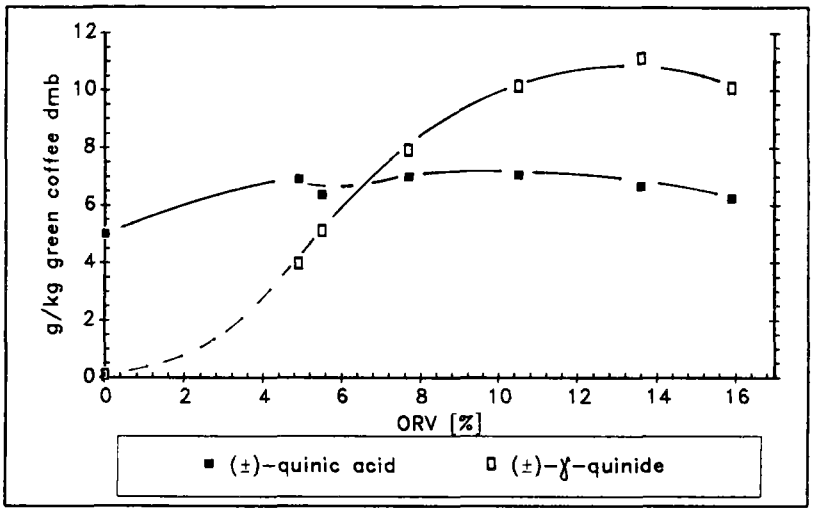


Fig.9. Variation of the (±)-quinic acid and the (±)-γ-quinide concentrations as a function of the roasting loss on dry matter basis (ORV)

(±)-quinic acid shows only a slight increase at the beginning of the roasting process and remains relatively constant up to higher degrees of roast, where it starts to drop (fig.9). The amount of (±)-γ-quinide increases from light to medium roasts and decreases slightly at dark roasts.

Opposite to (±)-quinic acid and its quinide all other stereoisomeric acids and lactones are showing a continuous increase with higher roasting degrees. The slopes are differing from case to case.

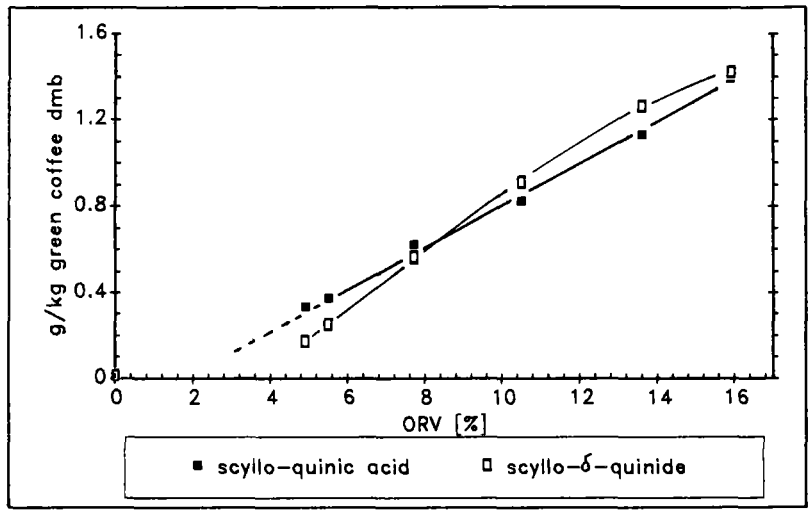


Fig.10 Variation of the scyllo-quinic acid and the scyllo-δ-quinide concentrations as a function of the roasting loss on dry matter basis (ORV)

Scyllo-quinic acid shows a linear increase, while the function of its δ-quinide seems to be concave to the ORV-axis (fig.10).

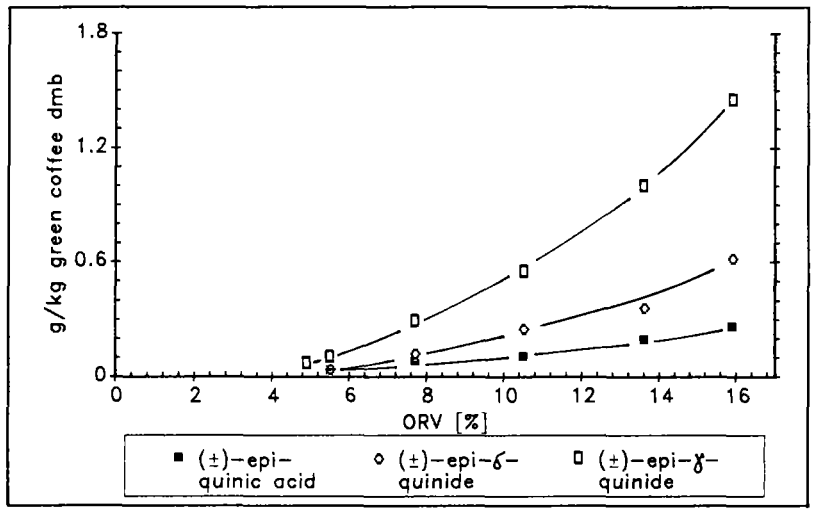


Fig.11. Variation of the (±)-epi-quinic acid, (±)-epi-γ- and (±)-epi-δ-quinide concentrations as a function of the roasting loss on dry matter basis (ORV)

The amount of (±)-epi-quinic acid raises with a more or less linear increase at higher roasting degrees (fig.11). (±)-epi-γ- and (±)-epi-δ-quinide are showing a stronger increase at higher roasting loss.

degree of roast ORV [%]	light (3-5)	medium (5-8)	dark (>8)
	[g/kg dmb]	[g/kg dmb]	[g/kg dmb]
neo-quinic acid	n.n. - +	n.n. - 0.06	+ - 0.22
meso-quinic acid I	+ - 0.07	0.04 - 0.17	0.10 - 1.14
(±)-quinic acid	7.17 - 7.75	6.63 - 9.47	7.60 - 10.38
(±)-*epi*-quinic acid	+ - 0.02	+ - 0.07	+ - 0.30
scyllo-quinic acid	+ - 0.34	0.27 - 0.67	0.43 - 2.87
meso-quinic acid II	n.n. - +	n.n. - 0.03	+ - 0.18
(±)-γ-quinide	1.35 - 4.08	2.29 - 8.49	4.22 - 16.64
scyllo-δ-quinide	0.08 - 0.29	0.17 - 0.59	0.27 - 1.60
quinide No.1	n.n.	n.n. - +	+
(±)-*epi*-δ-quinide	+ - 0.05	+ - 0.12	+ - 0.72
(±)-*epi*-γ-quinide	n.n. - 0.07	0.04 - 0.30	0.08 - 1.71
neo-δ-quinide	n.n. - +	n.n. - +	+ - 0.03
(±)-neo-γ-quinide	n.n.	n.n.	n.n. - 0.21

Fig.12. Amounts of quinic acids and quinides in coffee of different roasting degrees

Figure 12 gives an overview of the amounts of quinic acids and quinides quantified in coffees of different roasting degrees. Different sorts of coffee and roasters are included.
Of the newly discovered isomers in light roasted coffees mainly scyllo-quinic acid and its quinide could be established. At medium degrees of roast nearly all stereoisomeric quinic acids and quinides are generated. The higher the degree of roast the higher the contents of the isomeric compounds.

In roasted coffee the main stereoisomers of quinic acid are scyllo- and meso-quinic acid I. The main stereoisomeric quinides are scyllo-δ- and (±)-epi-γ-quinide.

Based on the observations made, we tried to prove, if quinic acids and quinides could be used as indicators for the degree of roast.
While the total amount of quinic acids and quinides is dependent on the concentrations of their precursors ((-)-quinic acid and the chlorogenic acids) in the green coffee bean, a relative parameter should be established, which is only dependent on the thermal treatment of the coffee.

Therefore a quotient called the degree of isomerisation (IG) was defined (fig.13). It represents the ratio between the sum of the isomeric quinic acids and quinides (neo-quinides as well as quinide No.1 excluded) and the sum of (±)-quinic acid and its quinide.

$$IG = \frac{\Sigma \text{ mol quinic acids \& quinides } ^{*}}{\Sigma \text{ mol (±)-quinic acid \& (±)-}\gamma\text{-quinide}}$$

* except neo-quinides and quinide No. 1

Fig.13. Definition of the degree of isomerisation (IG)

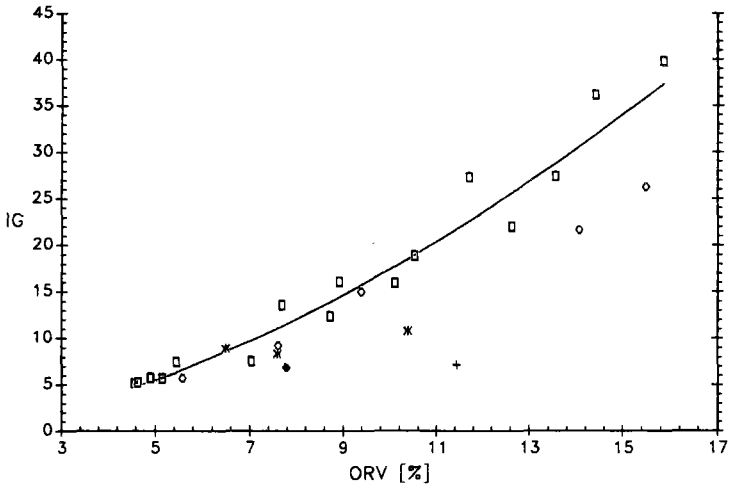


Fig.14. Degree of isomerisation (IG) as a function of the roasting loss on dry matter basis (ORV). □ = Rohkaffee-Röster, ◇ = drum roaster, ◆ = aerotherm quick roaster, * = quick roaster, + = industrial roast

Figure 14 shows the degree of isomerisation as a function of the roasting loss on dry matter basis. Coffee samples of known origins roasted in different ways were analyzed. For a better overview, only the types of the roasters are given. It can be shown that within a defined roasting procedure a correlation between the degree of isomerisation and the roasting loss is obtained. This coherence strictly exists only speaking of a definite procedure. Different roasting procedures are showing different functions.

An other common possibility to decide the degree of roast is to determine the colour of the coffees (fig.15). In the present case we measured it by reflectance of the ground coffee powder. Because the reflectance decreases with further roasting, its reciprocal times hundred was used for the following diagram to get a positive function. It is called darkness (Dy): Dy = 1/Y x 100. The diagram shows the degree of isomerisation as a function of the darkness. To the preceding coffee samples a short time roasting series was added. A well correlated logarithmic function was received, which is independent of the origin as well as the roasting type.

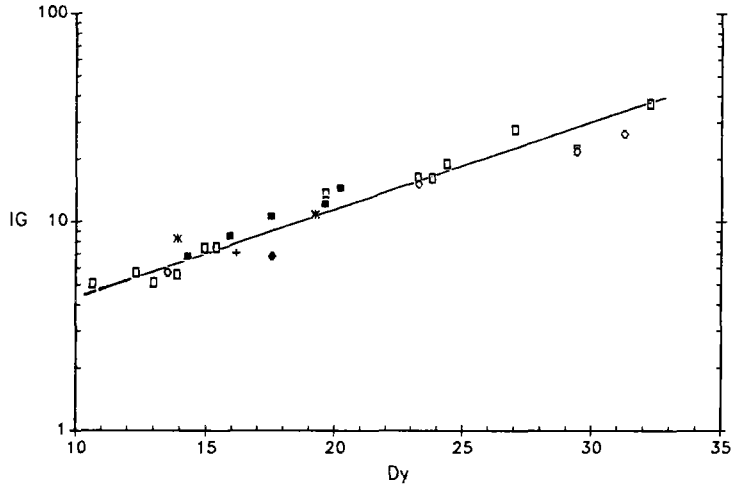


Fig.15. Degree of isomerisation (IG) as a function of the darkness (Dy). Symbols see fig.14; ■ = short time roaster

This was also the case with some commercial coffees. Only in the case of one very uneven roasted coffee and three espresso type coffees this procedure is not applicable. With this function it should be possible to estimate the roasting degree of the coffee even from extracts or beverages.

This presumption could be proved with two different industrial extracts of a KENIA ARABICA coffee, which are in a good agreement with the experimental function.

Conclusions

During the roasting process of coffee, all possible stereoisomeric quinic acids and quinides are generated. Of the five new acids and six quinides, the structure of three quinic acids and five quinides have been completely identified.
All new quinic acids and quinides increase continuously with higher roasting degrees.
A newly defined quotient (IG) enables the estimation of the roasting degree of coffee via its amount of stereoisomeric quinic acids and quinides.

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Summary

Quinic acid is one of the dominant acids in the coffee bean. It appears as free acid and as esters, mainly with caffeic acid, i.e. the chlorogenic acids.
Under thermal treatment only the racemisation of (-)-quinic acid and the generation of the corresponding (±)-γ-quinides have been reported and were already described in roasted coffee.
Under the roasting conditions of coffee as well as in model experiments with (-)-quinic-, n-chlorogenic acid and Potassium-caffeine-chlorogenate, which are not discussed at this time, all possible stereoisomers of quinic acid and quinide are generated. Their isolation, characterization and identification using GC/MS and NMR-spectroscopy will be briefly discussed. Seven compounds are described for the first time, pairs of enantiomers could not be separated.
More than 50 coffee samples of different degrees of roast, roasting types and pretreatment were investigated. 6 Quinic acids and 7 quinides (γ and δ) were quantified, 11 of them for the first time. Except for (±) - quinic acid and (±)-γ-quinide, the concentrations of all other isomers are increasing during the roasting process.
As a result a quotient IG, the degree of isomerisation, is defined. The IG is closely related to the colour and therefore to the roasting degree of coffee, independent of the origin and of the roasting type. It is possible to estimate the roasting degree of the coffee even from extracts or beverages.

TANNINS IN THE SUN-DRIED PULP FROM THE WET-PROCESSING OF ARABICA COFFEE BEANS

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INTRODUCTION

Coffee pulp is a by-product from the wet processing of coffee beans. Much of this pulp is currently dumped in water courses where it causes serious pollution (Bressani & Braham, 1980). For economic and environmental reasons attempts have been made to utilise coffee pulp as a feed for cattle, swine and poultry but with little success. When used at levels in excess of 10% of normal rations feed utilisation and growth rate are impaired due to the presence of ill-defined anti-nutritive or toxic factors.

Various components, including caffeine, low molecular mass phenols and tannins, have been blamed for these undesirable effects. Recently, it has been confirmed (Clifford & Ramirez-Martinez, 1991*a*) that arabica pulp used at 12-15% of normal rations would provide a level of caffeine previously shown in controlled studies to impair the growth of 100-day old calves (Bressani & Braham, 1980), whereas robusta pulp, due to a lower caffeine content, would be tolerated at such a proportion of the diet.

The low molecular mass phenols of coffee pulp have been characterised and quantified (Ramirez-Martinez, 1988; Ramirez- Martinez & Clifford, 1990; Clifford & Ramirez-Martinez, 1991*a*), although their effect on acceptability is still unclear. In contrast, although tannins are known to influence the acceptability of food and feed (Mehansho *et al.*, 1987), the coffee pulp tannins have not been well characterised. Early reports provided estimates obtained by non-specific methods for the contents of both condensed and hydrolysable tannins (Bressani, 1979; Bressani & Braham, 1980; Zuluago-Vasco & Tabacchi, 1980; Garcia *et al.*, 1985). More recently it has been shown that the methods routinely used to detect hydrolysable tannins are confounded by low molecular mass phenols such as protocatechuic acid and chlorogenic acids which are present in the pulp (Clifford & Ramirez-Martinez, 1991*b*). Using Porter's reagent (Porter *et al.*, 1986) it was demonstrated that coffee pulp, but not coffee beans, contained significant amounts of condensed tannins. The acetone-soluble tannin fraction formed some 1-3% db of sun dried coffee pulp. Attempts to quantify the unextractable tannins by exploiting the ability of Porter's reagent to convert insoluble tannins to soluble anthocyanidin pigments was only partially successful due to the chemical and physical lability of the pigments, and suggesting that other quantitative methods should be evaluated.

This paper reports our comparison of Porter's Reagents with quantitative methods based on substitution with dimethylamino-cinnamaldehyde in acidic media, and on the development of preparative-scale tannin extraction and fractionation procedures that will supply sufficient material for structure elucidation and biochemical studies.

MATERIALS AND METHODS

MATERIALS

Sun-dried coffee pulp obtained by wet processing arabica coffee beans (*C. arabica* var. *bourbon vermelho*) was supplied by the Estación Experimental Agrícola de Bramon, Venezuela.

(+)-Catechin and (-)-epicatechin were purchased from Sigma Chemical Company, Poole, UK. Other flavan-3-ols were isolated from unfermented tea leaf (Robertson and Bendall, 1983) and kindly provided by Mr S. C. Opie (See Opie *et al.*, 1990).

All other reagents were standard items from reputable commercial sources.

METHODS (Studies at the University of Surrey)

Extraction of acetone-soluble tannins

Coffee pulp (200 mg) was weighed into a test tube and to this was added 4ml of acidic acetone (50% aqueous acetone containing 0.5% formic acid). The tubes were capped and placed on a mixing wheel. The contents were mixed by rotation for 20 min, separated by centrifugation at 4000 rpm for 5 min, and the supernatant decanted into 25ml volumetric flasks. The insoluble residue was re-extracted five times, the supernatants bulked and diluted to volume with acidified acetone.

Porter's Reagents

Aliquots (1.00 ml) of the acidified acetone extracts were transferred to screw-capped (Teflon liner) test tubes and Porter's Reagents added (6ml butanol-concentrated hydrochloric acid 95 : 5 v/v, followed by 0.2ml 2% ferric ammonium sulphate). The contents were mixed thoroughly and the tubes placed in a boiling water bath for 40 min. An agitated but unheated control was prepared on a mixing wheel to check for the presence of pre-existing anthocyanins and/or anthocyanidins. The red pigment was diluted to a convenient volume with the butanolic Porter's Reagent and the absorbance at 550 nm converted to $E^{1\%}_{1cm}$ values after correction for the absorbance of the unheated control. In the absence of a suitable standard, the condensed tannin contents were calculated using $E^{1\%}_{1cm}$ values of 275 and 490. The smaller value assumes the tannins present are all 4->8 linked procyanidin dimers, whereas the larger is more appropriate for freshly prepared procyanidin polymers (Porter *et al.*, 1986; Porter, 1989).

Acidic Dimethylaminocinnamaldehyde reagents.

Three Dimethylaminocinnamaldehyde (DMAC) Reagents were prepared:

1) DMAC (0.1%) in methanol containing 4% conc. HCl;

2) DMAC (0.1%) in glacial acetic acid containing 4% conc. HCl;

3) DMAC (0.1%) in MeOH/Conc. HCl (3:1 v/v).

Reagents 1 and 2 were based upon vanillin reagents which have traditionally been used to quantify condensed tannins, (e.g. see Price *et al.*, 1978; Butler *et al.*, 1982) and estimate their degree of polymerisation, whereas Reagent 3 was that developed by McMurrough and McDowell (1978). DMAC was substituted for vanillin because it is less prone to participate in side reactions, and less sensitive to light (Porter, 1989).

In each case 1.00 ml of extract or standard (in 70% MeOH) was added to 5.00 ml of reagent, mixed and allowed to stand at room temperature for 10 min. The absorbance at 640nm was read against a blank in which water replaced the sample or standard.

Reversed phase HPLC

The pigments produced with Porter's Reagents and with the DMAC Reagents have been analysed on a 25cm x 4.7mm column packed with Chromasil C_{18} (5µ) eluted with a linear acidic acetonitrile gradient as follows: solvent A = 0·5% formic acid in water; solvent B 0·5% formic acid in 50% acetonitrile; 12% A

to 85% A in 41 minutes. Detection was at 540nm for Porter's pigments and sequentially at 280nm and 640nm for the DMAC pigments.

METHODS (Studies at the Universidad Nacional Experimental del Táchira)

Tannin fractionation

Although previous studies had established that 50% aqueous acetone containing 0.5% formic acid (acidified acetone) was a better solvent for coffee pulp condensed tannins than 70% methanol or 80% acetone, whether at room temperature or under reflux (Clifford and Ramirez-Martinez, 1991*b*), further trials were conducted. In this study water was compared with acidified acetone, 50% aqueous acetone containing 1% naphthalene, 50% aqueous acetone containing 1% sodium metabisulphite, and 50% aqueous acetone. The greatest yield of total phenols (as judged by the Folin-Denis Method) was obtained with 50% aqueous acetone, and this procedure was adopted for the development of the preparative scale fractionation procedures described in this paper.

In a preliminary study, sun dried Red Bourbon coffee pulp (200mg) was extracted with 50% aqueous acetone (1:20 m/v), and the resultant extract concentrated under reduced pressure to remove the acetone. The concentrated extract was partitioned against petroleum ether to remove chlorophyll and lipids, and separately against ethyl acetate to remove low molecular mass phenols including dimeric proanthocyanidins. Aliquots of each organic phase were evaporated to dryness and tested for the presence of condensed tannins, but were negative with Porter's Reagents. The remaining aqueous solution was concentrated to a paste.

Aliquots of this paste were added to a slurry of Sephadex LH-20 in 50% methanol, and after being allowed to equilibrate the resultant mixture was centrifuged, and the supernatant tested using Porter's Reagents for the presence of desorbed condensed tannins. The insoluble material was shaken with further volumes of 50% methanol until the supernatant was negative to Porter's Reagents, whereupon 75% methanol, 100% methanol, 50% acetone and 100% acetone were similarly applied.

On the basis of these preliminary studies the following procedure was developed for the fractionation of coffee pulp tannins:

To Sephadex LH-20 slurry (2g in 10 ml aqueous 50% methanol) was added 14.9g of the concentrated tannin-rich paste prepared as described above, and the mixture shaken for 30 min. to adsorb soluble tannins, followed by centrifugation. The insoluble matrix was subjected to 38 consecutive desorption/centrifugation steps. Desorption was by shaking for 5 min. with each of the following solvents - 50% methanol (18 x 20ml); 75% methanol (5 x 20ml); 100% methanol (10 x 20ml); and 50% acetone (5 x 20ml).

RESULTS AND DISCUSION

Dimethylaminocinnamaldehyde reagents

Table 1 summarises the results obtained with the three DMAC Reagents for standards ((+)-catechin, (-)-epicatechin, and (-)-epicatechin-gallate), and a tannin-containing extract from Red Bourbon coffee pulp. Limited tests were performed also on (-)-epigallocatechin-gallate and a mixture of (-)-epigallocatechin/(+)-gallocatechin.

It is evident that for each of the flavan-3-ols examined, pigment formation with DMAC is greatly influenced by the solvent in which the DMAC is dissolved. For a given reagent, the molar response of the flavan-3-ols may be influenced by their stereochemistry at carbons 2 and 3, the hydroxylation (3',4'-di or 3',4',5'-tri) of the B ring and by esterification at position 3. Unfortunately the ranking and magnitude of these effects differs for each DMAC Reagent, making these effects of little or no diagnostic value.

Moreover, even though apparently using identical procedures, different investigators report different behaviour. For example in the present study Reagent 1 (MeOH) with (-)-epicatechin (2,3-*cis*) gives a significantly ($p > 0.001$) greater molar response than with (+)-catechin (2,3-*trans*) whereas with Reagent 3 (MeOH/Conc. HCl) these two flavan-3-ols give statistically indistinguishable molar responses (11,360 ± 200 and 11,840 ± 285 respectively). In contrast, Delcour and De Varebeke (1985) reported for Reagent 3 that (-)-epicatechin did yield greater colour than (+)-catechin. While one contributory factor might be variations in the amount of water present during colour production (McMurrough and McDowell, 1978) Karchesy (1989) doubtless was correct in suggesting that there are many ill-defined factors influencing the behaviour of the DMAC Reagents.

TABLE 1. The molar response of various flavan-3-ols treated with various dimethylaminocinnamaldehyde reagents.

Flavan-3-ol	Dimethylaminocinnamaldehyde Reagent[a] Methanol	Acetic Acid	Methanol/HCl
(+)-catechin	15515 ± 543	7733 ± 749	11842 ± 285
(-)-epicatechin	17690 ± 661	7300 ± 330	11360 ± 200
(-)-epicatechin-gallate	11790 ± 113	7345 ± 328	8645 ± 457
(-)-epigallocatechin-gallate	16706	6177	7029
(-)-epigallocatechin/(+)-gallocatechin	13311	5202	8721

a = mean ± standard deviation

In view of the foregoing observations, it is hardly surprising that the apparent tannin content of a coffee pulp extract (Table 2) depends upon the particular combination of reagent and standard that is chosen. With values ranging from 1.3-3.0% it follows that standardisation is vital if results obtained in different laboratories are to be compared in a meaningful way.

TABLE 2. The apparent tannins content of an extract from Red Bourbon Coffee Pulp.[a]

	Dimethylaminocinnamaldehyde Reagents Methanol	Acetic Acid	Methanol/HCl	Porter's Reagents
(+)-catechin	1.61 ± 0.06	1.98 ± 0.16	1.27 ± 0.03	
(-)-epicatechin	1.41 ± 0.05	2.11 ± 0.10	2.47 ± 0.04	
(-)-epicatechin-gallate	2.99 ± 0.03	2.96 ± 0.13	2.47 ± 0.13	
E_{270}				6.77 ± 0.70
E_{490}				3.82 ± 0.43

a = Mean ± standard deviation

Whichever reagent/standard combination is adopted, it must be recognised that these values should be reported as *apparent tannin contents* since the response will depend also upon the degree of polymerisation of the flavan-3-ols being investigated. McMurrough and McDowell (1978) and Delcour and De Varebeke (1985) both reported that compared to monomers, oligomers on a weight basis yield progressively less colour as their degree of polymerisation increases. Although substitution may occur at positions 6 and 8, the sterically-favoured position 8 is preferred. Since there is a marked reduction in colour yield (50-65%) for dimers relative to (+)-catechin it has been concluded that only the 'upper' terminal residue (as normally depicted) is reacting and that steric factors prevent access to free sites in the 'lower' residues.

However, according to Delcour and De Varebeke (1985) the response by dimers is also influenced by the stereochemistry in their 'lower' residue and thus there is not a simple inverse relationship between degree of polymerisation and intensity of response with DMAC.

Since monomeric flavan-3-ols occur in coffee pulp in the range 0.19-0.86% (Ramirez-Martinez, 1988) there can be no doubt that DMAC-derived estimates of tannin content may be seriously distorted, but because of the complexity of the situation, the direction and magnitude of the distortion cannot be assessed.

Porter's Reagents

Table 2 also presents the equivalent data obtained by use of Porter's Reagents. It is clear that this method of analysis has the advantage not detecting monomeric flavan-3-ols. Indeed it has been shown by Porter *et al.* (1986) that all but the 'lowest' flavan-3-ol residue is converted by oxidative depolymerisation to the corresponding anthocyanidin(s). The

'lowest' residue is released unchanged. However, for several reasons, but especially because the anthocyanidins are labile, the yield of pigment is non-stoichiometric.

However Porter's Reagents, in direct contrast to the DMAC Reagents, yield progressively more pigment per unit weight as the molecular weight of the oligomer increases, albeit with the rate of that increase declining exponentially with increased polymerisation. For this reason the data in Table 2 have been calculated for two extreme situations. In the first it has been assumed that all the tannin is dimeric (6.0-7.5%), and in the second that all the tannin is of high molecular mass (3.4-4.3%).

Integration of these data for tannin content with previously published data. Bearing in mind the very different specificities discussed above, the current mean estimates obtained by DMAC Reagents (1.3-3.0%) and by Porter's Reagents (6.8% dimers, 3.8% polymers), are not inconsistent. Our previous analyses using Porter's Reagents of pulp from commercial species have given acetone-soluble tannin contents in the range 1-3% polymers. Since it was also demonstrated that the content of acetone-soluble tannin declines with age, these two sets of results are compatible, as are values of 1.6 and 2.6% obtained by Zuluago-Vasco and Tabacchi (1980) and Garcia *et al.* (1985).

HPLC of Porter's and DMAC Pigments

As reported previously (Clifford and Ramirez-Martinez, 1991*b*) Porter's Reagents generated two anthocyanindin pigments from the acetone-soluble tannins. The faster eluting, presumably delphinidin, dominated the slower, presumably cyanidin.

The pigments produced with each of the three DMAC Reagents were examined, and found to be similar. However those produced with Reagent 1 (MeOH) seemed to give sharper peaks. Although many peaks were detectable at 280nm, relatively few were observed also at 640nm. It is assumed that those peaks seen only at 280nm are not flavan-3-ol derivatives. Table 3 summarises the retention times and A_{280}/A_{640} ratio for those peaks detected at both wavelengths. These peaks differ quite significantly with respect to this ratio, and it is tentatively concluded that the smaller ratios indicate monomers, and that the ratio increases progressively with increasing degree of polymerisation.

TABLE 3. Retention time and A_{280}/A_{540} ratio for materials in Red Bourbon Coffee Pulp

Retention Time min.	A_{280}/A_{640} ratio
10.0	9
11.2	8
12.0	15
13.6	3
14.8	3
16.4	4
17.6	1
18.0	2
18.8	4
19.2	4
21.6	2
22.4	1
23.2	2

Tannin fractionation

The results presented in Table 4 show that selective desorption from Sephadex LH-20 has yielded at least 7 fractions that are positive to Porter's Reagents. Since flavan-3-ol dimers are readily soluble in ethyl acetate (Porter, 1989) they will have been removed during clean up of the crude acetone extract. It is reasonable to suggest therefore that each of these seven fractions are trimers or larger. So far as we are aware, this is the first report of such substances in coffee pulp.

TABLE 4. Tannin fractionation - Porter's Reactive Fractions.

Eluate Number	Solvent	Porter's Reaction
1-2	50% MeOH	+
3-6	50% MeOH	-
7-8	50% MeOH	+
9	50% MeOH	-
10-12	50% MeOH	+
13-14	50% MeOH	-
15-17	50% MeOH	+
18	50% MeOH	-
19-22	75% MeOH	+
23	75% MeOH	-
24-30	100% MeOH	+
31-32	100% MeOH	-
33	100% MeOH	-
34-37	50% Acetone	+
38	50% Acetone	-

Future developments

The oligomeric tannin fractions obtained in greatest yield will be examined by reverse phase HPLC to check their purity, and if sufficiently high, the fractions will be examined also by ^{1}H and ^{13}C nmr to obtain further information about the number and identity of the monomers present.

These data will be compared with data obtained by:

1) chromatography of the Porter's pigments, which provides information about monomer identity and ratio;

2) chromatography of pigments produced with DMAC Reagent 1 and measurement of the A_{280}/A_{640} ratio as an estimate of the degree of polymerisation; and

3) comparing the intensity of the pigments produced with Porter's Reagents and DMAC Reagent 1 also as an estimate of the degree of polymerisation.

If the results of chromatography and colorimetry procedures are consistent with those obtained by nmr spectroscopy, then the chromatographic and colorimetric procedures may be useful for the characterisation of tannin fractions where the amount of material isolated is too small to be characterised by nmr.

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SUMMARY
This paper compares critically several methods for the analysis of tannins in coffee pulp, and demonstrates that the result obtained is significantly influenced by the choice of method and standard adopted. The development of a method for the fractionation of coffee pulp tannins is also described.

RÉSUMÉ
Ce manuscrit compare critiquement plusieurs methodes pour l'analyse des tannines dans la pulpe du café, et démontre que le résultat est influencé beaucoup par le choix du methode et du niveau adopté. Le développement d'un methode pour la preparation des tannines individuels
de la pulpe du café est décrit aussi.

16-O-METHYLCAFESTOL
A QUALITY INDICATOR FOR COFFEE

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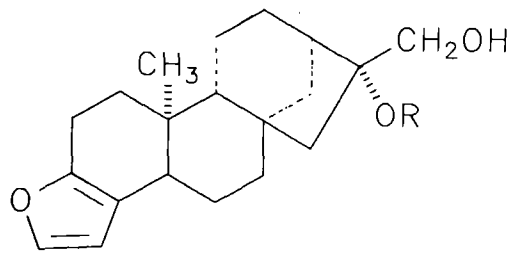
Introduction

Of all the species of coffee there are two that have acquired worldwide economic importance : these are Arabica and Canephora, variant Robusta. The Arabicas are valued much more highly by the trade because they have a far more pronounced and above all finer flavour than the Robustas. This is why they are the first choice of consumers in Germany and indeed the whole of northern Europe.
Coffee producers often draw attention to their special quality by the designations " Pure Highland Coffee " or " Pure Arabica Coffee ". Legal problems result, as it is not possible at present to verify statements of this kind.

The Arabica and Robusta species are easy to distinguish by the size of the beans, but this visible criterion is eliminated by roasting and subsequent mealing.
It is possible to detect quantities over 15 % of Robusta coffee in Arabica blends by sensory testing, but such results are only based on subjective impressions.

For a long time, therefore, efforts have been made to characterize the two coffee species in a differentiated and objective manner using chemico-analytical data.
any of the substances contained in the coffee were tested for suitability, but the disadvantage was that they could only be detected in varying quantities in the two species; it was not possible to determine small percentages of Robusta in Arabica blends.

In 1984 our study group discovered a new diterpene - 16-O-methylcafestol. Its structure was confirmed by synthesis. The new diterpene differs from the long-known diterpene cafestol in that it has a methoxy group in position 16 instead of the hydroxy group in cafestol.



R = H : Cafestol
R = CH_3 : 16-O-Methylcafestol

Fig. 1

Determination of 16-O-Methylcafestol in Coffee

A method was developed for determining 16-O-methylcafestol in crude and roasted coffees. After isolation of the coffee oil and extraction of unsaponifiable matter by boiling with ethanolic KOH the solution of unsaponifiable matter is cleaned on silica gel disposable extraction columns.
The 16-O-methylcafestol can be determined either by capillary gas chromatography or by high pressure liquid chromatography. The HPLC method has proved especially useful, for it does not require derivatization as CGC does.For HPLC measurement a C_{18} reversed phase column is used with acetonitrile/water (60/40) as an eluting agent. Detection is by the UV-method at 220 nm.

We analysed numerous crude Arabica and Robusta coffees for their 16-O-methylcafestol content. Whilst 16-O-methylcafestol was detected in all the crude Robusta coffees analysed, the new diterpene was not identified in any of the crude Arabica coffees by the analytical methods just described.

With 16-O-methylcafestol we have for the first time a substance that only occurs in one of the two coffee species and would therefore seem suitable as an indicator for characterizing coffee blends.

Stability of 16-O-Methylcafestol in the Roasting Process

It was necessary to study the stability behaviour of 16-O-methylcafestol in the roasting process. As the weight of the crude coffee changes on roasting, a reference quantity had to be ascertained to permit conversion of crude to roasted coffee. It proved convenient to determine certain mineral substances as these survive roasting without change.
Fig. 2 shows 16-O-methylcafestol content in comparison with cafestol content at various roasting temperatures. By measuring the diterpenes in quantities of coffee each containing 1 g of magnesium it is possible to acquire information on the stability behaviour of the two diterpenes.
Whereas cafestol is degraded upwards of a temperature of about 260 °C, 16-O-methylcafestol remains stable.
16-O-methylcafestol is therefore an ideal indicator of Robusta coffee in roasted Arabica blends.

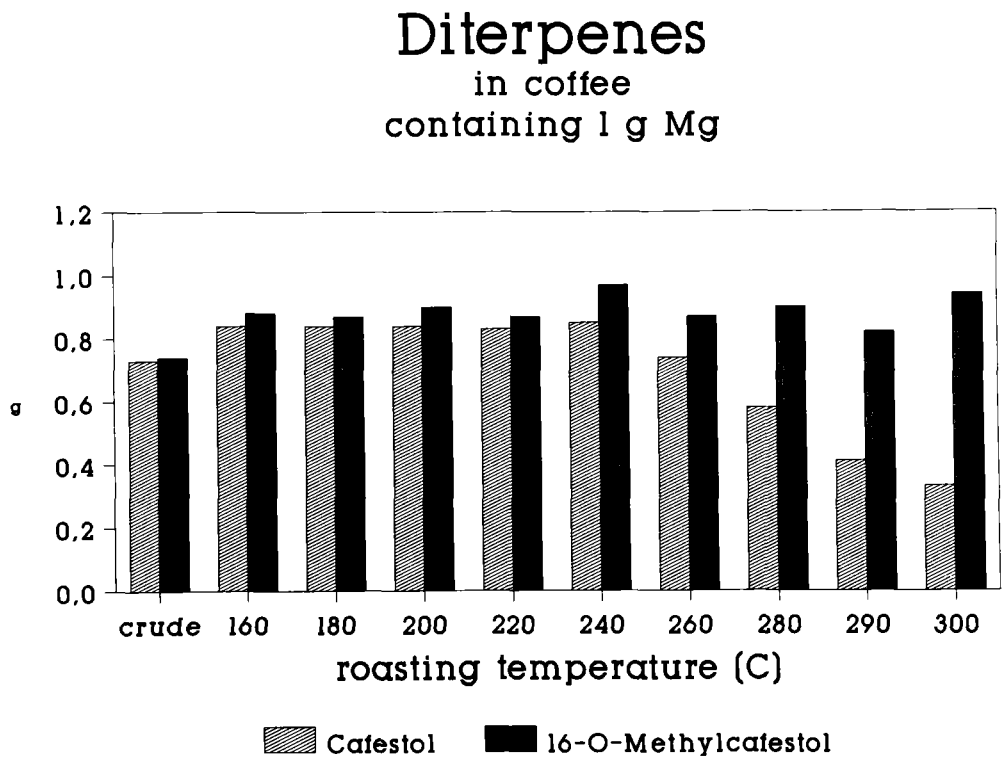
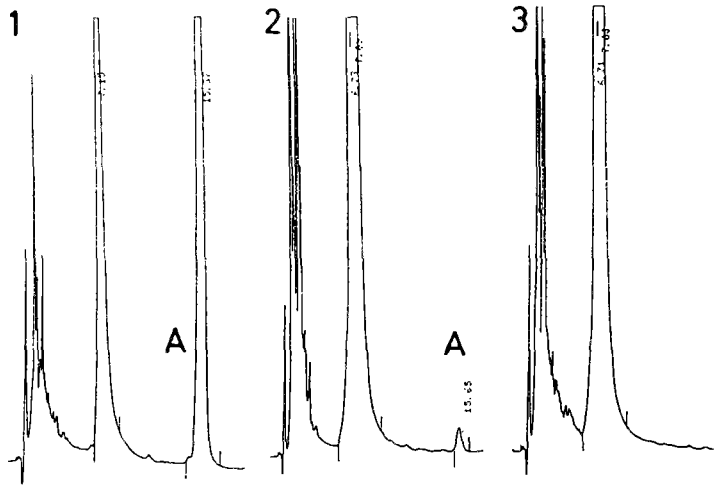


Fig. 2

Model Mixtures of Roasted Arabica and Robusta Coffees

To find out the smallest percentages of Robusta that can be detected in roasted Arabica blends, several blends were made up as models. We mixed an Arabica coffee from Colombia - roasted for three minutes at 260 °C - with 2, 5, and 10 percent of a Robusta coffee from Indonesia, which was roasted for 3 minutes at 270°C. It was not possible to detect 16-O-methylcafestol in the pure Arabica coffee, but even the blend containing only 2 percent Robusta produced a quantifiable signal (Fig.3). So for the first time we have a means of verifying the trade designations " Pure Highland Coffee" or "Pure Arabica Coffee" and integrating them in the food laws.



Roasted Coffee Samples (HPLC)
1: pure robusta coffee
2: arabica coffee with 2% robusta
3: pure arabica coffee
A: 16-O-methylcafestol

Fig. 3

Estimation of Parts of Robusta in Arabica Blends

Our next step was to find out wether it is possible to determine amounts of Robusta in Arabica blends of unknown origin.
We analysed 14 crude Robusta coffees of different provenance for their 16-O-methylcafestol content in the dry weight.

These coffees were subsequently roasted for three minutes at 285 °C and analysed again for their 16-O-methylcafestol content.
The 16-O-methylcafestol content of roasted coffee was found to correlate well with the amount contained in the corresponding crude coffee based on dry weight (Fig.4).
Determination of quantities of Robusta in unkown roasted Arabica blends therefore depends on the natural fluctuations in 16-O-methylcafestol.

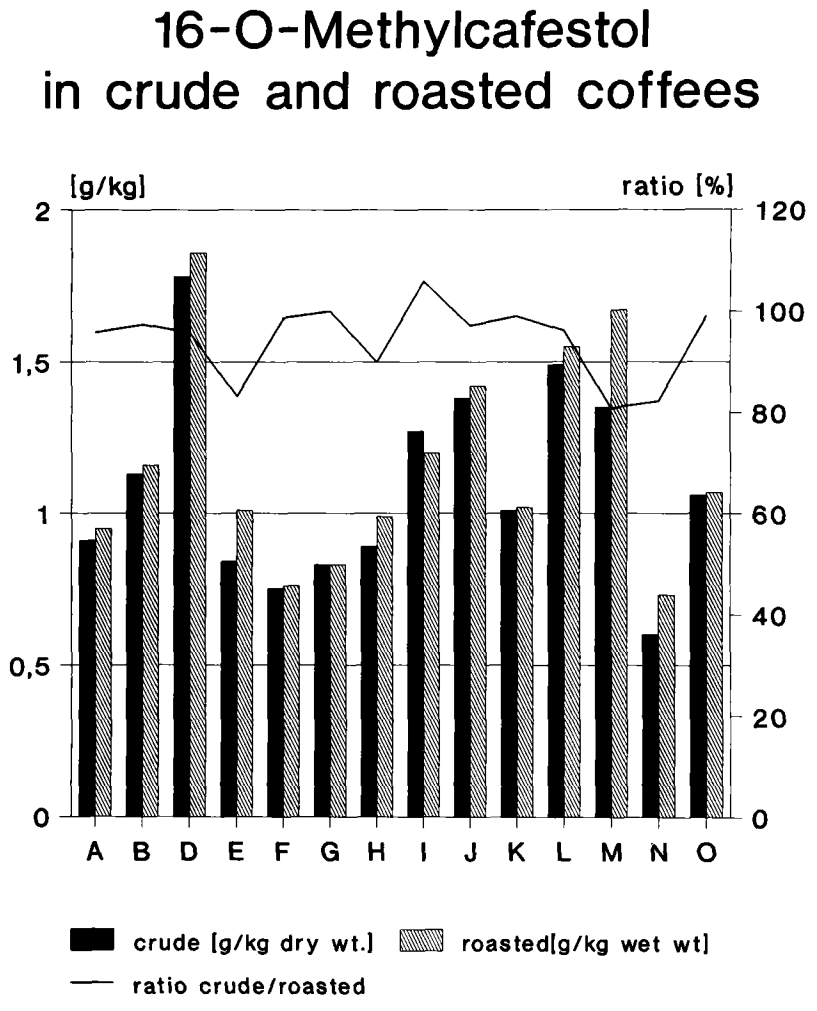


Fig.4

When 20 crude Robusta coffees of differing provenance were analysed by the HPLC method we have described, we found 16-O-methylcafestol contents between 0,6 and 1,8 g per kilogram of coffee based on dry weight. Both the median and the mean value were approximately 1,1 g. Over 80 percent of the values in the analysis were between 0,8 and 1,4 g per kilogram (Fig. 5).
A 16-O-methylcafestol value of 0,1 g per kilogram in an unknown roasted coffee blend therefore means there is 80 percent certainty that between 7 and 13 percent Robusta have been added.

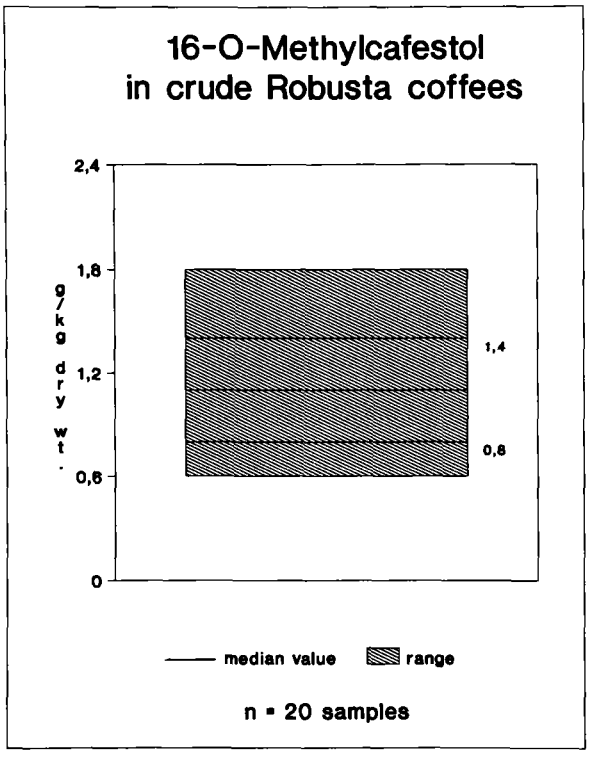


Fig. 5

16-O-Methylcafestol in Soluble Coffee

In addition to roasted coffee the market for soluble coffee is of great significance. 310.000 tonnes of soluble coffee are consumed worldwide. 13 million sacks of coffee are needed to produce this quantity. Naturally we found it interesting to analyse soluble coffees for their 16-O-methylcafestol content too.
Table 1 shows the very first results from samples from the trade. With analytical parameters differing from those used for crude and roasted coffee it was possible to detect 16-O-methylcafestol in five out of six samples. The values obtained were in the low milligram-per-kilogram range.

Sample 6 has the highest value at 115 mg per kilogram. This is a product that contains some roasted coffee in addition to soluble coffee.
Sample 2 was found to have the lowest value: 4,8 mg per kilogram.
In a model sample made up 95 percent from sample 1 and 5 percent from sample 2 it was still possible to determine a content of approximately 0,2 mg per kilogram.

16-O-Methylcafestol
in instant coffees
[mg/kg]

sample 1	-
sample 2	4.8
sample 3	9.0
sample 4	13.5
sample 5	23.7
sample 6	115

Table 1

Conclusions

Because of its stability during roasting and the possibility of detecting it in soluble coffee the new diterpene 16-O-methylcafestol is the ideal quality characteristic for reliably detecting the addition of Robusta to Arabica coffee.

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Summary

In 1985 a new diterpene has been identified in coffee, the 16-O-methylcafestol (OMC). The structure was confirmed by synthesis.
Furthermore a method for isolation and quantification of OMC was developed, OMC was detected only in Robusta coffees, not in Arabica coffees.
Specific modifications of the analytical method, earlier described, allow the determination of 16-O-methylcafestol even in instant coffees.
Due to sufficient stability of OMC during roasting process it can be used as an excellent indicator for coffee quality.

Resumen

En 1985 un nuevo diterpeno ha sido identificado en el Café, el 16-O-Metilcafestol (OMC). Su estructura fué confirmada por sintesis.
Ademas fué desarrollado un método para el aislamiento y quantificacion del OMC. OMC fué solamente detecado en cafés robusta y no en cafés arabica.
Modificaciones especificas del método analitico, descrito anteriomente, permiten la determinacion del 16-O-metilcafestol aun en cafés instantaneos.
Debido a la satisfactoria estabilidad del OMC durante el proceso del tostado puede ser este utilizado como un exelente indicador de la calidad del café.

ISOLATION AND IDENTIFICATION OF URSOLIC ACID FROM *COFFEA ARABICA* L. (COFFEE) LEAVES

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INTRODUCTION

The chemical composition of the coffee plant may influence the various technological processes leading to the production of consumable coffee. Our search for saponins that might explain the foaming property of roasted coffee beans yielded no saponins in the coffee plant parts (including roasted coffee beans), but it did indicate a relatively large amount of an unknown compound which came from the analytical method used for the isolation of saponins. It was identified as ursolic acid, which is frequently found in plants, especially in the waxy coatings of leaves and on fruits where they may serve as a protective function in repelling insect and microbial attack (Harborne, 1973). Stocker and Wanner (1975) reported on the changes of composition of unidentified C_{30} components of coffee leaf wax which constituted 0.4% of the total content. One of the unidentified C_{30} components was tentatively identified as ursolic acid as being the primary compound. Ursolic acid and the remaining unidentified compounds were found to be present at 19.8% to 27.1% of the wax of the leaf isolated in 66, 81 and 112-day old leaves .

EXPERIMENTAL

Plant Material: *Coffea arabica* L. trees were obtained as 6-week-old seedlings through the Plant Introduction Division of the U.S. Department of Agriculture (P.I. 394420) in 1976. They were grown at 23-32 °C in a greenhouse of Oklahoma State University, Stillwater, producing coffee fruits from 1977-1984 (Suzuki and Waller, 1984). Samples of *Coffea arabica* L. roots, and leaves, were obtained from a commercial plantation producing coffee for international commerce near Xalapa, Mexico and were air-dried, prior to use.

Extraction and purification: One Kg of coffee leaves (*Coffea arabica* L.) were extracted with ethyl ether in a Soxhlet apparatus for 24h. The extract was concentrated to 0.5L and extracted five times with 200 ml of 1% NaOH. The basic extract was acidified with conc. HCl to pH 3 and extracted exhaustively with ethyl ether. The organic layer was concentrated to 200 ml, then 1g of charcoal was added, and the mixture was boiled for 15 min and filtered. After evaporation of the solvent, the residue was dissolved in 20 ml of methanol-benzene (5:95) and the liquor was chromatographed on silica gel column (grade 12, 28-200 mesh) using the above solvent mixture for elution. Fractions were monitored by TLC silica gel using Liebermann-Burchard (Tschesche, 1961) reagent for detection. Chromatographically homogeneous fractions were collected and the solvent was evaporated. The dry residue was recrystallized three times from methanol-benzene, yielding 0.55g of a white crystalline product.

Acid hydrolysis to detect the presence of saponins: One milliliter of the butanol/methanol fraction was transferred to a 25-mL round-bottomed flask and evaporated to dryness under nitrogen. Five milliliters of 2*N* HCl (aqueous) were added to the residue and the solution was boiled for 5 h in a reflux system. Five milliliters of distilled water was added and the mixture was shaken in a 100-mL separatory funnel with 5 mL of ethyl acetate. The ethyl acetate was centrifuged at 2400 rpm for 15 min and the acetate layer was transferred to a 7.0-mL scintillation vial and evaporated; the aqueous layer was returned to the separatory funnel. The acid hydrolysate mixture was extracted twice more with 5.0-mL portions of ethyl acetate. The extracts were combined and evaporated to dryness, and the residue was dissolved in 0.5 mL methanol. Such solutions were used for the identification of triterpenoids of coffee or aglycones from saponins by one-dimensional TLC (Wyman-Simpson *et al.* 1991).

Triterpenoid acetate of unknown coffee triterpenoid: One-half gram of the unknown triterpenoid was dissolved in 10 ml of pyridine, and 10 ml of anhydrous, sodium acetate was added. The mixture was left overnight, poured into ice water, filtered and crystallized 3 times from methanol, yielding 420 mg of triterpenoid acetate.

Acetate of methyl ester of unknown coffee triterpenoid: One hundred milligrams of triterpenoid acetate was dissolved in 10 ml of ethyl ether and diazomethane in ether was added. After evaporation of ether the residue was crystallized from methanol (85 mg).

Methyl ester of unknown coffee triterpenoid: Fifty milligrams of unknown triterpenoid was dissolved in 20 ml of diethyl ether and diazomethane in ethyl ether was added. After evaporation of ether the residue was recrystallized from petroleum ether (43 mg).

Standards of ursolic acid and oleanolic acids: The ursolic acid and oleanolic acid were purchased from Sigma Chemical Co., St. Louis, MO and were recrystallized from methanol-benzene (5:95) three times. The crystalline acids were then treated to provide the triterpenoid acetates, acetate of methylester and methyl ester as described above.

Identification of the unknown compound from *Coffea arabica* L. leaves was based on their spectral characteristics and comparison of TLC with authentic samples wherever possible:

Mass Spectrometry Analysis: Fast atom bombardment (FAB) was performed on a ZAB-E mass spectrometer using the negative ion mode with glycerol and thioglycerol being used as the matrix. The linked scan (MS/MS) utilized a ZAB-SE equipped with a Cesium Ion Gun.

Nuclear Magnetic Spectrometry: ^{1}H-NMR and ^{13}C-NMR were performed on a 300 mhz Varian XL-300 nuclear magnetic spectrometer.

Thin layer chromatography: One-dimensional TLC was used to identify the triterpenoid from the butanol extracts of all samples and the aglycone portions from samples. Kieselgel 60 F_{254} plates (10 x 20 cm) with a coating thickness of 0.25 mm were used. The prepared samples and standards of purified triterpenoid from *C. arabica*, ursolic acid, oleanolic acid and their derivatives were dissolved in methanol. Two-microliter portions was spotted on plates for identification of the triterpenoid, the standards and their derivatives. Standards were prepared at a concentration of 1 µg/ µl. The solvent systems (all v:v:v) used separately for identification of the possibility of saponins were SS I, SS II, and SS III (Timbekova and Abubakirov, 1986). The solvent systems used for the identification of the unknown coffee triterpenoid were done using petroleum ether:chloroform:acetic acid 7:2:1; benzene: methanol 90:8 (Oleszek and Jurzysta, 1987); and benzene:methanol 90:10. The plates were sprayed with Liebermann-Burchard [1887 Tschesche (1961)] reagent and heated at 100°C for 2 min. Fluorescent spots were visualized under long-wave UV (366 nm).

RESULTS AND DISCUSSION

Thin-layer chromatography was performed on ground coffee (commercially obtained from a local grocery store), *C. arabica* leaves, and roots in a solvent system containing benzene:methanol (90:10) which showed a rather intensive spot for a triterpenoid or a steroid (Figure 1). Saponins were excluded on the basis of Solvents SS I, SS II and SS III. The samples were run in the petroleum ether:chloroform: acetic acid and the benzene:methanol (90:8); however, they did not move far enough from the origin, so the benzene:methanol (90:10) was developed for this purpose and used throughout the purification procedure.

The unknown compound was isolated and purified from *Coffea arabica* L. leaves yielding approximately 0.055%. It did not yield any sugar from acid-hydrolysis (Wyman-Simpson, 1991) indicating that it was *not a saponin.* Upon TLC of the unknown triterpenoid with ursolic acid and oleanolic acid standards, the results shown in Figure 2 were obtained. Clearly, more derivatization was needed and accomplished with the results shown in Figure 3. Since the methyl ester, acetate, and the acetyl methyl derivatives of ursolic acid, oleanolic acid, and the coffee unknown triterpenoid were identical (cf. Figure 3) in all of the TLC solvents tested, it was necessary to make use of infrared spectrometry, FAB-MS, FAB(Cs^+ ion gun)-MS, ^{1}H-NMR and ^{13}C-NMR spectrometry.

From the infrared spectrum (Figure 4) it was determined that the unknown contained at least one hydroxyl group and one carboxylic acid group. Negative ion fast atom bombardment (FAB) mass spectrometry (MS) of the samples with NaCl added (to produce more intense ions) provided spectra that are shown in Figure 5. From these spectra it was suggested that the unknown triterpenoid (Fig. 5a) was not oleanolic acid (Fig. 5c) because of the low intensity of the ions at *m/z* =207, $M^{+\bullet}$=456 and M^+-18 (*m/z*=438). In addition, the mass spectrum of the unknown (Fig. 5a) *does agree* with the ursolic acid standard spectrum (Fig. 5b). The mass spectrum of the methyl ester derivative of the unknown coffee triterpenoid (Fig. 5d) shows a $M^{+\bullet}$ at *m/z*=470 which is 14 mass units higher than the original (Fig. 5a), indicating that *only one carboxylic acid was present.* The base peak was *m/z*=262 whereas the base peak of Fig 5a was *m/z*=248 indicating that the methyl group was on the carboxylic group of ring E. The acetate derivative (Fig. 5e) showed the $M^{+\bullet}$ ion at *m/z*=498 with fragmentation occurring at $M^{+\bullet}$-15 (loss of methyl group), M^+60 (loss of acetyl group), and $M^{+\bullet}$-60-15 (loss of an acetyl and a methyl group) and a base peak of *m/z*=248, indicating that *only one hydroxyl group was present in the unknown.* To confirm this finding the mass spectrum of the acetate of the methyl ester of the unknown coffee triterpenoid (Fig. 5f) was found to yield $M^{+\bullet}$ ion at *m/z*=512 with the major fragment ion occurring at M^+-60 (loss of an acetyl group) and it gave a base peak of *m/z*=262. The partial fragmentation pattern of ursolic acid as well as oleanolic acid are shown in Fig. 6. The base peak at *m/z*=248 is formed via a reverse Diels-Alder reaction and consists of rings D, E, and part of ring C. The fragment formed from rings A and B are formed from a reverse Diels-Alder reaction to yield *m/z*=207. Further fragmentation of ion *m/z*=248 are the loss of a -COOH which gives an ion at *m/z*=203. Subsequent loss of a 5-carbon fragment from ring E provides the ion at *m/z*=133 which does not contain C-29 and C-30 which the methyl groups that are attached to C-19 and C-20 of ursolic acid. For oleanolic acid both C-29 and C-30 would be attached to C-20 only. It is clear that FAB-MS provides only part of the information needed to ascertain the structure.

The linked scan mass (MS/MS) spectra (metastables) of oleanolic acid (Fig. 7c), ursolic acid (Fig. 7b), and the unknown triterpenoid (Fig. 7a) are shown. It is clear that the metastables of ursolic acid (Fig. 7b) and the unknown from the leaves of coffee (Fig. 7a) are similar with respect to the major ions formed. There are small differences, however, that remain to be resolved.

The ^{1}H-NMR spectra (not shown) did not clearly show the differences among the samples that the ^{13}C-NMR spectra showed. The ^{13}C-NMR spectral data (Fig. 8) was determined on the derivatized samples since the original triterpenoid was soluble only with difficulty in CD_3Cl. Fig. 8 (a-c) shows the ^{13}C-NMR spectra of the derivatives prepared of standard ursolic acid (Fig. 8a), the unknown compound, with that of the standard, and the literature values. They agree very well (see particularly **C-18, 19, 20, 21, 22**). The data do *not* support the derivatives of oleanolic acid as being the compound identified. The same pattern was repeated in acetyl ursolate (Fig. 8b), standard and literature values and again the carbons were all the same. For acetyl methyl ursolate (Fig. 8c), the unknown and literature values indicated that it was not the oleanolic acid and derivative but, acetyl methyl ursolate. *It was concluded based on these data, that the unknown triterpenoid isolate was identified as ursolic acid* (Figure 9).

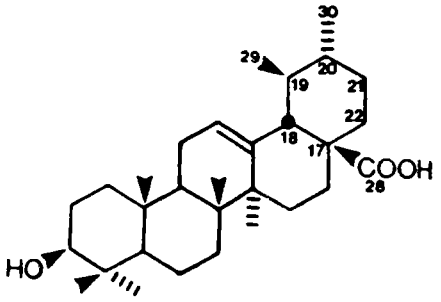

Figure 9. Structure of ursolic acid

Since ursolic acid is almost completely insoluble in water, and has little or no tendency to foam in water solution, it was concluded that some other compound(s) must be implicated in the foaming of roasted coffee beans. Ursolic acid is only slightly allelochemically active against the coffee plant (H. Stocker and T. Baumann, personal communication, 1991) and it is inactive against wheat (*Triticum aestivum)* and cheat (*Bromus secalinus)*; however, the indication is that it can serve as a synergistic agent with allelopathic reactions of mono-, sequi- and di-terpenes and mono-, sesqui-and di-terpenoids where it might act as a micellar host, which helps to retain the volatile plant constituents that may be important in development of the coffee plant, and releases them later during the development of the plants lifecycle (Williamson *et al.*, 1989).

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SUMMARY

The occurrence of ursolic acid in the mature leaves of *Coffea arabica,* is shown. It has been shown by TLC that coffee leaf ether extract contains triterpenoids which is predominately ursolic acid. The concentrated ether solution was extracted with dilute NaOH, acidified and extracted with ether. After evaporation of the organic solvent, the residue was chromatographed on silica gel using methanol-benzene (5:95). Fractions giving positive reaction with Liebermann-Burchard reagent were collected and evaporated. The residue was crystallized from benzene-ethanol (yielding: 0.05% DM), and its derivatives (acetate, methyl ester and methyl ester acetate) were prepared. Analysis by IR, FAB-MS, MS/MS, ^{13}C-NMR, and TLC show it to be ursolic acid. The occurrence of ursolic acid has not been found in any other part of the coffee plant but in leaves.

RESUMEN

Se demuestra la presencia de ácido ursólico en las hojas maduras de *Coffea arabica.* Usando TLC, se ha demostrado que el extracto de éter de las hojas del café contiene triterpenoides, los cuales se forman del predominante ácido ursólico. La solución concentrada de éter fue extraída con NaOH diluído, acidificada y extraida con éter. Después de la evaporacion del solvente orgánico, el residuo se sometió a cromatografía con sílica gel usando metanol:benceno (5:95). Fueron colectadas y evaporadas las fracciones que dieron reacción positiva con el reactivo de Libermann-Buchard. El residuo de benceno-etanol, fue cristalizado (produciendo .05% de peso seco), y sus derivados (acetato, acetato de metilo y acetato ester de metiol) fueron preparados. Análisis por IR, FAB-MS, MS/MS. ^{13}C-NMR y TLC mostraron la presencia de áscido ursólico. El ácido ursólico está presente solo en las hojas, no se ha encontrado en otra parte de la planta del café.

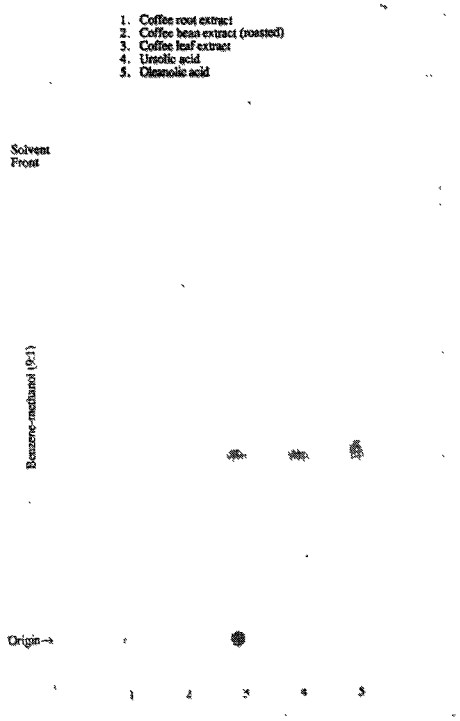


Figure 1. Photograph of a thin layer chromatogram of Coffee root extract (1), roasted coffee bean extract (2), and coffee leaf extract (3), developed in benzene:methanol (9:1) and sprayed with Lieberman-Burchard as seen under UV_{366nm}.

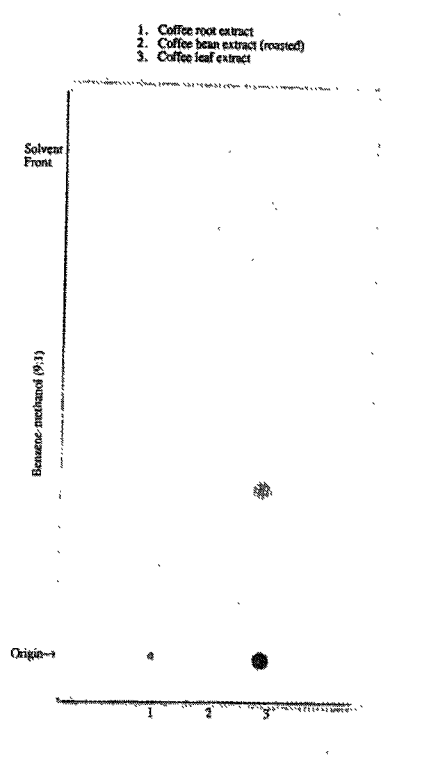


Figure 2. Photograph of a thin layer chromatogram of Coffee root extract (1), roasted coffee bean extract (2), coffee leaf extract (3), ursolic acid (4), and oleanolic acid (5), developed, sprayed, and visualized in a manner which was similar to Fig. 1.

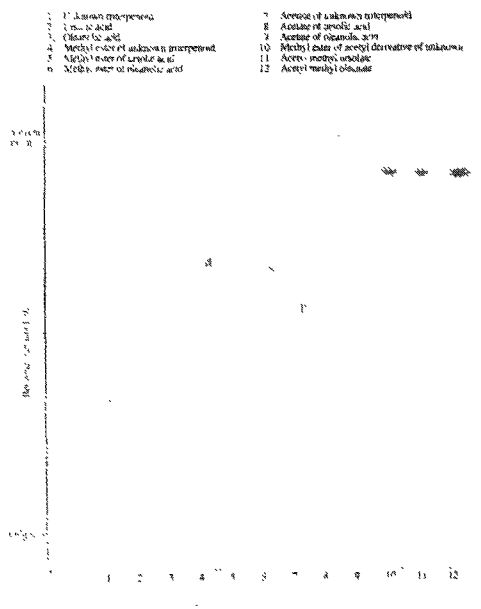


Figure 3. Photograph of a thin layer chromatogram of Coffee unknown triterpenoid (1), ursolic acid (2), oleanolic acid (3), methyl ester of unknown triterpenoid (4), methyl ester of ursolic acid (5), methyl ester of oleanolic acid (6), acetate of unknown triterpenoid (7), acetate of ursolic acid (8), acetate of oleanolic acid (9), methyl ester of acetyl derivative of unknown triterpenoid (10), acetyl methyl ester of ursolic acid (11), and acetyl methyl ester of oleanolic acid (12), developed, sprayed, and visualized in a manner which was similar to Fig. 1.

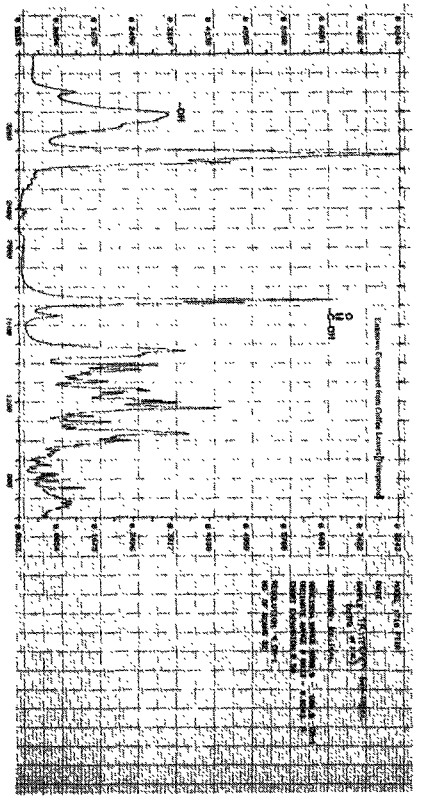


Figure 4. Infrared spectroscopy of the unknown triterpenoid from *Coffea arabica* L.

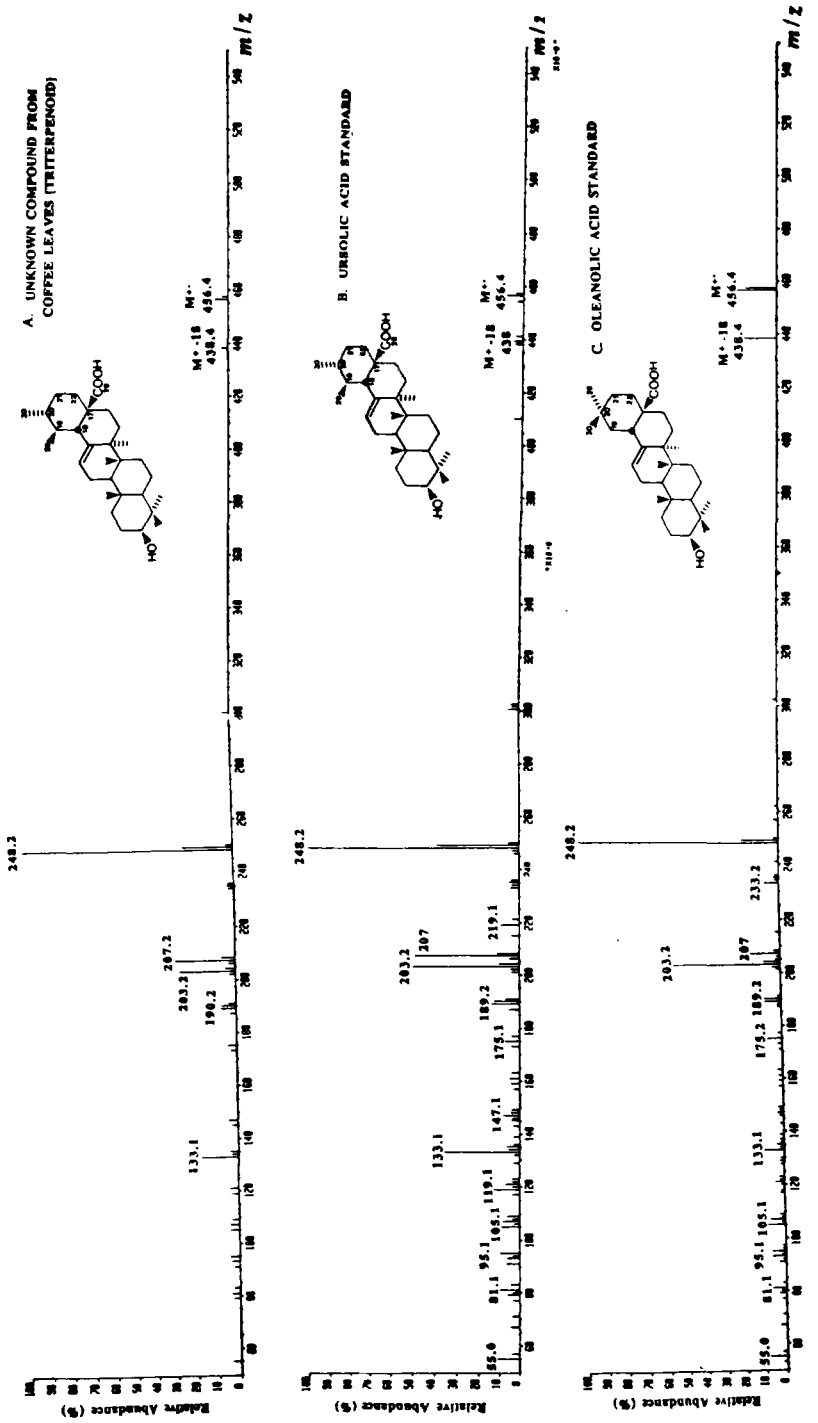


Figure 5: Negative Fast Atom Bombardment Mass Spectrometry, with NaCl added, produced for the following compounds:

A) Unknown Compound from Coffee Leaves (Triterpenoid)
B) Ursolic Acid Standard
C) Oleanolic Acid Standard

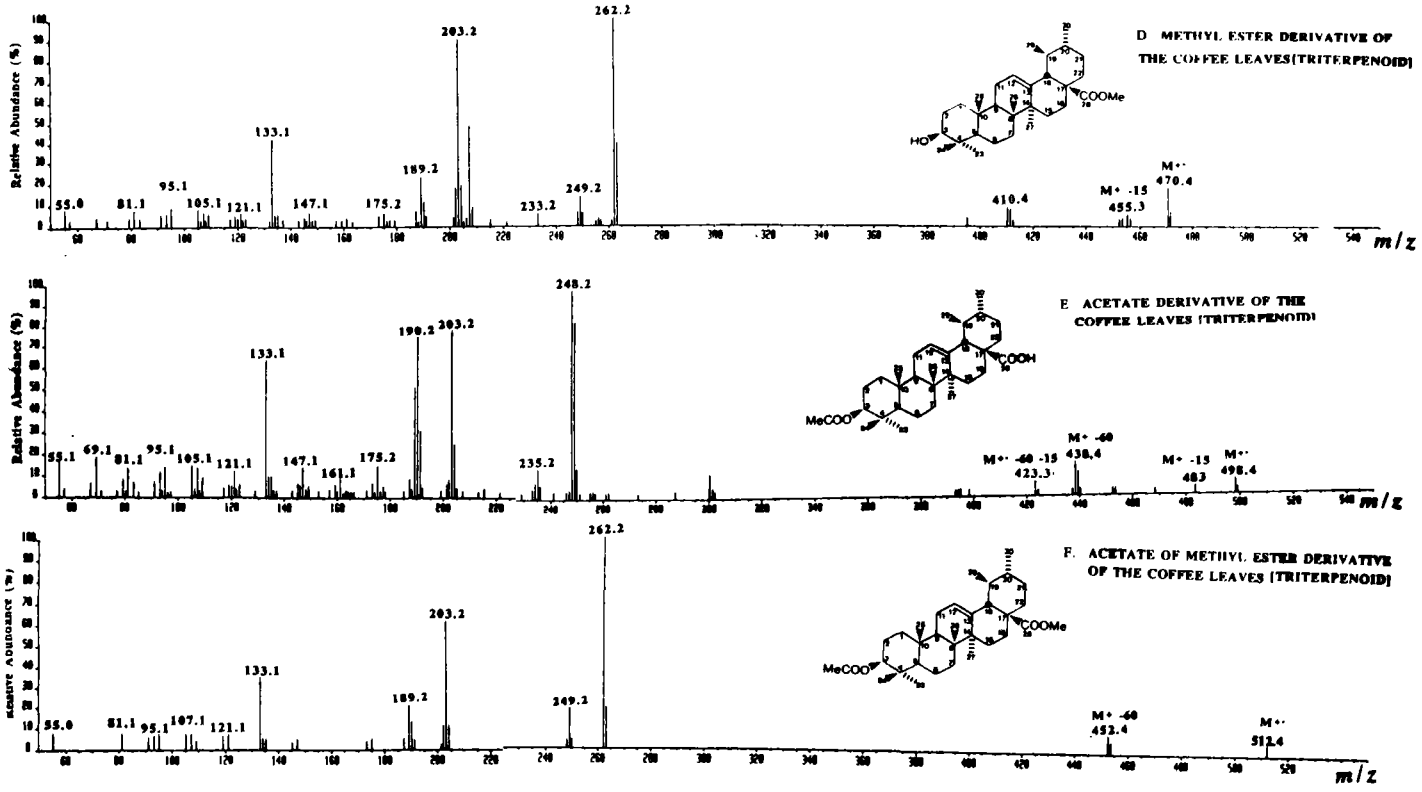


Figure 5 continued:

D) Methyl Ester Derivative of the Coffee Leaves (Triterpenoid)
E) Acetate Derivative of the Coffee Leaves (Triterpenoia)
F) Acetate of Methyl Ester Derivative of the Coffee Leaves (Triterpenoid)

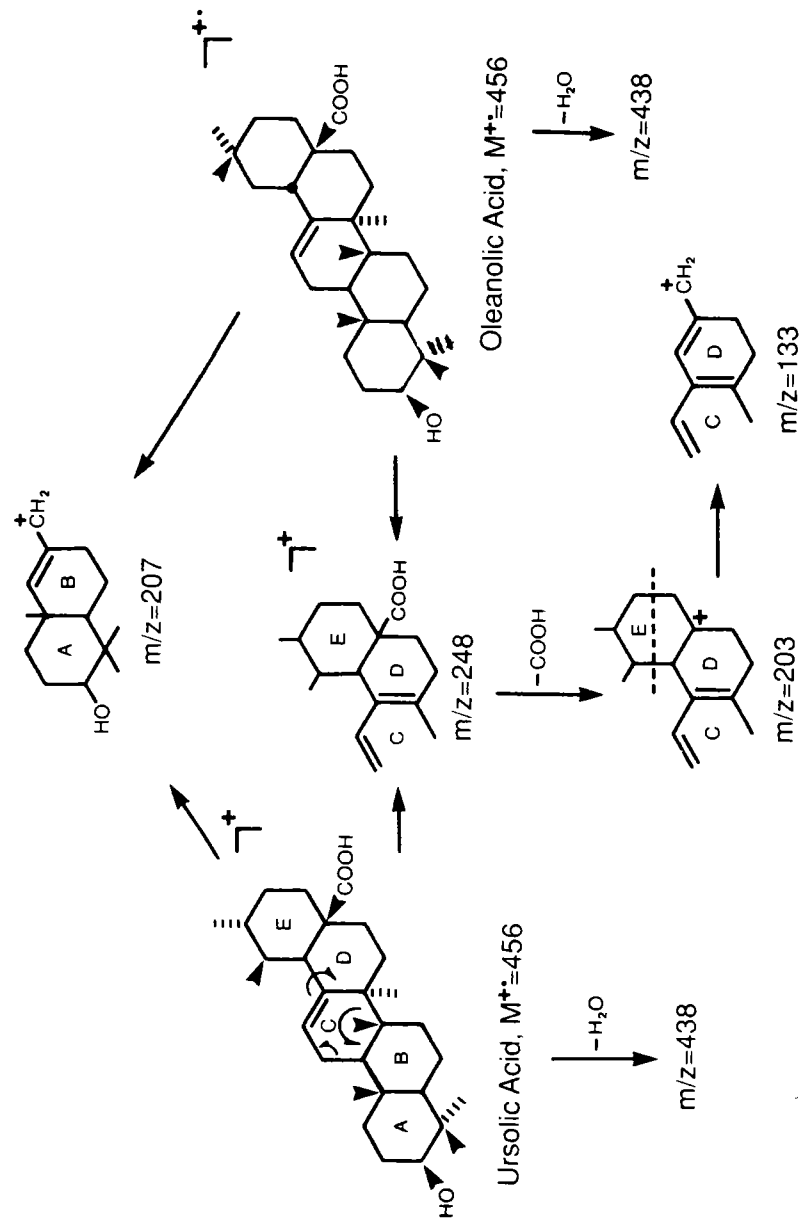


Figure 6: Partial Fragmentation Pattern of Ursolic and Oleanolic Acids (Differences are in intensity of the ions)

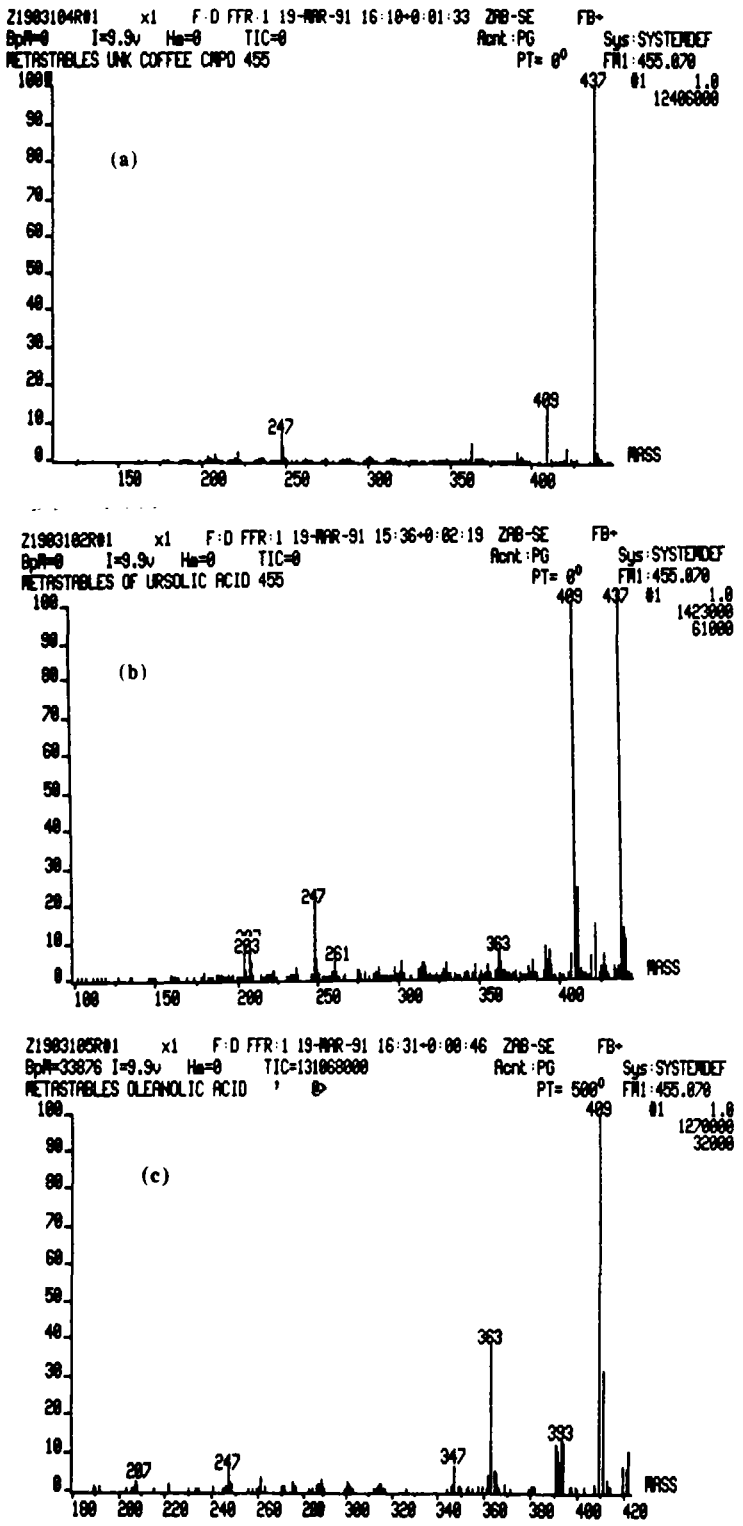
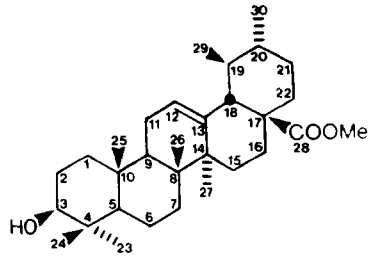
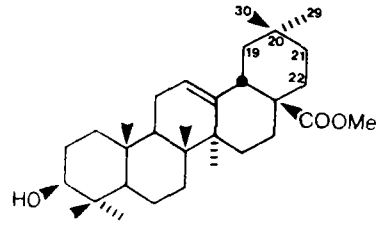


Figure 7: Metastable Ions (MS/MS) that occurred when the ZAB-2ES was run in the linked-scan mode of operation using the FAB (Cs^+) gun analysis:

a) Unknown Triterpenoid Coffee Compound
b) Ursolic Acid
c) Oleanolic Acid



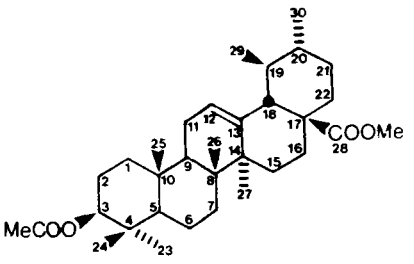
Methyl Ursolate



Methyl Oleanolate

Carbon	Methyl Ursolate			Methyl Oleanate
	Literature	Standard	Unknown	Literature
1	38.8	38.9	38.9	38.1
2	27.3	27.3	27.2	27.1
3	78.8	79.0	79.0	78.7
4	38.8	38.7	38.7	38.7
5	55.4	55.2	55.2	55.2
6	18.4	18.3	18.3	18.3
7	33.0	33.0	33.0	32.6
8	39.6	39.5	39.5	39.3
9	47.5	47.6	47.6	47.6
10	37.0	37.0	37.0	37.0
11	23.3	23.3	23.3	23.1
12	125.5	125.6	125.6	122.1
13	138.0	138.1	138.1	143.4
14	42.0	42.0	42.0	41.6
15	28.2	28.1	28.1	27.7
16	24.3	24.2	24.2	23.4
17	48.1	48.1	48.1	46.6
18	**52.8**	**52.9**	**52.9**	**41.3**
19	**39.1**	**39.0**	**39.0**	**45.8**
20	**38.8**	**38.6**	**38.6**	**30.6**
21	**30.7**	**30.7**	**30.7**	**33.8**
22	**36.7**	**36.6**	**36.6**	**32.3**
23	28.2	28.0	28.0	28.1
24	15.5	15.6	15.6	15.6
25	15.7	15.4	15.4	15.3
26	16.9	16.9	16.9	16.8
27	23.6	23.6	23.6	26.0
28	177.7	178.0	178.0	177.9
29	16.9	17.0	17.0	33.1
30	21.2	21.2	21.2	23.6
28-CO_2*CH_3	51.4	51.5	51.4	51.3

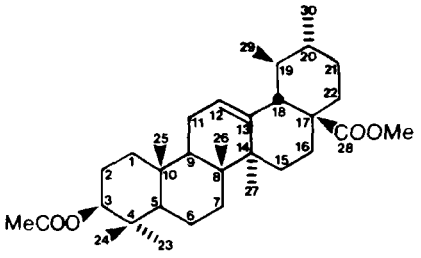
Figure 8a. ^{13}C NMR Spectral data for standard methyl ursolate, methyl oleanolate, and unknown from *Coffea arabica* L. leaves with literature values (Seo, *et al.*, 1975).



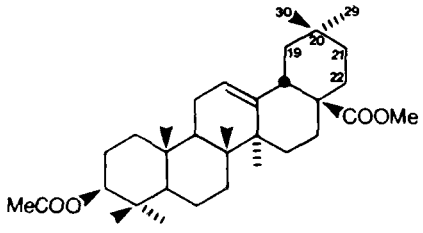
Acetyl Ursolate

Carbon	Acetyl Ursolate		
	Literature	Standard	Unknown
1	38.1	38.2	38.2
2	23.8	24.0	24.0
3	80.8	80.9	80.9
4	37.5	37.6	37.7
5	55.1	55.3	55.3
6	18.0	18.1	18.1
7	32.7	32.8	32.8
8	39.3	39.5	39.5
9	47.3	47.4	47.4
10	36.9	36.9	36.9
11	23.4	23.6	23.6
12	125.5	125.7	125.7
13	137.8	137.9	137.9
14	41.6	41.9	41.9
15	27.9	28.0	28.0
16	23.9	24.0	24.0
17	47.8	47.9	47.9
18	**52.3**	**52.5**	**52.5**
19	**38.8**	**39.0**	**39.0**
20	**38.7**	**38.8**	**38.8**
21	**30.4**	**30.6**	**30.6**
22	**36.5**	**36.7**	**36.7**
23	27.9	28.0	28.0
24	16.9	17.0	17.0
25	15.5	15.5	15.5
26	16.5	16.7	16.7
27	23.9	24.0	24.0
28	184.0	183.9	183.5
29	16.8	17.0	17.0
30	21.0	21.3	21.3
28-CO_2*CH_3	170.7	171.0	171.0
3-CHOCO*CH_3	21.1	21.2	21.2

Figure 8b. ^{13}C NMR Spectral data for standard acetyl ursolate, unknown from *Coffea arabica* L. leaveswith literature values (Seo, *et al.*, 1975).



Acetyl Methyl Ursolate



Acetyl Methyl Oleanolate

Carbon	Acetyl Methyl Ursolate			Acetyl Methyl Oleanate Literature
	Literature	Standard	Unknown	
1	38.3	38.3	38.3	38.1
2	23.6	23.5	23.6	23.6
3	80.7	80.9	80.9	80.7
4	37.6	37.7	37.7	37.5
5	55.3	55.3	55.3	55.2
6	18.1	18.2	18.2	18.2
7	32.8	32.3	32.9	32.6
8	39.5	39.5	39.5	39.3
9	41.4	47.5	47.5	47.5
10	36.8	36.9	36.9	36.9
11	23.2	23.3	23.3	23.0
12	125.4	125.4	125.5	122.1
13	138.0	138.2	138.2	143.6
14	41.9	41.9	41.9	41.6
15	28.1	28.1	28.1	27.7
16	24.2	24.2	24.2	23.6
17	48.0	48.1	48.1	46.6
18	**52.8**	**52.9**	**52.9**	**41.3**
19	**38.9**	**38.9**	**38.9**	**45.8**
20	**38.9**	**38.9**	**39.0**	**30.6**
21	**30.7**	**30.6**	**30.6**	**33.8**
22	**36.6**	**36.6**	**36.6**	**32.3**
23	28.0	28.0	28.0	28.0
24	16.9	16.9	16.9	16.8
25	15.5	15.5	15.5	15.3
26	16.9	16.7	16.7	16.8
27	23.6	23.5	23.6	25.8
28	177.6	178.0	178.0	177.8
29	17.1	17.1	17.1	33.1
30	21.2	21.2	21.2	23.1
28-CO_2*CH_3	51.3	51.4	51.4	51.4
3-CHO*$COCH_3$	170.5	171.0	171.0	170.5
3-CHOCO*CH_3	21.2	21.2	21.2	21.2

Figure 8c. ^{13}C NMR Spectral data for standard acetyl methyl ursolate, acetyl methyl oleanolate unknown from *Coffea arabica* L. leaves with literature values (Seo, *et al.*, 1975).

COMPARISON OF CAFFEINE, THEOBROMINE AND THEOPHYLLINE METABOLISM AND DISTRIBUTION BETWEEN COFFEE AND TEA PLANTS

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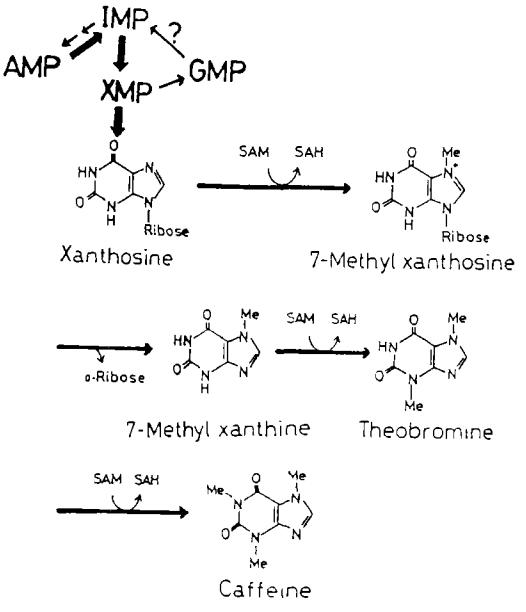
INTRODUCTION

In coffee *(Coffea arabica)* and tea *(Camellia sinensis)* plants, caffeine is synthesized from xanthosine by four sequential reactions (Suzuki and Waller, 1988; Waller and Suzuki, 1990; Scheme 1). Methyl groups are transferred from *S*-adenosyl-L-methionine (SAM) to xanthosine, yielding 7-methylxanthosine (Negishi *et al.*, 1985a,b, 1988), which undergoes further methylations via 7-methylxanthine and theobromine to caffeine (Suzuki and Takahashi, 1975a; Roberts and Waller, 1979). Although direct evidence has not yet been obtained, xanthosine seems to be derived from purine nucleotides. In the cells of these plants, the purine ring of caffeine is synthesized by the same series of reactions as those involved in the biosynthesis of purine nucleotides *de novo* (Anderson and Gibbs, 1962; Preusser and Serenkov, 1963; Suzuki and Takahashi, 1975b). Among exogenously administered compounds, adenine is the most effective precursor for the biosynthesis of caffeine (Suzuki and Takahashi, 1976a; Suzuki and Waller, 1984a), and, in plant cells, levels of adenine nucleotides are usually much higher than those of guanine nucleotides (e.g. Meyer and Wagner, 1985; Ashihara *et al.*, 1988). Thus adenine nucleotides seem to be primarily utilized as precursors for the synthesis of caffeine. The following pathway from adenine nucleotides to caffeine is postulated: AMP→IMP→XMP→xanthosine→7-methylxanthosine →7-methylxanthine→theobromine→caffeine (Fujimori *et al.*, 1991; Scheme 1).



Scheme 1. Biosynthesis of caffeine from purine nucleotides in tea and coffee (*C. arabica*) plants.
Legend: SAM: *S*-adenosylmethionine; SAH: *S*-adenosylhomocysteine; R5P: ribose 5-phosphate; PRPP: 5-phosphoribosyl-1-pyrophosphate. For SAM biosynthesis in tea plants, see Suzuki (1972). Redrawn from Suzuki and Waller (1988).

Biosynthesis of radioactive caffeine and theobromine in immature *C. arabica* fruit showed that a pathway from AMP and/or GMP was effective. Biodegradation of radioactive caffeine was shown to occur slowly, resulting in the formation of glyoxylic acid and urea which gives rise to CO_2 and NH_3 (Suzuki and Waller, 1984a,b; Waller and Suzuki, 1990). These results indicate that (a) biosynthesis of caffeine occurs mainly during the green stage of fruit development through methylation of N^7-methylxanthine and theobromine, and (b) caffeine degradation occurs primarily through the demethylation of theophylline, which accumulates after the fruit is full size and proceeds to ripen (cf. also Keller *et al.*, 1972; Roberts and Waller, 1979).

There are seasonal variations in the caffeine content and biosynthetic capacity for caffeine synthesis in tea leaves, and the decrease in autumn indicates metabolism and/or translocation of the alkaloid (Suzuki and Waller, 1986; Fujimori *et al.*, 1991). In tea seedlings, the biosynthesis of caffeine from [8-^{14}C]adenine occurs only in younger leaves, and more than 99% of the total caffeine in the seedlings is found in leaves (Ashihara and Kubota, 1986). However, biosynthesis of caffeine is also observed in other organs, such as flowers, of tea bushes (Fujimori and Ashihara, 1990). Moreover, the occurrence and contents of purine alkaloids in tea fruits and seeds during fruit development and seed germination have been reported recently (Suzuki and Waller, 1985; 1987a,b; Ashihara and Kubota, 1986).

The purpose of this report is to provide up-to-date, comprehensive knowledge of distribution and metabolism of purine alkaloids in coffee and tea. In contrast to the pathway leading to the formation of xanthosine from AMP, IMP, and XMP for caffeine biosynthesis (see the review by Suzuki and Waller, 1988; Negishi *et al.*, 1988; Fujimori *et al.*, 1991), that from GMP for caffeine biosynthesis remains obscure (Scheme 1); the possible pathway will be discussed from available evidence for the metabolism (catabolism) of guanine nucleotides in plants.

EXPERIMENTAL

For details the original articles must be consulted; or the chapter on *Metabolism and Analysis of Caffeine and other Methylxanthines in Coffee, Tea, Cola, Guanana and Cacao* provides a summary of work until 1987 (Suzuki and Waller, 1988).

RESULTS AND DISCUSSION

Distribution of Purine Alkaloids in *Coffea* and *Camellia* Plants

The discovery, chemical structure and occurrence of caffeine and associated compounds (Fig. 1) in plants as well as other organisms have already been adequately reviewed (for example, Kihlman, 1977; Arnaud, 1984).

	Trivial name	R^1	R^3	R^7 or R
1	Xanthine	H	H	H
2	7-Methylxanthine	Heteroxanthine	H	H	Me
3	1,3-Dimethylxanthine	Theophylline	Me	Me	H
4	3,7-Dimethylxanthine	Theobromine	H	Me	Me
5	1,7-Dimethylxanthine	Paraxanthine	Me	H	Me
6	1,3,7-Trimethylxanthine	Caffeine	Me	Me	Me
7	1,3,7,9-Tetramethyluric acid	Theacrine	-	-	-
8	2,1,9-Trimethyluric acid	Liberine	-	-	H
9	2,1,7,9-Tetramethyluric acid	Methylliberine	-	-	Me

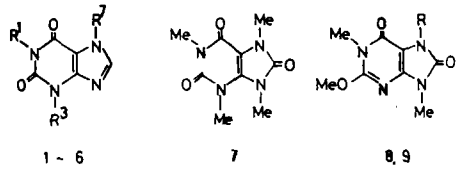
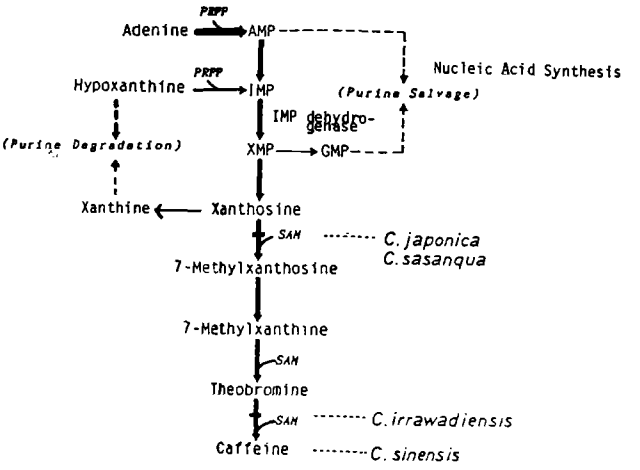


Figure 1 Structures of purine alkaloids in plants.



Scheme 2. Possible routes of purine alkaloid biosynthesis from adenine and hypoxanthine in *Camellia* plants.
Legend: PRPP: 5-phosphoribosyl-1-pyrophosphate, SAM: S-adenosyl methionine. Redrawn from Ashihara and Kubota (1987).

Several studies concerning the occurrence of purine alkaloids in caffeine-containing plants, especially with reference to the genera *Camellia* and *Coffea*, have been reported recently. In leaves of *Camellia sinensis* (tea) *and C. taliensis* the caffeine content exceeds 2% dry wt and the theobromine content is below 0.2%, whereas other *Camellia* species *(C. japonica* and *C. sasanqua)* contain little or no purine alkaloids in leaves (Nagata and Sakai, 1984, 1985a,b; Nagata, 1986). The major purine alkaloid in *C. irrawadiensis* leaves is theobromine (>0.5% dry wt), and little or no caffeine is present (Nagata and Sakai, 1985b). These findings indicate that metabolism of purine alkaloids differs among species of the genus *Camellia* (Ashihara and Kubota, 1987; Fujimori and Ashihara, 1990; Scheme 2).

Patterns of the purine alkaloids in leaves of *Coffea arabica* (Frischknecht *et al.*, 1982, 1986) are similar to those in tea leaves. Tea flowers and *C. arabica* unripe fruits (pericarp and seeds) show a similar alkaloid pattern, but a small amount of theophylline (<0.04% fresh wt) was also present in the pericarp of ripe *C. arabica* fruit (Suzuki and Waller, 1984b, 1985; Suzuki, 1985). Even in the fruit of tea, although it is of minor economic importance compared with that of coffee, the pericarp contains the most alkaloids (caffeine, 1%-2% fresh wt; theobromine, 0.05%-0.1%), but there are also considerable amounts in the seed coat and, to a lesser extent, the fruit stalk and the seed (Suzuki and Waller, 1985; Ashihara and Kubota, 1986).

Caffeine and chlorogenic acids as possible taxinomic criteria in *Coffea* plants covering 20 species have been reported by Clifford *et al.* (1989). The presence of caffeine in the beans of population A213 of *C. kianjavatensis*, one taxon of the *Mascarocoffea*, which traditionally have been viewed as caffeine-free, has recently been confirmed (Clifford *et al.*, 1991).

Besides theobromine, discovered in cocoa beans in 1842, and theophylline, isolated from tea leaves in 1888, the third dimethylxanthine, paraxanthine (Fig. 1), has been observed in *C. arabica* seedlings and callus cultures very recently (Chou and Waller, 1980a, b; Suzuki and Waller, 1982). Further, it should be added that three methyluric acids (Fig. 1) are present in the genus *Coffea* leaves and seeds (Wanner *et al.*, 1975; Petermann *et al.*, 1977; Petermann and Baumann, 1983; Kappeler and Baumann, 1986). As for 1,3,7,9-tetramethyluric acid, however, its occurrence was first reported in extracts of tea leaves (Johnson, 1937).

Metabolism of Caffeine in *Coffea* Plants: Dependence of Fruit/Leaf Development and Ageing

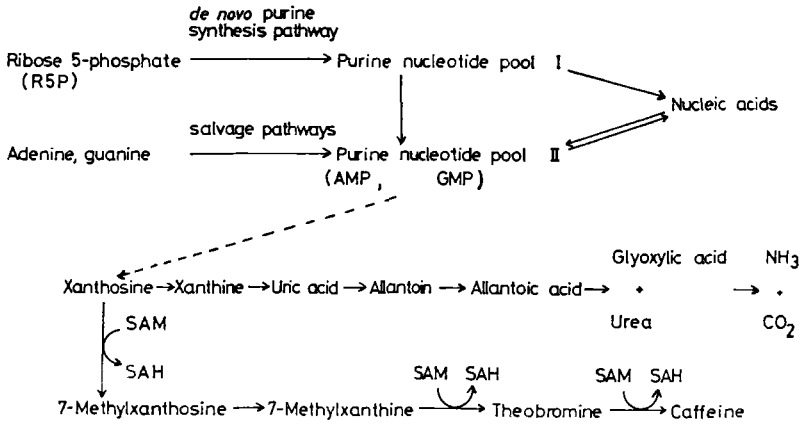
In coffee (*C. arabica*) plants, caffeine is synthesized from xanthosine via 7-methylxanthosine, 7-methylxanthine and theobromine and *S*-adenosylmethionine is the actual source of the methyl groups (Baumann *et al.*, 1978, 1983; Roberts and Waller, 1979; Waller *et al.*, 1983; Negishi *et al.*, 1985b; Scheme 1).

The purine ring of caffeine is synthesized by the same pathways as *de novo* purine biosynthesis, which was initially shown with nonpurine precursors in *C. arabica* leaves (Anderson and Gibbs, 1962) and then directly with purine precursors (adenine and guanine) in *C. arabica* fruits (Suzuki and Waller, 1984a; Table I). Immature fruits are metabolically more active in the formation of caffeine than the older, mature fruits (cf. also Roberts and Waller, 1979). Adenine and guanine may be converted into purine nucleotides (AMP and GMP) via the pathway of purine salvage (Scheme 3), as in tea leaves (Suzuki and Takahashi, 1976a). However, the pathway leading to the formation of xanthosine from these purine nucleotides remains to be characterized in coffee plants. By contrast, xanthine cannot serve as the direct precursor for the caffeine purine ring, and is metabolized by the conventional purine degradation pathway via uric acid even in tea and coffee *(C. arabica)* plants (Suzuki and Takahashi, 1975b; Waller *et al.*, 1981, 1983; Suzuki and Waller, 1984a; Scheme 3). Although the methylation of xanthosine to 7-methylxanthosine is the first step of a series of methylations in the pathway for caffeine biosynthesis, xanthosine is also metabolized by purine degradation pathway via xanthine (Negishi *et al.*, 1985b, 1988).

Table I. Biosynthesis: Incorporation of ^{14}C-Labeled Precursors into Methylated Xanthine in Coffee Fruits[a]

	nCi/fruit				
	radioactivity	radioactivity incorporated into			
precursor	uptake	7-MX		Tb	Cf
immature fruit					
[8-^{14}C]adenine	440	4.5 (1.0)[b]	6.9	(1.6)	147.2 (33.4)
[8-^{14}C]guanine	480	2.8 (0.6)	3.8	(0.8)	48.0 (10.0)
L-[*methyl*-^{14}C]methionine	450	trace (–)	0.7	(0.2)	17.1 (3.8)
mature fruit					
L-[*methyl*-^{14}C]methionine	460	trace (–)	0.6	(0.1)	4.3 (0.9)

[a]Each fruit was placed in a 0.1-ml vial with its petiole immersed in [8-^{14}C]adenine, [8-^{14}C]guanine, or L-[*methyl*-^{14}C]methionine (500 nCi in 50 μL of water). Administration was for 12 h and then incubation for 36 h in distilled water. 7-MX = N^7-methylxanthine. [b]Numbers in parentheses represent percent of the radioactivity incorporated. (Suzuki and Waller, 1984).



Scheme 3. Purine metabolism and caffeine biosynthesis in coffee (*C. arabica*) plants.
Legend: The pathway leading to the formation of xanthosine from purine nucleotides remains to be characterized. Redrawn from Suzuki and Waller (1988).

Biodegradation of caffeine to xanthine, which is further metabolized by the purine degradation pathway via uric acid, was first shown to occur in *C. arabica* leaves (Kalberer, 1964, 1965), but theophylline and theobromine have been identified as the first degradation products in immature and mature *C. arabica* fruits recently (Suzuki and Waller, 1984a, b). For [8-^{14}C]theophylline, the biodegradation products are 3-methylxanthine, allantoin, allantoic acid, urea, and an unknown compound, but no 1-methylxanthine (Suzuki and Waller, 1984a,b; de Romero and Waller, 1988). Further, it has been postulated that the appearance of theophylline in the pericarp of ripe fruit is probably due to a slowdown of theophylline catabolism at the mature fruit stage (Suzuki and Waller, 1984a, 1985).

Caffeine biodegradation to xanthine has also shown to occur in immature fruits of other *Coffea* species through demethylation of theobromine, theophylline and 3-methylxanthine (Mazzafera *et al.*, 1991). Purine alkaloid production and metabolism *in vitro* cultures of *Coffea* species have been adequately reviewed by Baumann and Frischknecht (1988).

Three methyluric acids, 1,3,7,9-tetramethyluric acid (7), O^2,1,9-trimethyluric acid (8) and O^2,1,7,9-tetramethyluric acid (9) (see Fig. 1), have been identified as the products of caffeine metabolism in leaves of *Coffea* plants such as *C. liberica, C. dewevrei,* and *C. abeokutae* (Wanner *et al.*, 1975; Petermann *et al.*, 1977; Petermann and Baumann, 1983). In these species, the young plant accumulating caffeine (6) transforms theobromine (4), but at later stages of growth caffeine is gradually replaced by 1,3,7,9-tetramethyluric acid and then by O^2,1,9-trimethyluric acid; O^2,1,7,9-tetramethyluric acid is presumably the direct precursor of O^2,1,9-trimethyluric acid (Petermann and Baumann, 1983; Baumann, 1986).

Caffeine Biosynthesis and Purine Metabolism in Tea Plants

The purine ring of caffeine in tea leaves is synthesized by the same pathways as *de novo* purine biosynthesis, which was initially shown with nonpurine precursors (Preusser and Serenkov, 1963) and then directly with purine precursors (see the review by Suzuki and Takahashi, 1977 and cf. also Negishi *et al.*, 1985a, c; Scheme 1).

Purine and nucleic acid metabolism concerning caffeine biosynthesis in caffeine-containing plants has been reported exclusively for tea plants, using L-[methyl-^{14}C]methionine (Ogutuga and Northcote, 1970; Suzuki and Takahashi, 1976b), *de novo* purine biosynthetic precursors such as glutamic acid γ-[^{14}C]methylamide (Konishi *et al.*, 1972a,b), [^{14}C]methylamine, and [1-^{14}C]glycine (Suzuki, 1973; Suzuki and Takahashi, 1976b), and purine precursors such as [8-^{14}C]hypoxanthine, [8-^{14}C]adenine, and [8-^{14}C]guanine (Suzuki and Takahashi, 1975b, 1976a; Ashihara and Kubota, 1986, 1987; Fujimori and Ashihara, 1990; Fujimori *et al.*, 1991).

Although the importance of the purine salvage pathways for caffeine biosynthesis has been demonstrated (Suzuki and Takahashi, 1975b, 1976a; Suzuki and Waller, 1984a; Ashihara and Kubota, 1986), it remains uncertain whether purine bases are the normal precursors (Scheme 2). Alternatively, among free purine

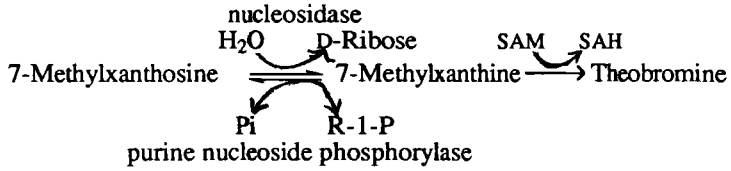
compounds, AMP is the major component in green tea, followed by ADP and GMP (Takino *et al.*, 1972), and adenine nucleotides seem to be utilized primarily as precursors for the synthesis of caffeine (Fujimori *et al.*, 1991).

Yabuki and Ashihara (1991) studied the metabolic fate of adenine nucleotides in noncaffeine-producing plant cells by tracing the flow of radioactivity from adenine nucleotides, prelabeled by incubation of suspension-cultured plant cells with [8-^{14}C]adenosine, into various derivatives of purines, and suggested that adenine nucleotides are rapidly catabolized in plant cells through the steps: AMP→IMP→inosine→hypoxanthine→xanthine (cf. also the review by Wasternack, 1982). The rapid conversion from AMP into other purine nucleotides (i.e. IMP, XMP, GMP, etc.) in tea plants, compared to the very low conversion of GMP into other purine nucleotides, has been described (Suzuki and Takahashi, 1976a, 1977; Scheme 1).

Caffeine Biosynthesis *in Vitro* with Enzyme Preparations

The complete description of a biosynthetic pathway should involve the characterization of each individual intermediate and each enzyme system involved; the enzymes described for caffeine biosynthesis and related metabolism in caffeine-producing plants until 1987 are summarized by Suzuki and Waller (1988).

Methyl groups are transferred from SAM to xanthosine, yielding 7-methylxanthosine (Negishi *et al.*, 1985a, b), which undergoes further methylations via 7-methylxanthine and theobromine to caffeine (Suzuki and Takahashi, 1975a; Roberts and Waller, 1979; Scheme 1). The products formed from 7-methylxanthosine were first identified as theobromine (Roberts and Waller, 1979), and then D-ribose and 7-methylxanthine when 7-methylxanthosine was incubated with unripe *C. arabica* fruit extracts; this confirmed the formation of 7-methylxanthine from 7-methylxanthosine through the action of nucleosidase, but not of purine nucleoside phosphorylase (Suzuki and Waller, 1980).



Recently, Negishi *et al.* (1988) detected *N*-methyl nucleoside hydrolase (*N*-methyl nucleosidase, N-MeNase), which hydrolyzes 7-methylxanthosine to produce 7-methylxanthine, in tea-leaf extracts and separated it from adenosine nucleosidase (ANase, EC 3.2.2.7) by DEAE-cellulose column chromatography. Among purine and *N*-methylpurine nucleosides, 3- and 7-methylpurine nucleosides were hydrolyzed preferentially by N-MeNase. ANase could not hydrolyze 7-methylxanthosine, although the enzyme showed high activity toward 7-methyladenosine. Negishi *et al.* (1988) suggested that N-MeNase catalyzes the hydrolysis of 7-methylxanthosine in the pathway of caffeine biosynthesis, whereas ANase is not directly concerned with it.

Caffeine Metabolism in Tea Plants: Seasonal Variations and Dependence of Development and Ageing

Even though there were fluctuations in the caffeine content of tea leaves, the changes under Kenyan (Owuor and Chavanji, 1986) conditions were not as large as those found under Malawi conditions where over 60% variations were noted in the course of the year (Cloughley, 1982). This is primarily because the seasonal mean temperature variations are not large under Kenya tea-growing conditions (Owuor and Chavanji, 1986). By contrast, the purine alkaloids decreased markedly in August and in October and November in Japan, while total nitrogen increased appreciably in November and December. These results suggest metabolism and/or translocation of the alkaloids in autumn, which remains(s) to be further characterized, and the absence of any role for them in the storage of nitrogen in winter (Suzuki and Waller, 1986; Waller and Suzuki, 1990).

Seasonal variations in the metabolic fate of adenine nucleotides prelabeled with [8-^{14}C]adenine were examined in leaf disks prepared at one-month intervals, over the course of one year, from the shoots of tea plants growing under natural field conditions (Fujimori *et al.*, 1991). Incorporation of radioactivity into nucleic aids and catabolites of purine nucleotides was found throughout the experimental period, but incorporation into theobromine and caffeine was found only in the young leaves harvested from April to June (Fig. 2). Moreover, significant activity of all three relevant *N*-methyltransferases was found in cell-free preparations from the leaves harvested in April and May. However, these activities disappeared completely from the leaves in July and August. In contrast, the activity of PRPP synthetase, which participates in the synthesis of nucleotides, was still maintained at detectable levels in July and August (Table 2). These results lead to the conclusion that the

biosynthesis of caffeine occurs in young leaves during the early stages of shoot development (April to June) and that one of the most important limiting factors for the synthesis of caffeine is the activity of various *N*-methyltransferases. Therefore, induction and repression of these enzymes seem to provide the primary mechanism for the control of the biosynthesis of caffeine in tea leaves (Fujimori *et al.*, 1991).

Table 2. Seasonal changes in the levels of activity of *N*-methyltransferases involved in the biosynthesis of caffeine and of 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase in tea leaves

Sampling time	*N*-methyltransferase activity [pkat g^{-1} fr. wt)			PRPP synthetase activity [pkat g^{-1} fr. wt)
	Xanthosine	7-Methylxanthine	Theobromine	
April	18±2	288±3	30±2	1115±45
May	13±3	195±13	20±3	763±72
June	<1	15±2	<1	67±3
July	nd	<1	nd	73±27
August	nd	nd	nd	42±10

Samples were harvested on the first day of every month. The average values and s.d. were obtained from more than six assays using two different samples. nd: not detected, (Fujimori, *et al.* 1991).

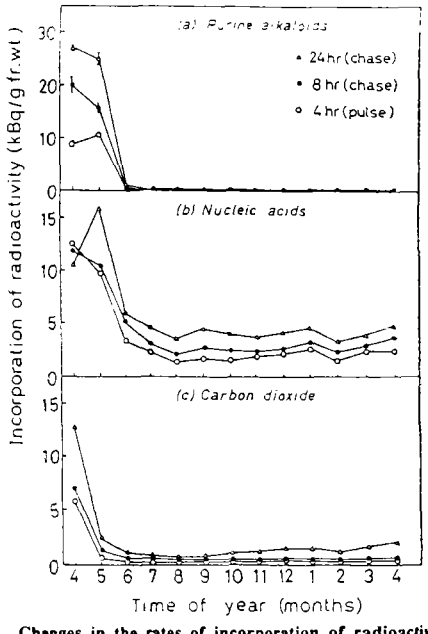


Figure 2 Changes in the rates of incorporation of radioactivity from [8-^{14}C]adenine into (a) purine alkaloids (i.e. theobromine plus caffeine), (b) nucleic acids and (c) carbon dioxide. The values are averages from duplicate samples.
Legend: ○, 4 h (pulse); •, 4 h (chase); Δ, 20 h (chase). Total uptake of radioactivity by leaf segments or disks (kBq g^{-1} fr. wt) was as follows: April (leaves from new shoot) 58.6 ± 2.1; May, 55.0 ± 3.1; June, 32.1 ± 1.0; July, 18.0 ± 0.7; August, 15.6 ± 0.5; September, 17.4 ± 1.4; October, 15.1 ± 1.4; November, 15.8 ± 1.2; December, 15.8 ± 1.2; January, 17.2 ± 0.8; February, 14.8 ± 1.6; March, 17.9 ± 1.7; and April (one-year-old leaves), 17.3 ± 0.7, (Fujimori, *et al.* 1991)

Synthesis and metabolism of purine alkaloids also appear to be closely associated with leaf development and ageing in tea seedlings (Ashihara and Kubota, 1986). An analysis for purine alkaloids in different parts of 4-month-old tea seedlings with high-performance liquid chromatography shows that more than 99% of the caffeine detected was in the leaves. The amount expressed per g fresh weight was higher in older leaves. Theobromine was found only in younger leaves. Zero or only trace amounts of theophylline were found in the seedlings.

Tracer experiments using [8-^{14}C]adenine indicated that (i) caffeine biosynthesis from [8-^{14}C]adenine occurs only in younger leaves, (ii) "salvage" of [8-^{14}C]adenine for nucleic acid synthesis takes place in all parts of the seedlings, (iii) considerable degradation of [8-^{14}C]adenine by conventional purine degradation pathway via uric acid takes place in roots and lower parts of stem tissue. These results strongly suggest that caffeine is synthesized in younger leaves and accumulated within the leaves (Ashihara and Kubota, 1986).

Biosynthesis of Purine Alkaloids in Flowers of *Camellia* Plants

In *C. arabica* caffeine is synthesized in the pericarp, transported to the seeds, and accumulated there during fruit development, and even transport from leaf to leaf has been demonstrated by application of doubly labeled caffeine (Baumann and Wanner, 1972). Biodegradation of caffeine is also considered to occur primarily in the pericarp of *C. arabica* fruit (Suzuki and Waller, 1985; Waller and Suzuki, 1990). In contrast, the caffeine found in the flower and dry fruit (pericarp and seed coats) of tea appears to be primarily that synthesized and accumulated in these tissues, which also contain important amounts of theobromine, the immediate precursor of caffeine biosynthesis, during their development (Suzuki, 1985; Suzuki and Waller, 1985).

Flower buds of new shoots of tea plants appear in August, and their fresh weight increases until October when the tree is in blossom. The amount of caffeine in the flowers increased substantially until October while that

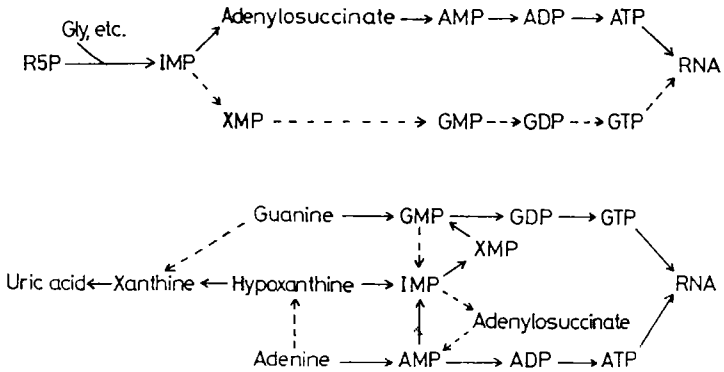
of theobromine changed only slightly. Caffeine was found both in stamens and in petals, but significant amounts of theobromine were found only in the stamens. The concentrations of purine alkaloids expressed per g fr. wt were higher in stamens and petals from flower buds of tea plants than those in stamens and petals after flowering (Suzuki, 1985; Fujimori and Ashihara, 1990). Tracer experiments using [8-^{14}C]adenine showed that caffeine is synthesized in stamens and petals, and the potential for synthesis is somewhat higher in the flower buds than it is in flowers after flowering (Fujimori and Ashihara, 1990). These results indicate that the decrease in the alkaloids in tea leaves in autumn (Suzuki and Waller, 1986) is not due to translocation of the alkaloids to the flowers.

The patterns of distribution of purine alkaloids in flowers of the various *Camellia* species (Fujimori and Ashihara, 1990) are the same as those in the leaves of the *Camellia* plants (Nagata and Sakai, 1985; Nagata, 1986). These results indicate that *Camellia* plants can be classified into three types with respect to the accumulation of purine alkaloids, as follows: caffeine-accumulating plants (e.g. *C. sinensis*); theobromine-accumulating plants (e.g. *C. irrawadiensis*); and plants that do not accumulate purine alkaloids (*C. japonica* and *C. sasanqua*).

Further, the metabolism of [8-^{14}C]adenine in isolated stamens and petals from the flower buds of four different species of *Camellia* revealed that the pathway from theobromine to caffeine does not operate in flowers of *C. irrawadiensis*, while the entire biosynthetic pathway for purine alkaloids is not functional in flowers of *C. japonica* and *C. sasanqua* (Fujimori and Ashihara, 1990), as it is in leaves of these *Camellia* plants (Ashihara and Kubota, 1987; Scheme 2).

Biosynthesis of Caffeine from Guanine Nucleotides: A Reappraisal

There is evidence to suggest that purine *de novo* and salvage reactions are compartmentalized separately in tea shoot tips, indicating the importance of the purine salvage pathways for caffeine biosynthesis (Suzuki and Takahashi, 1977; Suzuki and Waller, 1988; Scheme 4).



Scheme 4. A two-compartment model to explain labeling ratios in RNA purine nucleotides after feeding purine or nonpurine precursors to detached tea shoot tips. Reactions shown by *dashed lines* are assumed to be slow, allowing precursors of the de novo pathway to IMP (*top*) to be more incorporated into AMP of RNA than into GMP of RNA. In the salvage pathway (*bottom*), guanine is converted exclusively into GMP of RNA. Hypoxanthine is incorporated primarily into GMP of RNA, because conversion of IMP to GMP is much faster than that to AMP. At first, adenine labels largely AMP of RNA, but later GMP can also be formed if GMP synthesis from IMP is active in the salvage compartment. Hypoxanthine can also be degraded via xanthine and uric acid, but guanine is not readily degraded via xanthine and uric acid. Redrawn from Suzuki and Waller (1988) and Waller and Suzuki (1990).

Compared to the rapid conversion of adenine nucleotides to other purine nucleotides in tea plants (high incorporation of administered [8-^{14}C]adenine into the AMP and GMP of RNA), there was an exclusive incorporation of administered [8-^{14}C]guanine into the GMP of RNA (Suzuki and Takahashi, 1976a, 1977; Fig.

3). This suggests that the conversion of GMP into IMP catalyzed by the enzyme, GMP reductase (EC 1.6.6.8), is not an important reaction in the plants, and xanthosine is not likely derived from GMP via IMP and XMP for caffeine biosynthesis (Scheme 1). Thus, the pathway for xanthosine synthesis from GMP via IMP and XMP may not be operational. The results (Fig. 3) also indicate that, in contrast to the rapid decrease of administered [8-^{14}C]adenine in tea shoot tips, the decrease of administered [8-^{14}C]guanine is relatively slow. This may be a reflection of little activity of guanine deaminase (EC 3.5.4.3) which catalyzes the conversion of guanine to xanthine, as well as low activity of hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8), compared to high activities of xanthine dehydrogenase and of adenine phosphoribosyltransferase. These observations lead to the hypothesis that, regarding guanine nucleotides, xanthosine may be synthesized via guanosine, in a reaction catalyzed by the enzyme guanosine deaminase (EC 3.5.4.15), as follows: GMP → guanosine → xanthosine.

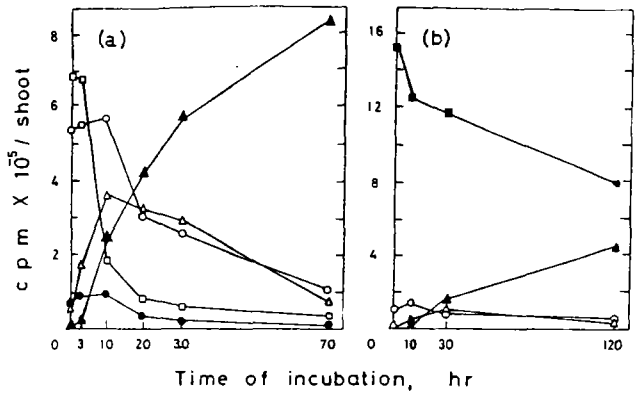


Figure 3a Distribution of radioactivity among adenine (□), guanine (■), free purine nucleotides (o), 7-methylxanthine (•) theobromine (Δ) and caffeine (Δ), when tea shoot tips were incubated with 5 μCi of [8-^{14}C]adenine (a) or with 5 μCi of [8-^{14}C]guanine (b). Groups of 4 excised shoot tips (2.5-2.6 g fr. wt) were each fed with 5 μCi of [8-^{14}C]adenine or with 5 μCi of [8-^{14}C]guanine within 1 hr and then incubated in H_2O in 50 ml flasks for various periods, (Suzuki and Takahashi, 1976a)

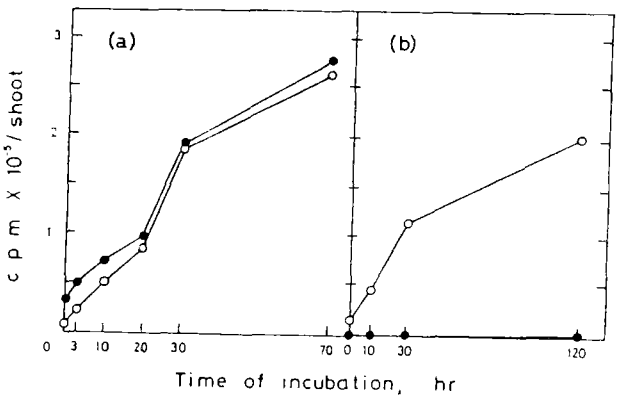
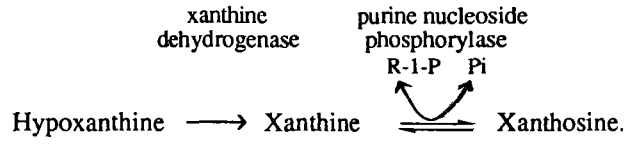


Figure 3b Incorporation of radioactivity from [8-^{14}C]adenine (a) and from [8-^{14}C]guanine (b) into the AMP (•) and GMP (o) of RNA in tea shoot tips. The experimental conditions were the same as those described in the legend to Fig. 3a, (Suzuki and Takahishi, 1976a)

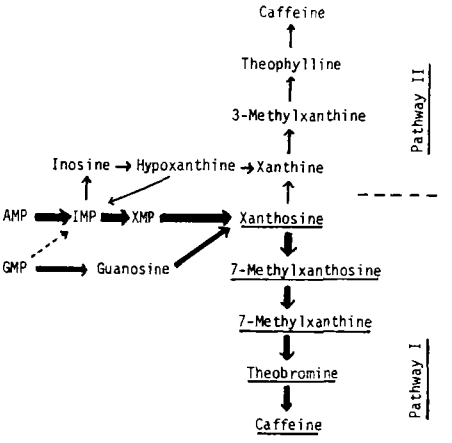
In noncaffeine-producing, cultured *Catharantus roseus* cells, there is evidence indicating the existence of the rapid catabolism of administered [8-^{14}C]guanosine via xanthosine and xanthine to CO_2 (an important route of guanine nucleotide catabolism) rather than via guanine and xanthine to CO_2 (Takasawa and Ashihara,unpublished observation; cf. also the review by Wasternack, 1982). These observations further suggest that induction and repression of three *N*-methyltransferases provide the primary mechanism for the control of the biosynthesis of caffeine in tea plants (Fujimori *et al.*, 1991), although IMP dehydrogenase (EC 1.2.1.14) is also important for the synthesis of xanthosine from AMP for caffeine biosynthesis (Scheme 2).

Theoretically (and under some favorable conditions), there is also a possibility of the synthesis of xanthosine from hypoxanthine and xanthine for caffeine biosynthesis:



However, this pathway for caffeine biosynthesis is highly unlikely and the xanthosine is synthesized from hypoxanthine via the purine salvage pathways through the steps: hypoxanthine → IMP→XMP→xanthosine (Suzuki and Takahashi, 1975b, 1977).

Finally, these observations (Scheme 5) are also consistent with studies of caffeine biosynthesis *in vitro* with enzyme preparations (see reviews by Suzuki and Waller, 1988 and Waller and Suzuki, 1990). Xanthine and hypoxanthine were totally inactive in these cell-free systems except that xanthine was methylated at the N^3 position by incubation with tea-leaf extracts at pH 7.5 (Negishi *et al.*, 1985a). However, it had been already demonstrated that 3-methylxanthine is almost or totally inactive as a substrate for dimethylxanthine (theophylline and/or theobromine) synthesis *in vitro* (Suzuki and Takahashi, 1975a; Roberts and Waller, 1979; Waller *et al.*, 1981). Thus, if caffeine is synthesized from xanthine via 3-methylxanthine and theobromine (and/or theophylline), this pathway is operative only as a minor route for caffeine biosynthesis but more possibly for theophylline biosynthesis (as demonstrated by Ogutuga and Northcote, 1970 and also cf. Suzuki and Takahashi, 1975a and Ashihara and Kubota, 1986; Scheme 5).



Scheme 5. Suggested pathways of purine alkaloid biosynthesis from purine nucleotides in tea plants.

Legend: The pathway from AMP via IMP and XMP to xanthosine appears to be the most important for caffeine biosynthesis (Pathway I). Pathway II is not important for caffeine biosynthesis, but it is considered to be important for theophylline biosynthesis, which is shown to occur in the lower, older leaves of 4-month-old tea seedlings (Ashihara and Kubota, 1986; cf. also Ogutuga and Northcote, 1970).

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SUMMARY

Caffeine is synthesized from xanthosine, via 7-methylxanthosine, 7-methylxanthine and theobromine, and S-adenosylmethionine is utilized as a donor of the methyl groups. Although the exact pathway has not yet been fully elucidated, xanthosine seems to be derived from purine nucleotides through the steps: AMP → IMP → XMP → xanthosine and/or GMP → guanosine → xanthosine. In contrast, there are seasonal variations in the caffeine content and biosynthetic capacity for caffeine synthesis in tea leaves and the decrease in autumn indicates metabolism and/or translocation of the alkaloid.

Biosynthesis of radioactive caffeine and theobromine in coffee fruit showed that a pathway from AMP and/or GMP was effective. Biodegradation of radioactive caffeine was shown to occur slowly, resulting in the formation of glyoxylic acid and urea which gives rise to CO_2 and NH_3. These results indicate that (a) biosynthesis of caffeine occurs mainly during the green stage of fruit development through methylation of N^7-methylxanthine and theobromine, and (b) that caffeine degradation primarily occurs through the demethylation of theophylline, which accumulates after the fruit is full size and proceeds to ripen.

RESUMEN

La cafeina es sintetizada a partir de la xantosina via 7-metilxantosina, 7-metilxantina y teobromina, el donador de grupos metilo es S-adenosilmetionina. Aunque la via exacta no ha sido totalmente elucidada, parece que la xantosina es derivada a partir de los nucleosidos de purina a través de las siguientes etapas: AMP → IMP → XMP → xantosina y/o GMP → guanosina → xantosina. En las hojas de té, el contenido de cafeína y la capacidad biosintética de la misma varía estacionalmente. La disminución de este alkaloide durante el otoño, sugiere que éste es metabolizado o translacado.

El seguimiento de la biosíntesis de cafeína radioactiva y de teobromina, mostró que la vía a partir del AMP y/o GMP fue efectiva. La cafeína radioactiva fue biodegradada lentamente, dando como productos el ácido glicoxylico y urea, a partir de los cuales se produce CO_2 y NH_3. Estos resultados indican que: a) la biosíntesis de cafeína se realiza durante el estado inmaduro (verde) del fruto a través de la metilación de N^7-metil-xantina y teobromina, y b) la degradación de la cafeína ocurre primariamente a través de la de-metilación de la teofilina, la cual es acumulada cuando el fruto alcanzó su tamaño final y empieza a madurar.

GASTROINTESTINAL PROTEASES AND THEIR INHIBITORS. LOW MOLECULAR-WEIGHT COFFEE COMPOUNDS AND TERPENES

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Principles of the methods

Trypsin, pepsin and chymotrypsin are proteases most widely used to determine the amino acid sequence of proteins. Trypsin is highly specific for peptide bonds containing lysine and arginine. Chymotrypsin hydrolyzes mainly bonds of aromatic amino acids but also at leucine. Pepsin exhibits some preference for aliphatic hydrophobic amino acid residues. Commonly specific synthetic amino acid derivatives or half-synthetic coloured proteins (azocasein) as substrates are employed. After proteolysis of the amino acid derivative the amount of the liberated residue can be determined colorimetrically.

Sometimes native proteins such as albumin and casein are used. The resulting peptide mixture after proteolytic hydrolysis is preseparated by acidic treatment and the soluble peptides in the supernatant are quantitatively measured by absorption. This technique cannot exclude the possibility that the precipitate contains appreciable amounts of hydrolyzed peptides, pretending a lower activity of the protease ("all or none principle").

To overcome these difficulties we developed a method for the determination of the substrate specificity of proteolytic enzymes
1.) with low molecular weight natural peptides instead of high molecular proteins, for example glucagon, melittin or insulin,
2.) without any precipitation of unhydrolyzed and hydrolyzed insoluble polypeptide material.

It follows that a) uncontrollable insoluble precipitates not appear after acidic or basis treatment, b) the whole hydrolyzed and unhydrolyzed material is separable by means of chromatography or high voltage electrophoresis. The best spot of a run serve as a guide peptide for quantitative determination.

The peptides of animal origin are valuable tools for the determination of the specificity of proteases on a molecular level. Every protease shows a specific pattern of peptides using one and the same substrate. The method is applicable to all gastrointestinal proteases, for example pepsin, chymotrypsin and trypsin, because of the known specificity of the enzymes.

Inhibition Experiments

It was recently well established, that alcohols inhibit proteolytic enzymes, such as chymotrypsin, trypsin and pepsin (1 - 5). Under the terpenes bisabolol from Matricaria chamomilla L. is a prominent inhibitor of pepsin, although normal meat digestion was not tested under these conditions.

Bisabolol and bisabolenes, which could stem from bisabolols, were isolated from many plants, but not from coffee till now. Nevertheless, the lack of proof that nonvolatile coffee compounds could be active against gastrointestinal proteolytic enzymes brought us to some in vitro inhibition experiments in this study. Three main components were tested: ferulic acid, chlorogenic acid and caffeine.

Before hydrolysis was started, one of these substances was incubated with pepsin (pH-Optimum 1 - 1.5) 15 min at 37°. Enzyme : substrate relation = 1 : 2. Hydrolysis was subsequently performed with the substrate melittin, the main peptide from bee venom. Enzyme : peptide relation was 1 : 200.

Results

Chlorogenic acid is undoubtedly inactive against pepsin, but the results given by the other two compounds were not so clear to interpret because of their moderate solubility in the incubation medium.

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Summary

It is well established that some alcohols and terpenes inhibit proteolytic enzymes of the gastrointestinal tract.

In the present work a method was established to interpret inhibition experiments undoubtedly. The technique was examined with the enzyme pepsin, bisabolol and some coffee compounds as substrates. Bisabolol was active, but chlorogenic acid was inactive against pepsin.

Zusammenfassung

Es ist wohl bekannt, daß einige Alkohole und Terpene proteolytische Enzyme des Gastrointestinaltraktes hemmen.

In der vorliegenden Arbeit wird eine Methode eingeführt, die es erlaubt, Hemmversuche unzweifelhaft zu interpretieren. Diese Technik wurde überprüft mit dem Enzym Pepsin und Bisabolol sowie einigen Kaffee-Verbindungen als Substrate. Bisabolol hemmte Pepsin, aber die Chlorogensäure war inaktiv gegen das Enzym.

ANTOCIANINAS EN PULPA DE CAFÉ DEL CULTIVAR BOURBON ROJO

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Introducción:

El cuestionamiento y la prohibición del uso en la industria de alimentos de algunos colorantes sintéticos ha renovado el interés en la utilización de pigmentos vegetales con este propósito (5). Una posibilidad es el uso de antocianinas, las cuales son responsables por casi todos los colores rojo, azul y púrpura presentes ampliamente en las plantas. Ellas se encuentran en todas las partes de las plantas superiores pero su presencia es mucho más obvia en las frutas y flores (1).

Las antocianinas son pigmentos solubles en agua cuya estructura base es el ión flavilio ó 2-fenilbenzopirilio (aglicón), a la cual se le une uno o más azúcares para formar el glicósido (antocianina). Los azúcares son por lo general glucosa, ramnosa, xilosa, galactosa y arabinosa. Casi todas tienen un azúcar sustituyente en la posición 3 donde el OH no es fenólico y en caso de que un segundo hidróxido sea sustituido por otro azúcar generalmente lo hace en la posición 5.

La información existente sobre la naturaleza de las antocianinas presentes en la pulpa de café es muy escasa y controvertida (6,8). En este trabajo se presentan los resultados preliminares de la separación de las antocianinas de la pulpa de café del cultivar Bourbón Rojo mediante el uso de cromatografías de papel y de columna de alta eficiencia (HPLC).

Materiales:

Muestras de café en cereza del cultivar Bourbón Rojo se obtuvieron de la Estación Experimental de Bramón, Estado Táchira. La pulpa se utilizó inmediatamente después del despulpado o se dejó secar al sol por aproximadamente 16 horas, se molió y se cirnió a través de un tamiz de 20 mallas.

Los reactivos y solventes utilizados fueron de grado analítico para las extracciones y cromatografía de papel y de grado HPLC para la cromatografía líquida de alta eficiencia.

Métodos:

Una porción de 50 g de pulpa de café se maceró por 5 min con 100 ml de etanol conteniendo 1% HCl en un homogenizador Sorvall, modelo 17105 (Newton, Conn., USA). El extracto se filtró a través de papel Whatman No. 1 bajo vacío y el residuo se reextrajo dos veces más con el mismo solvente. Los extractos se juntaron y se concentraron a un pequeño volumen

con un rotaevaporador Buchii bajo vacío a 40 °C.

Los pigmentos presentes en el extracto concentrado fueron separados por cromatografía de papel. El extracto se colocó a lo largo de una línea de 40 cm en uno de los extremos de hojas de papel de filtro (46 x 57 cm) Whatman No. 3. Se hicieron varias aplicaciones sobre la misma línea. Las hojas de papel de filtro se irrigaron en dirección descendente con una mezcla de ácido acético-agua-HCl concentrado en las proporciones 15:82:3 v/v (solvente AWH) hasta que la banda que migró más rápido alcanzó casi el borde del papel. Cuando el papel se secó las bandas de color rojo resultantes se cortaron y se eluyeron con etanol conteniendo HCl al 0,1%. Las fracciones correspondientes a cada banda se concentraron en un rotaevaporador hasta sequedad casi completa.

Las fracciones fueron sometidas a hidrólisis parcial ácida de acuerdo al método de Abe y Hayashi referido por Chen y Luh (2). Una porción concentrada de cada fracción fue hidrolizada por reflujo con 3 ml de HCl 1N en un balón de destilación de 25 ml inmerso en un baño de agua hirviendo. A intervalos de cierto tiempo (5, 10, 20, 30 y 60 min) se retiraron de la mezcla de reacción alicuotas de 0,4 ml e inmediatamente se colocaron en tubos de vidrio inmersos en un baño con hielo. Las muestras se puntearon en secuencia sobre papel de filtro Whatman No. 1 y se irrigaron por 14 h con el solvente AWH.

Cuando se logró la hidrólisis total, la muestra final se pasó a través de una minicolumna Sep-pak C18 y luego se lavó con agua destilada conteniendo HCl al 0,01%. Los pigmentos retenidos se eluyeron con metanol y se concentraron en un rotaevaporador Buchii bajo vacío a 40 °C. Estos pigmentos se inyectaron al cromatógrafo líquido para su comparación con un patrón de antocianidina (cianidina) obtenido de la piel de manzana roja comercial.

Los filtrados obtenidos a través del Sep-pak C18 después de la hidrólisis se concentraron a un pequeño volumen y luego se puntearon en papeles de filtro Whatman No. 1 junto con muestras auténticas de glucosa, xilosa, ramnosa y arabinosa. Los papeles de filtro se irrigaron en forma descendente con tres mezclas diferentes de solventes: n-butanol-ácido acético-agua (BAW-4:1:5); n-butanol-piridina-agua (BPW, 6:3:1); y fenol-agua (PW, 4:1). Los cromatogramas se asperjaron con una solución de anilina 2N en una mezcla de n-butanol y ácido fosfórico al 20% en la proporción 1:2 y en seguida se calentaron a 105 °C por 5 min.

Para los análisis por HPLC se utilizó un cromatógrafo líquido Perkin-Elmer (modelo serie 2/2) equipado con una válvula de inyección Rheodyne (modelo 7105), un detector UV-visible (modelo LC-75), un autocontrol, un integrador (modelo LCI-100) y una columna semi-preparativa Supelcosil LC-18 (250 x 10 mm). Una mezcla de dos solventes se utilizó como fase móvil: solvente A (metanol grado HPLC) y solvente B (solución acuosa de ácido fosfórico al 0,5%). La separación analítica por HPLC se logró combinando la elusión isocrática con elusión por gradiente lineal: 0-10 min, 30% A en B (isocrática); 10-20 min, 30 a 60% de A en B (gradiente lineal); 20-40 min, 60% de A en B (isocrática).

Resultados y discusión:

La cromatografía de papel del extracto total con el solvente AWH demostró la presencia de tres pigmentos en la pulpa fresca con valores de Rf de 0,07, 0,26 y 0,42. Los tres pigmentos fueron enumerados del 1 al 3 a partir de la mancha que se mueve más lentamente. Cuando las fracciones obtenidas mediante cromatografía de papel por bandas fueron examinadas por HPLC se observó que los tiempos de retención fueron de 24,4 min para el pigmento 1, 14,2 min para el pigmento 2 y 15,9 min para el pigmento 3. En la Figura 1 se presenta el cromatograma de los pigmentos de la pulpa de café del cultivar Bourbon Rojo que absorben a 520 nm. El pigmento 1 tiene el mismo tiempo de retención en HPLC y comportamiento en cromatografía de papel de la cianidina aislada de la piel de manzana roja comercial (7). El pigmento 3 es 3,3 veces más abundante que el pigmento 2. La absorbancia del pigmento 1 disuelto en metanol conteniendo 0,1% HCl disminuye gradualmente hasta desaparecer por completo. Asimismo se observó que en la pulpa secada al sol disminuye notablemente el contenido de los pigmentos 2 y 3 y el pigmento 1 está ausente.

Se utilizó el método de la hidrólisis parcial ácida para establecer la posición de unión y número de azúcares presentes en los pigmentos 2 y 3. La hidrólisis de un 3,5-diglicósido da dos monoglicósidos, los cuales son además hidrolizados al aglicón, de tal modo que en el cromatograma de papel aparecen cuatro manchas. De un 3-diglicósido se obtiene un cromatograma con tres manchas: el pigmento original, un monoglicósido intermedio y el aglicón. Los 3- ó 5-monoglicósidos dan cromatogramas con solamente dos manchas: el pigmento no hidrolizado y el aglicón. La Figura 2 presenta los cromatogramas de la hidrólisis parcial de los pigmentos 2 y 3. El patrón mostrado por el pigmento 3 es característico de un 3-diglicósido, mientras que el del 2 es el de un monoglicósido. El seguimiento en el tiempo de la hidrólisis parcial del pigmento 3 por HPLC y el comportamiento en cromatografía de papel del

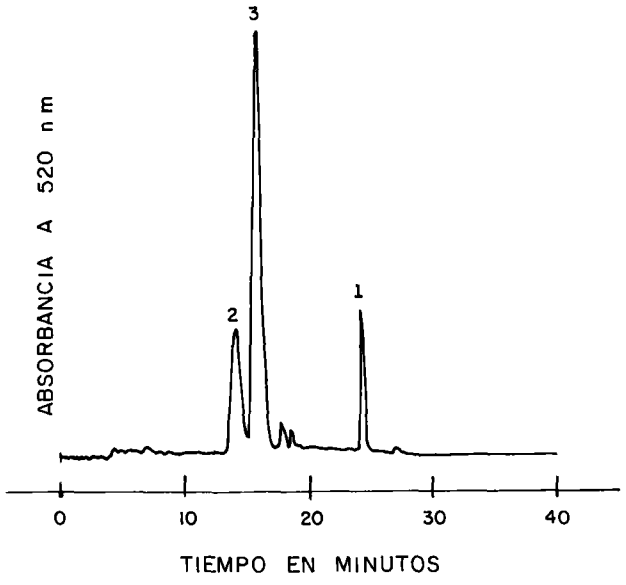

Figura 1. Cromatograma del extracto total de pulpa de café del cultivar Bourbon Rojo.

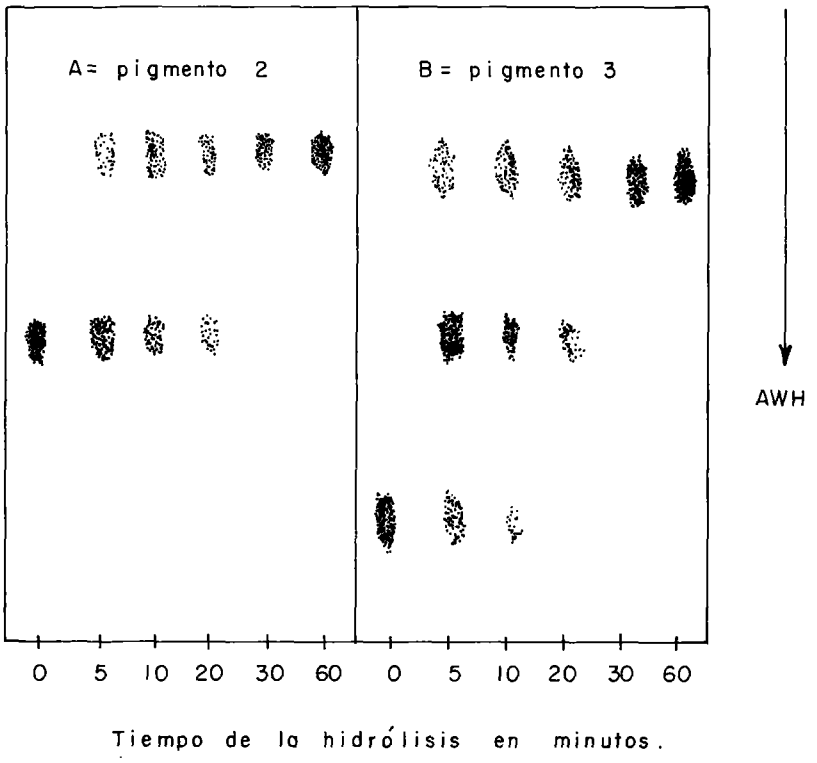

Figura 2. Hidrólisis parcial ácida de los pigmentos 2 y 3

monoglicósido resultante permite concluir que el pigmento 2 es un 3-monoglicósido. Estas conclusiones las refuerzan el hecho de que los dos pigmentos no exhiben fluorescencia cuando se examinan con luz ultravioleta de onda larga, característica ésta de las antocianinas 5-monoglicosídicas y 3,5-diglicosídicas (4).

La identificación de los azúcares presentes en las antocianinas de la pulpa de café se efectuó en los filtrados obtenidos después de la hidrólisis ácida total de los pigmentos 2 y 3. El Cuadro No. 1 presenta los Rg (relación entre la distancia recorrida por el azúcar con respecto a la distancia recorrida por glucosa) de los azúcares de los pigmentos y de los patrones.

Cuadro No. 1

Valores de Rg por cromatografía de papel de azúcares patrones y de los presentes en las antocianinas de la pulpa de café

Muestras de azúcar	Solventes BPW (6:3:1)	BAW (4:1:5)	PW (4:1)
Glucosa	1,00	1,00	1,00
Arabinosa	1,23	1,10	1,35
Xilosa	1,50	1,23	1,20
Ramnosa	1,97	1,47	1,52
Extracto total	0,99	1,05	0,99
Pigmento 2	0,98	1,02	0,98
Pigmento 3	0,97	1,03	0,98

De acuerdo a los resultados mostrados en el Cuadro No. 1 el azúcar presente en las antocianinas de la pulpa de café del cultivar Bourbon Rojo es glucosa. López y col. (6) encontraron dos antocianinas en la pulpa de café del cultivar Bourbon Rojo y las identificaron como cianidina 3-monoglicósido y cianidina 3,5-diglicósido. Aunque ellos no mencionan específicamente cual es el azúcar presente en las antocianinas, afirman que sólo encontraron glucosa como carbohidrato de los glicósidos de los flavonoides. Estos hallazgos concuerdan con nuestros resultados en lo que se refiere al aglicón y posición del azúcar en la primera antocianina (la cianidina 3-monoglicósido), pero difieren en cuanto a la posición del azúcar en la segunda antocianina. Zuluaga Vasco (8) también encontró dos antocianinas, presumiblemente en el cultivar Typica Rojo, que identificó como cianidina 3-glucósido y cianidina 3-glucoramnósido. Nuestros resultados no dejan lugar a duda de que las dos antocianinas detectadas en la pulpa de café del cultivar Bourbon Rojo son cianidina 3-glucósido y cianidina 3-diglucósido.

Es preciso señalar aquí que la identificación de las antocianinas basada únicamente en su purificación por técnicas de cromatografía de papel puede prestarse a confusión. En efecto, Harborne y Sherrath (3) encontraron que arabinosa se produce durante el proceso de purificación de las antocianinas como consecuencia de la acción del HCl sobre el papel de filtro. Así que la complementación de estas técnicas con las de HPLC analítica y preparativa es una manera de asegurar que los resultados sean reproducibles y confiables. A partir de esta concepción nos proponemos hacer un estudio comparativo de las antocianinas de la pulpa de café de dos especies, un híbrido y un sub-híbrido del cafeto.

Resumen:

Las antocianinas son los pigmentos responsables por el color de las cerezas de café rojas. Ellas consisten de un aglicón (antocianidina) sustituido con uno ó más azúcares. El aglicón de las antocianinas del Bourbón Rojo es cianidina, mientras que el azúcar sustituyente es glucosa. Las dos antocianinas detectadas en la pulpa del cultivar Bourbón Rojo son cianidina 3-glucosido y cianidina 3-diglucósido.

Summary:

Anthocyanins are the pigments responsible for the colour of the coffee red cherries. They are made of an aglycon (anthocyanidin) substituted with one or more sugars. The aglycon of the Red Bourbon anthocyanins was found to be cyanidin, whereas the substituting sugar was glucose. The two anthocyanins detected in coffee pulp of the cultivar Red Bourbon were cya - nidin 3-glucoside and cyanidin 3-diglucoside.

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COLOR MEASUREMENTS IN COFFEE AND COFFEE BREW

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For coffee there are only a few steps to be done from its growth in nature to the point of consumption as either roast & ground or as instant soluble coffee, but nevertheless, there is a wide variety of items to influence quality aspects.

In times of non-processed foods, we had integral ideas of their quality: we looked at them as a whole as we ate them as a whole. There was an integral description of taste, smell and sight as quality factors.

In the field of sensory evaluation, up to now, visual, haptic and flavor characteristics are the main points of food quality. Besides, we have more specialized classical and instrumental methods of analytical chemistry, which may serve well, e.g. for the identification of the residues of chemical food processing. On the other hand, they give us important close-ups on composition and components of foods and on the mechanism of reactions proposed to occur.

Especially regarding the coffee, in the 1977 colloquium of ASIC R.M.Pangborn[1] gave criteria for sensory evaluation of the coffee beverage, including visual characteristics and methods of presentation and measuring.

For chemists, visible analytics are allied with electronic spectroscopy, giving evidence of the energetic stages of the molecules under investigation. Coffee solutions do not show clear absorption spectra, as we can see in the next figure, thus, their data may be used only to give the exact determination of the visual phenomenon, we observe as color.

The observation of colors

Looking on the physiological part of the perception of colors in the human eye, there are receptors for red, green and blue working together with others for dark and light impressions[2]. Combining the visual responses in the mind, the overall color description may sound much more complicated, e.g. "pale blue grey" of a green coffee, or "clear light brown" for a cup of coffee.

Although the human sense is by far the most sensitive measuring equipment, an exact communication on these impressions might be difficult.

The highly esteemed brown color of coffee is obtained by roasting, and for many parts of the green coffee bean roasting is also the important factor in changing to solubles and in developing coffee flavor.

Thus, the structures of the brown pigments - and the main components they consist of - are topics of wide research, and studies are also performed in non-enzymic browning, the Maillard reaction, using model reactants.
Possibly produced colored substances[3] are given in Fig. 1.

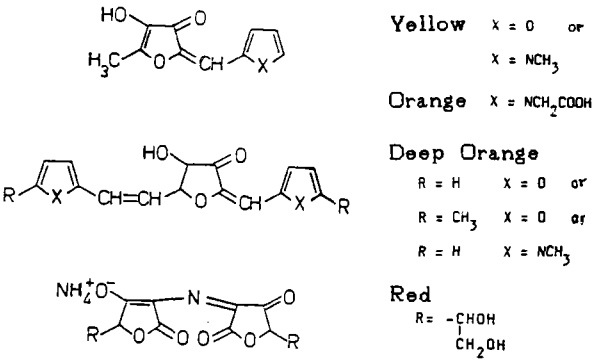

Fig. 1

Low molecular colored substances from model roasting reactions, furane type

These model reactions are used as well to elucidate the contributions of roasting to the generation of volatile and non-volatile aromas.
As connected with generation and degradation of melanoidins, the visual appearance of coffee may give as well a further criterion for coffee quality, and there is a need of communication about it.

Visual spectra obtained by reflectance at the solid coffee surface and by transflectance through a liquid coffee infusion are shown in Fig. 2.

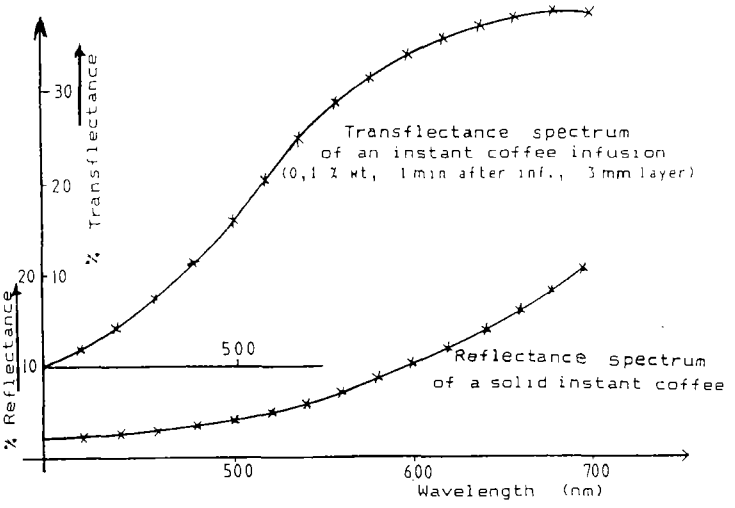

Fig. 2

Visual spectrum of solid instant coffee and of its aqueous infusion

From these spectra, there is neither evidence of the molecular constitution of the colored substances in solution nor do they give an imaginable impression of the real color to speak about, but they may be used to calculate colorimetric data[4].

Measurement of colors

Attempts to translate the human trichromatic perception of color into a universal color language started with a descriptive color atlas some 80 years ago, and had its first systematization in 1931 in the CIE (Commission Internationale d'Eclairage, International Commission of Illumination); the colors form a quasi triangular arrangement combined with a perpendicular axis for lightness.

Up to now, there had been many improvements in this field.
Today, specifying the colors of objects and materials and communication about it is done best with the psychometric L^*, a^*, b^* system CIE LAB 1976. Here, very many empirical data of color difference impressions are put into an easy to handle cartesian structure.

In CIE L^*, a^*, b^*, four colors are positioned on two perpendicular axes, red and green as end points of the a^*-axis and yellow and blue for the b^*-axis respectively, their intersection being uncolored. The third axis, vertical arising from the intersection, shows the lightness L^*, from down, black, through grey to top, white, with $L^* = 100$.
Each imaginable color is represented by a special point within this space, e.g. the brown of our coffee is to be found in the dark part between red and yellow.

This system is very helpful for dye production, to produce mixed colors by addition or subtraction of the primary ones, e.g. yellow and red give orange, and an addition of green is reducing a red color in direction to the uncolored, the grey.

For our purpose, color rendering may even better be illustrated in terms of the hue angle h_{ab} between the adjacent color axes, and the color intensity, Chroma, C^*, which is the radial distance to the uncolored axis.
The resulting color space is shown in Fig. 3, coffee brown being situated at a hue angle between 0 (red) and 90 (yellow) degrees, with varying chroma.

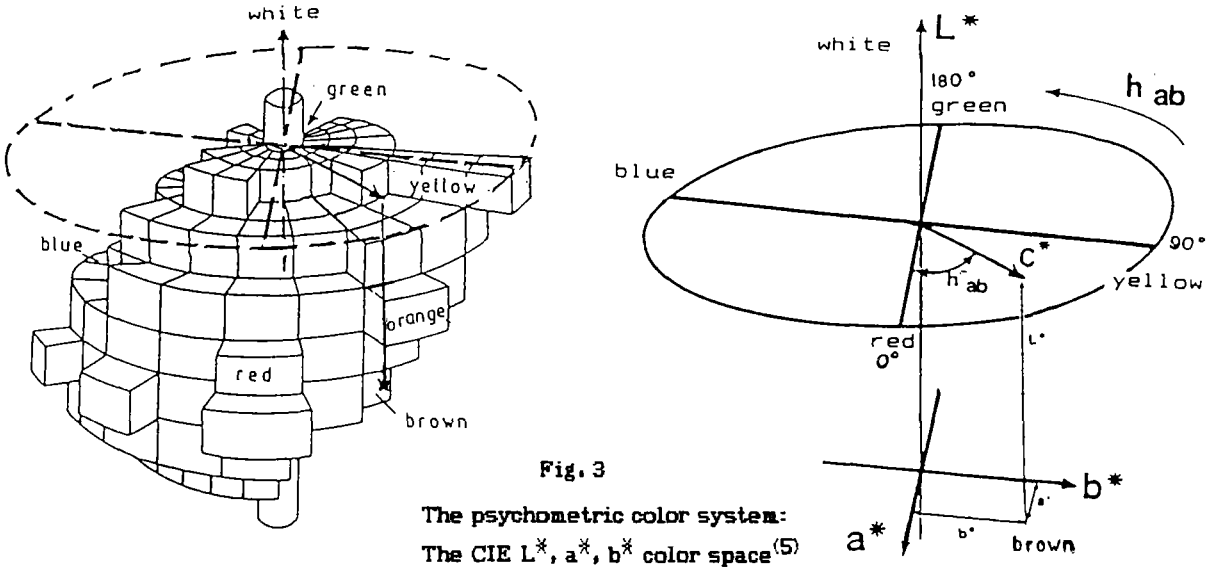

Fig. 3
The psychometric color system:
The CIE L^*, a^*, b^* color space[5]
and the CIE $L^*C^*h_{ab}$ system[6]

In the following, we are using lightness L^*, chroma C^*, and hue angle h_{ab}: The "clear light brown" of the coffee brew mentioned above is an infusion of great color intensity in a not so dark brown.

Going back to the process of coffee & color on their path from green coffee to the brew, roasting is the crucial point with strong color changing from the green coffee to coffee brown.
As this can easily be observed, in industrial roasting color measurement is widely used to follow the process, usually being performed in reflectance at a distinct wavelength, recorded as lightness[7], or recently as colorimetric data L and b, published at ASIC in 1987[8].

At this point, we may distinguish between an "outer" color of the coffee particles and the "inner" color as seen in the beverage, arising from the solution of colored solubles[9], maybe in roast coffee brew or instant coffee infusion.

Outside and inside need not be the same. Fig. 4 shows this fact with instant coffee, giving values for lightness, chroma and hue angle for some agglomerated and freeze dried extracts on the German market vs. those of infusions made thereof.

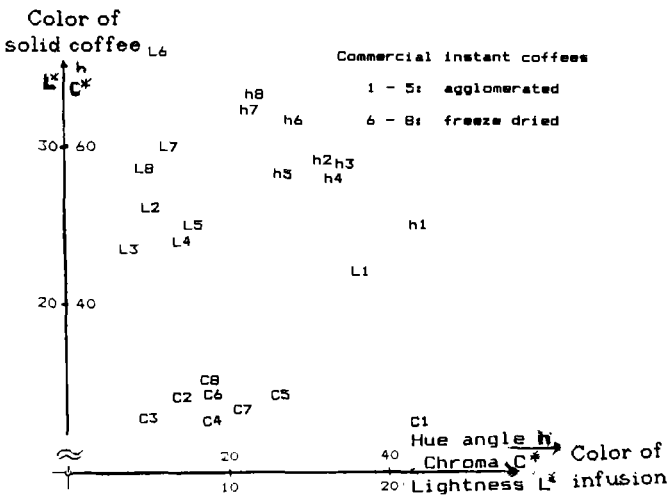

Fig. 4

Colors of commercial instant coffees

As we can see, there is no straight line through zero, but the points look more like traces of a chicken having stepped upon the ground; that means, there is no clear dependency between the color of instant coffee and its solution.
As far as it pertains to solid instant coffee, drying mostly influences the outer color, whereas in the liquid, the inner color is evidently more dependent on other parameters.

Procedure, equipment & limitations

Following the idea to have the color measurement of the beverage as close to the cup impression as possible, we choose an infusion in the usual concentration for instant coffee, in Europe +/- 1.5% per wt. Fig. 5 shows the measuring device.

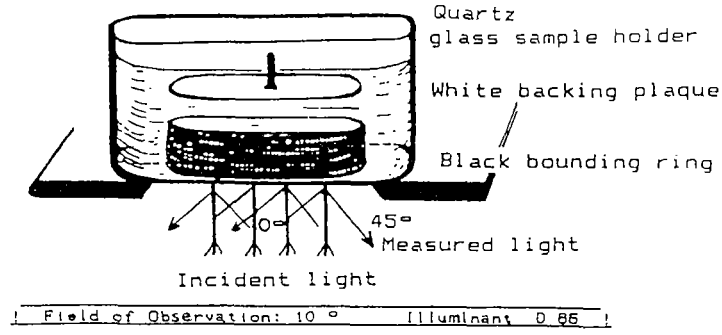

Fig. 5

Equipment for transflectance measurements of coffee liquors

We estimated the reflection of light through the bottom of a quartz glass sample holder on a white plate through a thin layer of coffee infusion - this is presumably the visual impression of coffee brew at the rim of a cup of white bone china. This type of optical measurement is called transflectance, because there is a transmission effect observed by reflection. Our procedure is in analogy to the method proposed by Angela Little in 1964[10] for translucent foods and by R.M. Pangborn et al. in 1971[11] for coffee brews.

To avoid the effects of uncomplete extraction of coffee color from R&G coffee, described by M.C. Merrit and B.E.Proctor in 1958[12], we took instant soluble coffees for the first research.

After preparation with freshly boiled water as usually recommended, the liquid extracts were measured in the course of natural cooling from infusion to near ambient temperature – usually coffee is drunk at 55^{O} to 60^{O} Celsius.

An example is given in Fig. 6, with data for lightness, chroma and hue angle.

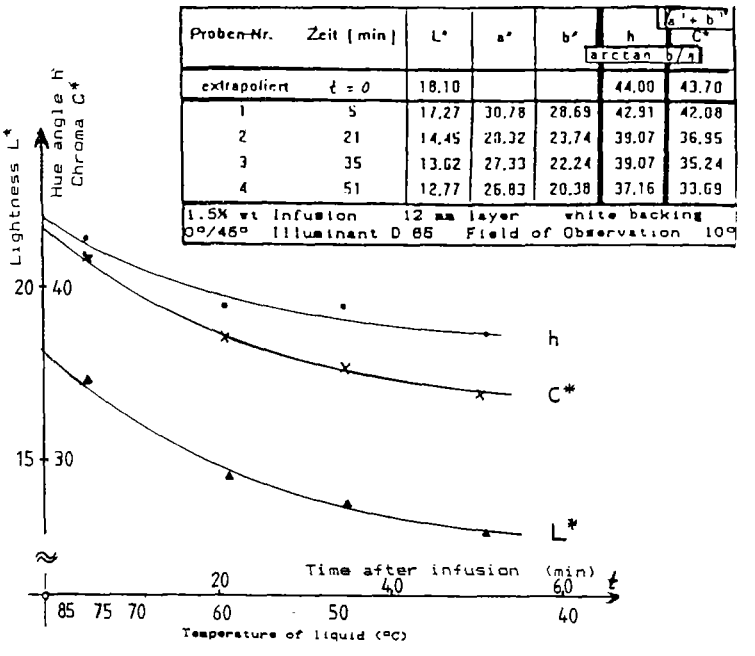

Fig. 6

The color of coffee beverage after infusion

During the time of observation, alterations of all colorimetric parameters can clearly be seen: The infusion is getting darker (decreasing L) with simultaneous decline in total color intensity (diminishing C), the yellow side being reduced more (h decreasing).
For standardization, we established the point "color of infusion" by extrapolation to the time of infusion.

With this procedure, we also looked at some other widespread presentations of coffee beverage, namely coffee and milk/cream/coffee whiter. Results are given in Table 1.

Coffee beverage m + m	L	h	C
black coffee 1 + 0	4.9	26.7	16.0
with skim milk powder, 1 + 1	39.7	70.1	35.7
with coffee whiter, 1 + 1	37.0	68.3	31.2
whole milk powder, 1 + 1	36.2	67.9	35.3
coffee cream 7.5% fat, 1 + 2.4	33.3	66.6	38.7
coffee cream 10% fat, 1 + 2.4	30.2	65.4	38.6
coffee cream 12% fat, 1 + 1.2	27.9	64.9	34.9

Table 1
Color of infusions of instant coffee with milk etc.

The results are completely different from the data of "black" coffee brew, and it is evident, that there are important other effects at work: An emulsion is formed, where the reflection is taking place at the

surfaces of the emulgated particles, depending on their quality and homogeneity. (This fact is known as well as a problem of standardization in instrumental color analysis of cocoa suspensions prepared with milk[9].)
Therefore we resigned measurements on coffee and milk, restricting the work on aqueous coffee infusions.

Observing the limitations of the method, investigations in the range up to double the cup concentration gave evidence, that at higher concentrations and after longer periods of liquid storage, secondary reactions are taking place. They are marked in increasing turbity, measurable in a drastical increase in optical density, and are thought to originate from agglomerations in the liquid.
Size enlargement of dissolved particles due to agglomeration may reach up to the wavelength range of the light of measurement – 700 nm in our case – thus resulting in diffuse scattering interactions, which obey special optical laws.

In the measurements we are performing here, effects of absorption and scattering are competitive. The latter is becoming more and more dominant with increasing depth of the optical layer, and with increasing concentration and particle size in the liquid.

In dye production, these scattering effects are helpfully described by the Kubelka-Munk equation, giving a spectral dependency of the ratio between absorption and scattering coefficients. We looked at this with our substrate as well; but spectra, like those of absorption, were of relatively poor resolution.

Things are proved by measurements in different thicknesses of layers.

Fig. 7 gives the colors of infusion in 0.3 and in 1.2 mm thickness, concentrations varying from 0.1 to 3%.

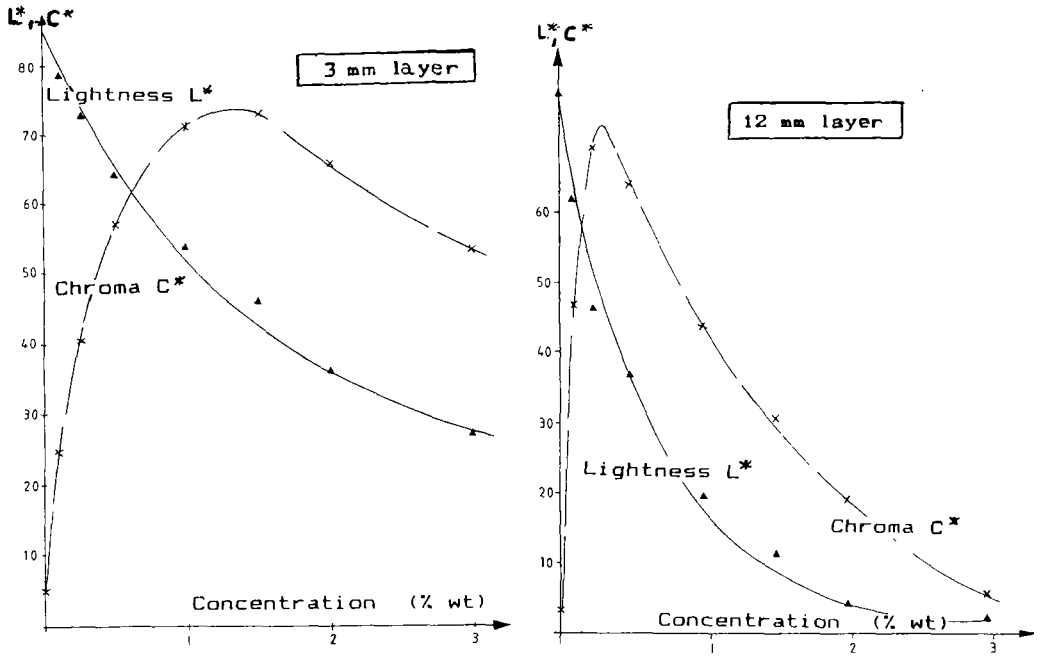

Fig. 7

Color measurements
in different layers of transflectance

As expected, in both systems the lightness is increasing with diminishing concentration up to the value of pure water, and, in lower concentrations, the chroma is increasing with concentration; beyond this, the chroma is decreasing again. The point of inversion depends on the thickness of the layer.
The observation of the "color of infusion" gives the overall impression of combined absorption and scattering, which may result in an inversion of the chroma at distinct concentrations.

To complete the method for color measurements of coffee beverage in aqueous infusion, another restriction has to be mentioned: The mineral content of the water for infusion, the influence of which is described by Pangborn in 1971[11].

Table 2 gives the variations in lightness of some commercial instant coffees, infusions being prepared with demineralised and with municipal Berlin drinking water (total dissolved solids 500 ppm, Calcium & Magnesium 120 ppm).

Table 2

Lightness of commercial instant coffees with different waters for infusion

Coffee	1	2	3	4	5	6	7	8
$L_{dem.}$	18.1	5.2	3.8	6.6	7.4	7.1	5.7	5.0
$L_{mun.}$	13.9	6.4	4.4	6.0	6.7	6.4	5.8	4.9

The data show irregular differences, found as well in chroma and hue angle.

Therefore, infusions for color matching should be done with demineralised water

- and with this last one, experimental conditions for color measurements are complete.

Using the procedure with R&G coffee brews, a dependency on grinding can be observed, to be discussed from a kinetic point of solubilization rates of the different fractions of solubles.

As one application of the procedure given above we looked at the hot storage of liquid coffees.

We know the sensoric alterations coffee is submitted to on keeping on the hot plate of a coffee machine. As H.G.Maier had pointed out in the 1987 ASIC colloquium[13], there is a marked increase in acidity, due to hydrolytic processes, with liberation of organic acids.

There are changes in the field of visual sensorics as well.
Fig. 8 shows the hue angle in transflectance measurements of cup infusions after different times of hot storage of the concentrated extracts of soluble coffee at ambient and three elevated temperatures.

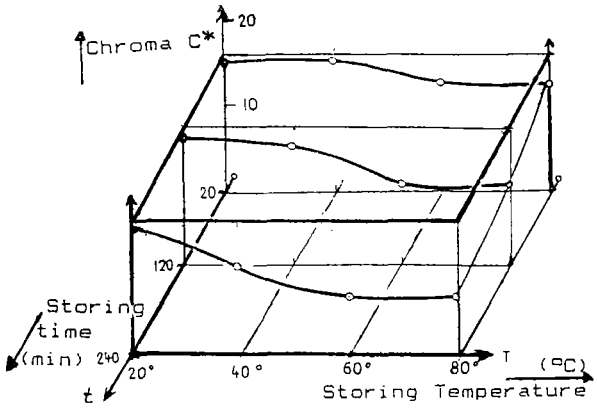

Fig. 8

Color of coffee infusions on hot storage

There is a clear decrease in chroma, indicating a reduction of the total color. A more detailed inspection of the colorimetric data visualized, that this was effected by a strong decrease in the red component of color, the a^*-value. Influence of pH/acidity may be discussed, but their eventual contributions are found to be too small. Thus, the changes in color of the liquor should be due to changes in the melanoidines, which possibly might be observed as well by other methods.

Data for lightness, chroma and hue angle of coffee beverages prepared under these conditions, as they are shown in the figures, are aggregate parameters standing for the colored principles of coffee, which are generated in roasting, and modified in the following operations, like grinding and brewing, and for the instant process as well through extraction and drying, and, beyond that all, influenced by changes with storage.

Conclusion

Observing the colors of coffee, their description in terms of the CIELab system is well intelligible; the use of colorimetric data is growing, as they may serve as indicators at several points of interest in coffee investigations: in a former ASIC report[8], to give a roasting gradient from the outside into the midth of the grain for roast process analysis, or, as in a poster presentation of this meeting[14], as an intrinsic physical property of coffee beans to describe the progress of roasting. Maybe, in the future, colorimetry may serve as well for green coffee visual sensorics to substitute the "pale blue grey" by an exact "L= , a= , b= " (first to be proved for its communicative value).

Colorimetric measurements of coffee liquids in thin layers are opening the possibility to estimate the visual appearance of the coffee beverage in its proper aqueous surrounding: Transflectance in cup concentration describes the original optical impression as a sensoric quality of the liquid, including all visible results of physical and chemical events in these infusions, whereas restricting the observation to absorption may be achieved in dilute systems and in very thin layers where scattering or trapping effects on particles are excluded.

In coffee investigations, methods of colorimetry may be used to give integrative data on the colored parts of the reaction systems, where absorption spectra fail to yield the appropriate results. Caution has to be used to clear up for other optical effects.

Acknowledgement to the Bundesanstalt für Materialprüfung Berlin, Dep. Farbmetrik, for helpful discussions, Mrs.D.Pahl for active assistance.

Summary

A method was presented to estimate the visual impression of a coffee brew in terms of colorimetry. Using instant coffee infusions for the investigations, procedure and limitations were developed: Transflectance measurements in thin layers of aqueous solutions, extrapolating the data to the time of infusion. Lightness, chroma and hue angle of the CIEL*a*b* system are chosen to describe what is to be seen as the overall color of a cup of coffee.
The same parameters can be used to determine the color of solid coffee in reflectance, maybe instant, R&G or even green coffee and the respective coffee beans, thus giving the opportunity to follow up the whole line of coffee processing.

Zusammenfassung

Es wird eine Methode vorgestellt, den visuellen Eindruck eines Kaffee-Aufgusses in den Begriffen der Farbmetrik wiederzugeben. Am Beispiel der Untersuchung von flüssigen Kaffee-Extrakten werden die Verfahrensweise und ihr Anwendungsbereich erläutert: Transflexions-Messungen in einer dünnen Schicht eines wäßrigen Aufgusses und Extrapolation der Daten auf den Aufguß-Zeitpunkt; aus dem psychometrischen CIE-L*, a*, b*-Farbsystem werden die Parameter Helligkeit L*, Chroma C* und Buntton-winkel h_{ab} gewählt, den Gesamt-Eindruck einer Tasse Kaffee wiederzugeben.
Die gleichen Parameter lassen sich auch verwenden, um die Farbe von festen Kaffee-Proben in Reflexion zu messen, sei es nun Instant-Kaffee, gemahlener Röst- oder gar Rohkaffee bzw. die entsprechenden Bohnen; so kann schließlich der gesamte Kaffee-Verarbeitungsprozeß verfolgt werden.

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SOLID WASTE SOLUTIONS FOR FOOD AND BEVERAGE PACKAGES

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Thank you very much and good morning. I am pleased to be with you and to have the privilege of sharing a few thoughts with you. I, of course, want to discuss certain aspects of the solid waste issue. In particular, as my title suggests, I want to talk about P&G's approach to managing solid waste. While it certainly seems as if we are dealing with a major problem and perhaps even a crisis, I have come to also view this problem as an opportunity and thus, my focus on solutions.

My approach will be to briefly give some overall perspective on garbage, including a brief but complete history. Then I will discuss Procter & Gamble's corporate policy and program on solid waste issues and finally discuss how we are using these principles in the food & beverage business.

Now, a few words about the discovery of garbage and its history. It all started in 400 BC when the Greeks developed the first town garbage dump. I would also indicate that refuse was a problem for prehistoric people. In fact, these people probably moved away from this garbage.

In 200 AD, the Romans thought up the brilliant idea of having sanitation workers. Two men would follow a cart and throw the garbage they found into it. Obviously, there's been little improvement on this idea in 1800 years. One of the few times garbage figured into medieval history was during a 1415 Portuguese attack on the Moroccan city of Ceuta. Two princes charged up what appeared to be a strategic point only to discover they had heroically captured the garbage dump.

In 1551, the first recorded example of packaging appeared. Andreas Bernhart, a German paper maker, began placing his paper in a wrapper bearing a design and his name and address. The first municipal refuse collection system was started by Benjamin Franklin in 1757. This was a very innovative system which avoided the need for landfill by having the slaves who collected the garbage wade into the Delaware River and toss it into the

current. 1776 was a landmark year in the history of garbage. In that year, we saw the first act of recycling in America. Patriots in New York knocked down a statue of King George III and remade it into 42,088 bullets. In 1834, the first solid waste legislation was passed. Charleston, West Va. enacted a law that protected vultures from hunters because they were critical in keeping the city's streets and yards clean from garbage.

The plastics industry was born in 1869 when American John Hyatt made "celluloid."

The first instance of "NIMBY" (Not In My Back Yard) appeared to be in 1894 when, to protest their up-river Washington's practice of using barges to float their garbage down the Potomac, the good citizen's of Alexandria, Va. began sinking the barges before they reached Alexandria.

In 1935, we see the birth of the <u>can</u> of beer (for which I am personally thankful). In 1938, the Xerox process was invented by Chester Carlson, a 32 year old Physicist. Styrofoam is invented by Dow in 1944. A single day garbage record was recorded for New York in 1945 when 6,334 tons of trash was dumped in the streets for V-J Day, end of WW II.

Moving along to 1962, Rachel Carson published "<u>Silent Spring</u>." This warned us of pesticide use and, more importantly, reminded us that when you throw something away, it may not really go away.

In 1968, the aluminum industry began aluminum recycling. As well, our standard beer can and soft drink can have been reduced in weight by at least 50%. In the 20 years since that time, 10.5 billion pounds have been recycled saving 60 million kilowatt hours and enriching collectors $3.75 billion. In 1973, a Harris Poll was recorded indicating who the American people really trust. Fifty-two percent felt they could trust their garbage collector; only 18% felt they could trust their President. And we all remember the wonderful 4-day historic tribute we paid to the Statue of Liberty in 1986. We left behind 2,079 tons of garbage. With the 1987 sailing of the garbage barge "Mobro" with its 3,100 tons of Islip garbage, we finally realized we had a problem and this brings up up to present.

As you may have already heard, we're seeing more legislation dealing with solid waste than we can keep track of. Last year, we had more than 1,000 state and local bills on solid waste. They year it will be more. Also, as you have probably heard, our loss of landfills is one of the primary reasons for the solid waste crisis. In 1985, we had more than 5,500 landfills in this country and by the year 2000, we're going to have less than 1,500. Landfills are filling up and they're being closed at a rapid rate and new ones can't be easily sited.

Clearly awareness of this issue is growing at a huge rate. In 1980, only about 10% of the German people cared about the environment. By 1988 environmental protection was just as high of a concern to the German people as unemployment, the highest concern in that country. We're seeing the same growth of concern in the U.S. We're just a few years behind Europe, that's all. It's growing very, very fast, and I think we can expect the same kind of growth of concern in this country.

Really garbage ought to be simple to understand. In fact, all archeologists study garbage; the garbage shown in this landfill is just fresher than most. Bill Rathge from the University of Arizona actually fathered the field of garbology in 1973 when he began studying landfills. Rathge's work has helped clarify many misconceptions about the landfill. For example, he has consistently demonstrated that garbage doesn't really biodegrade in a landfill. In fact, landfills are designed to prevent biodegradation. I will be summarizing some of the key information generated by Rathge's work but for those that are interested, I would recommend you read his article in the May 1991 National Geographic Magazine.

So, let me tell you just a couple of important facts about what's in trash. Each American generates about 3.6 pounds of municipal solid waste a day. And the way this is fractionated in the waste stream, about 36% of it is paper and paperboard. It is important to note that about 20% is yard waste -- your grass clippings, and your yard waste. And about 9% is food waste. Those things don't even have to be in our garbage cans -- they can be composted. Another thing to note is that plastics are only 7% of the waste stream. This is another piece that the American consumer has a little bit out of perspective and needs some education on. That doesn't mean we shouldn't do something about plastic, but it's not the problem that people make it out to be.

If you look at how this fractionates in terms of broad categories, you see that containers and packaging are about 33% of the municipal solid waste stream. And of that 33%, most of it is paper and paperboard, and only 4% is plastic.

I think the bottom line here is that nobody is going to escape. This isn't a problem that can be handled by just one sector of our society. It belongs to all of us. And I think all of us are going to have to play a role.

In fact, we as a large consumer products manufacturer, feel that environmental quality is an important new consumer need. In other words, it is as much a consumer need in products now, as building in better cleaning for detergent powders; better absorbability for diapers and better tasting coffee and beverages. And consumers frequently remind us that the consumer is really king and we had better learn how to meet their needs in this important area of business.

Through our early experience in Germany and Europe, we came to view environmental quality as a new consumer need. Similarly for the food and beverage business, I submit that environmental concerns will become as important as other attributes like taste, nutrition and convenience. Certainly, product safety is critical and packaging must perform its critical role in preserving food safety.

Well, what's the answer? As I was telling you before, the solid waste issue has been addressed by the scientific community, and the experts are beginning to agree on what the solutions are. So, because we have at least the scientific community agreeing, this is an issue on which we can began to act with some kind of confidence that we are doing the right thing. And what the solution is, is something called Integrated Waste Management. Integrated Waste Management is a set of four solutions that we use in a particular order, because the ones at the top are better to use than the ones at the bottom, in terms of the health of our environment. So at the top, we have source reduction. And source reduction of course means, make less of it, use less to make it. Don't make it in the first place. And this is a story we need to do a better job of telling especially relative to plastics. The second one is recycling, reusing, and composting. When we talk about composting, we mean a very specialized system that can convert up to two-thirds of the waste stream to soil-like material. These are all methods by which you can take the waste and return it back to some useful life. The third one is waste-to-energy incineration. I think all the experts agree that we can't take care of the whole waste stream by the first two methods as much as we would like to, and that there will be some need for waste-to-energy incineration in this country. And at the very bottom, there is landfill. In other words, try and do something else with it before we put it in a landfill.

In particular, I would like to emphasize recycling and composting. We can ultimately manage a large portion of our waste stream with composting which is a natural technique that will lead to the breakdown of organic materials to soil like material. This is, of course, what happens to leaves and yard clippings if we let nature do its job. In fact, upwards of 60% of the waste stream is compostable. We at P&G are trying to stimulate recycling and composting by conducting projects with local communities, supporting research and designing our packages to fit these waste streams.

There is a philosophy behind solid waste management, since this issue is really being worked at the local level. The better any city does the first two things on the list, the less they will have to use the bottom two things.

Well, how are we doing now? Currently we landfill more than 80% of our solid waste. And in light of the data that I mentioned on landfill closings, we obviously can't continue to do that. The EPA has set goals for 1992 of reducing the solid waste stream by 25% through source reduction and recycling; increasing incineration to 25% and reducing landfill to 50%. These are interim goals. We obviously would like to get down to where we landfill only about 10%.

Now, I'd like to talk for a few minutes about what kinds of things manufacturers can do to make their products more environmentally acceptable. I'm going to show some examples of products. First, Concentration -- concentrating products is a very good way to save on packaging. Downy triple concentrate is a way of saving two-thirds on packaging. Concentrated cleaning powders are coming along. These, as well, save on packaging and save on space. Consumers like this because they don't have to carry home as big a box.

Combination Products -- Being able to put two or three products in the same box is another way of significantly saving on packaging. In other words, Tide with bleach saves the need of a bottle of bleach and a box of detergent. Bold saves a need for a bottle for softener and a box of detergent. Pert Plus is an example of a product in which there are two products in one; you don't need two bottles in your shower, you just need the one.

The refill concept has successfully built our business in Europe. As I said, the European consumer is several years ahead of the American consumer in being concerned about this issue. And the German consumer has really realized what this product offers in terms of saving on solid waste. Lenor is the equivalent of Downy fabric softener in Europe. What this concept involves is that the consumer buys the 4-liter bottle of Downy or Lenor. They take it home and when it's gone they don't throw the bottle away. Instead, they save it and buy this small plastic packet of concentrate. They put it in the used Lenor bottle, fill it with water and have a reconstituted bottle of softener. This saves 85% on packaging.

Obviously, we are very anxious to do this same kind of thing with the American consumer. We have introduced this concept in Canada, in four brands. In the U.S. we have just introduced into test market in the Washington and Baltimore area, this version of the refill concept. In the U.S., we have had to use this carton instead of the pillow pack because the American consumer is not familiar with the pillow packs. In Canada and Europe, milk and other products are sold in pillow packs, so the consumers are used to them. In the U.S. this is an unfamiliar kind of package. So, we had to use something a little more familiar.

The initial results are very encouraging. We've got our fingers crossed and we're hoping that the American consumer will start to want to use things like this very soon.

More than 70% of our cartonboard is recycled. We're trying to use recycled materials wherever we can. Spic and Span is now being marketed in a 100% post-consumer recycled PET bottle. This is the kind of plastic that 2-liter pop bottles are made from. And just last spring, we announced that we also now have a technology to be able to use post-consumer recycled plastic in our detergent bottles, which are high density polyethylene (HDPE). The technology here is much more complicated. So right now we can only use about 20-30% post-consumer in these bottles. But we are expecting to be able to improve that technology very rapidly and grow to a higher percentage of use.

We are coding all of our bottles with the SPI code, which will tell recyclers and consumers what kind of resins the bottle is made from. You'll start to see these little

embossed symbols on the bottom of plastic bottles and this will be very helpful in getting our recycling systems going.

Not surprisingly this same approach fits food and beverage products. Let me tell you how we approached this issue. Basically, I used a variation of integrated waste management to benchmark our packages because in our business the primary solid waste issue is packaging. I surveyed all of our packaging for 3 attributes: source reduction, recyclability, and recycle content. What we found was that we needed to use a variety of approaches because we use a variety of packaging; including single resin plastics, like PET and HDPE, plastic laminates, metallic laminates, paperboard, corrugated, composite cans, aluminum, tin plated, steel, glass, aseptic boxes.

So what have we done so far? What I found surprised me is that close to 80% of our packages already possess a significant solid waste attribute. Source reduction - we, like many manufacturers have light weighted many of our packages. Frequently using aesthetic pleasing designs like this Tiffany bottle we use for Puritan and Crisco. In addition, we have found that by using different packaging we can significantly reduce the amount of packaging like we did for our Folgers brick pack.

We also found that a number of our packages are already recyclable like this Folgers Coffee can and tin plated steel cans. In addition, our glass bottles are recyclable and all indications are that our plastic bottles like this Jif PET jar, Citrus Hill HDPE jug and the Folgers Coffee plastic jar will be recyclable. In fact, in cooperation with our packaged soap efforts we are planning to put our PET and HDPE food and beverage containers into Spic & Span, Tide, Cheer, and Era. While some people believe there is a glut of potential recycled material, I know in some cases we and others are actually having difficulty getting enough recycled material.

Finally, recycle content. As you know, aluminum, steel and many paper products already contain recycled material. These are just a few examples of products in which we are using recycled paper. And, where possible, we are making sure our various packages contain recycled material. For recycled paper, it's important to note that this is not in direct contact with the product. However, because aluminum, steel and glass are actually melted, any potential contaminants are removed and recycled material from these sources is used in direct food and beverage contact.

While this is not a complete list of our products, I think you have an idea of how we are approaching our products. Well, you might ask, where do you go from here? More of the same, i.e., source reduction, recyclability, recycle content and ultimately packages will be compostable. But I'm certainly not saying every package needs to have every attribute, but I do believe that most of our packages will have to possess at least one of these attributes. And solid waste attributes will need to be considered along with other key Food and Beverage attributes like taste, convenience and nutrition.

I would like to conclude by discussing a few of the key issues facing our industry. Certainly plastics recycling and reuse of post-consumer plastics is a huge one. We need to do more to support plastics recycling and reuse of plastics in a safe way. There are already major efforts that some of your companies may be involved with. Coca-Cola and Pepsi have taken a major step and propose to use a very specialized post-consumer material that has been converted into material that is indistinguishable from virgin plastic. This material has been approved by the U.S. FDA. And I know that soft drink plastic bottles are being reused in Europe.

Laminates - Clearly, we love them in the food and beverage industry. They provide critical barriers for our products. Although they are truly a wonderful way to achieve source reduction, their lack of recyclability will be an issue. A fortune awaits the manufacturer who develops materials that do the job of laminates that are easily recyclable.

In summary, solid waste solutions for our business are really not much different than other business. Indeed, solid waste solutions need to become part of your business. We cannot simply say food and beverage products are different. Packaging preserves our products and make them safe. Sure it does, but we need to make environmental attributes an important part of our business strategies and set about solving these problems. Finally, I want to indicate that each of you can play a critical role in helping with this issue. Encourage your respective companies to make the solid waste environmental issue part of their business efforts. Finally, participate in recycling or composting efforts. This is an issue for which each of us can make a difference.

Thank you very much. I would be happy to attempt to answer your questions.

SUMMARY

SOLID WASTE SOLUTIONS FOR FOOD AND BEVERAGE PACKAGES

J. T. ROTRUCK

There is now very little question that solid waste will be a major issue for the 1990's. Numerous pieces of evidence support this: Landfills are closing, solid waste is a frequent media topic, many state and local governments have passed laws dealing with solid waste, recycling programs are ubiquitous, etc. While the use of the word "crisis" in discussing solid waste has been routine, there are workable approaches for dealing with the issues. Procter and Gamble's approach for dealing with solid waste and its application to food and beverage products will be used to demonstrate solid waste solutions. We have used the integrated waste management approach to measure solid waste environmental attributes and to develop strategies for future packaging changes. Key issues facing food and beverage packages will be summarized.

RESUME

EMBALLAGES ET PRODUITS ALIMENTAIRES/BOISSONS : DES SOLUTIONS POUR LES DECHETS SOLIDES

J. T. ROTRUCK

Il est maintenant pratiquement certain que les déchets solides vont constituer un problème important pendant les années quatre-vingt dix. On peut citer de nombreuses preuves à l'appui : Les décharges publiques ferment les unes après les autres ; les déchets solides attirent frequemment l'attention des journalistes ; de nombreuses autorités locales ont promulgué des arretés concernant les déchets solides ; les programmes de recyclage se multiplient, etc... Rien que le terme de "crise" soit régulièrement employé pour parler des déchets solides, il existe des solutions réalistes a ces problèmes. L'approche de Procter and Gamble en ce qui concerne le traitement des dechets solides et sa mise en oeuvre dans le domaine des produits alimentaires et des boissons seront utilisées pour démontrer des solutions aux problemes de déchets solides. Nous avons utilisé l'approche de gestion intégrée des déchets pour mesurer les attributs environnementaux des déchets solides et pour développer des stratégies pour les futurs changements d'emballages. Les principaux problemes affectant les emballages des produits alimentaires et des boissons seront résumés.

ÉTUDE MICROSCOPIQUE DE QUELQUES TYPES DE CAFÉS DÉFECTUEUX. II : GRAINS À GOÛT D'HERBE, DE TERRE, DE MOISI ; GRAINS PUANTS, ENDOMMAGÉS PAR DES INSECTES

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La classification des grains de café s'effectue selon plusieurs critères (1,7) dont la taille, la densité, la couleur et l'absence de faux-goûts, les lots devant présenter la plus grande homogénéité possible. En principe, les défauts de couleurs sont éliminés dans les lots de bonne qualité, et sont sortis par des trieuses automatiques ou à la main. Les défauts de goût sont plus sournois, car ils n'entraînent pas forcément des altérations de couleur, et sont souvent découverts à la torréfaction.

Depuis quelques années, nous avons entrepris une étude systématique des grains de café défectueux. Après l'étude sur les grains de café riotés (4,5), nous avons présenté lors du précédent colloque de l'ASIC à Paipa (Colombie) un travail concernant les défauts de couleur (6). L'étude actuelle porte surtout sur des grains présentant des défauts organoleptiques.

Matériel et méthodes:

Les grains examinés sont des Arabica originaires du Kenya, du Mexique et de Porto Rico. Tous ont été préparés comme précédemment décrit (2,3) et examinés au microscope électronique à balayage Philips 505, à 20 kv, ou au microscope photonique Leitz Orthoplan.

Résultats:

Grains présentant un goût d'herbe:

Dans tous les cas, ce sont des grains de taille adulte, qui n'ont pas terminé leur maturation. En effet, la réaction au Xanthydrol appliquée sur des coupes obtenues par congélation, montre la présence d'une faible coloration bleue, confirmant la présence d'une petite quantité de sérotonine, au niveau de la cuticule des grains, et une nette coloration violette au niveau des "protein bodies" du parenchyme, correspondant à la présence de tryptophane. Nous avions précédemment démontré que les grains totalement immatures ne présentent aucune coloration bleue au niveau de la cuticule, mais que les

grains ayant terminé leur maturation ne contiennent plus de tryptophane dans le parenchyme (2). Il s'agit donc bien de grains en cours de maturation. Quelques grains peuvent présenter une faible infection microbienne (bactéries,levures et rares moisissures) au niveau du tégument argenté. Mais dans tous les cas, leur épiderme est propre, et le contenu du parenchyme parfaitement correct. Ces grains peuvent être difficiles à détecter, car ils ne présentent pratiquement pas d'altération de couleur.

Grains à goût terreux:

Les lots de grains reconnus comme ayant un goût de terre sont assez peu homogènes au point de vue couleur. On y observe des grains de teinte normale, et des grains présentant des couleurs variant entre le brun clair et le noir. L'examen microscopique démontre que les grains verts sont des grains corrects. Les grains ayant des défauts de couleur sont tous infectés par divers microorganismes tels que: bactéries (Fig. 1, levures (Fig. 2), et de nombreuses moisissures: Fusarium (Fig. 3), Geotrichum (Fig. 4), une population très importante de type Eurotium (Fig. 6, 5, 8, 11) avec des cléistothèques caractéristiques (Fig. 9), et diverses souches d'Aspergillii: Aspergillus flavus (Fig. 7), A. fumigatus (Fig. 7), A. niger (Fig. 10). Cependant, cette infection reste située exclusivement au niveau du tégument argenté, aussi bien en surface que dans le repli central. L'épiderme est dépourvu de microorganismes. On y observe des petits filaments de polysaccharides (Fig. 12) qui sont toujours observés après une fermentation. Le parenchyme des grains est correct pour les grains non infectés (Fig. 13) mais dans le cas des grains atteints, le contenu cellulaire est fortement dégradé. La figure 14 montre que le contenu protéinique et saccharidique des cellules a disparu. Seule subsiste la couche périphérique des lipides (L).

Les parois cellulaires sont intactes. Il est à remarquer que les moisissures de type Eurotium sont cosmopolites, se développent sur des matières organiques pauvres en eau et ont donc dû pousser sur des grains secs, alors que les Aspergillii nécessitent une teneur en eau nettement plus élevée. Quant aux Fusarium, la plupart vivent dans le sol, ou sont phytopathogènes.

Grains à goût moisi:

Dans les lots examinés, tous les grains présentent une modification de couleur et paraissent grisâtres. Tous sont fortement infectés par des moisissures, aussi bien au niveau du tégument argenté (Fig. 15), en surface et dans le repli central, qu'au niveau de l'épiderme (Fig. 16). Au niveau du tégument argenté, la couche de mycélium peut former un vrai tapis (Fig. 18, repli central) qui retient de très nombreuses spores (Fig. 15). Dans certaines zones moins infectées, on peut constater que de nombreuses bactéries (Pseudomonas) forment une sorte de symbiose en recouvrant le mycélium (Fig. 17). Ces grains sont totalement dépourvus de moisissures de type Eurotium ou de type Fusarium. On observe essentiellement des Aspergillii. On peut observer de belles têtes sporifères de type Aspergillus dans le repli central des grains (Fig. 19) et quelques très rares cleistothèques (Fig. 20). Les Aspergillii sont essentiellement A. tamarii, A. niger, A. ochraceus, A. flavus. On observe deux types de moisissures osmophiles: Aspergillus penicilloides, et Wallemia sebi. Ces grains sont dépourvus de levures. Une partie de la flore fongique est donc différente de celle observée chez les grains présentant un goût terreux. Dans tous les cas, le parenchyme de ces grains est fortement dégradé. La figure 21 montre une coupe transversale d'une partie de grain, à l'aspect vidé. Les figures 22 et 23 illustrent deux gradations dans la lyse partielle de ces grains. La figure 22 montre que les parois cellulaires sont intactes, mais que dans le cytoplasme ne subsiste plus que la couche de lipides périphériques, alors que dans la figure 23, on peut encore observer quelques protein bodies. Dans les cas extrêmes ne subsistent que les parois cellulaires.

Grains endommagés par des insectes

Il s'agit dans cette étude essentiellement de grains endommagés par le scolyte du grain, Stephanoderes hampei, parasite qui commet encore le plus de dégâts au récoltes. Comme le montrent les figures 24 et 25, ces dégâts peuvent être importants en surface et à l'intérieur. En règle générale, les parties non rongées sont libres de toute infection microbienne. La figure 26 montre un tégument argenté intact et propre, la figure 27 un épiderme particulièrement propre. Mais dans toutes les zones rongées, on observe la présence de très nombreux microorganismes. Les figures 28 et 29 montrent

que ces zones rongées sont tapissées par un mycélium plus ou moins dense. On note la présence de spores de Fusarium (Fig. 30) de Geotrichum et de bactéries (Fig. 31, 33), de levures (Fig. 32). Mais on y trouve également les spores de toutes les espèces déjà citées d'Aspergillus niger, ochraceus, flavus, fumigatus. Par contre le parenchyme non rongé présente un aspect normal. Le cytoplasme est correctement rempli.

Grains puants:

Plusieurs lots de grains puants examinés présentaient des infections multiples: bactéries, levures et moisissures, et il était difficile de trouver le responsable de ce défaut particulier. Finalement nous avons reçus des grains puants du Kenya, infectés par une seule bactérie: Bacillus brevi.

Aucune levure n'était détectable, et on a pu montrer rarement la présence de Geotrichum. Cette bactérie forme un tapis aussi bien à la surface du tégument argenté (Fig. 34) qu'à la surface de l'épiderme (Fig. 35). Le parenchyme est également dégradé. Tous les saccharides et les protéines non incluses dans des "protein bodies" ont disparu (Fig. 36: aspect général du parenchyme; Fig. 37: détail des "protein bodies" restants. La couche lipidique périphérique est toujours présente, et les parois cellulaires paraissent intactes.

Conclusions:

Mis à part le goût d'herbe qui est dû manifestement au manque de maturité des grains, les différents mauvais goûts sont dus à des infections microbiennes. Dans les grains présentant un goût terreux, ce sont les souches de moisissures de type Eurotium qui sont dominantes. Les levures sont également en nombre très élevé. Dans les grains à goût de moisi, ce sont les Aspergillii qui sont dominants. Les Eurotium sont absents ainsi que les levures. Les Eurotium s'attaquent à des produits secs. Les Aspergillii nécessitent une teneur en eau plus élevée pour se développer. Nous avons donc deux types d'infection distincts. Le goût puant semble dû à une infection bactérienne importante, les rares moisissures de type Géotrichum détectées n'ayant aucune influence organoleptique. Quant aux grains abîmés par les insectes, ils montrent une infection strictement localisée au niveau des zones rongées. On pourrait supposer que les grains à goût moisi ont été infectés massivement avant le séchage ou durant celui-ci, alors que les grains à goût de terre sont soit des grains secs ramassés à terre, ou restés sur les caféiers depuis une récolte précédente, ou encore infectés lors du stockage.

Remerciements

Je remercie très vivement Mme N. Braendlin, Nestlé, pour avoir déterminé le type de bactéries infectant les grains puants, et son étude sur les souches de moisissures, ainsi que Mme M. Weber pour son excellent travail photographique.

Résumé

Les grains de café à goût d'herbe sont des grains de taille adulte n'ayant pas terminé leur maturation (faible présence de sérotonine). Tous les autres défauts sont dus à une forte infection microbienne: bactéries, levures et moisissures spécifiques pour chaque défaut. le parenchyme des grains est fortement dégradé. ces types d'infection apparaissent sur les arbres, durant le processus de séchage, ou lors du stockage. Les grains endommagés par les insectes sont également très infectés.

Summary

Grassy flavour: unripe beans with adult size (serotonin missing). All other flavour defects are due to strong infection by several micro-organisms: bacteria for the stinker beans, yeasts and mouls for the earthy flavour, mouls and bacteria for the mouldy flavour. These types of infection occur in the fields, during inappropriate drying processes or during storage. Insect-damaged beans are strongly infected as well.

Remarque:

Par convention, la barre située au coin inférieur droit des microphotographies indique la grandeur en μm. Lorsqu'elle ne comporte aucune indication, elle représente 1 μm.

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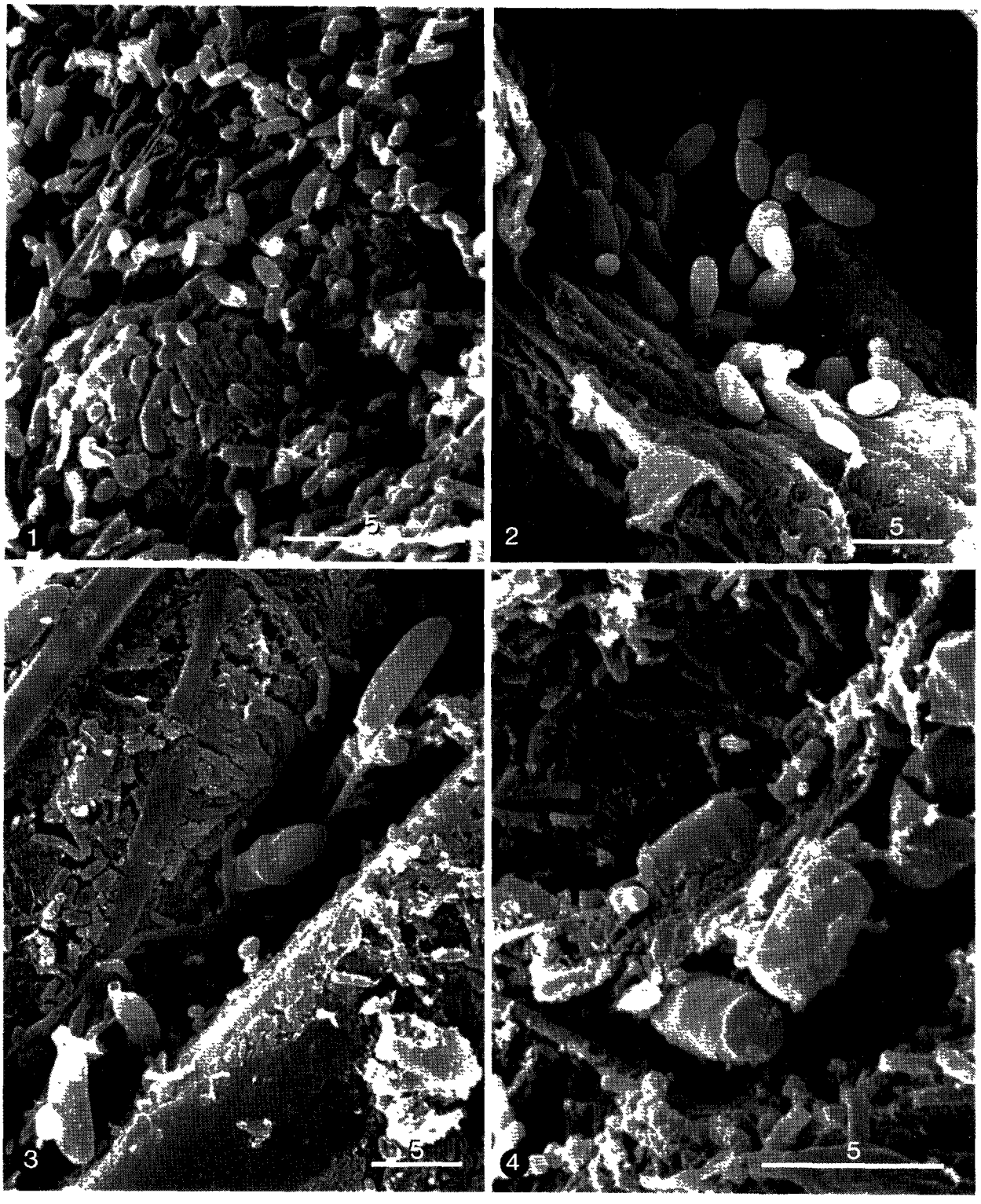


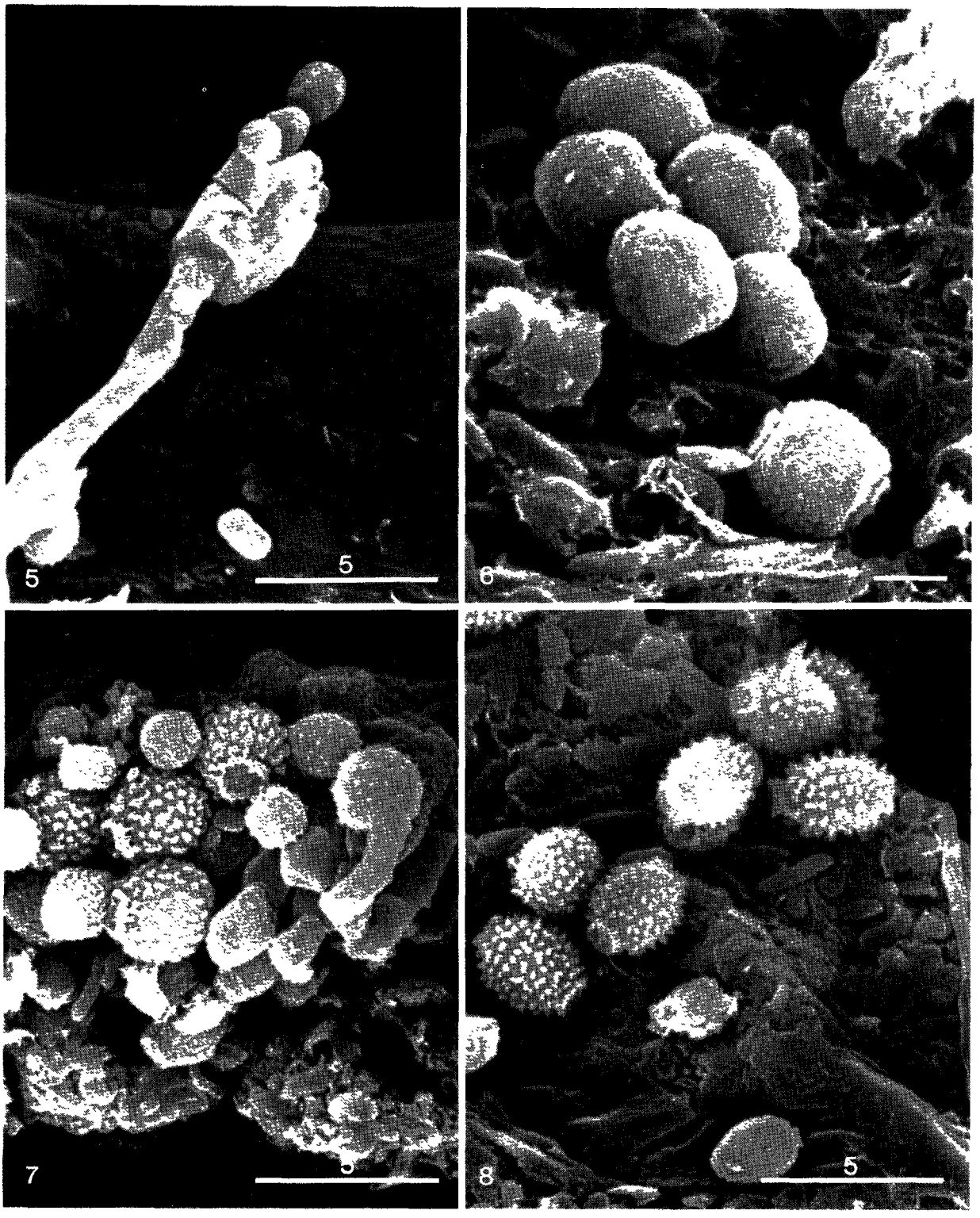
Grain à goût terreux

Fig. 1: Bactéries

Fig. 2: Levures

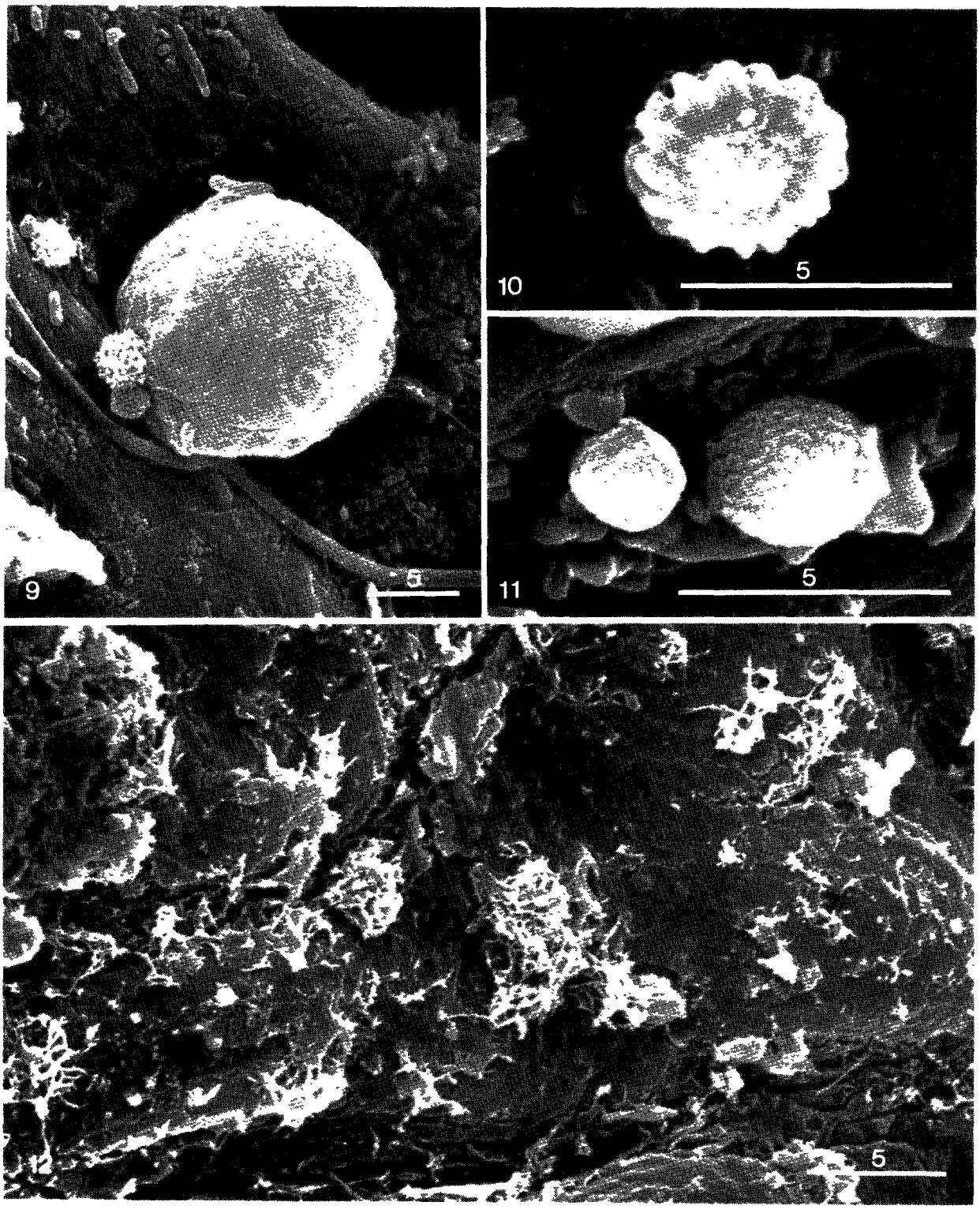
Fig. 3: Spores de Fusarium

Fig. 4: Arthrospores de Geotrichum



Grain à goût terreux

Fig. 5: Tête sporifère de Pénicillium
Fig. 6: Spores du même Pénicillium
Fig. 7: Spores d'Aspergillus flavus, A. fumigatus
Fig. 8: Spores d'A. restrictus



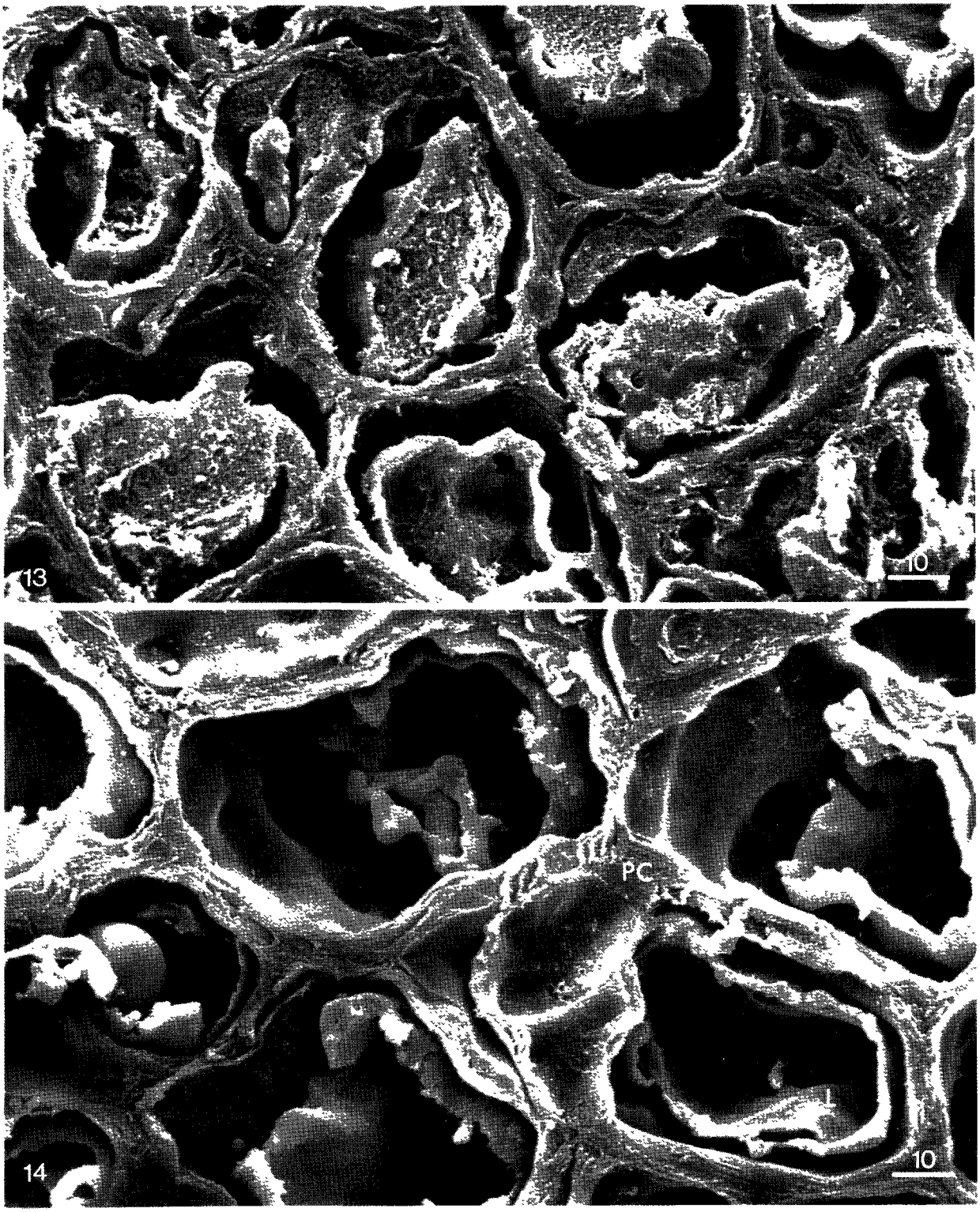
Grain à goût terreux

Fig. 9 : Cléistothèques

Fig. 10: Spores d'A. niger

Fig. 11: Eurotium

Fig. 12: Epiderme propre



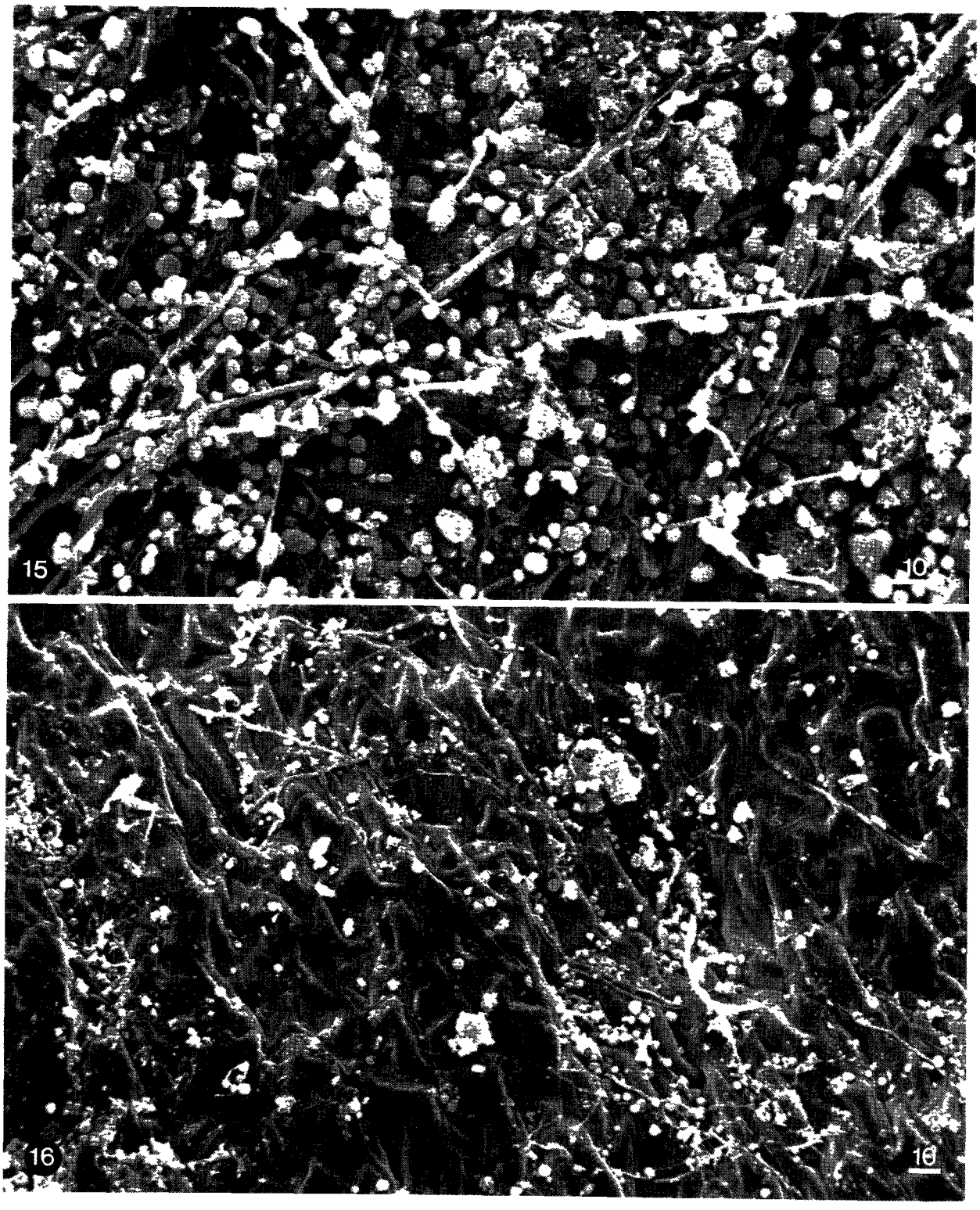
Grain à goût terreux

Fig. 13: Parenchyme normal

L: Couche lipidique

Fig. 14: Parenchyme dégradé de grain infecté

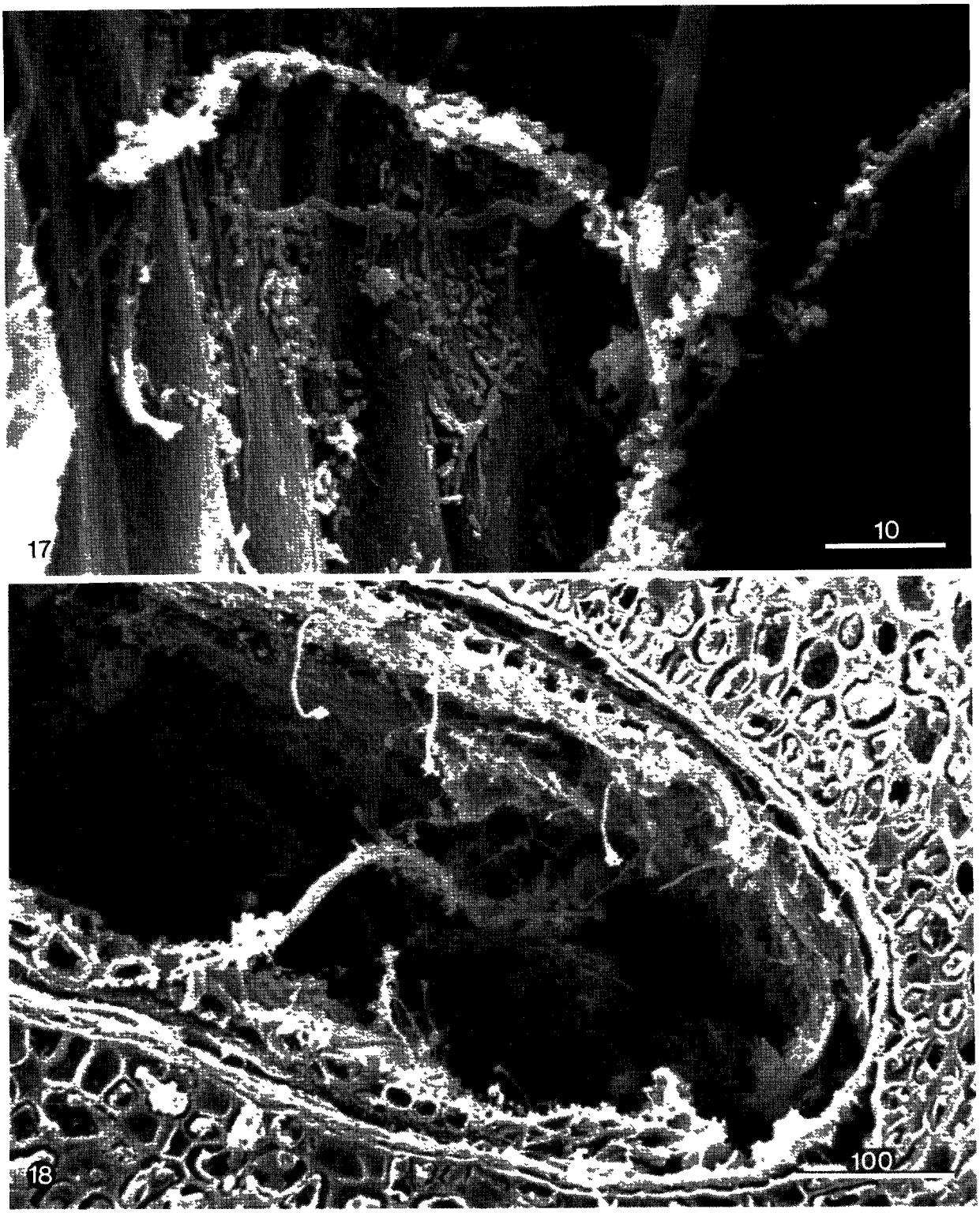
PC: Parois cellulaires



Grain à goût moisi

Fig. 15: Tégument argenté recouvert de spores et de mycélium

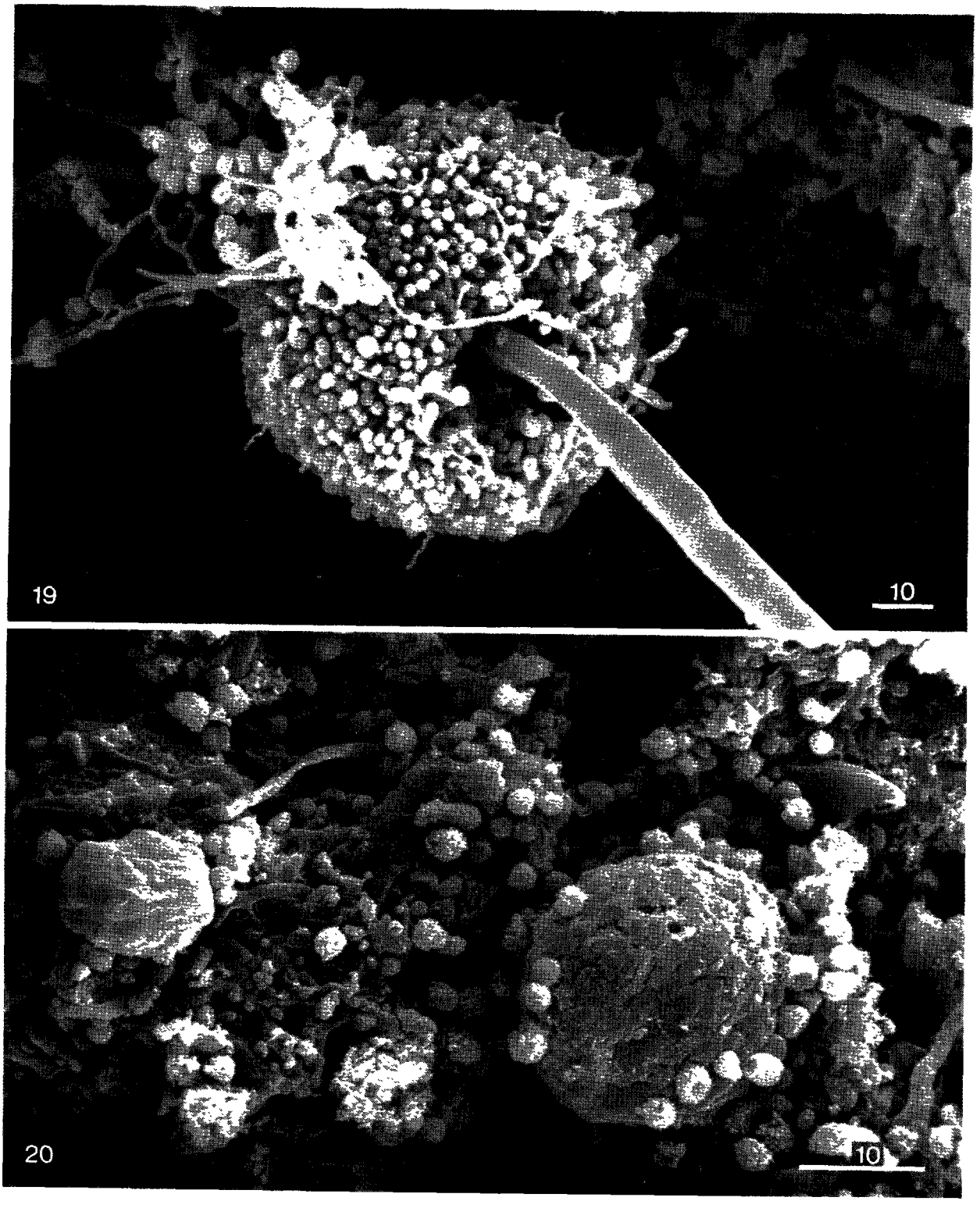
Fig. 16: Epiderme recouvert de mycélium et de spores



Grain à goût moisi

Fig. 17: Repli central. Bactéries sur du mycélium

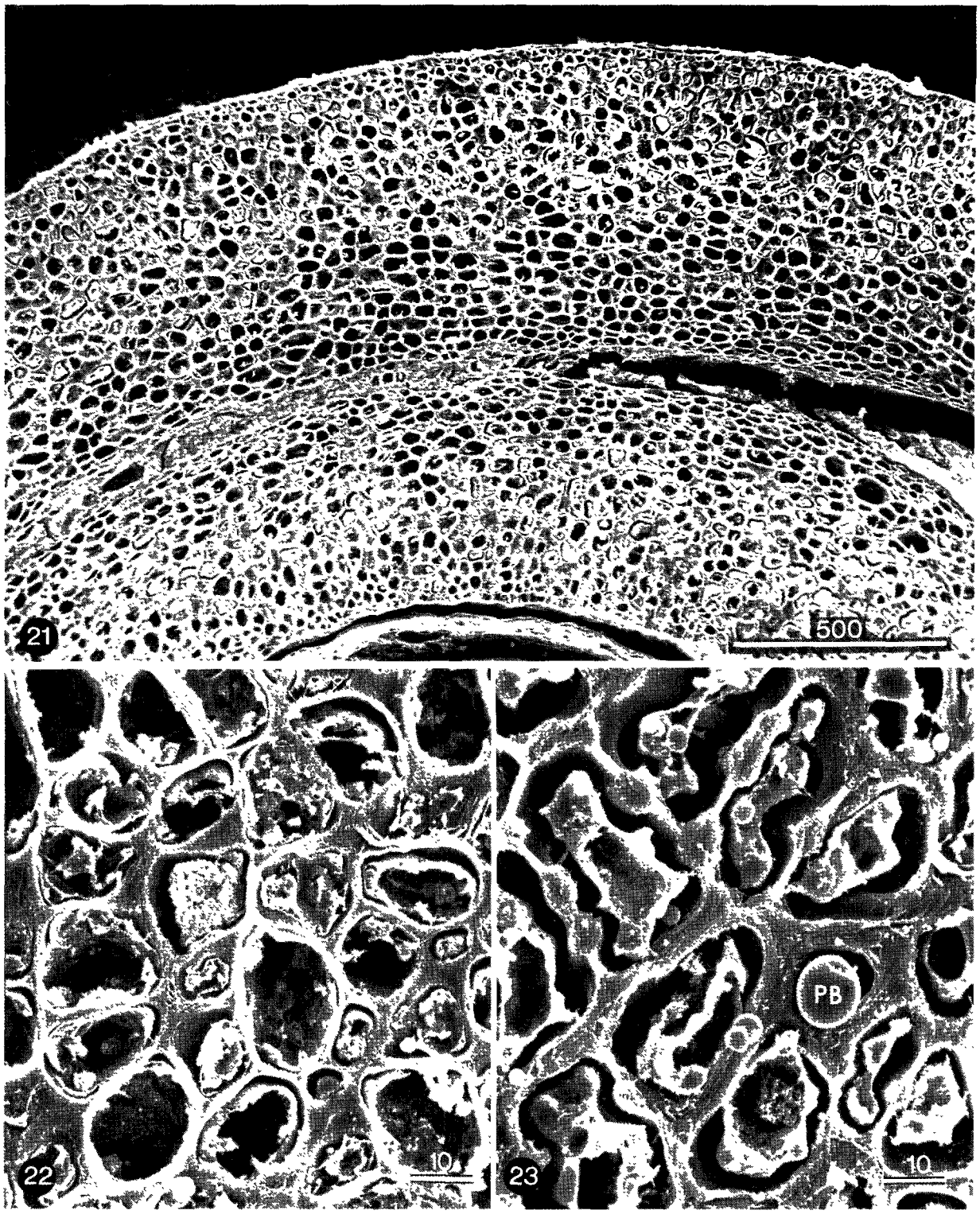
Fig. 18: Repli central tapissé de moisissures



Grain à goût moisi

Fig. 19: Repli central. Tête sporifère d'Aspergillus

Fig. 20: Repli central. Quelques rares cléistothèques

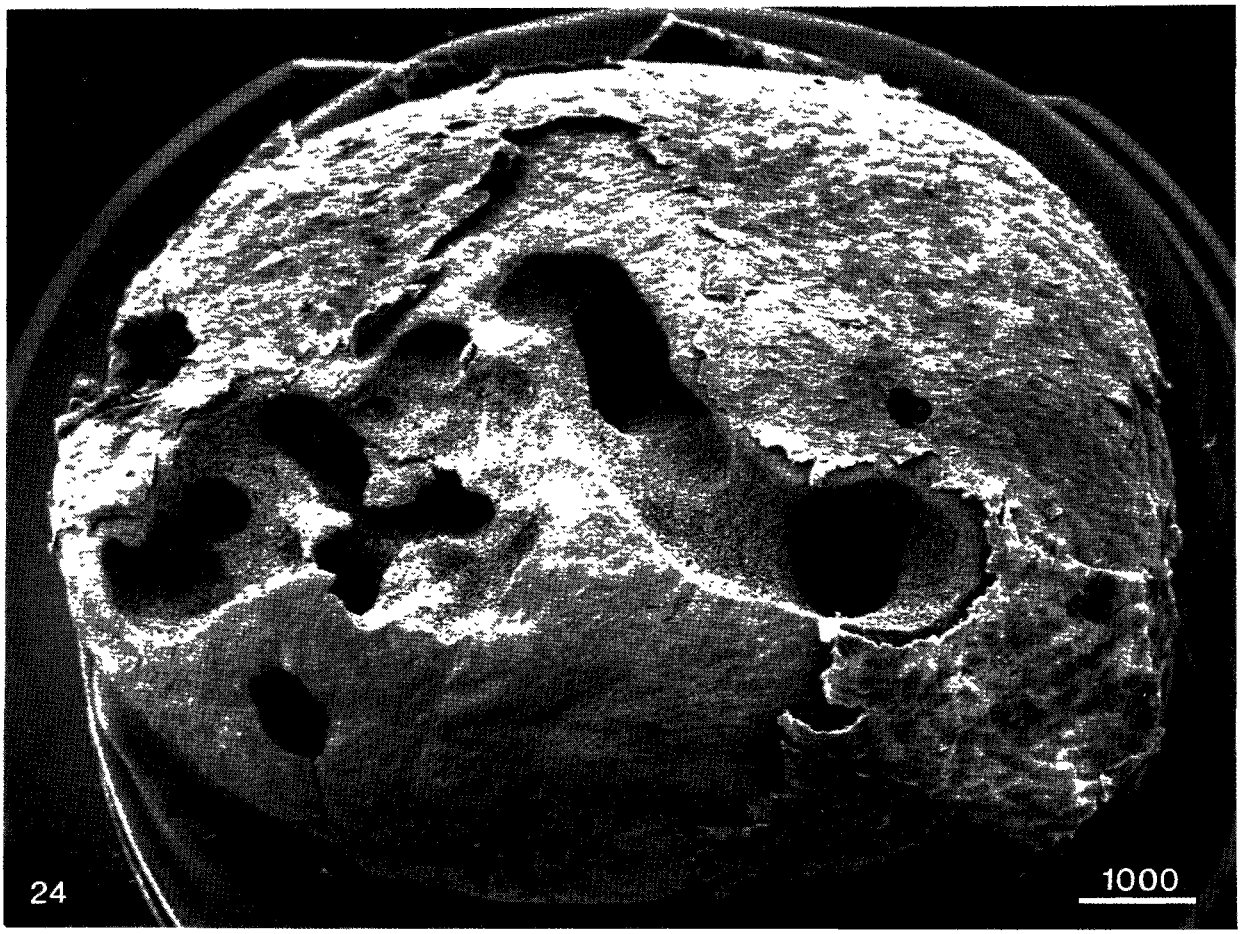


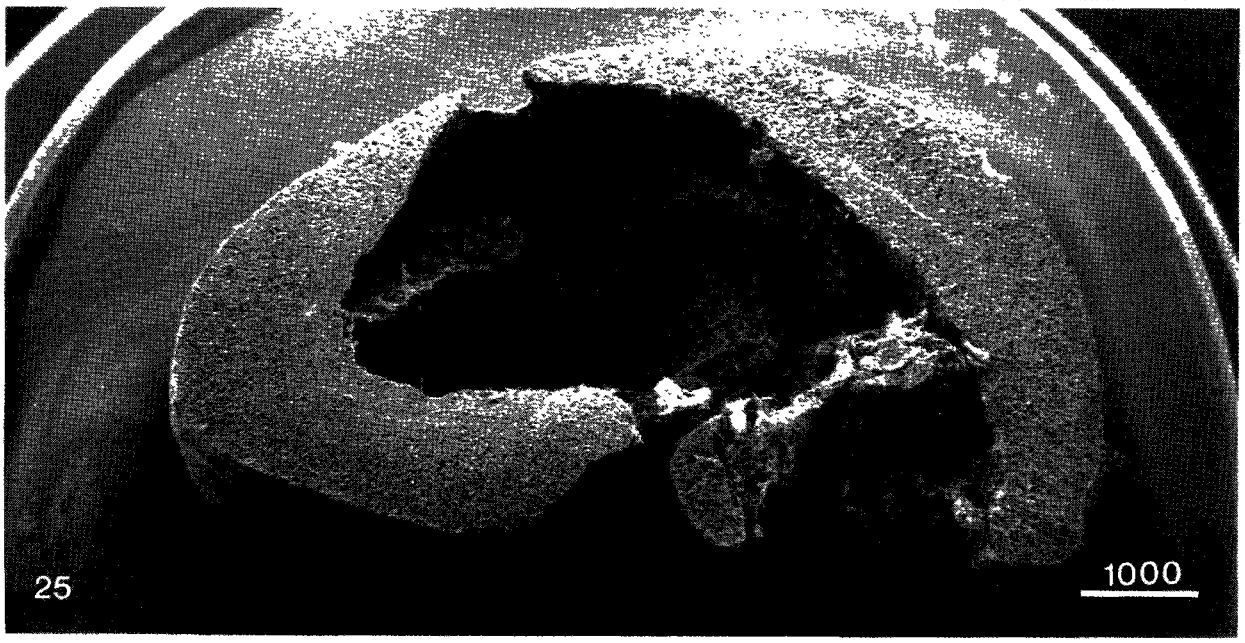
Grain à goût moisi

Fig. 21: Coupe transversale dans un grain. Parenchyme dégradé

Fig. 22: Détail. Cellules fortement vidées

Fig. 23: Parenchyme moins atteint PB: protein body

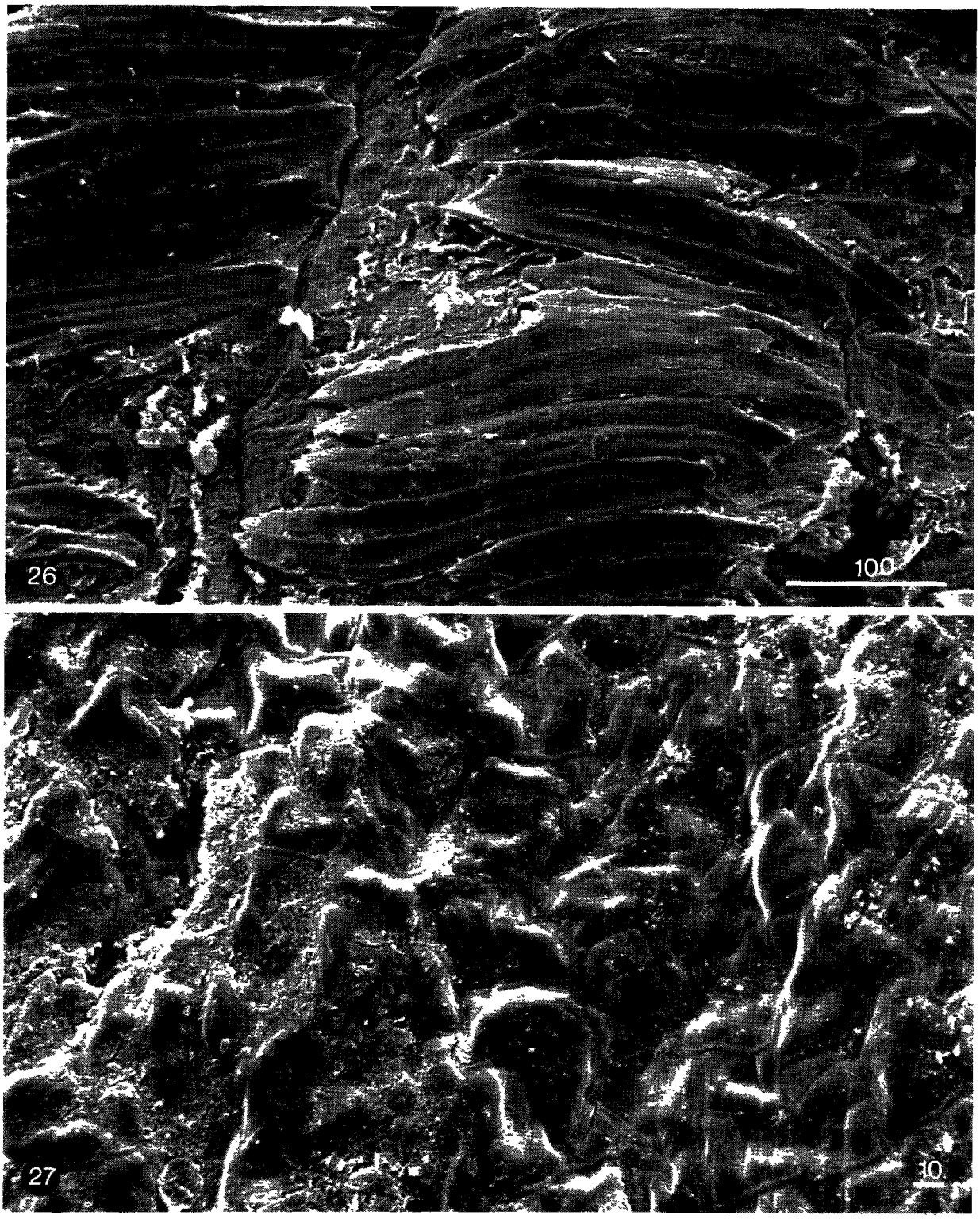




Grain endommagé par des insectes

Fig. 24: Grain entier

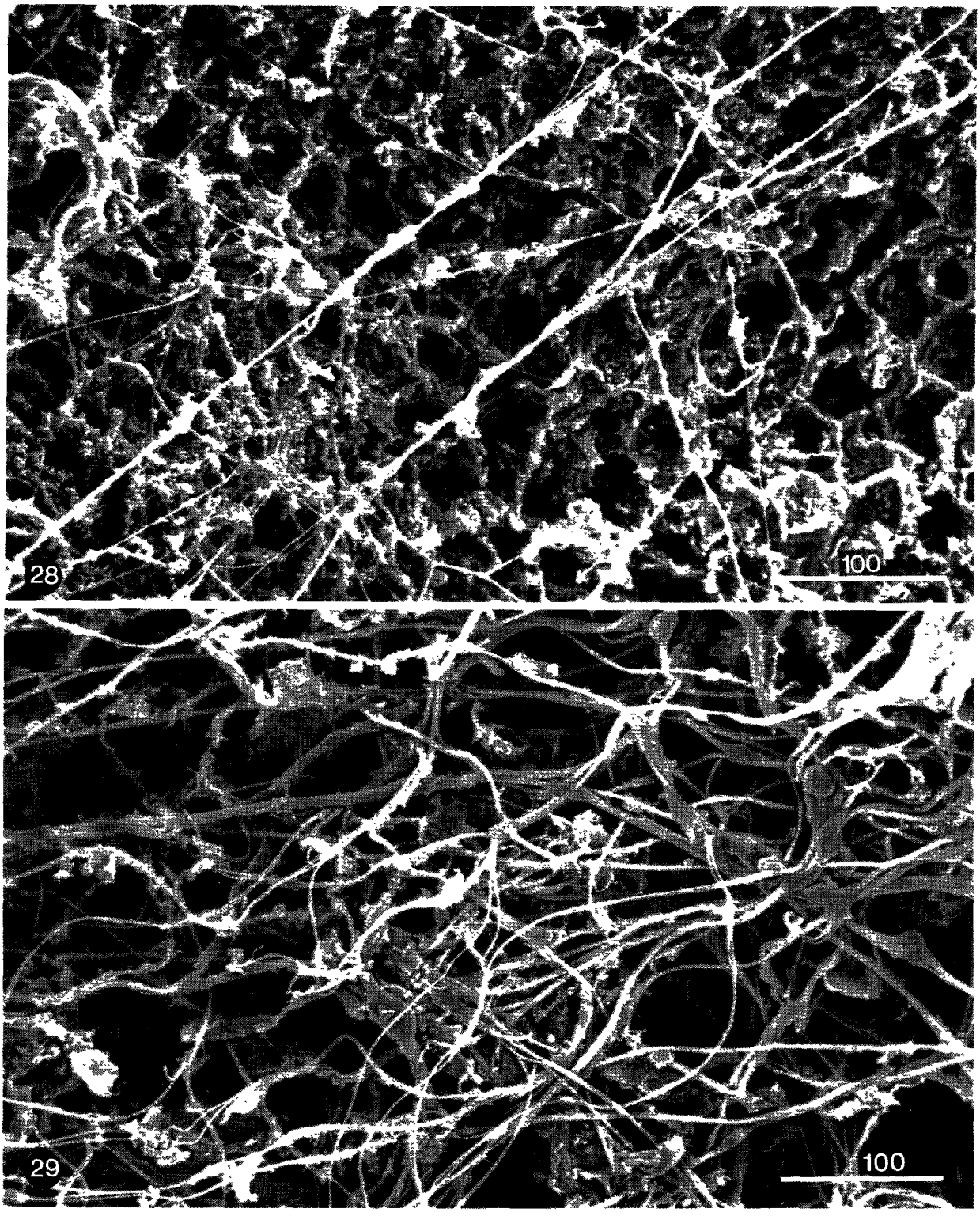
Fig. 25: Coupe transversale d'un grain



Grain endommagé par des insectes

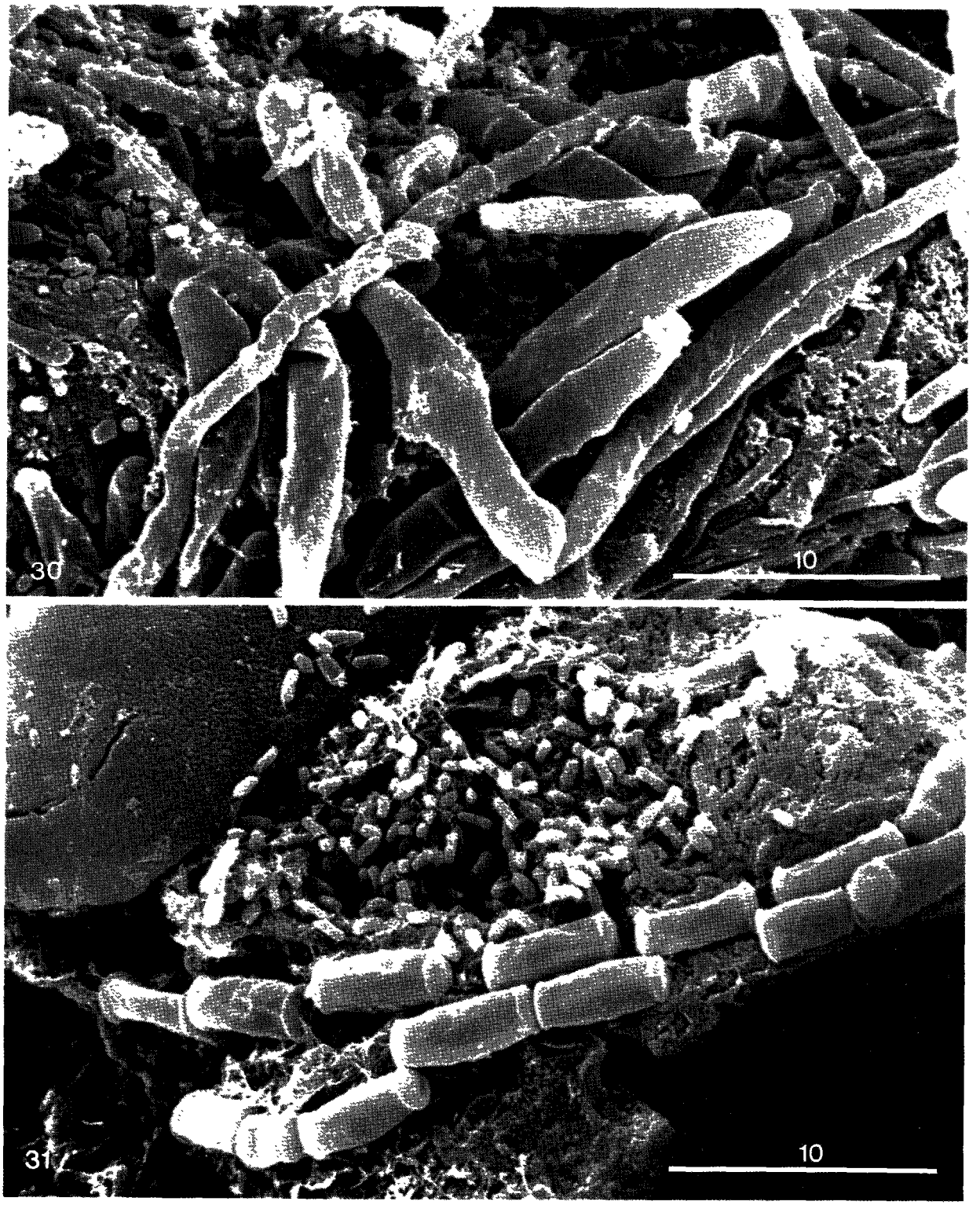
Fig. 26: Zone non rongée. Tégument argenté propre

Fig. 27: Zone non rongée. Epiderme propre



Grain endommagé par des insectes

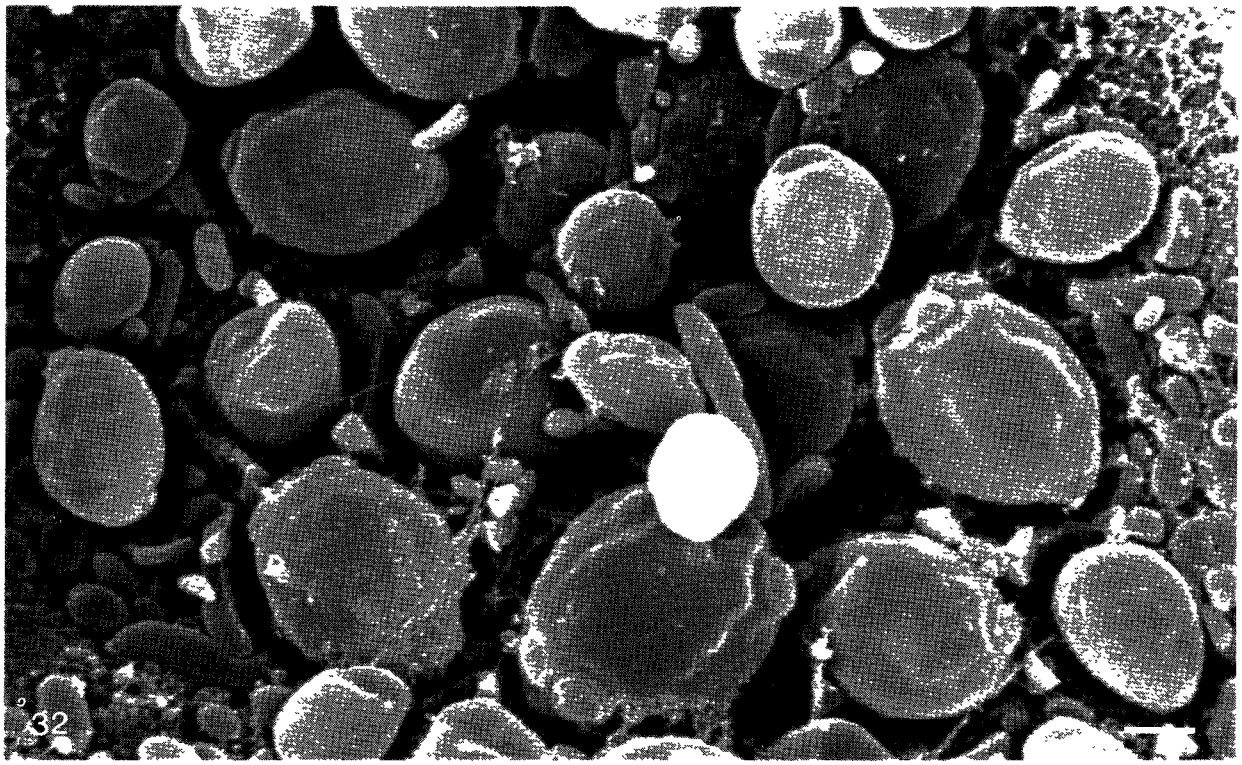
Fig. 28 et 29: Zones rongées, envahies par du mycélium

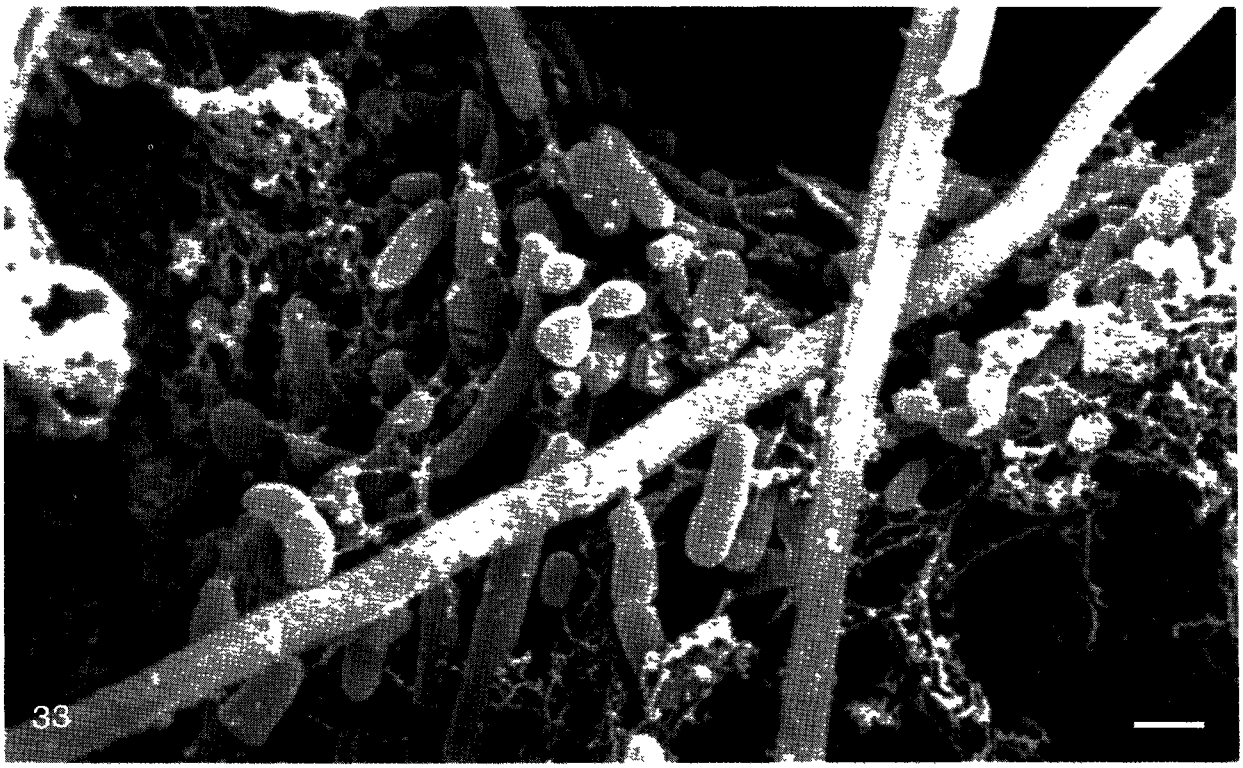


Grain endommagé par des insectes

Fig. 30: Zone rongée. Spores de Fusarium

Fig. 31: Zone rongée. Arthrospores de Geotrichum

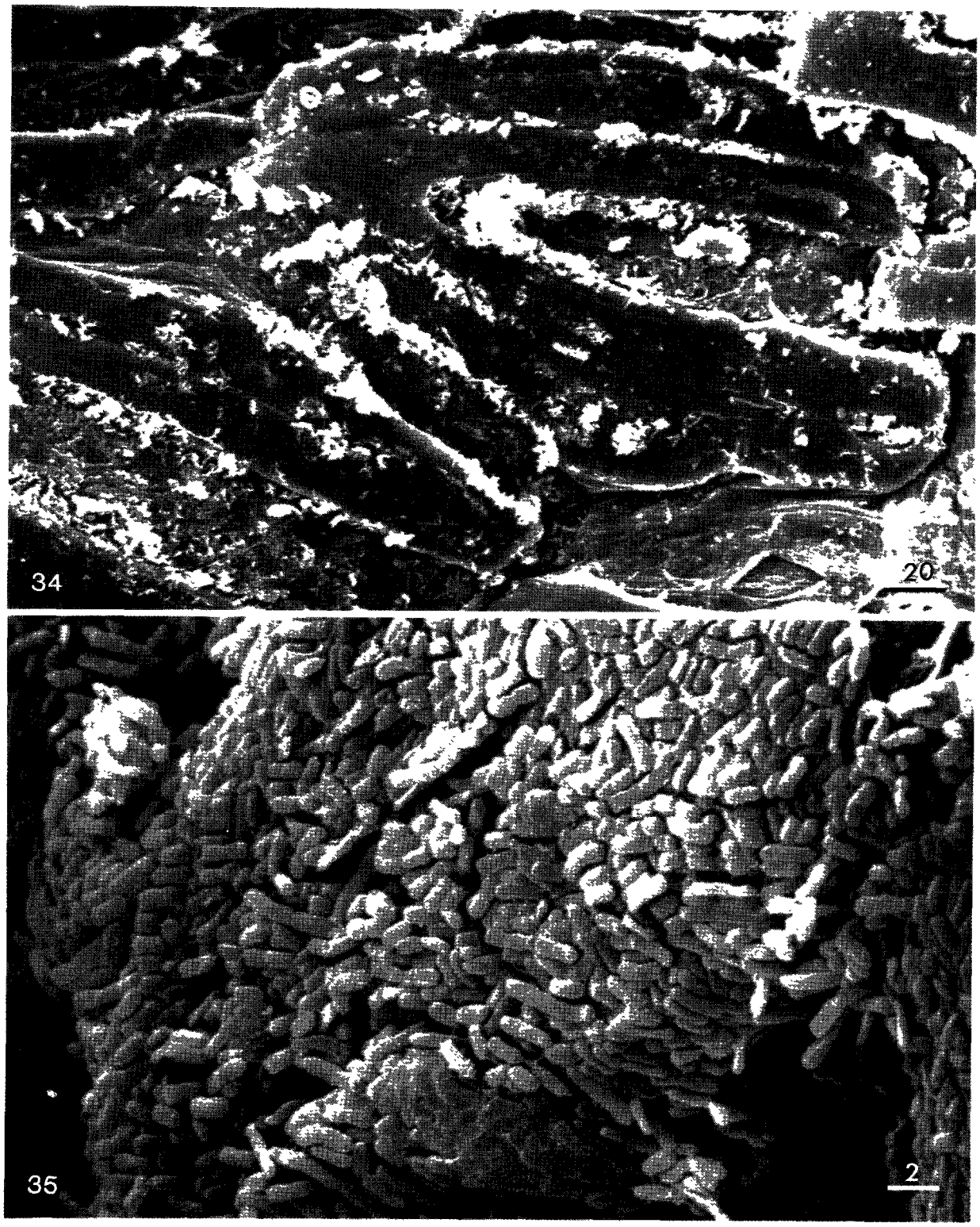




Grain endommagé par des insectes

Fig. 32: Zone rongée. Levures

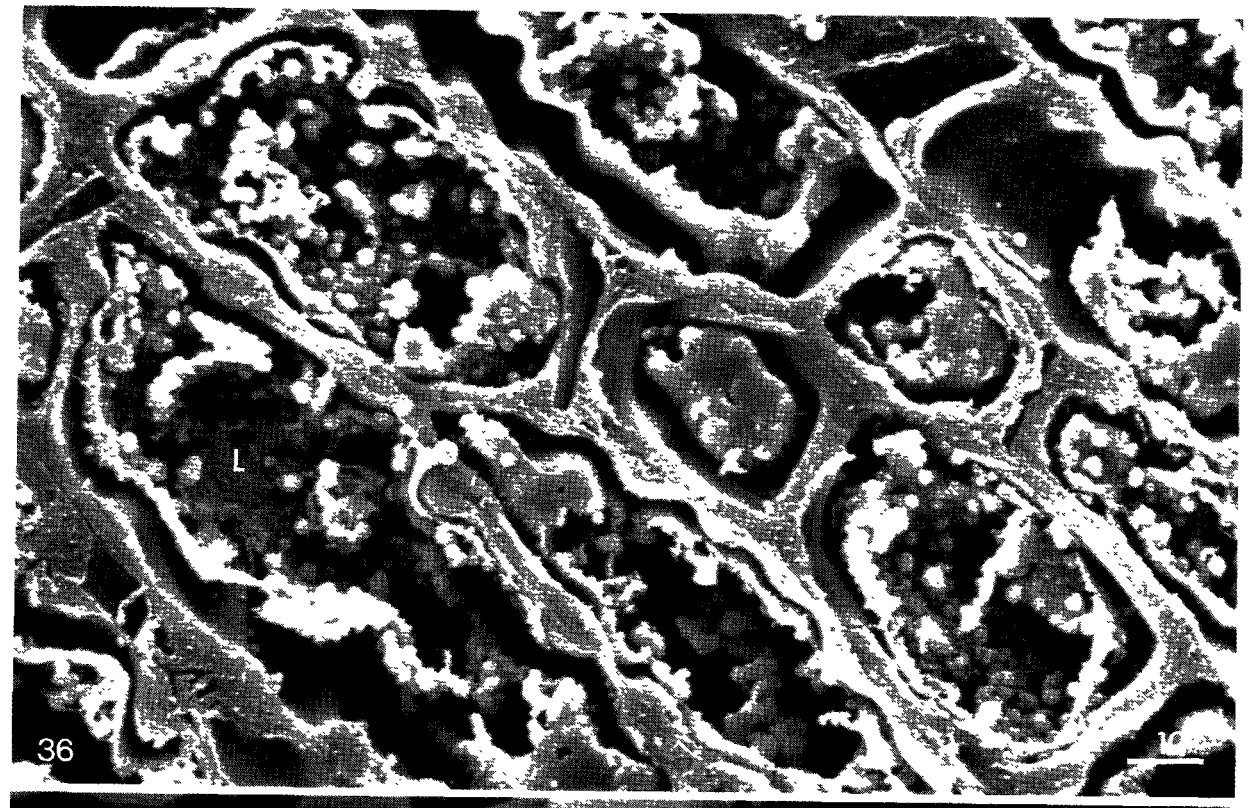
Fig. 33: Zone rongée. Bactéries

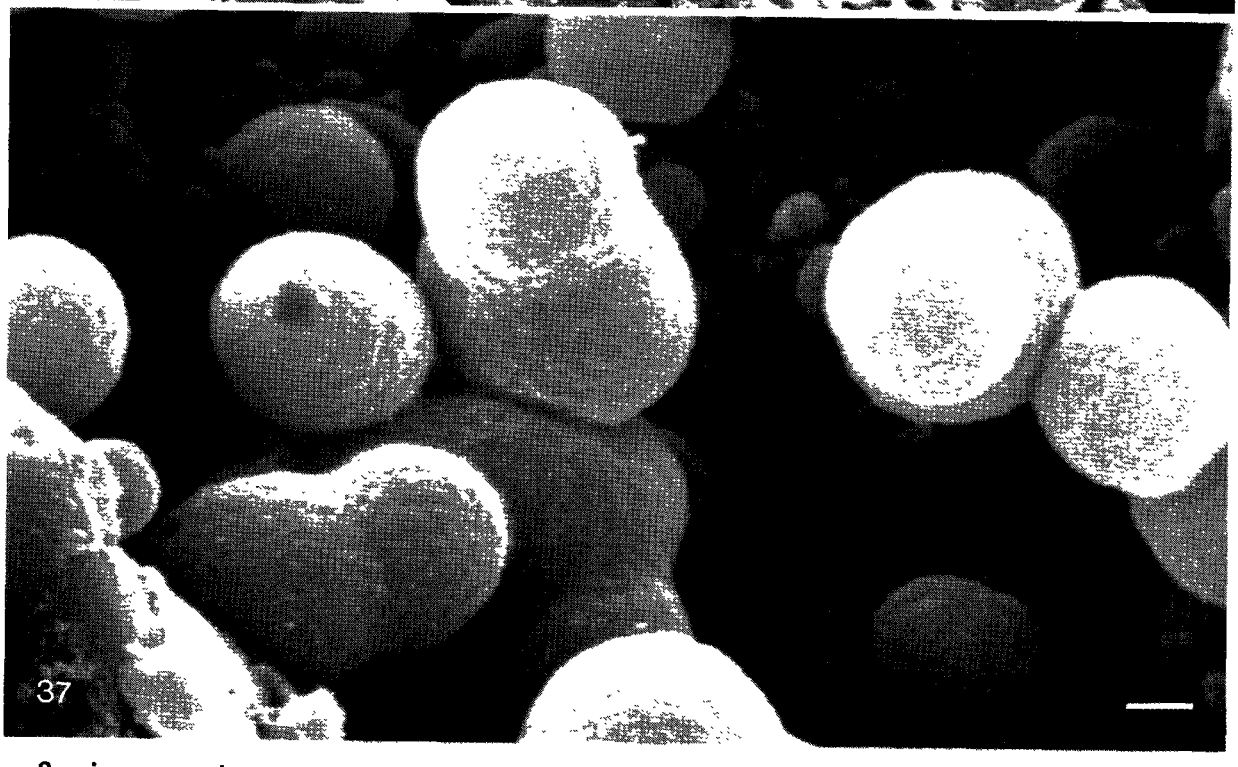


Grains puants

Fig. 34: Tégument argenté recouvert de Bacillus brevis

Fig. 35: Epiderme central tapissé de Bacillus brevis





Grains puants

Fig. 36: Parenchyme fortement atteint.
Visibles: Lipides (L) et protein bodies

Fig. 37: Détail des protein bodies.

GROWTH IN USE OF AUTOMATED FLUID BED ROASTING OF COFFEE BEANS

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In the past decade there has been a marked growth in the use of fluid bed roasters, because of shorter roasting times, increased productivity, taste upgrading, lower weight losses, more bean swelling, higher solubles yields plus improved and increased flavor and aroma.

The Industrial-Commercial quality objectives sought differ from the small gourmet roaster retailer/wholesaler.

Today we have available a small tabletop economical totally automated "on-premises" electric roaster (3.5 Kg —7 min.) simply constructed and maintained at 1/4 the cost of the RFB-6 (3 Kg —3 min.) introduced from Europe a few years ago.

These economical simple electric or gas heated automated roasters offer a new "Benchmark of Quality," especially in clean taste without tars, freshness in taste and higher levels of flavor and aroma.

In all cases, roasting degree is controlled by sensitive temperature probes and programmed instruments producing accuracy, reproducibility, regardless of location, lighting, time of day or manner of employee.

Automation reduces labor needs and removes the fallibility of human errors. Automation also improves safety.

Sivetz—U.S. Patent 3,964,175—licensee Neuhaus-Neotec

Introduction

In 1975 in Hamburg, Germany at ASIC, I presented a pioneering style of fluid bed coffee bean roasting machine that markedly differed from the Lurgi or Wolverine. That was the beginning of two commercial ventures: mine out of the USA, and Neotec out of Germany. In both cases the first five years dealt with getting established and attempting to sell progressive state-of-the-art methods to an industry long established in cylinder roasting machines. In spite of obvious advantages in fluid bed roasting over cylinder roasting, the industry remained mentally and mechanically bound to cylinder roasting.

The Lurgi machine used excessive air flows and small batches, and was not very well accepted in the trade, whereas the Wolverine shallow tray beds gained initial acceptance in drying steamed Robusta beans a subsequently roasting them, at Hills Brothers in California. This mode of use suffered a severe setback when Jacobs in Germany was unsuccessful in marketing "high yield" roasted coffee beans in the mid 1980s.

Meantime, Probat had entered into manufacturing a radial chamber using hot air fluidization in a large 10-foot rotating bowl with perimeter cooling. This series of models called e.g. RZ-4000, roasted 4,000 Kg per hr or 1 bag/min.

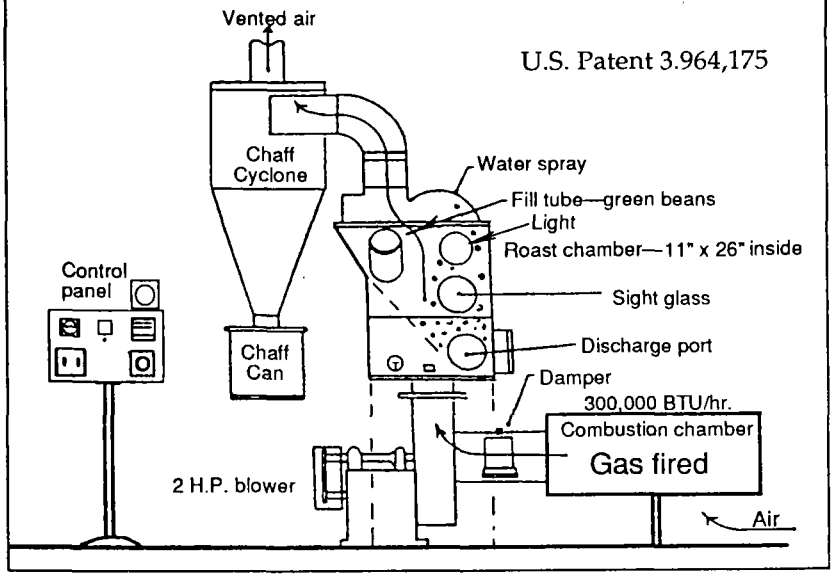
Virtually all these mentioned roasters were applied to industrial large operations where the end products were ground, degassed and packaged usually in brick packs with an outer carton. Their quality was invariably poor and the coffee lacked aroma and fresh flavor. Therefore, the highly automated roaster was more for productivity than quality, e.g. Finnish market.

However, with the dramatic growth for freshly roasted coffee beans in the USA since the early 1980s, there was a demand for small roasting machines in the USA. Whereas larger and faster fluid bed roasting machines came into demand for other reasons: increased efficiency, better tasting beans, less labor with good quality control and reproducibility, the use of 13 oz R & G coffee in the traditional 16 oz (one lb) cans, and improved solubles yields in brewing, as well as upgrading Robustas, and allowing great production flexibility from smaller batch runs offering more blends, more roasts and more variety.

Sivetz Rotating Air-Fluidized Bean Bed

Coffee Roasting Machine

with automatic temperature controls. e.g. roasts 33 lbs in 8 min.



Growth in Use of Fluid Bed Roasters

Table 1 shows the progressively greater use of fluid bed roasters in the gourmet trade in the USA by Sivetz fabrications, and elsewhere in the world by Neotec fabrications and sales.

The Sivetz philosophy was to sell initially to new retailers, to train them to coffee roasting and technology, and let them spread the use of freshly roasted, top quality coffees among the deteriorating supermarket mass produced users. This course of events grew rapidly from the west coast of the USA eastward, and swept in the older traditional metal cylinder roasters both from Europe and some made in the USA.

Sivetz's customers had not much motivation for fast roasts in a retail shop, so initially these fluid bed roasters operated on say 12 minute roast times. Later, by 1986, with increased air flows and baffled circulation of the beans, 6 minute roasts were attainable. Controls were reliable and the equipment was sturdy. Many early entrants into use of Sivetz roasters became very successful and profitable, including purveyors to hotels and restaurants as well as to office coffee use.

Retail roasting requires only small batch machines, e.g. a few Kg and say 5 min. Such machines are best suited to using electrical heating. Although some Japanese machines entered the USA market, their fabricators did not make a suitable performing machine, especially taking a long time to roast and not getting good bean flavor development. The attempt by Wolverine to sell small hot air blast machines also was unsuccessful.

By 1990 Sivetz had developed and sold numerous styles of electric machines for in-store use. Their configurations were conical chambers with central hot air bean spouting, and enclosed in glass tubes for visual display of the whole roasting process. Finally miniaturized configurations of the RFB or rotating fluid bed were sold by Neuhaus Neotec, and now by Sivetz.

This configuration allowed automatic filling and automatic roast bean discharge.

The system allowed exact bean end temperature control, hence controlled flavor and aroma development by operators that had little to no skills in this field. It allowed a retail clerk to roast for inventory or "to order" on demand within a few minutes, delivering to the customer honestly fresh roasted beans.

The consequence of this event teaches the consumer how truly fresh coffee should be procured and subsequently used, yet does not require the retailer to make a great investment in machinery nor does it require a skilled special and highly paid "roast master."

The delivery of fresh top quality at a retail level is achieved at a reasonable cost to both the retailer and the customer. Everyone gains, except the mass manufacturer producing packaged coffees and offering them, months after roasting to an uneducated and dissatisfied consumer.

The *yardstick of quality* has been established at the local retail shop.

The division between retail operations and massive roasting operations becomes obscured, when someone who has never roasted before, starts his retail operation with a 20 Kg fluid bed roaster, then upsizes to a 1 bag roaster and within 8 years upgrades again to a 3 bag roaster—a true case in New York City.

Sivetz prefers to sell and make smaller roasting machines to new users operating in a local market, whereas Neuhaus Neotec has chosen to supply highly automated fast 3-minute roasting machines to the established mass marketed coffee firms, like Hills-Nestlé, General Foods, etc.

Roasting 200 Kg green beans in 3 minutes demands a much more sophisticated and much more expensive system, than roasting 20 or 200 Kg beans in 6 minutes.

Roasting Time

Strange as it may seem, many people in the roasting business believe that coffee beans are roasted by time. That is untrue.

When coffee beans are heated over 400°F (200°C), a chemical process is started within each bean, causing heat to be developed and released into the bean. Sugars, starches and proteins are altered dramatically to other compounds. Water, carbon dioxide and aromatics are volatilized.

TABLE 1

CHRONOLOGY OF SIVETZ AND NEOTEC ROASTER SALES 1979-1991—Cumulative

Fluid Bed System...to 200 Kg batch

	NEOTEC	SIVETZ
YEAR	Country	All USA
1979	U.K.	23
1980	Greece	35
1981		52
1982		60
1983		69
1984	Germany & Netherlands	87
1985	Switzerland & Netherlands	103
1986	Belgium	112
1987	Switzerland & Finland	119
1988	U.K.	226
1989	Germany	233
1990	Japan & USA (8)	243
1991	Austria & USA (3)	250

In a fluid bed of beans, that batch (not layer) temperature can be very accurately measured with a thermocouple or thermometer, and that exact temperature corresponds to the degree of roast or taste in the chemical process that bean has undergone, and also the color of the bean.

Both Neuhaus Neotec and I have checked this relationship of resulting bean color to sensed bean temperature and beverage flavor, and have found their relationship to be totally relatable. This means that the subjective eye judgement when roasting, causes considerable variations in results, whereas the thermocouple sensor when set to cause system heating to stop is very accurate (within 1°F) and very reproducable regardless of who or where the roasting is done.

In other words *we have an absolute measurement of the degree of roast*, whether it is in San Francisco, New York City, Chicago, Europe, Australia, or Asia.

Make no mistake, *this is a profound control system*, and in fact has not been much used before the Sivetz and Neotec fluid bed roasters were commercialized. The system is in fact even today only at the threshold of growth and use.

In this regard TIME of ROAST becomes largely a matter of how the roasting system is designed and operated.

Roast times of say between 4 and 10 minutes usually produce excellent tasting coffees.

Roast times less than 3 minutes tend to give more acidity and less uniform flavor development.

Roast times longer than 15 minutes tend to give a baked flatter taste.

Influence of Bean Types

One *word of caution* in the relating of beverage flavor, bean color and end bean temperature in the roast, and that is, not all coffee beans are the same.

For example, Sumatra beans are very hard, and if roasted to 450°F (232°C) or less, they will not come to full flavor development nor appropriate color. So one must allow for the nature of the bean, before setting the end bean temperature.

On the other hand, a 471°F (244°C) end set bean temperature with Brazilian beans grown at 1800 feet above sea level, can almost burn or certainly be considered over roasted, whereas a Colombian bean at the same temperature can make a good espresso taste and beverage; it will appear near black in color. However, the Sumatra at that temperature is a dark brown and has no suggestion of a burnt taste.

Therefore, some skill in the properties of green coffees in so far as their altitude of growth and origin, come into play in setting end bean temperatures.

Notably, green beans with low moistures due to over drying in a dry climate or after decaffeination have less moisture to lose, hence for a given set of roasting conditions will cause the beans to roast more quickly. However, whether the initial moisture is 8% or 12%, the final end roast bean temperature will control the final bean taste and color.

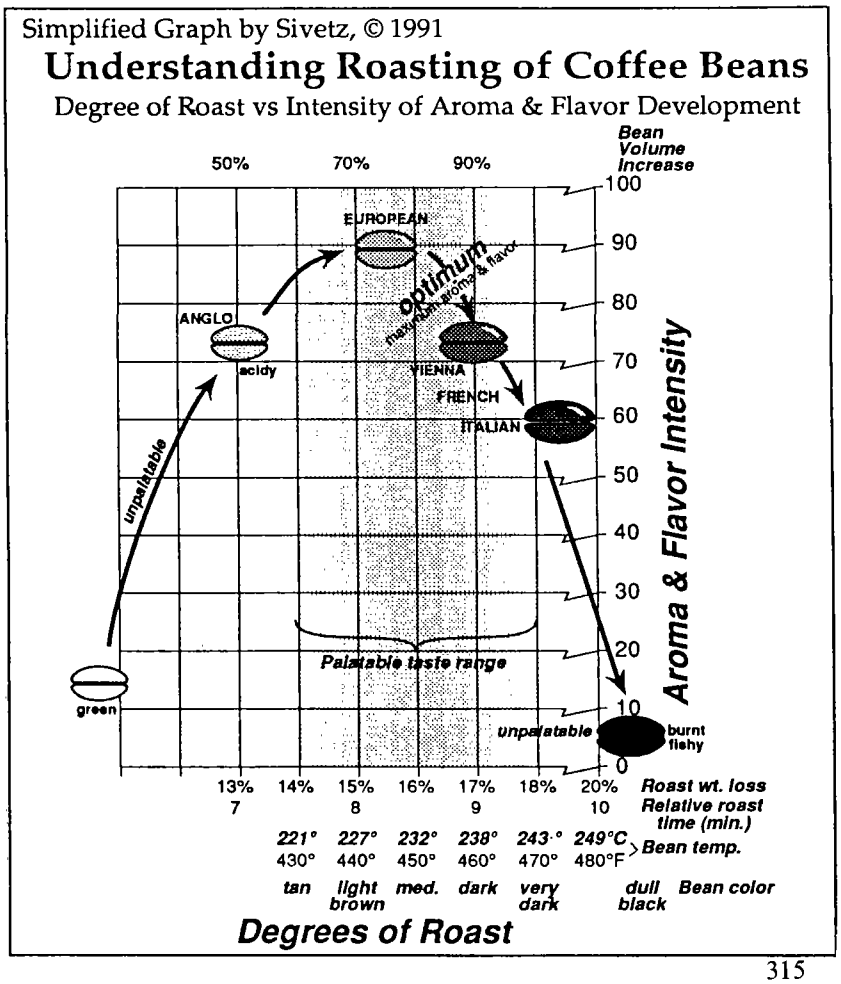
Faster Roasting

Faster roasting times have been in commercial use for 50 years, yet it is only now that the advantages of say 5 minute roasts are being accepted and more widely used not only in larger roasting operations, but also in retail shops.

Traditional cylinder roasting times were usually 15 to 20 minutes, and high roast chamber temperatures near 800°F were not uncommon. These conditions gave a "baked" taste with little aroma, and much tar deposits and higher roast weight losses.

What we have seen occur in the past 15 years, has been a reduction in roast times, e.g. a Sivetz fluid bed roaster can deliver excellent product in 7 to 8 minutes, whether it is a 15 Kg , 35 Kg , 70 Kg or larger batch bed. A Neotec fluid bed roaster can roast in less than 3 minutes, although it is generally acknowledged that this is not gourmet quality coffee. The Wolverine shallow oscillating bean bed suffered from producing a non-uniform bean roast, and lost favor from especially the Jacobs experience in Germany in the mid-1980s. The 5 minute Burns continuous roaster has prevailed, but is only suitable to firms that can use 5 or 10 tons/hr of production. The accelerated roasting rates gained by the Gothot-PROBAT cylinder mixer were confounded by high air temperatures and an internal mixing vane. The PROBAT RZ bowl batch roaster, a monstrous machine that processed 1 bag a minute or 3600 Kg per hr was a mass production roaster using some principles of air levitation, but had its share of problems, as illustrated in the Gevalia, Stockholm explosion of 1989.

In general we can see roasting times falling from 16 to 4 minutes in 15 years. Old inefficient roasting machines still exist that roast in 1 hour. Faster raters of roasting are generally beneficial, but whether they are or not always needs to be examined in terms of the exact process conditions and the ultimate type of product quality being sought, by that processor.



Sivetz patented fluid bed roasters establish absolute degrees of roast by end bean temperature controls totally relating to bean color and taste.

Small Automated Roasting Systems

In the idealized marketing situation where freshly roasted bean quality is transferred to the knowledgeable consumer, we have many small roasting machines, operated automatically, on the retail premises. In such a manner the retail clerk becomes the "roast master"; and the roast bean inventory is near nil; so staling is near nil. The key factor in this arrangement is to have a low enough investment in the roaster equipment, that this market system can be profitable, especially when franchised or used with multiple retailing outlets managed by one firm for green coffee purchases and overall quality control. Such a system exists today for the first time. Refer to the Sivetz table top 3.5 Kg (8 lb) 8 minute automatic roaster. The thermocouple probe accurately sensing the coffee bean temperature at all times is one of the key factors in making for simple automated roasting machines. The retail clerk loads the roaster with the selected green coffee beans from wall stored green coffee displayed stocks, and scale.

He or she *sets the roasting parameters* on the control panel:

- End roast bean temperature (degree of roast).
- Time for water spray, to stop pyrolysis and/or further cool.
- Time of roast bean dump into cooling can.
- Time roaster blower and cooling blower operate.

The net result is that the Sivetz fluid bed roaster goes through the roasting and cooling cycle *automatically*, while the clerk attends to other matters. The clerk can return ten minutes later, and pick up the roasted and cooled beans, and all operating parts of the roaster have shut down.

The clerk can custom roast, or roast for a small inventory of different bean types. Since the clerk is not always busy with buyers, the *roaster-clerk* saves labor. Also the *roaster-clerk* is more knowledgeable about the coffees being sold. Further, high quality uniformly roasted beans full of fresh coffee flavor and aroma, demand the best brewing methods to preserve those flavors in cup. Such good quality aromatic beans will drive out poor brewing methods like the paper filter/drip machines used in USA and elsewhere, and there will be a trend toward convenient espresso brewers like the Krup Novo. One does not have to brew espresso strength coffee from this machine, nor does one have to make insipid weak beverage either, but one can make a mid strength, e.g. European strength and 3 to 4 fl oz (90 to 115 ml), not 6 to 8 fl oz mugs.

Gourmet Retailing

Although Neuhaus-Neotec (Sivetz licensee) has made a package 3 Kg automated roaster, its size and cost (US $40,000) is "out-of-line" with most retail gourmet investments. .....the Sivetz 14 Kw 8 lb 8 min automatic roaster at $10,000 today fits the need of the Gourmet retailing market.

Failure of Mass Production to Deliver Freshness

It is fair to say today, in view of the efforts made by most major roasters both in the USA & Europe to produce and market GOURMET QUALITY coffees, that they have been unsuccessful. This is because mass production with low pricing as objectives, are inconsistent with local fresh roasting of quality beans and freezer preservation of same. It does no good to inertly seal, in freezer, stale canned, pouched, or bagged mass produced roasted beans. These are often too lightly roasted, burnt, oxidized—full of defects and Robusta blends. We are moving into an era of consumer enlightenment, and knowledge that tells them they do not have to drink stale objectionable tasting coffees, because they now know what good quality freshly roasted beans taste like.

Roasting Better Tasting Coffees

There is mounting and extensive evidence on an industrial scale, that the use of rotating bean *fluid bed roasting* gives more flavor and better tasting coffee beans with higher solubles yields and higher extracted solubles concentrations both for beverages as well as for soluble coffee production.

RFB roasters help "clean up" lower grades of coffee beans by:

- Levitating out of the spouting beds, broken and deformed beans, as well as dirt, dust, chaff, twigs, leaves, and other light matter.
- Settling out at acute angled wall above perforated plate, heavy particles like stones, metals, wires, etc. can be removed after the roasted beans are discharged.
- Flushing out immediately chaff and smokey tars. Otherwise these tars deposit on bean surfaces giving a harsh bitey taste to the subsequent beverage. Chaff does not smoulder and burn in roaster.

TABLE 2—RELATING END BEAN TEMPERATURE VS COLOR, TERMINOLOGY, TASTE AND WEIGHT LOSS

Range of palatable bean roasts 450°F to 475°F

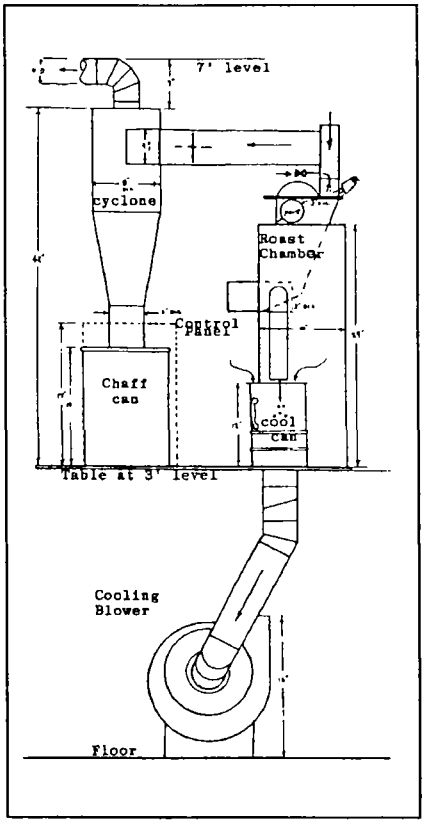
TEMPERATURE °C	°F	Term	Roast Loss*
220°C	428.0°F		wt. %
222	431.6		
224	435.2		
226	438.0—440°F	Anglo tan color	13.5
228	442.5		
230	446.0		
232	449.6—450°F	American light brown	15.0
234	453.2		
236	456.8		
238	460.4—460°F	European brown	16.5
240	464.0		
242	467.6		
244	471.2		
246	474.8—475°F	Espresso dark brown	20.0
248	478.4		
250	482.0		
252	485.6		
254	489.2—490°F	burnt, black unpalatable, oily	25+

NOTE: Measured bean temperatures in a Sivetz fluid bed roaster are very accurate, reproducible, and are aided by automatic temperature (thermocouple) sensors and controllers. Similar bean temperature accurate measurements are impossible in a cylindrical type roaster.

*based on 12 wt % moisture in green beans

TOTALLY AUTOMATIC TABLE TOP FLUID BED ROASTER AND COOLER

8 lb/8min–14 Kw–230V



The vigorous green and roast bean movement results in 2 benefits:

- The metal walls and sight/light glasses remain clean.
- Rubbed beans release their skins, and their waxy surfaces become polished and smooth, thus improving air to bean heat transfer, as well as final roast bean appearance.

RFB roasters use relatively low inlet air temperatures, e.g. 500°F (260°C) can produce a dark roast of 475°F (246°C), without scorching or "tipping" the beans. *This cannot be done in a cylinder roaster.*

In fact, the same color grounds from a cylinder roaster will taste "burnt."

In general fluid bed "once-through" air roasters give a smooth (not harsh, not bitter) tasting beverage. When RFB roasters have inlet air temperatures near 700°F (350°C), they can produce 3 to 4 minute roasts but it is not recommended.

INSTANT COFFEE Advantages of RFB Roasting for Extractions

In addition to more and better flavors, there occurs 15% more swelling of the beans, or a 15% lower bulk density. The water extractant more readily permeates the expanded and more ruptured cells of the roasted granules.

This gives faster solubles extraction, and higher solubles concentrations.

This gives "darker" roasts without developing burnt tastes nor losing solubles yields.

Faster solubles yields at lower processing temperatures gives more and better flavored extracts, hence more and better spray and freeze dried powders.

When the RFB roaster levitates out broken, defective and light granules, a denser more wholesome roasted batch of beans results, giving more flavor and aroma.

A noticeable increase in solubles yields is achieved.

Summary

In the past decade there has been a marked growth in the use of fluid bed roasters, because of shorter roasting times, increased productivity, taste upgrading, lower weight losses, more bean swelling, higher solubles yields plus improved and increased flavor and aroma.
The Industrial-Commercial quality objectives sought differ from the small gourmet roaster retailer/wholesaler.
Today we have available a small table top economical totally automated "on-premises" electric roaster (3.5 kg-7 min) simply constructed and maintained at 1/4 the cost of the RFB-6 (3 kg-3 min) introduced from Europe a few years ago.
These economical simple electric or gas heated automated roasters offer a new "Benchmark of Quality", especially in clean taste w/o tars, freshness in taste and higher levels of flavor and aroma.
In all cases, roasting degree is controlled by sensitive temperature probes and programmed instruments producing accuracy, reproducability, regardless of location, lighting, time of day or manner of employee.
Automation reduces labor needs and removes the falability of human errors.
Automation also improves safety.
The major causes of fires and explosions in all types of roasters is explained in terms of the combustible gases liberated.

Résumé : Un petit torréfacteur à lit fluide.

Au cours des dernières années on a eu de plus en plus recours à ce type d'appareil pour plusieurs raisons : rapidité d'exécution, amélioration de l'arôme, gonflement du grain plus important et aussi extraction plus satisfaisante des matières solubles.
La qualité du café obtenu avec cet appareil est très différente de celle du produit traité dans un torréfacteur industriel.
Nous offrons aujourd'hui un petit torréfacteur automatique, électrique, d'une capacité de 3,5 kg, au quart du prix du RFB-6 vendu en Europe il y a quelques années : la durée de l'opération est de sept minutes.
La torréfaction est contrôlée par des sondes et des instruments sensibles, ce qui permet de garantir une bonne exécution de l'opération, sans tenir compte du lieu, de l'éclairage ou encore de la manière de procéder.
L'automatisme évite toute erreur humaine et renforce les conditions de sécurité, la cause principale des incendies et des explosions dans les installations industrielles de torréfaction étant due aux gaz libérés pendant la pyrolyse.
En bref, ces petits torréfacteurs, chauffés à l'électricité ou au gaz, permettent d'obtenir un café d'excellente qualité par sa saveur et son arôme.

Resumen : Tostador de café en cama fluida con control automático.

En los ultimos 10 años apareció un aumento significante en el empleo de tostadores en cama de fluidez debido a su brevedad de tostadura, mayor productividad, mejor sabor, menor perdido de peso, mayor hinchazón, del grano, mayor rendimiento de solubles, y mejor y mayor gusto y aroma.
Los objectivos deseados de calidad industrial y comercial son diferentes de los del pequeño tostador encontrado a venta al por menor y al por mayor.
Hoy dia tenemos a nuestra disposición un tipo compacto de tostador eléctrico que es económico y totalmente automático para usarse en el propio establecimiento (3,5 kg-7 min), sencillamente construido y de facil manutención, por 1/4 del costo del RFB-6 (3 kg-3 min) introducido en Europa.
Estos sencillos tostadores automáticos, eléctricos o a gas, ofrecen un nuevo "Símbolo de Calidad", especialmente por su puro sabor sin alquitrán, la puridade del sabor y más altos niveles de gusto y aroma.
En todos los casos el grado de tostación esta controlado por sensibles, sondas de temperatura e instrumentos programados que producen precisión y uniformidad de tostadura de grano, sin hacer caso de la localidad, illuminación, hora del dia, o abilidad del empleado.
La automación reduce la necesidad de mano de obra y elimina la posibilidad de error humano, e aumenta la seguridad.
Las mayores causas de incendios y explosiones en todos tipos de tostadores se explican en terminos de la liberación de gases combustibles de granos.

Zusammenfassung : Automatisches Fluessig-Bett-Roesten von Kaffeebohnen.

Im letzten Jahrzehnt hat es einen deutlichen Wachstum im Gebrauch dieser Machinen gegeben auf Grund kuerzerer Brennzeiten mit folglicher vergroesserter Produktivitaet, Geschmacksbereicherung, weniger Gewichtsverluste, groesserer Schwellung und loeslicher Ertrag der Bohnen, mit verbessertem Geschmack und Aroma.
Die gesuchten Industrie-Handel Qualitaets Ziele weichen von denen der kleinen Feinschmecker Roester im Einzel/Grosshandel ab.
Heutzutage steht uns ein kleiner oekonomischer und vollstaendig automatisierter Kaffee-Roester fuer den Einzelhandel zur verfuegung (3,5 kg/7 min). Er ist einfach und kostet nur ein Viertel des Preises einer RFB-6, die vor wenigen Jahren in Europa vorgestellt wurde.
Dieser oekonomische (elektrisch oder Gas geheizte) automatische Brenner bietet Ihnen einen neuen "Hoehepunkt in Qualitaet" an. Besondere Merkmale sind : Sauberkeit, Frische und eine hohe Stufe des Geschmacks und Aromas.
In allen Faellen ist der Roestungsgrad des Brenners vollstaendig von empfindlichen Sonden und Instrumenten, die Genauigkeit und exakte Wiedergabe unabhaengig von Ort, Beleuchtung, Tageszeit oder Faehigkeit des Bedieners der Machine ermoeglichen,kontrolliert. Automatisierung beseitigt die fehlbarkeit des menschlichen Einflusses.
Automatisierung verbessert auch die Sicherheit. Die Hauptgruende von feuern und Explosionen in allen Typen der kommerziellen Kaffeebrenner sind generall und Explosionsgase, die waehrend der Pyrolyse frei werden, zurueckzufuehren.

COFFEE GRINDING DYNAMICS : A NEW APPROACH BY COMPUTER SIMULATION

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1. Introduction

The classical representation of coffee usually pictures it in beans, either green or roasted.

The bean preserves the particular qualities which render coffee a such characteristic product; but for coffee to be consumed as food, it is necessary to destroy the beautiful form that nature has given the seed and to transform it in powder.

Indeed, the final product of the efforts undertaken by botanists and agronomists, on one hand, to produce a perfect green coffee, and by chemists and technologists, on the other, to transform it in high quality roasted beans, is the fragrant cup of coffee we all appreciate.

There is no doubt that each stage of processing plays an important role in obtaining an excellent result. Nevertheless, it is worth to be underlined that 80% of the material that is produced will be unavoidably discarded in form of spent coffee grounds.
Hence, the selective transfer of the chemical compounds from the solid phase into solution is a key factor in determining the beverage's tastiness.

This is particularly true in the case of the espresso, where the brevity of the contact period conditions the kinetics of transfer in the liquid phase of the major sensorially effective substances.

Equally important is the influence of the surface area exposed to the action of hot water.

The study of the extraction process has been conducted by many authors, also from the kinetic point of view {1}{2}{3}, and several of them draw attention on the influence of the ground powder's fineness on the yield of the beverage{4}{5}.
However, apparently little consideration has been given to the physical phenomenon of the preparation of coffee powder, namely the process of comminution of roasted coffee beans{3bis}.

This led us to assume that the operation of roasted coffee grinding had had approximately the same evolution of the operation of espresso coffee percolation: that is, that it had been developed through a process of successive trials by various persons of extraordinary talent in the course of years, but without any systematic scientific approach.

We therefore started the study of the process of grinding from explorative and descriptive stage, by studying first the material to be processed (the bean), then a tool typically used in industrial practice (the grinder), and later by applying a method employed in construction science to a structural model of roasted coffee designed by a computer.

2. Cellular Structure of a Coffee Bean

Like all seeds, the coffee bean is made of tissues composed of cells with cellulose walls.

Numerous authors {6} have investigated the bean's macroscopic morphology, with plicas and flaking surfaces (fig. 1), as well as its microscopic morphology (fig. 2), with cells approximately prismatic in shape, hexagonal in cross section and having a characteristic mean size of 30 μm.

This structure is preserved even after roasting, although it undergoes a considerable geometric expansion presumably due to the pressure of pyrolysis gases.

This leads us to assume that the cellular walls are subject to a system of internal stresses, at least for a certain period after roasting: providing an explanation for the friablity of the roasted bean which easily flakes off under the slightest action of forces and so produces macroparticles a cubic millimeter large. These macroparticles may be rightly considered to be homogeneous and isotropic: they constitute the material to be ground and to be examined by computer simulation, as we shall see in paragraph 4.

Fig. 1 Green coffee bean macrostructure

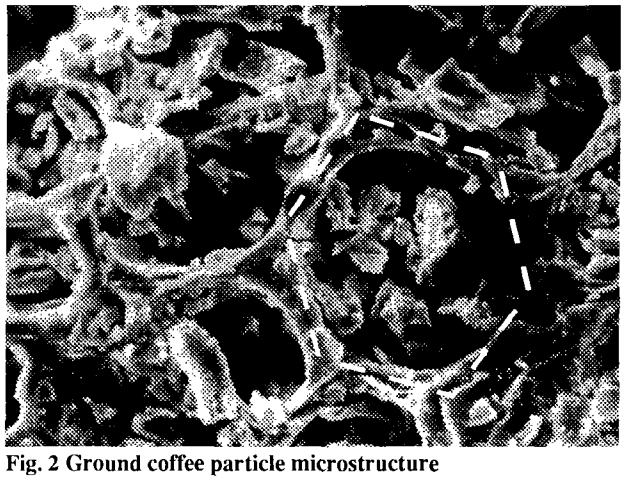

Fig. 2 Ground coffee particle microstructure

Examinations performed on optical microscope (fig. 3) show that the distribution of particle sizes is extremely wide, with a clear numerical preponderance of the smaller sized particles.

A possible explanation for this fact will be presented as the conclusion of paragraph 4.

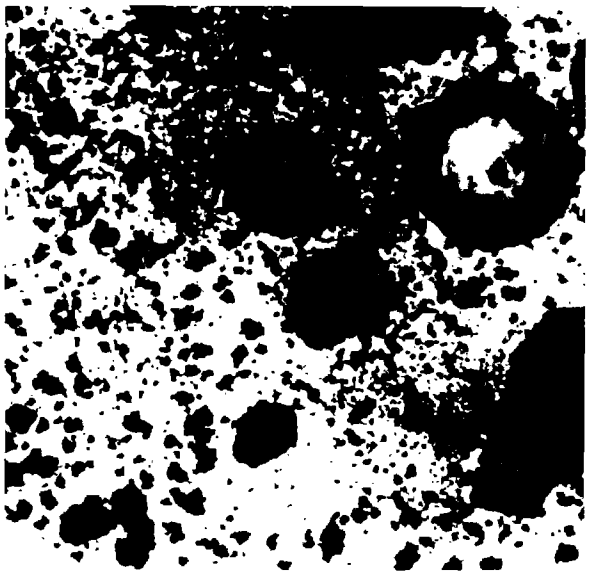

Fig. 3 Roast and ground coffee powder

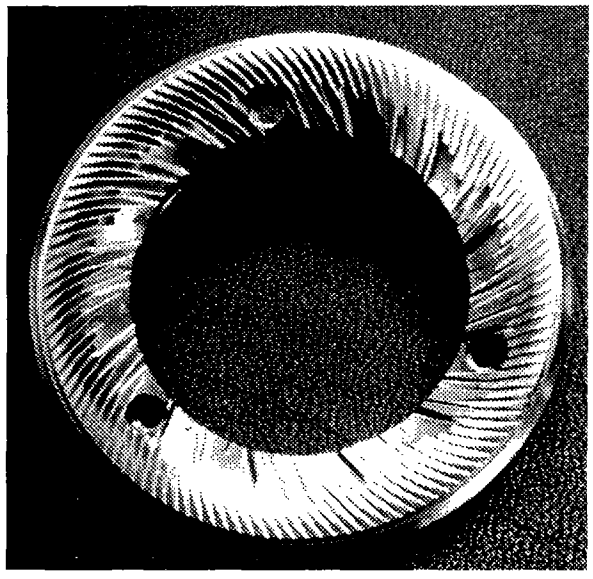

Fig. 4 Grinding tool

3. Examination of a Technological Device

Obviously, many different grinding methods exist, but all have in common one fundamental concept: the application of forces (actions and reactions) on a material {7}{8}.

These forces are responsible for inducing stress configurations that exceed the elastic and plastic behaviour of the material and are sufficient for local fracture.
As a result of the expenditure of the power supplied by the machine, the linkage forces present within the material are overcome and new surface is created, that also means increase of potential energy.

The better studied size reduction machines (ball mills) are used in the mining industry and operate in water medium: this of course prevents their use for coffee grinding, due to its solubility in water.

In coffee practice a wide range of machine configurations are found, all based on cutting tools operating in air or inert gas medium: but, since no literature (excepted the commercial one) was found, our approach consisted in examining a typical tool used in industry as if it were a physical product of nature.

The technological instance we studied is employed in various types of grinding machines, for both coffee bar or industrial use, to produce finely ground espresso powder.

It consists of a pair of opposed toothed tools: the upper one is static and the lower one is rotary. The tools are identical for reasons of construction economy; their active and reactive structure (fig. 4) is formed by teeth or more precisely, by subradial grooves.

We will not dwell upon the metrological methods used to survey the topology of the object. These results were put in a CAD (Computer Assisted Design) programme {9} which permitted a geometrically precise reconstruction of the tool and its visualization from all possible points of view (figg. 5 and 6).

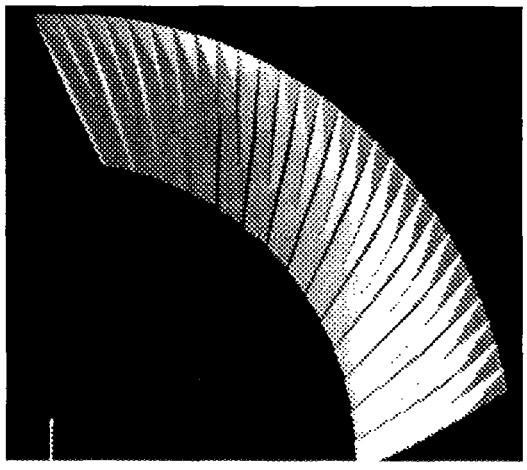

Fig. 5 CAD image of the grinding tool

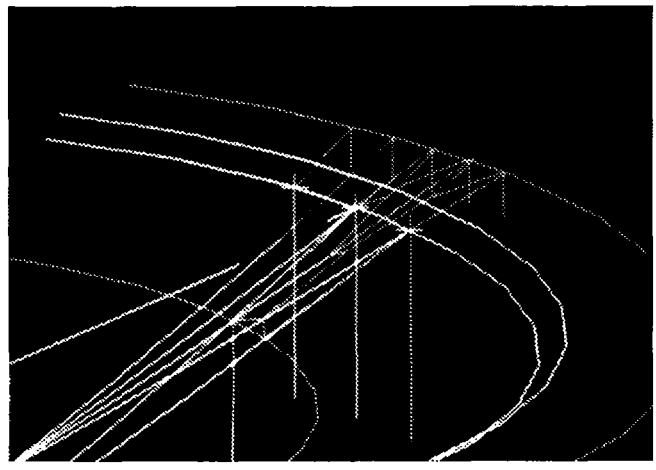

Fig.6 Reconstruction of lines and planes of the grinding tool

The building system is obviously based on the repeated action of rotating abrasive wheels, which function by motion of translation along an axis with a given eccentricity, inclination and tilting (fig. 7).

A particular moment of the action is when a coffee particle moves between the pair of tools: because of the funnel shape of the groove in which it is moving (fig. 8), every particle, larger than the gap, will be caught between a pair of teeth sooner or later.

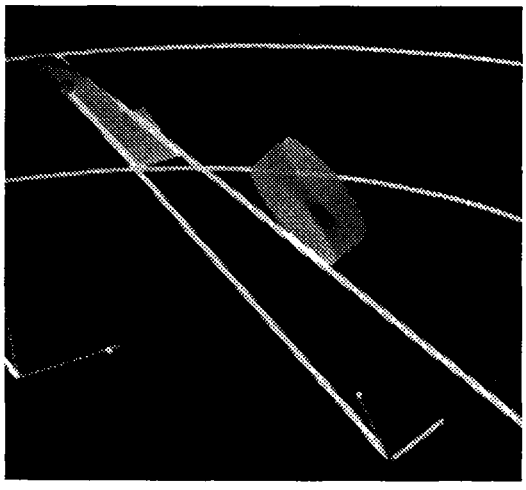

Fig. 7 Tooth building system, as interpreted by CAD

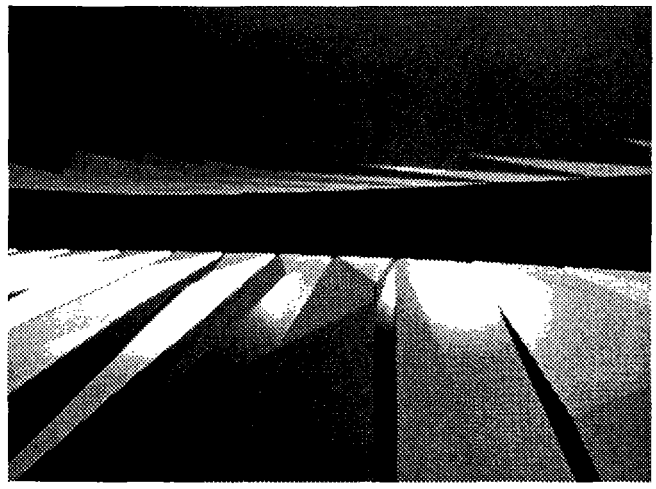

Fig. 8 CAD image of the gap between the pair of tools

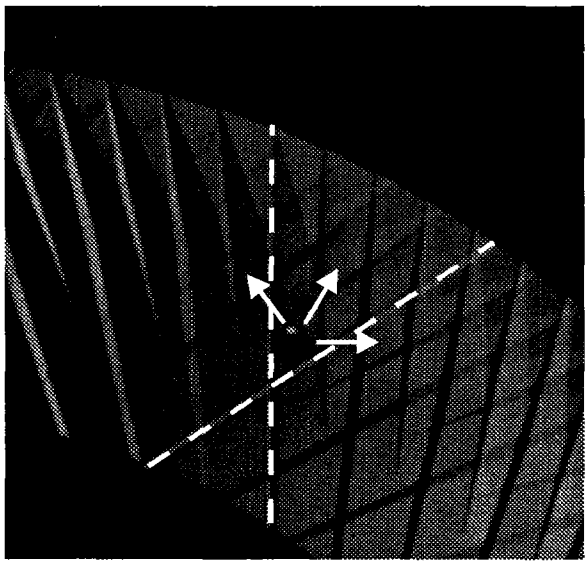

Fig. 9 "Scissors Action" of the tools

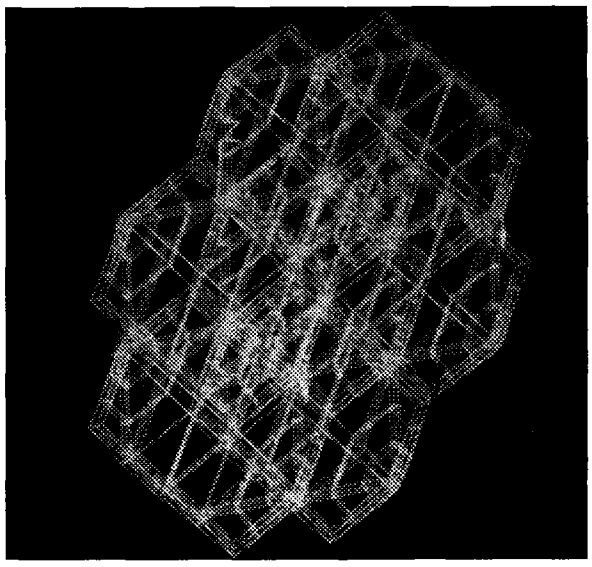

Fig. 10 3-D coffe particle simulation

At this point a question arises: where does the centrifugal motion of the particle originate from?

The answer provided by the CAD is clear: the eccentricity of the teeth's shape forms a pair of scissors (fig. 9) which, in addition to applying normal shear to the particle, also applies a force determined by the composition of vectors: this force is directed in radial centrifugal direction.

However, a single particle moving freely between the grinders is clearly an abstraction, for in reality there is a multitude of various sized particles interacting one against the other. Hence, the concentrated force of the tooth is opposed by a distributed pushing force of a population of other particles.

The cutting operation is repeated a number of times by the pair of tools until the particles are reduced to sizes so small that the powder escapes through the adjustment gap exixsting between the grinding wheels. It is this adjustment gap that determines the overall fineness of the product.

4. Application of Forces on a Cellular Structure

Up to now, we have considered the coffee particle as a material point, on which the laws of kinematics act. But we have also seen that the real structure of roasted coffee is made up of cells.

In order to study the effect of the forces applied by the teeth on a cellular particle, another computer programme was used {10}.

This programme was designed to simulate load stresses in a complex structure (a mechanical part, a building) and to determine its deformation and failure.

The calculation method employed is called the "finite element method"{11}. It functions by breaking up a complex structure into a threedimensional lattice of elementary girders.

This is how it works: the natural cellular structure of roasted coffee has been reproduced by us into a regular geometric construction, consisting of a series of right hexagonal bicuspid prisms (fig. 10). Then the laws of statics, and precisely of construction science, were applied to this model.

Construction science studies real structures.

Let's consider the most classical of all: a girder supported by two bearings and subject to a concentrated load (fig. 11a).

The question is what the stress value and distribution are within the structure.

The conventional approach considers the structure as being unidimensional, with a uniform cross section, with weight force and vectorial reactions (fig. 11b).

If the structure is as simple as this one, the equation system for the calculation of strains is simple; otherwise, the problem becomes rapidly so intricate that it cannot be solved by the classical calculation.

An interesting alternative offered by the computer consists in considering the structure composed of a lattice of elementary girders (fig. 11c): then simple equations are applied to each of these girders.

The same process is repeated beam after beam and by considering also the adjacent elements till the state of strain and stress is determined.

The major advantage of this method lies in the fact that it allows to calculate a complex threedimensional structure by repeating, thanks to the capacity of the computer, the same simple equations instead of having to work out one single complicated equation, specific to each structure and nearly always irresolvable.

By means of computer graphics we may visualize the strain in the complex structure, and represent different stress values by different colours.

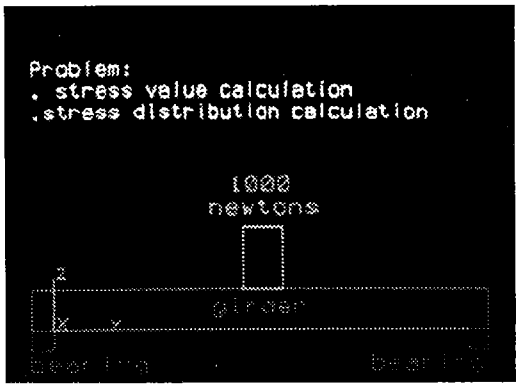

Fig. 11a Real girder

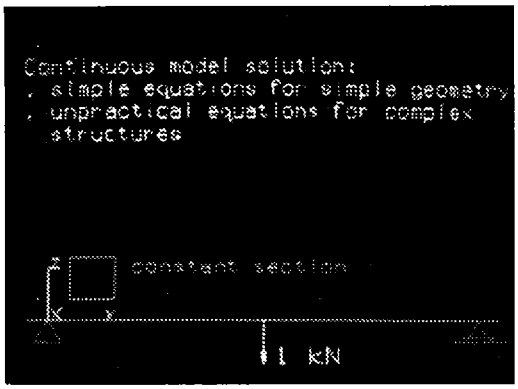

Fig. 11b Idealized girder

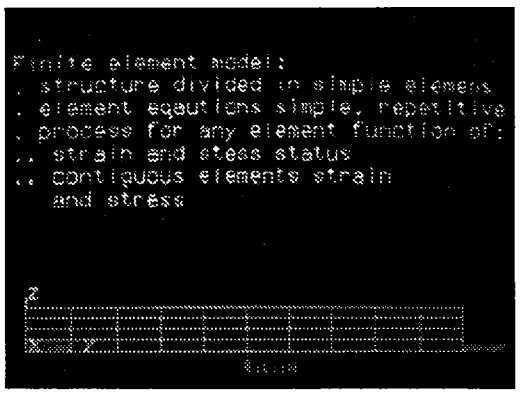

Fig. 11c Girder as reticular structure

Fig. 12a shows the structure not subject to load, while fig. 12b shows it loaded and already deformed.

Stress values are shown on the colour scale in the left part of fig. 12c: the more stressed points are coloured in yellow or red (we apologize for the loss in quality on this black and white reproduction).

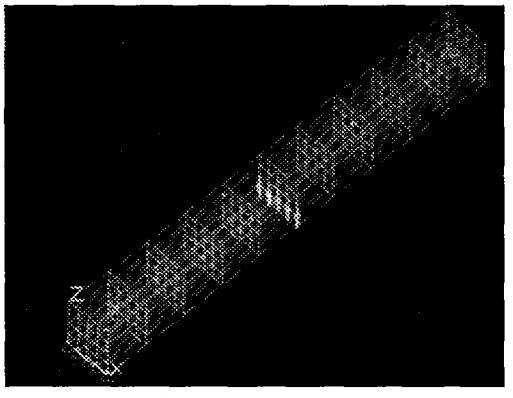

Fig. 12a 3-D reticular girder

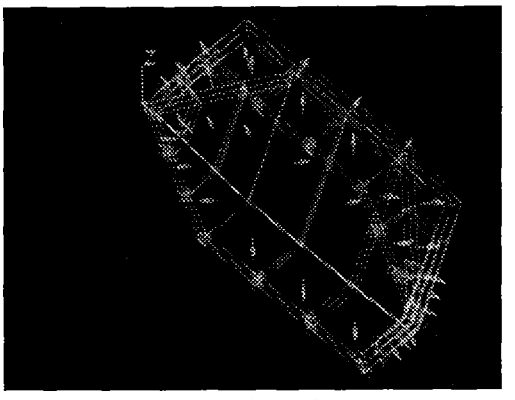

Fig. 13a Half coffee cell simulation

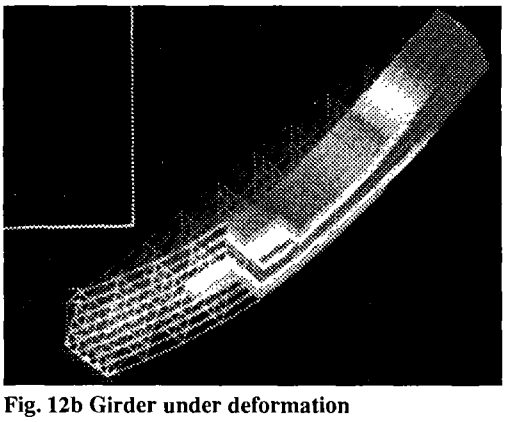

Fig. 12b Girder under deformation

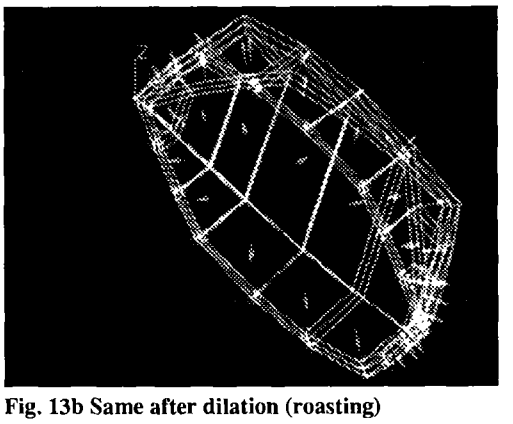

Fig. 13b Same after dilation (roasting)

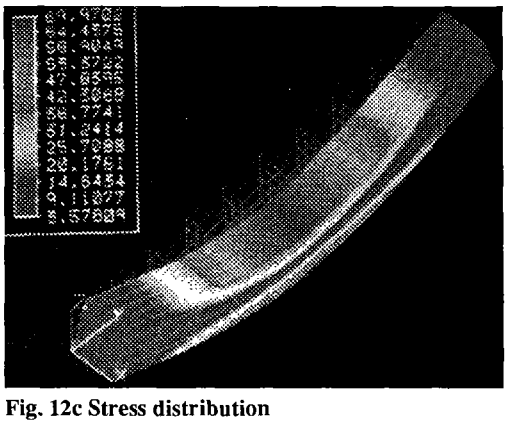

Fig. 12c Stress distribution

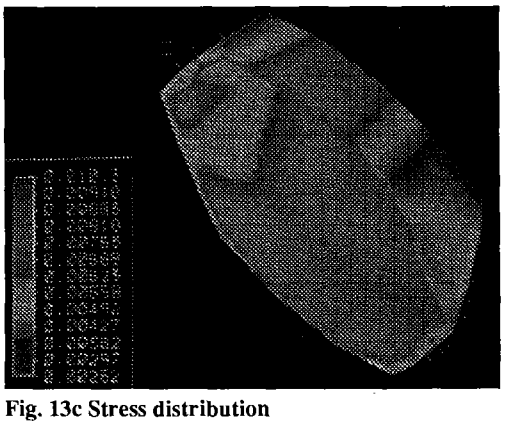

Fig. 13c Stress distribution

An example of this application is the effect of the internal pressure of roasting gases on an external cell wall of a ground coffee particle. Each wall has been simulated by a system of elementary girders (fig. 13a) and the pressure has been simulated by set of vectors acting on the reticular structure.

The effect is not simply an expansion (fig. 13b), which provides an explanation for the increase in specific volume on roasting, but also a state of internal stress of the wall, thus becoming friable (fig. 13c).

Another interesting aspect is the application of forces on a particle composed of a few dozen cells. Our programme is able to calculate a threedimensional structure, but in order to permit an easier visually intuitive understanding, we limited ourselves to working on a plane structure.

A concentrated force simulating the reaction of a tooth reacts to distributed forces. The unloaded structure (fig. 14a) undergoes deformation till it becomes a loaded structure.

The system of stresses is far from being uniform and reaches values of possible failure in well-defined points (fig. 14b).

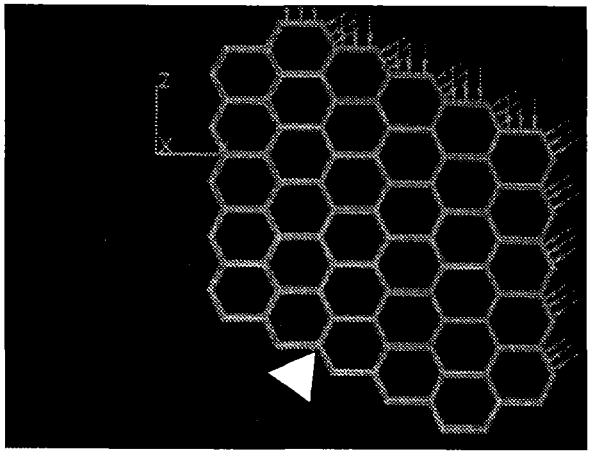

Fig. 14a 2-D coffee particle

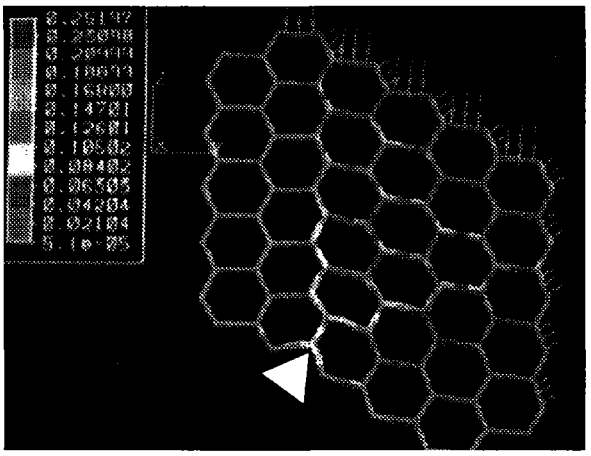

Fig. 14b Detail of stress distribution

The computer has not been programmed to cause the structure to fail, but it is possible to eliminate elementary girders that reach stresses capable of causing failure (figg. 15a and 15b) and to continue calculating the deformation of the "cracked" structure produced by these stresses.

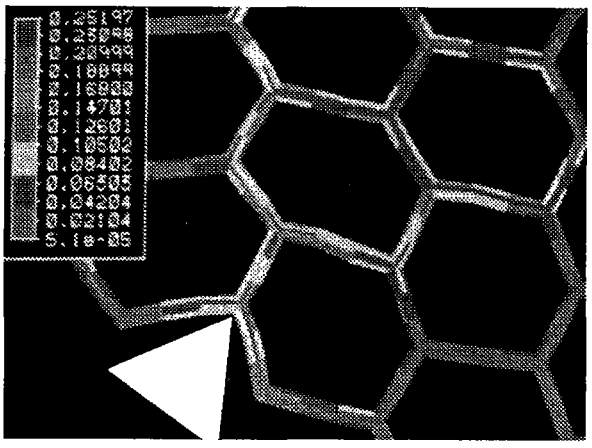

Fig. 15a Detail of stress distribution

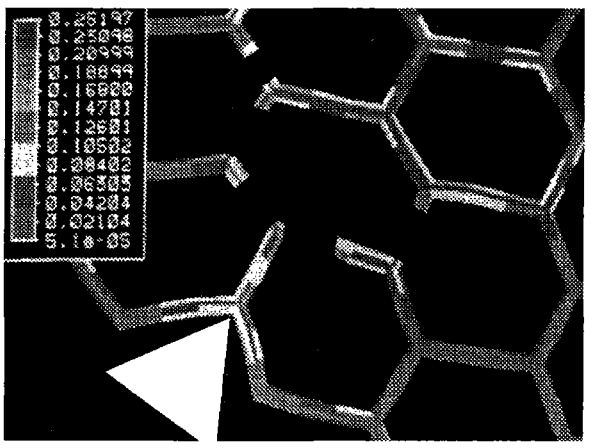

Fig. 15b Stressed girders removed

Fracture lines gradually begin to appear (fig. 16a).

Unlike what we may have expected, these lines do not divide the particle in a symmetrical way. By repeating this operation a number of times, several very small particles are formed, together with a very large one (fig. 16b).

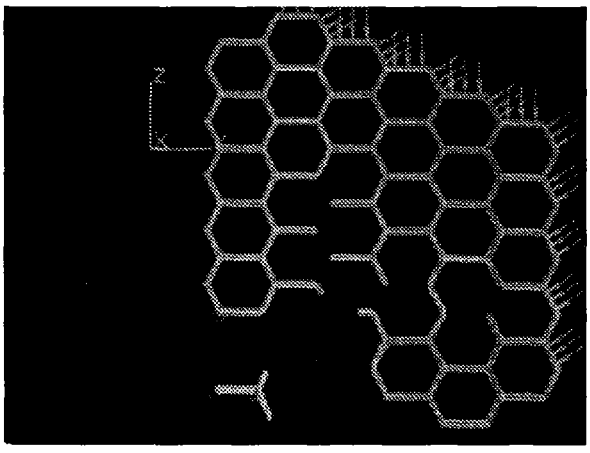

Fig. 16a Fracture lines pattern

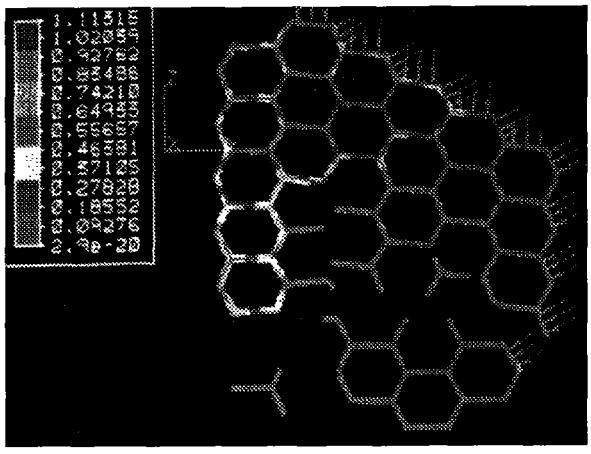

Fig. 16b Subparticles formation

5. Conclusions

The conclusion we feel entitled to draw from this simulation is that the model reproduces reality fairly well, as it reveals how the application of forces by the grinder's teeth cause the formation of a consistent quantity of fine powder.

This is corroborated by microscope examinations of ground powder and by laser scattering particle size analysis.

Even by practical experience espresso bartenders know very well how important the impalpable fraction with a smaller than 20 μm diameter is, in order to get a correct percolation.

A similar systematic approach should also be used in investigating a further complex phenomenon: the hydraulic one.

In the present work, we have limited ourselves to building a transparent percolation chamber under conditions of espresso brewing (fig.17a).

This chamber enables us to observe:
- hot water, as it reaches an soaks (fig. 17b) the cake of ground coffee,
- the pressure increase, further compacting the cake (fig. 17c)
- a first drop of coffee appearing: at first, it is transparent being a pure solution (fig. 17d), but the percolated liquid gradually grows more and more opaque because of the presence of emulsion (fig. 17e);
- at the end, foam appearing (fig. 17f).

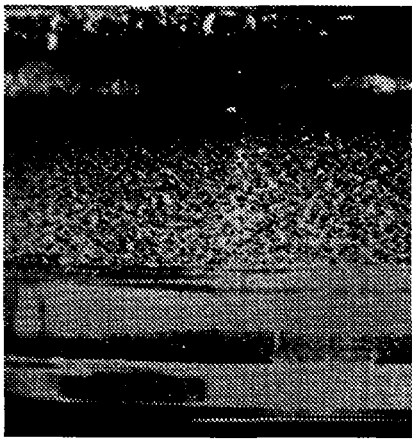

Fig. 17a Ground coffe cake reached by hot water

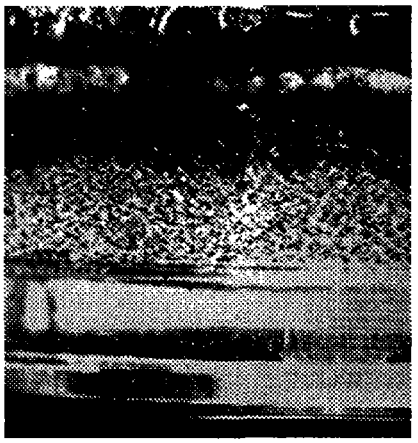

Fig. 17b Coffe powder imbibition

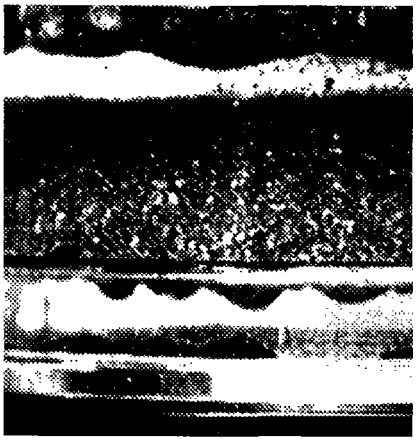

Fig. 17c Building up of hydraulic pressure

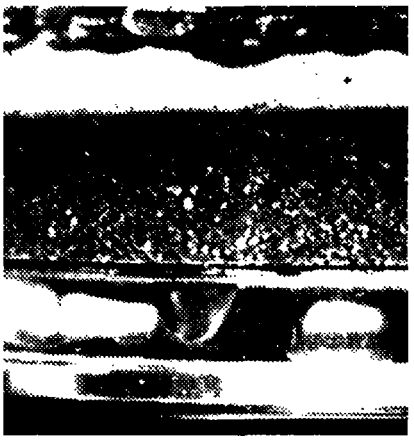

Fig. 17d Percolation of drops through metal filter

Fig. 17e Opacification of the percolating liquid

Fig. 17f Foam development

This one is also a highly interesting field of study:

- dynamics of fluid percolation in a bed of particles subject to physico chemical evolution.

We hope to explore this field in the near future.

6. Acknowledgements

The Authors are grateful to Dr. E. Dentan for the micrography of the green bean and to Dr. J. Heathcock for the realization of the micrography of ground coffee, and wish to thank Mr. L.Norbedo for directing the educational audiovisual based on the present scientific report.

7. Video Presentation

This article, as presented at the S. Francisco 1991 ASIC Conference, has been transfered into a VHS tape (PAL standard) and is available at cost price, both in english and italian version, writing to:

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Via Flavia 110 - 34147 TRIESTE - (Italy)
Phone ++39/40/89951 - Fax ++39/40/831110
att.n Dr. Marino Petracco

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COFFEE GRINDING DYNAMICS: A NEW APPROACH BY COMPUTER SIMULATION

The technological operation "comminution of roasted coffee beans" has been investigated with two different aims:

- to characterise the application of forces to the material to be ground by means of shearing tools
- to understand the inner mechanics of how structures made of cells break into fragments.

After the geometrical survey of a typical flange grinding tool, a CAD computer program has been used to reconstruct the primary topological lines and surfaces, and the movement of a punctiform particle inserted in such a system has been studied.

The inner cellular texture of the coffee bean has been then assimilated to a reticular structure of elementary girders, and submitted to the action of simulated forces: computing of stress and strain situation was performed by finite elements method.

The visualisation of the possible fragment sizes so produced suggests an explanation for the broad granulometrical spectra of the coffee powder normally obtained by industrial grinders.

DYNAMIQUE DE LA MOUTURE DU CAFE': UNE NOUVELLE APPROCHE PAR ORDINATEUR

Nous avons étudié le procédé technologique "comminution de grains de café torrefié" avec deux objectifs:

- caractériser l'application des forces, imposées par des outils coupants, sur le matériel à moudre
- comprendre la mécanique intime de la rupture en fragments de structures cellulaires.

Après relèvement géométrique d'un outil typique (meule dentée), on a utilisé un programme de CAD dans le but de reconstituer ses lignes et surfaces primaires, et on a étudié le mouvement d'une particule ponctuelle dans ce système.

On a ensuite considéré la texture cellulaire de l'intérieur du grain comme une structure réticulaire, constituée par des poutrelles élémentaires et soumise à des forces simulées: le calcul des efforts et des déformations a été fait par la méthode des éléments finis.

En visualisant les dimensions possibles des fragments ainsi produits, on peut suggérer une explication pour la large granulométrie des poudres de café communément obtenues par les moulins industriels.

KAFFEEMAHLENDYNAMIK: EIN NEUER ANNÄHERUNGVERSUCH MIT COMPUTERSIMULIEREN

Wir haben das technologische Verfahren "Zerkleinerung von Röstkaffee-Bohnen" zu zwei verschiedenen Zwecken untersucht:

- Charakterisieren des Kräfteaufbringens auf Materialien, die durch schneidende Werkzeuge gemahlen werden müssen
- Verständnis der innersten Mechanik des Zerstückelns von Zellstrukturen.

Nach der geometrischen Ermittlung eines typischen Zahnradwerkzeuges haben wir mit Hilfe eines CAD-Programms die Grundlinien und -fläche rekonstruiert. Auch die Bewegung eines punktförmigen Teilchens in diesem System wurde untersucht.

Wir haben später die Zelltextur der innersten Bohne mit einer von elementaren Balken gebildeten und mit Scheinkräften geladenen Netzstruktur verglichen: Beanspruchung und Verformung wurden mit der Finit-Element-Methode berechnet.

Die Veranschaulichung der so hergestellten möglichen Bruchstückgröße könnte eine Erklärung für die breite Partikelmeßverteilung liefern, die gewöhnlich von industriellen Mahlmaschinen erzeugt wird .

THE PHYSICAL PROPERTIES OF THE VOLATILE COMPOUNDS IN ROASTED COFFEE

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This paper concerns itself with the physical properties of the volatile compounds of roasted coffee and its beverages, which subject has not received as much attention as their organic chemistry. Physical properties are important, since they determine behaviour in processing, especially in instant coffee manufacture, and indeed in sensory analysis.

The two key solution physical properties of each volatile compound are,

1), relative volatility in aqueous solution taken at infinite dilution, α_j^{∞}
2), liquid molecular diffusivity or diffusion coefficient, D_j

Additional subscripts, 'w', 's', can be used to indicate whether the solution is just water or contains soluble solids. Values of α for a particular compound will depend on temperature within a limited range only to a small extent, and will be influenced by the concentration of coffee solubles present (i.e. whether low as in a cup of coffee, or higher as in coffee extracts in process), and in the case of basic/acidic volatile substances by the pH value of the solution reflected by their pK_a values. When considering coffee aroma concentrates, relative volatility values in pure water are usually the figures needed; infinite dilution is usually valid up to about 1000 ppm. Values for liquid diffusivity are of course, very strongly influenced by the viscosity of the solution in which they are present.

The potential uses of these particular physical properties in processing were first clearly considered and developed by the late Professor Thijssen (and colleagues) from 1965 onwards until nearly his untimely death in 1986. The main equations that have been derived, have been well described in the literature (1), (2), (3). The equation for percentage Extraction is not as yet clearly defined; i.e. %Extraction = Water/Coffee ratio x 1/ f (γ_j^{∞}). The activity coefficient γ_j^{∞} is a derivative of relative volatility as will be described shortly, though the beneficial effect of high water/coffee ratio has been demonstrated in practice by Pictet (4).

There are now believed to be over 700 different volatile compounds in roasted coffee, and in the beverages made from them (though differing in amount and number, according to the type of processing adopted). The determination of these two physical properties can be either preferably direct or predictive, as shown in table 1 below.

Table 1. DETERMINATION OF PHYSICAL PROPERTIES

Property	Direct Measurement	Predictive from
α_{j}^{∞} (dimensionless)	By GC for partition coefficient (air-water)	1) Vapour Pressure and saturation aqueous solubility 2) Pierrotti correlations
D_j ($m^2 s^{-1}$ units)	Various methods	Molal Volume and Wilke-Chang eqn.

It will be noted that prediction of relative volatility requires knowledge of two other dependent physical properties; vapour pressure of the pure compound and activity coefficient, the latter being best determined by simple measurements of saturation water solubility of the compound, (or solution of interest), both at the required temperature.

On examining the information available from the literature for each of the 700+ volatile compounds in roasted coffee, there appears to be no central compilation of such information, so that recourse is needed to a variety of sources. Whilst relative volatility at infinite dilution in either water or coffee solutions is determinable fairly easily by GC methods, values for only a few volatile compounds in coffee have actually been published; and these by Buttery and colleagues (5) (6) for some pyrazines, alcohols and unsaturated aldehydes. Boiling points (and therefore vapour pressures) are generally well documented, certainly at normal atmospheric pressure and usually at one other lower pressure, enabling prediction through log vp. versus 1/absolute temperature types of relationship at other required temperatures. Published data for saturation water solubility are however, surprisingly scarce, apart from qualitative statements, for all but the better know organic compounds. The reciprocal of the mole-fraction of the organic compound present in the solution at saturation gives the activity coefficient immediately, and so enabling calculation of relative volatility. Where the compound is completely miscible with water, this predictive route is, of course, not available, though can be if azeotropes exist.

In a previously published paper (3), the available information is tabulated for 1) the volatile compounds known present in roasted coffee at the highest amount, and for 2), a miscellany of those stated to be of especial flavor/aroma importance (e.g. furfuryl mercaptan, kahweofuran, etc.). In the present paper, information is tabulated for as many sulphur-containing substances as possible, in particular all the thiazoles and thiophenes so far identified as being present in roasted coffee. These compounds are considered to be of especial aroma/flavour importance, though generally present in exceedingly small quantities (justifying 'infinite dilution'), often a few ppb (parts per billion). There are 28 known different triazoles in coffee, and the information is listed in table 2 at the end of the paper; similarly for thiophenes in table 3, and for some various thiols and thioethers (sulphides) in table 4. A catalogue or accession number has been included (in this case from Macmillan's Dictionary of Organic Compounds, 1982, though no doubt supplements will issue); molecular formula and weight; boiling points at atmospheric pressure, and at one other pressure; saturation water solubility at a given temperature, qualitative and quantitative information where available. Compounds are listed in order of increasing molecular weight. Ideally, such listings would include values for relative volatility itself (either directly or predictively determined) at some useful temperature, say at 50°C for evaporative stripping/condensation of coffee extracts, or 20°C; and similarly for liquid diffusivity. It will be seen that relative volatility values at infinite dilution, in a homologous series of compounds, increase with molecular weight, surprising at first sight, since though the vapour pressure of the pure compounds will be decreasing (boiling point rising), their activity coefficients will be markedly increasing as a result of their increasing antipathy to water (decreasing solubility). For example, a predictive value for the relative volatility in water at infinite dilution of thiophene is obtained as follows; The saturation water solubility at 25°C has been given as 0.1% w/w, so that the activity

coefficient is given by the reciprocal of

$$\frac{0.1/84}{0.1/84 + 99.9/18}$$

which equals 4600. Since the vapour pressures of thiophene and water at the same temperature are 90 and 23.7 mm Hg respectively, the relative volatility is given by

$$4600 \times 90/23.7 = 17,468$$

Such a high figure is not unusual for a very poorly soluble substance. In the same way, thiophene with a simply calculated molal volume from Le Bas figures of 88 indicates a liquid diffusion coefficient of 1.05×10^{-11} $m^2 s^{-1}$

It is a moot point as to whether the column for relative volatility in a table would be most conveniently completed by a research student, taking GC measurements or solubility measurements. Mass spectra information needed or used for identification in GC/MS work is in contrast, well published.

Finally, such considerations are equally applicable to the wines and spirits industry, and similarly in respect of the orange juice industry.

Reference

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(2). Clarke (R.J.). Chapters 5 and 6 in Coffee, volume 2 (Technology), Ed. by R.J. Clarke and R. Macrae, Elsevier Applied Science Publishers (Barking), 1987, p. 109-199.

(3). Clarke (R.J.). Café Cacao Thé, vol.XXXIV, (4), 1990, p.285-294.

(4). Pictet (G.). Chapter 8 in Coffee, Volume 2 (Technology) as above, p.221-256).

(5). Buttery (R.G.), Ling (L.C.) and Guadagni (D.G.). J. Ag. Food Chemistry, vol.17, 1969, p. 385-389.

(6). Buttery (R.G.), Bomben (J.L.), Guadagni (D.G.) and Ling (L.C.). J. Ag. and Food Chemistry, vol.19, 1971, p. 1045-1048.

Summary

The physico-chemical characteristics of the 700+ known volatile compounds in roasted coffee have not been so well investigated as their organic chemistry. Indeed much data is lacking and no centralized compilations appear available. This paper emphasises their importance in processing operations involved in instant coffee manufacture (extraction, evaporation, and spray-drying). GC techniques and sensory analysis. Compilations of some key physical properties are presented for all the thiophenes, thiazoles and many other substances of aroma/flavour interest.

Résumé

Les caractéristiques physiques-chimiques des 700+ composés volatils identifiées en café torrefié, n'ont eu pas aussi bien étudiés que leur chimie organique. En effet, beaucoup des données est manquées; pas des compilations centralisées paraissent être disponsibles. Cet exposé souligne leur importance dans les traitements comportés en industrie de café soluble (extraction, evaporation, séchage), les techniques GC et l'analyse sensorielle. Des compilations de quelques propriétés physiques importantes sont presentés pour tous les thiophenes, thiazoles et plusieurs des autres composés d'interêt arome/flaveur.

TABLE 2. PHYSICAL PROPERTIES OF THIAZOLE AND DERIVATIVES FOUND IN ROASTED COFFEE VOLATILES

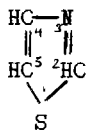
DOC No.	SUBSTANCE NAME	MOLECULAR Formula	Weight	BOILING POINT 1) at 760mm 2) other °C	WATER SOLUBILITY (Satn.) Qual./Quant.
					
J01911	THIAZOLE	C_3H_3NS	85	118.2 61/100	Miscible Azeotrope
	Alkyl Derivatives (24)				
M03696	2-methyl-	C_4H_5NS	99	129 65-70/80	
M03697	4-methyl-	ditto	ditto	130-3 70/59	
M03698	5-methyl-	ditto	ditto	141 57/32	
-	2-ethyl-	C_5H_7NS	113	158 67/46	
-	4-ethyl-	ditto	ditto	145 -	
-	5-ethyl-	ditto	ditto	162 100/100	
D07132	2,4-dimethyl-	ditto	ditto	150 72/50	
D07133	2,5-dimethyl-	ditto	ditto	151-3 86/80	
D07134	4,5-dimethyl-	ditto	ditto	158 75/47	
-	2n-propyl-	C_6H_9NS	127	172 -	
E01082	2-methyl-4 ethyl-	ditto	ditto	- 167-9/719	
-	2-methyl-5 ethyl-	ditto	ditto	- 108/100	
E01083	4-methyl-5 ethyl-	ditto	ditto	173-4/755 78-9/25	
E01081	4-methyl-2 ethyl-	ditto	ditto	160/729 116/173	
-	5-methyl-4 ethyl-	ditto	ditto	- 169/70 44-6/1	
T04135	2,4,5- trimethyl-	ditto	ditto	164-6 64-6/20	

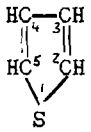
TABLE 2 (Continued) Alkyl Thiazole Derivatives

-	4n-butyl-	$C_7H_{11}NS$	141	-
-	2,4-diethyl-	ditto	ditto	-
-	2,5-diethyl-	ditto	ditto	-
-	2,4-dimethyl- 5-ethyl-	ditto	ditto	123/100 185 -
-	2,5-dimethyl- 4-ethyl-	ditto	ditto	- 32/11
-	4,5-dimethyl- 2-ethyl-	ditto	ditto	- -
-	4,5- dimethyl- 2i-propyl-	$C_8H_{13}NS$	155	- -
-	5-ethyl- 2n-propyl-	ditto	ditto	- -
	Acyl thiazoles			
-	2-acetyl-	C_6H_5NOS	128	- -
-	2-acetyl- 4-methyl-	C_7H_7NOS	142	- -
-	2-propionyl- 4-methyl-	C_8H_9NOS	156	- -
	Benzotriazoles			
B00549	Benzotriazole	C_7H_5NS	135	231 131/34

Notes. (1) Accession numbers (DOC) taken from Macmillan's Dictionary of Organic Compounds, 5th.Edn. with supplements 1982, Chapman and Hall (London); together with boiling point and other data as available. Other sources of information have been the CRC Handbook of Data on Organic Compounds, 2nd Edn. 1985 by R.C.Weast and J.G.Graselli and Perry's Chemical Engineer's Handbook, 5th.Edn. 1985, McGraw-Hill (New York). There is specific information on Thiazole and its derivatives by Metzger in Vol. 34 of Heterocyclic Compounds, 1979, Interscience-Wiley (New York).

(2) Listing of Compounds taken from Van Straten, S., Maarse, M., and Visscher, C.A., 1986, Volatile Compounds in Foodstuffs. Coffee: Qualitative Data, T.N.O., Zeist, The Netherlands. Also Silwar, R., Kampschroer, H. and Tressl, R., Chem. Mikrobiol, Technol. Lebensm., 1987, 10, 176-187; and Silwar, R., Bendig, I, Walter, G., and Dommers, D., Lebensmittelchem. Gerichtl. Chem., 1986, 40, 84-88.

TABLE 3. PHYSICAL PROPERTIES OF THIOPHENE AND DERIVATIVES FOUND IN ROASTED COFFEE VOLATILES

DOC No.	SUBSTANCE NAME	MOLECULAR Formula	Weight	BOILING POINT 1)at 760mm 2)other °C	WATER SOLUBILITY (Satn.) Qual./Quant.
					
T020603	THIOPHENE	C_4H_4S	84	84 50/234	sl.sol 0.1%w/w
	Alkyl Derivatives (6)				
M03746	2-methyl-	C_5H_6S	98	110-2 9/10	
M03747	3-methyl-	ditto	ditto	115 11/10	
V0023	3-vinyl	C_6H_6S	110	- -	
P0527	2n-propyl-	$C_7H_{10}S$	126	157-60 55/20	
E01086	2-methyl-4-ethyl-	ditto	ditto	162-4 67/30	
-	2-butyl-	$C_8H_{12}S$	140	181-2 -	
	Bi-				
T01966	thiopheno (2,3,b)-	$C_6H_4S_2$	140	- 75-80/1	
	Ketones(4), Acyl Derivatives(9) and Diones(2)				
-	thiophene-3-one	C_4H_4OS	100	- -	
-	tetrahydro2-one	C_4H_6OS	102	- -	
-	tetrahydro-3-one	ditto	ditto	- -	
-	2-methyl-tetrahydro-3-one	C_5H_8OS	116	- -	
A00424	2-acetyl-	C_6H_6OS	126	213 96/13	
A00425	3-acetyl-	ditto	ditto	208/748 78-9/5	
-	2-acetyl-3-methyl-	C_7H_8OS	140	- 74/4	
-	2-acetyl-4-methyl-	ditto	ditto	- 85/4	

A00355	2-acetyl-5-methyl-	ditto	ditto	232-3/ 98-100/3
P02431	2-propionyl-	ditto	ditto	- 100-1/11
-	1-(thienyl-2) propane-1,2-dione	$C_7H_6O_2S$	154	- -
-	1-(thienyl-3) propane-1,2-dione	ditto	ditto	- -
	Aldehydes(2) and Alcohols(1)			
T02066	2-formyl-	C_5H_4OS	112	- 93-4/20 44-5/1
-	2-formyl-3-methyl-	C_6H_6OS	126	- -
M02642	2-hydroxymethyl-	C_5H_6OS	114	- 95-6/12
	Esters(3)			
-	thenyl formate	$C_6H_6O_2S$	142	- -
M03750	methyl 2-thiophene carboxlate	ditto	ditto	- -
-	thenyl acetate	$C_7H_8O_2S$	152	- -
	Others(3)			
D03950	kahweofuran (2-methyl-3-oxa-thiabicyclo (3,3,0)octa-1,4-diene)	C_7H_8OS	140	- -
-	4-methyl-kahweofuran	$C_8H_{10}OS$		- -
B00576	benzothiophene	C_8H_6S	134	221 103-5/20

Notes.(1) Accession numbers (DOC) taken from Macmillan's Dictionary of Organic Compounds, 5th Edn. with supplements (1-7) 1982, Chapman and Hall (London); together with boiling point and other data as available. Other sources of information have been also as in the footnote to Table 2, except that specific information on Thiophene and its derivatives is available in Volume of Heterocyclic Compounds, 1984, Interscience-Wiley (New York).
(2) Listing of Compounds from source as in Table 2.

TABLE 4. PHYSICAL PROERTIES OF MISCELLANEOUS ORGANIC SULPHUR VOLATILES IN ROASTED COFFEE

DOC No.	SUBSTANCE NAME -S- -S-S- -S-S-S-	MOLECULAR Formula	Weight	BOILING POINT 1) at760mm 2) other °C	WATER SOLUBILIIY (satn.) Qual./Quant.
	Alkyl thiols				
M00472	methane thiol [methyl mercaptan]	CH_4S	48	5.9 -	sl. sol 2.2%w/w
E00500	ethane thiol	C_2H_6S	62	35 -	sl.sol 1.5%w/w
P02365	1-propane thiol	C_3H_8S	76	68	-
	Alkyl-furyl thiols				
F00829	(furyl-2)methane thiol [furfuryl mercaptan]	C_5H_6S	114	160 84/65	-
-	(5-methyl-furyl-2) methane thiol	C_6H_8S	128	- -	-
	Alkyl thioethers - sulphides				
D07117	methylthiomethane [dimethyl sulphide]	C_2H_6S	62	38 -	-
E01079	methylthioethane [methylethyl sulphide]	C_3H_8S	76	67 -	-
D06100	methyldithiomethane [dimethyl disulphide]	$C_2H_6S_2$	94	110 -	-
-	methyldithioethane	$C_3H_8S_2$	108	- -	-
D03363	ethyldithioethane	$C_4H_{10}S_2$	122	154 46/11	-
D10617	methyltrithiomethane [dimethyl trisulphide]	$C_2H_6S_3$	126	165-170 -	-
-	methyltrithioethane	$C_3H_8S_3$	140	- -	-
	Alkyl-furyl thioethers(15)				
F00829	2-(methylthiomethyl) furan	C_6H_8OS	128	- 62-3/28	-
	Insufficient physical property data on remaining compounds.				

Notes. See notes (1)(2) after tables 2 and 3.

ACID HYDROLYSIS OF SPENT COFFEE GROUNDS TO PRODUCE D-MANNOSE AND D-MANNITOL

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INTRODUCTION AND BACKGROUND:

Spent coffee grounds are the major waste product generated in the soluble coffee process. From about 35 to 60% and typically 45 to 50% of the starting weight of roasted coffee is usually extracted by hot water in multi-column percolators. Based on these yields, about 0.8 to 1.9 lbs. of spent grounds on a dry basis are generated per lb. of soluble coffee produced. The amount of spent grounds per manufacturing site can be in multi-million pound quantities per year.

The subject of spent coffee grounds disposal was investigated by M. Bond (1) and reviewed by R. Pfluger (2). Disposal has included sewer discharge, sanitary landfill, and incineration. In some regions of the world, spent grounds have been used as an additive to cattle feed. In the U.S. spent grounds have been burned to recover some of the fuel value present in the oil fraction. According to Pfluger the fuel value of 40 lb. grounds at 50% moisture is equivalent to 1 gal of #6 fuel oil. The additional investment and operating costs needed in energy recovery becomes greatly favored when the costs of trucking and dumping increase dramatically as available dump sites decrease. Various applications for the utilization of waste coffee grounds have been proposed or examined and most of these have proven uneconomic or impractical.

This paper suggests, based on the chemistry of commercially extracted spent grounds, that this waste should be considered a chemical feedstock for the production of higher value products specifically D-mannose and upon reduction: D-mannitol (3). D-Mannitol, a specialty chemical, has numerous food, pharmaceutical and non-food applications and has a retail value of about $3.00/lb for the U.S.P. crystalline grade.

The relatively high price for D-mannitol reflects the combined raw material

and processing costs involved in its manufacture. Starting with invert sugar, hydrogenation produces a mixture of about 26% mannitol and 74% sorbitol but the yield of crystalline mannitol is only about 17%. The remaining 9% mannitol is difficult to recover from the sorbitol liquor (4).

Numerous technolgical attempts have been made to increase the yield efficiencies. Makkee et al (5) reviewed various production schemes in the literature based on D-glucose or D-glucose\D-fructose mixtures as starting materials. They proposed a combination process using glucose isomerase and hydrogenation with copper on silica catalyst to produce syrups containing up to 65% mannitol and 35% sorbitol starting from glucose or invert sugar (6). The enzyme, however, must be protected against traces of copper ion by addition of the chelating agent EDTA.

Another approach is to generate high mannose feedstocks. The reduction of mannose produces mannitol quantitatively and would result in significantly greater yields upon crystallization.

Casebier et al. (7) separated mannose from other sugars in the liquor of wood pulp hydrolysates by selective crystallization of the mannose bisulfite adduct followed by regeneration of mannose. This process generates large amounts of chemical waste. Sherman and Chao (4) separated mannose from a mixture of sugars (e.g pulp hydrolysates) at up to 70% purity using absorbtion on zeolitic molecular sieves. Saittagaroon et al. (8) generated an acid hydrolysate from defatted copra meal which upon reduction contained 66% mannitol, 24% sorbitol, and 5 % galactitol.

Wolfrom and co-workers (9,10) and Bradbury and co-workers (11,12) examined the polysaccharide fraction in green coffee beans. Both Robusta and Arabica contain a mannan (about 22% d.b.), an arabinogalactan (ranging from about 14-18%), and cellulose (ranging from about 7-9%). The mannan in coffee was confirmed by Bradbury to be a linear beta 1-4 linked polymer similar to cellulose but of lower mol. wt., ranging from about 20 to 40 mannose units. Thaler (13) studied the polysaccharide chemistry of coffee extracts prepared in the pilot plant with increasing degrees of extraction. He demonstrated that large amounts of galactan and mannan and small amounts of arabanan and glucan were extracted with increasing yield.

An approximate composition of the polymeric carbohydrate in a roasted coffee blend and spent grounds after commercial percolation (e.g. 45-50% extracted) is given in Table 1 below (3):

TABLE 1

Approximate Polymeric Carbohydrate Composition (Dry Basis)

Component	% by Wt. R&G Coffee	% by Wt. Spent Grounds
Cellulose	8%	15%
Mannan	20%	25%
Arabinogalactan	13%	5%
Total polymer	41%	45%

As can be seen from the above data, commercial spent grounds contain large amounts of cellulose and mannan and smaller amounts of arabinogalactan.

Grethlein (14) published on the conversion of cellulosic material (e.g. paper) in municipal waste to glucose via high temperature short time acid hydrolysis. The development of a continuous plug flow reactor (CPFR) by Grethlein and Thompson (15) at Darmouth College demonstrated the feasibility of this approach. This technology was deemed appropriate for evaluation in acid hydrolysis of spent coffee grounds.

EXPERIMENTAL METHODS:

The initial kinetic data for hydrolysis reaction was generated using a CPFR in which an acidified slurry of milled coffee grounds (0.8 mm or below) was rapidly heated by the direct introduction of high pressure steam into the reactor and quenched by rapid cooling upon flashing the slurry through an orifice back to atmospheric pressure. Concentrated sulfuric acid was injected into the reactor just prior to heating. Residence time in the reactor was calculated based on the mass flow rate (g/min.) of the slurry and the reactor volume. A known wt. of slurry was titrated with standardized sodium hydroxide to pH 7 to determine acid concentration and the amount of salt generated upon neutralization. The slurry was filtered and the hydrolytic conversion was based on the level of soluble and insoluble solids determined upon drying at 105C, before and after hydrolysis. Soluble solids in the filtrate were corrected for salt.

Sugars in the hydrolysate were analyzed in a separate sample of filtrate, after precipitating the sulfate using barium carbonate, using high performance liquid chromatography (HPLC); Altex R.I. detector; Aminex HP-95P carbohydrate column (Biorad Cat. #125-0098) at 80C, solvent: water at 0.5 ml/min. Oligosaccharides were analyzed using HPLC: R.I. detector, Waters Carbohydrate Analysis Column (part number 84038), solvent: acetonitrile/ water (70:30 v/v) with flow rate of 2 ml/min. (ambient temperature).

The potential sugar yield from the spent grounds was determined by the quantitative saccharification method of Saeman using acid hydrolysis (16) and analysis by HPLC for the sugars produced as above.

RESULTS AND DISCUSSION:

Hydrolysis Kinetics:

The data generated with 1% sulfuric acid is given in Table 2 below. The mannose and glucose yields (dry basis spent grounds) were converted to fractional conversion by dividing by maximum yield based on quantitative saccharification.

TABLE 2

EXPERIMENTAL KINETICS DATA– 1% SULFURIC ACID

Temp.(C)	Time (sec)	% Acid (H_2SO_4)	%Mannose Yield	% Conv. Mannan	%Glucose Yield	% Conv. Cellulose
160	8.6	1.1	1.7	5.4	0.1	0.6
160	9.6	1.3	1.3	4.3	0.1	0.5
160	31.2	1.1	4.1	13.2	0.1	0.8
180	8.2	1.1	8.4	27.1	0.3	1.5
180	8.2	0.9	13.4	43.5	0.8	4.7
190	8.0	1.0	18.5	60.0	0.5	2.5
200	7.7	0.9	24.2	78.5	1.7	9.5
200	7.8	1.0	25.1	81.3	0.9	4.8
200	9.0	1.1	28.1	91.1	0.9	4.8
200	19.2	1.0	25.9	84.2	2.2	12.4
200	19.2	1.0	26.4	85.7	2.1	11.8
200	19.2	1.0	25.5	82.8	2.5	13.9
200	8.0	0.9	28.7	93.2	1.9	10.7
200	9.1	1.2	29.8	96.8	1.7	9.3
207	16.4	1.4	30.6	99.4	2.6	14.6
210	7.5	0.9	25.0	81.2	1.6	9.0
220	7.3	0.9	24.2	78.6	2.7	15.0
240	6.7	0.8	22.0	71.3	7.0	39.0
240	7.2	0.9	19.0	61.5	7.9	43.9
240	7.4	0.7	20.1	65.3	11.6	64.7

The maximum mannose yield was 30.8% and the maximum glucose yield was 17.9% for the grounds examined in this study. Data generated with limiting amounts of sulfuric acid are given in Table 3. This data demonstrated that mannan oligosaccharides were generated in the first stages of hydrolysis.

Table 3

Oligosaccharides Generation by Limiting Acid Concentration

Acid level Wt. %	Temp. (C)	Time sec	Distribution of Manno-saccharides (%) by area DP1	DP2	DP3	DP4	DP5	DP6	DP7	DP8	DP9
0.25	200	8	51.8	20.9	13.5	8.3	2.6	1.4	0.7	0.7	--
0.10	220	8	36.3	23.0	15.4	10.2	6.7	4.3	2.7	1.3	--
0.05	220	8	14.5	15.8	15.7	14.3	13.1	11.5	8.2	4.8	2.3
0.025	220	8	12.6	14.0	14.9	14.7	14.3	12.7	8.9	4.7	3.3

The reaction kinetics for mannan hydrolysis is a "psuedo" first order consecutive reaction similar to cellulose (14):

Cellulose --> Glucose --> Degradation Products

For Mannan the true reaction is:

Mannan Polymer--> Mannan Oligosaccharides--> Mannose--> Degradation Prod.

However, at higher temperatures and excess acid this is simplified to:

Mannan Polymer --> Mannose --> Degradation Products

The concentrations of reactants and products are given by the following equations (17).

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

$$A = A_o e^{-k_1 t}$$

$$B = k_1 A_o [e^{-k_1 t} - e^{-k_2 t}]/(k_2 - k_1)$$

$$C = A_o [1 + (k_2 e^{-k_1 t} - k_1 e^{-k_2 t})/(k_1 - k_2)]$$

At maximum B, $t = \ln [(k_1/k_2)]/ (k_1 - k_2)$

Rate constants (k's) are in the form $k_i = K_i (Ac)^{n_i} e^{-E_i/RT}$

where: K = constant (min^{-1}), E = activation energy
A_c = acid concentration (wt. %), R = gas law constant
n^c = exponent for acid concentration, T = absolute temperature
t = residence time (min.)
A = the fraction of polymer available for hydrolysis
B = the fraction of polymer converted to sugar
C = the fraction of sugar decomposed

By applying non-linear least squares curve fitting to our experimental data for 1% acid, kinetic constants were calculated for the mannan hydrolysis reaction, assuming an Arrehnius form of the rate constant.

The results shown in Table 4 are similar to those published by Grethlein and Thompson (15), except that insufficient data were available to estimate the effect of acid concentration on the rate constants, therefore these

kinetic constants were determined only for 1% sulfuric acid $(A_c)^{n_i=1}$. Comparing the kinetic constants for mannan hydrolysis at 1% sulfuric acid with those for cellulose hydrolysis (as reported by Grethlein and Thompson), we see that the activation energies (E_2) and the pre-exponential constants (K_2) for glucose and mannose degradation are quite similar, and calculation of the rate constants show that these two sugars degrade at similar rates. See Table 6.

Table 4 Kinetic model Parameters

Parameter	Mannan (this study)	Cellulose (Grethlein/Thompson)
K_1 (gener.)	$1.7 \times 10^{25}(min^{-1})$	$1.2 \times 10^{19}(min^{-1})$
K_2 (degrad.)	$2.4 \times 10^{14}(min^{-1})$	$3.8 \times 10^{14}(min^{-1})$
E_1 (gener.)	51667 cal/gm mole	42500 cal/gm mole
E_2 (degrad.)	32352 cal/gm mole	32700 cal/gm mole

The hydrolysis of mannan however is significantly faster than that of cellulose. The activation energy (E_1) is about 20% higher for mannan conversion than for cellulose conversion but the pre-exponential factor (K_1) is 1 million times greater. This leads to reaction rates for mannose generation which are 50-400 times more rapid than for glucose generation. The significance of this finding is that, under the right conditions a virtually pure mannose hydrolysate can be obtained since the majority of the mannan can be hydrolyzed with very little concurrent hydrolysis of cellulose to glucose. See Table 5 for hydrolysate sugar composition.

Table 5

Relative Sugar Composition of Selected Hydrolysates

Temperature	190 C	200 C	200 C	210 C
Wt. % sulfuric	1.0	0.5	1.0	0.5
Residence (sec.)	8.0	7.6	7.8	7.4
%Sugar Yield (d.b)	19.5	23.0	26.5	27.2
% Cellobiose	0.3	0.5	0.5	0.7
% Glucose	2.3	2.0	3.2	3.1
% Xylose	1.1	1.1	1.1	1.3
% Galactose	1.3	1.2	0.7	0.7
% Mannose	95.0	95.3	91.5	94.2
% Total	100	100	97	100

Our limited data suggest that the cellulose in coffee grounds behaves similarly to the cellulose in wood as the reaction kinetics reported by Grethlein for wood pulp predict coffee cellulose hydrolysis reasonably well.

Figure 1 is a plot of the experimental data for conversion of cellulose and mannan at 200 C and 1% sulfuric acid. Figure 2 is a plot of the predicted conversion for the mannan in spent coffee grounds at 1% sulfuric acid based on the mathematical model. Table 6 gives the predictions of the model for both mannan and cellulose [(Grethlein/Thompson (15)] with 1% sulfuric acid.

The kinetic model described by Grethlein (14) assumes that the hydrolysis of amorphous cellulose and hemicellulose (e.g. xylan) to sugars is almost instantaneous above 180 C. BeMiller (18) in a review article on acid hydrolysis of starch describes studies by Rogovin and Konkin et al. who found that "the hydrolysis of mannan was 2-2.5 times that of cellulose in homogeneous medium and 60 times times greater in heterogeneous hydrolysis."

Figure 1

Conversion of Cellulose and Mannan
200°C - 1% Sulfuric Acid

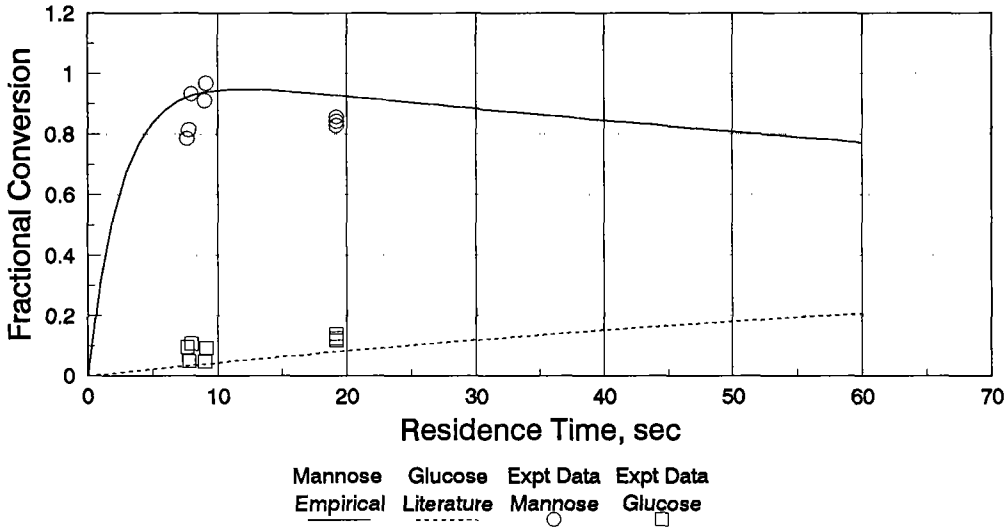


Figure 2

Mannose Yield - 1% Sulfuric Acid

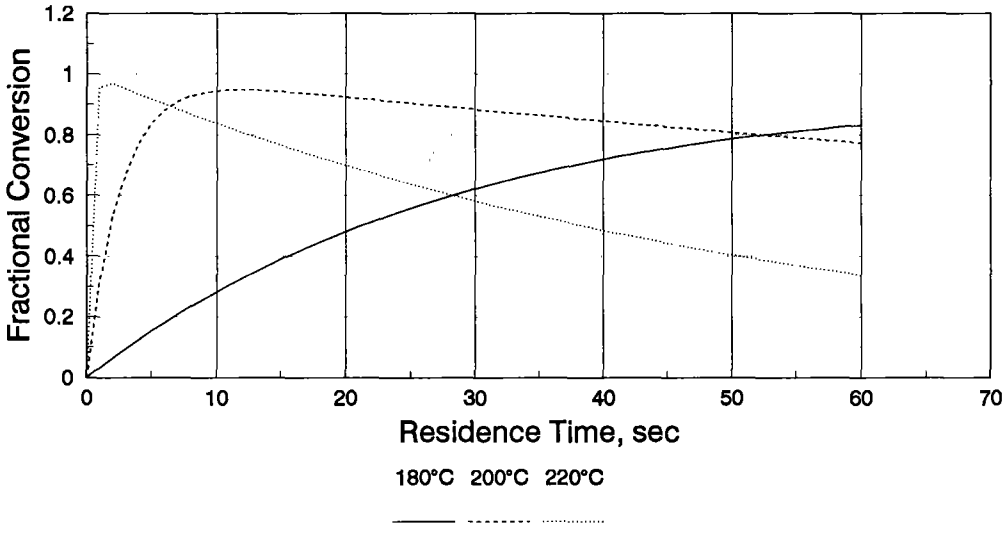


Table 6

Predictions of Mathematical Model for the Hydrolysis of Cellulose and Mannan with 1% Sulfuric Acid

Temp. C	Mannan to Mannose Max. Conv.	Mannan to Mannose Time to Max Conv.	Cellulose to Glucose Max. Conv.	Cellulose to Glucose Time to Max Conv.
180	0.898	1.8 min.	0.285	20.5 min.
200	0.949	11.7 sec.	0.361	3.5 min.
220	0.973	1.5 sec.	0.444	41 sec.
240	0.986	0.2 sec.	0.519	9 sec.

Rate Constants (min^{-1})

Temp. C	Mannose Generation	Mannose Degradation	Glucose Generation	Glucose Degradation
180	2.0×10^{0}	5.9×10^{-2}	3.8×10^{-2}	6.1×10^{-2}
200	2.3×10^{1}	2.7×10^{-1}	2.8×10^{-1}	2.9×10^{-1}
220	2.1×10^{2}	1.1×10^{0}	1.8×10^{0}	1.2×10^{0}
240	1.7×10^{3}	3.9×10^{0}	0.5×10^{0}	4.3×10^{0}

The data from this study suggest that the mannan in spent coffee grounds is not amorphous because its hydrolysis follows a kinetic model similar to that of crystalline cellulose. Ivory nut mannan like coffee mannnan is a beta 1-4 linked polymer with a similar molecular weight distribution: two fractions of 10 and 40 Dp and has been compared to cellulose because of their similar chemical structure and formation of crystalline polymorphs. Chanzy et al (19) examined the electron difractograms of laminar single crystals of mannan. They found that cellulose microfibrils will act as nucleation sites for the crystallization of ivory nut mannan resulting in a "shish-kebab" morphology. So although we have not independently confirmed its crystalline structure in roasted coffee, it is reasonable that it is similar to ivory nut mannan in this regard.

Process Description:

A process flow sheet for the pilot plant production of mannose from spent coffee grounds is shown in Figure 3. The key elements of the process are: slurry preparation and acidification, reaction in a plug flow tubular reactor (at the desired time and temperature), reaction quenching by flashing to atmospheric pressure, and slurry neutralization with calcium hydroxide, followed by hydrolysate separation and concentration of the mannose syrup.

The reduction of mannose to mannitol is a conventional industrial process. Figure 4 is a flow sheet for a continuous process described by Kasehagen (20). A catalyst comprising of reduced nickel supported on kieselguhr is added to the mannose syrup at a level of 2% nickel basis sugar to form a slurry which is then passed upward into a tubular reactor under a pressure of 1600 psi and temperature of 150C. Hydrogen gas is fed into the reactor at a flow rate of 10 times that of the slurry. The residence of the slurry in the reactor is 45 to 90 minutes. The unreacted hydrogen gas is separated near the end of the reactor and recycled with make up gas into the entrance of the reactor. The slurry after hydrogenation is filtered free of the catalyst; the filtrate is then demineralized using cation and anion exchange resins in sequence and the mannitol crystallized.

The feasibility of preparing mannitol was demonstrated by reducing a small sample of the 190 C hydrolysate in Table 5 using the sodium borohydride method of Bradbury et al (21). The product was crystallized once, washed with ethanol, and analyzed as 97.5% mannitol by gas chromatography.

Figure 3

Flowsheet for the Production of Mannose Hydrolysate Syrup

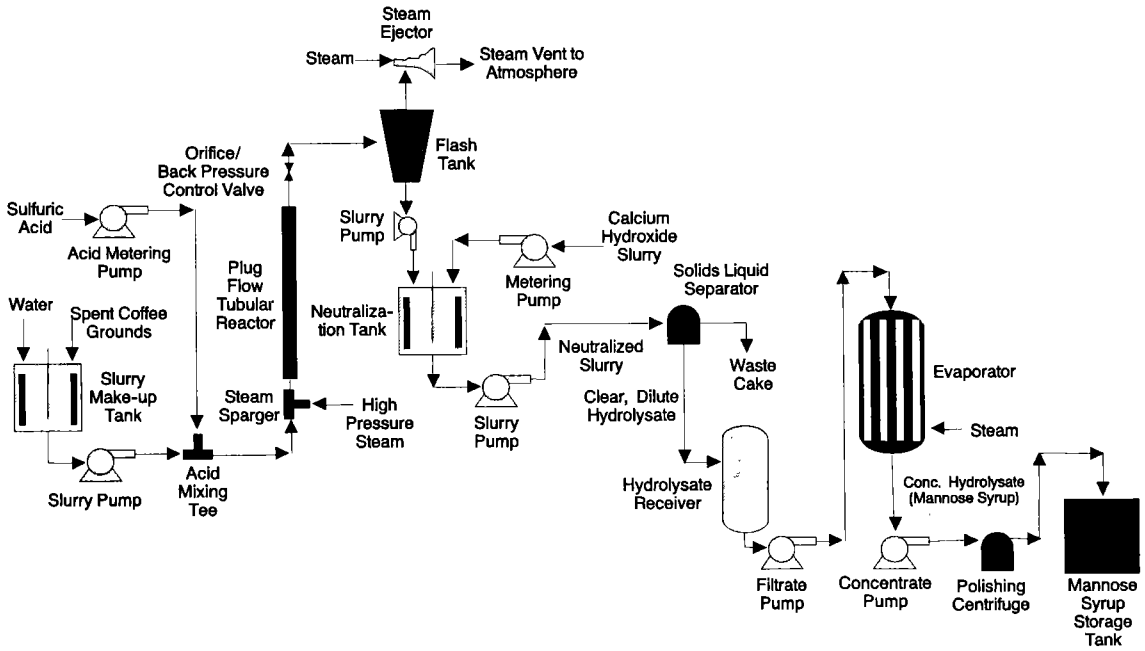
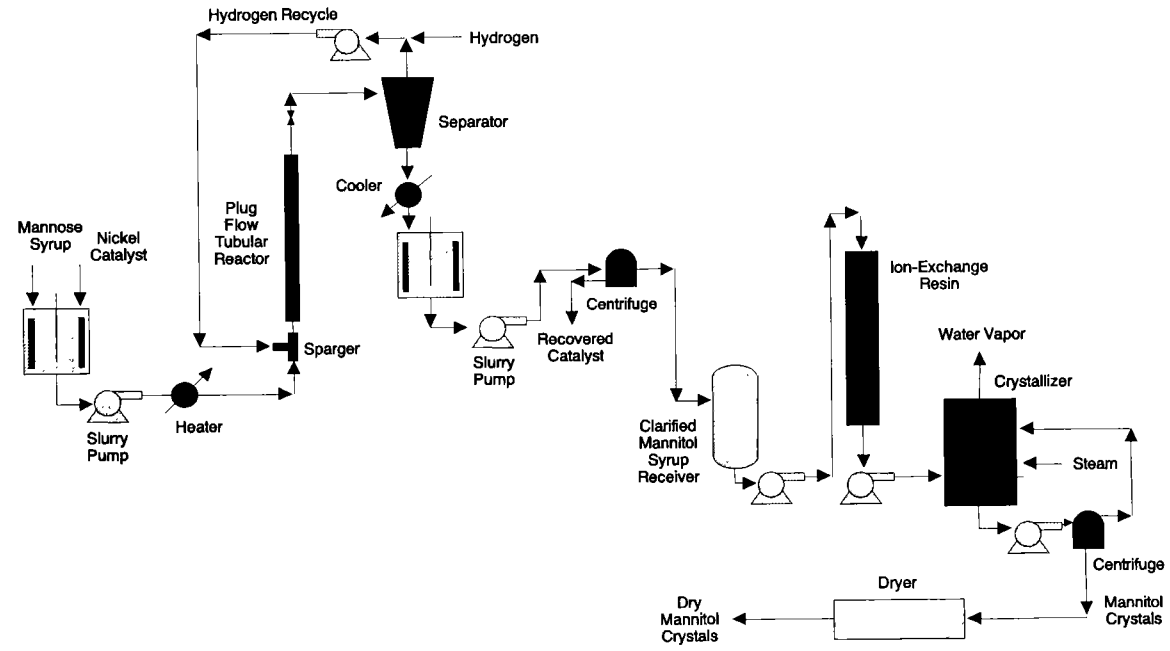


Figure 4

Flowsheet for the Production of Mannitol



CONCLUSIONS:

This study demonstrates that spent coffee grounds resulting from commercial percolation are high in mannan which can be selectively hydrolyzed from cellulose using high temperature short time hydrolysis conditions in an isothermal plug flow reactor. Syrups can be produced with up to 90-95% mannose on a sugar basis. These can be reduced to form food grade or USP grade crystalline mannitol, a valuble specialty chemical. Based on the data about 1/5 to 1/4 lb. of mannitol can be prepared per pound of spent grounds on a dry basis. One suggestion for disposal of the filter cake, which contains organics including nitrogeneous material and about 25% calcium sulfate, is that it be used as a soil enricher.

Acknowledgements:
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SUMMARY:

Spent grounds resulting from the manufacture of soluble coffee consist of about 45-55% polymeric carbohydrate on a dry basis, mostly mannan and cellulose with a small amount of arabinogalactan. This study examined the kinetics of high temperature short time hydrolysis of commercial percolator spent grounds with sulfuric acid in a tubular plug flow reactor.

Temperatures studied ranged between 160C and 240C, residence times between 6 and 30 sec., and slurry acid levels between 0.05% and 2%. The kinetic differences in the hydrolysis of mannan and cellulose permit selective hydrolysis of the mannan polymer and produce hydrolysates with up to 90-95% mannose on a sugar basis. It is suggested that the hydrolysates which contain a proponderance of mannose are effective feedstocks in the production of mannitol, a speciality chemical.

SOMMAIRE:

Hydrolyse acide du marc de café pour la production de mannose et de mannitol.

Le marc provenant de la production du café instantané est constitué d'environ 45 à 55% d'hydrate de carbone polymerique (base sèche), principalement mannane et cellulose avec une petite quantité d'arabino-galactane. Cette étude couvre la cynétique de l'hydrolyse haute température - courte durée du marc provenant de percolateurs industriels à l'aide d'acide sulfurique dans un réacteur tubulaire à écoulement piston.

Cette étude inculuait des températures entre 160 et 240°C, des temps de résidence entre 6 et 30 secondes, et des niveaux d'acide entre 0.05 et 2% dans le marc en solution. Les différentes cynétiques de l'hydrolyse de la mannane et de la cellulose permettent l'hydrolyse sélective du polymère de mannane et la production d'un hydrolysat contenant jusqu'à 90 à 95% de mannose (base sucre). Il est suggéré que les hydrolysats contenant principalement du mannose constituent une alimentation efficace pour le procédé de production du mannitol (produit chimique fin).

A MODIFIED SECOFFEX PROCESS FOR GREEN BEAN DECAFFEINATION

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Jacobs Suchard Corporate Research & Development
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Ladies and gentlemen,

Through my presentation I want to inform you about a recently finished development about the decaffeination of green coffee based on a modified Secoffex process.

Mankind expenses an enormous amount of energy, capital and effort in order to remove caffeine, the stimulant responsible for changing our mood, increasing concentration and our degree of alertness. Apparently there are many people not interested in striving for greater alertness at "Night & Day". Following the first patent application from Ludwig Roselius issued to HAG (in Bremen) in 1905, there has been numerous process developments in decaffeination technology; the most important of which are:

- *Solvent extraction of caffeine from pre-wetted beans with, for example, dichloromethane or ethyl acetate.*
- *Decaffeination using carbon dioxide.*
- *Aqueous extraction using a solution of green coffee extract or "water process"*

Whereas the world's most famous decaffeinated blends like HAG and SANKA are today produced on the basis of a solvent-free natural CO_2-process, we concentrated on the so-called Swiss water process.

The "water process" using water for decaffeination, was developed by the Coffex Company in 1938 in Switzerland. However, commercialisation of the process was slow and it was not until the late Seventies, that Coffex were able to realize commercial production: with the introduction at Schaffhausen with a capacity of 4 000 t/per year.

The new water process was operated batchwise with caffeine removed by leaching with fresh water added to the extraction vessel. Consequently, the extract also contained water soluble solids washed from the green beans. The green coffee extract containing caffeine was then passed through a bed of activated carbon to remove the caffeine from the solution by adsorption. In order to increase the selectivity of the activated carbon for caffeine, the activated carbon was preloaded with a sugar solution. Following the adsorption step the carbon must be regenerated to remove caffeine. The decaffeinated green bean extract was then returned to the extraction vessel where impregnation of the green beans with the water soluble solids was achieved by evaporation of the water.

Jacobs Suchard obtained the Coffex Company in 1980 and following the acquisition a program of further development and optimisation on Secoffex began at its Research Centre (Fig. 1).

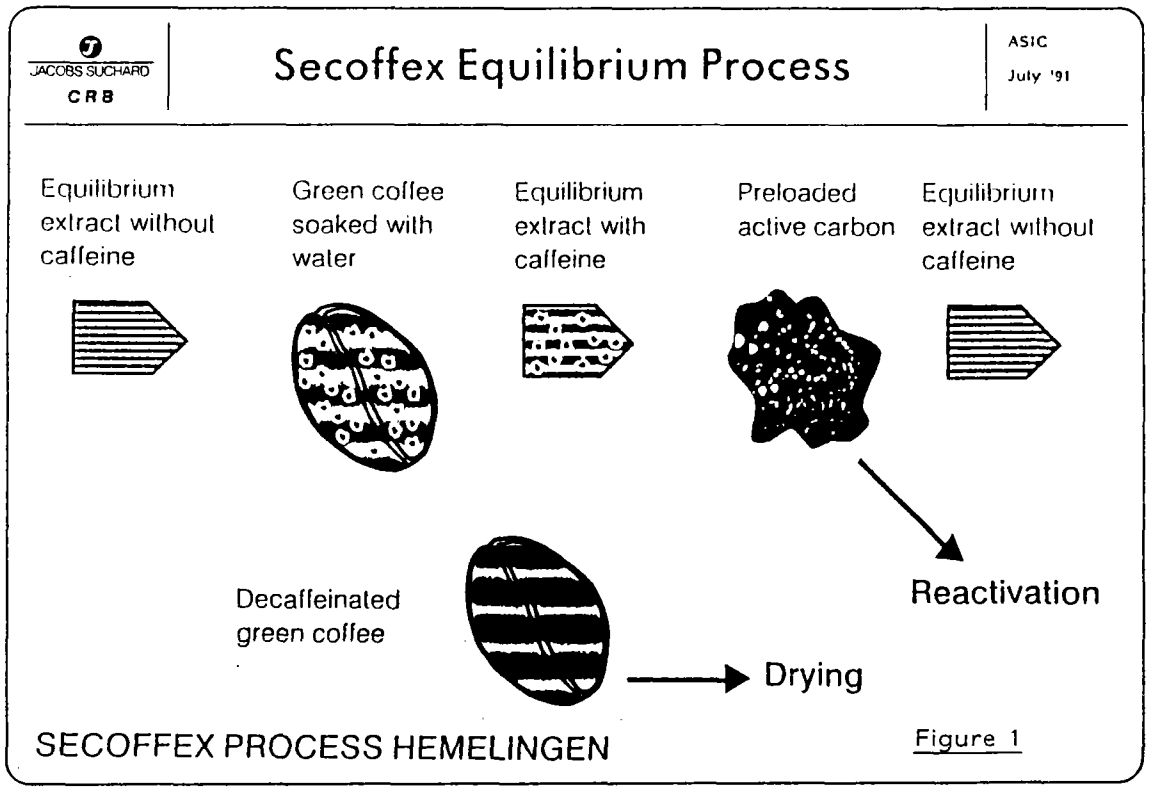


Figure 1

The net result of this program was to move the process from a batch to a continuous operation avoiding the extraction of green bean solids and consequently the latter re-integration step. The extraction of caffeine is now accomplished with a solution of "caffeine-free" green coffee extract with a concentration of soluble solids in equilibrium with the soluble solids concentration of the green beans.

First studies concerned the evaluation of an optimum in relation to the water balance (Fig. 2).

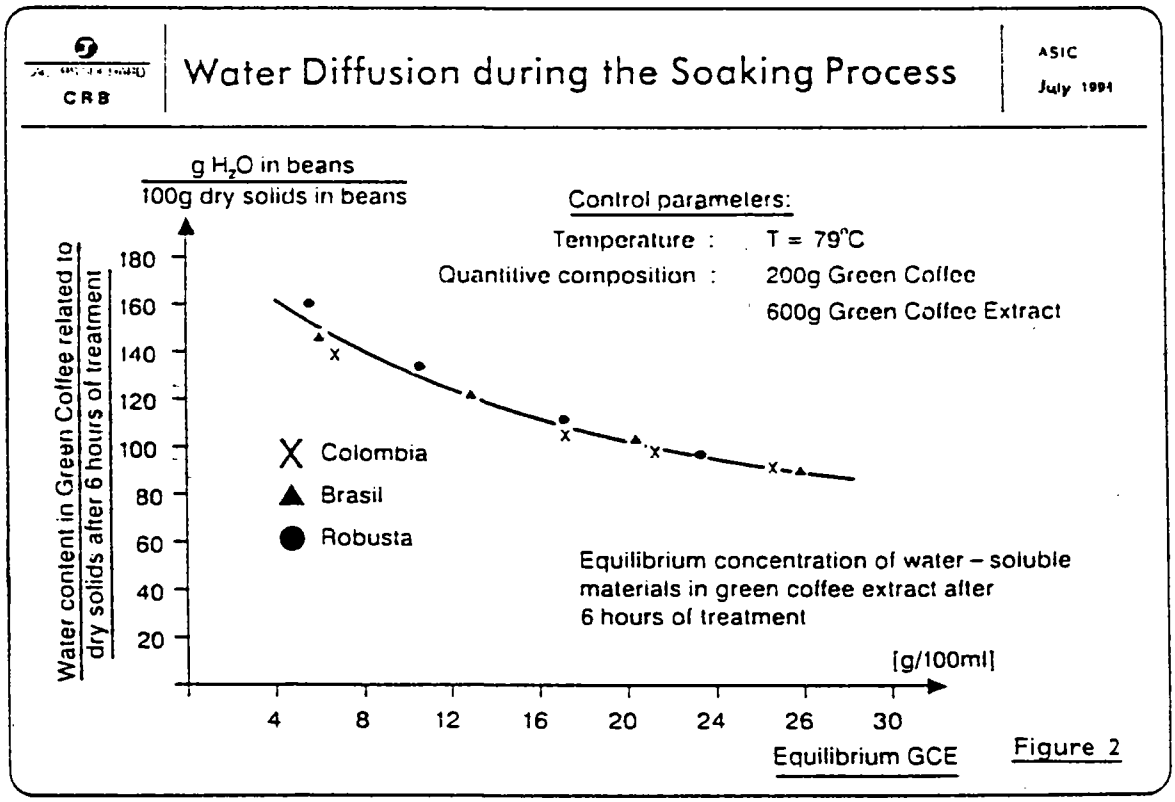


Figure 2

Following this equilibrium relations between the bean's water content (which later has to be removed) and the concentration of soluble materials (which in fact influences the total coffee losses) we finely fixed the operation parameters: about 16 - 24 %.

The later process control is mainly based on automatic in line caffeine-concentration measurement and process conditions can easily be varied by cycle time, degree of filling, feed flow rate and temperature. The rate of extraction of caffeine depends on the temperature of the green coffee extract (Fig. 3).

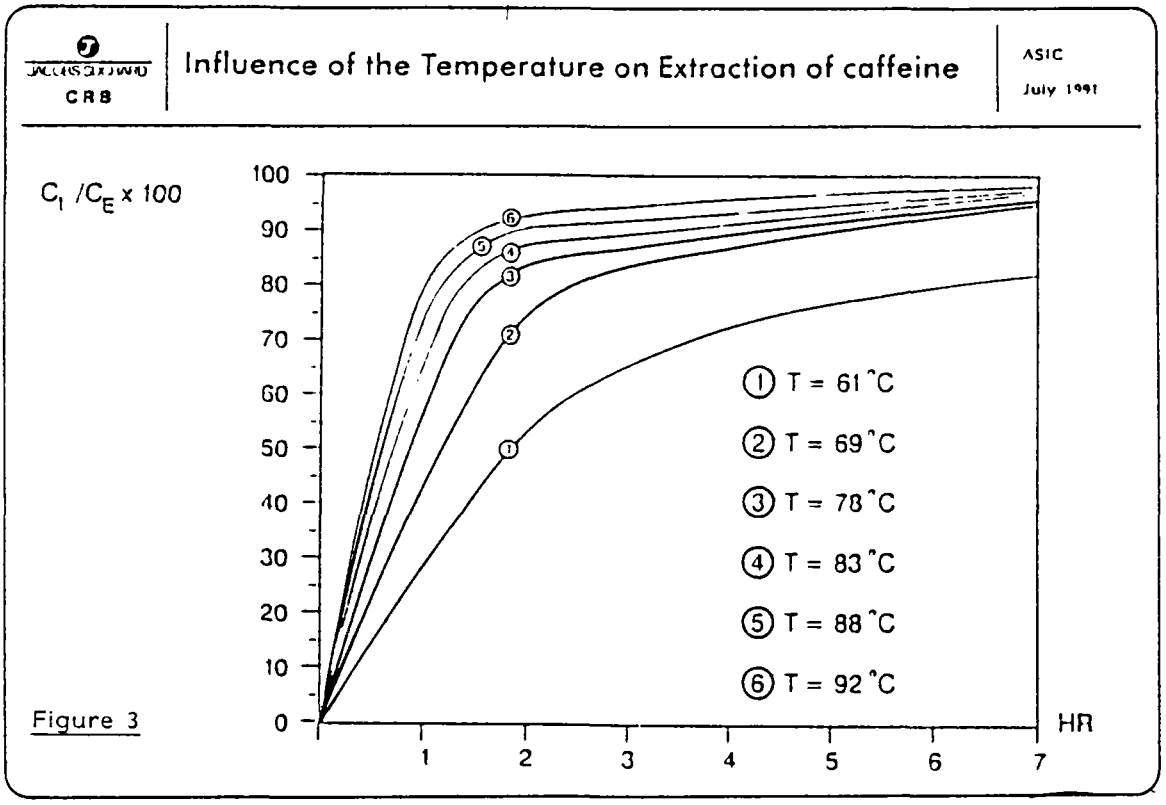
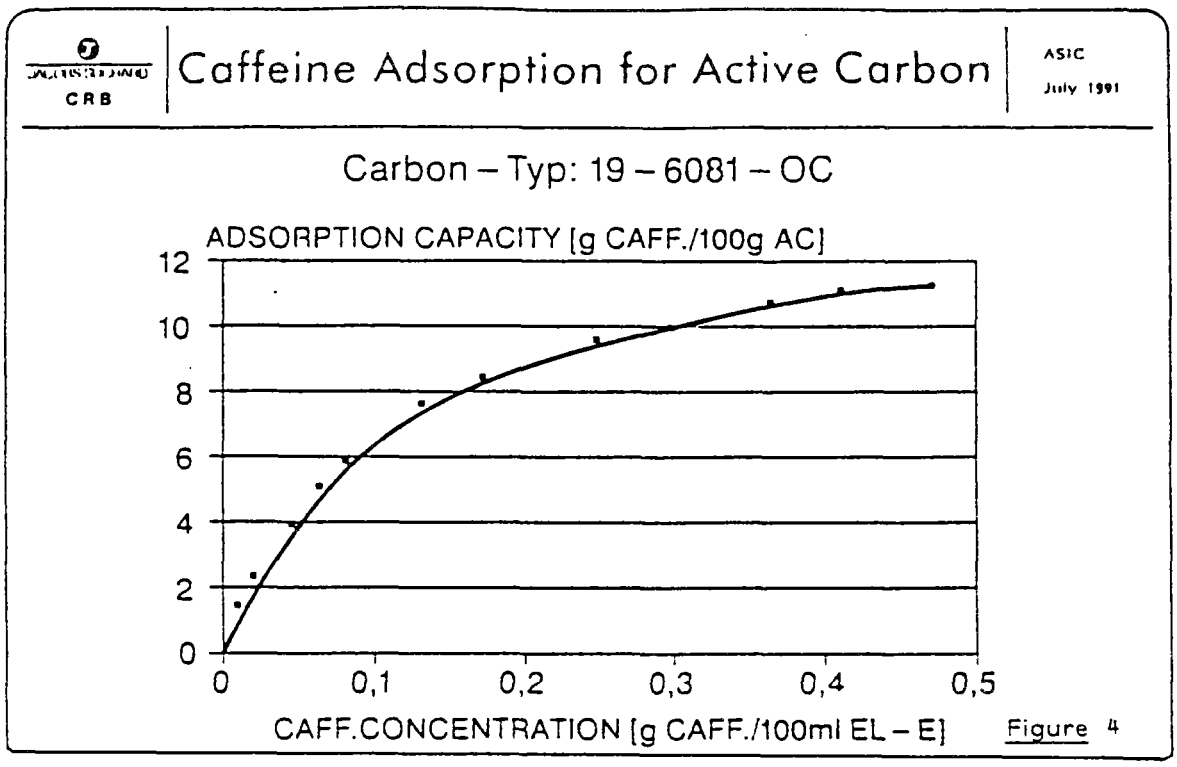


Figure 3

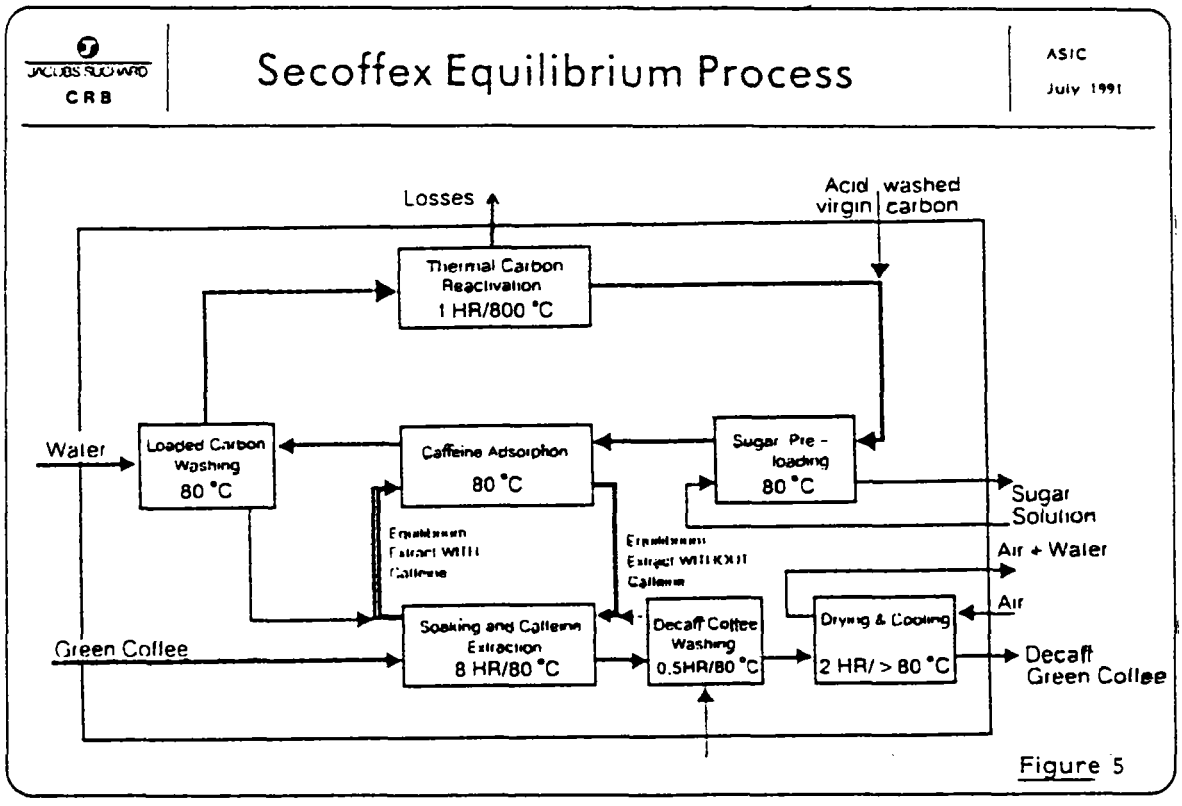
Today the operating temperature of green coffee extract of 80 C is an optimum between capacity, quality and bacteriological stability - and this temperature is a pseudo optimum for the adsorption of caffeine on activated carbon as well.

Experimental adsorption isotherms are useful for the selection of the most appropriate adsorbent. The adsorption capacity of the activated carbon used in our process is shown in Fig. 4.



The isotherm illustrates the amount of adsorbed caffeine per unit weight of carbon in dependence of the residual equilibrium concentration of caffeine remaining in the green coffee extract. Normally we run the process between 8 and 10 % caffeine loading.

The figure 5 shows the process flow sheet for the pilot plant.



Residence times and process conditions have been optimized to achieve

- *highest selectivity*
- *lowest impact on taste*
- *avoiding bacteriological contamination*
- *minimizing carbon consumption*

Now the equipments had to be selected:

In order to achieve a continuous process Jacobs have installed caroussel extractors for both the extraction and adsorption of caffeine (Fig. 6).

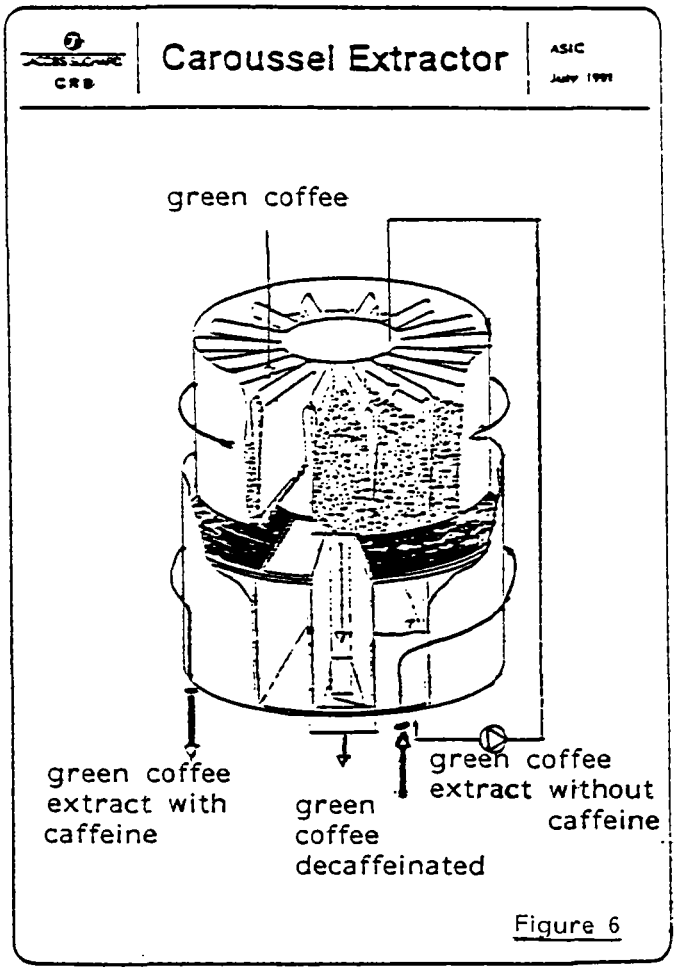


Figure 6

In this continuous countercurrent multiple step process the beans are decaffeinated down to a caffeine content of less than 0.1 % and the green coffee extract is decaffeinated in the caroussel adsorber down to less than 0.02 %. Caroussel extractors are well suited for these stepwise countercurrent processes.

For the reactivation Jacobs selected a three stage fluidized bed system which offers flexibility, safety and low carbon losses (Fig. 7).

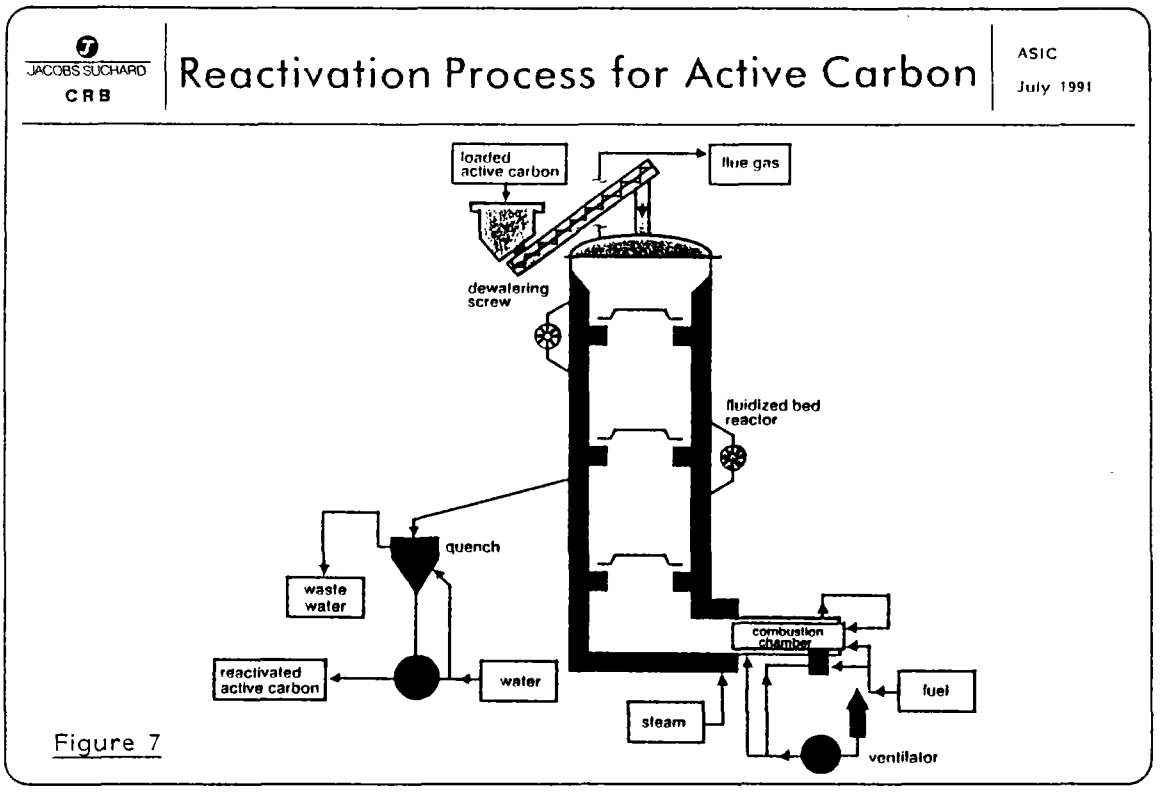


Figure 7

The reactivation of a spent carbon is a complex process which can be broken down into three stages:

- *drying at about 100 C*
- *desorption and pyrolisis of adsorbed materials between 100-800 C*
- *activation at higher temperature of up 800 C (that is, gasification of residual organics and oxidation of carbon residuals by a regenerating gas).*

After reactivation the carbon is quenched in water and transported back to the adsorption system.

The heating occurs under stoichiometric gas burning. A sophisticated air cooling and cleaning system was installed in order to recover energy and to avoid air pollution as far as possible.

After having accomplished the development of the Secoffex process on laboratory and pilot plant scales the commercialization of the new process was realized (Fig. 8).

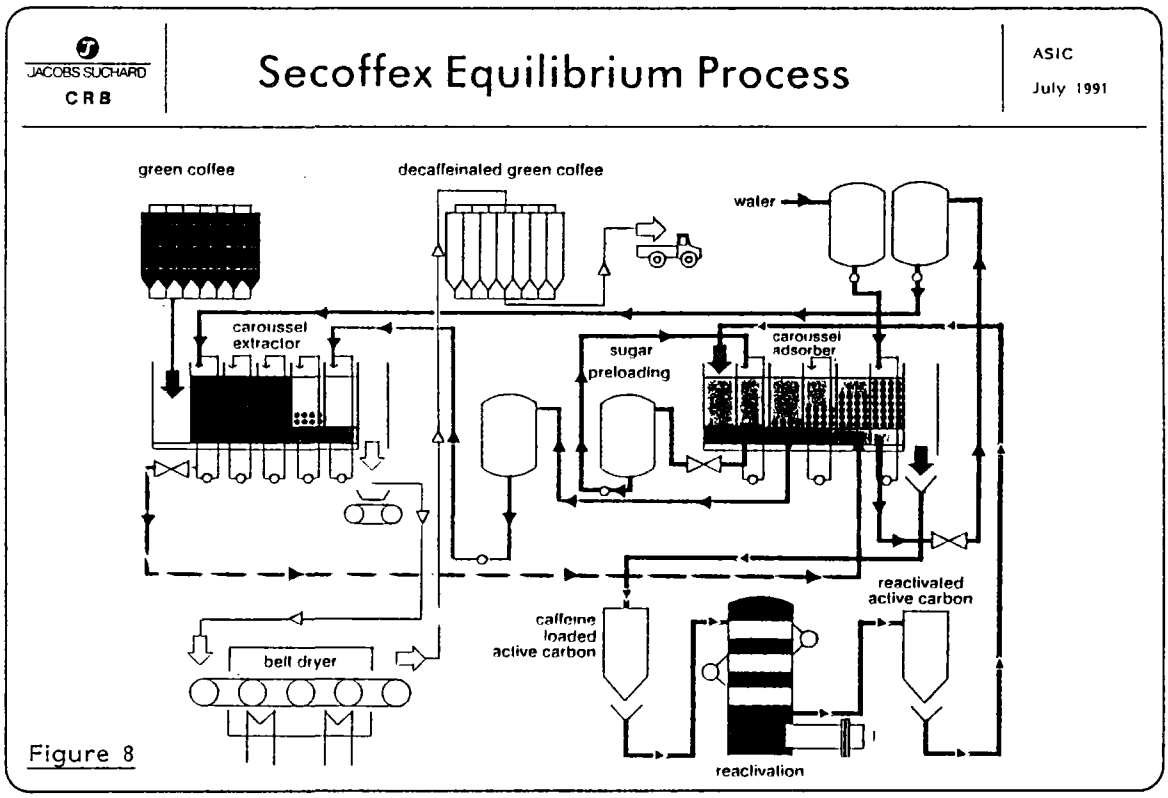


Figure 8

The commision of two plants, one in Vancouver, one in Bremen, took place in 1988/89 combined with the successful launch of a new decaffeinated premium brand: "Night & Day".

DECAFFEINATION WITH SUPERCRITICAL CARBON DIOXIDE

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INTRODUCTION

Because carbon dioxide (CO_2) is physiologically benign, it is an attractive extraction solvent for use in food processing. However, since it is a gas at normal ambient conditions, processes using carbon dioxide operate at elevated pressures to permit better extraction efficiencies. Above its critical point, about 1070 psi (73 atmospheres) and 31°C (88°F), carbon dioxide is in effect neither a gas nor a liquid but a "supercritical" fluid which has enhanced solvency for many substances.

The use of supercritical carbon dioxide for extraction of a variety of natural products was pioneered in Europe, beginning at commercial-scale about twenty years ago. Current commercial applications include decaffeination of coffee and tea as well as the extraction of spices, hops, nicotine, pharmaceutical chemicals, flavors and colors from natural materials. In addition, the technology has been proposed and pilot tested for other applications, including extraction of cholesterol from dairy products and animal fats, extraction of fat from cocoa and partial removal of oil from potato chips and other snack foods.

Operating a caffeine extraction plant above the CO_2 critical point allows efficient decaffeination and permits recovery of caffeine as a saleable byproduct. This technology offers the following advantages over methods using other solvents such as methylene chloride, ethyl acetate, or water extraction:

- **No Harmful Residue.** Carbon dioxide is a safe, nontoxic material that is completely and easily removed from the decaffeinated beans as well as from the aqueous solution of byproduct caffeine.
- **Superior Quality.** Product flavor and appearance are very close to that of undecaffeinated coffee and superior to the product from other decaffeination methods. Consumers recognize this process as a "natural" decaffeination technique and have demonstrated willingness to pay more for this quality.
- **Low Process Losses.** Losses of coffee solubles (other than caffeine) are quite low, less than other decaffeination methods, resulting in greater product yield.

The Supercritical Processing Group of Liquid Carbonic has developed a proprietary coffee decaffeination process using supercritical carbon dioxide. This paper presents the results of a technical and economic evaluation of the process conducted by Fluor Daniel. A conceptual process design is described, along with estimates of capital and processing costs. The effects of variations in plant capacity and design are discussed.

DECAFFEINATION PLANT DESIGN BASIS

A Base Case conceptual design was developed for a plant to process 32 metric tons per day (Te/D) of whole, green Colombian arabica coffee beans, removing 97 percent of the caffeine. The design was based on Liquid Carbonic's proprietary process, their patent applications, and pilot plant data. The key parameters in the Base Case design are:

Feedstock:	Green Colombian arabica coffee beans in 70 kilogram burlap sacks; 12% moisture, 1.25% caffeine (dry basis).
Annual capacity:	10,560 metric tons (23,232,000 pounds) of green coffee feed; about 32 metric tons (70,400 pounds or 460 bags) per day for 330 days per year operation (90% onstream factor).
Product:	97% decaffeinated green (unroasted) Colombian coffee beans at 10% moisture.
Byproduct:	An aqueous stream containing crude caffeine to be sold for further processing for caffeine recovery.
Location:	Southeastern U.S.A., adjacent to an existing coffee processing facility.
Extraction conditions:	
Pressure	2000-5000 psig (14-35 MPa) (130-330 atmospheres)
Temperature	70-130°C (158-266°F)
Residence time	6-12 hours
Separation conditions:	
Pressure	700-1500 psig (5-10 MPa) (45-100 atmospheres)
Temperature	15-50°C (60-120°F)

In order to explore the potential economies of scale for the process, capital and processing costs estimates were prepared for two other plant capacities:

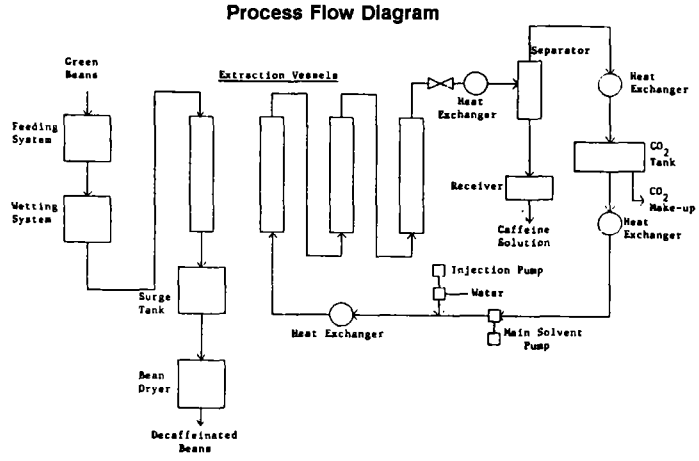
Case II:	50% of the Base Case: 16 metric tons/day
Case III:	200% of the Base Case: 64 metric tons/day

PROCESS DESCRIPTION

A simplified flowsheet for the process is shown in Figure 1, and the process is described below.

FIGURE 1

Process Flow Diagram



Green Bean Handling and Preparation. Green coffee beans are received at the plant site in burlap bags and stored in a warehouse until needed for production. The bags are opened manually and emptied into the bean feeder hopper. The beans then feed alternately into one of two pressure pots on the green beans conveying system, a dense phase system that transfers the beans to the green beans surge tank located at the top of the process plant, about 70 feet above the ground. The surge tank holds sufficient feed for one 8-hour shift's operations.

A vibratory feeder discharges one batch of beans from the surge tank into the wetting mixer, a steam jacketed double cone mixer. After the addition of steam and hot water from the water tank, the wetting mixer is tumbled to allow the water to absorb uniformly into the dry beans, raising the moisture content to 25-45% and the temperature to 70-100°C. The wetting mixer is then stopped, opened, and allowed to discharge by gravity onto the wetted beans conveying system, a screw type conveyor running directly above the extractor vessels. This conveyor transports and discharges the wetted beans into the next extractor to be loaded. After the extractor vessel is filled, it is sealed and purged with CO_2 to expel any air trapped in the vessel.

Extraction Section. The extraction module consists of four extractor vessels which operate in a semi-continuous mode, with three vessels in extraction service at any one time and the fourth vessel being either filled with fresh beans or discharging extracted beans. The three vessels in the extraction train are cycled such that a counter-current flow (relative to the beans) of supercritical solvent is maintained. Fresh solvent is introduced into the vessel that has been in the train the longest, first contacting the most extracted beans. The solvent exits this vessel flowing through the second vessel in the train and then into the last vessel, the one which was most recently put into the train and contains the least extracted beans. This scheme is effectively a counter-current extraction with the lean solvent contacting the most extracted beans and the freshest beans seeing the most saturated solvent.

Solvent is allowed to bleed into the newly filled and purged extractor vessel, bringing it to the extraction pressure. This vessel is brought into the extraction train at the end, contacting the most saturated solvent. The first extractor (now containing completely extracted beans) is then valved out of the extraction train leaving three vessels on line with the newly filled vessel being the last in the train. The first extractor, now out of the extraction train, begins the depressurization and unloading sequence. Complete decaffeination requires 6-12 hours under design extraction conditions. For example, if the decaffeination time were 8 hours and with three extraction vessels in the train, a vessel would complete its cycle and be coming off line (and a new vessel going on line) every 2 hours and 40 minutes.

Separation Section. The rich solvent laden with caffeine leaves the last vessel in the extractor train and flows through the extract filters to remove any particles which may have become entrained in the advent. Flow continues through a pressure control valve where an isenthalpic expansion to the separation pressure occurs, lowering both the solvent pressure and temperature. The separator heater, a steam-heated heat exchanger, is used bring the solvent to the desired separation temperature before it flows into the separator. At the separator conditions, the solubility of caffeine and other components in the CO_2 is greatly reduced. The water, caffeine and a minor amount coffee solubles drop out as a liquid phase, collecting in the bottom of the separator and then are transferred to the extract receiver. Both the extract receiver and the separator are jacketed for steam and maintained at a temperature to prevent the crystallization of the caffeine. The extract transfer pump moves the caffeine solution out of the plant to a tank where the solution is accumulated for sale as a byproduct or for disposal.

Solvent Recovery. Carbon dioxide vapor leaving the separator flows through a solvent filter and the knock out drum where any remaining entrained particles or liquid droplets are removed. The clean CO_2 vapor is condensed to the liquid phase in the solvent condenser, a shell and tube heat exchanger. The liquid flows directly into the solvent hold tank.

The solvent hold tank is an insulated horizontal surge tank holding liquid CO_2 in volume approximately equivalent to the volume of the extraction train and associated piping. The solvent level within the tank is maintained by makeup with fresh carbon dioxide from a bulk liquid CO_2 storage tank via the solvent transfer pump. This compensates for normal CO_2 process losses through vents, seals, and exit streams. The solvent hold tank is maintained at 700-1000 psi (5-7MPa) and 13-30°C (55-85°F), holding the carbon dioxide at its vapor-liquid saturation point.

Liquid CO_2 flows from the solvent hold tank through the solvent sub-cooler, a shell and tube heat exchanger cooled with refrigerant. This decreases the solvent temperature and prevents cavitation in the solvent pumps. The solvent pumps increase the CO_2 pressure to the extraction conditions, changing it into a supercritical fluid. The water injection pump injects potable water into the carbon dioxide stream, saturating the solvent with water to maintain bean moisture level in the extractors. A static mixer assures complete mixing of the water and carbon dioxide. The solvent then flows through the recycle solvent heater, a steam-heated shell and tube heat exchanger, which brings the solvent to extraction temperature. The solvent leaves this heat exchanger and enters the first extraction vessel in the extraction train.

Decaffeinated Bean Handling and Drying. At the end of the caffeine extraction cycle, an extractor is valved out of the extraction train and depressurized. High pressure carbon dioxide is released through a pressure control valve. The extractor pressure is decreased to the solvent recycle pressure, allowing the CO_2 within the beans to diffuse out. After the high pressure CO_2 has been recovered in this manner, the CO_2 remaining in the extractor vessel is vented to atmospheric pressure through the vent scrubber. The vessel is then opened and the decaffeinated beans are discharged to the decaffeinated beans conveying system, a screw conveyor that runs directly

under the extractors and discharges to the dryer feed surge tank. From this tank, the dryer feeder continuously feeds wet decaffeinated beans to the dryer.

The decaffeinated beans dryer is a steam heated, single pass belt-type dryer that continuously drys the wet (25-45 percent moisture) decaffeinated beans, discharging them at approximately 120°C (248°F), with a moisture content of approximately 10 percent. After discharging from the dryer, the beans are transported by a belt-type conveyor for cooling and transfer to the warehouse area for storage prior to roasting or packaging for reshipment to customers.

Equipment Considerations. Equipment must be designed in accordance with the ASME UPV Code for cyclic high pressure service, and also designed for ease of cleaning as required for food processing. Process equipment, piping, and systems in contact with wet beans or water bearing process streams are constructed of type 304 stainless steel. Other equipment, such as green bean handling or drying, are constructed of carbon steel. High pressure equipment is constructed of carbon steel clad with type 304 stainless steel. Large bore openings are used on the extractor vessels with hydraulically operated quick opening, high pressure closures. Appropriate alarms, interlocks, and emergency venting systems must be used to allow safe operation at high pressure.

CAPITAL COST ESTIMATES

Capital cost estimates were developed using primarily equipment-factored estimating techniques. When using this estimating approach, the estimates for the various systems which comprise the decaffeination facility have been developed based on machinery and equipment prices that were derived from process design and detailed equipment specifications. These duty specifications were priced on an individual basis for each system using current in-house data or vendor quotations. Material costs for concrete and steel and the field labor man-hours associated with each equipment item were factored based on individual equipment designs. The factors for bulk material costs and labor man-hours reflect Fluor Daniel's recent experience for equipment in systems with similar configurations and unit capacities. Adjustments were made to reflect a Southeastern U.S.A. site and to mid-1990 open shop construction costs. Equipment and material were assumed to be procured on a worldwide basis and the construction labor productivity and wage rates are based on Fluor Daniel's previous experience in the site area.

The capital cost estimates given below include direct and indirect field costs, office costs, sales tax, and contingency. The estimates do not include permitting, royalties, working capital, owner's costs, piling, or land acquisition costs. We have included all the process facilities required for green bean handling, decaffeination, and bean drying. However, because the estimate basis states that the plant would be built at an established site with associated coffee handling facilities, we have not included complete utilities and offsites, which would be required if this were a grassroots project. We have assumed that these utilities and offsites requirements would be available from the existing facilities. The capital cost estimates include interconnecting piping, communications systems, and utilities distribution and conditioning within the battery limits, but exclude such items as site preparation, roads, fencing, process water treating, firewater, and other general facilities.

The contingency is included as an allowance for unexpected costs associated with the design and construction of these facilities. As developed for this estimate, contingency is based on a mathematical risk analysis which focuses on the critical areas of uncertainty with the estimates. Factors which contribute to the contingency include: accuracy of pricing, accuracy of quantities, accuracy of labor rates, accuracy of productivity factor, and engineering design development. Items which are not considered in developing the contingency value include client involvement, government regulations, major design changes, labor strikes and unusual field conditions. The value used for contingency in this estimate is typical of those developed for conventional industrial projects.

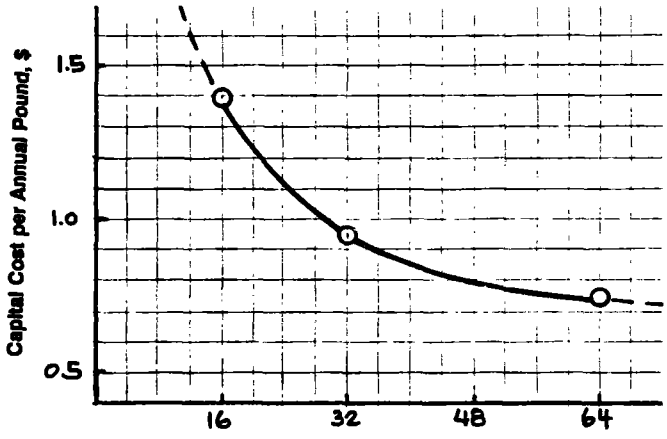
The overall capital cost estimates for the three cases are:

	Te/D	$10^6$$	$/annual lb.
Base Case	32	22.0	0.95
Case II	16	16.2	1.40
Case III	64	34.5	0.74

The third column above is the capital cost per annual pound of coffee processed. This parameter, calculated by dividing the capital cost estimates by each unit's annual capacity, provides a measure of the relative economy of scale. Figure 2 is a plot of the capital cost per annual pound versus the plant capacity and shows that for a capacity much lower than the Base Case plant the capital cost per pound of coffee processed begins to increase rapidly.

FIGURE 2

Capital Cost per Annual Pound vs Plant Capacity



ESTIMATED PROCESSING COSTS

The estimated processing requirements and costs for the Base Case are presented in Table 1. The quantities, unit costs, and annual costs for each item are shown. The electric power demand is based on using all electric drivers for rotating equipment. Typical U.S. industrial practice was used for the other items. No credit has been taken for the possible sale of the caffeine byproduct. In the second column the annual processing costs are divided by the plant capacity to show the processing costs on a per pound of coffee basis.

TABLE I

Estimated Processing Costs - Base Case

	10^3 $/year	¢/lb product
Utilities		
Electric power: 2000 kW @ 4.3 ¢/kWh	680	2.9
Steam: 34.8 x 10^6 Btu/h @ \$4.90/10^6 Btu	1,350	5.8
Cooling water: 5000 gpm @ 7.4 ¢/10^3 gal.	175	0.8
Carbon dioxide: 1.1 Te/h @ 2.2 ¢/lb.	415	1.8
Process water: 20 gpm @ \$1.00/10^3 gal.	10	--
Subtotal	2,630	11.3
Labor		
Operating Labor: 2 per shift @ \$15/h	340	1.5
Supervision @ 25% of direct labor	85	0.4
Subtotal	425	1.9
Other		
Maintenance @ 4% of capital cost	880	3.8
Taxes and insurance @ 1.5% of capital cost	330	1.4
Plant overhead (G&A) @ 25% of direct labor	85	0.4
Subtotal	1,295	5.6
Total Processing Costs	**4,350**	**18.8**

The largest portion of the 18.8¢/pound processing cost is the utilities, especially steam and electric power consumption which alone claim nearly half the total processing cost. The next largest item is plant maintenance at 3.8¢/pound or about 20% of the processing cost.

For comparison the operating costs for the two alternate cases are shown in Table 2, below. This table presents the annual costs for each item as well as the overall per pound processing costs, though for simplification it does not show the utility quantities.

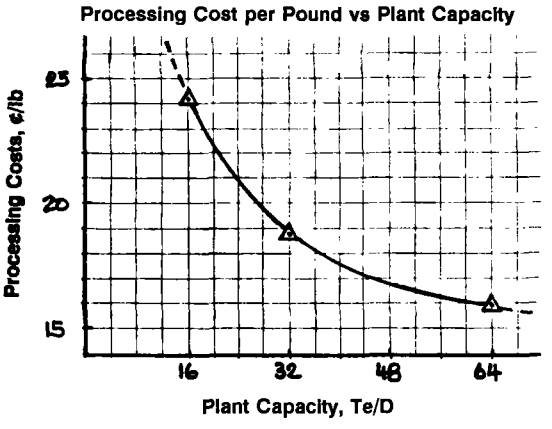
TABLE 2

Estimated Processing Costs - Alternate Cases

	Case II	Case III
Plant capacity, Te/D	16	64
	10^3 $/Y (¢/lb)	10^3 $/Y (¢/lb)
Utilities		
Electric power	430 (3.7)	1,085 (2.3)
Steam	675 (5.8)	2,700 (5.8)
Cooling water	90 (0.8)	350 (0.8)
Carbon dioxide	210 (1.8)	835 (1.8)
Process water	5 (-)	20 (-)
Subtotal	1,410 (12.1)	4,990 (10.7)
Labor		
Operating labor	340 (2.9)	340 (0.7)
Supervision	85 (0.7)	85 (0.2)
Subtotal	425 (3.6)	425 (0.9)
Other		
Maintenance	650 (5.6)	1,380 (3.0)
Taxes and insurance	240 (2.1)	520 (1.1)
Plant overhead (G&A)	85 (0.7)	85 (0.2)
Subtotal	975 (8.4)	1,985 (4.3)
Total Processing Costs	**2,810 (24.2)**	**7,400 (15.9)**

Figure 3 is a plot of the processing costs for each of the three cases versus the plant capacity and shows that for capacities much less than the Base Case plant the processing costs begin to increase significantly. In contrast, a two-fold increase above the Base Case in plant throughput reduces the processing cost by less than 3¢ per pound.

FIGURE 3



PROJECT ECONOMIC ANALYSIS

An economic analysis was performed to determine the necessary sales price increment for the decaffeinated beans (above the cost of the feed beans) and the relative profitability for each case. Using a simplified calculation procedures described below, the required product sales price increment was determined for each case to give a 15% after tax return on investment. The results of these analyses are presented in Table 3 with the values shown in millions of dollars per year.

All of the direct costs and the allowances for taxes, insurance and plant overhead costs are taken directly from Tables 1 and 2. The allowance for plant overhead includes local administration, payroll burdens, and sales expenses and for the purposes of this study has been estimated at 100% of the operating labor costs. Taxes and insurance are calculated at 1.5% of the total installed costs.

The Annual Capital Recovery Factor (ACRF) is taken as an annual charge against the project cash flow, equal to 20% of the capital costs. The ACRF is an empirical factor which is often used in preliminary evaluations such as this to account for depreciation, return on investment (ROI) and other capital-related items. By evaluation of past discounted cash flow (DCF) analyses of various chemical and industrial projects we have determined that an ACRF of about 20% is appropriate as an average annual charge when the project target ROI is 12-15%, after tax. Thus, this simplified economic analysis approximates the same project cash flows which would result from a DCF calculation performed to give a 12-15% ROI.

The ACRF simplification is appropriate for this type of initial evaluation and provides an adequate estimation of the project economics. Subsequent, more detailed studies may then use DCF analyses to fine tune the economics, accounting for the effects of escalation, inflation, project investment leveraging, and any tax incentives which the ACRF approach does not consider.

As with the previous data the total annual project costs have been divided by the annual production rate to give an overall cost (including project return and profits) per pound of decaffeinated coffee. These values represent the required sales price increment, over the cost of the raw green beans, which the decaffeinator must charge in order to cover all his direct and indirect costs, recover his capital investment and realize a modest profit.

Similarly to the data discussed previously, the overall project economics show that for capacities below the Base Case, the costs per pound begin to increase rapidly, while for higher capacities the economy of scale allows the per pound cost to decrease.

TABLE 3

Overall Project Economics, $10^3$$/Y

	Base Case	Case II	Case III
Utilities	2,630	1,410	4,990
Labor & Supervision	425	425	425
Maintenance	880	650	1,380
Taxes & Insurance	330	240	520
Plant Overhead	85	85	85
Annual Capital Recovery Factor	4,400	3,240	6,900
Total	8,750	6,050	14,300
Total, ¢/lb	**37.7**	**52.1**	**30.8**

DESIGN CONSIDERATIONS

This preliminary design includes a number of basic assumptions which would be reviewed in the course of designing an actual commercial plant. Some of these issues are discussed here.

Continuous Operation. The semi-continuous operating mode was selected for this design based on known practices in similar high pressure processes. This is a conservative and practical scheme and represents good business practice. However if a truly continuous method of adding and removing coffee beans from the high pressure zone could be achieved, the capital-intensity of the process could be reduced significantly. For the same throughput, the plant cost would be decreased by the cost of one expensive, heavy-walled extractor vessel and its associated equipment. Alternatively the plant capacity could be increased by some value approaching one third for about the same capital investment, thus significantly reducing the per pound costs.

Caffeine Recovery. As a simplification, this conceptual design does not include facilities for recovery of the caffeine from the aqueous steam leaving the extract receiver vessel. Preliminary examinations suggest that caffeine byproduct sales could contribute significantly to project cash flow and improve the overall process economics. The byproduct sales value would have to be balanced against the capital and operating costs of the caffeine recovery facility.

Plant Location. The location of any plant will have an impact on project economics and must be carefully evaluated on a case-by-case basis. For this study we selected a typical southeastern U.S.A. location which offers relatively low labor and utility costs. The project economics are somewhat more favorable there, compared to higher-cost areas, such as the northeastern U.S.A. Comparison of offshore locations would be more complex.

Equipment Selection. Certain choices of equipment may have impacts upon the capital and/or processing costs of the plant. For example, the simplifying decision in this study to use electric drivers for the large duty solvent pumps would be examined more closely for an actual design. Depending on local circumstances and specific design philosophy, it is possible that a detailed comparison would show that steam turbine drivers would be preferable for the major duties.

Another possibility would be to use a recovery turbine at the pressure reduction point between the extract filters and the separator. The large decrease in pressure at that point in the process offers a significant potential for energy recovery. However, it is not clear whether this concept could be applied practically and the issue would need to be addressed during actual design.

CONCLUSIONS

The Liquid Carbonic proprietary coffee decaffeination process offers a natural nontoxic method of producing high yields of superior product. A commercial-scale plant designed to process about 10,000 Te/Y of coffee will require $22 million of capital investment. The processing costs would be approximately 19¢ per pound and the overall economics would require an ex-plant product sales price increment of about 38¢ per pound over the price of the raw green beans. While that size plant is economical, a larger facility would cost less per pound of capacity and would result in an overall sales price increment of about 31¢ per pound over the raw green beans.

The process has been demonstrated at pilot scale and is ready for commercial application.

SUMMARY

The advantage of coffee decaffeination using supercritical carbon dioxide is that it employs a nontoxic physiologically benign solvent in a natural process which produces a high yield of superior quality product. A process design, based on pilot plant data, is presented for commercial-scale coffee decaffeination using Liquid Carbonic's proprietary supercritical carbon dioxide extraction process. Capital costs, processing costs and preliminary project economics are estimated for three plant capacities. Battery limits capital costs of $22 million and overall processing costs including capital recovery, taxes, and profit result in a decaffeination cost increment of about $0.38 per pound ($0.83 per kilogram) for a plant capable of decaffeinating 32 metric tons per day of whole, green coffee beans. Capital costs increase to $34.5 million and production costs decline to about $0.31 per pound ($0.68 per kilogram) for a 64 metric ton per day capacity plant.

En la descafeinización de granos de café verde con dióxido de carbono supercrítico se emplea un solvente fisiológicamente sin riesgo y el proceso produce un producto de calidad superior. Un diseño de proceso supercrítico de Liquid Carbonic para una planta de capacidad comercial se presenta. Los costos y las economías del proyecto están estimados para tres capacidades de planta. Para el caso básico, una planta de 32 toneladas métricas por día, el costo de construcción es $22 millones. El costo de operación que incluye ganancias, impuestos, y otros costos de capital, se ha estimado a $0.38 por libra ($0.83/kg.). Para una planta más grande, de 64 toneladas métricas por día, el costo de construcción aumenta a $34.5 millones y los costos de operación disminuyen a $0.31 por libra (0.68/kg).

LES TECHNIQUES MODERNES DE REPRODUCTION ASEXUÉE. IMPACTS SUR L'AMÉLIORATION GÉNÉTIQUE DES CAFÉIERS

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INTRODUCTION

La sélection de structures hétérozygotes performantes et leur reproduction conforme par voie asexuée est un moyen rapide et efficace pour améliorer les plantes pérennes à cycle long.

Cette méthode permet en effet d'exploiter au mieux la diversité génétique de ces végétaux et de valoriser tout individu exceptionnel né du hasard des croisements, mutations ou manipulations génétiques.

Ce type de sélection clonale, utilisé de tout temps chez les plantes fruitières et forestières, nécessite, pour être efficient, une technique de reproduction asexuée bien éprouvée.

Le but de cet exposé est de montrer l'importance de la reproduction asexuée dans l'amélioration des caféiers, mais aussi comment la sélection clonale, inconnue chez l'arabica, pourrait être introduite chez ces caféiers grâce aux progrès des biotechnologies végétales (20, 23, 59), les avantages d'une telle sélection clonale chez les caféiers allogames ayant été démontrés depuis longtemps (15, 26, 29).

Cet exposé comprendra essentiellement deux parties :

1) Un rappel bref des données et stratégies d'amélioration des caféiers utilisées à ce jour de façon à montrer aux non-sélectionneurs en particulier pourquoi et comment la voie asexuée conduit à une amélioration plus rapide et plus efficace des caféiers.

2) Description et analyse des techniques modernes de micropropagation de caféiers (microbouturage et embryogénèse somatique) sans détails techniques.

En conclusion, nous analyserons les conditions et les limites d'utilisation de cette micropropagation ainsi que les moyens qui seraient nécessaires pour un emploi plus accru de ces techniques en caféiculture.

DIVERSITE GENETIQUE NATURELLE CHEZ LE G. *COFFEA*

Le premier point qu'il convient de rappeler concerne la diversité génétique naturelle des caféiers ; celle-ci devrait en effet jouer un rôle déterminant dans leur amélioration future (3, 7).

Il existe plusieurs espèces de caféiers qui peuvent fournir un produit consommable. Elles diffèrent entre elles au niveau de nombreux caractères d'intérêt génétique : système de reproduction, niveau de ploïdie, adaptation écologiques, composants chimiques, résistance aux maladies, etc. (2, 5, 6, 16, 32, 35, 54, 67).

Cette richesse génétique peut encore être élargie grâce à l'interfertilité qui existe entre plusieurs de ces espèces (8, 41, 42, 51).

Cette diversité génétique qui traduit la très grande souplesse d'adaptation de ces végétaux n'a pratiquement pas été utilisée par les sélectionneurs.

Seules deux espèces, *C. arabica* et *C. canephora* sont cultivées à grande échelle. Leurs caractéristiques sont souvent complémentaires. Leur amélioration, qui d'abord a été faite séparément, puis de façon associée pour les besoins nouveaux de la sélection, comporte donc un volet intraspécifique et un volet interspécifique.

C. CANEPHORA

1) Amélioration intraspécifique

Le *C. canephora* comprend plusieurs variétés réparties en deux groupes : guinéens et congensis, qui présentent entre eux des phénomènes d'*heterosis* (9).

Ces caféiers autoincompatibles sont à allogamie exclusive et leurs descendances toujours hétérogènes, sauf si les géniteurs sont des haploïdes doublés (10, 28, 30).

Il existe chez ces caféiers un procédé de bouturage, mis au point depuis 1953, simple, peu coûteux, bien adapté au contexte économique des pays producteurs de robusta (27, 48).

Grâce à cette possibilité de reproduction asexuée, la sélection clonale a pu être privilégiée et l'amélioration de ces caféiers a fait rapidement des progrès considérables (15, 23, 26, 29, 64).

Dans la stratégie utilisée, le renouvellement des clones se fait par le biais de populations nouvelles, améliorées par croisements avec intégration des données récemment acquises : *heterosis* de groupe, sélection récurrente (26, 40).

Ces populations nouvelles constituent autant de relais permettant de passer d'un niveau à un autre supérieur.

En conclusion, ici : une technologie de reproduction végétative utilisable à grande échelle a permis de valoriser les structures hétérozygotes exceptionnelles et de faire progresser très rapidement l'amélioration génétique de ces caféiers.

2) Amélioration interspécifique

Chez les caféiers canephora, les voies interspécifiques ont été utilisées soit pour les besoins d'une adaptation à une écologie donnée (c'est le cas des hybrides congusta (69), soit pour les besoins d'une amélioration de la qualité (c'est le cas pour les hybrides arabusta créés en Côte d'Ivoire dans les années 70 (18, 19).

La stratégie qui a été suivie pour la création de ces arabusta est tout à fait classique : doublement chromosomique des canephora, croisement avec arabica et sélection au sein de la F_1 hétérogène obtenue des individus les plus remarquables, qui sont alors reproduits par bouturage. La technique de bouturage évoquée pour les canephora s'est révélée en effet applicable à arabusta (19, 53).

En conclusion : ici aussi l'emploi de la reproduction asexuée a permis d'améliorer très rapidement les qualités du robusta traditionnel et de créer pour ainsi dire un nouveau cultivar de caféier.

C. ARABICA

1) Amélioration intraspécifique

Chez les caféiers arabica, les orientations intraspécifiques ont prédominé pendant longtemps. Le *C. arabica* étant autogame, le but ici a été d'obtenir par sélection généalogique ou par back cross une variété fixée, capable d'être reproduite fidèlement par graines.

Dans les stratégies utilisées, on constate que des structures hétérozygotes de valeur apparaissent dès la F_1 Leur fixation pour ainsi dire par voie asexuée aurait permis d'éviter toutes les opérations d'autofécondation ou de rétrocroisement situées en aval et d'accélérer d'autant l'amélioration de ces caféiers (15, 65, 66).

L'intérêt de ces génotypes F_1 exceptionnels a été réactualisé avec la découverte au Kenya et en Ethiopie de phénomènes d'*heterosis* dans certains croisement de *C. arabica*. Des tentatives de récupération de ces F_1 par voie végétative ont été faites, puis abandonnées au profit de fécondations manuelles dirigées, après castration (1, 35, 50, 66).

En raison des particularités biologiques des caféiers, de telles opérations impliquent des contraintes considérables. Dans un certain futur, ces castrations pourront être évitées, avec l'emploi de stérilité mâle induite, comme cela a déjà été réalisé par le groupe de Plant Genetics Systems en Belgique (Nature 347, n° 6265, p. 737-741).

Avec ou sans castration, l'obtention par voie sexuée de ces F_1 exceptionnels implique que les deux géniteurs soient homozygotes, ce qui n'est pas toujours le cas.

2) Amélioration interspécifique

Chez le *C. arabica*, les voies interspécifiques ont été imposées par les besoins de création d'un arabica résistant aux rouilles. La rouille a en effet envahi tous les pays producteurs de l'Amérique Latine en raison de l'identité génétique des caféiers arabica de ces pays.

Par ailleurs, l'expérience a montré qu'il n'existait pas chez l'espèce arabica de génotype résistant à toutes les races connues de rouille ; par contre, il en existe chez les *C. canephora*. La création d'un caféier arabica résistant aux rouilles implique le passage obligé par des combinaisons hybrides interspécifiques, en l'occurence avec *C. canephora* (15, 34, 47, 65, 66).

La stratégie utilisée à l'heure actuelle vise au transfert de la résistance canephora par l'intermédiaire d'un grand nombre de back cross sur le parent récurrent *C. arabica*, de façon à obtenir *in fine* une variété facilement reproductible par graines.

Ce processus est très long, plus de 30 années. On a constaté que des structures hétérozygotes performantes apparaissaient dès les premiers back cross. La fixation de ces génotypes de valeur, par reproduction asexuée, devrait permettre d'éviter les étapes situées en aval et d'accélérer l'amélioration des caféiers (65, 66).

On doit en outre noter que, en raison du niveau d'hétérozygotie encore élevé du parent non récurrent, ces structures hétérozygotes ne peuvent être reproduites par fécondation dirigée, comme précédemment.

Dans ce contexte de recherche de *C. arabica* résistant aux rouilles, il faut signaler les génotypes à résistance horizontale polygénique, et dont la reproduction ne peut être envisagée que par voie asexuée (34, 47).

En définitive, chez les *C. arabica*, quelles que soient les stratégies adoptées, l'emploi de la voie végétative apparaît comme le meilleur moyen pour accélérer l'amélioration de ces caféiers.

En conclusion générale, la reproduction asexuée apparaît en toutes circonstances comme le meilleur recours pour une amélioration rapide et efficace des caféiers, qu'ils soient allogames ou autogames.

Quels sont alors les procédés de reproduction asexuée utilisables chez les caféiers ?

Les techniques de bouturage, bien adaptées à la reproduction végétative des canephora et arabusta, se sont révélés peu applicables au *C. arabica* (64).

Quant au greffage, il n'a pratiquement jamais fait l'objet d'une utilisation à grande échelle chez le caféier. Son usage a été limité aux collections de germplasm ou à des essais de lutte contre les nématodes (17, 52).

Les techniques de micropropagation applicables aux caféiers arabica apparaissent dès lors come très prometteurs.

MICROPROPAGATION

La micropropagation de caféiers comprend deux volets : microbouturage et embryogénèse somatique.

1) Microbouturage.

Le microbouturage *in vitro* de caféiers a été mis au point en 1980 (11, 21, 24). La technique de base a été par la suite transférée au Costa Rica où elle a été adoptée aux conditions particulières des pays tropicaux par Berthouly (com. pers.). Au Costa Rica, le microbouturage a été utilisé pour la multiplication d'hybrides F1 issus de fécondation manuelle entre Catuai et Catimor.

Le principe du microbouturage est simple : un nœud de tige orthotrope chorophyllienne débarassé de stipules pour faciliter la sortie des bourgeons est mis sur un milieu minéral de base additionnée d'une cytokinine (12, 21, 24).

L'explant produit une tige qui est découpé en microboutures de un nœud avec une paire de feuilles. Ces microboutures mises sur milieu frais produisent à leur tour de nouvelles tiges, qui à leur tour seront découpées en microboutures et ainsi de suite, tandis que les explants initiaux, recyclés chaque fois sur milieu frais, produisent de nouvelles tiges.

Les tiges de développement suffisant sont enracinés par trempage de leur base dans un mélange d'auxines et repiquage sous mist, dans un substrat poreux. Cette rhizogénèse en conditions non *in vitro* conduit à un système racinaire puissant et bien développé.

Les microboutures enracinées sont transplantées en pépinière où elles se développent en plantules aptes à la mise en plein champ.

L'expérience montre que les caféiers issus de microboutures ont, par rapport aux seedlings de même origine génétique, un développement et une entrée en production plus précoce.

Le taux de multiplication 10 boutures/80 jours, variable selon les génotypes, est trop faible et peut encore être amélioré. Un coût trop élevé des caféiers issus de microboutures peut, en effet, constituer un frein à leur usage, en particulier pour la reproduction de type compact dont les densités/ha sont très élevées.

Pour information, disons que des tiges orthotropes *in vitro* peuvent aussi être obtenues à partir d'apex, de bourgeons néoformés sur fragments d'entre-noeud ou encore par reversion de plagiotrope (12, 13, 24, 31, 37).

Le noeud reste cependant l'explant le plus pratique, le plus sûr, le plus rentable, donc le plus utilisé.

2) Embryogénèse somatique

Avec un taux de multiplication plus élevé que celui du microbouturage, l'embryogénèse somatique apparaît comme un procédé de reproduction de caféiers très prometteur. La garantie de conformité offerte par l'embryogénèse somatique serait par contre moindre. En fait, nous manquons totalement de données sur le comportement en champ des caféiers issus d'embryogénèse somatique.

Ce procédé est connu chez les caféiers depuis plus de 20 ans. Le premier cas d'embryons somatiques de caféiers a en effet été découvert en 1970 chez *C. canephora* (63). Depuis de nombreux travaux ont été effectués sur ce thème et aujourd'hui, il est possible d'obtenir des embryons somatiques de caféiers à partir d'explants très différents : fragments de feuille, de tige, parois d'ovule, d'anthère .. en conditions physico-chimiques également très diverses (25, 36, 39, 43, 44, 46, 49, 55, 57, 58, 59).

L'explant le plus employé est un fragment de feuille, matériel abondant, facile à renouveler, sans traumatisme de la plante donneuse.

La stratégie la plus utilisée comporte deux phases dites « induction » et « différenciation ». Au cours de la première phase, sur milieu d'induction, il y différenciation de cellules somatiques en cellules embryogènes : la 2e phase a lieu sur milieu de différenciation, dont le rapport A/C est toujours inférieur à celui du milieu d'induction ; au cours de cette seconde phase de différenciation, il y a développement des cellules embryogènes en embryons somatiques bien organisés.

Selon les conditions de culture, ces embryons somatiques apparaissent au bout de 3 à 6 mois.

Selon Sondahl et Sharp (58, 60, 61), le déroulement de l'embryogénèse somatique de caféiers se fait suivant deux modalités différentes qui ont été appelées Low Frequency Somatic Embryogeneis (LFSE) et High Frequency Somatic Embryogenesis (HFSE). On pourrait résumer ces deux modalités comme suit :

- dans le cas de LFSE, les cellules embryogènes différenciées au cours de la phase d'induction se développent directement en embryons somatiques bien constitués.

- dans le cas de HFSE, les cellules embryogènes donneraient naissance d'abord à un cal embryogène qui ensuite se développerait en embryons somatiques.

La réalité en fait est beaucoup plus complexe ; ainsi, la filiation premières cellules embryogènes différenciées et cal ou tissu embryogène à haute fréquence n'a jamais été établie.

Au plan pratique, on doit noter :

- les cals ou tissus à haute fréquence se différencient plus tardivement que les LFSE, environ deux mois plus tard.

- les cals à haute fréquence peuvent être induits directement sans différenciation préalable de LFSE.

- les cals à haute fréquence conduisent toujours à une production d'embryons somatiques en grand nombre, donc à taux de multiplication très élevé, les différenciations pouvant être réalisées en milieu solide ou en milieu liquide.

Bauman et son équipe ont décrit une modalité d'embryogénèse somatique de caféiers qu'ils ont appelée *self control somatic embryogenesis* (SCSE). Celle-ci apparaît en fait comme un cas de HFSE induit en milieu liquide avec multiplication des embryons auto-entretenue par embryogénèse adventive (4).

Les séquences et combinaisons hormonales utilisées par les différents auteurs, dans cette stratégie à deux phases, sont variables d'un chercheur à un autre, ce qui traduit l'extrême souplesse de réponse à l'embryogénèse somatique chez ces végétaux.

Quelles que soient leur origine et modalités de différenciation (LFSE, HFSE, SCSE), les embryons somatiques de caféier, au terme de leur développement, comprennent : deux feuilles cotylédonaires, une zone hypocotylaire terminée par une zone racinaire. Le rapport de dévelopement hypocotyle/cotylédon est fonction de la richesse du milieu de différenciation en cytokinine ; une teneur trop élevée en cytokinine conduit à un développement exagéré des cotylédons.

Semés sur milieu de germination, ces embryons somatiques se développent en plantules qui, après passage en pépinière, acquièrent un développement suffisant pour être mises en plein champ où elles conduisent à des caféiers normaux et fertiles.

LA PRODUCTION EN MASSE D'EMBRYONS SOMATIQUES

L'emploi de l'embryogénèse somatique comme technique de multiplication à grande échelle de caféiers de valeur implique la maîtrise de la technologie de production en masse de ces embryons somatiques et de leur germination en plantules récupérables en champ.

La stratégie de production en masse d'embryons somatiques de caféiers repose sur deux constatations :

1) Les embryons somatiques de première génération produisent spontanément, par bourgeonnement, des embryons somatiques de 2e génération, qui à leur tour produiront une troisième génération et ainsi de suite. Ce processus, qui peut être auto-entretenu, est appelé embryogénèse adventive ou embryogénèse secondaire.

2) A certain stade de leur développement, les cellules embryogènes obtenues sur milieu d'induction peuvent se multiplier en tant que telles pour donner de nouvelles cellules à potentialités embryogènes.

La stratégie de production en masse d'embryons somatiques basée sur l'exploitation de l'embryogénèse adventive a été développée par Staristsky sur *C. canephora*. Des paquets d'embryons sont mis en suspension ; il y a production continue d'embryons à la fois par bourgeonnement et à partir de cellules embryogènes disséminées dans le milieu. Par filtration, on récupère les embryons complètement développés pour les passer en germination tandis que le reliquat remis sur milieu frais continue à produire de nouveaux embryons (62).

La deuxième stratégie basée sur l'aptitude des cellules embryogènes à se multiplier en tant que telles est plus classique et d'une meilleure rentabilité en embryons somatiques. Ici, les cals embryogènes obtenus en fin de phase d'induction ainsi que les cals à haute fréquence obtenus sur milieu de différenciation sont mis en suspension.

Les cellules embryogènes se multiplient tout en restant accolés sous formes d'agrégat, de microcals embryogènes. Ces structures embryogènes, transférées sur milieu de développement, produisent des embryons qui parfois restent accolés, formant de véritables bouquets d'embryons à différents stades de développement.

Le manque de synchronisme dans le développement des embryons est fréquent. Des améliorations ont parfois été obtenus par addition d'acide abcissique au milieu de différenciation. La filtration périodique avec mise en culture d'agrégat de dimension comparables constitue le meilleur moyen de vaincre partiellement cet asynchronisme.

La technologie de production en masse d'embryons somatiques de caféiers est aujourd'hui parfaitement maîtrisée par plusieurs laboratoires publics et privés.

Des embryons somatiques de caféiers peuvent aussi être obtenus en une seule étape. Dans cette embryogénèse à une phase, l'explant reste sur un milieu unique, tout le temps jusqu'à différenciation d'embryons complètement formés.

La composition de ces milieux uniques est différente selon les auteurs (25, 55, 68).

Cette embryogénèse sur milieu unique peut paraître pratique ; en fait, elle est mal adaptée aux technologies de production en masse et à l'induction d'embryogénèse chez les types récalcitrants, en raison d'une gamme de combinaisons équilibrées auxine/cytokinine plus limités que dans la stratégie à deux phases.

En résumé, l'embryogénèse somatique de caféier apparaît comme un processus simple, facile à réaliser. A l'heure actuelle, beaucoup de données concernant les méthodologies d'obtention d'embryons somatiques sont disponibles ; par contre, nous manquons totalement d'informations sur le comportement en champ de caféiers issus de différentes techniques d'embryogénèse somatique. De telles données sont cependant indispensables pour déterminer les limites et le conditions d'utilisation de ce procédé comme moyen de reproduction asexuée de caféiers cultivés.

CONCLUSION

La reproduction asexuée constitue, en toutes circonstances, le meilleur relais pour une amélioration rapide et efficace des caféiers cultivés, qu'ils soient autogames ou allogames.

Jusqu'à preuve du contraire, l'emploi à grande échelle des techniques de micropropagation ne devrait concerner que les caféiers arabica. Il existe en effet pour les canephora arabusta un procédé de bouturage peu coûteux, bien adapté au contexte économique des pays producteurs de

robusta et qui a fait ses preuves depuis bien longtemps. Ce procédé de bouturage peut encore être amélioré.

Les caféiers canephora ont cependant une excellente réactivité en culture de tissus. Cette espèce pourra donc être utilisée pour des travaux d'approche, de méthodologie, d'évaluation, d'induction *in vitro*, de variabilité...

Microbouturage et embryogénèse somatique présentent chacun leur lot d'avantages et d'inconvénients.

Le microbouturage qui offre toutes les garanties de conformité sera donc la seule technique utilisable pour les conservations de germplasm et échange de matériel. L'expérience a par ailleurs montré que les caféiers issus de microbouturage avaient un développement et une entrée en production plus précoces que les seedlings de même origine génétique. Le taux de multiplication du microbouturage, encore trop faible, risque de gréver le prix de revient de la plantule et le coût à la plantation, surtout s'il s'agit de multiplier les types compacts à forte densité/ha.

L'embryogénèse somatique, avec un taux de multiplication très élevé, pourrait conduire à une plantule de coût moindre, mais en embryogénèse somatique, le passage inévitable par une phase cal indifférencié plus ou moins longue peut être source de variations somoclonales plus ou moins importantes.

A ce sujet, on peut cependant noter que chez les caféiers le produit final de consommation est une boisson qui résulte de transformations technologiques et de mélanges et où, *in fine*, les caractéristiques individuelles disparaissent dans le cadre d'un produit final standard. En conséquence, un certain taux de variation sera donc tolérable, surtout si ces variations n'affectent pas les qualités agronomiques, rendement/ha, qualité, résistance aux maladies, etc.

Pour être concurrentiels, les caféiers issus de micropropagation doivent être dotés de réelles plus-values génétiques (productivité, qualité, résistance aux maladies ...), or les recherche sur l'embryogénèse somatique ont toujours été effectuées sur variétés commerciales, reproduites par graines et dont les embryons somatiques ne présentent aucun avantage génétique.

Pour promouvoir l'emploi de caféiers issus de micropropagation, il faudrait une plus grande coopération entre laboratoires de recherches des pays industrialisés et les sélectionneurs de terrain qui sont les premiers à connaître les structures hétérozygotes dotées de plus-value et capables de faire les frais d'une reproduction asexuée par micropropagation.

Une telle coopération est tout aussi indispensable pour le suivi du comportement en champ des caféiers issus d'embryons somatiques.

Les biotechnologies végétales et leurs applications pratiques sont en progrès constant dans les pays industrialisés grâce aux contributions apportées par les firmes privées soucieuses des avantages que peut fournir chaque gain génétique.

Dans les pays en voie de développement, l'amélioration des caféiers est du ressort des seuls organismes publics dont les moyens de travail sont souvent réduits et nettement insuffisants. Une plus grande intégration d'organismes privés au niveau de ce secteur devrait pouvoir accélérer les solutions génétiques à bien des problèmes concernant l'amélioration des caféiers. Ce serait, en quelque sorte, une façon de « *renvoyer l'ascenseur* » à ces pays producteurs.

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Résumé

La sélection de structures hétérozygotes de valeur et leur reproduction par voie asexuée constitue la voie d'amélioration la plus efficace et la plus rapide des végétaux arbustifs pérennes à cycle long.
Ce type de sélection permet d'exploiter tout individu exceptionnel de valeur apparu au hasard de croisements, de mutations, de variations somaclonales, de manipulations génétiques.
Pour être efficace, toute sélection clonale nécessite donc une technique de reproduction asexuée éprouvée, facile d'utilisation.

Chez les caféiers allogames (Robusta), grâce à une technique de multiplication végétative simple, peu coûteuse, bien adaptée au contexte des pays en développement, d'importantes améliorations génétiques ont été obtenues.

Chez les caféiers autogames (Arabica), l'amélioration basée sur des croisements intra et interspécifiques a pour but l'obtention de génotypes résistants à la rouille (Hemileia vastatrix) et débouche souvent sur des structures hétérozygotes intéressantes jusque là négligées.

Depuis quelques années, grâce au développement de la biotechnologie, des méthodes modernes de reproduction par voie asexuée des caféiers Arabica (microbouturage et embryogenèse somatique) ont été appliquées avec succès par des laboratoires privés et publics. Ces procédés devraient permettre la réalisation d'importants progrès dans l'amélioration génétique du caféier Arabica.

Summary : Modern techniques of asexual reproduction : influence on genetic improvement of coffee.

Selection and asexual reproduction of valuable heterozygous structures is certainly a quick, efficient mean for genetic improvement of perennial, long cycle woody plants. Such a strategy, which will help to exploit any exceptional valuable genotype derived from crosses, mutations, genetic manipulations, needs to be used with an easy manageable asexual technique of reproduction. Important genetic improvements have been carried out in allogamous coffee (Robusta) thanks to an horticultural technique of vegetative reproduction, cheap, easy to reproduce, well adapted to economic conditions of developping countries, producers of Robusta. In Arabica, breeding (through intra and interspecific crosses) aims at obtaining genotypes resistant to rust (Hemileia vastatrix) and often comes to heterozygous structures with added value which so far were neglected, as an asexual technique of reproduction was then not available. Since a few years, with development of biotechnology, modern techniques of asexual reproduction of Arabica plants, by micorcuttings and somatic embryos have been performed by public and private laboratories. The utilization of these techniques should help to make important progresses in genetic improvement of Arabica coffee.

ADVANCES IN GENETIC MANIPULATION OF THE COFFEE PLANT

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Introduction

Coffea arabica L. and *Coffea canephora* Pierre are the most cultivated coffee species worldwide. Culturally, coffee drinking has become a must for people in both producing and consuming nations and efforts are being made to widen coffee consumption especially in eastern Europe and China. For producing countries, green coffee trading is of prime importance as a source of hard currencies. Coffee breeding is therefore mandatory for the improvement of quality and other characteristics to raise competitiveness in coffee production. Classical means of crossing have yielded higher producing varieties, cultivars with specific pathogen resistance, besides an array of cultural traits as witnessed by the series of ASIC meetings.

Modern ways to generate improved coffee plants have emerged during the last two decades. *In vitro* culture techniques are well developed for this species, so that different laboratories are in position to routinely induce somatic embryogenesis to regenerate intact coffee plants. *C. arabica* plants derived from protoplasts can be now regenerated (ACUÑA & PEÑA 1991), opening the way for the application of direct DNA transfer techniques and protoplast fusion experiments, aiming at manipulating the genome of promissory varieties. The regeneration of haploid plants derived from anther/ovule cultures is also an area of intense research both because of its use in genetic studies and improvement. A thorough discussion on the application of biotechniques in coffee can be found in SÖNDAHL (1989).

Agrobacterium tumefaciens Smith & Townsend, Conn., causes crown gall, a neoplastic disease, in many dicotiledoneous plants by transfering DNA, the T-DNA in the genome of its host plants. The T-DNA resides in the megaplasmid known as tumor inducing plasmid or *Ti*-plasmid. Genes responsible for the induction and maintenance of crown gall tumors and for the synthesis of opines are carried in the T-DNA. The latter compounds serve the bacterium as a source of carbon and nitrogen and are only synthesized after introduction of T-DNA into the host genome. This natural plant transformation system has been modified in a manner, that it does not cause crown gall. This was achieved by deletion or inactivation of tumor genes in the T-DNA. The capability to stably introduce DNA has been left intact (WEISING et al.(1988). Several plant species are now being transformed by its use (HOOYKAAS 1989), and this could be extended to coffee. Although coffee is a dicotiledoneous plant, and therefore a potential host for *Agrobacterium*, natural interactions between this two organisms are not commonly observed. It may be expected that this interaction can be forced in the laboratory as we have already reported (OCAMPO et al. 1989).

We present here data supporting the assumption that *A. tumefaciens* is able to genetically transform coffee tissues. This system could be further adapted for transfering heterologous genes of interest to coffee plants.

Materials and Methods

Plant material

Seeds of *C. arabica* cv. caturra were used for this study. The seeds were surface sterilized in 2.5% sodium hypochlorite for 5 minutes and then were extensively washed with sterile water. They were germinated over water-agar at room temperature in the darkness until the length of the hypocotyls were about two times that of the seed.

Bacterial strains

Wild-type *A. tumefaciens* strains were kindly provided by the culture collection of Centro de Investigaciones Microbiológicas CIMIC, Universidad de los Andes, Santa Fe de Bogotá. These strains were isolated from local host plants and were designed 17613, 3565, 3569, 391. The strain A281 was obtained from Centro Internacional de Agricultura Tropical CIAT, Cali, and is known as supervirulent causing rapidly appearing tumors in a variety of plants. It carries the plasmid pTiBo542 (JIN et al. 1987). The oncogenicity of strains was tested in tomato (*Lycopersicum esculentum* Mill.) plants. Tumors developed in tomato within a month.

Inoculation

Hypocotyls were inoculated by puncturing them with a needle, that was smeared with colonies of each strain of *Agrobacterium*, while the hypocotyls were still attached to the seed. They were then plated on MS medium (MURASHIGE & SKOOG 1962) without adding growth hormones. The medium also contained carbenicillin (200 µg/ml, Sigma) and cefotaxime (400 µg/ml, Hoechst) to inhibit bacterial growth. This medium composition was used throughout. After about tree weeks, when tumors were apparent, they were excised and plated again.

Opine assay

About 100 mg tumor tissue was used for opine detection. The tissue was squeezed in an Eppendorf microtube and cleared by centrifuging at 14000 rpm in the microfuge. 4 µl of the supernatant were assayed for the presence of opines after paper electrophoresis as described by REYNAERTS et al. (1988). Authentic standard compounds were coelectrophoresed to confirm the assay.

Results

All strains of *A. tumefaciens* tested incited neoplastic growth at the sites of inoculation. The tumorous nature of this tissues was tested by their capacity to grow on rich media in the absence of growth stimulating plant hormones (Fig.1). This effect could be evidenced around 4 weeks after inoculation and the tumors survived under the experimental conditions described here for over 6 months after they were excised from the germinated seed. Control, non-inoculated tissue survived only between 15 and 30 days and died thereafter. The appearance of neoplastic growth varied in efficiency depending on the strain used (Table 1). The supervirulent strain A281 showed the highest efficiency among the strains tested. Some of the tumors stopped growing after a few months and died finally.

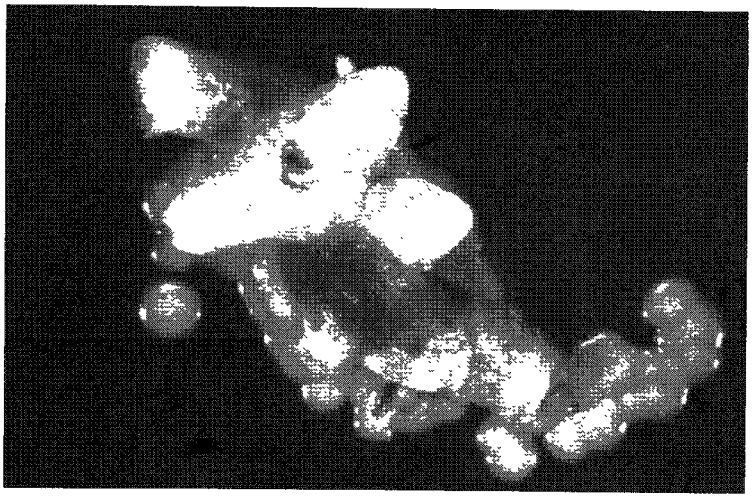

Fig. 1 Hypocotyl explant from C. arabica showing neoplastic growth, seen as clear outgrowth. Darken tissue is in the process of necrosis. Some Agrobacterium colonies can also be seen.

TABLE 1

Tumour formation by *A. tumefaciens* strains in coffee hypocotyls

Strain	# of samples	# of tumours	% of tumours
17613	30	5	16.6
3565	30	3	10.0
3569	30	4	13.3
391	30	5	16.6
A281	30	13	43.3

Although evident to the naked eye, tumors growed slowly and the amount of tissue obtained was in terms of fresh weight scarce, making molecular analysis difficult. Opines could only be assayed in the samples shown in table 2.

TABLE 2

Opines assay in coffee hypocotyl tumours

Agrobacterium strain	Octopine	Nopaline	Agropine
3565	(-)	(-)	(-)
391	(-)	(-)	(+)
A281	(-)	(-)	(+)

The test was positive for agropine in the tumor tissue incited by strains 391 and A281. All other opines tested gave negative results for the conditions of the assay reported here.

Discussion

The approach of this study was the utilization of wild-type *A. tumefaciens* strains to test the potential use of this bacterium for the genetic transformation of coffee. The system was choosed, as the tumor phenotype is a relatively easy way to determine if *Agrobacterium* is capable to achieve this goal. The infections were tried in many different coffee tissues under *in vitro* conditions hoping to have manipulatable systems to carry out this type of study. As stated above, crown galls are not observed in natural coffee plantations and attempts to incite galls *in planta* have failed as reported in the survey of DE CLEENE & DE LEY (1976) and under our hands.

We succeeded by infecting hypocotyls of *in vitro* germinated coffee seeds (Fig. 1). The production of tumors could be evidenced (Table 1) and agropine could be detected in two of the treatments (Table 2).

Although our observations are at a very preliminary stage, it seems in the light of the data presented here, that indeed *A. tumefaciens* can be developed as a transformation system for coffee. Strains with higher efficiency for gene transfer to coffee cells should be identified. On the other hand, the hypocotyl system is not optimal since regeneration of complete plants starting with this tissues has not been reported to our knowledge. Trials in our laboratory have been unsuccessful so far.

Studies are under way making use of embryogenic cell suspensions as starting material, together with disarmed strains carrying vectors with selectable marker genes like *bar* (confering resistance to phosphinothricin) and NPTII (coding for neomycin phosphotransferase), and screenable marker genes like GUS that codes for ß-glucuronidase.

Other techniques of gene transfer to plants are now at the hands of the experimenters. Direct DNA delivery methodologies have been developed for plants not amenable to *Agrobacterium* transformation, particularly for monocots like cereals (DAVEY et al. 1989). Direct DNA transfer methods have the advantage of bypassing biological barriers. DNA uptake by regenerable coffee protoplasts mediated by chemical or physical means is now possible to be worked out (ACUÑA & PEÑA 1991). The use of microproyectiles or biolistics (KLEIN et al. 1987) offer another possibility. This procedure evades biological complications by shooting DNA glued to heavy metal particles. Several biolistic devices have been developed and are used routinely. Transgenic plants have been recovered already by the use of biolistics (POTRYKUS 1989).

In frame of our studies on the molecular mechanisms of resistance in coffee against the coffee leaf rust, *Hemileia vastatrix* Berk & Br., we are interested in having a transformation system for coffee at hand. It would allow basic studies concerning the biology of the coffee plant to be performed, with the aim at identifying and cloning *Coffea* genes and their corresponding regulatory regions. Heterologous genes could also be transferred in order to confer resistance, by example, to the coffee borer *Hypothenemus hampei* Ferrari, like BT-endotoxin genes from *Bacillus thuringiensis*, and proteinase inhibitor genes. This insect has become a menace to coffee growing countries.

The area of genetic manipulation promises applications for coffee, perhaps not in the near future, but this is related to the efforts that shall be made in research and should be strengthen.

Acknowledgement

We wish to thank Dr. M. Quijano-Rico for presenting this contribution to the 14th ASIC Conference on Coffee Science in San Francisco.

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Abstract

Methods for the genetic manipulation of the coffee plant are being developed. We are focusing our attempts to apply plant transformation procedures based on *Agrobacterium tumefaciens*. The availability of such a system will allow the use of cloned genes of interest in plant biology to be transfered to the coffee plant. This will permit the access to new methodologies in coffee breding, and will also allow to use the modern techniques of molecular biology as an approach to study the biology of the coffee plant. We have observed infection of coffee hypocotyls with wild-type *Agrobacterium* strains. In those tissues, *A. tumefaciens* incites tumors, able to grow in a chemically defined medium (MS) but without hormones added. Methods for regeneration of coffee from these tissues have not been reported, so we are also working in this field. The capacity to incite tumors is an indication of the potential use of *Agrobacterium* to transform coffee. We are now using *bar*, NPTII and GUS genes as markers. Other tissues and cells are being used, like embryogenic cell suspensions. We report here advances obtained in the adaptation and optimization of procedures to genetically transform the coffee plant.

PROTOPLAST CULTURE AND REGENERATION IN *COFFEA* SPECIES

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INTRODUCTION

Cultivated coffee species are restricted to a few cultivars. Some of the breeders' objectives are to increase the variability inside the genus using biotechnology techniques.

One way is the use of protoplast technology characterized by the culture of single cells. It can induce more or less somaclonal variation. It also allows to perform fusion experiments for the transfer of genomic and/or cytoplasmic information from relative species. Some preliminary conclusions drawn from interspecific sexual crosses lead to suppose that environmental specificity of coffee species could be determined by cytoplasmic information. Therefore somatic hybridization could be useful to transfer this information without modifying the nuclear genome. Another possible way of use of protoplast is the transformation with foreign DNA allowing to regenerate transformed plants without problems of chimera.

Until now only a few results have been obtained on coffee protoplast regeneration. One can note the work of Söndahl et al (1980) on callus induction of Coffea arabica protoplasts but without any regeneration; likewise in 1984 Orozco and Schieder mentioned the obtention of callus from protoplast of Coffea arabica and Coffea canephora and the sexual hybrid "arabusta". In 1986 at the Minneapolis symposium, Yasuda et al mentioned the obtention of plantlets from protoplasts of Coffea arabica.

Only one publication (Schöpke et al 1987) reported plantlet regeneration of protoplasts from somatic embryos of Coffea canephora.

Success in regenerating plants from protoplasts of woody species has, until now, been low (Davey and Power, 1988). We describe hereafter the techniques for protoplast isolation, culture and plantlet regeneration through somatic embryogenesis in Coffea arabica, Coffea canephora and the sexual hybrid "arabusta".

MATERIAL AND METHODS

Cultivars of the different Coffea species (C. arabica cv Caturra, C. canephora, R5 and the interspecific hybrid "arabusta" 1312.2) were provided by IRCC/CIRAD (FRANCE) and are cultivated in the greenhouse or maintained by microcuttings in vitro. After a disinfection of 2 minutes in HgCl2 solution (1g/l) and two washes with bidistilled sterile water, leaf fragments (1 cm2) are cultivated in vitro on solid medium 1 (modified MS medium: Murashige and Skoog 1962); with 2,4 Dichlorophenoxy acetic acid (2,4D) 0,3 mg/l and Kinetin 1,5 mg/l (Tab 1) autoclaved at 120°C for 20 minutes and incubated in a room culture of 26°C with a photoperiod of 16 hours given by fluorescent lights 75μEm-2s-1. Induced callus containing meristematic cells are subcultured on liquid medium 2 (Pierson et al. 1983) with Isopentenyl adenine 5 mg/l and Indole butyric acid (IBA) 1mg/l (Tab 1), and subcultured every month (for details see Zamarripa et al, 1991).

Small aggregates (2 g) of 2 weeks old cell suspension are incubated for 6 hours in Petri dishes (diameter 90 mm) with 15 ml enzyme solution containing (w/v) Cellulase Onozuka R 10 2%, Macerozyme Onozuka R 10 1%, Pectolyase Y 23 0.2% M.E.S. (2N (morpholino) ethane sulfonic acid) 0,5mM, CaC12,2H20 25mM, Mannitol O.5M at pH 5.5, on a rotary shaker 50 rpm. The enzyme solution and protoplast medium are sterilised by filtration on a 0.25μm filter. The solution is strained on nylon mesh 25 μm and centrifugated at 100 G for 5 minutes; protoplast are washed twice with liquid medium 3 (modified Blaydes 1966, version 2 Tab 1) and cultivated in darkness at a concentration of 200,000 protoplasts/ml in 3 ml of medium 3 with 2,4 D 0.5 mg/l and Naphthalene acetic acid (NAA) 0.5 mg/l and Kinetin 0.5 mg/l in Petri dishes diameter 30 mm.

Monthly, protoplasts are subcultured by renewing half of the medium, with medium 4 containing Benzyl amino purine (BAP) 1mg/l (Tab.1) with a progressive reduction of the osmotic pressure by a decrease of glucose concentration from 92 g/l to 54, 36 and 18 without any variation of saccharose concentration (20 g/l). After the fourth month, the culture is diluted every month by half with medium 5 (Yasuda et al. 1985) with BAP 1 mg/l (Tab 1). Seven months old microcallus are transferred to the same solid medium (Agar 8 g/l) and subcultured every two months on this medium. Two months later globular embryos appear on the callus and after two months cotyledons develop; then they are transferred to solid medium 6 (Tab. 1) with Indole acetic acid (IAA) O.5 mg/l and Kinetin 0.1 mg/l (Tab 1). At two leaves stage, embryos are transferred to pots with 1/3 sand and 2/3 compost in the greenhouse.

RESULTS AND DISCUSSION

The choice of embryogenic type of cells induced on leaf fragments (Fig. 1.A) is important to develop a competent cell suspension for protoplast isolation.

Protoplast yield is high with meristematic cell suspension (Fig. 1.B) but decreases if somatic embryos are induced. No significative differences are observed in isolation of protoplast of the three cultivars.

First divisions of coffee protoplasts appear after 2 to 3 days of culture; then the development is quite slow. Clusters of only 6 cells appear after 1 month (Fig. 1.C) and another 6 months are required to obtain microcallus of 2 or 3 mm big enough to be transferred on to solid medium in the case of Coffea "arabusta" and Coffea canephora. The conversion of protoplast into calli reaches about 30%, then 50% of the calli regenerate similarly to the process of "high frequency somatic embryogenesis" as described by Söndahl and Sharp 1977 (Fig. 2.A.B.C). The development of globular embryos into plantlets is easy (Fig. 3.A.B.C) for the genotype of "arabusta" 1312.2 maintained on the medium 6 with IAA O.5 mg/l and agar 8 g/l but more difficult for the genotypes of canephora and arabica. More recent results with Coffea arabica give the regeneration of plantlets in six months after protoplasts' isolation. It is necessary to transfer at cotyledon stage to medium 6 with BAP 0.2 mg/l and Gelrite 2 g/l, then transfer to the same medium as "arabusta" but with gelrite. In these conditions the rooting and the development are better. The transfer to the greenhouse

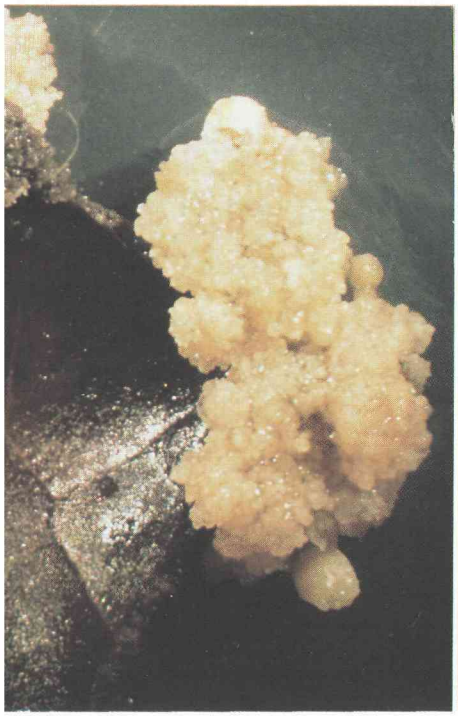
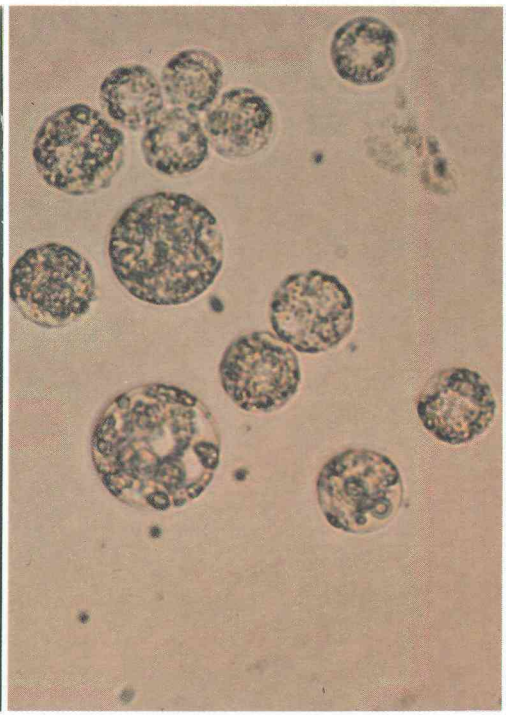
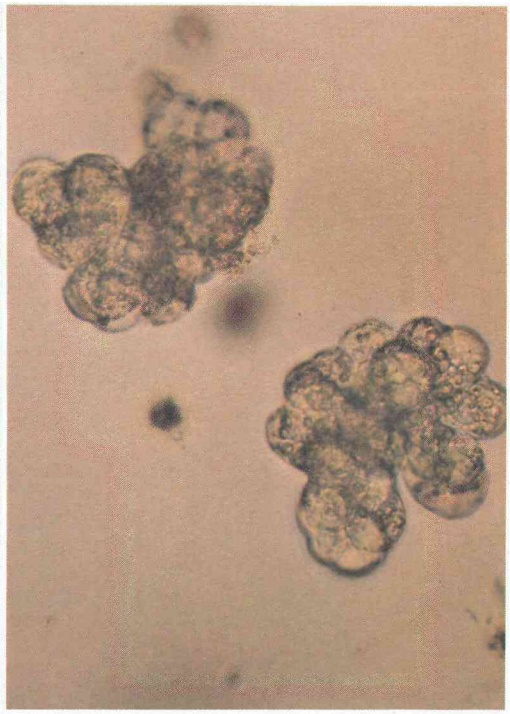


Fig. 1.A. Induced calli on leaf fragment

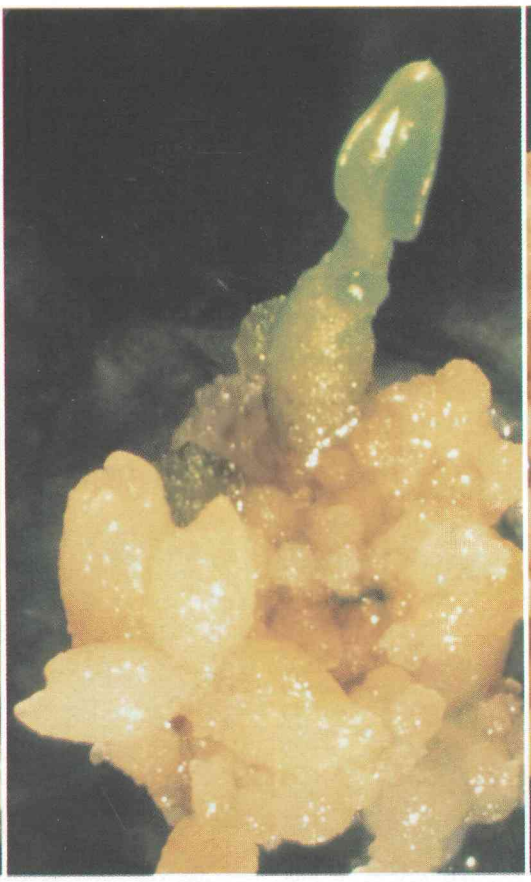


B. Isolated Protoplasts _____ 25 μm

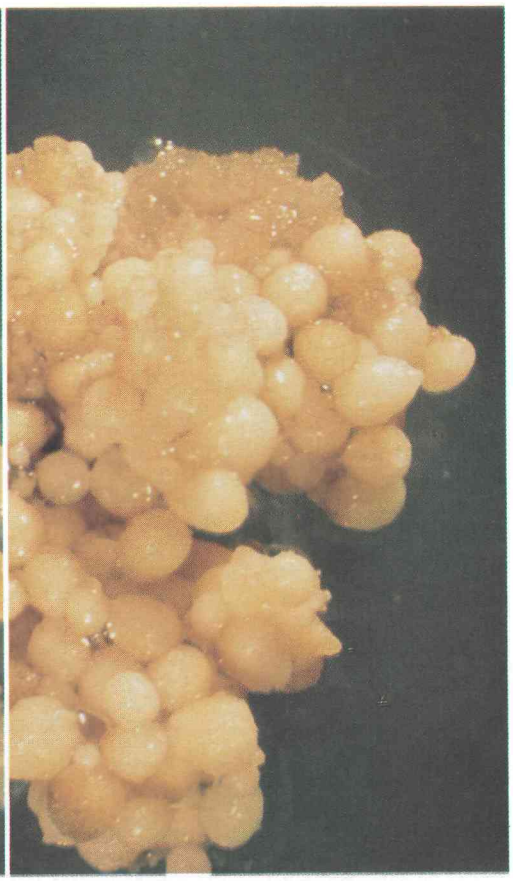


C. Clusters of 6 cells from protoplast _____40 μm

_____ 400 μm



_____ 500 μm



_____ 500 μm

. 2. Embryogenic calli from protoplast
A. Coffea "arabusta" 1312.2

B. Coffea arabica caturra

C. Coffea canephora R5

_____ 1,5 mm _____ 3 mm _____ 5 mm

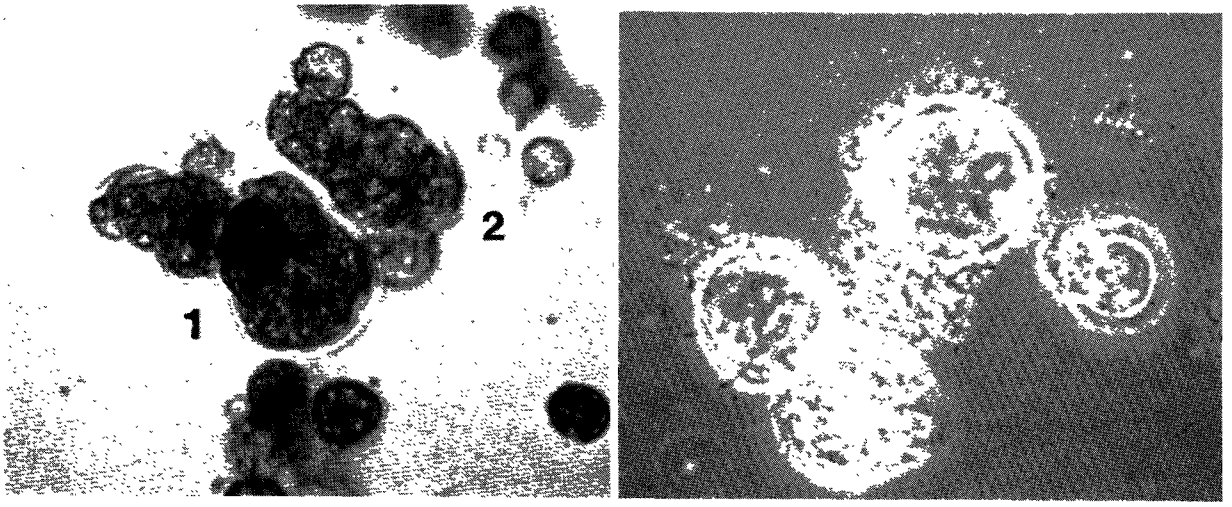
Fig. 3. Germination of somatic embryos and plantlet regeneration
A. "Arabusta" 1312.2 B. Coffea arabica caturra C. Coffea canephora R5

Fig. 4. Regenerated plantlets for protoplast of Coffea "arabusta" and Coffea canephora R5

depends on the qualities of the embryos, a well shaped apex with two leaves is necessary (Fig. 4). Some progress has been made in the rapidity of embryo development in Coffea arabica by transferring the embryos at torpedo stage to medium with gelrite.

Some preliminary results of protoplast co-culture with different Agrobacterium strains have been obtained. We used this transformation technique in order to avoid some technical or legal problems linked to other transformation techniques.

The strain used is disarmed Agrobacterium tumefaciens plasmid PGV 2260 35 GUS-Intron with kanamycin resistance and the Gus-Intron marker of Glucuronidase constructed by Vancanneyt et al. 1990 (kindly provided by L. Jouanin, INRA, FRANCE). One or two days after isolation, protoplasts are mixed with a 12 hours' old culture of bacteria at the concentration of 100 million per milliliter of protoplasts suspension (Fig. 5.A). 48 hours later the protoplast culture is washed in medium 3, then Ceporine (100 mg/l) is added and renewed during two subcultures. One month later, Glucuronidase reaction is developed (see Jefferson 1987); the X-Gluc (5-bromo-chloro-3-indolyl beta-D-glucuronic acid), substrate of the enzymatic reaction is added to protoplast culture at 50 ug/ml and incubated overnight at 35°C. Preliminary experiments show a specific blue coloration of protoplast colonies (Fig 5.B). These first results should mean transient expression of the GUS gene in Coffea arabica caturra protoplast and must be confirmed at later stage development. DNA extraction and analysis will be made on callus after their development.



____ 12 μm ____ 50 μm

Fig. 5.A. Protoplast coculture with Agrobacterium tumefaciens
B. Glucuronidase activity in two months old transformed calli.
1. Transformed calli with integrated GUS gene (blue activity)
2. Grey calli without integration of GUS gene

CONCLUSION

The techniques developed here are suitable for the culture and regeneration of protoplasts in Coffea species as routine. This gives the opportunity to develop other tools for breeding as protoplast fusion between relative species. The first hopeful preliminary results in protoplast transformation have to be confirmed but are encouraging and open the way to introduce new traits in Coffea as it is currently made for others.

Tab.1: CULTURE MEDIA

(AMOUNT mg/l) pH 5.5

	MEDIUM 1	MEDIUM 2	MEDIUM 3	MEDIUM 4	MEDIUM 5	MEDIUM 6
MACRO-NUTRIENTS	MS*	MS/2	BLAYDES+	BLAYDES	YASUDA#	MS/2
MICRO-NUTRIENTS	MS	MS/2	BLAYDES	BLAYDES	YASUDA	MS/2
Na2EDTA	37.3	37.3	37.3	37.3	37.3	18.65
FeSO4,7H2O	27.8	27.8	27.8	27.8	27.8	13.9
MESO-INOSITOL	100	100	100	100	100	100
NICOTINIC ACID	1		1	1	1	1
PYRIDOXIN HCL	1		1	1	1	1
THIAMINE HCL	1	10	1	1	1	1
BIOTINE	0.01					0.01
CALCIUM PANTHOTENATE	1					1
L.CYSTEINE	20	50				
CASEINE HYDROLYSATE		100			250	
2,4.DICHLOROPHENOXY ACETIC ACID	0.3		0.5			
NAPHTALENE ACETIC ACID			0.5			
KINETIN	1.5		0.5			
ISOPENTENYL ADENINE		1				
INDOLE BUTYRIC ACID		5				
BENZYL AMINO PURINE				1	5	
SUCROSE	30000	30000	20000	20000	30000	10000
GLUCOSE			92000	92000		

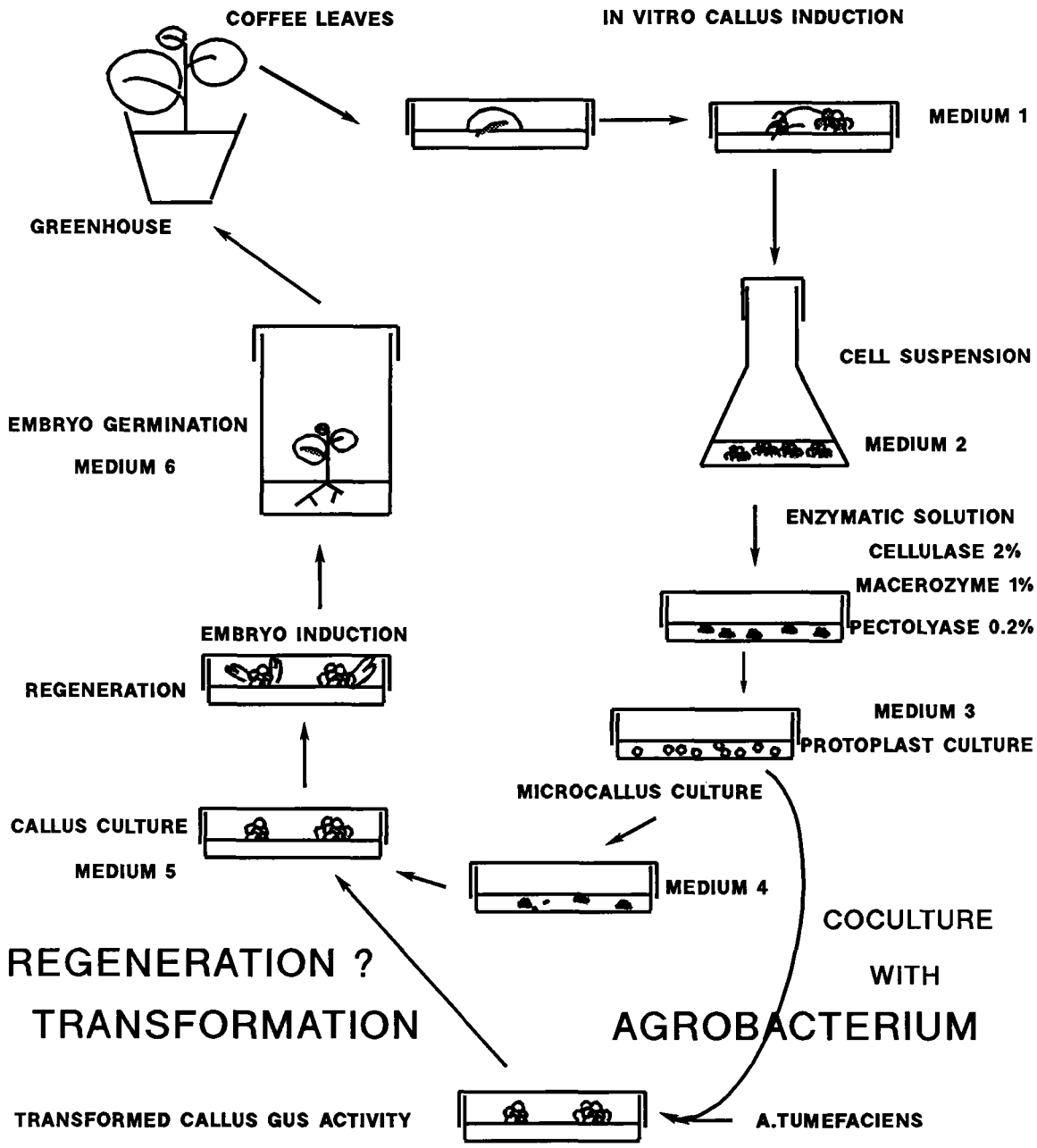
*MURASHIGE T. and SKOOG F. 1962

+BLAYDES D.F.1966

#YASUDA et al 1985

PROTOPLAST TECHNOLOGY

REGENERATION AND TRANSFORMATION



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Zamarripa, A., Ducos, J.P., Bollon, H. Dufour, M., Pétiard, V.
Production d'embryos somatiques de caféier en milieu liquide : effets densité d'inoculation et renouvellement du milieu (A paraître dans Café Cacoa Thé, n° 4., 1991).

SUMMARY

Plant regeneration is still a necessary step for the obtention of transgenic plants. Hereafter we describe the regeneration of plantlets from protoplasts of different Coffee species. Protoplasts of Coffea arabica, Coffea canephora and the interspecific hybrid "arabusta" are isolated from embryogenic cell suspensions derived from leaf callus. A standard enzyme mixture of cellulase, macerozyme and pectolyase is used for isolation; and protoplasts are cultivated in modified Blaydes medium with 2,4 dichlorophenoxy acetic acid, kinetin and naphthalene acetic acid. Cultures are diluted by half, monthly, with the same basal medium containing benzyl amino purine (BAP). Six or seven months later, calli are subcultured onto Yasuda solid medium with BAP. After two subcultures, embryos are detectable and transferred to solidified Murashige and Skoog medium for germination. Two-leaf stage plantlets are then transferred to the greenhouse for acclimatization.

RESUME

La régénération des plantes est encore une étape nécessaire à l'obtention de plantes transgéniques. Nous décrivons, ci-dessous, la régénération de plantules à partir de protoplastes de différentes espèces de caféier. Les protoplastes de Coffea arabica, Coffea canephora et de l'hybride interspécifique "arabusta", sont isolés à partir de suspensions embryogènes dérivées de cals de feuilles. Une solution enzymatique standard de cellulase, macerozyme et de pectolyase est utilisée pour l'isolement; puis les protoplastes sont cultivés dans un milieu modifié de Blaydes contenant de l'acide 2,4 dichlorophenoxyacétique, de la kinétine et de l'acide naphtalène acétique. Chaque mois, les cultures sont diluées de moitié avec le même milieu de base contenant de la benzyl amino purine (BAP). Six ou sept mois plus tard, les cals sont repiqués sur du milieu solide Yasuda avec de la BAP. Après deux repiquages, des embryons sont détectables et transférés sur du milieu solide de Murashige et Skoog pour la germination. Au stade deux feuilles, les plantules sont transférées à la serre pour acclimatisation.

DÉVELOPPEMENT D'UN PROCÉDÉ DE MULTIPLICATION EN MASSE DU CAFÉIER PAR EMBRYOGENÈSE SOMATIQUE EN MILIEU LIQUIDE

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INTRODUCTION

La multiplication végétative du caféier est possible, soit traditionnellement (bouturage, greffage, etc.) soit par micropropagation in vitro. Le bouturage, voie la plus utilisée dans la multiplication végétative, est cependant limité par le nombre réduit et la nature des boutures que peut produire un caféier. De plus, le bouturage exige l'installation de parcs à bois, ce qui engendre des contraintes de conservation et de superficie.

Depuis 1970, plusieurs auteurs: Staritsky (1970), Söndahl et Sharp (1977), Dublin (1980; 1981) Pierson et al.(1983), Yasuda et al. (1985), Garcia et Menendez (1987), Berthouly et al. (1987), ont montré que la micropropagation et l'embryogenèse somatique peuvent être appliquées au caféier.

Ces méthodes pourraient accélérer la diffusion de nouvelles variétés et conduire à un coefficient de multiplication plus élevé. En effet, une seule microbouture de caféier peut fournir au bout d'un an près de 20.000 plantules. Dans le même temps, une bouture dans des conditions horticoles ne fournira que 100-200 plantes (Dublin, 1984). Garcia et Menendez (1987) travaillant sur l'embryogenèse somatique en milieu solide mentionnent comme "production record" 60 à 65 embryons par explant après 14 semaines de culture.

L'embryogenèse somatique est la voie la plus favorable à la production d'embryons à grande échelle, grâce surtout à la culture en milieu liquide. Chez d'autres espèces, les résultats sont très prometteurs; par exemple, Pétiard et al. (1987) rapportent une capacité de production de 80.000 embryons de carotte par litre et par jour dans un bioréacteur de 10 litres.

Etant donné la longueur du cycle de reproduction, ainsi que le mode de bouturage du caféier, installer rapidement des plantations homogènes nécessite actuellement des délais longs et de grandes surfaces consacrées à la multiplication. Le développement d'une méthode de multiplication basée sur l'embryogenèse somatique en masse et en milieu liquide pourrait donc se révéler très intéressante, par exemple pour des clones sélectionnés de Coffea canephora ou pour des hybrides intra ou interspécifiques.

Cette étude décrit un procédé de production en masse d'embryons somatiques de caféier qui peut se décomposer selon les étapes suivantes (fig. 1):

a) Etablissement des souches de tissus embryogènes en milieu liquide
b) Phase de multiplication de tissus embryogènes
c) Conservation des souches par congélation
d) Phase de production d'embryons (en fiole d'Erlenmeyer ou en bioréacteur)
e) Phase de développement d'embryons

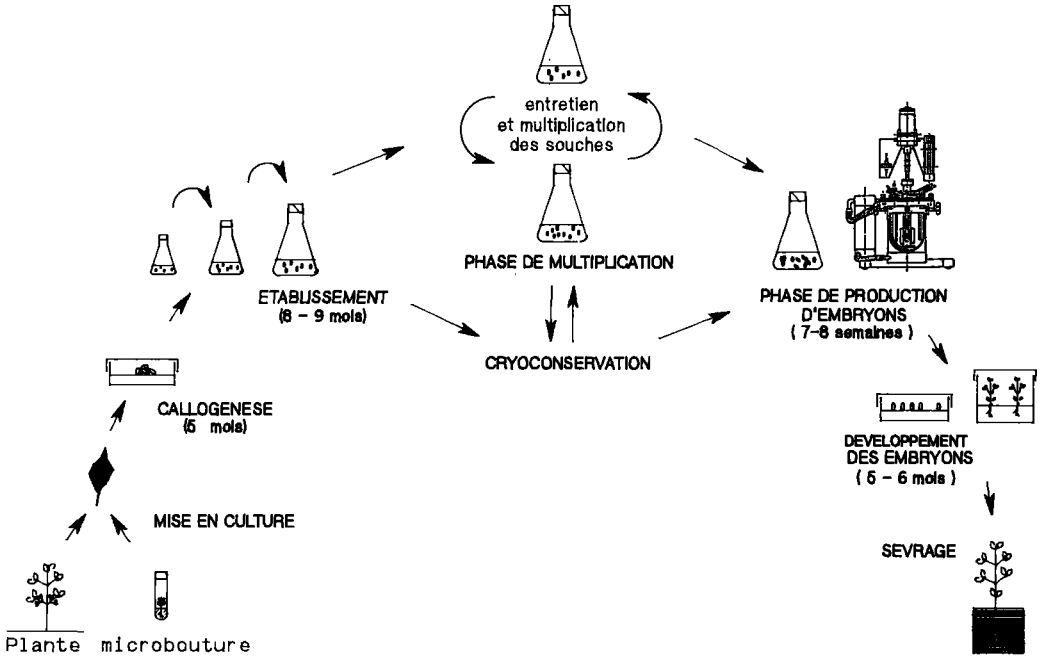

Fig. 1 - Schéma du procédé de multiplication du caféier en milieu liquide

ETABLISSEMENT DE SOUCHES DE TISSUS EMBRYOGENES

A ce jour 5 souches ont été obtenues à partir de 2 variétés de C. arabica (Catuai et Caturra), 2 clones de C. canephora (R2 et R5) et 1 clône de l'hybride Arabusta (1312-2). Tous les génotypes ont été fournis par l'IRCC/CIRAD.

Pour l'induction des cals, des fragments de feuilles ont été placés sur le milieu de callogenèse préconisé par Dublin (1984). La culture a été menée à l'obscurité. Les explants ont été repiqués sur un deuxième milieu semi-solide contenant de la BAP à 1 $mg.l^{-1}$ (Yasuda et al. 1985) et transférés à la lumière. Les cals obtenus, de couleur jaune crème et de texture friable, ont été transférés dans des fioles d'Erlenmeyer de 50 ml avec 20 ml de milieu. Un milieu proche de celui rapporté par Yasuda et al. (1985) a été retenu (Zamarripa et al. 1991). Par la suite, du milieu neuf est rajouté progressivement et les suspensions sont transférées successivement dans des fioles d'Erlenmeyer de 100 ml, puis de 250 ml. Les cultures sont placées sur agitateur à mouvement giratoire (110 rpm). La température est maintenue constante à 22-23°C.

Un des facteurs importants à considérer dans l'établissement d'une souche est la densité d'inoculation lors du transfert des cals en milieu liquide; celle-ci doit être au moins supérieure à 10 g $MF.l^{-1}$. Une densité inférieure provoque l'embryogenèse et ne permet pas de maintenir les souches sous forme indifférenciée. Le temps nécessaire entre le transfert en milieu liquide des cals embryogènes et l'obtention d'une culture en suspension stable à potentiel embryogène est de l'ordre de 8 mois.

PHASE DE MULTIPLICATION DE TISSUS EMBRYOGENES

Le maintien des souches s'effectue par repiquage tous les 21 jours. Pour chaque repiquage la biomasse est récoltée sur filtre de nylon (diamètre des pores : 50 μm) puis elle est transférée dans un Erlenmeyer de 250 ml contenant 100 ml de milieu à une densité de 10 g $MF.l^{-1}$.

Les souches se présentent sous forme d'agrégats de taille hétérogène dont la moyenne est de 430 μm pour la variété Catuai, de 630 μm pour l'hybride 1312, de 760 μm pour R2 et de 940 μm pour le clône R5. La croissance de ces souches est stable et linéaire et le coefficient de multiplication de la biomasse est faible: de 2,5 à 3,5 en 21 jours. Dans les suspensions, au sein des agrégats, aucun embryon n'est discernable (fig 2.a) Elles ne contiennent pas non plus des proembryons isolés analogues à ceux décrits dans le cas de la carotte par Halperin (1967).

Au niveau cytologique on observe des agrégats hétérogènes composés de deux types cellulaires (observations réalisées sur les variétés catuai, caturra et R2):
a) La périphérie des agrégats est constituée généralement de nodules dont les cellules présentent un rapport nucléocytoplasmique élevé, des réserves amylacées plus ou moins importantes et des mitoses nombreuses.
b) Au centre des agrégats on observe une différenciation des cellules par vacuolisation excessive et dans certains cas une accumulation de gros grains d'amidon. (fig. 2.b).

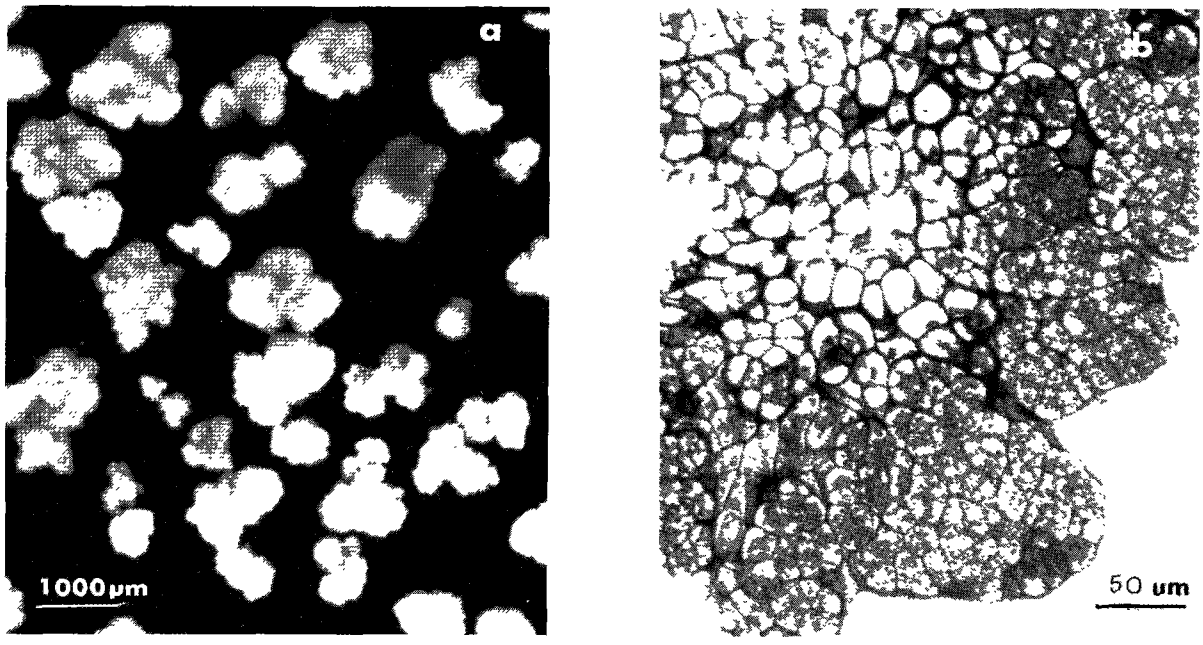

Fig. 2 - Aspect morphologique d'une souche de tissus embryogène (génotype R_2)

a - Aspect macroscopique b - Aspect microscopique

CRYOCONSERVATION DES SOUCHES EMBRYOGENES

La congélation a été effectuée en utilisant une méthode de congélation simplifiée (Tessereau et al. 1990). Les agrégats cellulaires sont mis à incuber dans le milieu standard contenant 1,0 M de saccharose pendant 24 heures, puis sont introduits dans des ampoules cryobiologiques et simplement placés 24 heures dans un congélateur ménager à -20°C avant d'être immergés dans l'azote liquide pour stockage indéfini. Après un réchauffement rapide dans un bain-marie thermostaté à 40°C, la souche subit une diminution progressive de la concentration en saccharose du milieu avant son transfert dans les conditions standard de culture. La capacité à réduire le TTC (2, 3, 5-tryphenyl tetrazolium chloride) en rouge de formazan a été utilisée pour évaluer la viabilité des cellules après congélation en déterminant le nombre d'agrégats colorés et l'absorbance à 485 nm du rouge de formazan extrait (Towil et Mazur, 1975).

En utilisant cette méthode, des expérimentations répétées ont montré que 80 % des agrégats après congélation à -20°C et 70 % après stockage dans l'azote liquide (N2L) étaient colorés (Fig. 3). Exprimé par l'absorbance à 485 nm, le taux de viabilité chute à 62 % après traitement à - 20°C et à 50 % après conservation dans l'azote liquide.

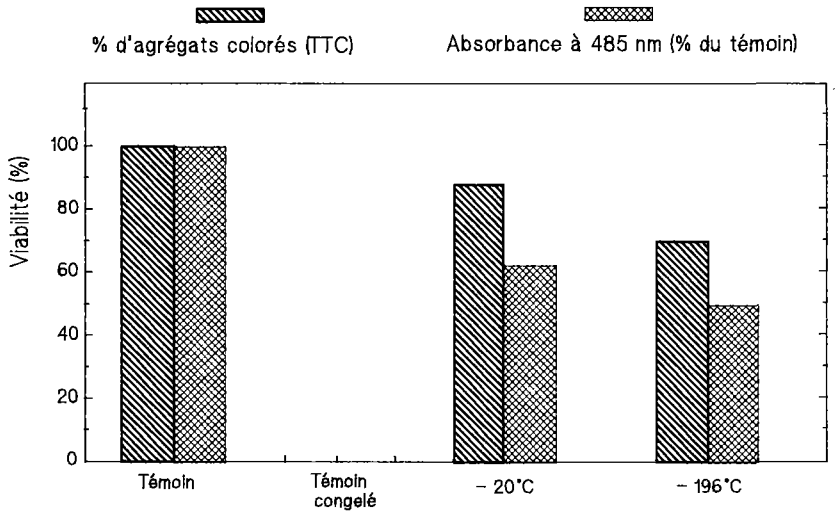

Fig. 3 - Evolution de la viabilité de la souche R_2 au cours de la congélation

Les cinétiques de production d'embryons obtenues à partir de souches cryoconservées ou non, se sont révélées similaires. Le potentiel embryogène (6500 embryons par litre et par jour) est maintenue et, de plus, aucune différence significative n'apparaît pour ce qui est du stade de développement des embryons produits. Cela montre que la capacité de différenciation n'a pas été modifiée par le traitement de cryoconservation.

Cette méthode caractérisée par l'emploi d'un agent cryoprotecteur unique (et ne nécessitant pas l'utilisation d'un équipement sophistiqué, comme cela est habituellement le cas) s'est avérée efficace sur toutes les souches de caféier testées. Ainsi, elle permet l'établissement d'une collection de souches embryogènes dans l'azote liquide constituant un stock stable à partir duquel, à tout moment, il est possible de lancer des programmes de production en masse de caféier.

PRODUCTION D'EMBRYONS EN MILIEU LIQUIDE

Toutes les expériences concernant l'optimisation de la production d'embryons sont réalisées dans des fioles d'Erlenmeyer de 250 ml avec 100 ml de milieu. Le milieu utilisé pour l'expression de l'embryogenèse est celui rapporté par Dublin (1984).

Exemple de cinétique embryogène

La Figure 4 montre l'évolution de différents paramètres : concentration en embryons totaux, en torpilles, en agrégats cellulaires et en matière fraîche, observées lors d'une embryogenèse initiée avec une densité d'inoculation de 0,5 g MF.l.$^{-1}$ et soumise à un renouvellement hebdomadaire du milieu.

Les deux premières semaines correspondent à la formation de proembryons. Une production massive d'embryons débute ensuite: la concentration en embryons totaux augmente de 2 x 10^3 à 240 x 10^3 embryons par litre entre les 14ème et 42ème jours, date à laquelle cette concentration atteint un plateau. La production moyenne mesurée sur les 6 premières semaines est de 5700 embryons par litre et par jour. Les premiers embryons atteignent le stade torpille après 28 jours et leur concentration atteint 90 x 10^3 par litre à 6 semaines, soit environ 38 % des embryons totaux. A cette date, plus de 90 % de la biomasse est constituée par des embryons groupés en bouquets par leur pôle racinaire. Cette caractéristique est commune à tous les génotypes étudiés à ce jour. La formation d'embryons est asynchrone, ce qui conduit à une hétérogénéité dans les stades embryonnaires. Il est à noter que la production varie en fonction des conditions de culture (densité d'inoculation, périodicité de renouvellement du milieu, agitation, obscurité, etc.).

Effet de la densité d'inoculation et du renouvellement du milieu

Pour l'établissement de ce procédé une étape importante a été franchie lorsque nous avons mis en évidence le fait que l'embryogenèse est fortement dépendante de la densité d'inoculation. Comme le montre la Figure 5, celle-ci détermine directement la production finale en embryons, laquelle varie à l'inverse de la densité et peut être quasi nulle pour des densités élevées de l'ordre de 5 à 10 g MF.l^{-1}.

Aux faibles densités, non seulement la production spécifique est plus élevée mais de plus les embryons sont mieux développés.

L'effet de la densité d'inoculation (soit production d'embryons et leur développement, soit inhibition de l'embryogenèse) est un phénomène observé pour toutes les souches étudiées. D'une manière générale, la densité optimale d'inoculation pour nos souches est comprise entre 0,2 et 1,0 g MF.l.$^{-1}$

L'inhibition, liée à des densités d'inoculation élevées, a été rapportée dans le cas de la carotte (Nouaille et Pétiard, 1988) et dans le cas du riz (Ozawa et Komamine, 1989). Ce type de limitation pourrait être la conséquence de l'accumulation dans le milieu de substances inhibitrices excrétées par les tissus. Une concentration critique de ces substances serait atteinte, d'autant plus rapidement que la densité d'inoculation est élevée.

Cette inhibition peut être partiellement supprimée par des renouvellements du milieu. La figure 6 présente l'effet du renouvellement régulier du milieu sur la production d'embryons à 6 semaines. Ainsi, avec un renouvellement par semaine, la production est multipliée par 6. Un renouvellement toutes les 2 semaines ne provoque qu'une augmentation de 50 % et est insuffisant pour obtenir des embryons au stade torpille. Ce stade est observé seulement avec un renouvellement hebdomadaire ou bihebdomadaire (17 et 29 % respectivement).

Par ailleurs, l'effet du renouvellement est d'autant moins efficace que la taille de l'inoculum est grande.

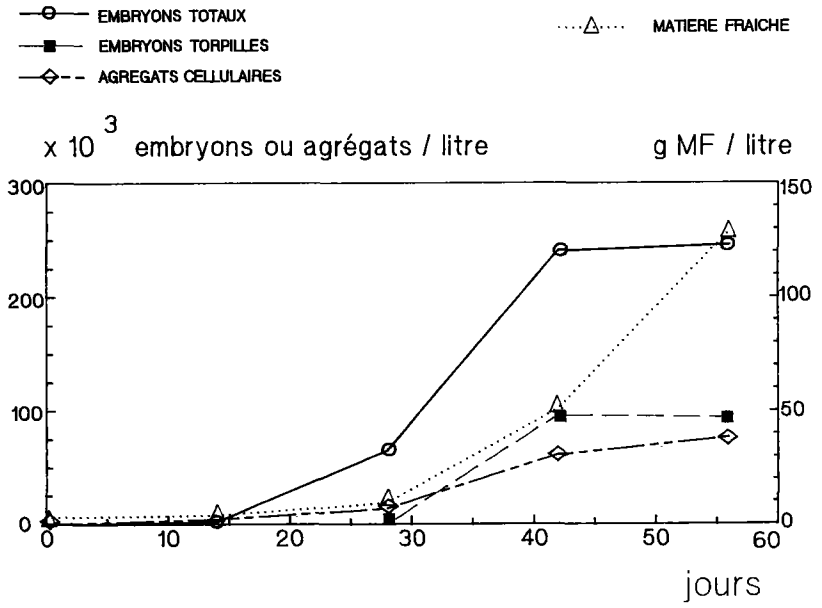

Fig. 4 - Cinétique d'embryogenèse du caféier en fiole d'Erlenmeyer (densité d'inoculation : 0,5 g MF.l^{-1} milieu renouvelé tous les 7 jours, génotype R_2).

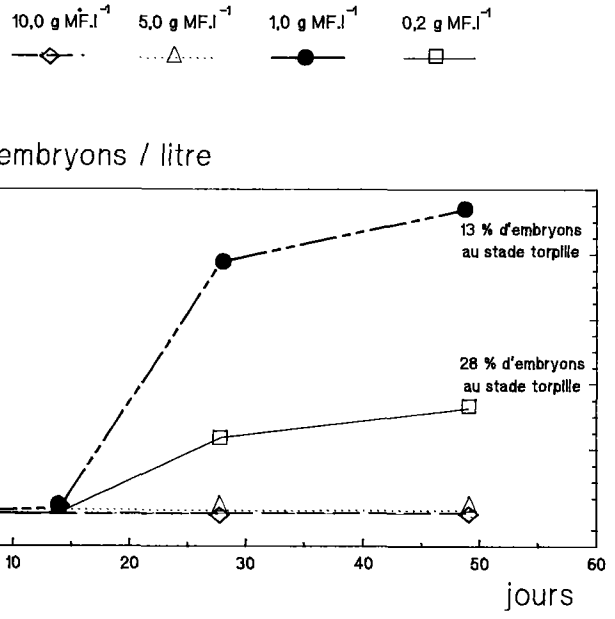

Fig. 5 - Effet de la densité d'inoculation sur la production d'embryons de caféier (milieu non renouvelé, génotype R_2)

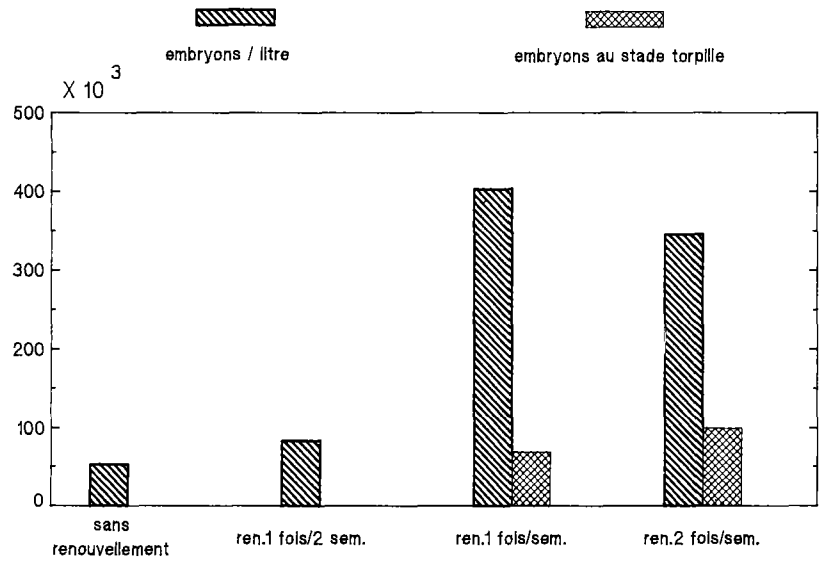

Fig. 6 - Effet de la fréquence du renouvellement du milieu sur la production d'embryons de caféier

Scaling-up en bioréacteur

Des essais d'embryogenèse ont été entrepris en bioréacteur de 3 litres agité mécaniquement (Setric SGI). La Figure 7 montre un exemple d'une cinétique d'embryogenèse de la souche R2 dont les conditions opératoires ont été les suivantes : une agitation mécanique de 60 rpm., une aération du milieu de 0,04 volume d'air par volume de milieu par minute (V.V.M.) et une température de 26°C.

La cinétique d'embryogenèse s'est révélée très comparable à celle obtenue en fioles d'Erlenmeyer. Sur l'exemple rapporté, une production importante d'embryons débute au-delà du 20ème jour. La concentration en embryons atteinte au 49ème jour est de 200 x 10^3 embryons par litre, la production moyenne est donc de 4000 embryons par litre et par jour. A ce même jour, les embryons ayant atteint le stade torpille représentent 20% des embryons totaux.

DEVELOPPEMENT DES EMBRYONS

Après 7 à 8 semaines de culture en milieu liquide, les embryons associés en bouquets ou isolés manuellement, sont transférés sur un milieu semi-solide contenant de la BAP à 0,225 mg l^{-1} (Zamarripa et al. 1991). Après 4 semaines, les embryons sont repiqués dans un 2ème milieu identique au premier mais dépourvu de BAP. A cette date les embryons initialement semés en bouquets sont individualisés aisément. Au bout de 8 semaines, les premières paires de feuilles peuvent être observées. Le taux de conversion des embryons en plantules au stade 2 feuilles est de 50 à 70 % au bout de 12 semaines. Les embryons provenant soit de bioréacteur, soit d'une souche cryoconservée, présentent un taux de conversion similaire. L'acclimatation en serre peut s'effectuer dès que les plantules ont 2-3 paires de feuilles (Fig. 8). Le pourcentage de développement varie de 80 à 95 % au bout de 20 semaines.

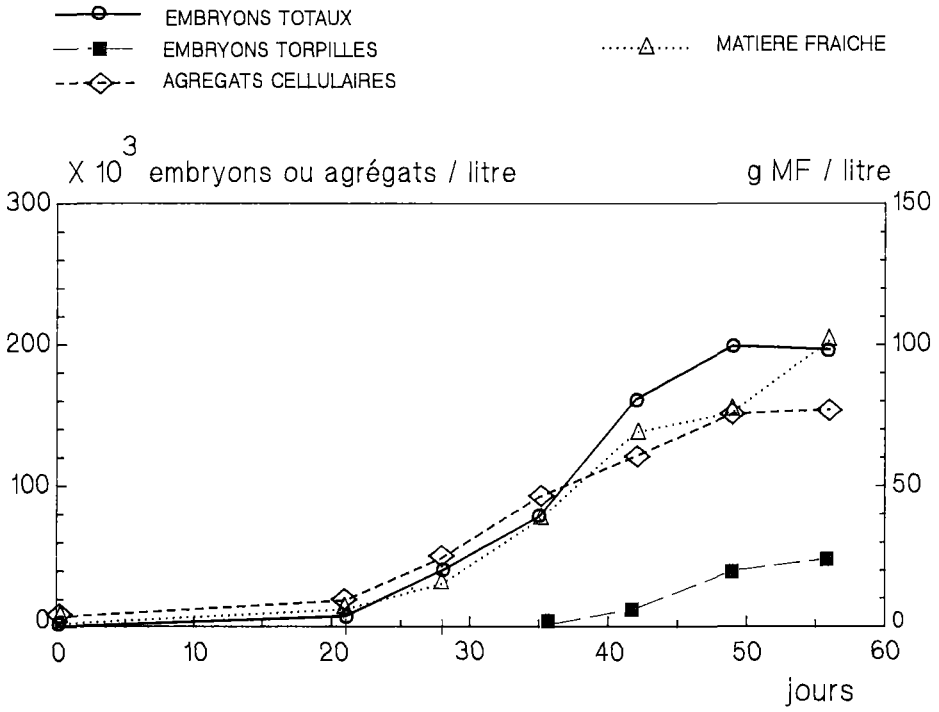

Fig. 7 - Cinétique d'embryogenèse du caféier en bioréacteur de 3 litres.
(densité d'inoculation : 1,0 g MF.l^{-1}, milieu renouvelé tous les 7 jours, génotype R_2).

CONCLUSIONS

Cette étude a permis de montrer qu'il est possible de produire des embryons somatiques en milieu liquide chez les espèces de caféier les plus importantes au plan agronomique. Ce procédé peut avoir des implications à plusieurs niveaux pour la création variétale et pour la multiplication. Il pourrait tout d'abord être utilisé dans les schémas de sélection afin de conserver des génotypes intéressants.

La cryoconservation des souches pourrait éviter les risques de perte ou de dérive génétique et, qui plus est, permettre le stockage dans un faible volume à l'abri des contaminations. Sachant que le coût de la conservation en plantation représente 24 % du coût global de l'amélioration du caféier (Charrier et al. 1989) on entrevoit aisément l'avantage de techniques simplifiés, de conservation à long terme du caféier.

Un calcul simple permet d'illustrer la puissance du procédé de multiplication. Si l'on considère la production en bioréacteur de 3 litres (inoculé avec seulement 3g.MF, c'est à dire le contenu d'une fiole d'Erlenmeyer à la fin d'une phase de multiplication) 600 000 embryons peuvent être obtenus tous les 2 mois. Compte tenu des pertes lors de la phase de développement et lors du transfert à la serre, 400 000 individus environ, correspondant à 100 hectares de plantation, peuvent être produits bimestriellement.

De plus, en tout état de cause, le coût de production par embryogenèse somatique serait largement inférieur à celui de la micropropagation. Reste à évaluer la conformité des plantes au champ en ce qui concerne leurs caractères agronomiques et le maintien de la qualité du café obtenu. Les études préliminaires concernant cette conformité sont en cours.

En conclusion, cette méthode de multiplication végétative en masse peut maintenant être envisagée, afin d'installer rapidement des plantations de caféiers sélectionnés (hybrides ou transformés).

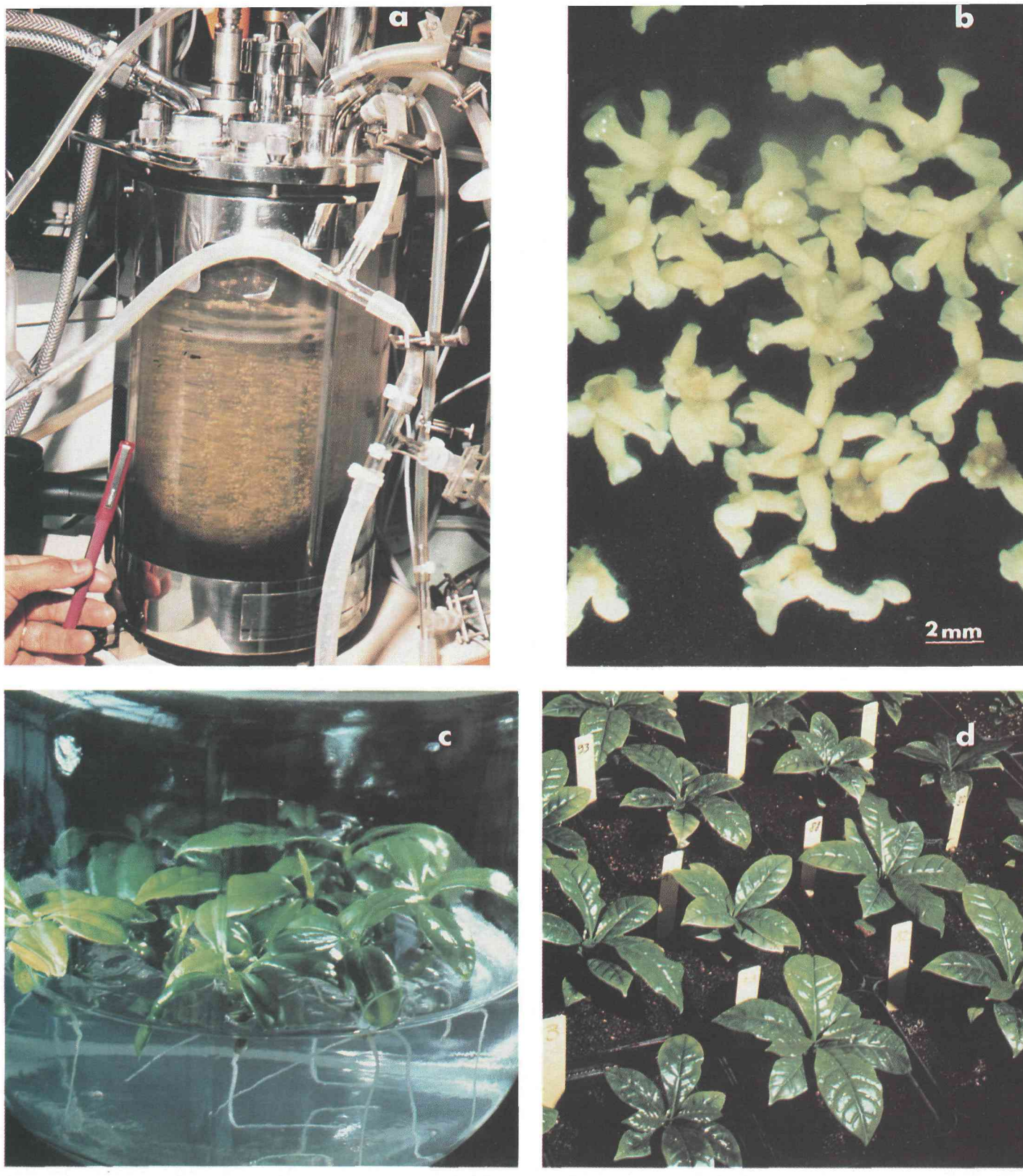

Fig. 8 - Production et développement des embryons somatiques de caféier

a. - Culture des embryons somatiques en bioréacteur (7 semaines après le début de l'embryogenèse).
b. - Aspect des embryons en milieu liquide
c. - Plantules en milieu semi-solide 16 semaines après leur sortie du bioréacteur.
d. - Sevrage des plantules (3 mois après leur transfert en serre)

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RESUME

DEVELOPPEMENT D'UN PROCEDE DE MULTIPLICATION EN MASSE DU CAFEIER PAR EMBRYOGENESE SOMATIQUE EN MILIEU LIQUIDE

Cette étude décrit les étapes qui permettent, à partir d'un génotype de caféier, d'obtenir la production en masse d'embryons somatiques puis de plantules viables en serre. L'obtention de souches de tissus embryogènes de C.arabica, C.canephora et de l'hybride arabusta est rapportée. Une méthode de cryoconservation a été appliquée sur une souche et a permis de conserver son potentiel embryogène. La production d'embryons en milieu liquide est très dépendante de la densité d'inoculation utilisée: la production et le développement des embryons sont inhibés par une densité d'inoculation élevée, reflet d'un probable autoantagonisme supprimé partiellement par un renouvellement régulier du milieu de culture. Ainsi, en fiole d'Erlenmeyer, à faible densité d'inoculation (1 g.MF.l.$^{-1}$), 460 000 embryons sont produits en 7 semaines; en bioréacteur la production est de l'ordre de 4000 embryons/l/jour. La conversion des embryons en plantules est également décrite. L'étude de la conformité des plantes obtenues est en cours. Ces résultats montrent que la production massive d'embryons somatiques et de plantules de caféier est dorénavant réalisable.

SUMMARY

MASS PROPAGATION OF COFFEA spp. BY SOMATIC EMBRYOGENESIS IN LIQUID MEDIUM

This study describes the procedure which, starting with a genotype of coffee tree, allows a mass production of somatic embryos and their development into plantlets in the greenhouse. Cell suspension cultures of C. arabica, C. canephora and of the hybrid arabusta with a stable embryogenic potentiality have been obtained. Cryopreservation has been applied to the cell suspension culture. It is shown that embryogenic potentiality is retained after freezing. The production of somatic embryos is highly dependent on the inoculum density: the production and the development of embryos are inhibited when the inoculum density is high, probably due to self-antagonism. This inhibition is partially suppressed when the medium is periodically renewed. At a low inoculum density of 1.0 g FW.l^{-1}, 460,000 embryos/l were produced in 7 weeks, in Erlenmeyer flasks; in a bioreactor, the production is about 4,000 embryos/l/day. The further step of embryo-to-plantlet conversion is also described. The conformity of the regenerated plants is currently assessed. These results show that mass production of coffee somatic embryos and plants can now be considered.

CONTRIBUTION À L'AMÉLIORATION DE *COFFEA CANEPHORA* PIERRE PAR HYBRIDATION INTERSPÉCIFIQUE AVEC *COFFEA LIBERICA* BULL EX. HIERN

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INTRODUCTION

L'étude des relations génétiques entre diverses espèces du genre *Coffea*, entreprise en Côte d'Ivoire à partir de 1975 par l'ORSTOM, a permis de mettre en évidence des possibilités d'amélioration de l'espèce *C. canephora* par hybridations interspécifiques avec les espèces diploïdes (LOUARN, 1982).

C. canephora se croise avec douze espèces différentes au moins, en particulier, avec *C. congensis*, *C. salvatrix*, *C. spA*, *C. humilis*, et *C. liberica* (LOUARN, 1982).

L'exploitation des descendances hybrides dépend de leur fertilité: les congusta, issus du croisement de *C. canephora* x *C. congensis* sont exploitables directement du fait de leur fertilité relativement bonne, contrairement aux descendants du croisement de *C. canephora* x *C. liberica* (LOUARN, 1980). L'utilisation de ces derniers nécessite au préalable, une restauration de leur fertilité, dont le rétrocroisement sur *C. canephora* peut être un moyen.

Les observations effectuées sur Les hybrides rétrocroisés (BC1) portent sur le comportement végétatif, la fertilité et la productivité.

Les résultats, présentés dans cette communication mettent l'accent sur les possibilités d'amélioration de la fertilité, condition essentielle pour l'intégration des hybrides interspécifiques de *C. canephora* x *C. liberica* dans les schémas de sélections clonales élaborés pour l'amélioration de *C. canephora*.

MATERIEL ET METHODES

Avec un effectif total de 1592 plants, issus de 76 descendances et trois clones de *C. canephora* (témoins) (tableau 1), le matériel végétal est essentiellement constitué d'individus de première génération de rétrocroisements (BC1) obtenus à partir du croisement de l'hybride F1 (*C. canephora* x *C. liberica*) par *C. canephora*. 66 combinaisons dont 10 directes et réciproques

(soit un total de 76 croisements) ont été réalisées avec 13 géniteurs de *C. canephora* (9 clones et 4 souches de caféiers sauvages de la Nana) et 22 descendants d'hybrides F1 qui proviennent d'essais d'observations implantés à Man (Côte d'Ivoire).

Tableau 1 : Tableau récapitulatif des effectifs et des et des descendants, et des géniteurs utilisés.

type de croisements	géniteurs canephora	géniteurs hybrides F1	nombre de croisants	effectif
F1 x caneph	8*	4* + 13	34	652
caneph x F1	8* + 5	4* + 5	42	890
témoins	-	-	-	50
total	13	22	76	1592

* = nombre de géniteurs utilisés comme mâles et femelles dans les croisements.

L'étude expérimentale a été réalisée à Divo (Côte d'Ivoire), dans un essai planté en randomisation totale en 1984, avec une densité de 2222 plants à l'hectare.

Les variables observées concernent:
- la vigueur, estimée par le diamètre au collet, la hauteur et la longueur du plus grand rameau plagiotrope, mesurés sur des arbres âgés de trois ans.

- les fertilités mâle et femelle: la fertilité mâle est exprimée par le taux de grains de pollen à cytoplasme coloré au carmin acétique à 2%. Les études ont été réalisées sur un échantillon de 248 descendants (80, du croisement F1 x canephora et 168, du croisement réciproque) et 12 individus des clones témoins, récolté au hasard dans la parcelle.

La fertilité femelle est exprimée par les taux de caracolis et de loges vides et le coefficient de remplissage des fruits. Les observations ont été faites à partir d'échantillons de 200 à 300 fruits immatures (âgés de six à sept mois) prélevés et coupés transversalement au scalpel. Les cerises sont alors classées en cinq catégories: A (2 loges pleines), B (un caracoli plein et une écaille), C (un loge pleine et une loge vide), D (un caracoli vide et une écaille), E (2 loges vides).
 * le taux de grains caracolis (tCAR) = (B/(2A+B+C))*100
 * le taux de loges vides (tVID) = (C+D+2E/(2A+B+D+2C+2E))*100
 * le coefficient de remplissage des fruits = 2A+B+C/A+B+C+D+E

- la productivité, deux paramètres liés à la productivité ont été observés: l'intensité de la floraison et le temps d'épanouissement des fleurs. Le poids de cerises fraîches de par arbre a été noté chaque année, l'analyse porte sur leur cumul.

Les analyses portent sur les descendants des deux types de croisements (F1 x canephora et canephora x F1) (tableau 1), qui sont répartis en deux groupes. Ces deux groupes sont comparés entre eux et aux clones témoins. Les variables sont traités par l'analyse de la variance à un critère de classification appliqué aux effectifs inégaux (programme BONSTAT réalisé par CAUDRON).

RESULTATS

1 - Comportement végétatif des arbres

Le tableau 2 (annexes) présente les résultats d'analyse des variables de la vigueur. Il ressort un effet groupe hautement significatif ($p = 0.01$) quelque soit le caractère. Les clones témoins sont plus vigoureux que les descendants. La différence de vigueur entre les descendances des deux types de croisements est nette. Le croisement *C. canephora* x F1 produit des individus plus vigoureux que ceux du croisement réciproque (F1 x *C. canephora*).

2 - Fertilité

2. 1 - Fertilité mâle

Les résultats enregistrés, donnent un taux de viabilité pollinique moyen de 81% (cv = 13%). L'analyse statistique ne montre pas de différences significatives ($p = 0.1$) entre les groupes (les témoins d'une part et les deux groupes de descendances d'autre part).

Quatorze pourcent des génotypes, essentiellement issus des descendances, ont très peu, ou pas de pollen; cela n'a aucune influence sur l'environnement pollinique. Les problèmes de fécondations constatés ne pas sont dus à une quelconque déficience du pollen, mais, plutôt liés au mauvais fonctionnement de la plante.

2. 2 - Fertilité femelle

Les résultats enregistrés sur différents échantillons traités en 1987, 1988 et 1990 (Tableaux 3, 4 et 5 en annexe) montrent que les paramètres de la fertilité femelle sont influencés par les facteurs climatiques. Les classements ne sont pas pour autant modifiés (il n'y a pas d'effet d'inter-action). L'analyse de ces paramètres donne un effet groupe hautement significatif ($p = 0.01$). Les clones témoins sont plus fertiles que les descendants. La différence de fertilité entre les descendants des deux types de croisements n'est pas très marquée; cependant, l'utilisation de *C. canephora* comme géniteur femelle semble apporter un léger gain de fertilité.

Comparés aux hybrides F1 (LOUARN, 1987), les BC1 issus du croisement F1 x canephora apportent un gain moyen de fertilité de 7%; ceux du croisement réciproque, une amélioration de 13% (la comparaison est faite avec la moyenne des trois années au cours desquelles l'étude a été réalisée).

3 - Productivité

3. 1 - La floraison

Deux notations ont été faites: une, en 1986 (deux ans après la mise en place de l'essai) et une autre en 1989 (cinq ans après) sur l'intensité de la floraison (quantité de fleurs produites lors d'une floraison). Les résultats de ces campagnes sont présentés au tableau 6 des annexes. On constate que les descendances ne fleurissent pas beaucoup, 8 et 11% des arbres en 1986 et 3% seulement en 1989 ont très bien fleuri; alors que 46 et 44% des individus témoins ont eu une importante floraison dans les mêmes périodes. Les descendances du croisement *C. canephora* x F1 ne se comportent pas mieux que celles du croisement réciproque.

Le second paramètre relatif à la floraison, est le temps qui s'écoule entre le déclenchement de la floraison et l'épanouissement des fleurs. Les résultats

présentés au tableau 7 (annexes), montrent que les clones témoins fleurissent totalement les sixième et septième jours après le déclenchement de la floraison par la pluie. Chez les descendants, plus de 50% des arbres fleurissent le sixième jour. Il reste une proportion d'arbres dont les fleurs ne s'ouvrent que plus tard vers le neuvième ou le dixième jour. Ce sont généralement des fleurs de taille inférieure à la normale, et chez lesquelles le changement de couleur caractérisant la maturité du bouton floral n'intervient pas.

3. 2 - Production

La moyenne de production sur quatre ans (1986, 1987, 1988 et 1989) des descendants du croisement F1 x canephora est de 3 kg de cerises fraîches par arbre (cv >100%), et de 4 kg (cv >100%) pour ceux du croisement réciproque. Les témoins ont produit en moyenne, 16 kg (cv = 60%) pendant la même période. La différence hautement significative (p = 0.001) se traduit sur l'histogramme (fig.1) par la proportion très élevée d'arbres dont la production est nulle ou faible chez les descendants. Néanmoins, l'histogramme fait apparaître quelques arbres hauts producteurs chez ces descendants, ce qui est important pour la sélection.

DISCUSSION - CONCLUSION

Les résultats obtenus peuvent se résumer en trois points essentiels:

1 - L'effet du sens du croisement

Le comportement des deux groupes de descendants est différent suivant le sens du croisement. Contrairement aux hybrides F1 dont l'obtention est plus aisée en utilisant *C. liberica* comme parent femelle (LOUARN, 1980), ici les meilleurs résultats sont obtenus avec les descendants dont le parent femelle est *C. canephora*. Ces résultats qui doivent être précisés en utilisant effectivement les mêmes géniteurs dans les deux sens du croisement, s'expliquent par le fait que d'une part, *C. canephora* ayant une méiose plus régulière que l' hybride F1 produit des gamètes femelles plus équilibrés, et d'autre part, le pollen destinée à la fécondation est sélectionné naturellement.

2 - La fertilité

L'évaluation de l'environnement pollinique des arbres a permis de se rendre compte de l'amélioration effective de la fertilité pollinique des hybrides. En effet, après seulement une génération de rétrocroisement, les hybrides ont atteint un niveau de fertilité pollinique équivalent à celui des témoins.

La fertilité ovulaire a été observée sur trois ans. Les résultats fluctuent d'une année à l'autre. L'influence du climat sur le taux de grains caracolis a été déjà observée à Madagascar (CHARRIER, 1971). Concernant la variation dans le temps du taux de loges vides, LE PIERRES et CHARMETANT (1985) ont montré que ce paramètre est très influencé par l'environnement. Le dernier paramètre étudié exprime directement le niveau de fertilité des descendants. Sa fluctuation dans le temps vient du fait qu'il tient compte directement des taux de caracolis et de loges vides. Le classement reste cependant, inchangé. Les clones témoins ont moins de caracolis, moins de loges vides et des coefficients de remplissage des fruits plus élevés que les descendants, quelle que soit l'année.

3 - La floraison et la production

Le niveau de productivité des descendants est faible par rapport à celui des clones témoins. Ceci est la marque d'une structure génétique hybride

encore imparfaite. En dehors de toute influence de l'environnement, les faibles intensités des floraisons enregistrées (chez les descendants) ne peuvent qu'engendrer de faibles productivités. Chez *C. canephora*, moins de 40% des fleurs évoluent en fruits (SNOECK, 1981), cette proportion devrait être encore plus faible chez les hybrides. Par ailleurs, le décalage de floraison entre *C. canephora* et *C. liberica*, entraînant l'épanouissement des fleurs en plusieurs étapes chez les hybrides, perturbe le bon déroulement de la pollinisation.

La première génération de rétrocroisements montre une amélioration de la fertilité de 7 et 13% respectivement, pour les descendants du croisement F1 x canephora et pour le croisement réciproque, par rapport aux hybrides F1. Mais, elle est encore insuffisante, comparée à celle des clones témoins. Cependant, des possibilités de sélection individuelle existe, compte tenu de la grande variabilité observée dans les descendances. Etant donné la faible proportion de ces génotypes, il convient de poursuivre le processus d'amélioration par la création d'une deuxième génération de rétrocroisement. Le choix des géniteurs hybrides doit tenir compte des caractères intéressants recherchés chez *C. liberica* comme par exemple, la monocaulie et la ramification plagiotrope prépondérantes, la floraison sur vieux bois, la faible teneur en caféine (0.5 à 1.9% de la matière sèche) et la tolérance à la sécheresse (LOUARN, 1980).

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RESUME

L'amélioration de *Coffea canephora* par hybridation interspécifique a débuté en 1975 par la mise en place d'un programme d'hybridation de cette espèce avec diverses espèces du genre *Coffea* et en particulier avec *C. liberica*. Les descendances F1 de *C. canephora* x *C. liberica* ont montré quelques défauts de fertilité dont la restauration a nécessité la création d'une seconde

génération d'individus (BC1), obtenus par rétrocroisement des hybrides F1 sur le parent *C. canephora*.

L'étude des BC1 porte sur la vigueur, la fertilité et la productivité. Les observations montrent un effet du sens du croisement, les meilleurs résultats étant obtenus avec les descendants du croisement *C. canephora* x F1. La fertilité quoique faible par rapport aux témoins est améliorée par rapport aux hybrides F1. La productivité est également faible, mais la variabilité observée permet le choix d'individus producteurs.

SUMMARY

Inter-species hybridization to improve *Coffea canephora* started in 1975 with a program to hybridize this species with various species of the genus *Coffea*, in particularly with *C. liberica*. F1 descendants of *C. canephora* x *C. liberica* showed certain fertility problems. To restore this, a second generation of individuals (BC1), was created by back-crossing F1 hybrids with the parent *C. canephora*.

Vigor, fertility and productivity have been observed for BC1. Progeny performance is different when *C. canephora* is used as male or female. Crosses with *C. canephora* as female are the best. Fertility is still low but has been improved compared with F1 hybrids. Productivity shows a great range of variability allowing the choice of high-yielding genotyps.

ANNEXES

TABLEAU 2 : ANALYSE STATISTIQUE DES CARACTERES DE VIGUEUR

	F1 x can (HC)		can x F1 (CH)		Témoins (T)		eff	classement
	m	cv (%)	m	cv (%)	m	cv (%)		N. et K. (5%)
diam. collet (cm)	43	31	45	29	52	33	* *	HC < CH <T
hauteur (cm)	133	29	147	27	158	24	* *	HC < CH <T
long. rameau plagiot.(cm)	78	36	84	33	101	23	* *	HC < CH <T

Légende: HC = hybride F1 x C. canephora
CH = C. canephora x hybride F1
m = valeur moyenne
cv = coefficient de variation

FERTILITE FEMELLE
(analyse des différents paramètres)

TABLEAU 3 : TAUX DE GRAINS CARACOLIS

année	F1 x can (HC)		can x F1 (CH)		Témoin (T)		effet	classement
	m	cv (%)	m	cv (%)	m	cv (%)		N. et K. (5%)
1987	51	32	48	38	40	21	* *	HC = CH > T
1988	59	30	57	31	51	20	* *	HC = CH > T
1990	50	32	45	42	39	29	* *	HC > CH > T

TABLEAU 4 : TAUX DE LOGES VIDES

année	F1 x can (HC)		F1 x can (CH)		Témoins (T)		effet	classsement
	m	cv (%)	m	cv (%)	m	cv (%)		N. et K. (5%)
1987	16	88	12	74	5	46	* *	HC > CH > T
1988	12	78	11	84	7	58	* *	HC = CH > T
1990	10	53	11	58	7	58	* *	HC = CH > T

TABLEAU 5 : COEFFICIENT DE REMPLISSAGE DES FRUITS

année	F1 x can (HC)		can x F1 (CH)		Témoins (T)		effet	classement
	m	cv (%)	m	cv (%)	m	cv (%)		N. et K. (5%)
1987	1.14	21	1.22	17	1.37	6	* *	HC < CH < T
1988	1.13	15	1.16	16	1.25	7	* *	HC < CH < T
1990	1.23	11	1.27	16	1.36	9	* *	HC = CH < T

TABLEAU 6 : INTENSITE DE LA FLORAISON NOTEE EN 1986 ET 1989.
Répartition des arbres suivant leur niveau de floraison dans les groupes de descendants.

	année	F1 x can (HC)	can x F1 (CH)	Témoins (T)
% d'arbres à floraison nulle (0-100 fl)	1986	9	7	6
	1989	8	3	0
% d'arbres à floraison faible (100-1000 fl)	1986	25	22	10
	1989	28	24	2
% d'arbres floraison moyenne (1000-10000)	1986	58	60	38
	1989	61	70	54
% d'arbres à floraison importante (> 10000)	1986	8	11	46
	1989	3	3	44

TABLEAU 7 : FLORAISON 1989. Répartition des arbres pour différentes périodes d'épanouissement des fleurs, dans chaque groupe de descendants.

	F1 x can (HC)	can x F1 (CH)	Témoins (T)
proportion d'arbres n'ayant pas de fleurs*	5	3	0
% d'arbres à fleurs ouvertes** à partir de 6 jours après le déclenchement	51	56	42
% d'arbres à fleurs ouvertes à partir de 7 jours après le déclenchment	28	37	58
% d'arbres à fleurs ouvertes à partir de 8 jours après le déclenchement	7	2	0
% d'arbres à fleurs ouvertes à partir de 9 jours ou plus après le déclenchement	9	2	0

* : les arbres n'ont aucune, ou ont très peu de fleurs; ce qui ne permet pas d'apporter une appréciation.
** : on considère ici les arbres avec 70 à 80% de fleurs ouvertes.

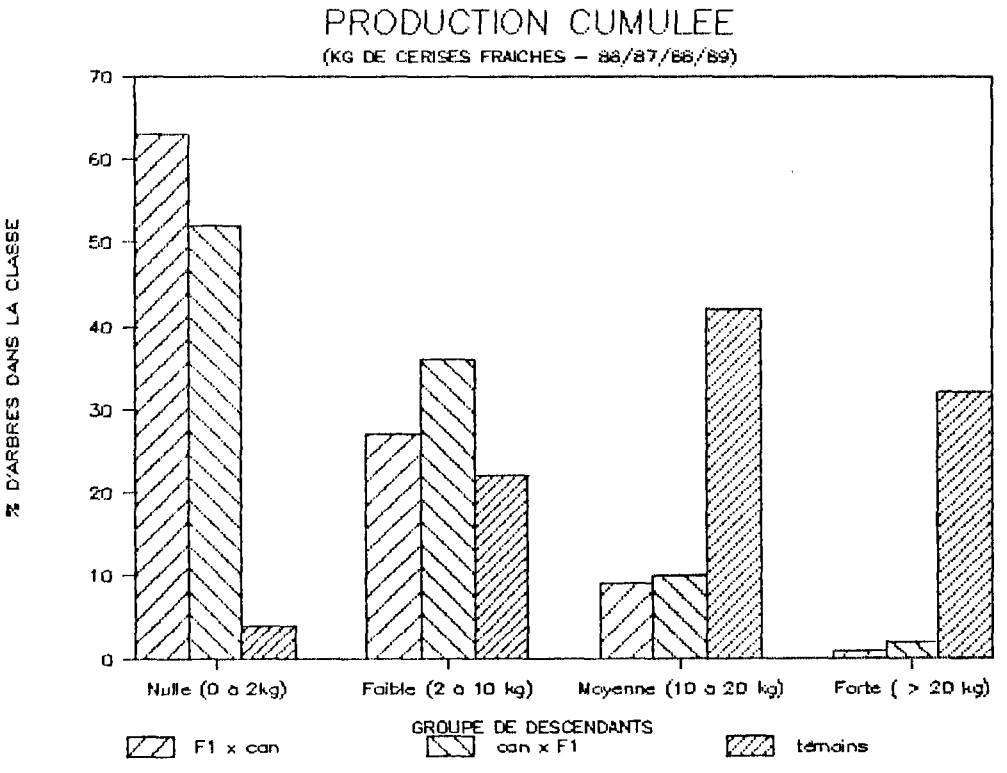

Fig. 1 : HISTOGRAMME DES PRODUCTIONS. Classement des arbres suivant leur niveau de production dans les différents groupes de descendants.

PLANT CELL CULTURE FOR PRODUCTION OF NATURAL INGREDIENTS

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INTRODUCTION

Ingredients used in foods and beverages are natural if directly derived from a plant or animal source or through fermentation of a plant or animal source[1]. The function of the ingredient may be nutritional or flavor when used in food substances. Treating the source or ingredient by means of a synthetic chemical reaction may designate the process and product as artificial and not natural.

Plants exhibit heterogeneous (differentiated) features such as stems, leaves, roots, flowers, bark, and seeds each with a function or purpose for reproduction and survival. The plant's physiological functions involve producing primary metabolites such as amino acids, oils, starch, and sugars essential for plant sustenance and growth, and secondary metabolites with a "secondary" but not necessarily less important purpose such as to attract a pollinating insect to one of the flowers or to ward off the infestation of a predator that could be an insect or a microbe. These primary and secondary metabolites can also be important products of the plant much as the fruit resulting from a sexual cross (e.g. the coffee cherry) or else a component of the root system (e.g., the potato tuber) are important plant products. The secondary metabolites valuable to the plant may be present as a complex of many compounds or as a single component. Examples of valuable plant metabolic products, the opiate alkaloids, include the secondary metabolites morphine and codeine (derived from Papaver somniferum), as important medicinals. Quinine, a valuable anti-infective agent, is derived from the bark of the Cinchona. In more recent times, a component (Taxol) extracted from the bark of the Pacific yew, Taxus brevifolia, has promise as an anti-cancer drug of broad spectrum activity[2].

In the food flavors area, the unique flavor, vanilla is extracted from the vanilla bean (Vanilla planifolia and others), cocoa is extracted from Theobroma cocao and processed to cocoa products, and coffee beans are processed to produce a valuable and desired beverage.

Supply of a plant secondary metabolite may often be uncertain because the plant source of the metabolite is difficult to grow or severely subject to disease and weather, resulting in lower yield, and higher costs. Further, the land or origin of the plant may also present sociological or political hindrances that negatively affect access. The plant may not be cultivated easily and only exist in the wild, and it may grow too slowly to allow adequate supply. The concentration of the secondary metabolite(s) in the plant of interest may be low or the compound may be unstable making recovery of the product difficult and costly. For example, if strawberries are worth $1/kg and a component is present in strawberries at less than 0.1%, then the cost to isolate the component starts at $1000/kg of component. If the component can be manufactured less expensively and more reliably in a manner that doesn't involve agriculture, then it is possible to consider alternatives to crop cultivation to obtain the secondary metabolite. In many cases, pharmaceuticals that are originally plant sourced are synthesized in the laboratory if this approach is practical for new drugs.

For food ingredients and flavors, synthetic chemistry is not an acceptable alternative if the product and process must be remain natural. The trend toward natural by consumers has been apparent for many years in the U.S.A. and experts do not expect it to abate. The signs are that the natural trend is increasing in the Pacific Rim and also in Europe.[3] The world market for food flavors is near $1 bil. A total of $300mm of the U.S. flavor market involves natural flavors growing at 8%/year.

Plant Cell Culture Technology

The cultivation of plant cells in culture to produce valuable secondary metabolites by natural processes represents a modern alternative to economically obtain valuable secondary metabolites for pharmaceutical, specialty chemical, food ingredient, and flavor applications. Plant cell culture may supplement or replace materials from cultivated or non-cultivated crops.[4]

The technique can result in the same natural product that the plant produces in a specific tissue. In theory, any part of the plant can product the compound of interest in culture since plants are totipotent (i.e., cells of stem, leaf, or root can all be induced to regenerate to a whole plant; therefore, they presumably have the genetic potential to synthesize any compound synthesized by the whole plant). In practice, cells are cultivated from several parts of the plant; cells selected for development offer the most promise to make the compound of interest at the greatest rate and in the highest concentration.

Whole plant tissue, once freed of all contaminating microorganisms (obtaining completely aseptic plant tissue without destroying it is an art), is processed through stages of differentiation (causing cells to undergo changes and differ within a biomass) and de-differentiation yielding aggregates of cells or sets of individual cells that are nearly alike, perhaps so alike, that they are clones. The process of differentiation and de-differentiation is as a result of exposure to particular environmental conditions including various liquid, solid, and gaseous states levels of nutrients, and hormonal stimulants. This process replicates many changes that occur naturally within the intact plant. Once a desired cell state is reached, the aggregates can be separated and propagated for multiplicative growth under culture conditions analogous to conditions employed to cultivate bacteria, yeast, fungi, and mammalian cells. Since the plant cells selected have the genetic potential to produce the full spectrum of components, produced in the whole plant or part of the plant, each cell or cell aggregate becomes a potential "factory" for any plant product. Under proper process conditions and exposure to desired nutrients, the individual plant cells initiate production of the secondary metabolite(s) and products of interest, a perfectly natural process.

The potential power of this technology is apparent. Products difficult or expensive to source from crops and impossible or unacceptable for synthesis in the laboratory can be cultivated by natural processes in closed aseptic vessels under full process control. If yields and production rates are sufficiently high, the plant cell culture method will be economically competitive.

Tables 1 and 2 indicate a range of colors, flavors, oils, sweeteners, and spices possible to produce by plant cell culture. Successful production of these compounds by plant cell culture depends on the technical difficulty in establishing culture for the particular product, the cost involved to develop a process to make the product, and the value received (price per unit weight), and market size once success is achieved. For example, a product valued at below $100/kg is worth producing by plant cell culture if the volume is sufficiently high, e.g., greater than 100,000 kg/yr.[5]

There may be other reasons to use plant cell-derived products. For example, a natural anthocyanin red color, stable to light and acidity in beverages, is worth developing in view of difficulties in the availability of natural colors for foods and beverages.

Having a array of natural fruit flavors from tissue culture is valuable, since in many cases, the natural character of current products that are non-cell culture derived come into question (such as cherry) and the content of flavor in the intact fruit is often too low to justify extraction (e.g., raspberry). Having natural licorice flavor by tissue culture would be advantageous for marketing to particularly industries. Certain of the essential oils may be valuable if the components prove to be of special health benefit and cell culture can allow the concentration of such components to be increased in culture (see Figure 1 for cases where the concentration of components in cell culture exceeds that in the intact plant).

Plant cell culture could be an attractive route to derive clinically-important proteins in certain cases. For example, plant cell culture could be preferred if production from mammalian culture is prohibitive due to dangers of virus infestation. Proteins correctly glycosylated and unfolded in plant cell culture would be more desirable than proteins produced by microbes that are incorrectly glycosylated, causing an undesirable immune response when administered to humans as a drug. Production of products by plant cells in aseptic closed vessels would be preferred to production in an animal subject to virus infestation or to production in a crop in an open field where conditions are more difficult to control. Artificial sweeteners produced in plant cell culture may be too costly compared to other means; however, production of such sweeteners often proteins, by the plant cell culture route is nevertheless still plausible.

ESCAgenetics Corporation has considerable experience in a number of plant cell culture systems as indicated in Table 3. Recently it has focused on vanilla because of its business value (and also on the pharmaceutical taxol because of its promise and striking fit to ESCAgenetics' core plant cell culture technology). In the case of vanilla, the process is being scaled to commercialization since the company predicts economical manufacturing based on results at the 72 liter scale. In the case of Taxol, ESCAgenetics has recently reported significant progress in development of a cell culture system to produce this valuable drug.[6]

Vanilla flavor production begins with cultivation of the vanilla orchid in locales such as Madagascar, the Cayman Islands, and Indonesia. The vanilla plant grows as a vine and produces an orchid that must be hand-pollinated in order to produce the bean (the bean is the fruit of the plant and contains thousands of seeds). Once the bean forms, it ripens to a degree on the plant, is then picked, and is subjected to a sweating, drying and conditioning process involving natural enzymatic processes within the bean over a curing

period that can last 6-8 months. The beans are then packed and shipped elsewhere where they are extracted with ethanol to yield vanilla extract. This process results in a product valued at $50/gal extract or more, and between $2000 and $4000/kg dry basis vanillin in the bean. A multitude of components such as vanillin, para-hydroxy benzyaldehyde, vanillic acid, and acetic acid appear in bean extract and contribute to its rich character.[7]

ESCAgenetics plant cell culture technology provides cells that can be multiplied and then produce natural vanilla flavor. The time to produce vanilla flavor by ESCAgenetics technology is of the order of a few weeks, not 6 to 8 months (the time required to cure the beans) and not several years, the time required to grow vanilla orchid plants. The plant cell culture process involves taking sterile explants from an aseptic plant established in the laboratory, inducing formation of a true undifferentiated material where all cells are nearly the same (the callus) and then obtaining the desired cell suspension for propagation and flavor production after transfer of the true callus to solution. Both the callus and cell suspension are available as starting points to be quickly propagated to larger batches of cells and subsequent formation of the flavor complex. A key milestone indicating progress with plant cell culture technology is to be able to produce at large scale at a yield and rate matching that achieved at small scale. This has been accomplished by ESCAgenetics for PhytoVanilla and is critical to establishing a commercial process. Natural Vanillin concentration levels achieved in ESCAgenetics large-scale culture per unit weight of cell exceed those in the bean, thereby establishing the basis for attractive economies for PhytoVanilla and PhytoVanillin, (PhytoVanilla is the flavor complex from culture, a component of which is natural PhytoVanillin also isolated as product).

The markets for PhytoVanilla and PhytoVanillin include those existing products using vanilla extract and where use of a lower cost PhytoVanilla would be permitted and accepted. Opportunities exist to substitute for synthetic vanillin in products where change to a natural vanillin is desired for purposes of labeling. Finally, there will be many new products where a natural PhytoFlavor would be advantageous.

The process of plant cell culture to produce secondary metabolites does not require genetic engineering to be effective. However, it is plausible to think of modifying the pathway of a cell so in a selective manner, to increase or decrease flux through a pathway, improve the uptake of glucose or oxygen, and thereby effect yield increases (if these changes help overcome limitations in productivity). However, product can be made economically without use of such technology through techniques available today as employed at ESCAgenetics.

POTENTIAL APPLICATIONS OF PLANT CELL CULTURE TO COFFEE PROCESSING

Plant cell culture can potentially benefit the coffee industry through development of large scale proprietary technology for production of high quality coffee beverages. Culture of coffee plant cells in bioreactor vessels should be technically feasible. Coffee cell cultures has been reported in the literature and there is substantial information on use of different techniques to try and improve flavor characteristics of poor quality coffees.[8,9,10] Even though such work focused on only a few compounds known to be present in coffee, effort could be applied to a broad spectrum of organic compounds that affect flavor and aroma, keying on those which result in consumer appeal, and using plant-cell culture to produce compounds potentially useful in coffee flavor development.

Flavor precursors in coffee beans picked at the proper maturity can be augmented by flavor complexes produced in culture added to the system at any point in the process. In the wet process, the added flavor precursors can be added to wet pulping, to fermentation, as a spray during drying and at other points prior to, during and subsequent to roasting. Additions of key precursors may result in higher levels of key compounds during a roasting time and temperature regimen.[11]

The many factors in coffee quality studied to date would also bear on achieving flavor improvement (e.g., control of aroma loss, avoiding excessive oxidation, control of microbiology, and factors related to obtaining extended shelf life).

Current industry practices to enhance overall coffee quality include physical, chemical or enzymatic processes which produce thermally-sensitive or highly unstable flavors or aromas. Processing can cause disproportionation of organic substances native to the coffee cell and other stable or natural enhancers of flavors can be further degraded as a result of addback of flavor, steam collection, and recovery processes. Therefore, review of key process points where flavor is changed, and where addition of products from plant cell culture is beneficial would be of of value and a way to improve coffee through proprietary technology.

The flavor spectrum of coffee is described by many sensory attributes such as those indicated in Table 4.[11] A coffee flavor produced by tissue culture as a concentrated natural flavor or as a way to upgrade inferior coffee would have to pass an organoleptic panel against a control of a rich coffee flavor. This can be a challenging task since coffee aroma is known to consist of more than 500 compounds.

The natural balance and levels of these flavor notes are found to more acceptable to the consumer in high quality green or roasted coffee beans, such as the high grown arabicas. Poor quality coffees may lack many of the smooth, mellow descriptors of the higher grades of coffee. In poor coffees, the right compounds may be present, but not at the proper concentration to provide the right flavor and this represents an area where precursor supplementation from plant cell culture could be helpful. Examples of critical compounds often examined in regard to establishing a balanced coffee flavor include those listed in Table 5.[11]

Plant cell culture bioreactor systems such as those in development at ESCAgenetics could be applied to treat coffee pulp or slurries of roasted or green coffees to affect flavor.

Extraction and concentration techniques could be developed based on supply of tissue culture derived product to enable the processor to addback enhanced aromas and flavors to products they produce and sell. This essence derived from plant cell culture would be regarded as natural occurring without the stigma of being "synthetic". The improved flavor/aroma mixture could be designed to be more stable (e.g. less easily volatized) and similar to the chemical and physical makeup of the higher quality coffee beans.

Concept such as these in Table 6 would have to be developed in the laboratory and then scaled to production. Given in depth understanding of coffee chemistry and flavor/aroma, the coffee industry can supply this natural and innovative technology through a company dedicated to giving the consumer higher quality flavor and aroma products and options. ESCAgenetics technology can be used to break new ground in generation of natural coffee enhancers much as it is breaking ground in vanilla flavor, fruit flavors, specialty flavors, ingredients, processing aids, and new production technologies to provide important pharmaceuticals.

Plant cell culture (PhytoProduction) results in natural products and offers many flavor/aroma enhancement opportunities to the Coffee industry ranging from green coffee processing to the production of marketable products. The technology can result in better coffee quality and could be an improvement over use of synthetic enhancers or additives that have been either inadequate, prohibited or expensive. In closing, the genetic engineering of coffee is the subject of a separate area of research at ESCAgenetics and is covered in another paper at this conference. ESCAgenetics is searching for possible pathways that can link the exciting technologies of plant cell culture and genetic engineering of coffee.

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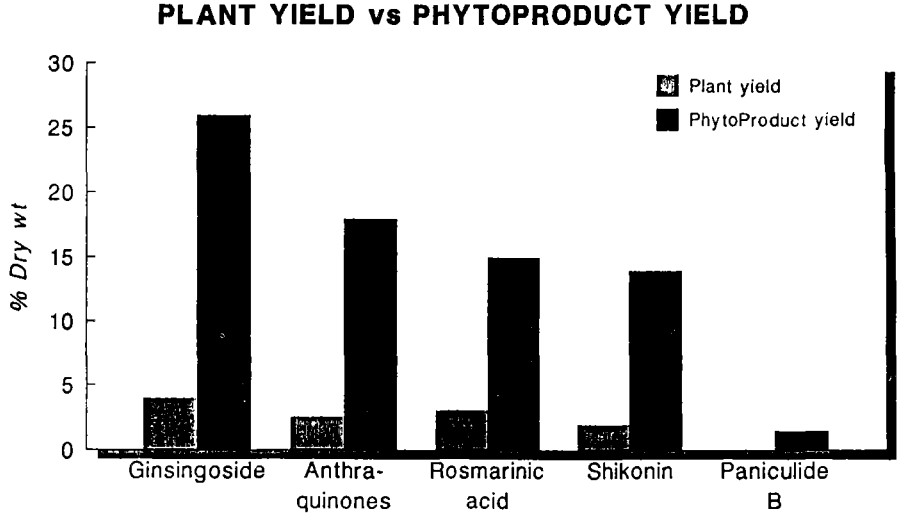
LIST OF TABLES

F I G U R E 1



Concentration of Components in Cell Culture and in the Intact Plant.

T A B L E 1

Food Colors	Fruit Flavors	Vegetable Flavor	Special Flavor
• Anthocyanins	• Strawberry	• Tomato	• Vanilla
• Betacyanins	• Cherry	• Celery	• Cocoa
• Saffron	• Grape	• Asparagus	• Licorice
	• Peach	• Capsicum	
	• Raspberry		
	• Banana		
	• Pineapple		
	• Mango		

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T A B L E 2

Oils	Sweeteners	Spices
• Mint	• Stevioside	• Cinnamon
• Garlic	• Miraculin	• Cardamom
• Onion	• Monellin	• Rosemary
• Rose	• Thaumatin	• Sage
• Patchouli		• Turmeric
• Jasmine		

ESCAGENETICS

TABLE 3

FLAVORS

- Vanilla
- Strawberry
- Concord grape
- Cherry
- Raspberry
- Licorice

ESCA GENETICS

TABLE 4

EXAMPLES OF SENSORY ATTRIBUTES TO DESCRIBE COFFEE FLAVOR

caramel	groundsy
cereal	woody
earthy	nutty
dark roasted	winey
smooth	buttery
mellow	

ESCA GENETICS

T A B L E 5

EXAMPLES OF COMPOUNDS IMPORTANT FOR A BALANCED COFFEE FLAVOR

acetadehyde	isobutyraldehyde
isovaleraldahyde	diacetyl
furan	2,3 pentandione
furfuraldehyde	furfural
dimethyl sulfide	

ESCAGENETICS

TABLE 6

POTENTIAL APPLICATION OF PLANT CELL CULTURE TO COFFEE PROCESSING

- Produce a broad spectrum of organic compounds by plant cell culture that are key to flavor and aroma.
- Add flavor precursors derived from cell culture to different steps in bean processing to obtain higher levels of key compounds that may affect flavor.
- Examine the effects of plant cell culture additives on aroma loss, flavor degradation and stability, microbiology, and product shelf life.
- See if plant cell culture techniques can be helpful in upgrading coffee bean product quality.
- Develop process concepts that are scalable to production.

ESCAGENETICS

ÉVALUATION D'HYBRIDES ARABUSTA F1 (CAFÉIERS DIPLOÏDES DOUBLÉS × *COFFEA ARABICA*) EN CÔTE D'IVOIRE DE 1982 À 1989

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La création, l'amélioration et la sélection des hybrides Arabusta (Coffea canephora 4X=44 x C.arabica), ont été entreprises en Côte d'Ivoire au début des années 60 par CAPOT (1,2,3). Son but était de mettre à la disposition de la caféiculture ivoirienne une variété:

- productive et rustique, adaptée aux conditions de basse altitude de l'ouest africain,
- qui, comparée aux caféiers Robusta, améliore sensiblement la qualité du produit, tant dans sa présentation (granulométrie) que pour ses qualités gustatives.

Ce programme de recherche a suscité de nombreuses études et de fructueuses collaborations (5,7,17), en particulier entre l'IRCC et l'ORSTOM en Côte d'Ivoire. D'autres pays s'y sont intéressés aussi (12,14).

Le matériel végétal obtenu a été fixé par bouturage horticole (8) et soumis à une sélection clonale; sa multiplication par microboutures a été étudiée (9,19). Au début des années 80, alors que le succès avait semblé imminent (6,11), le bilan d'une vingtaine d'années de recherche était dressé: cet hybride, satisfaisant quant à la qualité du café produit (4,6,13), avait une productivité jugée insuffisante et irrégulière.

Les facteurs d'explication avancés (18) étaient divers:

- d'une part, l'irrégularité des associations chromosomiques au cours de la méïose, liée à l'état hybride, entraînait des anomalies dans la fructification;
- d'autre part, l'amélioration, par sélection dans des générations successives, de la fertilité des géniteurs diploïdes doublés, semblait avoir peu d'effet sur celle des hybrides;
- de plus, la base génétique des géniteurs, Robusta en particulier, était restreinte;
- enfin, le manque de rusticité de l'hybride pouvait, en partie, être lié à l'inadaptation des géniteurs arabica aux conditions de Côte d'Ivoire.

MATERIEL ET METHODES

10 000 nouveaux hybrides furent donc créés de 1980 à 1983. Leur composition a été présentée à l'ASIC en 1985 (17). Les buts principaux, outre la possibilité d'opérer une sélection individuelle dans un grand nombre de plants, étaient:

- d'utiliser les géniteurs C.canephora autotétraploïdes sélectionnés les plus fertiles,
- d'élargir la base génétique des géniteurs, C.arabica et diploïdes doublés (C.canephora, Congusta),
- d'évaluer l'intérêt de la sélection en basse altitude de C.arabica pour l'amélioration de l'hybride.

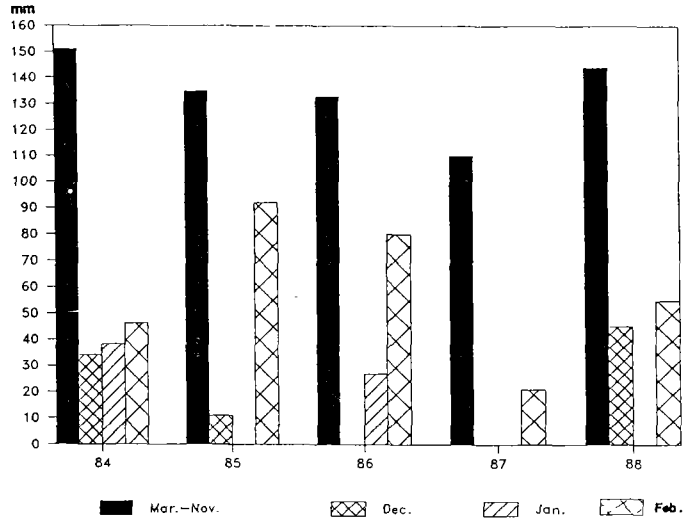
Environ 6000 hybrides Arabusta ont ainsi été plantés de 1982 à 1984 sur la station IRCC de Divo. Huit essais ont été installés, en randomisation totale arbre par arbre. Le clone Arabusta sélectionné 1313 servait de témoin constant pour l'évaluation de plus de 200 familles hybrides, à effectifs variables. Ce clone témoin a aussi servi de référence dans la sélection individuelle.

Chaque arbre a été suivi individuellement pendant trois à quatre récoltes, suivant les essais. La récolte était pesée au champ à chaque passage. Différents paramètres de vigueur et d'architecture ont été mesurés. La fertilité était estimée par coupage de 200 cerises immatures par arbre et évaluation des pourcentages de grains caracolis et de loges vides. Les caractéristiques technologiques ont été déterminées à partir d'un échantillon de 0,5 à 1 kg de cerises mûres par arbre. La sensibilité aux aléas (rouille orangée, sécheresse) était notée par appréciation visuelle au champ, lors de la présence de symptômes.

CONDITIONS DE L'EXPERIENCE

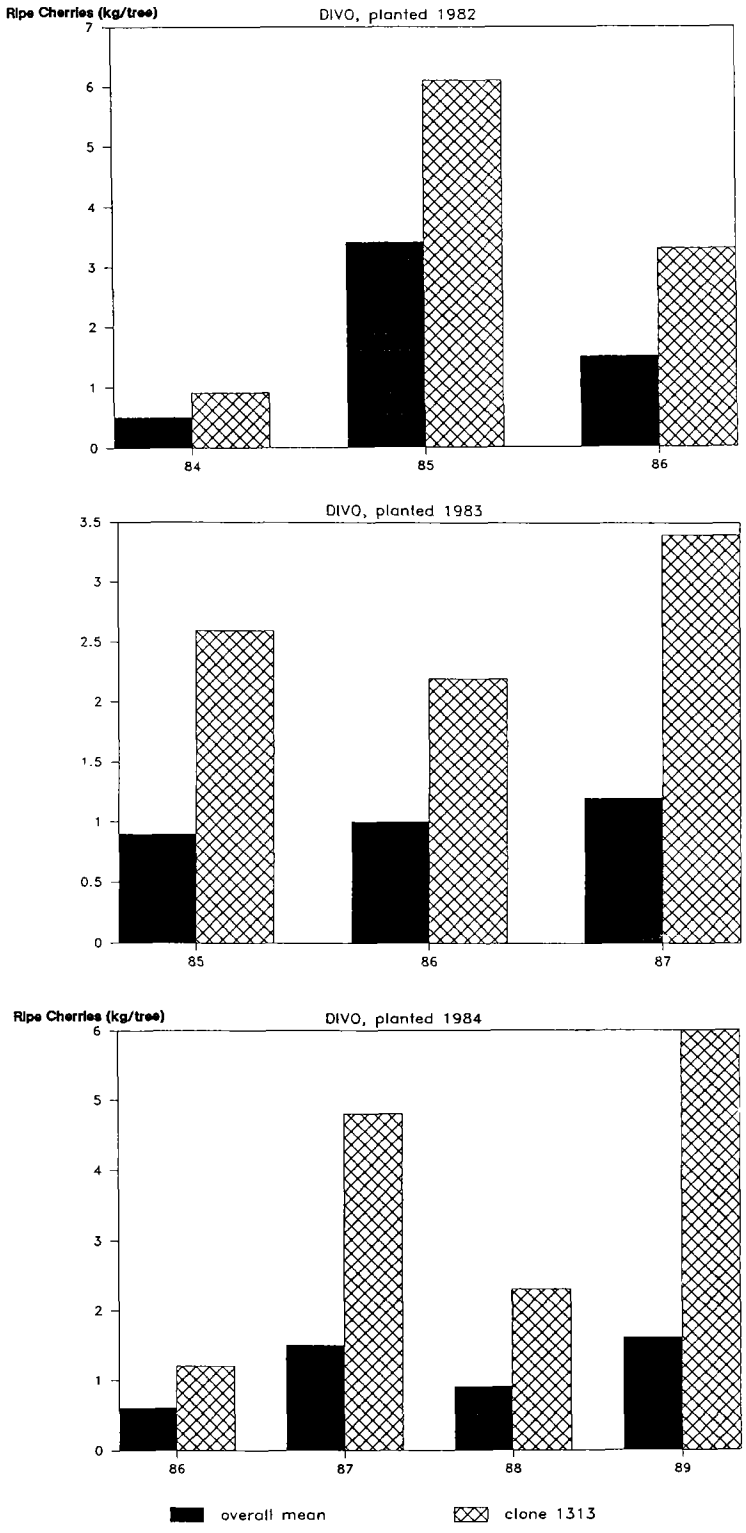
La figure 1 indique les conditions climatiques dans lesquelles se sont déroulées les essais. On note que la rigueur de la saison sèche (décembre à février), est de plus en plus prononcée de 1986 à 1988. Parallèlement, la pluviosité moyenne en saison des pluies (en noir) a fortement diminué pendant la même période.On atteignait, en 1988, des conditions tout à fait critiques pour la caféiculture. 1988-89 fut plus favorable.

Figure 1: Pluviométrie, Divo, de 1984 à 1989



(moyennes mensuelles)

Figures 2, 3, 4: Productions annuelles du clone témoin et moyenne générale des essais
(kilogrammes de cerises fraîches par arbre)

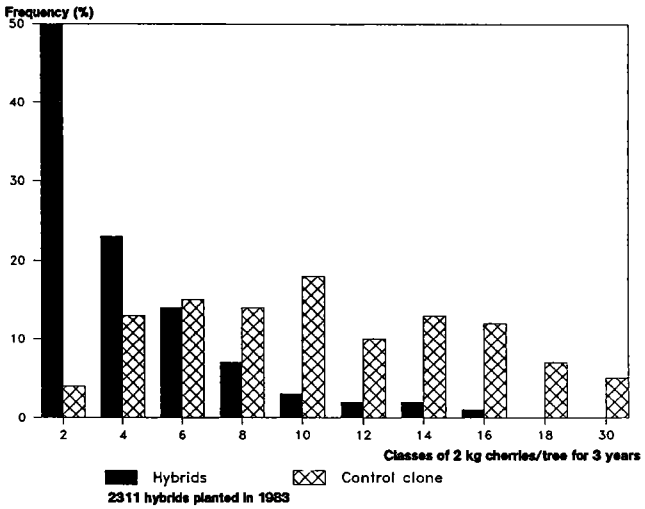


Les productions annuelles du clone témoin 1313 et la moyenne des essais sont présentées sur les figures 2, 3 et 4 pour les essais plantés en 1982, 1983 et 1984. Elles illustrent bien les conséquences de ces conditions climatiques sur la productivité moyenne, et la sensibilité à l'environnement (sécheresse), de ce matériel (15,16).

RESULTATS

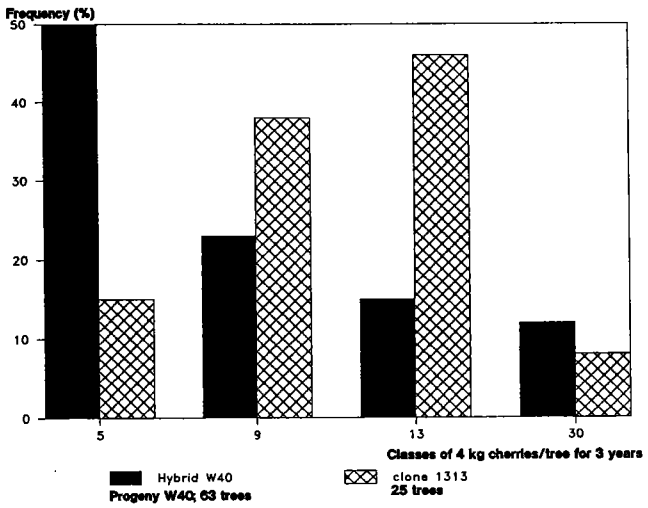
La figure 5 indique, en fréquences, la distribution de la productivité dans les essais plantés en 1983. Plus de 2300 plants hybrides sont comparés à 72 arbres du clone témoin. La comparaison des deux distributions indique que les chances de sélectionner des individus "élites"sont très faibles.

Figure 5: Distribution de la productivité des hybrides et du clone témoin, plantés en 1983



Dans le meilleur des cas, certaines familles hybrides se sont révélées statistiquement équivalentes au clone témoin. Pour la descendance W40, par exemple, on obtient le type de distribution de la figure 6. La proportion de plants peu productifs est encore importante, mais un petit nombre d'individus ont une production, au mieux, équivalente à celle des meilleurs arbres du clone témoin.

Figure 6: Distribution de la productivité dans la descendance W40 et le clone témoin



Le seuil de sélection, pour des individus améliorateurs, a donc été fixé, dans chaque essai, à la moyenne du meilleur clone témoin augmentée de deux écart-type.

Tableau 7: Individus hybrides sélectionnés par comparaison au clone témoin

Année plant. (nbre récol.)	Nbre d'arbres total	Individus sélectionnés > m + 2 e.t.	>m + e.t.(*)
1982 (3)	1140	0	3
1983 (3)	2812	4	19
1984 (4)	1880	5	7

(*) arbres à production irrégulière exclus

De cette manière, comme le montre le tableau 7, neuf arbres seulement sur les 6000 testés (soit 1,5 pour mille) ont été retenus. Si leur production cumulée est bien supérieure à la moyenne du meilleur clone témoin, ils présentent le même défaut que celui-ci: leur production est très fluctuante d'une année sur l'autre, et certaines productions annuelles sont quasi nulles. Or, un des buts de la sélection est d'obtenir un matériel rustique et aussi peu sensible à l'environnement que possible. Le seuil de sélection a donc été abaissé à la moyenne du clone, augmentée d'un écart-type seulement, en éliminant les arbres à production fluctuante. Le taux de sélection global atteint ainsi près de six pour mille.

Tout ce matériel, dans les conditions de Divo, présentait au moins une résistance partielle forte à la rouille orangée, mais seuls les arbres indemnes (absence de taches sporulantes) ont été retenus. De même, les arbres attaqués par le foreur du tronc (Bixadus sierricola) ont été éliminés de la sélection.

Une des préoccupations majeures des sélectionneurs, concernant cet hybride interspécifique, a toujours été sa stérilité, exprimée par différents paramètres (10,13). Les nombreux échantillons prélevés sur les hybrides présentés ici confirment que la restauration de la fertilité des tétraploïdes Robusta, par brassage en générations successives, n'a aucune influence sur les hybrides Arabusta obtenus. Les Congusta tétraploïdes (C.canephora x C.congensis) donnent des descendances plus hétérogènes, ce qui doit permettre une sélection individuelle plus aisée; malheureusement, le manque de rusticité du Congusta se retrouve dans les hybrides. En revanche, les observations réalisées sur les fruits montrent que les hybrides faits avec Congusta ont un meilleur remplissage des fruits (grains normalement formés) que ceux à parent Robusta, ces derniers étant, de ce point de vue, supérieurs aux hybrides issus de C.liberica (observations LE PIERRES, non publié).

La figure 8 présente l'évolution des pourcentages de grains caracolis sur l'ensemble des échantillons prélevés dans les essais de 1983 (en noir). Jusqu'à présent, ce paramètre paraissait très stable chez les Arabusta. Ici, en liaison avec les conditions climatiques, l'évolution est sensible: 142 arbres de différentes familles, plantés en 1983, ont été échantillonnés systématiquement chaque année; l'analyse de l'effet année est hautement significative, et le test de Neumann et Keuls à 5% sépare nettement les trois années.

Le taux de grains caracolis, lié à la fertilité ovulaire, passe en deux ans de 58% à 77%. Les taux de loges vides sont liés bien plus fortement encore à l'environnement. L'indépendance de ces deux expressions de la stérilité (18), de même que l'influence du sens du croisement (10,20) ne se vérifient pas toujours dans ces essais.

Figure 8: Evolution du taux de grains caracolis dans les essais plantés en 1983 à Divo

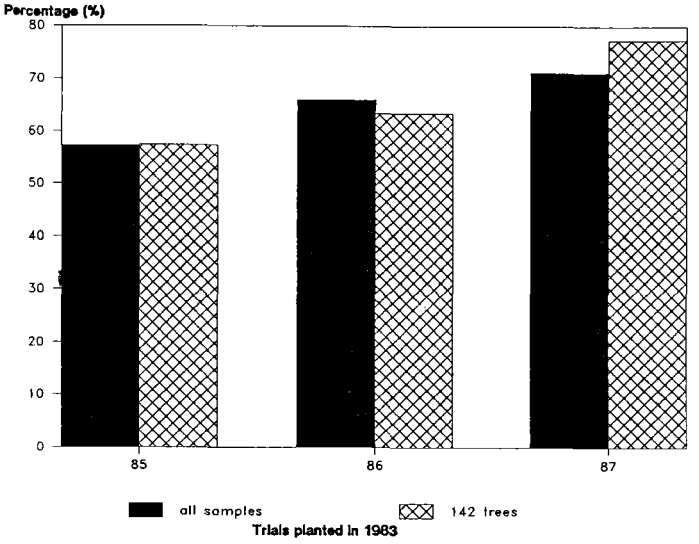
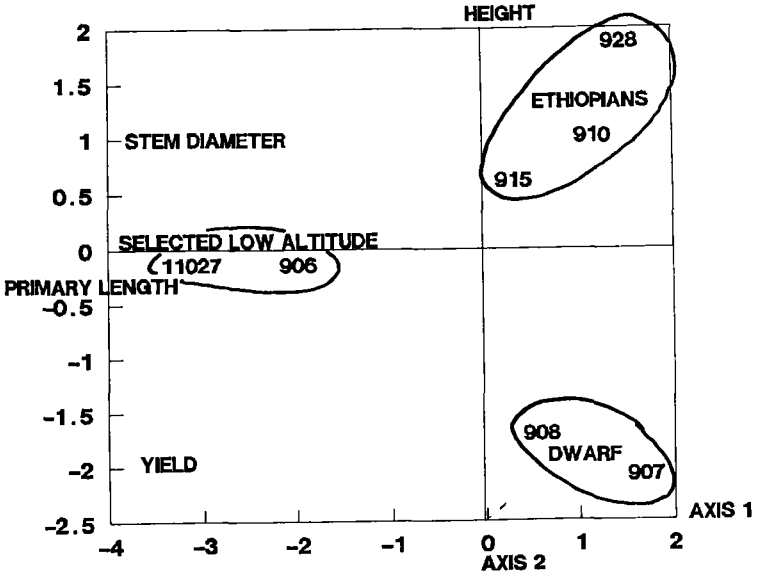


Figure 9: Analyse factorielle en composantes principales des hybrides issus de sept géniteurs arabica



7 Arabica parents representing 582 hybrids.
4 variables
Axes 1 and 2 represent 79 % of total variation

En ce qui concerne les géniteurs arabica, une analyse factorielle en composantes principales (Figure 9) a été réalisée sur 7 géniteurs représentés par 582 hybrides plantés en 1984. Les caractères suivants participent à l'analyse: hauteur de l'arbre, longueur de la plus grande primaire, diamètre au collet, et production. Les génotypes "Ethiopiens" proviennent de la prospection ORSTOM/IRCC de 1966. Ils ont été largement utilisés comme géniteurs de la plupart des Arabusta jusqu'en 1980. Cette analyse met en évidence deux voies d'amélioration de la productivité:

- d'une part (sur la gauche) l'amélioration et la sélection de l'adaptation en basse altitude (906 est la variété Guinée Pita sélectionée en Guinée, et 11027 est une sélection ORSTOM de Divo dans des hybrides entre Ethiopiens),
- d'autre part, l'utilisation de variétés productives (ici, 907 = Catimor et 908 = Porto Rico).

La combinaison des deux devrait donc être un facteur d'amélioration des hybrides, et a été entreprise. De plus, l'introduction du gène Caturra dans les hybrides permet d'obtenir des arbres de format réduit, et relativement productifs.

CONCLUSIONS

Cependant, une conclusion s'impose, dans les conditions de Côte d'Ivoire: l'hybride interspécifique Arabusta F1 est, malgré sa vigueur, caractérisé par une faible fertilité et une grande sensibilité à l'environnement. Cela se traduit par des taux de grains caracolis toujours supérieurs à 50%, des taux de loges vides fluctuants, mais importants (15 à 40%). Le rendement technologique varie entre 10 et 15%, et la productivité, si elle peut, certaines années, dépasser 2 tonnes de café vert par hectare, est très variable d'une année sur l'autre, et trop faible pour justifier un développement: les seuils de sélection évoqués plus haut varient entre 1000 et 1400 kg de café marchand par hectare et par an, alors que, pour le Robusta et dans les mêmes conditions, ils dépassent deux tonnes de moyenne annuelle.

Du point de vue génétique, cet hybride interspécifique peut être utile pour, d'une part, introgresser l'arabica par certains caractères de résistance aux maladies et aux insectes des caféiers diploïdes, et, réciproquement, améliorer la qualité des cafés produits en basse altitude.

Cependant, l'avenir de l'Arabusta passe, de toute évidence, par l'amélioration de l'hybride lui-même, à travers un cycle de rétrocroisements sur C.arabica, autofécondations, et sélection de génotypes adaptés en basse altitude. Cette voie a été entreprise depuis quelques années, mais n'en est qu'à ses débuts. Par ailleurs, pour pousuivre l'élargissement de la base génétique des hybrides, le croisement direct entre Carabica tétraploïde et espèces diploïdes est possible (21), et plus rapide que le passage par le doublement chromosomique des diploïdes.

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CHARMETANT P., LE PIERRES D., YAPO A.. EVALUATION D'HYBRIDES ARABUSTA F1 (Caféiers diploïdes doublés x C.arabica) EN COTE D'IVOIRE DE 1982 A 1989.

Plus de 6000 hybrides Arabusta F1 ont été testés sur la station de Divo, avec un clone témoin Arabusta F1.
Après avoir exposé les conditions climatiques dans lesquellles se sont déroulés les essais (saison sèche de plus en plus marquée de 1985 à 1989) et leurs conséquences sur la fertilité et les productions du témoin et de l'ensemble des hybrides, les grandes lignes des résultats obtenus sont présentées.
La productivité de ce type de matériel est faible, et les 38 individus sélectionnés apportent une amélioration insuffisante pour justifier un développement agronomique de cet hybride.
La sélection en basse altitude de C.arabica, combinée à l'utilisation de variétés productives et naines de cette espèce, peuvent améliorer productivité et rusticité de l'hybride.
Cependant, seule l'amélioration de l'hybride lui-même, à travers des cycles de croisements et d'autofécondations, peut permettre d'espérer la mise au point, en Côte d'Ivoire, d'une variété qui produise un café de qualité sensiblement supérieure à celle du Robusta.

RÉSULTATS RÉCENTS DU PROGRAMME DE SÉLECTION RÉCURRENTE RÉCIPROQUE CHEZ *COFFEA CANEPHORA* EN CÔTE D'IVOIRE

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INTRODUCTION

Le nouveau programme de sélection récurrente réciproque mis en place chez *Coffea canephora* en Côte d'Ivoire a été présenté lors de la dernière réunion de l'ASIC à Paipa (LEROY et CHARRIER, 1989). Il repose sur l'existence de deux pools génétiques chez cette espèce, les Guinéens et les Congolais (BERTHAUD, 1986; CHARRIER et BERTHAUD, 1988). Les premiers résultats confirment l'intérêt des hybrides entre les deux pools génétiques pour la sélection de clones et de descendances (LEROY et *al.*, 1991).

Nous présenterons ici les méthodes de sélection adoptées, basées sur les études génétiques, et les résultats obtenus pour la productivité de descendances et l'utilisation des régressions production-vigueur, la part des aptitudes générales et spécifiques à la combinaison pour les caractères sélectionnés, ainsi que l'héritabilité des caractères liés à la productivité. Les conséquences de ces résultats sur le déroulement du schéma de sélection et le brassage des populations seront enfin discutées.

MATERIEL ET METHODES

Des descendances plantées au champ en 1986, selon un dispositif en randomisation totale arbre par arbre, sont analysées:

- des descendances à parents constants (deux Guinéens croisés par cinq Congolais) dans un essai comportant également trois clones témoins vulgarisés (126, 202 et 461) et des descendances intragroupes. A l'exception d'une famille à 19 individus, les effectifs par descendance ou clone sont supérieurs à 50 plants. 1360 plants ont été observés (essai 1).
- un essai de descendances intergroupes (13), intraguinéennes (2) et de deuxième génération (3 descendances obtenues par croisements entre des génotypes hybrides), comparées aux trois mêmes clones témoins vulgarisés. Les effectifs sont compris entre 54 et 100 plants par descendance, au total 1808 plants en essai (essai 2).

Dans ces deux essais, les données suivantes ont été analysées: paramètres de vigueur, composantes de la production, productivité individuelle des arbres,

sensibilité à la sécheresse et à la rouille orangée. Les données ont été traitées en analyse de variance à un facteur pour des effectifs inégaux avec classement par Newman et Keuls modifié pour les effectifs inégaux (KRAMER, 1956).

L'analyse de covariance sur les moyennes des arbres voisins (PAPADAKIS, 1937) a été utilisée pour tenir compte de l'hétérogénéité du terrain. Par ailleurs, une analyse de covariance de la production avec comme covariable la vigueur de la même plante a été réalisée, dans le but de sélectionner des arbres ayant un rapport production/vigueur favorable (CHARMETANT et LEROY, 1989). Ces deux analyses améliorent la séparation des moyennes par l'analyse de variance en diminuant le coefficient de variation résiduel.

Enfin, une analyse à deux facteurs (mâle et femelle) dans l'essai à parents constants a permis d'évaluer les aptitudes générales et spécifiques à la combinaison des caractères étudiés et de calculer leur héritabilité.

RESULTATS ET DISCUSSIONS

A) Variables analysées

1) Vigueur

Le tableau 1 présente la vigueur moyenne des familles et clones en essai, exprimée par le diamètre à la base des arbres à 30 mois. Seules les meilleures et la plus mauvaise famille ont été représentées, ainsi que les trois clones témoins. Les coefficients de variation observés sont comparables pour les clones et les descendances, ils sont compris entre 11 et 20% .

TABLEAU 1: VIGUEUR DES PLANTS
DIAMETRE AU COLLET A 30 MOIS (MM): VALEUR DE QUELQUES FAMILLES ET CLASSEMENT PAR LE TEST DE Newman et Keuls à 5%

ESSAI 1			ESSAI 2		
CLONE 461	64,9	A	222 X 02213	70,9	A
444 X 054 C	63,4	A B	466 X 410	67,3	A
410 X 121 H	63,1	A B	CLONE 461	63,7	A B
155 X 111 H	61,2	A B	392 X 410	60,7	A B C D
410 X 069 H	59,8	A B	392 X 02214	59,7	B C D
155 X 121 H	59,7	A B	444 X 410	59,1	B C D
CLONE 202	52,9	B	CLONE 202	51,8	C D
CLONE 126	49,6	B C	CLONE 126	48,4	D
155 X 414 G	47,0	C	119 X 107 F	44,6	D

Certaines familles intergroupes sont donc aussi vigoureuses voire plus vigoureuses que les clones témoin 202, 126 et 461. Il faut noter que dans l'essai 1, la famille la plus vigoureuse résulte du croisement de deux génotypes congolais. Les familles les moins vigoureuses des deux essais sont issues du croisement de deux génotypes guinéens ou de celui de deux clones hybrides intergroupes.

2) Productivité

Le tableau 2 présente la productivité cumulée des trois témoins clonaux, des meilleures et de la plus mauvaise des familles sur trois récoltes.

TABLEAU 2: PRODUCTIVITE DES PLANTS
CUMUL TROIS RECOLTES (KG C.M./ha/an):
VALEUR DE QUELQUES FAMILLES ET CLASSEMENT PAR Newman et Keuls à 5%

ESSAI 1			ESSAI 2		
CLONE 461	1590	A	CLONE 461	1560	A
410 X 057 H	1360	B	466 X 410	1460	A
410 X 121 H	1330	B	392 X 410	1225	B
444 X 054 C	1290	B	392 X 02214	980	C
155 X 121 H	1050	C	444 X 410	970	C
410 X 111 H	930	C	222 X 02213	840	CD
CLONE 126	660	D	CLONE 126	580	EFG
CLONE 202	550	D	CLONE 202	430	FGHI
444 X 414 H	235	E	054 X 414	200	HI
155 X 414 G	135	E	155 X 416 G	155	I

Le clone 461 s'avère toujours le plus productif, dans l'essai 1 il est même significativement plus productif que toutes les autres familles en essai. Les coefficients de variation observés sont compris entre 33 et 110%, mais les descendances les plus productives n'ont pas de coefficients plus élevés que les clones. Ceci souligne l'effet considérable du milieu qui rend plus difficile la sélection individuelle d'arbres productifs. La productivité importante du clone 461 est en partie due à une entrée en production plus précoce, caractéristique du matériel clonal. Cependant, de nombreuses descendances intergroupes ont des productivités très importantes qui pourraient justifier leur vulgarisation, puisqu'elles produisent plus que certains clones vulgarisés (126, 202) et que de nombreux arbres dans plusieurs familles ont des productions individuelles supérieures à la moyenne du clone 461, comme le montre le tableau 3.

Cependant, les familles issues de croisements entre génotypes des deux pools génétiques ne sont pas toujours productives, certaines de ces descendances sont parmi les plus médiocres dans les deux essais.

TABLEAU 3: NOMBRE D'ARBRES LES PLUS PRODUCTIFS
PAR RAPPORT A LA PRODUCTION DU CLONE 461

PRODUCTION	NOMBRE D'ARBRES SUPERIEURS A MOYENNE DU 461	MOYENNE + 1 écart-type	MOYENNE + 2 écart-type
ESSAI			
1	153 (13)	48 (10)	10 (6)
2	118 (9)	36 (6)	11 (3)

( ): entre parenthèses, nombre de familles dont sont issus les arbres productifs dans ces deux essais.

Les analyses de variance de la productivité ont été reprises avec l'analyse des plus proches voisins de Papadakis. Cette analyse permet d'améliorer la précision en prenant en compte la microhétérogénéité du sol. Les analyses n'ont permis d'expliquer qu'une faible part de la variation, moins de 10% quelque soit le caractère utilisé pour construire la covariable.

3) Corrélations entre vigueur et productivité

Afin de déterminer des variables de vigueur prédictives de la productivité cumulée sur plusieurs récoltes, les corrélations entre caractères ont été étudiées. Les corrélations les plus élevées entre les caractères de vigueur et

de production sont présentées dans le Tableau 5.

TABLEAU 5: COEFFICIENTS DE CORRELATION ENTRE LES CARACTERES DE VIGUEUR ET LA PRODUCTIVITE OBSERVES DANS DEUX ESSAIS DE DESCENDANCES DE *Coffea canephora*

ESSAI 1 RECOLTE CUMULEE		Prob.	ESSAI 2 RECOLTE CUMULEE		Prob.
VG878*	0,438	0,00	VG878	0,325	0,00
DC189	0,560	0,00	DC189	0,519	0,00
VG879	0,514	0,00	VG879	0,452	0,00
AC879	0,040	N.S.	AC879	0,138	0,00

* VG878: accroissement de diamètre au collet entre 6 et 18 mois
voir le Tableau 4 pour la signification des sigles

Toutes les corrélations totales individuelles observées sont significatives à 1%, à l'exception de l'accroissement relatif de diamètre au collet entre 6 et 30 mois dans l'essai 1. Cette variable est certainement trop liée à l'état des plants à la plantation, et non au développement des plants qui conditionne la productivité des arbres.

C'est le diamètre au collet à 30 mois qui est le mieux corrélé avec la productivité, avec un coefficient de détermination supérieur à 25%. Cette donnée qui représente le développement de l'arbre avant sa première production pourra donc être utilisée pour la prédiction générale des potentiels de productivité des familles.

4) Covariances productivité/vigueur

L'analyse de la productivité en utilisant la vigueur comme covariable a également été réalisée pour des données de diamètres au collet et d'encombrement des arbres. Les résultats sont présentés dans le Tableau 4.

TABLEAU 4: SIGNIFICATION ET PART DE LA VARIATION POUR LA PRODUCTIVITE EXPLIQUEE PAR L'ANALYSE DE COVARIANCE SIMPLE

PARCELLE	CO-VARIABLE	POURCENTAGE DE VARIATION EXPLIQUE
ESSAI 1	DC189	23,5%
	VG879	19,1%
	AC879	N.S.
	ENC90	N.S.
ESSAI 2	DC189	16,7%
	VG879	11,4%
	AC879	N.S.
	ENC90	6,6%

Co-variables: DC189: diamètre au collet à 30 mois
VG879: différence de diamètre au collet entre 6 et 30 mois
ENC90: diamètre de l'arbre perpendiculairement à la ligne de plantation
AC879: accroissement relatif de diamètre au collet entre 6 et 30 mois
* N.S.: non significatif à 5% de probabilité

L'analyse de covariance dans des essais en randomisation totale arbre par arbre, tels que ceux qui sont présentés, est un outil puissant puisqu'elle permet d'expliquer jusqu'à 25% de la variation de la productivité dans le cas du diamètre au collet à 30 mois de l'arbre lui-même pris comme covariable. Les autres variables de vigueur expliquent une part moins importante de la variabilité.

Dans les deux parcelles étudiées, l'analyse de covariance simple sur le diamètre au collet à 30 mois modifie le classement des moyennes des clones et descendances en essai. Dans l'essai 1, le clone témoin 461 était significativement plus productif que toutes les descendances. Après analyse de covariance, une descendance lui est équivalente par le test de Newman et Keuls à 5%. Cette descendance, productive et moins vigoureuse que le témoin, pourra être sélectionnée et plantée à des densités plus élevées que celles couramment appliquées actuellement.

En revanche, dans l'essai 2, on observe le phénomène inverse: une descendance aussi productive que le clone 461 s'avère significativement moins productive que lui après analyse de covariance. L'utilisation des covariables est donc intéressante. Elle permet d'adapter les sélections à des conditions de densité, de fertilité du terrain ou de techniques culturales différentes.

B)Etude des aptitudes à la combinaison

Cette étude a été menée sur l'essai à parents constants par analyse de variance à deux facteurs croisés (mâle et femelle), avec étude de l'interaction mâle par femelle. Celle-ci donne l'indication de l'importance des phénomènes d'aptitude spécifique à la combinaison. Pour tous les paramètres de vigueur tels que le diamètre au collet à 30 mois et les différences de diamètre entre 6 et 30 mois, on observe des aptitudes spécifiques et générales à la combinaison significatives.

En revanche, pour la productivité cumulée des arbres sur trois récoltes, l'interaction est non significative, ce qui signifie que l'aptitude générale à la combinaison prédomine pour la productivité des hybrides entre les deux groupes Guinéens et Congolais. On observe de très grandes différences pour la productivité des descendants des géniteurs: 4 kg de cerises fraîches en moyenne par arbre sur trois récoltes pour les descendants du clone 444, et 8 kg pour les descendants du clone 121.

Une analyse de variance à effets mixtes a été effectuée pour les données de vigueur et de productivité des arbres: facteur femelle fixe et facteur mâle aléatoire. En effet, le nombre de femelles (2) ne permet pas de considérer ce facteur comme étant aléatoire, alors que les cinq mâles représentent une plus grande variabilité dans le groupe congolais. Les héritabilités au sens strict calculées sont présentées dans le Tableau 6.

TABLEAU 6: HERITABILITES AU SENS STRICT CALCULEES PAR LE MODELE A EFFETS MIXTES

CARACTERE	HERITABILITE
DC189**	0,1
VG879**	0,1
AC879**	0,1
C8889*	0,51
CUM90*	0,15
C8890**	0,52

*: C8889: récolte cumulée 1988 et 1989
CUM90: récolte 1990
**: voir Tableau 4

La très faible héritabilité des caractères de vigueur dans cette analyse confirme l'importance des aptitudes spécifiques à la combinaison pour la

vigueur des descendants. En revanche, les caractères de productivité ont des héritabilités plus élevées, la transmission de la productivité est de type additif dans cet essai. Ces valeurs élevées sont peuvent être dues au caractère très typé des génotypes mâles comme les clones 121 et 444. Les valeurs d'héritabilité très voisines observées pour le cumul sur deux ou trois récoltes sont dues à des corrélations très élevées entre ces deux variables (production faible en 1990).

CONCLUSIONS

Pour la sélection d'arbres et de descendances productives, ces essais sont intéressants puisqu'un grand nombre de plants a une productivité importante, grâce à la grande vigueur des hybrides entre les deux groupes. Certains de ces arbres pourront donc être clonés et éventuellement proposés à la vulgarisation. Certains caractères de vigueur, comme le diamètre au collet à 30 mois, sont assez bien corrélés avec la productivité et permettent des prédictions sur la productivité future des arbres observés.

Les héritabilités assez faibles qui sont observées pour la vigueur montrent l'importance de l'observation des descendances pour connaître la valeur des géniteurs. Pour le choix des génotypes qui seront brassés pour créer la population améliorée, point de départ du deuxième cycle de sélection, il est donc nécessaire de connaître avec précision les descendants de chaque géniteur croisé avec plusieurs testeurs.

La forte aptitude générale à la combinaison observée pour la productivité, déjà observée par d'autres auteurs (BOUHARMONT et *al*, 1986) permet de sélectionner de nombreuses têtes de clones dans les descendances les plus productives en tenant compte des relations entre vigueur et productivité pour adapter les sélections aux densités de plantation.

Enfin, le choix définitif des arbres et hybrides à sélectionner, et des géniteurs à brasser pour créer la population améliorée sera également basé sur les données technologiques et organoleptiques du café produit, ainsi que la résistance aux maladies et insectes des caféiers.

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RESUME

Les premiers essais plantés en 1985 et 1986 dans le cadre du programme de sélection récurrente réciproque permettent d'évaluer la vigueur et la productivité des hybrides entre Guinéens et Congolais, et les possibilités de sélection de clones et de descendances.

L'étude des aptitudes à la combinaison permet de calculer les héritabilités et de connaître la transmission des caractères sélectionnés dans les hybrides intergroupes et dans chacune des deux populations.

Les conséquences de ces résultats pour le brassage des géniteurs et la création des populations améliorées sont discutées.

SUMMARY

From the first trials planted in 1985 and 1986 as part of the reciprocal recurrent programme, the vigor and yield of hybrids between Guinean and Congolese, and the possibilities of selecting clones and descendants, have been assessed.

From studies of general and specific combining ability, the inheritability of some selected characters can be calculated.

The consequences of these results on mixing parents and creating improved populations are discussed.

QUALITÉS TECHNOLOGIQUES ET ORGANOLEPTIQUES DE QUELQUES CLONES DE *COFFEA CANEPHORA* EN CÔTE D'IVOIRE

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INTRODUCTION

Dans le contexte actuel de la commercialisation du café, le handicap majeur du café Robusta est lié à sa qualité. Le traitement post-récolte y joue un rôle essentiel. Pourtant, les études réalisées par Cramer dans les années cinquante ont montré que le facteur génétique est loin d'être négligeable (CRAMER, 1957). Dans les programmes d'amélioration classiques menés en Côte d'Ivoire (IFCC, 1963; CAPOT, 1977), les problèmes de qualité du café étaient essentiellement abordés sous l'angle technologique.

Dans le nouveau schéma d'amélioration mis en place en Côte d'Ivoire depuis 1984 (BERTHAUD, 1986; LEROY et CHARRIER, 1989), la qualité du café produit est le principal objectif de sélection.

Les résultats des premiers essais plantés pour ce programme en 1985 et 1986 (LEROY et *al.*, 1991) permettent de fixer un seuil de sélection élevé pour la productivité, l'effort de sélection porte donc sur d'autres caractères, comme la résistance aux parasites et à la sécheresse, et la qualité du café produit. Cette qualité comprend les caractéristiques technologiques (essentiellement granulométrie et taux de caféine) et organoleptiques des cafés produits.

Les résultats présentés, sur les qualités technologiques et organoleptiques de quelques cafés issus des deux populations en sélection (Guinéens et Congolais) et de leurs hybrides, montrent la variabilité disponible pour le sélectionneur.

MATERIEL ET METHODES

Les données technologiques ont été obtenues sur des arbres en collection dans la station IRCC de Divo. Les valeurs présentées sont des moyennes de plusieurs mesures (2 à 5).

Les dégustations ont été faites à partir d'échantillons de café vert récoltés à Divo entre octobre et décembre 1989. Quatre échantillons de 1,5 kg de cerises ont été récoltés sur 10 génotypes: trois génotypes Guinéens, quatre génotypes Congolais et trois clones hybrides sélectionnés entre ces deux groupes.

Les échantillons ont été traités par voie humide et séchés au soleil. Après déparchage, ils ont été envoyés au laboratoire de technologie de l'IRCC à Montpellier. Ils ont été dégustés en ranking par série de quatre , avec comme témoin le clone 126 (clone hybride). Pour chaque préparation dégustée (soit 10 clones par 4 répétitions, c'est à dire 40 cafés), les données suivantes ont été notées:

- perte de poids à la torréfaction en %
- augmentation de volume à la torréfaction en %
- corps et force (note de 1 à 3)
- acidité (note 1 ou 2)
- amertume (note de 1 à 3)
- astringence (note 1 ou 2)
- note de synthèse

Pour chaque préparation dégustée, un indice de torréfaction de 1 à 5 a été attribué (5=café trop torréfié).

Les données quantitatives ont été traitées en analyse de variance à un facteur, avec classement des moyennes par le test de Newman et Keuls à 5%

Une analyse factorielle des correspondances a été faite sur les données qualitatives et quantitatives après transformation.

RESULTATS

1) Granulométrie et taux de caféine

Les données sont présentées dans le Tableau 1. La granulométrie est exprimée en grammes pour 100 fèves à 12% d'humidité et le taux de caféine en % de la matière sèche.

TABLEAU 1: TAUX DE CAFEINE ET GRANULOMETRIE MOYENNE DES DIX GENOTYPES ETUDIES

CLONE	155 G*	410 G	160 G	178 C*	182 C	477 C	464 C	095 H*	461 H	126 H
TAUX CAFEINE	3,1	2,5	3,2	2,1	2,3	2,6	2,0	3,8	3,5	2,4
GRANULO METRIE	11	10	11	16	13	19	14	15	12	16

*G:Guinéen C:Congolais H:Hybride

Les génotypes Guinéens ont en moyenne des taux de caféine supérieurs aux Congolais (2,93% contre 2,25%) et une granulométrie inférieure (10,8 grammes pour 100 fèves contre 15,32), ce qui est en accord avec des données générales sur ces deux groupes (BERTHAUD, 1986). Cependant, les effectifs insuffisants ne permettent pas de mettre en évidence des différences significatives par analyse de variance.

2) Données de la torréfaction

Les variables quantitatives mesurées lors de la torréfaction (diminution de poids, augmentation de volume et note de synthèse) ont également été traitées en analyse de variance. Les principaux résultats sont présentés dans le Tableau 2. Nous avons considéré les valeurs moyennes pour les 12 à 16 valeurs individuelles obtenues pour chacun des groupes.

TABLEAU 2: VALEURS MOYENNES ET CLASSEMENT PAR Newman et Keuls à 5% DES TROIS TYPES DE GENOTYPES POUR LA PERTE DE POIDS ET L'AUGMENTATION DE VOLUME A LA TORREFACTION, LA NOTE DE SYNTHESE DES DEGUSTATIONS

	VALEURS MOYENNES			ANALYSE DE VARIANCE	
	C	G	H	F	CLASSEMENT
DMPDS*	13,0	14,9	12,8	***	G > C = H
AGVOL**	82,8	101,4	90,0	***	G > H > C
NOTES	5,02	4,59	4,96	N.S.	

***: significatif à 1% N.S.: non significatif
* DMPDS: perte de poids à la torréfaction
**AGVOL: augmentation de volume à la torréfaction

Les génotypes guinéens présentent donc une forte diminution de poids à la torréfaction, associée à une plus forte augmentation de volume que les autres génotypes. Pour ce dernier caractère, les génotypes hybrides présentent des valeurs intermédiaires entre celles des génotypes des deux groupes. La note globale donnée à chaque boisson par les dégustateurs, résultante de nombreux paramètres, ne permet pas de mettre en évidence de différences significatives entre les différents types de génotypes.

3)Analyse du test de dégustation

Les résultats du test par clone sont présentés dans le Tableau 3.

TABLEAU 3: RESULTATS DES TESTS DE DEGUSTATION: VALEURS MOYENNES POUR LES GENOTYPES ETUDIES

CLONE		CORPS	ACIDITE	AMERTUME	NOTE DE SYNTHESE
410	G	2,50	1,00	2,00	4,28
155	G	2,25	1,25	2,00	5,10
160	G	2,25	1,00	2,50	4,40
178	C	1,75	1,00	1,50	4,65
182	C	2,00	1,25	1,75	5,43
477	C	1,75	1,00	1,25	5,35
464	C	2,00	1,00	2,00	4,65
095	H	1,25	1,00	1,25	4,05
461	H	2,25	1,25	1,75	5,05
126	H	2,00	1,00	2,00	5,78

L'analyse de la note par clone permet de mettre en évidence des différences hautement significatives. Le génotype hybride 126, avec une note moyenne de 5,78, a été particulièrement apprécié des dégustateurs. En revanche, le clone hybride 095, très riche en caféine (3,8%), a été très peu apprécié. Un essai d'analyse de covariance avec le degré de torréfaction comme covariable n'a pas donné de résultats significatifs.

Les données du test de qualité ont été traitées en analyse factorielle des correspondances après séparation en classes des variables quantitatives (perte de poids et augmentation de volume).

La note de synthèse a été laissée en variable supplémentaire dans l'analyse. En effet, il s'agit d'une donnée subjective prenant en compte l'ensemble des autres variables analysées. La note d'astringence a été écartée en raison de sa très faible variabilité.

Les variables actives dans l'analyse étaient donc au nombre de cinq: chute de poids, augmentation de volume,corps, acidité et amertume. Le pourcentage d'explication pour les trois premiers axes est de 61%, ce qui est important pour une telle analyse (figure 1).

La représentation sur les axes 1 et 2 permet de séparer les Guinéens et les Congolais selon un axe de corps et d'amertume, dont les valeurs élevées sont liées aux génotypes Guinéens . Les génotypes hybrides sont intermédiaires entre les deux groupes, et présentent une très grande variabilité pour les caractères étudiés.

La note de synthèse apparaît au centre du nuage de points du groupe Congolais, les génotypes de ce groupe sont donc plus appréciés. En revanche, elle s'oppose aux corps et amertumes élevés, caractéristiques de la plupart des génotypes guinéens.

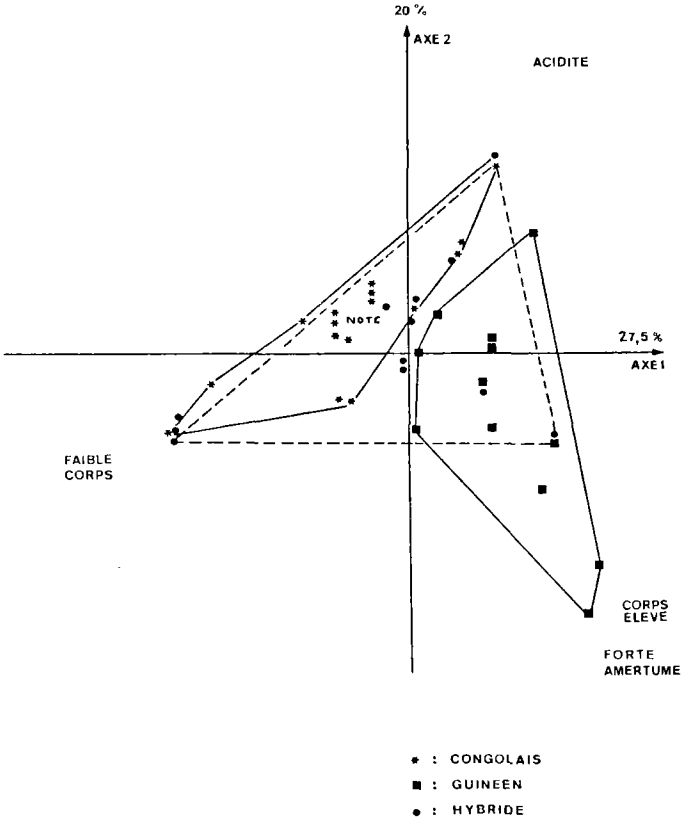

Figure 1 : ANALYSE FACTORIELLE DES CORRESPONDANCES DES DONNEES DE LA TORREFACTION ET DES TESTS ORGANOLEPTIQUES

Les coordonnées des points de l'analyse factorielle des correspondances pour les trois premiers axes ont été reprises pour effectuer une analyse factorielle discriminante (figure 2). Chaque café a été classé dans son groupe d'origine (Guinéens, Congolais, Hybrides).

65% des cafés sont bien classés par cette analyse. Le résultat le plus important est cependant la bonne séparation observée entre Guinéens et Congolais. Aucun génotype Congolais n'est classé parmi les Guinéens, un seul Guinéen, très éloigné des deux barycentres, est classé parmi les Congolais. Cette analyse confirme la bonne séparation des deux populations. Les Hybrides se trouvent répartis dans les trois groupes définis au départ.

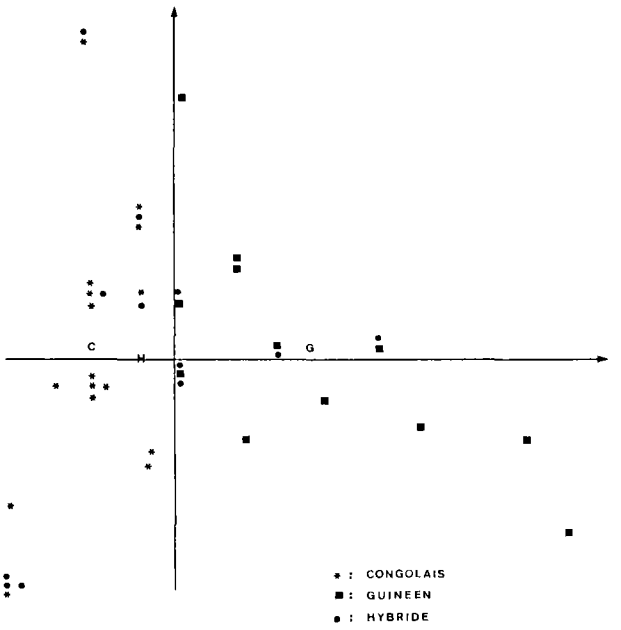

Figure 2 : ANALYSE FACTORIELLE DISCRIMINANTE
A PARTIR DES COORDONNEES DE L'AFC

DISCUSSIONS CONCLUSIONS

Les caractéristiques technologiques des cafés analysés dans cette étude (granulométrie et taux de caféine) ont des héritabilités moyennes avec une transmission de type additif (CHARRIER et BERTHAUD, 1975 et 1988; BOUHARMONT et al., 1986). Dans le programme d'amélioration, la sélection pour ces deux caractères est donc possible. Des granulométries supérieures à 14 grammes pour cent fèves et un taux de caféine inférieur à 2,5% de la matière sèche seront recherchés pour le choix de têtes de clones dans les hybrides.

L'analyse des caractères liés à la torréfaction (diminution de poids et augmentation de volume) montre des différences très importantes entre les génotypes étudiés. La forte augmentation de volume constatée pour certains génotypes Guinéens est un caractère supplémentaire recherché par l'industrie qui pourra faire l'objet d'une sélection dans les descendances et les clones en essai.

Les tests organoleptiques proprement dit ont mis en évidence de fortes différences d'appréciation entre les différents breuvages. L'analyse factorielle des caractères de torréfaction et de dégustation permet de séparer les deux groupes de cafés en sélection. Les premiers résultats présentés indiquent une tendance au corps et à l'amertume chez les Guinéens, opposé à une certaine neutralité organoleptique pour les Congolais. Les hybrides ont une variabilité importante pour l'ensemble de ces caractères.

Les résultats enregistrés permettent d'orienter la sélection afin de proposer aux planteurs et aux industriels la production de café Robusta aux qualités organoleptiques améliorées. La stratégie de la sélection pour la qualité organoleptique des Robustas en Côte d'Ivoire repose donc sur trois axes:

- sélection chez les Guinéens de cafés moins amers et possédant un corps moins prononcé
- sélection pour l'acidité chez les Congolais
- sélection dans les hybrides pour l'ensemble des caractères technologiques et organoleptiques.

D'autres critères de la qualité du café, tels que le taux d'extraction à la lyophilisation et la teneur en MIB (VITZTHUM et al., 1990) pourront être introduits ultérieurement dans les critères de sélection.

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RESUME

Depuis 1984, un programme de sélection récurrente réciproque a été entrepris en Côte d'Ivoire pour améliorer le caféier Robusta, *Coffea canephora*.
Les principales caractéristiques technologiques et organoleptiques des cafés produits par quelques génotypes des deux populations sont présentés. La bonne productivité observée des hybrides entre Guinéens et Congolais permet de mettre l'accent sur la sélection pour la qualité dans le programme d'amélioration, et d'envisager la commercialisation de cafés Robusta plus appréciés des consommateurs.

SUMMARY

The main technological and organoleptic characteristics of the coffee produced by a few genotypes of the two populations are presented. As a result of the good yield observed in Guinean-Congolese hybrids, selecting for quality in the selection programme can be stressed, and the marketing of the popular Robusta varieties can be considered.

ÉTUDE COMPLÉMENTAIRE DE LA DIVERSITÉ GÉNOTYPIQUE ET PHÉNOTYPIQUE DES CAFÉIERS DE L'ESPÈCE *COFFEA CANEPHORA* EN COLLECTION EN CÔTE D'IVOIRE

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1 INTRODUCTION

L'évaluation de la diversité génotypique et phénotypique des collections de *Coffea canephora* constitue la base de l'amélioration de cette espèce en Côte d'Ivoire. Ainsi, la mise en évidence des groupes Guinéen et Congolais par BERTHAUD (1985), à partir de l'étude de populations sylvestres, a abouti à la mise en place d'un schéma de sélection récurrente réciproque (LEROY et CHARRIER, 1989) utilisant la vigueur hybride entre ces deux groupes.
L'évaluation génotypique et phénotypique des caféiers s'est étendue depuis à l'ensemble des collections comprenant également des caféiers cultivés (LEROY et CHARMETANT, 1987 ; ADIBOLO et BERTRAND, 1988 ; MONTAGNON, 1989 ; MONTAGNON et al., sous impression). Une analyse globale peut donc être réalisée. Les diversités génotypique, évaluée par marqueur enzymatique, et phénotypique sont traitées successivement. Les conséquences des résultats sur la sélection sont discutées.

2 MATERIEL ET METHODES

2-1 Matériel :

Le tableau 1 dresse la liste des populations sylvestres ou origines cultivées étudiées dans ce travail.

Les individus des populations sylvestres sont représentés par deux arbres en lignes et ceux des origines cultivées par quatre arbre en ligne.

Vingt clones du groupe congolais d'un essai de géniteurs plantés en 1988 ont également été étudiés. Chaque clone est représenté par 20 arbres en randomisation totale.

Tableau 1 - Description des groupes de caféiers étudiés

POPULATIONS SYLVESTRES			ORIGINES CULTIVEES		
DENOMINATION	EFFEC (*)	PAYS (**)	DENOMINATION	EFFEC	PAYS
Cameroun	16 +	CAM	Inéac	44 ++	ZAI
Doungba	09 +	RCA	Lula	28 ++	ZAI
Libengue	45 +	RCA	Ebobo	30 +	RCI
Ndongue	99 +	RCA	Aboisso	27 ++	RCI
Kouoin	10 +	RCI	Robusta A1	21 ++	???
Gbapleu	10 +	RCI	Boukoko	05 +++	CAM
Logbonou	26 +	RCI	Ouganda	06 +++	OUG
Fouroung	37 +	RCI	C10 Man	15 +++	ZAI
Maraoué	22 +	RCI	Niaouli	20 +++	BEN
Bossematie	09 +	RCI	Kouillou		
Ira 1	31 +	RCI	de Madagascar	32 +++	GAB
Ira 2	25 +	RCI	Togo	17 +++	TOG
Bafingdala	19 +	RCI			
Gbapleu 2	13 ++	RCI			
Pelezi	42 ++	RCI			
Gonate	14 ++	RCI			
Piné	42 ++	GUI			

(*) + : Données électrophorètiques BERTHAUD (1986)
++ : Données électrophorètiques MONTAGNON (1989)
+++ : Données électrophorètiques sur demande à l'auteur
(**) CAM = Cameroun, RCA = République Centrafricaine, RCI = Côte d'ivoire, TOG = Togo, ZAI = Zaïre, GAB = Gabon, BEN = Bénin, ??? = Origine inconnue, GUI = Guinée, OUG = Ouganda.

2-2 Observations et méthodes d'analyse :

2-2-1 Diversité génotypique :

Six systèmes enzymatiques ont été étudiés par électrophorèse permettant l'observation de neuf loci (BERTHOU et TROUSLOT, 1977) :

- Phosphoglucodeshydrogénase PGD : 2 loci
- Phosphoglucoisomérase PGI : 1 locus
- Isocitrate deshydrogénase ICD : 1 locus
- Phosphatase acide PAC : 1 locus
- Estérase EST : 3 loci
- Phosphoglucomutase PGM : 1 locus

Les fréquences allèliques des populations pour chaque locus ont été traitées par le logiciel BIOSYS (SWOFFORD et SELANDER, 1981). La distance génétique de NEI (1978), convenant à ce type d'étude intraspécifique (de VIENNE et DAMERVAL, 1985) a été utilisée pour l'établissement de dendrogrammes.

2-2-2 Diversité phénotypique :

Seules les origines sélectionnées, plantées depuis plusieurs années, ont été analysées en collection. La description est essentiellement botanique et concerne : la longueur (LONGF) et la largeur (LARGF) des feuilles, le rapport RAPPF = LONGF/LARGF illustrant la forme de la feuille, l'angle des rameaux secondaires avec l'axe primaire dans le tiers supérieur de l'arbre (ANGLE), le nombre d'entre-noeuds orthotropes (ENORT) et la granulométrie (GRANU). Une note visuelle de ramification a également été prise en compte.

Concernant l'essai de géniteurs, les variables étudiées concernent plus particulièrement l'architecture de l'arbre : Hauteur (HAUT), longueur de la plus longue primaire (LPRIM), port de l'arbre illustré par FORME = HAUT/LPRIM, nombre d'entre-noeuds orthotropes (ENORT) et plagiotropes (ENPLA), longueur des entre-noeuds orthotropes (LGORT = HAUT/ENORT) et plagiotropes (LGPLA = LPRIM/ENPLA) et le nombre d'entre-noeuds total (ENTOT = ENORT X ENPLA). Enfin, une note visuelle de sensibilité à la sécheresse se rapportant au degré de noircissement des feuilles et de défoliation a été prise en compte. Toutes ces variables sont observées sur des caféiers de deux ans.

Tant pour la collection que pour l'essai de géniteurs, des analyses multivariées ont été réalisées avec le logiciel du CIRAD, CSTAT, pour l'Analyse en Composantes Principales (ACP) et STATITCF pour l'Analyse Factorielle Discriminante (AFD).

3 RESULTATS DISCUSSIONS

3-1 Diversité génotypique étudiée par électrophorèse :

Le dendrogramme obtenu à partir de la distance génétique de NEI (1978) pour tous les groupes de caféiers en collection est présenté Fig. 1. La séparation Guinéen - Congolais est nette et confirme les résultats précédents (BERTHAUD, 1985). Pelezi a fixé un allèle rare, l'allèle F, au locus PAC (MONTAGNON et al., sous impression), ceci explique son détachement des autres populations guinéennes.

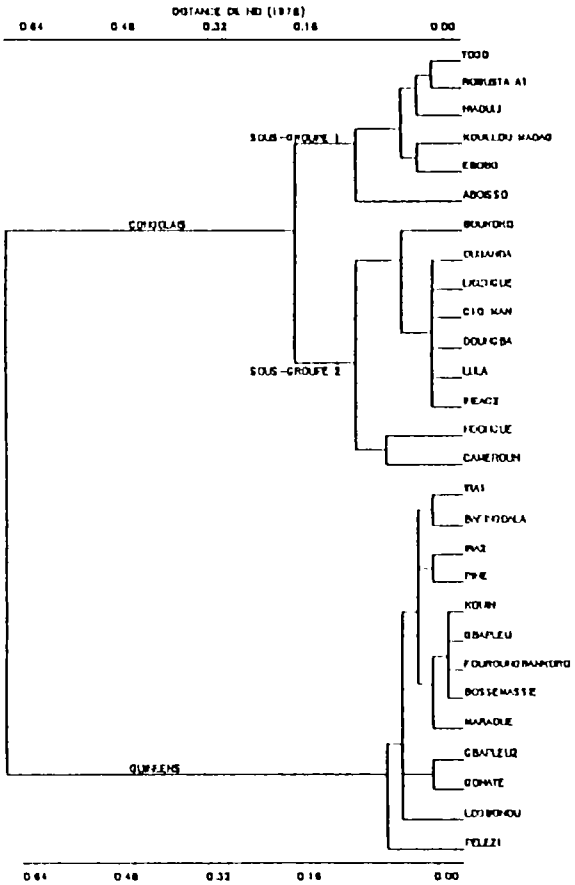


Figure 1. - Dendrogramme obtenu avec la distance génétique de NEI (1978) sur tous les loci analysés

Deux sous-groupes se distinguent au sein du groupe congolais. L'un, sous-groupe 2, se compose des origines cultivées ou populations sylvestres d'Afrique Centrale. L'autre, sous-groupe 1, renferme des origines cultivées dont aucune n'est originaire d'Afrique Centrale.

L'origine génétique de cette séparation se trouve au locus ESTB dont l'allèle G est fixé pour le sous-groupe 2 alors que sa fréquence est inférieure à 0,10 dans le sous-groupe 1. Cette structure conforte les hypothèses d'existence de sous-groupes au sein des congolais émises à partir de l'observation de groupes originaux : Ebobo (BERTHAUD, 1985) ou Robusta A1 et Aboisso (MONTAGNON et al., sous impression). Cependant, cette séparation ne reposant que sur un locus (ESTB) n'est pas satisfaisante. D'autre part, les origines cultivées ont été brassées, on ne peut établir de conclusions définitives qu'à partir de l'étude de populations sylvestres qui ne figurent pas dans le sous-groupe 1. Il est toutefois remarquable que les populations sylvestres et les origines sélectionnées d'Afrique Centrale soient relativement proche génétiquement.

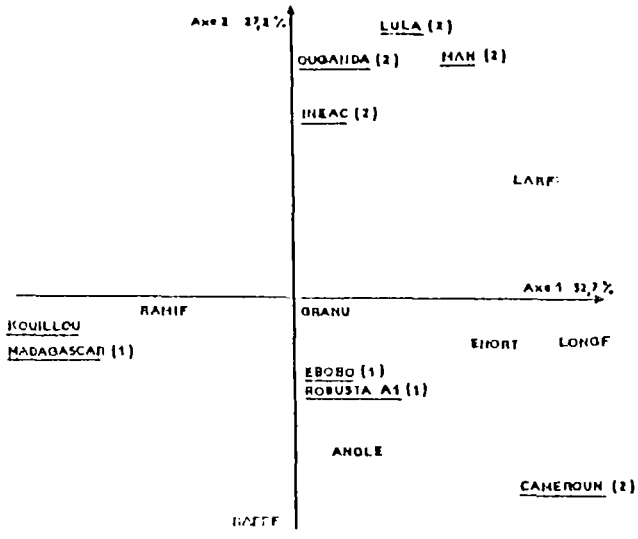
Cette mise en évidence de deux sous-groupes doit donc être considérée comme un indice de structuration génétique de l'espèce *Coffea canephora*. La recherche de nouveaux marqueurs enzymatiques de cette séparation et l'étude de populations sylvestres d'Afrique Centrale Atlantique (du Togo au Gabon), non encore prospectées devront confirmer ou infirmer cette hypothèse.

3-2 Diversité phénotypique du groupe Congolais :

3-2-1 Etude botanique en collection :

Les deux premiers axes de l'ACP expliquent 72 % de la variabilité totale. Les corrélations des variables avec les différents axes (Fig. 2) permettent de définir l'axe 1 comme celui de la taille des feuilles et l'axe 2 comme celui de la forme des feuilles illustrée par la variable RAPPF. Les feuilles sont d'autant plus effilées que RAPPF augmente. L'AFD réalisée avec les coordonnées factorielles de l'ACP (Fig. 2) en identifiant chaque origine permet de les discriminer sur les axes 1 et 2 :

- Petites feuilles : Kouillou de Madagascar
- Feuilles moyennes effilées : Robusta A1 et Aboisso
- Grandes feuilles larges : C10 Man, Inéac, Lula, Ouganda
- Grandes feuilles effilées : Cameroun



( ) Sous groupe génétique défini par électrophorèse

Figure 2. - Représentation des variables et de quelques groupes de caféiers de types congolais (soulignés) sur les axes 1 et 2 de l'Analyse Factorielle Discriminante menée sur la collection

Les deux premiers types de feuilles correspondent à des origines du sous-groupe 1 défini précédemment et les deux derniers au sous-groupe 2.

Dans cette étude, la taille des feuilles est corrélée positivement au nombre d'entre-noeuds orthotropes et négativement à la ramification. La granulométrie ne sépare pas les groupes étudiés.

3-2-2 Etude architecturale en essai de géniteurs :

A partir de l'étude génotypique, les clones observés dans cet essai ont été repérés selon leur appartenance au sous-groupe 1 ou au sous-groupe 2. Ces deux sous-groupes sont bien séparés par l'axe 1 de l'ACP (Fig. 3). Cet axe sépare des arbres possédant un grand nombre d'entre-noeuds, grands, d'un port buissonnant et relativement résistant à la sécheresse (sous-groupe 1) à des arbres plus petits et allongés, à faible nombre d'entre-noeuds et relativement sensibles à la sécheresse (sous-groupe 2).

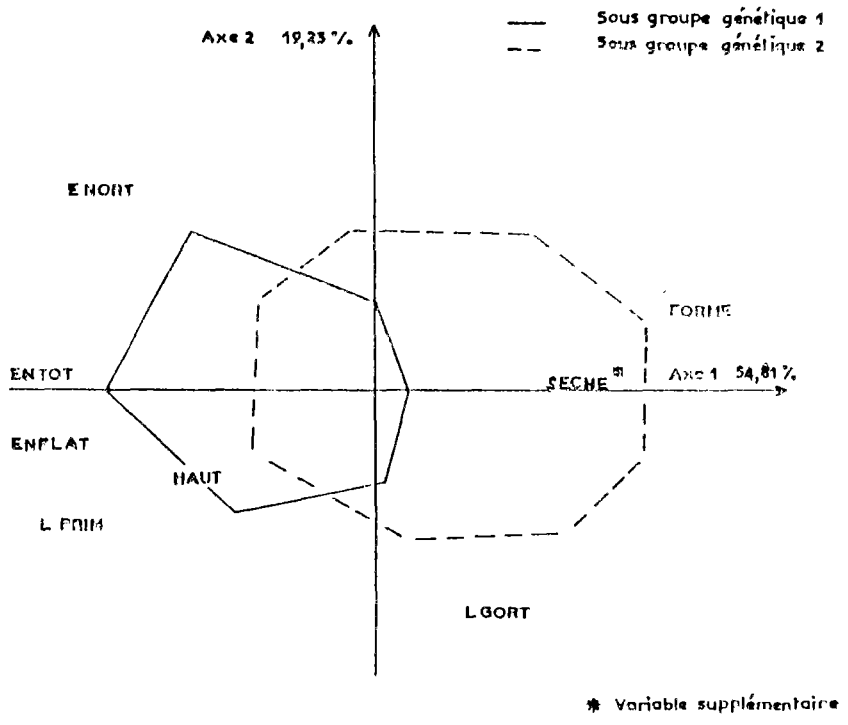


Figure 3. - Représentation des variables et des deux sous-groupes génétiques congolais définis par électrophorèse sur les axes 1 et 2 de l'Analyse en Composantes Principales menée sur l'essai de géniteurs

3-2-3 Synthèse des deux études phénotypiques :

Il existe une grande variabilité phénotypique au sein des Congolais. La taille et la forme des feuilles d'une part, le nombre d'entre-noeuds, le port de l'arbre d'autre part, sont de bons descripteurs de cette variabilité.

Les sous-groupes 1 et 2 définis précédemment ont des types morphologiques bien différenciés :

Sous-groupe 1	Sous-groupe 2
Nombre d'entre-noeuds élevé	Faible nombre d'entre-noeuds
et entre-noeuds courts	et entre-noeuds longs
Grands arbres buissonnants	Arbres plus petits et allongés
Ramification importante	Faible ramification
Feuilles petites à moyennes	Feuilles grandes et larges

La description du sous-groupe 1 congolais se rapproche de celle des Guinéens (BERTHAUD, 1985 ; LEROY et CHARMETANT, 1987). PORTERES (1959) et CORDIER (1961), n'ayant pas accès à la diversité génétique, avait défini deux groupes morphologiques au sein de *Coffea canephora* : les Kouillou et les Robusta. Les Kouillou recouvraient le groupe Guinéen et le sous-groupe 1 congolais. Les Robusta se limitaient au sous-groupe 2 congolais.

4 CONCLUSION

Deux sous-groupes ont été mis en évidence dans le groupe génétique congolais de l'espèce *Coffea canephora*. Ce sont deux sous-groupes phénotypiques. Le faible nombre de loci discriminants et l'absence de populations sylvestres du sous-groupe 1 interdit de conclure définitivement à deux sous-groupes génotypiques. De nouvelles prospections en Afrique Centrale Atlantique et l'observation de nouveaux systèmes enzymatiques devraient permettre de préciser le caractère génétique de cette séparation.

Les individus du sous-groupe 1 possèdent une architecture et un niveau de résistance à la sécheresse recherchés en sélection. Ce dernier point mérite cependant des études spécifiques plus approfondies. Le schéma de sélection récurrente et réciproque intègrera ces nouvelles données. La phase de brassage intragroupe des Congolais se fera séparément dans chacun des deux sous-groupes. Les géniteurs Guinéens seront croisés systématiquement avec un testeur Congolais de chaque sous-groupe. Il pourra également être intéressant de tester les combinaisons hybrides entre ces deux sous-groupes.

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RESUME

L'analyse globale de la diversité génotypique et phénotypique sur 28 populations sylvestres et origines cultivées couvrant l'aire de répartition de *Coffea canephora* permet de mettre en évidence deux sous-groupes à l'intérieur du groupe Congolais. Ce sont deux sous-groupes phénotypiques dont la séparation génétique demande à être confirmée. Le sous-groupe 1 a des caractéristiques morphologiques proches des Guinéens (petites feuilles, grand nombre d'entre-noeuds, bonne ramification, port buissonnant). Le sous-groupe 2 correspond à la description classique des Congolais (grandes feuilles, faible nombre d'entre-noeuds, faible ramification).

SUMMARY

Phenotypic and genotypic diversity among 28 wild population and cultivated origins of *Coffea canephora* was evaluated. Two sub-groups were defined within the Congolese genetic group. They were separated on a phenotypic basis, further informations are needed to confirm the genotypic separation. Trees of sub-group 1 show guinean traits while sub-group 2 fits the classical description of the congolese group.

EL PROGRAMA DE SELECCIÓN Y EVALUACIÓN DE VARIEDADES DE CAFÉ EN AMÉRICA CENTRAL

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INTRODUCCION

El Programa Cooperativo para la Proteccion y Modernización de la caficultura en América Central, Panamá, México y República Dominicana (PROMECAFE), inició sus actividades en 1978, considerando el interés de los países del Area de aúnar esfuerzos, para lograr el mejoramiento integral de la caficultura a nivel regional, con el apoyo del Instituto Interamericano de Cooperación para la Agricultura (IICA), quien ha colaborado con el Programa en la coordinación y administración de los recursos, a través de sus oficinas en cada uno de los países miembros.

Una de las acciones iniciales de PROMECAFE, se relacionó con el analisis del germoplasma existente en la colección del CATIE, en Costa Rica, con el propósito de identificar genotipos con resistencia y/o tolerancia a la Roya Anaranjada (Hemileia vastatrix Berk et Br.), para su posterior distribución y evaluación en los países de la región, en vista del apararecimiento de esta enfermedad en Nicaragua en 1976. Por otra parte, se establecieron contactos con Centros de Investigaciones de Cafe como el CIFC en Oeiras, Portugal, el IAC de Campinas en Brasil y otros , con el propósito de gestionar la introducción de nuevas fuentes de germoplasma.

Esta actividad inicial se convirtió en el Programa de selección y reproducción de variedades resistentes a la Roya, como un componente del Proyecto Regional de control de Plagas del Cafeto, que fué financiado por la Oficina Regional para América Central y Panamá (ROCAP) de la Agencia Internacional para el Desarrollo (AID) de los Estados Unidos de América, que se inició en 1982.(IICA/PROMECAFE, 1987.)

El objetivo general de esta actividad se definió en términos de identificar nuevas variedades de café que combinen las características de resistencia a la roya, alta producción y buena calidad de grano para posteriormente multiplicar a corto plazo, los materiales mas sobresalientes.

MATERIALES Y METODOS

Como base de operaciones del programa, se estableció en el CATIE, Turrialba, Costa Rica, la Unidad Central de Mejoramiento, considerando que en este Centro, existía una de las colecciones de Café más completa del mundo y la infraestructura para la realización de actividades de campo y laboratorio. Esta Unidad, tenía como propósito evaluar el germoplasma de la colección de café, así como efectuar la introducción y evaluación inicial de nuevas accesiones y su distribución posterior a los programas nacionales, para su evaluación y selección, bajo las condiciones ecológicas de cada país.

Introducción de Germoplasma

El Banco de Germoplasma de Café del CATIE, se inició en la década del 50, en vista de la necesidad de tener una colección de variedades de café, para servicio de los países latinoamericanos y también por la preocupación que se planteó desde esa época, por el posible aparecimiento de la Roya en el Continente Americano.

Hasta en la década del 70, se introdujeron los primeros materiales segregantes derivados del cruzamiento entre el Caturra y el Híbrido de Timor, producido por el Centro de Investigaciones de las Royas ∫766Xdafeto (CIFC) en Portugal, que es considerado una fuente de variabilidad genética de mucha importancia para la selección de progenies con fenotipo de Caturra y resistencia a la Roya. (Bettencourt, 1981, 1982.).

Con el inicio del Proyecto financiado por USAID/ROCAP en 1982, se hizo énfasis en la introducción de nuevo germoplasma y se tomó como estrategia del Programa de Selección y Reproducción de Variedades , trabajar con materiales avanzados (F4 en adelante), para que los materiales que se distribuyera a los países, tuviera características fenotípicas uniformes y permitiera la selección de los mejores genotipos en el corto plazo, considerando el tiempo de duración del proyecto que era limitado.

Las introducciones realizadas por PROMECAFE en los últimos 12 años, suman 669 accesiones de diferente origen geográfico, que representan una fuente de diversidad genética muy importante. La mayoría de las accesiones provienen del Programa de la Universidad Federal de Vicosa , del Instituto Agrónomico de Campinas de Brasil, del Centro de Investigación de las Royas del Café (CIFC) de Portugal, del Instituto Frances de Café y Cacao (IRCC) y de CENICAFE de Colombia. (IICA/PROMECAFE, 1990). En la actualidad, el Banco de Germoplasma cuenta con más de 1600 accesiones origen, que constituyen una reserva genética, muy valiosa para el mejoramiento del Café.

Ensayos Regionales de Adaptación

En la estrategia operativa del Programa, se consideró que los materiales introducidos a Turrialba, tenían que ser aumentados y al mismo tiempo evaluados por sus características fenotípicas, tamaño y forma del grano, etc. con el propósito de eliminar en una primera instancia, aquellos materiales que tuvieran caracteristicas indeseables, antes de distribuirlos a los programas nacionales, a través de los experimentos regionales o bien como material para lotes de observación y selección.

Desde 1980, se han distribuido a los países de la región, nueve ensayos regionales que contienen 117 tratamientos o progenies, para su evaluación a nivel de país.

En este trabajo se presentan resultados de experimentos selectos de Honduras, Nicaragua y Costa Rica, que fueron sembrados en condiciones diferentes de altura sobre el nivel del mar y con diferentes regimenes de pluviosidad. En el experimento PROMECAFE No. 1, se incluyeron progenies derivadas del cruzamiento Caturra 19/1 x Hibrido de Timor 832/1, (CIFC Hw. 26/13), el Híbrido de Timor, (CIFC-1343/86, las variedades Mundo Novo, Geisha cv. 496, Caturra y Catuai; así como hibridos sintetizados en Turrialba, entre materiales pertenecientes a diferentes grupos fisiologicos respecto a su reacción a las razas de Roya.

En los ensayos de evaluacion de progenies de la Serie 86, los materiales provenían del programa de mejoramiento genético de la Universidad Federal de Vicosa, Brasil/CIFC de Portugal (Hibrido Hw. 26/5). Estos materiales se recibieron en Brasil, en la generacion F-3, procedentes de Angola, donde el CIFC tenía un progama de selección y evaluación de poblaciones segregantes,con resistencia a la Roya. En Turrialba, las progenies se recibieron en la generación F-5, razon por la cual, presentan uniformidad para diferentes caracteristicas fenotipicas y de resistencia a la Roya.

En los ensayos de Honduras y Nicaragua la producción se registró en kilogramos de café cereza por planta y en Costa Rica los datos se tomaron por parcela y se convirtieron a toneladas métricas por hectárea. El fruto vano se determinó, colocando 100 cerezas maduras en un recipiente con agua y el número de cerezas flotantes representa el porcentaje de frutos vanos. En Honduras para la calificación de vigor, se utilizó una escala de 1 - 10, donde 1 es deficiente y 10 es excelente y se registra después de la cosecha.

Cuadro 1

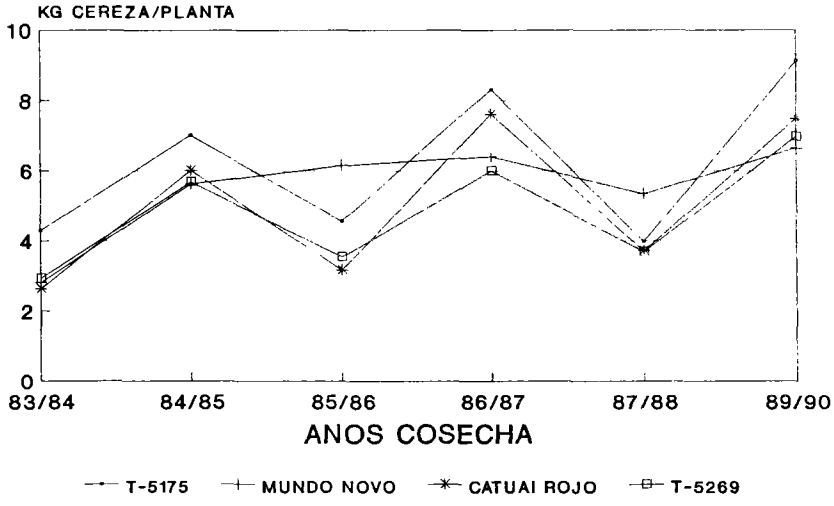
PRODUCCION PROMEDIO DE SEIS COSECHAS, FRUTOS VANOS, RESPUESTAS A LA PODA Y VIGOR. PMC.1. LOS LINDEROS - IHCAFE, HONDURAS

GENOTIPO	PROD. PROM. CEREZA KGR/pl	CATUAI %	FRUTOS VANOS (%)	RESP* PODA	VIGOR**
T - 5175	6.21	121.7	6.6	4.8	5.4
MUNDO NOVO	5.49	102.6	3.7	4.8	4.7
GEISHA	5.23	102.5	7.3	4.2	4.6
CATUAI ROJO	5.10	100.0	4.6	4.9	5.9
T - 5269	4.79	93.9	8.4	4.7	5.1

Media General: 4.44 Kg/planta
Altitud : 1100 msnm Precipitacion: 1864 mm
FUENTE: Flores et al - Memoria IX Reunion Regional de Mejoramiento de Cafe. IICA. PROMECAFE. 1990

* Escala de 1 - 5: 1= Respuesta pobre; 5 = Respuesta excelente
** Dato tomado después de la Cosecha 87/88
Escala 1 - 10: 1= Deficiente; 10 = Excelente

GRAFICO 1. EVALUACION DE GENOTIPOS SELECTOS EN RELACION AL CATUAI



LOS LINDEROS, IHCAFE, HONDURAS.

Cuadro 2

PRODUCCION PROMEDIO DE SEIS COSECHAS, VIGOR Y FRUTOS VANOS DE PROGENIES SELECTAS DE LA SERIE 86 EN RELACION AL CATUAI. PMC No. 3

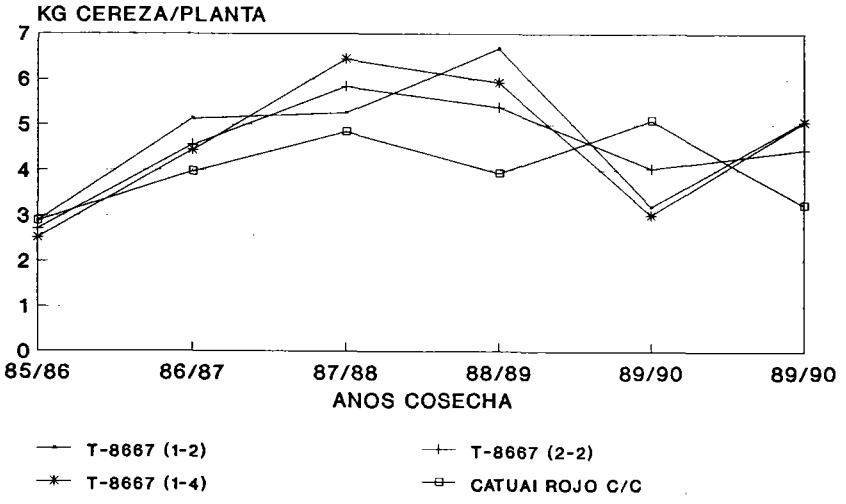
LOS LINDEROS. IHCAFE, HONDURAS

PROGENIES	PROD. PROM. KGR.CER./PL	% DE CATUAI	VIGOR * ESCALA 1 - 10	FRUTO VANO %
T - 8667 (1-2)	4.71	117	6.7	3.0
T - 8667 (1-4)	4.57	114	6.3	3.3
T - 8667 (2-2)	4.50	112	6.4	3.4
T - 8673 (4-5)	4.25	106	6.2	3.9
CATUAI ROJO	3.99	100	5.9	1.6

Media General: 3.54 Kgr/planta
Altitud: 1100 msnm Precipitación: 1864 mm

* Escala de 1 - 10: 1 = deficiente; 10 = excelente
Dato tomado después de la Cosecha 90/91

GRAFICO 2. EVALUACION DE PROGENIES DE T-8667, EN RELACION AL CATUAI.



LOS LINDEROS, IHCAFE, HONDURAS.

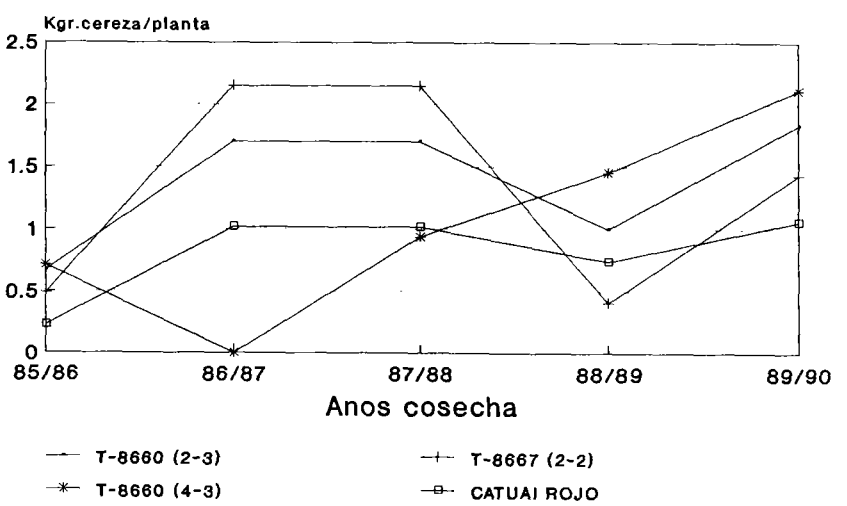
Cuadro 3

COMPORTAMIENTO DE PROGENIES SELECTAS DE LA SERIE 86 EN RELACION AL CATUAI. JINOTEPE, MAG, NICARAGUA

PROGENIES	PROD. PROM. KGR.CER./PL	CATUAI %	FRUTO VANO %
T - 8660 (2-3)	1.38	169.5	5.5
T - 8667 (2-2)	1.32	162.8	6.3
T - 8660 (4-3)	1.23	151.0	5.8
T - 8667 (4-5)	1.04	127.9	6.2
CATUAI ROJO	0.81	100.0	6.6

Media General: 0.69 Kgr/planta
ALTURA: 485 msnm PRECIPITACION: 1500 mm
FUENTE: BAYLON, M. PIZZI, W. MEMORIA IX REUNION REGIONAL DE FITOMEJORAMIENTO- IICA/PROMECAFE - 1990

GRAFICO 3. EVALUACION DE PROGENIES DE LA SERIE 86 EN RELACION AL CATUAI.



Jinotepe, MAG, Nicaragua.

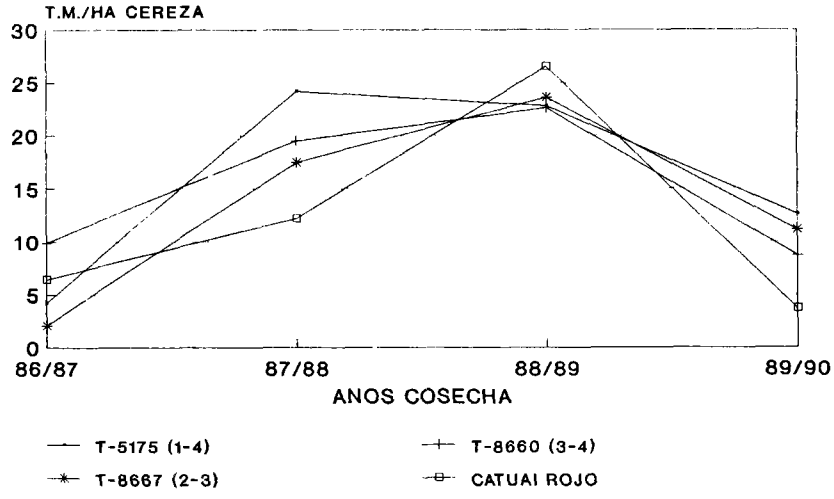
Cuadro 4

EVALUACION DE LINEAS DE CATIMOR EN RELACION AL CATUAI - PROMEDIO DE CUATRO COSECHAS. PALMARES, ICAFE-MAG, COSTA RICA

GENOTIPO	PROD. PROM. T.M. Cereza/ha	CATUAI %	PL. PODADAS %
T - 5175 (1-4)	15.9	131	33.3
T - 8660 (3-4)	15.1	124	27.1
T - 8667 (2-3)	13.6	111	14.5
CATUAI ROJO	12.2	100	39.6

ALTITUD: 1000 msnm PRECIPITACION: 1765 mm
FUENTE: AGUILAR, G. MEMORIA, IX REUNION REGIONAL DE MEJORAMIENTO DE CAFE - IICA - PROMECAFE - 1990
MEDIA GENERAL: 13.04 Kgr/planta

GRAFICO 4. EVALUACION DE MATERIALES SELECTOS EN RELACION AL CATUAI



PALMARES, ICAFE-MAG, COSTA RICA

RESULTADOS Y DISCUSION

En el cuadro 1, se presenta la producción promedio de seis cosechas en café cereza de genotipos selectos del experimento PROMECAFE No. 1, que fue sembrado en el Centro Experimental Los Linderos del Instituto Hondureño del Cafe en Honduras. En el mismo cuadro, se presenta el porcentaje de producción en relación al Catuaí, porcentaje de frutos vanos, respuesta a la poda y vigor. El Catimor T - 5175, superó en rendimiento al Catuai, el mejor testigo en un 21.7 por ciento, en promedio de las seis cosechas. El porcentaje de frutos vanos fue ligeramente superior al Catuai (6.6 % vs. 4.6 %), sin embargo, esta por abajo del límite de selección establecido (8 %). En relación, a la respuesta a la poda, no se observó diferencias entre el Catuaí y el T-5175, después de 6 cosechas consecutivas sin manejo de tejido; lo mismo se observó en el caso del vigor vegetativo.

En la gráfica No. 1 se puede observar, el comportamiento de la producción atraves de los 6 años de cosecha. El cultivar Mundo Novo, es el que presenta menos variación bianual y el Catimor T-5175 se comporta en forma similar al Catuaí, lo cual es un buen indicador de las bondades de este material, que además de tener alta productividad, presenta muy buenas características agronómicas y resistencia a las razas de roya existentes en la colección del CIFC, donde se ha clasificado en el grupo fisiológico "A".

En el cuadro 2, se presenta la producción promedio de seis cosechas por planta, porcentaje sobre Catuaí, vigor y porcentaje de frutos vanos de progenies selectas de la serie 86, incluidas en el experimento PROMECAFE No.3, sembrado en el Centro Experimental Los Linderos, en Honduras. Las progenies T-8667(1-2), T-8667(1-4) y T-8667(2-2) produjeron 17, 14 y 12 por ciento mas que Catuaí, en promedio de las seis años cosecha. El vigor de estas progenies fue ligeramente superior al Catuaí, de acuerdo a la evaluación efectuada despues de la sexta cosecha. En relación al fruto vano, presentan un porcentaje entre 3 y 4 por ciento, que se considera excelente.

En la gráfica 2, se puede observar el comportamiento de la producción a través de los diferentes años. Las progenies de T-8667 presentan un comportamiento superior al Catuaí.

En el Cuadro 3, se presenta la producción promedio por planta en cinco cosechas de progenies derivadas de T-8660 y T-8667, en la localidad de Jinotepe, Nicaragua; las cuales rindieron entre 28 y 70 por ciento más que la variedad testivo Catuaí. El porcentaje de fruto vano no mostró diferencias significativas, en relación al testigo. En la gráfica 3, se puede observar el comportamiento a través de los años de las tres progenies experimentales, en relación al Testigo. En la grafica 3, se puede observar el comportamiento bianual de los materiales en estudio.

En Costa Rica, (Cuadro 4) en la localidad de Palmares, las progenies T-5175 (1-4), T-8660 (3-4) y T-8667 (2-3), rindieron 31, 24 y 11 por ciento más que el testigo Catuaí rojo en producción de grano en promedio de cuatro cosechas. En la gráfica 4, se puede observar el comportamiento de estos materiales, en relación al testigo, a través de los años de cosecha 86/87 a 89/90.

El excelente comportamiento observado por estos materiales genéticos con resistencia a la Roya, en comparación con el cultivar Catuaí, en ambientes tan diversos como el caso de Jinotepe, Nicaragua, ubicado a 485 msnm con una precipitación de 1500 mm. anuales y los Linderos en Honduras con 1100 msnm y una precipitación anual de 1874 mm., es un indicador de su capacidad de adaptación a diferentes ambientes sin detrimento del potencial productivo.

Los datos obtenidos en experimentos en ejecución en los demás países miembros de PROMECAFE, confirman el potencial de estas nuevas variedades que han sido denominadas, PROMECAFE 1 (T-5175), y PROMECAFE 2 (T-8667), que representan una alternativa de producción para los pequeños y medianos productores de café de la región. Por otra parte, se ha observado que estos materiales tienen muy buena respuesta a la poda y caracteristicas de grano y de calidad de taza, similares a las mejores variedades comerciales disponibles.

En la cosecha 90/91, se identificaron plantas de estos dos materiales en base a producción, uniformidad fenotipica, caracteristicas del grano, porcentaje de frutos vanos, etc.. las cuales se distribuyeron a los países de la región, en forma individual para evaluacion de las progenies correspondientes, así como en la forma de un " compuesto " o una mezcla de las líneas, para la siembra de lotes de observación, selección y aumento y de esta forma, se evita tener una población, con una base genética muy reducida, siguiendo la metodología utilizada en la formación de la variedad " Colombia ". (Castillo y Moreno, 1988.)

Un aspecto importante, en relación al comportamiento de estos materiales en las condiciones prevalecientes en America Central, es que en 1982, cuando se inició el programa, se tenía poca confianza en que los materiales resistentes a la Roya, llegaran a superar a las variedades comerciales, considerando la experiencia negativa que tuvo el programa de mejoramiento genetico de la Universidad de Vicosa, en Brasil a finales de la década de los 70.

Bettencourt (1982) sugirió, que bajo las condiciones ecologicas (clima, suelos....) y de manejo del cultivo en America Central, que son completamente diferentes del sistema de

cultivo en Brasil, los materiales podrían tener un comportamiento superior, principalmente en el aspecto de longevidad y vigor, lo cual ha sido confirmado con los resultados presentados en este trabajo.

CONCLUSIONES

1. Los resultados obtenidos en estos experimentos, son una evidencia, del potencial de las nuevas variedades PROMECAFE 1 Y PROMECAFE 2 en productividad, vigor, resistencia a Roya, etc..en comparacion con las variedades comerciales Catuaí y Caturra y por lo tanto representan una alternativa de producción para los pequeños y medianos productores de café de la región.

2. El buen comportamiento de los materiales, observado a traves de los diferentes ambientes donde se establecieron los ensayos, es un indicador de que no existe una interaccion genético-ambiental especifica, para los materiales en referencia y que su adaptabilidad puede considerarse, similar al Catuai.

3. El Instituto Hondureño del Café, liberó en 1990, la variedad " IHCAFE 90 ", que es una seleccion local del T - 5175, apartir de la informacion obtenida en el experimento PROMECAFE No.1.

4. El esfuerzo cooperativo que se ha venido realizando en el seno de PROMECAFE, para la evaluación y selección de nuevas variedades de café, ha dado sus primeros frutos, gracias al esfuerzo de los Fitomejoradores de los Programas nacionales, quienes han permitido la obtención de resultados confiables para la selección de los materiales mas promisorios.

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COFFEE VARIETY AND SELECTION PROGRAM IN CENTRAL AMERICAN COUNTRIES

Juan José Osorto

In 1980, PROMECAFE started an intensive Coffee variety evaluation and selection program, in order to identify superior genotypes, with rust resistance, high yielding ability and good cup quality. The program included, several uniform variety trials, that were planted in different countries of Central America. In this paper, we are reporting a summary of the main results, obtained after six years of harvest in Honduras, five years in Nicaragua and four years in Costa Rica. The materials included in the evaluation originated from different sources: CIFC-Portugal, UFV-Brasil, and CENICAFE-Colombia. The materials identified with Turrialba number T-5175 and T-8667 overcome the commercial varieties up to 18 to 20 percent in grain production. This materials has been named PROMECAFE, 1 and 2 respectively and a mixture of progenies has been distributed in 1990 to the member countries for multiplication and distribution to coffee farmers. Related to seed size, these materials have the same characteristics as **Catuai** the check variety. Cup quality is similar to the commercial varieties.

RESUMEN

EL PROGRAMA DE SELECCION Y EVALUACION DE VARIEDADES DE CAFE EN AMERICA CENTRAL

Juan José Osorto

En 1980, PROMECAFE inició un programa intensivo de evaluación y seleccíon de variedades de café, con el propósito de identificar genotipos superiores, resistentes a la Roya (Hemileia vastatrix Berk et Br), de alta *productividad y buena calidad de taza. El programa incluyó* ensayos uniformes de variedades que fueron sembrados en los países de América Central. En este trabajo, se presenta un resumen de los principales resultados obtenidos, después de 6 años/cosecha en Honduras, 5 años en Nicaragua y 4 años en Costa Rica. Los materiales incluídos en los ensayos, se obtuvieron de diferentes fuentes: CIFC-Portugal, UFV-Brasil y CENICAFE-Colombia. Los materiales identificados con los números T-5175 y T-8667 superaron a las variedades comerciales en un 18 a 20 porciento en producción de grano. Estos materiales han sido designados PROMECAFE-1 y PROMECAFE-2 respectivamente, y una mezcla de progenies ha sido distribuuida en 1990 a los países de la región para su multiplicación y distribución a los productores. En relación al tamaño de las semillas, estos materiales tienen las mismas características de **Catuaí** la variedad testigo. La calidad de taza es similar a las variedades comerciales.

STABLE TRANSFORMATION OF FOREIGN DNA INTO *COFFEA ARABICA* PLANTS

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The long-term prospects for improving coffee characteristics at the genetic level will be significantly enhanced when methods of introducing specific DNA sequences into desired coffee cultivars become available. This report describes the first demonstration of stably transformed coffee plantlets using a model DNA system.

In order to obtain stably transformed Coffea, a number of approaches may be taken which will lead to coffee plants that contain stably integrated foreign DNA. Development of commercially acceptable cultivars based on this technology ultimately will require additional efforts to identify specific biochemical targets leading to improved varieties, and development and testing of the genes which will successfully modify those characteristics. One important step in embarking on such a program is to develop a methodology by which DNA can be successfully introduced into coffee and its effects can be measured.

The objectives of this study were to develop a method to stably introduce foreign DNA into Coffea. We chose to use electroporation to accomplish this. Electroporation is a procedure in which DNA is introduced into plant protoplasts by subjecting them to an electrical charge, and protoplasts are subsequently recovered and allowed to develop. This method has been successfully applied to a number of plant systems. In order to apply the method to Coffea, it was first necessary to develop protoplasts that were at the same time receptive to the electroporation protocols while retaining the ability to regenerate and form embryos which ultimately develop into plants.

This presentation summarizes our effects in developing such a protoplast system, and examples of how we used them to develop stably transformed Coffee plantlets.

PART I: REGENERATION OF PROTOPLASTS INTO PLANTS VIA SOMATIC EMBRYOGENESIS

PREPARATION OF COFFEA PROTOPLASTS WHICH RETAIN PLANT REGENERATION CAPABILITY

Production of Coffea Callus - Seedlings of Coffea arabica were obtained from a local nursery. Young leaves were surface sterilized by washing in a 1% liquinox solution for five minutes, a 7% calcium hypochlorite wash for 3 minutes while shaking at 150 rpm, and rinsing three times with sterile water. Leaf sections, approximately 1 cm^2, were cultured top surface down on coffee callus medium (Table 1). The explants were cultured at 28-30°C in the dark or under dim light.

TABLE 1

COFFEE CALLUS MEDIA[a]

MS salts[b]		Sucrose	40,000
B_5 vitamins[c]			
myo-Inositol	100	Kinetin	20 uM
Casein hydrolysate	100	2,4-D	5 uM

[a] all media were adjusted to pH 5.6, all quantities in mg/L unless otherwise noted
[b] Murashige, et al., Physiol. Plant., 15: 473, 1962
[c] Gamborg, et al., Exp. Cell Res., 50: 151, 1968

Production of Embryogenic Suspension Cultures - Proliferating leaf callus was transferred to 50 ml of liquid cell culture medium (CM, Table 2) in 250 ml delong flasks and incubated at 27 +/- 2°C on a gyratory shaker at 150 RPM until small white-to-cream colored cells with dense cytoplasm were visible. These small cells were repeatedly subcultured until stable suspensions of small cells were obtained. The cultures were maintained on a 7-10 day transfer schedule in 50 mls of CM medium.

TABLE 2

CM MEDIA[a]

$Ca(NO_3)_2$ $4H_2O$	800	Thiamine-HCl	2.0
$MgSO_4$ $7H_2O$	300	Nicotinic acid	1.0
$NH_4H_2PO_4$	230	p-Aminobenzoic acid	0.1
KNO_3	2000	myo-Inositol	1010.
NH_4Cl	500	Pyridoxine HCl	0.5
		Choline chloride	5.0
Na_2EDTA $2H_2O$	8.591	D-Biotin	0.001
NH_4VO_3	0.023	Cyanocobalimin	0.001
$CuSO_4$ $5H_2O$	0.02	Folic acid	0.01
$NiSO_4$ $5H_2O$	0.045	Casein hydrolysate	100.
$MnCl_2$ $4H_2O$	14.7	Glycine	2.0
$NaMoO_4$ $2H_2O$	0.388	Cystine	1.0
H_3BO_3	2.0	Methionine	1.0
$ZnSO_4$ $7H_2O$	1.1	Adenine sulfate	40.0
$CrK(SO_4)_2$ $12H_2O$	0.098	Hypoxanthine	10.0
Na_2SeO_3	0.018	Thymidine	10.0
KI	0.75	L-Sodium malate	10.0
$Na_3Citrate$ $2H_2O$	29.4	Sucrose	40000.
$FeSO_4$ $7H_2O$	29.04	Kinetin	10 uM
		2,4-D	5 uM

Protoplast Isolation and Culture - A 5-7 day post-transfer culture was shaken and allowed to settle for a few seconds before withdrawing a 10 ml aliquot of small cells. The cells were collected by centrifugation for five minutes at 50-100 xg, resuspended in 30 ml of enzyme solution (Table 3), and incubated overnight at 27-30°C on a gyratory shaker at 50 rpm. Protoplasts were filtered through a 74 micron mesh screen, harvested by centrifugation, washed with protoplast rinse solution (enzyme solution salts without enzymes or bovine serum albumin), and then floated over an osmotically-adjusted 41% (v/v) Percoll cushion. Protoplasts banding above the cushion were collected in the rinse buffer, rinsed in the rinse solution, and diluted with a given volume of electroporation buffer (Table 4). Protoplasts for electroporation experiments were diluted to a final concentration of 2-4 x 10^6/ml with electroporation buffer and held on ice until used.

TABLE 3[a]

ENZYME SOLUTION

$MgSO_4$ $7H_2O$	300 mg/L	Mannitol	0.5 M
$Ca(NO_3)_2$ 4 H_2O	800 mg/L	MES	5 uM
$NH_4H_2PO_4$	230 mg/L	Cellulysin	20000 mg/L
KNO_3	2000 mg/L	Macerase	5000 mg/L
NH_4Cl	500 mg/L	Pectolyase Y23	2500 mg/L
		Bovine serum albumin	1000 mg/L

[a] the enzyme solution was treated for one hour with 1% neutralized activated charcoal, filter sterilized, and stored frozen

TABLE 4

ELECTROPORATION BUFFER

Mannitol	0.65 M
$MgCl_2$	10 mM
MES	5 mM

PLANT REGENERATION THROUGH INDUCTION OF COFFEA SOMATIC EMBRYOS

Embryogenesis was pre-induced by transferring cells to a medium composed of MS salts (without hormones), B_5 vitamins, and 3% sucrose. Full induction was accomplished by transferring to liquid induction medium (LIM = 0.5 x MS major salts with KNO_3 increased to 38 mM, B_5 vitamins, and 2% sucrose), and then to LIM plus 0.8% Difco Noble agar. Single embryos were then dissected out of the resulting embryo clusters and subcultured on the same medium. Embryos that germinated and formed a root were transferred to GA-7 containers (Magenta Corp.) containing sterile vermiculite moistened with Hoagland's solution (Hoagland and Arnon, Calif. Agric. Exp. Stn. Circ., 347: 1, 1950) for the formation of plants. After a period of acclimation, the plants were transferred to soil and grown in a growth chamber (28°C, 12 hours/day photoperiod).

Plantlets which were not exposed to electroporation have been successfully recovered from protoplasts using these protocols. Regenerated plants from these protoplasts are currently 4 feet tall. They have not yet flowered.

PART II: TRANSFORMATION OF PROTOPLASTS AND RECOVERY OF TRANSFORMED PLANTS

In order to obtain stably transformed coffee cells, we first investigated the relationship of transient transformation to stable transformation in model plant systems (tobacco and tomato). By measuring both cell viability subsequent to electroporation, expression of a transiently expressed enzyme (chloramphenical acetyl transferase), and production of stable transformed cells using a kanamycin resistant gene across a range of applied voltages, in these two model systems, we were able to demonstrate that maximum stable transformation was seen at lower voltages than for transient expression. We applied this method to coffee cells by first optimizing transient expression using a CAT gene construct, and then calculated an appropriate range of applied voltage for recovery of stably transformed protoplasts using a Kanamycin resistance gene. By using this procedure, electroporation conditions which should yield stably transformed coffee were determined.

Electroporation Conditions - Coffee protoplasts were electroporated by suspending the prepared protoplasts in electroporation buffer, combining with 20-40 ug/ml pGA472 (An, et al., EMBO Journal 4: 277, 1988) with an additional 20-40 ug/ml sterile calf thymus DNA as carrier.

10^6 protoplasts in 1 ml were incubated for 10-12 minutes in an ice-cooled disposable 0.4 x 1 cm plastic cuvette to which aluminum heat duct tape (3M) had been attached to the 0.4 cm internal lateral surfaces as electrodes. Electroporation was achieved by discharging a 330 volt x 350 microfarad capacitor across the electrodes at voltages ranging from 150 to 240 v/cm. (Final resistance = 800 - 1050 ohms; RC = 215 - 250 milliseconds).

Protoplast Recovery - The protoplasts were then diluted 1:1 with CP four days after electroporation, and again three weeks later with CM plus kanamycin (100 mg/L). The cultures were maintained as for suspension cultures, except for the addition of continuous kanamycin selection. Cultures were then transferred to induction media as described above.

TABLE 5

CP MEDIA

CM media, with modifications:

Plus		Minus	
Calcium pentothenate	1.0	Hypoxanthine	
		Thymidine	
L-Arabinose	10.0		
Galactose	10.0	New concentration	
Mannose	10.0	Sucrose	10000
Rhamnose	10.0		
Trehalose	10.0		
Xylose	10.0		
Glucose	36000.		
Mannitol	54000.		

[a] all media were adjusted to pH 5.6, all quantities in mg/L unless otherwise noted.

Coffee protoplasts were electroporated over a range of 150-240 volts/cm. After 3-4 months of kanamycin selection, very small cell clumps were observed in samples treated at 220 and 200 volts/cm, respectively. Kanamycin-resistant tissue was not obtained from any "no DNA" controls. While the specific transformation rate is unknown, it was estimated that Coffea transformation was about 1×10^{-6} per electroporated protoplast. Stable electroporative transformation of tobacco (selection performed on solid medium) was as high as 1×10^{-4} per electroporated protoplast.

The kanamycin-resistant Coffea cultures were then transferred to liquid induction media and plated onto solid induction media, as described above. Embryos that formed were allowed to develop and transferred to vermiculite to develop further.

Analysis of Transformed Tissues - Twenty ug of DNA from regenerated leaf tissue (Dellaporta, et al., in Molecular Biology of Plants - Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pg. 35-36, 1984) were digested with Pst I, Sst II and Hind III and processed for Southern analysis (Maniatis, et al., in Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory , Cold Spring Harbor, New York. pp. 382-389, 1982). Similarly digested pGA472 DNA and DNA from untransformed Coffea plant tissues were used as positive and negative control DNAs, respectively. Filters were probed with a ^{32}P-radiolabelled (Feinberg et al., Analytical Biochemistry, 132: 6-13, 1983) 1.2 kbp Bam HI x Hind III restriction fragment representing the NPT-II Kanamycin gene from pPK65 (Koziel, et al., J. Molecular and Applied Genetics, 2: 549, 1984).

No hybridization of the kanamycin probe to the untransformed control Coffea DNA was observed. The positive control pGA472 DNA hybridized to fragments of 1.8KB and 400 bp, as expected from the pGA472 sequence. Two independently derived Coffea transformants were analyzed. The first showed hybridization to bands of 1.8 Kb and 40 bp, with an additional band at 2.8 Kb. The second transformant showed hybridization at 400 bp, 1.6 Kb, and 5.5 Kb. It is assumed that the hybridizing bands of the unexpected sizes represent 3 kanamycin gene sequences that have integrated into the Coffea tissue and remain functional but have undergone rearrangements and do not have the same colinearity with the vector, pGA472.

The transformed Coffea plantlets above developed leaf tissues, which were analyzed as described. They established only feeble root systems and therefore have not developed into plants capable of flowering. It is believed that the reasons for this are that the cells were in culture too long by the time they were finally regenerated. We believe that the use of healthy, vigorous embryogenic cultures will results in better efficiencies and greater progression of plant development than was shown in this initial study. Opportunities to create coffee with improved quality characteristics, such as coffee with naturally reduced caffeine but with other flavor qualities intact, or plants with improved disease resistance characteristics can be envisioned using DNA transformation.

We believe that this first example of successful stable transformation of Coffea opens the doors to develop those opportunities.

Summary - Genetically altered Coffea arabica plantlets have been obtained. Coffea suspension cultures were established and were used to obtain protoplasts. These protoplast were transformed with a kanamycin-resistance gene by an electroporation procedure and regenerated into cells. Embryos were formed from the transformed cells and regenerated into cells. Embryos were formed from the transformed cells and regenerated into plantlets. The regenerated embryos have been shown to contain the inserted foreign DNA, indicating that stable transformation of Coffea has been achieved. Several opportunities for improving coffee quality characteristics can now be pursued by applying this transformation technology to problems of commercial significance.

CHLOROGENIC ACID IN LEAF DISKS, SUSPENSION-CULTURED CELLS, AND PROTOPLASTS OF COFFEE (*COFFEA ARABICA* L.). PHYSIOLOGICAL ROLE AND SUBCELLULAR LOCALIZATION

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INTRODUCTION

A remarkable correlation exists between the concentrations of chlorogenic acid (mainly 5-CQA) and caffeine found in different coffee species (CARELLI et al. 1974). For example, green Robusta beans sequester chlorogenic acid and caffeine at levels of about 8 to 12 % and 1.2 to 4 % (d. wt), respectively. In Arabica beans, the concentrations of both compounds are distinctly lower, i.e. 4 to 8% and 0.6 to 2 % respectively. The values are quoted from CLIFFORD (1985) for chlorogenic acid and from CHARRIER (1975) for caffeine. Finally the beans of some wild coffee species have a comparingly low content (0.3 to 1.9 %) of chlorogenic acid and are virtually caffeine-free (CHASSEVENT 1972; CHASSEVENT et al., 1974; CLIFFORD et al. 1989) .

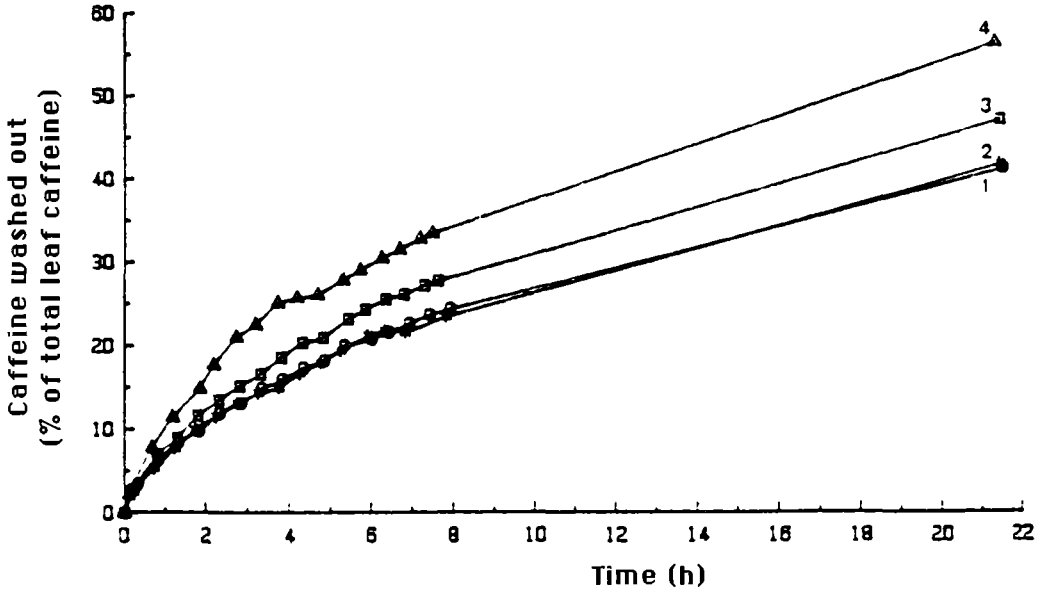
This coherence between the two compounds is not only manifested in the various tissues within the coffee plant but is also very clearly seen in suspension-cultured cells. When chlorogenic acid formation is stimulated by external factors such as light and polyethylene glycol, a concomitant increase in caffeine accumulation will occur (BAUMANN and RÖHRIG, 1989). Moreover, a mutant coffee cell line producing no caffeine could by no means be forced to synthesize chlorogenic acid (unpublished results).

So far, a metabolic connection between the two compounds is not known and even not recognized. However, it appears from studies by PFRUNDER et al. (1980) and KAPPELER (1988) that there is at least a physiological link because of the well-known caffeine-chlorogenate complex (SONDHEIMER et al. 1961, HORMANN and VIANI 1972; KAPPELER et al. 1987). We used leaf disks, suspension-cultured cells, and leaf protoplasts to study the role of this 1:1 complex in subcellular compartmentation of caffeine as well as to gain information about the localization of 5-CQA.

RESULTS AND DISCUSSION

When leaf disks, punched out with a corkborer and infiltrated under reduced pressure with water, are placed in a comparingly large volume of water, caffeine migrates only very slowly out of the tissue into the aqueous surrounding (Fig 1).

Fig. 1: Washout curves of leaf disks (16 ea., ø 1 cm) taken from 5- (**1**), 6.5- (**2**), 10- (**3**), and 13- (**4**) week-old leaves.



Even after 21.5 h a fraction of 40 to 60 %, depending on leaf age, of the total leaf caffeine is still retained in the tissue.

In Table I evidence is presented that complex formation between caffeine and chlorogenic acid may be responsible for the limited washout of caffeine.

Table I. Caffeine theoretically not complexed versus caffeine washed out

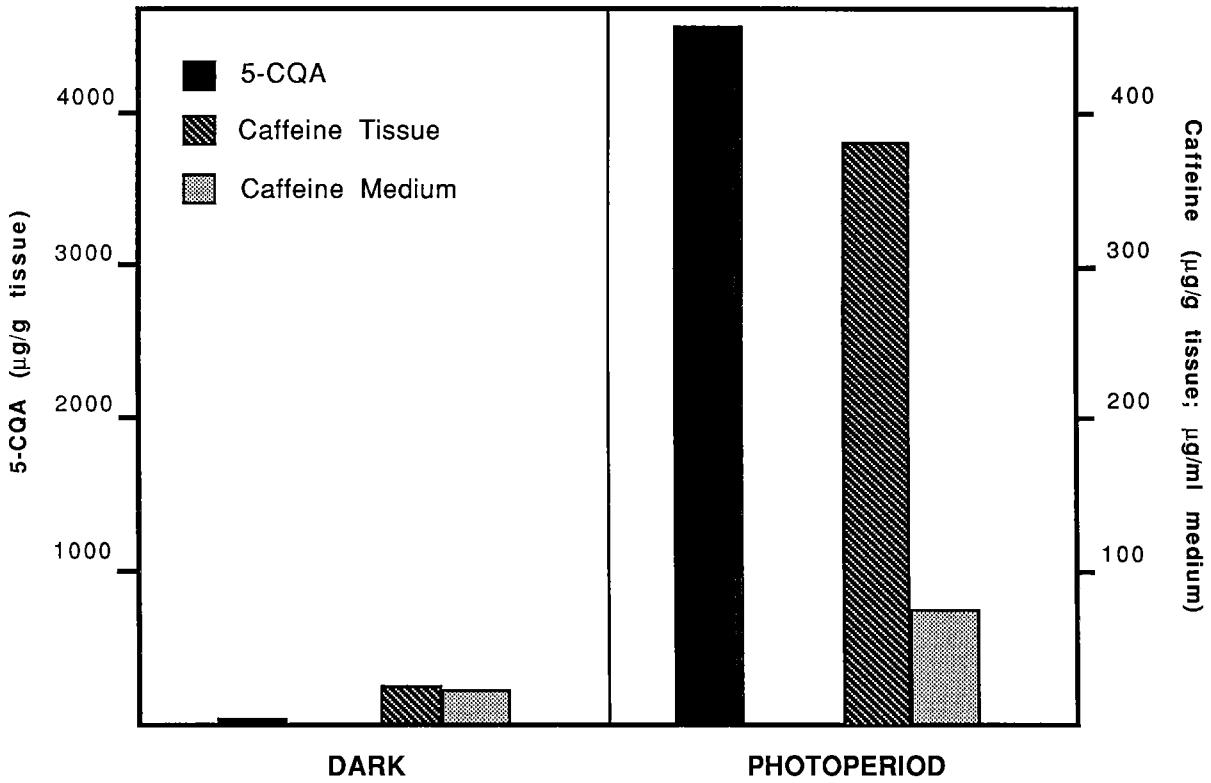
Leaf age (weeks)	Caffeine (% of total leaf caffeine) theoretically not complexed **(A)**	washed out **(B)**	Difference **B-A**
3	78.5	44.9	-33.6
5.5	57.9	59.2	1.3
8	59.1	63.7	4.6
11.5	41.9	40.9	-1.0
14	37.6	39.3	1.7
16.5	39.5	54.4	14.9

In one leaf of a pair the concentrations of the two partners were determined; these data together with the complexation constant were used to calculate the theoretically

complexed and free fractions of caffeine (KAPPELER, 1988). The other leaf was subjected to the washout procedure described above. With the exception of the youngest and oldest leaves, theory matches practice. It is important to mention that the equilibrium between the complex and the reactants is apparently not re-adjusted during the washout, since the washout curve reaches a constant level at about 70 % after 3 d. Furthermore it has to be noticed that throughout the entire washout experiment, caffeine is the only UV-absorbing substance discharged into the water. Chlorogenic acid remains in the leaf disks as long as the tissue is not impaired by the washout conditions used.

A more direct proof of the role of chlorogenic acid may be given by the use of suspension-cultured coffee cells (Fig. 2).

Fig. 2: Formation of 5-CQA and caffeine in cell suspensions (*Coffea arabica*) after 12 d.



The cells are growing in an aqueous medium and are therefore under a permanent washout condition. When grown in darkness, they produce only a small amount of chlorogenic acid. In contrast, they accumulate chlorogenic acid by a factor of one hundred when put into light (photoperiod, 12h/12h). In both cases, chlorogenic acid is detected exclusively in the cells and not in the culture medium. As regards to caffeine concentrations, Fig. 2 shows an almost equal distribution between cells and medium in the dark-grown cultures. However, when cells grown in the light accumulate a high concentration of chlorogenic acid, caffeine is not only increasingly produced but also compartmented intracellularly to a great extent.

Finally, protoplasts isolated from young coffee leaves (Table II) contain, on the basis of chlorophyll (cytoplasmic marker), only about half the amount of chlorogenic acid of the leaf cells. Since in the preparation the ratio of chlorophyll-free (epidermal) to

chlorophyllous (mesophyll) protoplasts is comparable to that found in leaf cross sections, tissue-specific compartmentation of 5-CQA is not responsible for the ratio decrease (5-CQA/chlorophyll) during isolation. We propose that the fraction of 5-CQA missing in protoplasts after their isolation is localized in the apoplast and is released during cell wall degradation. This fraction must be very effective in caffeine compartmentation, since the protoplasts are virtually devoid of caffeine (Table II).

Table II. Chlorogenic acid (5-CQA) and caffeine in leaves and protoplasts of *Coffea arabica*

	Leaves	Protoplasts
	Ratio (by weight)	
$\frac{\text{5-CQA}}{\text{Chlorophyll}}$	9.6	5.3
$\frac{\text{Caffeine}}{\text{Chlorophyll}}$	3.6	0.07
	Content (%)	
5-CQA	100	56
Caffeine	100	1.9

The protoplastic fraction of 5-CQA is considered to be in the vacuole. However, direct proof has to be given by vacuole isolation, which, on a large scale, still imposes some problems.

MATERIAL AND METHODS

Washout studies and compartmentation analyses were done as described by KAPPELER (1988). Tissue culture experiments and HPLC analyses were carried out according to BAUMANN and RÖHRIG (1989). For protoplast isolation, almost fully expanded coffee leaves were selected from the first or second leaf pair and cut in a Petri dish with a scalpel into 1 mm stripes while immersed in buffered osmoticum (0.7 M mannitol, 6 mM $CaCl_2$, 0.1% BSA, 10 mM cysteamine and 10 mM MES/NaOH, pH 5.5). Then the stripes (100 to 130 mg f. wt) were transferred into 7.5 ml buffered osmoticum in a Petri dish (5 cm) lined with a solid layer consisting of 4 % agar, 4 % gelatine, and 1 % activated charcoal (KELLER and MATILE, 1985). After preincubation (1 h, 30°C, 60 rpm, darkness), the osmoticum was exchanged for the enzyme mixture with 2% cellulase Onozuka R-10 (Serva, Heidelberg, Germany) and 0.1% pectolyase Y-23 (Seishin Pharmaceutical Co., Ltd., Tokyo, Japan) in 7.5 ml buffered osmoticum. Digestion was finished after 3.5 to 4 h at 35°C (darkness, 60 rpm). The protoplasts were separated from the undigested material by filtration through a 48 µm nylon grid and sedimented by centrifugation for 4 min at 100 g. The pellet was washed 3 times with 5 ml and finally resuspended in 1 ml buffered osmoticum. Chlorophyll was determined spectrophotometrically (LICHTENTHALER and WELLBURN, 1983).

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SUMMARY
Chlorogenic acid (5-CQA) has an important role in cellular caffeine compartmentation as illustrated for coffee leaves and suspension-cultured cells. Protoplast isolation studies indicate that about half of the amount of 5-CQA is located in the apoplast and that this fraction is very effective in caffeine compartmentation by complex formation.

ZUSAMMENFASSUNG
Am Beispiel von Kaffeeblättern und Zell-Suspensionskulturen konnte gezeigt werden, dass der Chlorogensäure (5-CQA) eine wichtige Rolle in der zellulären Kompartimentierung des Coffeins zukommt. Studien mit isolierten Protoplasten weisen darauf hin, dass etwa die Hälfte der Menge an 5-CQA im Apoplasten lokalisiert ist, und dass diese Fraktion für die Kompartimentierung des Coffeins durch Komplexbildung ausschlaggebend ist.

PREMIERS RÉSULTATS D'UN ESSAI COMPARATIF DE VARIÉTÉS DE CAFÉIERS ARABICA SELON TROIS DISPOSITIFS EXPÉRIMENTAUX DANS LA RÉGION DU KIRIMIRO (BURUNDI)

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Introduction

Les cultivars de caféier arabica diffusés au Burundi par l'I.S.A.Bu.[1] proviennent d'une sélection généalogique réalisée par l'I.N.E.A.C.[2], à Mulungu au Zaïre, parmi les Mibirizi (Mi) et d'autres introductions en provenance de l'étranger telles le Jackson du Kenya (J) et le Bourbon de Mayaguez de Porto Rico (BM) (SNOECK et PETIT, 1964).

Les essais comparatifs de variétés réalisés à Rubona au Rwanda, puis au Burundi à Gisha et à Kayanza ont permis de mettre en évidence le bon comportement de cinq lignées qui sont actuellement diffusées au Burundi (figure 1). Les Mibirizi 49/1848 et 68/1589 sont considérés comme tolérants à la sécheresse, bons producteurs et rustiques. Les Bourbons Mayaguez 71/2147 et 139 sont de bons producteurs peu sensibles à l'anthracnose des fruits (Colletotrichum coffeanum Noack), ils possèdent de bonnes caractéristiques granulométriques et organoleptiques. Le Jackson 2/1257 est lui aussi un bon producteur peu sensible à l'anthracnose, mais il est plus adapté aux altitudes élevées ; sa granulométrie et sa liqueur sont toutefois moins bonnes que celles des Bourbons (ISAR, 1963;GAIE et FLEMAL,1988).

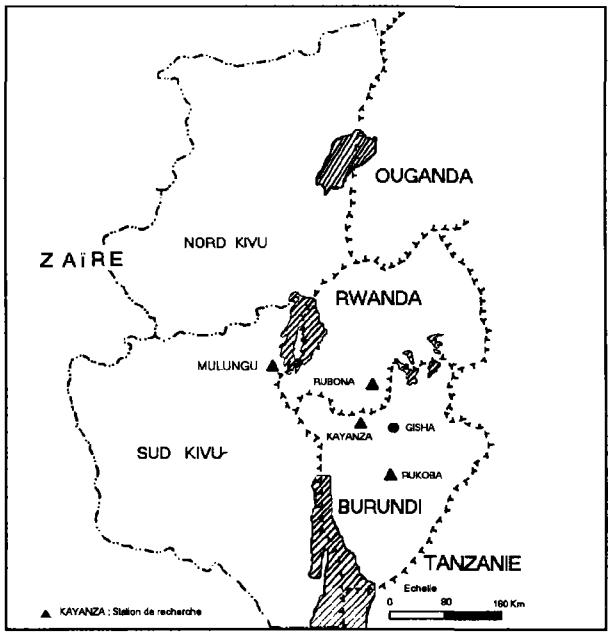

Figure 1 : Stations de recherche sur caféier arabica au Zaïre, Rwanda et Burundi.

[1] I.S.A.Bu. = Institut des Sciences Agronomiques du Burundi

[2] I.N.E.A.C. = Institut National pour l'Etude Agronomique du Congo Belge

Il faut y ajouter le Mibirizi bouts bruns (BB),appelé ainsi en raison de la coloration de ses jeunes feuilles, qui n'est pas une véritable lignée mais une population conservant encore une certaine hétérozygotie génétique.

Un important travail de sélection est poursuivi par l'ISABU, en collaboration avec l'I.R.C.C., pour répondre aux problèmes grandissants que sont l'incidence des maladies (Colletotrichum coffeanum Noach et Hemileia vastatrix) ou la nécessité d'intensifier la culture. L'institut dispose d'une vaste collection de caféiers arabica, comportant actuellement 406 variétés en provenance de 12 pays. Cette réserve génétique est en cours d'évaluation dans deux essais de triage et vingt essais comparatifs (BITOGA et al.,1990).

Les essais de sélection du caféier arabica au Burundi étaient généralement conduits selon un dispositif expérimental classique en blocs aléatoires complets à six répétitions.

D'autres schémas expérimentaux pourraient être envisagés pour ce type d'expérience, notamment le bloc aléatoire complet avec témoin systématique qui fut utilisé à Rubona, ou le dispositif complètement aléatoire récemment étudié dans le cadre d'essais de sélection du cacaoyer et du caféier robusta (LOTODE et LACHENAUD, 1988 ; CHARMETANT et LEROY, 1989).

Matériel et méthode

Un essai de sélection a été planté en 1986 au centre expérimental de Rukoba dans le Kirimiro, zone d'aptitude caféicole modérée du Burundi. Il a pour but d'évaluer le comportement de 15 variétés dans cette région et de comparer l'efficacité de trois dispositifs expérimentaux.

Le centre de Rukoba se situe à 3° 23' de latitude Sud et 29° 54' de longitude Est et est à une altitude de 1650 m. La pluviométrie annuelle moyenne fluctue entre 1150 et 1250 mm avec trois mois et demi de saison sèche où la pluviométrie est inférieure à 50 mm ce qui constitue la principale contrainte clima-tique du centre. La température annuelle moyenne varie de 18,0°C à 20,0 °C .

D'après la classification INEAC, le sol est un hygro-xéroferralsol développé dans un substrat de micaschistes. La texture est argilo-sableuse, le pH (H_2O) = 4,0, la capacité d'échange cationique = 4,0 méq/100 g,le taux de saturation en bases = 11,2 % et l'indice de Kamprath = 81 %. Les conditions pédologiques sont donc particulièrement difficiles pour le caféier arabica.

Les 15 variétés reprises dans l'essai sont présentées dans le tableau 1. Elles proviennent de sept régions du monde ; deux d'entre elles ont été triées à Rubona, six ont été sélectionnées à Mulungu, trois à Ruiru, trois à Balehonnur et une à Santa Tecla.

TABLEAU 1 : Origine et lieu de sélection des 15 variétés.

Variétés	Sigle	Lieu de sélection	Origine
Jackson 2/1257	J2	Mulungu (Zaïre)	Kenya
Bourbon Mayaguez 71	BM71	Mulungu (Zaïre)	Porto Rico
Bourbon Mayaguez 139	BM139	Mulungu (Zaïre)	Porto Rico
Mibirizi bouts bruns	MiBB	Mulungu (Zaïre)	Guatemala
Mibirizi 49/1848	Mi49	Mulungu (Zaïre)	Guatemala
Mibirizi 68/1589	Mi68	Mulungu (Zaïre)	Guatemala
Ainamba Babaca Kaffa 5691	ABK 5691	Rubona (Rwanda)	Ethiopie
Ainamba Babaca Kaffa 5718	ABK 5718	Rubona (Rwanda)	Ethiopie
Blue Mountain	Blue Mountain	Ruiru (Kenya)	Jamaïque
K7	K7	Ruiru (Kenya)	Inde
SL28	SL28	Ruiru (Kenya)	Kenya
Mysore	Mysore	Balehonnur (Inde)	Inde
S288	S288	Balehonnur (Inde)	Inde
S795	S795	Balehonnur (Inde)	Inde
Tekisic	Tekisic	Santa Tecla (San Salvador)	San Salvador

Le Jackson 2 a été choisi comme témoin.

Trois dispositifs expérimentaux sont comparés dans l'essai : en blocs aléatoires complets, complètement aléatoire (dénommé aussi "en randomisation totale") et en blocs aléatoires complets avec un témoin systématique répété toutes les 3 lignes (tableau 2).

TABLEAU 2 : Caractéristiques des trois dispositifs expérimentaux.

Dispositif	Nombre de répétitions	Parcelle élémentaire		Superficie du dispositif (y compris les bordures des blocs) m^2
		type	surface m^2	
Blocs aléatoires complets (BAC)	6	une ligne de 10 caféiers	37,50	3825
Complètement aléatoire (CA)	40	un caféier	3,75	2632
Blocs aléatoires complets avec témoin systématique (BTS)	4	une ligne de 10 caféiers	37,50	3375

Les caféiers ont été plantés à un écartement de 1,5 m dans la ligne et 2,5 m entre les lignes soit une densité de plantation égale à 2666 caféiers /ha. Les plants sont conduits en multicaulie sur 3 tiges. Ils reçoivent 60 unités d'azote/ha/an ainsi qu'une pulvérisation annuelle d'oxyde de zinc pour lutter contre la carence en zinc généralisée sur l'essai.
Le sol est totalement paillé avec Eragrostis appliqué à environ 30 tonnes de matière verte/ha/an.
Les observations ont été réalisées par caféier, elles ont porté sur les trois premières productions , le développement végétatif des arbres dans le dispositif complètement aléatoire et une estimation de la sensibilité des variétés à l'anthracnose des fruits.
Le développement végétatif des arbres a été évalué par des mesures de la hauteur et du diamètre à la base de la plus haute tige et du rayon moyen de la couronne foliaire établi par trois observations sur chaque caféier.

Résultats

Les résultats des observations du développement végétatif des caféiers au cours des trois années dans le dispositif complètement aléatoire sont présentés dans le tableau 3.

TABLEAU 3 : Paramètres du développement végétatif des 15 variétés,en cm.

Variétés	Hauteur moyenne			Diamètre moyen			Rayon moyen couronne		
	1989	1990	1991	1989	1990	1991	1989	1990	1991
Blue Mountain	119 a	168 a	203 a	1,98 a	2,70 a	3,33 a	40,3 ab	46,4 b	54,1 c
M1 B.B.	118 ab	165 ab	202 a	1,96 a	2,86 a	3,45 a	35,7 bcd	47,0 b	59,4 bc
M1 49	116 abc	162 abc	195 ab	1,93 a	2,70 a	3,50 a	34,7 cd	45,1 b	57,0 bc
BM 139	114 abcd	156 bcd	195 ab	1,83 abc	2,60 a	3,31 a	36,9 abc	48,5 b	58,9 bc
M1 68	113 abcd	159 bcd	196 ab	1,79 abcd	2,63 a	3,22 a	33,7 cd	44,8 b	59,2 bc
Tekisic	111 bcd	154 cd	188 bc	1,85 abc	2,68 a	3,41 a	33,2 cd	45,0 b	56,0 bc
K7	110 cd	157 bcd	194 ab	1,89 ab	2,73 a	3,35 a	36,1 bcd	46,4 b	59,4 bc
BM 71	110 cde	156 bcd	194 ab	1,89 ab	2,82 a	3,50 a	35,8 bcd	48,3 b	62,7 ab
Mysore	109 def	154 cd	186 bc	1,87 ab	2,60 a	3,21 a	35,9 bcd	46,6 b	55,0 c
S795	108 def	148 d	183 bc	1,73 bcde	2,56 a	3,25 a	41,7 a	56,1 a	67,5 a
SL28	107 def	148 d	181 c	1,79 abcde	2,71 a	3,36 a	35,9 bcd	48,3 b	59,7 bc
J2	102 ef	151 cd	188 bc	1,67 cde	2,50 a	3,16 a	33,7 cd	45,1 b	55,3 c
S288	99 f	127 e	161 d	1,53 e	2,18 b	2,79 b	33,1 cd	43,2 b	51,6 c
ABK 5718	89 g	123 e	154 de	1,70 bcde	2,66 a	3,38 a	32,7 cd	47,5 b	63,0 ab
ABK 5691	82 g	117 e	146 e	1,62 de	2,55 a	3,13 a	31,5 d	45,8 b	59,9 bc
F.obs à 14 et 550 dl.	29,16***	29,73***	33,00***	5,53***	2,71***	3,24***	4,79***	3,59***	5,28***
CV.résidu.(%)	10,9	11,2	9,8	16,8	18,5	15,9	21,9	20,2	17,5

La variété S288 est totalement inadaptée aux conditions locales : elle se développe mal et 15 % des caféiers sont morts. Les variétés ABK sont petites mais néanmoins vigoureuses avec un bon diamètre de tige et un grand rayon de la couronne. La variété S795 se distingue par le rayon de la couronne le plus grand. La figure 2 schématise le développement végétatif des variétés ABK5691, S795 et du témoin Jackson 2 qui se distinguent par une croissance différente.

La production cumulée des trois premières années est présentée dans le tableau 4.

TABLEAU 4 : Production cumulée (1989+1990+1991) en kg CM./ha/an des 15 variétés dans les trois dispositifs.

Variétés	Dispositif BAC	Dispositif CA (1)	Dispositif BTS	Tous les caféiers des 3 dispositifs (1)
Mysore	301 abc	338 a	317 ab	316 a
SL 28	321 ab	281 abcd	322 a	310 ab
S 795	328 ab	289 abc	277 abc	302 ab
K 7	293 abc	317 ab	269 abc	293 ab
Blue Mount.	297 abc	265 abcd	250 abc	275 abc
ABK 5691	337 a	209 bcd	230 abc	270 abc
ABK 5718	258 abc	262 abcd	291 abc	269 abc
BM 139	257 abc	292 abcd	254 abc	266 bc
BM 71	245 abcd	276 abcd	282 abc	264 abc
MI B.B.	216 bcd	247 abcd	208 abc	222 cd
J2	290 abc	231 bcd	195 c	213 d
Tekisic	226 abcd	178 cde	173 c	197 d
Mi 68	197 cd	162 de	201 bc	188 d
Mi 49	187 cd	178 cde	175 c	181 d
S 288	145 d	163 e	167 c	156 e
Fobs. à 14 et k2 dl.	5,19*** 70	6,72*** 599	4,37*** 42	20,35*** 2345
Moy. Gén.	260	246	241	244
CV.résid. (%)	23,5	46,5	20,8	46,2

(1) Les tests statistiques F de Snedecor, test de Newman-Keuls, coefficient de variation résiduelle sont effectués sur les données transformées ln (x+1).

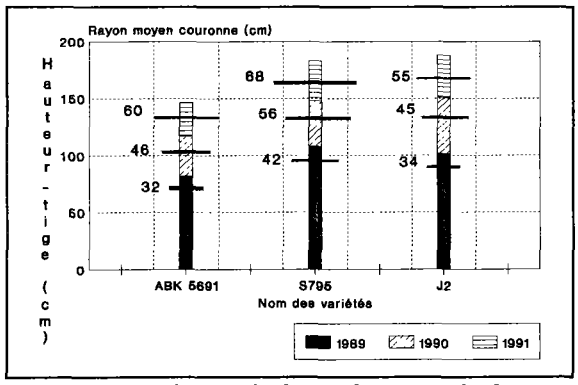

Figure 2 : Schéma de la croissance de 3 variétés (hauteur et rayon de la couronne).

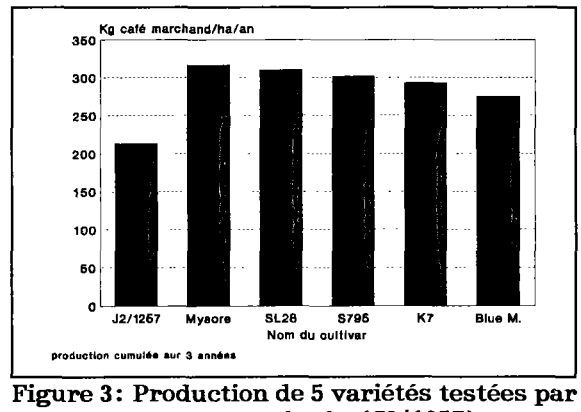

Figure 3: Production de 5 variétés testées par rapport au témoin (J2/1257).

Le niveau de production des variétés est très faible, il est au mieux de 316 kg de café marchand/ha/an pour la variété Mysore sur les trois premières années de production. Le groupe des meilleurs producteurs comporte neuf variétés parmi lesquelles se trouvent 2 variétés indiennes, Mysore et S795, les trois variétés sélectionnées au Kenya, SL28, K7 et Blue Mountain, les deux variétés éthiopiennes, ABK 5691 et 5718, et seulement deux cultivars diffusés par l'ISABU, BM 139 et BM 71.

Les variétés ont un comportement uniforme dans les trois dispositifs à l'exception de l'ABK 5691 et du Jackson 2 qui produisent plus dans le dispositif BAC.

La production des cinq meilleurs variétés est comparée à celle du témoin dans la figure 3. La variabilité de la production est élevée : dans le dispositif CA, le coefficient de variation résiduelle sur les données transformées vaut 66 % avec de forts écarts entre variétés. Une transformation de variables permet de réduire ce coefficient à 47 %.
Dans les dispositifs BAC et BTS, sa valeur est aussi élevée comparativement à d'autres essais sur caféier arabica menés à l'ISABU, et est comprise entre 21 et 25 % .

La production est influencée par l'incidence de l'anthracnose des fruits qui se manifeste avec une intensité différente selon les variétés. Une observation générale a été réalisée afin de comparer les variétés. Elle a permis de les classer en quatre catégories:

très sensible : S795 ;
sensible : SL28, Mysore, K7, J2, Blue Mountain, Tekisic, BM 71, S288 ;
peu sensible : Mi BB, Mi 49, Mi 68, ABK 5691, BM 139;
très peu sensible: ABK 5718.

EFFICACITE DES DISPOSITIFS EXPERIMENTAUX

Pour étudier les dispositifs expérimentaux, la variable retenue dans l'analyse statistique est la production cumulée observée par caféier ou par ligne de dix caféiers.

Comparaison entre le dispositif complètement aléatoire et le dispositif en blocs aléatoires complets

1°/ le dispositif complètement aléatoire

Dans ce dispositif, la dissymétrie de la distribution des données et l'hétérogénéité entre variances peuvent être tellement importante que les conditions d'application de l'analyse de la variance ne sont plus respectées. Cette dissymétrie et cette hétérogénéité imposent d'éliminer des données très "anormales" d'une variété ou d'une répétition, et/ou de recourir à une transformation de variables, ici Y=ln (X+1), pour stabiliser les variances entre les variétés .

Les productions individuelles des caféiers ont été soumises à l'analyse de la variance à un facteur à l'état brut mais aussi après transformation de variables et suppression d'une variété (S288) à forte variabilité,dont le coefficient de variation est de 140 % (tableau 5). La transformation et la suppression de la variété S288 permettent de diminuer l'écart-type résiduel et donc d'augmenter la valeur du Fobs et d'assurer de meilleures conditions d'application de l'analyse de la variance. La distribution globale des résidus devient symétrique avec un coefficient de symétrie b1 qui s'approche de la valeur idéale 0. La transformation logarithmique "normalise" bien la distribution des résidus mais il persiste une inégalité de variances.

Pour le dispositif CA, le recours aux transformations de variables apparaît nécessaire afin de mieux satisfaire aux conditions d'application de l'analyse de la variance à un facteur de classification.

TABLEAU 5 : Résultats des analyses statistiques des données de production cumulée pour le dispositif complètement aléatoire.

Paramètres	Toutes les variétés		Sans la variété S288	
	données non transformées	données transformées ln (x+1)	données non transformées	données transformées ln (x+1)
Fobs. à	4,83***	6,72***	4,66***	5,10***
k1 dl.	14	14	13	13
k2 dl.	585	585	546	546
CV. résid. (%)	66,4	46,5	62,7	42,6
E-T. résid.	163	61	157	60
	Validation du modèle ETUDE DES RESIDUS			
Normalité des résidus				
- diagramme	log-normal	normal	log-normal	normal
- symétrie (b1)	1,95***	0,00NS	2,09***	0,04 *
- aplatissement (b2)	8,16***	3,22NS	9,29***	3,34 NS
Egalité des variances				
X2 à 14 dl.	68,77***	57,35 ***	56,93***	30,89**
(test Bartlett)				
Var.max/Var. min	5,1	6,7	4,6	4,2

2°/ le dispositif en blocs aléatoires complets

L'analyse statistique de ce dispositif a été réalisé selon trois méthodes : un modèle en blocs aléatoires complets avec des parcelles élémentaires de 10 caféiers, ou suivant un modèle complètement aléatoire avec des parcelles élémentaires de soit 10 caféiers soit 1 caféier (tableau 6).

Pour une même unité d'observation de 10 caféiers, le dispositif en blocs aléatoires complets est plus précis que le dispositif complètement aléatoire: la valeur de F obs est plus élevée et l'écart-type résiduel est plus faible. Les blocs sont donc ici bien utiles et leur efficacité relative est évaluée à 133 % par rapport au dispositif complètement aléatoire (100 %).

Pour une unité d'observation constituée d'un caféier et en considérant que la répartition est quasi-complètement aléatoire, l'analyse est réalisée sur des données transformées pour mieux normaliser les distributions et stabiliser les variances (paragraphe précédent). Le test de comparaisons multiples de moyennes (test de Newman-Keuls) est légèrement plus performant en travaillant sur des unités d'observation de 1 caféier en raison d'un nombre plus élevé de degrés de liberté et de répétitions.

TABLEAU 6 : Résultats des analyses statistiques des données de production cumulée pour le dispositif BAC analysé comme dispositif en blocs aléatoires complets et comme dispositif complètement aléatoire.

Paramètres	dispositif blocs aléatoires complets	disp. complètement aléatoire 10 caféiers	disp. complètement aléatoire 1 caféier ln (x+1)
Fobs. à 14 dl. et k2 dl. CV. résid.(%)	5,19*** 70 23,5	3,91*** 70 27,1	8,30*** 885 44,7
Différence significative entre 2 moyennes en %	27,1	31,2	16,0
C.M.M. Test de Newman-Keuls (95 %)	ABK 5691 a S 795 ab SL28 ab Mysore abc Blue M. abc K7 abc J2 abc ABK 5718 abc BM 139 abc BM 71 abcd Tekisic abcd Mi B.B. abcd Mi. 68 cd Mi. 49 cd S288 d	ABK 5691 a S 795 ab SL28 abc Mysore abc Blue M. abc K7 abc J2 abc ABK 5718 abcd BM 139 abcd BM 71 abcd Tekisic abcd Mi B.B. abcd Mi. 68 bcd Mi. 49 cd S288 d	ABK 5691 a S 795 ab SL28 ab Mysore ab Blue M. ab K7 ab J2 abc ABK 5718 abc BM 139 abc BM 71 abc Tekisic abc Mi.B.B. bc Mi.68 c Mi. 49 c S288 d

Contrôle localisé de l'hétérogénéité de l'essai

Pour les dispositifs en blocs aléatoires complets, on peut essayer de maîtriser l'hétérogénéité ponctuelle par l'utilisation d'un témoin répété systématiquement dans la répétition. Ce système est avantageux si les parcelles sont petites ou s'il n'y a pas de gradient de fertilité spécifique ou encore si la variabilité des conditions expérimentales est répartie aléatoirement dans le champ d'expérience. YATES a introduit une méthode statistique adaptée à l'utilisation de témoins systématiques : l'analyse de la covariance. Une augmentation de l'efficacité du test statistique, de 38 à 528 % a été observée par MELTON et FINKNER (1967) par l'utilisation de témoins systématiques sur les productions d'Alfa dans les sols de la région de New Mexico.

1°/ Pour le dispositif complètement aléatoire

DAGNELIE (1981;1987), LOTODE et LACHENAUD (1988) conseillent de réaliser l'analyse de la covariance dont la covariable est la moyenne de parcelles voisines dans le but d'améliorer la précision de l'essai. Cette technique appelée méthode des plus proches voisins ou de Papadakis a été utilisé avec succès dans un essai de sélection d'hybrides de cacaoyer en Côte d'Ivoire (LOTODE,1988).
Deux indices de fertilité ou de valeur de l'environnement pour la petite zone entourant l'arbre ont été employés :

$I_1 = 1/8 \sum_{i=1}^{8} V_i$ $\quad V_i$ = valeur de la production cumulée pour l'arbre voisin i

$I_2 = 1/8 \sum_{i=1}^{8} (V_i - \overline{VH}_i)$ $\quad \overline{VH}_i$ = moyenne des valeurs de la production cumulée sur l'ensemble des arbres (du dispositif) de la variété située en i.

TABLEAU 7: Comparaison des résultats obtenus entre l'analyse statistique du dispositif CA par la méthode classique (analyse de la variance) et celle de la méthode des plus proches voisins (analyse de la covariance) sur les données transformées ln(X+1).

Paramètres	Méthode classique	Méthode des plus proches voisins Indice 1 (I1)	Indice 2 (I2)
Fobs à 14 et k2 dl.	6,72*** 585	6,77*** 584	6,87*** 584
E-T. résid.	61,0	60,7	60,6
Efficacité relat.	100 %	100,9 %	101,1 %

N.B.: Efficacité relative = rapport des carrés des écarts-types résiduels, exprimé en % .

Les moyennes ajustées par les deux indices de fertilité sont très voisines des moyennes non ajustées. Dans le dispositif CA de Rukoba, l'utilisation de la méthode des plus proches voisins pour la production cumulée n'apporte guère d' amélioration de la précision de l'essai, l'efficacité relative étant de 101 % par rapport à la méthode classique (tableau 7).

2°/ Pour le dispositif en blocs aléatoires complets

L'ajustement des données en fonction du témoin systématique (analyse de la covariance) n'apporte guère d'amélioration dans la précision de l'essai (tableau 8) et très peu de changement dans la détermination des groupes de moyennes homogènes (efficacité relative = 100,8 % *par rapport à aucun ajustement*). *Ces résultats peuvent être expliqués par une bonne* homogénéité du champ d'essai et par une trop importante variabilité du témoin choisi (coefficient de variation par caféier de 76 %).

TABLEAU 8 : Résultats des analyses statistiques du dispositif BTS sur la récolte cumulée 1989+1990+1991.

Paramètres	Non ajustés	Analyse de la covariance (covariable = moyenne pondérée des productions des 2 témoins les plus proches)
Fobs. à 13 et k2 dl.	4,13*** 39	4,26*** 38
E-T. résid.	51,88	51,68
Eff. relative	100,0 %	100,8 %

Discussion sur les 3 dispositifs comparés

En fonction des résultats des analyses statistiques et des caractéristiques de l'expérimentation, un tableau comparatif a pu être établi sur les avantages et les inconvénients respectifs des trois dispositifs.

1°/ Caractéristiques de l'expérimentation :

Dispositif	Avantages	Inconvénients
CA BAC BTS	Superficie réduite de l'essai.	Superficie plus grande que dispositif CA. Superficie encore plus grande que le dispositif BAC pour un même nombre de blocs.
CA BAC et BTS	Conviennent mieux pour des sites plus hétérogènes.	nécessite un sol très homogène.
CA BAC et BTS	Les caféiers sont dans une situation proche de la situation réelle de plantation industrielle ou paysanne; faible concurrence entre les variétés.	*La concurrence devrait être marquée; des* variétés de format et d'architecture différentes se retrouvent intimement mêlés.
CA BAC et BTS	Visualisation comparative aisée entre variétés (vigueur).	Pas de visualisation comparative aisée entre variétés; il faut ajouter des parcelles supplémentaires.
CA BAC et BTS	Facilité de récolte, par ligne de 10 caféiers.	Récolte individuelle plus laborieuse ;source d'erreurs plus grande; nécessité d'un bon suivi des récoltes.

2°/ Analyse statistique:

Dispositif	Avantages	Inconvénients
CA		Sur les données initiales, les conditions d'application de l'analyse de la variance ne sont pas vérifées d'où nécessité de transformer les données (ln,...).
BAC et BTS	Les conditions d'application de l'analyse de la variance sont vérifées.	
CA	Le test de comparaisons multiples de moyennes, donne plus de groupes homogènes.	
BAC et BTS		Le test de comparaisons multiples de moyennes donne un peu moins de groupes homogènes de moyennes.

BAC = dispositif en blocs aléatoires complets.
CA = dispositif complètement aléatoire.
BTS = dispositif en blocs aléatoires complets avec témoin systématique.

L'efficacité relative des trois dispositifs peut être aussi comparée sur base de la différence significative entre 2 moyennes, exprimée en % de la moyenne générale (tableau 9). Le dispositif CA est le plus sensible et permet de mettre en évidence des différences significatives entre 2 moyennes de l'ordre de 20 %, mais les données ont dû subir une transformation de variables. Le dispositif BTS qui comporte 4 répétitions est le moins précis (différence significative de 30 %).

TABLEAU 9 : Comparaison de la différence significative entre 2 moyennes que l'on peut mettre en évidence en % de la moyenne du dispositif.

Dispositif	Coefficient de variation résiduelle	nombre de répétitions	Différence significative entre 2 moyennes en %
CA (*) 15 variétés 585 degrés de liberté	46,5	40	**20,4**
BAC 15 variétés 70 degrés de liberté	23,5	6	**27,2**
BTS 14 variétés 39 degrés de liberté	21,3	4	**30,4**

(*) les données ont subi une transformation logarithmique.

CONCLUSIONS

Au terme des trois premières années d'observation de cet essai comparatif de 15 variétés, planté dans une région d'aptitude caféicole modérée, des différences très hautement significatives sont apparues entre les moyennes de production des variétés. Les productions les plus élevées ont été obtenues avec Mysore, SL28, S795, K7 et Blue Mountain ; les cultivars diffusés par l'ISABU sont très mal classés à l'exception des deux Bourbons. Le mauvais comportement des cultivars Mibirizi dans cette zone est de nature à modifier les recommandations actuelles de l'ISABU. En effet, les Mibirizi sont considérés comme résistants à la sécheresse et donc plus appropriés pour une diffusion dans les zones de culture marginales (JOSIS et al.,1983). Cette opinion ne se vérifie pas pour le moment, elle devra éventuellement être modifiée si les résultats se confirment. En attendant, il semble prudent d'y distribuer exclusivement les cultivars Bourbons plus productifs et qui possèdent également une meilleure granulométrie.

Le niveau de production de l'essai est très faible, atteignant à peine 300 kg de café marchand/ha/an pour les meilleures variétés. A titre de comparaison, la production atteinte par le Mysore après trois ans à Gisha était d' environ 1000 kg de café marchand/ha/an.
Ce faible niveau de production s'explique par les contraintes importantes que subissent les caféiers: saison sèche longue, sols très peu fertiles et très forte incidence des maladies cryptogamiques. Un programme d'amélioration génétique par hybridation s'impose afin de créer des variétés mieux adaptées et plus résistantes aux maladies.

La comparaison statistique des trois dispositifs expérimentaux envisagés dans l'essai a permis de tirer certaines conclusions.

Le dispositif complètement aléatoire avec des parcelles d'un caféier présente l'avantage d'une superficie réduite mais exige beaucoup plus de soins dans les observations et les récoltes. Il peut être adopté si les conditions d'expériences et la variabilité des variétés testées sont homogènes. Dans notre expérimentation, l'analyse statistique de la production cumulée a nécessité une transformation logarithmique des données pour satisfaire aux conditions d'application de l'analyse de la variance.

Le dispositif en blocs aléatoires complets avec parcelle de 10 caféiers et un témoin systématique exige une superficie plus grande et, dans les conditions de notre essai, l'emploi d'un témoin systématique ne s'est pas avéré efficace du point de vue de l'analyse statistique. D'autres essais sur le caféier arabica et le théier sont menés à l'ISABU en utilisant cette technique du témoin systématique. De faibles augmentations d'efficacité, allant de 0 à 13 %, y ont été observées pour la première année de production (OTOUL,1990). L'utilisation d'un témoin peu variable (matériel clonal) devrait être beaucoup plus efficace.

Le dispositif en blocs aléatoires complets avec parcelle de 10 caféiers convient bien pour des sites plus hétérogènes et son interprétation statistique reste classique et aisée.

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Résumé

Un essai a été planté en 1986 dans le centre de recherche de Rukoba au Burundi afin de comparer quinze variétés de caféiers arabica, d'origine diverse, dans une écologie d'aptitude modérée à l'arabicaculture. Les caféiers sont évalués selon trois dispositifs expérimentaux : soit des parcelles élémentaires de dix caféiers en six blocs aléatoires complets ou en quatre blocs aléatoires complets avec un témoin répété systématiquement, soit des parcelles élémentaires de un caféier répétées quarante fois de façon complètement aléatoire.

Les observations ont porté sur le développement végétatif des caféiers et sur leur production en drupes au cours des trois premières années.

L'analyse statistique des résultats a mis en évidence des différences très hautement significatives entre les moyennes des variétés pour les paramètres observés.

Plusieurs variétés introduites dépassent les cultivars diffusés dans le pays au point de vue production. Les cultivars Mibirizi, réputés résistant à la sécheresse, sont pour le moment les moins productifs dans cette zone de dures conditions écologiques. Le très faible niveau général de production démontre la nécessité de recourir à un programme d'amélioration génétique par hybridation.

Le dispositif complètement aléatoire (un caféier/parcelle) est le plus efficace pour la comparaison statistique des moyennes mais il exige plus de soins dans les observations et il est nécessaire d'effectuer une transformation logarithmique des données de production pour l'analyse statistique.

Pour une même unité d'observation (10 caféiers/parcelle), l'analyse statistique selon un dispositif en blocs aléatoires complets est plus efficace que si l'on supprime l'effet blocs.

Le contrôle de l'hétérogénéité locale dans le dispositif complètement aléatoire par la méthode de "Papadakis" ou l'utilisation d'un témoin systématique dans le dispositif en blocs aléatoires complets n'apporte qu'une très faible amélioration de la précision de l'essai. Le dispositif avec le témoin systématique est le moins précis pour comparer les moyennes.

En fonction des résultats de l'étude, quelques recommandations d'ordre général sont formulées pour le choix des dispositifs en fonction des conditions de terrain, de matériel végétatif , d'organisation du travail et de l'analyse statistique.

Summary

In 1986, a trial was begun to test fifteen varieties of Arabica coffee from different origins at the Rukoba Research Center in a zone considered moderately adapted for Arabica coffee in Burundi. The coffee trees were evaluated in three experimental designs : a randomized complete block design with 6 replications of ten trees/plot, a randomized complete block design with 4 replications (ten trees/plot) with a systematic control, and a completely randomized design with 40 replications of one tree/plot. Observations were made on the vegetative growth and the cherry yield during the first three years.

The statistical analyse of these results show very highly significant differences between the coffee varieties. Some introduced varieties yielded more than local varieties. The local varieties Mibirizi, well known dry resistant, were less yielded in this zone with hard ecological conditions. The poorer level of yield proved the necessity to begin a program of crop improvment by hybridization.

The completely randomized design with one tree/plot was the most efficient for the statistical comparison of means, but it required more care in observations and it was necessary to use a logarithmic transformation of the data for the statistical analysis.

For the ten trees/plot, the statistical analysis according to the randomized complete block design was more efficient than when block effect is suppressed.

The control of micro-heterogeneity in the completely randomized design by the "Papadakis" method or the use of a systematic control in a randomized complete block design improved the precision of the trial somewhat. The randomized complete block design with a systematic control was the less accurate to compare the means of varieties.

Some recommendations are made concerning the choice of experimental design in relation to the site, the crops, the organization of work, and the statistical analysis.

ESSAIS MULTILOCAUX D'ENGRAIS SUR CAFÉIER ARABICA AU BURUNDI. MÉTHODOLOGIE DE MISE EN PLACE ET D'OBSERVATION

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Introduction

L'influence des éléments minéraux apportés au sol par les engrais sur la production des caféiers Arabica est étudiée dans des essais installés dans des centres (ou stations) de recherche répartis dans les principales régions du Burundi.

En plus de ceux-ci, des essais multilocaux ont été installés en 1988 chez des planteurs de café afin de tester, en milieu réel, les résultats obtenus dans ces centres. Ce qui permet d'avoir ainsi plusieurs conditions de sol et de climat.

Les formules et les doses d'engrais à appliquer aux caféiers dans ces essais ont été calculées à partir des analyses de sol à l'aide du programme informatisé mis au point pour l'étude de la fertilisation minérale des caféiers. Les formules recommandées sur base de ces analyses ont ensuite été synthétisées en fonction des niveaux des éléments et des équilibres entre les bases échangeables du sol afin de pouvoir ne recommander qu'un nombre limité de formules d'engrais aux services de vulgarisation.

La présente note reprend la méthodologie utilisée pour effectuer le choix des plantations, le dispositif expérimental adopté dans ces plantations, ainsi que les observations qui y sont effectuées.

Choix des plantations

La culture du caféier Arabica couvre près de 60.000 ha répartis en petites exploitations familiales de 100 à 300 caféiers chacune.

Sur base de la durée de la saison sèche, de la hauteur des précipitations, du volume et de la fertilité des sols, quatre classes d'aptitude ont été définies. Ce qui a permis de distinguer sept régions plus ou moins aptes à la culture des caféiers Arabica au Burundi.

A partir des cartes pédologiques établies par G. SOTTIAUX et al. (1988), les types de sol les plus représentatifs de chaque région ont été identifiés et un ou plusieurs secteurs d'observation par région ont été sélectionnés. Le nombre de secteurs choisis étant proportionnel à l'importance de la culture des caféiers dans la région concernée. Une vingtaine de secteurs ont ainsi été sélectionnés (figure 1).

Dans chaque secteur, 10 plantations ont été choisies afin de pouvoir y tester les différentes formules et doses d'engrais. Le choix des plantations a été basé sur les trois critères suivants :

- La plantation devait avoir au moins 60 caféiers observables et homogènes du point de vue de la taille et de l'aspect végétatif.
- Ces caféiers devaient avoir environ 3 ans d'âge. Ceci afin d'avoir des plants qui entrent dans leur première année de production et encore suffisament jeunes pour assurer une réponse rapide aux engrais.
- La plantation devait être bien entretenue et appartenir à un paysan qui continuerait l'entretien régulier des caféiers (désherbage, égourmandage, ...).

On admet que la fertilisation ne peut être réalisée que si les autres techniques culturales sont correctement effectuées. Un effort doit être fourni pour que les entretiens soient assurés de manière constante dans tous les essais afin que l'entretien ne constitue pas un facteur de variation dans l'analyse statistique.

Pour des raisons de facilité du suivi, les plantations se situent dans un rayon de moins de 5 km du centre du secteur.

Un observateur a été affecté à chaque secteur. Il est chargé des observations et des récoltes dans les dix plantations retenues. Il s'assure aussi que le planteur entretienne convenablement sa plantation.

Dispositif expérimental

Chaque plantation constitue un essai complet et statistiquement analysable. Le dispositif expérimental adopté est la randomisation simple de 3 traitements en 20 répétitions. La parcelle élémentaire est constituée d'un seul caféier. Il faut donc 60 caféiers observables par essai.

Les traitements sont matérialisés sur les caféiers observés par une marque de peinture de couleur :

- noire pour les caféiers ne recevant pas d'engrais (témoins),
- rouge pour les caféiers recevant la dose 1 d'engrais,
- jaune pour les caféiers recevant la dose 2 d'engrais.

La répartition aléatoire des traitements par répétition est réalisée une seule fois pour tous les essais. Ce qui permet d'établir des fiches d'observation et de récolte identiques pour l'ensemble des plantations et de faciliter l'analyse des données. La répartition des objets au hasard par répétition dans chaque plantation est assurée sur le terrain par les différences qui existent dans la forme des champs de caféiers et le choix de plants qui seront observés. Un plan de la disposition des caféiers observés a été établi pour chaque parcelle d'essai.

A cause de la très petite dimension des parcelles paysannes (moins de 100 caféiers homogènes par plantations), il n'y a pas de bordure entre les caféiers observés.

Choix de la formule d'engrais

Dans chacune des plantations observées, un échantillon composite de sol a été prélevé dans les 20 premiers cm de terre. Sur base de l'analyse physique et chimique de ces échantillons, une formule d'engrais est proposée.

La formule et la dose d'engrais ont été calculées à l'aide d'un programme informatique réalisé par SNOECK D. et SNOECK J. en 1987. Celui-ci compare les niveaux des éléments et les équilibres entre cations à des seuils qui sont fonction de la texture du sol (teneur en argile plus limon fin).

En prenant en compte tous ces facteurs, le programme propose diverses formules. Les doses recommandées sont calculées pour atteindre des niveaux considérés comme normaux dans la partie du sol qui reçoit les engrais.

Afin d'identifier les tendances de fertilisation, les formules d'engrais recommandées ont été regroupées par classe de sol, par climat et par altitude. Seul le facteur sol s'est révélé intéressant. L'observation des analyses de sol révèle un déséquilibre marqué dans le rapport Mg/K qui s'explique par l'utilisation régulière du paillage par les caféiculteurs.

Compte tenu du fait que la capacité d'échange cationique est généralement supérieure à 5 méq%, que les niveaux de phosphore sont généralement suffisants et que le paillis apporte suffisamment de potassium, deux formules d'engrais suffisent pour l'ensemble des essais :

- si la saturation en base est supérieure à 40%, l'azote seul sera recommandé. Les doses de 130 et 260 kg d'urée par hectare et par an sont comparées au témoin dans les essais multilocaux.

- dans les autres cas, on compare l'azote aux mêmes doses que ci-dessus plus de la chaux dolomitique à 500 kg/ha/an (contenant 35% de CaO et 25% de MgO).

Les premiers épandages d'urée ont été fait en mars 1988. Ensuite, les engrais sont apportés en octobre et de nouveau en mars. La dolomie est apportée une fois par an, en janvier.

Observations et récoltes

L'influence des engrais est observée sur l'aspect végétatif des caféiers et sur leurs rendements.

Aspect végétatif

Des observations sont réalisées mensuellement sur quatre caféiers par traitement pour suivre l'apparition ou l'évolution du die-back (par l'observation des chutes de feuilles) et des carences en éléments minéraux. Les carences en azote, magnésium et zinc sont les plus fréquement rencontrées au Burundi.

L'importance des symptômes est évaluée selon une échelle d'intensité des attaques de 0 à 5 pour chacune des observations.

La rouille et l'anthracnose, qui sont considérées comme des maladies d'importance secondaire au Burundi, peuvent cependant avoir localement des effets importants. Il a donc été décidé d'en suivre l'évolution et la répartition géographique. Le nombre de feuilles attaquées par Ascochyta est aussi suivi sur 3 branches situées à 3 niveaux (haut, milieu et bas).

L'importance des attaques des principaux insectes prédateurs du caféier au cours d'une année est observée sur base du nombre de colonies de pucerons et de cochenilles par arbre et du nombre de feuilles attaquées sur 3 branches à 3 niveaux de l'arbre (haut, milieu et bas) pour les mineuses et les thrips.

Rendements

Les rendements sont mesurés à partir de la pesée des cerises récoltées arbre par arbre. Une première récolte à blanc fut enregistrée en 1989. La récolte de 1990 a donné les premiers résultats significatifs.

Afin de contrôler l'efficacité des récoltes, deux estimations de celles-ci sont effectuées un mois avant le premier passage de cueillette des cerises.

La première est basée sur l'appréciation visuelle de l'âge et de la charge en fruits des caféiers. Elle consiste à donner une note de 1 à 3 pour la taille (l'âge) des caféiers (selon que les plants sont petits, moyens ou grands) et une valeur de 0 à 5 pour la charge en fruits (notée **"ch-fr"** dans les équations ci-dessous). L'estimation est obtenue en effectuant le produit de ces 2 valeurs. Cette méthode, bien que subjective, est cependant rapide et permet de détecter des erreurs obtenues lors des comptages et des récoltes.

La seconde estimation est obtenue par comptage de tous les glomérules de chaque caféier observé. Cette technique, quoique assez fastidieuse, donne des résultats qui concordent de façon satisfaisante avec les résultats qui ont été obtenus par la pesée des cerises.

L'analyse de la régression linéaire entre les récoltes et les deux types d'estimations a été réalisée pour chaque secteur. Des coefficients de corrélations hautement significatifs ont été observés. Les équations obtenues pour l'ensemble des essais à partir de la moyenne des équations de régression de chaque secteur sont (pour une production estimée en kg de café marchand par ha) :

Production = 199,7 x (âge x ch-fr) + 41
(Pour des coefficients de corrélation variant de 0,36 à 0,78 selon les secteurs).

Production = 2,70 x Comptage + 250
(Pour des coefficients de corrélation variant de 0,40 à 0,88 selon les secteurs).

Comme il y a 500 à 600 observations par secteur, le coefficient "r" est toujours très hautement significatif ($r > 0,29$).

Si l'on impose le facteur constant = 0, les équations deviennent :

Production = 195,7 x (âge x ch-fr) avec $0,51 < r < 0,72$
Production = 2,86 x Comptage avec $0,64 < r < 0,91$

Sur base des comptages effectués dans les essais, on calcule qu'un glomérule contient en moyenne 5 cerises, qu'il y a 720 cerises par kilogramme. Le rendement moyen obtenu lors du traitement du café est de 0,162 kg de café marchand par kg de cerises.

Sachant qu'il y a environ 2 500 caféiers par hectare, le coefficient de régression obtenu par calcul est donc égal à (5 / 720 x 0,162 x 2500) = 2,81. Ce qui est très proche du coefficient obtenu à partir des comptages ci-dessus.

Efficacité des engrais

Calcul statistique

On constate que, dès la deuxième année d'application d'engrais, une différence de plus de 30 % de récolte a été observée dans 27 % des plantations entre les caféiers témoins et les caféiers ayant reçu l'une des deux doses d'engrais (simple ou double). Parmi celles-ci, 65 % d'entre elles avaient déjà manifesté cette différence lors des estimations.

Comme les récoltes des caféiers ont été réalisées arbre par arbre et que chaque parcelle constitue un essai en randomisation totale de trois objets (témoin, dose 1 et dose 2 d'engrais) en 20 répétitions, l'analyse statistique de chaque parcelle d'essai a pu être effectuée. Vingt et une parcelles parmi les 167 qui ont pu être observées cette année ont montré une réponse significativement positive à l'engrais. Ce qui correspond à 13 % du nombre total d'essais et 47 % des essais où une différence de plus de 30 % avait été observée. Par contre, quatre plantations (2 % du total) ont montré une réponse significativement négative aux apports d'engrais. Parmi celles-ci, deux plantations qui avaient des pH élevés et un niveau de saturation en base suffisant, ont reçu, par erreur, de la chaux magnésienne. Les deux autres plantations ont été installées sur des régosols qui ne conviennent pas à la caféiculture. Le tableau 1 reprend les résultats de récoltes dans les plantations où un effet significatif des engrais sur les productions a été observé. On constate que les variations de productions se retrouvent de façon assez régulière dans les estimations de récolte.

Calcul économique

Sachant qu'il faut 50 grammes d'urée à 75 francs/kg par caféier et par an pour la dose 1 d'engrais (ou 100 g pour la dose 2), le coût de l'engrais (avec 0,75 F/pied de main d'oeuvre comprise) est de 4,5 F/arbre. En se fixant un seuil de rentabilité (recette/coût) égal à 2 et si l'on ne tient pas compte de l'amortissement dû aux deux premières années d'application d'engrais, il faut que les caféiers fertilisés produisent au moins 250 g pour la dose 1 (ou 460 g pour la dose 2) de cerises par arbre de plus que le témoin.

Pour cette année, on observe que 46 % des plantations se sont révélées rentables à la dose 1 d'urée et 42 % à la dose 2 dans les régions où l'azote seul est recommandé.

Dans le cas où la chaux magnésienne est nécessaire, il faut rentabiliser un investissement supplémentaire de 9,5 FBu par plant, puisqu'il faut mettre 250g de chaux à 35 F/kg. Des différences de 780 g (pour la dose 1) à 1000 g de cerises (pour la dose 2) entre les caféiers témoins et ceux recevant des engrais sont alors nécessaires. Seulement 10 % des plantations se sont révélées rentables au cours de cette année.

Discussions et conclusions

Les essais multilocaux permettent de tester les formules d'engrais dans un plus grand nombre de situations que les essais sur centre. Ils montrent si une régionalisation des formules basée sur les diverses caractéristiques des sols est possible.

De plus, la répartition des secteurs dans l'ensemble du pays donne l'occasion de réaliser un inventaire et un suivi phyto-sanitaire de la caféiculture à l'échelle du pays.

Cependant, une formation préalable des agents de terrain est nécessaire et le suivi de leurs travaux n'est pas toujours facile. Cet handicap a été compensé par des recoupements dans les informations fournies par les agents. En particulier en ce qui concerne les récoltes.

Les estimations de récoltes permettent de contrôler les différences de productions entre les traitements d'un même essai. Il ne faut cependant pas s'y fier pour comparer plusieurs plantations entre elles lorsque les observations n'ont pas été effectuées par la même personne. En effet, pour un caféier ayant la même production dans deux régions différentes, l'observateur habitant une région apte à la caféiculture aura tendance à donner une cotation plus faible que celui qui est dans une région d'aptitude médiocre.

Note : 100 F Bu = 3,23 F Fr = 0,60 US $

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Carte des principaux secteurs d'observation

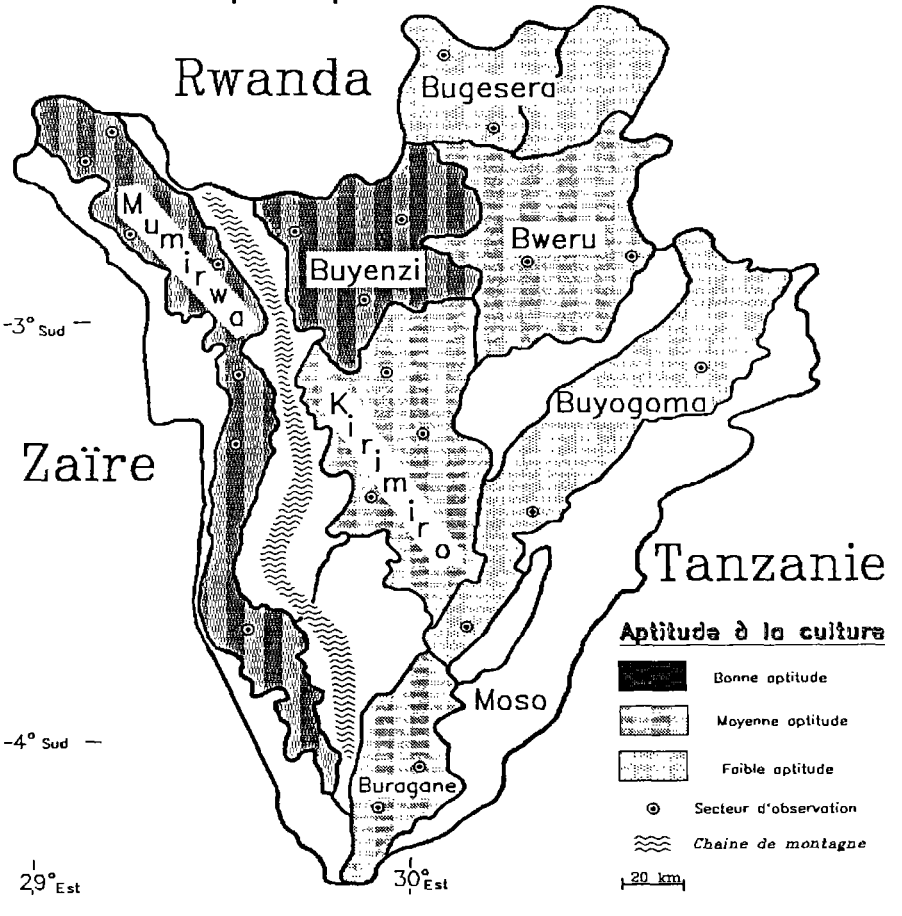

TABLEAU 1 : Essais statistiquement significatifs pour la récolte.

	MABAYI			MUGINA			KANYOSHA - 1			KANYOSHA - 2		
	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée
	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte
Témoin	10,9	605 c	2108 b	-	577	601 b	5,1	202 b	1408 b	4,7 b	285	1505 b
dose 1	12,0	807 a	2867 a	-	736	643 b	7,3	318 a	1978 a	8,3 a	400	1959 a
dose 2	11,3	691 b	2609 a	-	766	965 a	5,2	240 b	1419 b	8,1 a	366	1994 a
F obs	1,4 NS	4,6 *	3,1 *	-	1,2 NS	3,1 *	2,2 NS	5,1 **	4,0 *	5,8 **	2,5 NS	3,2 *
C.V.	18 %	30 %	40 %	- %	54 %	61 %	66 %	47 %	45 %	54 %	44 %	56 %

	BUBANZA			RUGAZI - 1			RUGAZI - 2			RUGAZI - 3		
	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée
	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte
Témoin	11,0	467	1049 b	8,9	518	1124 b	8,2	313	1391 b	7,3	166	1035 b
dose 1	11,2	481	1087 b	8,9	436	1540 a	7,3	246	1791 a	6,9	175	1709 a
dose 2	12,0	564	1489 a	8,1	497	1440 a	7,9	265	1369 b	7,3	148	964 b
F obs	0,3 NS	0,5 NS	3,9 *	0,6 NS	0,5 NS	3,7 *	0,8 NS	0,8 NS	4,1 *	0,3 NS	0,4 NS	7,1 **
C.V.	34 %	58 %	45 %	29 %	58 %	36 %	27 %	63 %	35 %	30 %	51 %	35 %

	BURAMBI			MAKAMBA			RUTANA			KARUZI - 1		
	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée
	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte
Témoin	5,0	80	286 b	11,1	1127 a	2645 a	4,1	339	1050 b	6,2	435	799 a
dose 1	5,5	91	434 a	10,1	960 b	2564 a	4,8	460	1528 a	5,5	332	568 b
dose 2	6,2	100	300 b	8,9	799 c	1882 b	4,5	417	1460 a	6,1	445	882 a
F obs	0,6 NS	0,6 NS	3,4 *	2,3 NS	3,6 *	3,0 *	1,1 NS	1,9 NS	3,1 *	0,7 NS	2,8 NS	3,2 *
C.V.	56 %	65 %	57 %	31 %	39 %	44 %	33 %	48 %	46 %	34 %	40 %	53 %

	KARUZI - 2			MUSONGATI - 1			MUSONGATI - 2			VUMBI - 1		
	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée
	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte
Témoin	4,6	316	680 b	6,8	568 a	1197 a	4,9 b	641 b	1465 b	4,9	366	1605 b
dose 1	5,7	474	1194 a	5,5	360 b	675 b	5,9 a	947 a	2161 a	5,4	453	1861 b
dose 2	5,9	453	1006 a	5,8	394 b	797 b	4,9 b	564 b	1400 b	6,1	513	2269 a
F obs	1,8 NS	1,6 NS	3,4 *	1,7 NS	3,6 *	3,7 *	2,9 *	11,3 **	5,8 **	1,9 NS	1,8 NS	3,1 *
C.V.	40 %	74 %	66 %	36 %	56 %	66 %	28 %	36 %	45 %	36 %	54 %	53 %

	GISURU - 1			GISURU - 2			GISURU - 3			VUMBI - 2		
	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée
	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte
Témoin	7,3 b	693 b	1358 b	5,3 b	559 c	1354 c	9,1	1298	2849 b	3,6	218	935 b
dose 1	8,8 a	951 a	2090 a	5,8 b	696 b	1857 a	10,3	1538	3774 a	4,7	225	1278 a
dose 2	7,5 b	787 b	2014 a	7,1 a	824 a	1566 b	9,9	1429	3390 ab	3,9	161	930 b
F obs	3,4 *	3,1 *	3,1 *	5,5 **	3,8 *	8,1 **	1,2 NS	0,8 NS	3,1 *	2,8 NS	1,8 NS	4,2 *
C.V.	26 %	41 %	39 %	29 %	42 %	33 %	25 %	42 %	38 %	38 %	55 %	39 %

	GASORWE - 1			GASORWE - 2			GASORWE - 3			MUYINGA - 1		
	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée	Estimations		Pesée
	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte	Visuelle	Comptage	récolte
Témoin	3,1	144 b	1483 b	7,0	405	977 b	7,2	535	369 b	8,4	925	3071 a
dose 1	4,0	249 a	2248 a	7,9	543	1354 a	8,6	727	661 a	7,9	732	2524 ab
dose 2	3,3	210 a	2210 a	6,5	401	918 b	8,0	634	462 b	7,2	568	1913 bc
F obs	1,5 NS	4,9 *	5,1 **	1,4 NS	2,9 NS	3,9 *	1,7 NS	2,1 NS	4,8 *	1,1 NS	2,8 NS	3,3 *
C.V.	48 %	53 %	58 %	38 %	45 %	47 %	29 %	46 %	44 %	34 %	64 %	56 %

	MUYINGA - 2		
	Estimations		Pesée
	Visuelle	Comptage	récolte
Témoin	5,8	383 c	1316 b
dose 1	6,7	480 b	1460 b
dose 2	7,2	582 a	1806 a
F obs	2,3 NS	4,9 *	3,3 *
C.V.	33 %	42 %	40 %

Résumé

Des essais multilocaux ont été installés dans plusieurs régions du Burundi. Ces essais ont pour but de tester diverses formules d'engrais chez des planteurs de caféiers.

Les secteurs d'observations ont été choisis dans les régions les plus représentatives du BURUNDI sur base des climats et des cartes pédologiques existantes.

Dix plantations jeunes et bien entretenues ont été choisies dans chaque secteur. Chacune d'entre elles constitue un essai dans lequel des caféiers recevant une dose simple ou double d'engrais sont comparés à des caféiers témoins. Les formules et doses ont été calculées à l'aide d'un programme informatisé sur base des analyses de sols. Le dispositif statistique adopté est la randomisation totale d'un caféier par parcelle élémentaire en vingt répétitions.

Les observations portent sur la résistance aux aléas et sur les productions. Pour limiter les risques d'erreurs, des recoupements entre observations ont été prévus. De même, des estimations de récoltes sont effectuées anvant la cueillette des cerises.

On observe que la réponse aux engrais apparait dès la deuxième année après la mise en place des essais dans plus de 25 % des essais.

Summary

Multilocal trials have been installed in various regions in Burundi in order to compare different fertilizer formulae. Those trials are conducted in coffee plantations owned by small coffee growers.

The observation sectors were chosen in the most representative coffee regions of the country. The choice was based on climatic and pedological maps.

In each sector, 10 coffee plantations having young trees and well maintained were selected. Each plantation consists of a trial in which trees receiving a single or a double dose of fertilizer are compared to trees not fertilized. Formulae and doses of fertilizer were calculated by means of the computerized program based on soil analyses.

The statistical design is complete randomized blocks of single tree per plot and 20 replications.

Observations are done on resistance to pests, diseases, deficiencies in elements and yields. Controls are done in order to minimize errors that could occur during observations. Estimation of yields are done before harvesting.

A response to fertilization could be observed after only 2 years of fertilizer application on more than 25 % of the trials.

MISE EN PLACE ET ÉVALUATION DE LA MÉTHODE DU DIAGNOSTIC SOL DANS UN BASSIN CAFÉIER DU MEXIQUE

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En 1989, à la conférence ASIC de Païpa, SALLEE et al. nous avaient présenté le projet de recherche développement mis en place avec le Ministère de l'Agriculture (la *SARH*), dans le bassin caféier Xalapa - Coatepec de la région centre de l'Etat de Veracruz.

Parmi les thèmes de recherche agronomique, avait été diagnostiquée l'amélioration du système de production traditionnel jusqu'à son point d'équilibre économique, la structure de ces caféières ne permettant pas de rentabiliser une conduite intensive.

Depuis trois ans, un "paquet" technique cohérent s'est défini pour augmenter la rentabilité du système traditionnel sans alourdir le coût de production. Il s'agit:

- d'adopter une politique de taille cyclique,
- d'aménager l'ombrage,
- d'améliorer la fertilisation en quantité, qualité, fréquence et mode d'application,
- de replanter les manquants.

En ce qui concerne la fertilisation, le projet de recherche s'est attaché à mettre en place un système de fertilisation raisonnée sur la base d'un diagnostic sol, méthode qui fait l'objet d'un consensus actuellement en caféiculture (Carvajal, 1984, Snoeck et al., 1988 et 1989, Valencia et al., 1989).

Nous vous présentons, ici, cette démarche de recherche-développement.

PROBLEMATIQUE DE LA FERTILISATION DANS LA REGION

1. Au plan technique, la vulgarisation agricole recommande l'application, 2 fois par an, de 250 grammes de NPK 18-12-06 par caféier quel que soit le type de sol ou la structure de la caféière. Cette généralisation est en contradiction avec la grande variabilité des

sols mis en évidence par Rossignol et al. en 1987: andosols, sols ferralitiques, brunizems, sols vertiques, lithosols, rendzines, etc. La formule employée, avec sa forte proportion de phosphore, est en désaccord avec les résultats de recherche d'autres pays caféiculteurs. Enfin le système de dose par pied est incompatible avec une gestion en unités fertilisantes par hectare, surtout pour les fortes densités de plantation.

2. Deuxièmement, la dépense en fertilisants représente la majeure partie du coût de production hors récolte et dans la majorité des cas, c'est le plus gros besoin de trésorerie au cours du cycle agricole (cf. Pasquis et al. à ce colloque).

PREMIERE APPROCHE: REALISATION D'UNE ENQUETE DIAGNOSTIC REGIONALE

La méthode de recommandation sur diagnostic sol s'est donc imposée. Il s'agissait de la mettre en place dans une optique de développement.

Dans une première phase, une enquête diagnostic a été réalisée (Rouzet et al., 1990).

1. Cette étude a obligé à la création d'un logiciel de recommandations de fertilisation en espagnol, variante du programme de Snoeck et al., adapté aux conditions de la région.

2. Elle a permis une meilleure connaissance des problèmes majeurs des sols à vocation caféière:

   - acidification et désaturation de la zone fertilisée comme il est possible de l'apprécier sur la figure 1, où sont comparées les distributions des fréquences de pH dans et hors du rond de fertilisation,
   - déficience généralisée en potasse, clairement visible sur la figure 2 qui présente la courbe des teneurs en potasse en fonction de la texture, sur le fond des courbes théoriques de Forestier (1964).

3. Il a aussi été observé que la formule 18-12-06, n'est valable que dans très peu de cas; la majorité des recommandations n'incluent pas de phosphore et les niveaux de potassium entraînent des équilibres N/K de 1/1, 1/2 jusqu'à 1/3.

4. Les niveaux de fertilisation ont été mesurés; en moyenne les producteurs appliquent NPK 200-75-55 en unités par hectare et par an. Le nombre d'unités d'azote est trop élevé pour les conditions de rendement (en moyenne 800 kg de café marchand/ha/an), d'ombrage et de densité de plantation (en moyenne 1600 caféiers/ha),

5. La remise des recommandations aux producteurs, sans suivi rapproché, a permis une première caractérisation des facteurs limitants du développement de la méthodologie:

   - pas ou peu de disponibilité en engrais simples sur le marché local, pour réaliser les mélanges recommandés, surtout pour la potasse et le magnésium,
   - pas de trésorerie au moment de l'achat des intrants et de grandes difficultés pour obtenir des crédits bancaires de campagne,
   - pas d'assistance technique valable pour induire le changement de technologie,
   - une certaine méfiance vis à vis des recommandations.

Figure 1 : FREQUENCE COMPAREE DES pH DANS L'INTERLIGNE ET LE ROND DE FERTILISATION.

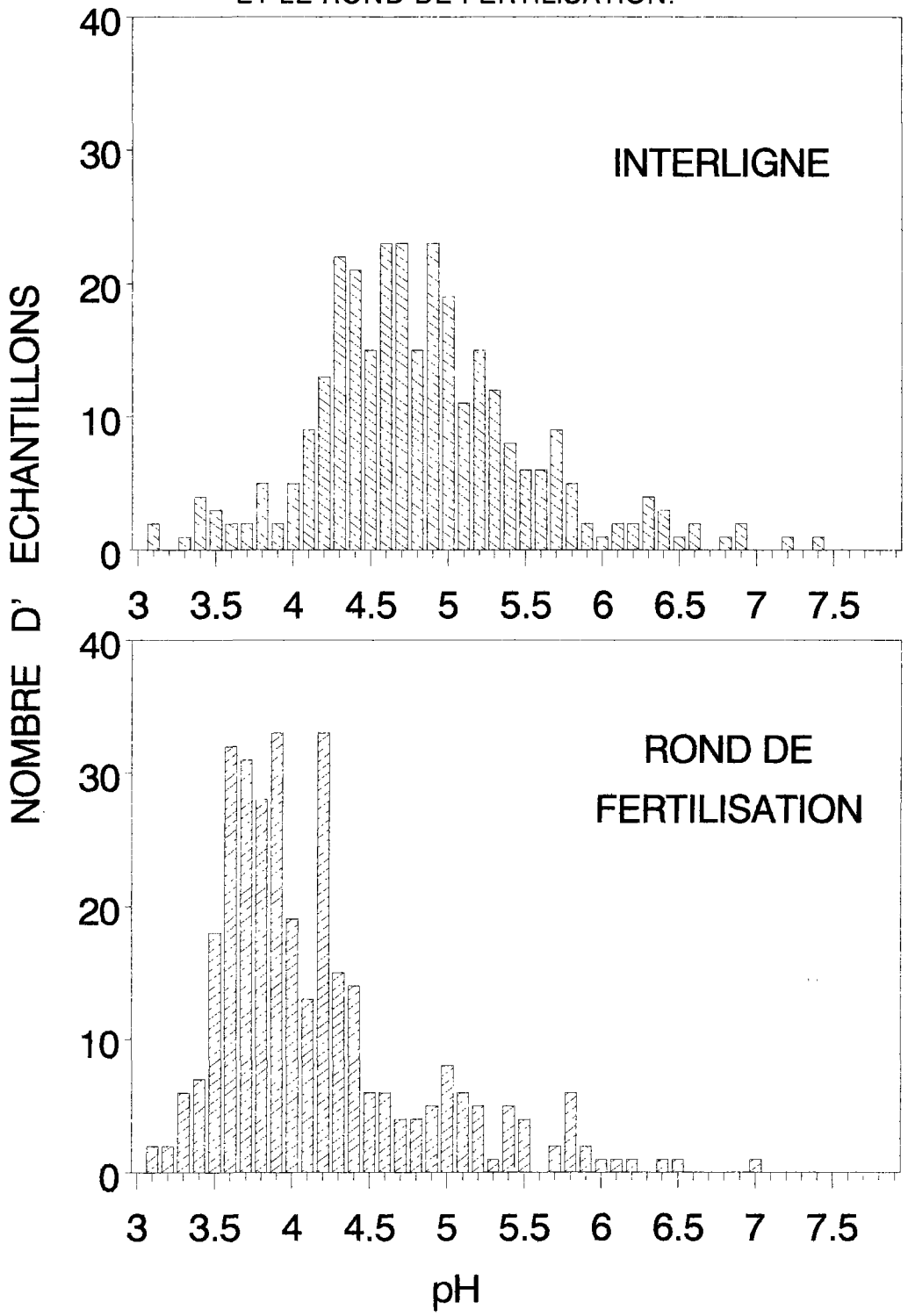
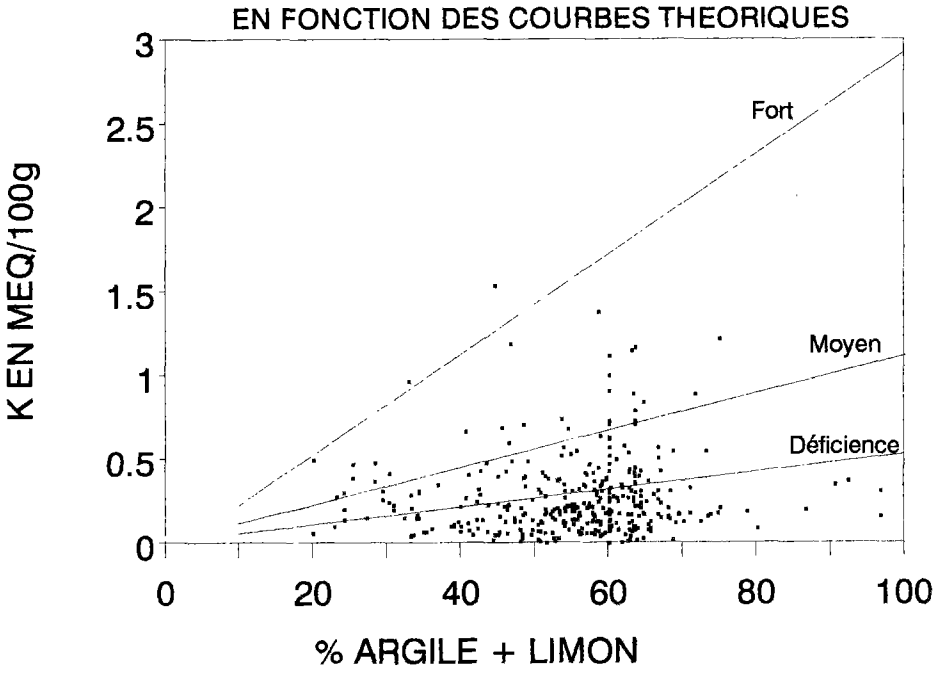


Figure 2 : POTASSIUM ECHANGEABLE DANS 365 ANALYSES EN FONCTION DES COURBES THEORIQUES



6. D'autre part l'étude des facteurs explicatifs du rendement a permis de mettre en évidence que les facteurs écologiques (sol, climat) et agronomiques (densité de plantation, intensité de l'ombrage, etc.) ont autant d'influence que la composition chimique des sols. Ce point confirme la nécessité d'adopter un ensemble cohérent d'améliorations simultanées dans les parcelles traditionnelles.

7. Enfin, au niveau scientifique, ont été soulevés les problèmes de la validité , premièrement, des recommandations de chaulage, conséquence logique des niveaux de pH rencontrés dans les ronds de fertilisation et, deuxièmement, du protocole de prise d'échantillons servant au diagnostic sol.

DEUXIEME PHASE : GENERALISATION DE LA METHODE

En fonction des premiers résultats, la méthode a été étendue à une organisation paysanne de petits producteurs, la ROCA[1], organisée en coopérative exportatrice de café. Les associés se sont portés volontaires pour tester l'innovation en grandeur réelle, sur les bases suivantes.

Pour le crédit, très difficile à obtenir de la part des banques en ces temps de restructuration de l'économie mexicaine, la même coopérative a prêté, sur fonds propres, à ses associés le montant individualisé et par parcelles calculé sur la base des recommandations.

Pour la disponibilité des engrais, des accords ont été pris avec FERTIMEX[2], entreprise para-publique de distribution des engrais qui a accepté de jouer le jeu de l'innovation en attendant la création par la coopérative d'une centrale d'achats d'intrants.

1. Red de Organizaciones Cafetaleras Autogestivas, réseau de groupements de caféiculteurs de la région Xalapa - Coatepec
2. Fertilizantes de Mexico

Pour l'assistance technique, impossible à attendre des institutions, dans le courant de désengagement actuel, une participation modeste des agriculteurs a été demandée qui a permis d'engager deux agronomes indépendants chargés des analyses de sol, des calculs de crédits individuels, de l'organisation des achats de fertilisants, de la formation, etc.

Pour valider la méthode, cinq parcelles démonstratives ont été mises en place chez des producteurs volontaires dans les cinq communautés touchées par le programme. Ces essais extrêmement simples sont constitués de deux parcelles comparant les recommandations issues du diagnostic sol à la fertilisation traditionnelle à base de 18-12-06.

Pour évaluer la cohérence technico-économique des recommandations de chaulage, 19 essais ont été mis en place, comparant, en deux parcelles, les recommandations issues du diagnostic sol, avec et sans chaulage.

Enfin, dans la mesure du possible, les agriculteurs volontaires ont souscrit à l'ensemble du "paquet" technique proposé, grâce à l'appui des agronomes à leur service.

RESULTATS DE LA GENERALISATION

La généralisation de la méthode a permis d'obtenir des résultats très concrets.

1. Les essais ont très vite rempli leur rôle démonstratif, bien que les chiffres comparatifs de récolte ne soient pas disponibles avant plusieurs années; ce résultat a surpris les techniciens habitués à l'inertie des plantes pérennes. Trois mois après la première fertilisation, les différences d'aspect végétatif entre parcelles traditionnelle et diagnostic sol étaient surprenantes, en faveur du diagnostic sol.

2. Au niveau technique, le programme de recommandations a été couplé à une échelle d'unités d'azote de 60 à 300 unités/ha/an. Le nombre d'unités est déterminé en fonction d'une enquête agro-écologique. Les critères pris en compte sont: les facteurs limitants écologiques, la densité de tiges par hectare, l'ombrage, le rendement et la stratégie du producteur. Les quantités de phosphore et de potasse restent déterminées par le calcul des corrections plus les exportations.

3. Au niveau économique, sur les 320 hectares où s'est appliquée la méthode, le coût global a été réduit de 36% par rapport à la fertilisation traditionnelle: 77 US $/ha/an contre 120 US $, soit un gain total au niveau de l'organisation de 13.760 US $ qui a permis l'acceptation du paiement direct de l'assistance technique par les paysans.

4. Au niveau technologique, dans les usines de traitement du café de l'organisation, les responsables notent une différence claire entre le café des associés qui suivent le programme de fertilisation et le café acheté à l'extérieur. La différence porte sur les cerises noires, sèches, dûes à ce que les kenyans appellent "overbearing die-back", dont le symptôme est le dessèchement prématuré des rameaux plagiotropes qui portent beaucoup de fruits. Cette observation, empirique, a été faite dans les cinq usines de l'organisation ROCA et devra être confirmée statistiquement dans les essais; cependant la conviction des usiniers a été si forte qu'il a été question de fermer les ventes aux non-associés.

5. Au niveau méthodologique, il a été possible d'observer que les trois problèmes amont, crédit, engrais et assistance technique, une fois levés, la méthode de fertilisation sur diagnostic sol est unanimement

adoptée et acceptée avec enthousiasme. C'est une porte d'entrée excellente pour la reproduction d'un projet de développement avec d'autres organisations de caféiculteurs.

6. Enfin, il s'est avéré que le problème d'un mélange particulier à chaque parcelle, réalisé par le producteur lui-même, n'en est pas un dans nos conditions. La recherche qui avait tendance à la régionalisation d'une formule et sa réalisation par les usines d'engrais, sous estimait la capacité des producteurs.

CONCLUSION

La fertilisation du caféier sur la base du diagnostic sol, telle qu'elle a été proposée par Snoeck et al. est une méthode bien adaptée à l'amélioration des caféières traditionnelles du bassin Xalapa - Coatepec.

Mais il a fallu compléter le programme en fonction des conditions écologiques, pédologiques, agronomiques et économiques, locales. Dans notre cas c'est donc un outil de recherche qu'il est nécessaire de faire passer par une phase de recherche-développement.

Les trois problèmes les plus limitants à la mise en place d'une fertilisation raisonnée sont:

1. l'absence de trésorerie et de crédit au moment de l'achat des intrants,
2. la non disponibilité des engrais simples sur le marché local,
3. et l'absence d'assistance technique valide pour entreprendre et suivre une innovation technique.

De plus, cette opération de recherche-développement a joué son rôle d'interface avec la recherche puisqu'elle pose les problèmes de l'efficacité des chaulages recommandés, de la toxicité de l'aluminium dans les andosols ou du protocole d'échantillonage.

Enfin il est intéressant de noter l'enthousiasme des caféiculteurs pour la méthode de diagnostic sol qui est le résultat le plus spectaculaire de l'opération.

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RESUME

MISE EN PLACE ET EVALUATION DE LA METHODE DU DIAGNOSTIC SOL DANS UN BASSIN CAFEIER DU MEXIQUE

Dans le cadre du projet DIMAC, Développement Intégré dans les Marges de l'Aire Caféière Xalapa-Coatepec, présenté en 1989 à l'ASIC Paipa, a été mis en place la méthode de diagnostic sol pour la fertilisation du café. Il s'agit d'optimiser la dépense de fertilisants qui représente la majeure partie du coût de production, hors récolte.

Une première phase d'étude nous a permis de caractériser les tendances des sols de la région et d'analyser les problèmes relatifs à la méthode de diagnostic sol: la non-disponibilité des fertilisants simples (K et Mg), la difficulté d'obtention du crédit au moment des achats d'intrants, l'absence de suivi technique, et enfin la méfiance des producteurs vis à vis de la méthode non développée sur place.

Sur ces bases et grâce à un réseau de 5 parcelles d'essai/démonstration, comparant la fertilisation traditionnelle (500g de NPK 18-12-6 par arbre et par an) aux recommandations issues du diagnostic sol, il a été possible d'étendre la méthode à environ 300 hectares de caféières en 1990. Le coût global a été réduit de 36% par rapport à la fertilisation traditionnelle. Les essais, pour lesquels les chiffres comparatifs de récolte ne seront pas disponibles avant plusieurs années, ont déjà remplis leur rôle démonstratif; les différences de croissance des caféiers sont parfois surprenantes et de plus il a été noté une amélioration de la qualité des cerises, en faveur du diagnostic sol.

RESUMEN

IMPLEMENTACION Y EVALUACION DEL METODO DE DIAGNOSTICO DE SUELO EN UNA CUENCA CAFETALERA MEXICANA

En el cuadro del proyecto DIMAC, Desarrollo Integrado en las Margenes del Area Cafetalera Xalapa-Coatepec, presentado en 1989 en el ASIC Paipa, se implementó el método de diagnóstico de suelo para la fertilización

del café. Se quiere optimizar el gasto de fertilizantes que representa la mayor parte del costo de producción, fuera del corte.

Una primera etapa nos permitió, caracterizar las tendencias de los suelos de la región y analizar los problemas relativos al desarrollo de la metodología: la indisponibilidad de los fertilizantes simples (K y Mg), las dificuldades de obtención del crédito al momento de la compra de los insumos, la falta de seguimiento técnico y el recelo de los productores en una metodología no desarrollada localmente.

Sobre estas bases y gracias a una red de 5 parcelas de ensayo/demostración, comparando la fertilización tradicional (500g de NPK 18-12-06 por cafeto y por año) a las recomendaciones del diagnóstico de suelo, haya sido posible extender el método a 300 hectáreas en 1990. Se ahorró 36% del costo total, en comparación con la fertilización tradicional. Los ensayos, cuyos resultados comparativos de producción no estarán listos antes de unos años, ya desempeñaron su papel demostrativo; las diferencias de crecimiento de los cafetos son sorprendentes y además se notó un mejoramiento en la calidad de las cerezas, a favor del diagnóstico de suelo.

SUMMARY

IMPLEMENTATION AND EVALUATION OF THE SOIL DIAGNOSIS METHOD IN A MEXICAN COFFEE AREA

As part of the DIMAC project (Integral Development in the Margins of Xalapa - Coatepec coffee area), presented in 1989 in ASIC, has been settled the soil diagnosis method for coffee fertilization. The objective is optimizing the fertilizer despense which represents the greatest part of coffee producing cost without pruning.

The first stage permitted a characterization of general soils tendencies in the area, and an analysis of the problems on implementing the method with productors: unavailability of fertilizers (potash and magnesium), imposibilities of credit to buy them, lack of technical help and distrust of a foreign method.

Upon these basis and with the help of 5 demonstrative trials, comparing traditional fertilization (500 g of NPK 18-12-06 per tree per year), it has been possible to extend the soil diagnosis method on 300 hectares. The global save has been of 36%, compared with traditional one. The trials, which statistics results will not be ready until several years, have already achieved his demonstrative end; growth differences between the two treatments are surprising and the quality of cherries showed to be better in the soil diagnosis treatment.

L'ANTHRACNOSE DES BAIES DU CAFÉIER ARABICA (CBD, COFFEE BERRY DISEASE) AU CAMEROUN : ÉPIDÉMIOLOGIE ET LUTTE CHIMIQUE

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INTRODUCTION:

Cultivé au Cameroun sur les hauts plateaux de l'Ouest sur environ 150 000 ha, le caféier arabica (*Coffea arabica* L.) représente pour ce pays une source potentielle de revenu non négligeable (production annuelle moyenne de 20 000 tonnes de café marchand). Toutefois, la diminution importante du prix d'achat au planteur et la transformation des systèmes de culture, caractérisée par une diversification de plus en plus marquée vers les cultures vivrières de rente et parfois le maraîchage, dans le cadre d'une association culturale qui s'effectue au détriment du café, constitue actuellement un grand handicap (Losch et *al.*, 1990).

De plus, 40% de cette caféière, soit environ 60 000 ha, est située au dessus de 1500 m, à des altitudes très favorables à une maladie des fruits, l'anthracnose des baies, appelée "Coffee Berry Disease" (C.B.D.). Les dégâts dus à cette maladie sont d'autant plus importants que cette caféière de haute altitude est constituée presque exclusivement de variétés locales très sensibles (var. Jamaïque,...).

Cette maladie, apparue au Cameroun qu'en 1958 (Muller, 1964), toujours localisée actuellement au seul continent africain, peut détruire jusqu'à 80% de la récolte selon les années et les lieux.

C'est à une souche particulière de *Colletotrichum coffeanum*, au pouvoir pathogène bien défini, rencontrée essentiellement sur fruit, qu'est attribué le C.B.D. (Mc Donald, 1926; Rayner, 1952; Hindorf, 1972; Muller, 1978). Pour cette souche, la dénomination "*Colletotrichum coffeanum* Noack *sensu* Hindorf" peut être adoptée (Muller, 1978).

C'est sur fruits verts, où un diagnostic sûr peut être donné, que cette maladie présente un faciès typique, notamment sous sa forme "active" caractérisée par des lésions déprimées, de couleur brun foncé, montrant de nombreuses acervules libérant de grandes quantités de spores. Ces lésions entraînent une pourriture humide de la pulpe et des grains, pouvant provoquer rapidement une destruction totale des fruits qui se présentent, au stade ultime, sous la forme de petits sacs noirs, encore accrochés aux rameaux, s'écrasant facilement sous les doigts. Les rameaux et les feuilles restent macroscopiquement sains, ce qui apparaît comme une caractéristique de cette maladie.

Il apparaît vraisemblable que c'est essentiellement à partir des baies malades issues d'une campagne précédente, et non récoltées, que la maladie se développe (Muller, 1980). A partir des nouveaux fruits atteints, sources efficaces de contaminations secondaires, ce sont notamment les fortes pluies qui assurent la dispersion des spores (éclaboussures et ruissellements). Au cours d'une même campagne, la présence de baies issues de floraisons isolées pourraient ainsi servir de sources permanentes d'inoculum.

Au Cameroun, la présence de nombreux génotypes sauvages éthiopiens, issus de la prospection ORSTOM-IRCC (1966), représente une source de résistance, au potentiel important, qui est en cours d'exploitation. Toutefois, c'est la lutte chimique qui, bien que contraignante et coûteuse, est toujours largement utilisée. Le calendrier de traitement employé (5 ou 7 applications à partir de la 2ème semaine selon la nature des produits fongicides) permet ainsi de protéger les fruits jusqu'à la 22ème semaine environ.

Ainsi, chaque année, nous mettons en place des essais destinés à comparer au champ l'efficacité fongicide, vis à vis du C.B.D., de molécules proposées par l'industrie phytosanitaire. La recherche des meilleurs paramètres, permettant de tester ces molécules, nous a amené à suivre et à analyser les évolutions des populations de baies saines et malades sur des parcelles témoins et traitées. Quelques résultats obtenus en 1990 sont exposés dans cette communication.

MATERIELS ET METHODES:

Matériel végétal:

Une exploitation familiale d'environ 1200 caféiers arabica (*Coffea arabica* L.), située à 1800 m d'altitude, dans la localité de Santa (CAMEROUN-Province du Nord-Ouest), a été choisie pour l'intensité très élevée de la maladie observée pendant trois années successives. La pluviométrie de cette zone, de l'ordre de 2500 mm par an, est répartitie essentiellement sur 6 mois, d'avril à octobre. Cette plantation est constituée presque exclusivement de caféiers d'origines locales, comme la variété Jamaïque, très sensibles au C.B.D..

Dispositif expérimental et observations:

Dans cet essai, 11 objets ont été mis en comparaison, 10 objets recevant un traitement fongicide et 1 objet étant un témoin non traité. La randomisation totale arbre par arbre, avec 100 répétitions par objet, a été utilisée comme dispositif expérimental.

Sur chacun des 1100 caféiers en observation, 5 branches ont été tirées au hasard sur 5 étages, toutes directions confondues, et marquées pour les comptages.

Sur chacune des branches marquées, des comptages hebdomadaires des nombres de baies saines et malades ont été réalisés de la 8ème semaine à la 24ème semaine après la floraison.

Traitements fongicides:

9 fongicides ont été comparés au Dacobre 500, produit de référence, et à un témoin non traité (TNT). La liste des fongicides mis en essai est reportée Tab.1. Les traitements ont été réalisés à l'aide de pulvérisateurs à dos à pression entretenue. Un volume moyen de 0,8 l de bouillie fongicide, par caféier à chaque traitement, a été pulvérisé.

Les traitements ont débuté 2 semaines après la floraison principale qui a eu lieu les 11/12 avril. Les 3 premiers traitements ont été séparés de 4 semaines; ce délai a été ensuite réduit à 3 semaines pour le 4ième traitement et à 2 semaines entre les derniers traitements. Selon les fongicides utilisés (5 ou 7 applications), ce calendrier de traitement permet la protection des baies jusqu'à la 20ème-21ème semaine après la floraison.

Analyse des résultats:

Afin de comparer l'efficacité des différentes formulations testées, nous avons adopté le pourcentage de perte de baies calculé selon la formule ci-dessous:

$$\% \text{ pertes} = \frac{BT1 - BT17}{BT1} \times 100$$

où: BT1 = Baies totales au 1er comptage;
BT17 = Baies totales au 17ème comptage.

Tab.1: Liste des fongicides mis en essai, matières actives, pourcentages de produit commercial utilisés (% P.C.) et nombre de traitements.

FONGICIDES	MATIERES ACTIVES	% P.C.	NB DE TRAITEMENTS
Dacobre 500 (DAC-référence)	chlorothalonil oxychlorure de cuivre	0,4	7
Octave (OCT)	prochloraz manganèse oxychlorure de cuivre	0,4	5
Kocide 500 (KOC)	chlorothalonil hydroxyde de cuivre	0,4	5
Arabicobre (ARA)	chlorothalonil oxychlorure de cuivre	0,4	5
Exp 02314 A (EXP)	/	0,33	5
Nordonil (NOR)	chlorothalonil oxyde de cuivre	0,4	5
Nordyrène (NYR)	anilazine oxyde de cuivre	0,27	5
Anilox 350 (ANI)	anilazine hydroxyde de cuivre	0,5	5
Dacobre WDG (WDG)	chlorothalonil oxychlorure de cuivre	0,4	5
Pennfluid (PEN)	mancozèbe	0,75	5

RESULTATS ET DISCUSSION:

Comparaison de l'efficacité de diverses formulations fongicides:

L'analyse de variance du pourcentage de perte de baies après transformation des données (tab.3), montre des différences significatives entre les traitements. A l'aide du test de comparaison de moyenne de NEWMAN-KEULS, on différencie 5 groupes (tab.4).

Le produit avec lequel les chutes de baies ont été les plus élevées (PEN) n'apparaît pas significativement différent du témoin non traité (TNT); le produit ayant permis de récolter le plus grand nombre de baies (EXP) apparaît significativement différent de tous les autres. Grâce à ce dernier produit, les chutes ont été réduites de 50% par rapport au témoin non traité (tab.2). A l'aide du pourcentage de perte de baies, une excellente séparation des différents traitements a donc été obtenue.

Si entre le témoin et le traitement le plus efficace, aucune différence significative du nombre de baies au 1er comptage (8ème semaine aprés la floraison) n'est observable, par contre, pour notamment 2 traitements (OCT et KOC), environ 15 % de baies de plus sont enregistrés par rapport au témoin qui comptait un nombre de baies comparable au traitement "EXP 02314 A" (28 000 Baies sur TNT et EXP contre 32 000 baies sur OCT et KOC).

Toutefois, en prenant le nombre de baies de départ comme covariable, nous n'avons pas obtenu de différences notoires, dans le classement des traitements selon leur efficacité, par rapport à l'analyse précédente.

Lors de la 1ère observation (8 semaines après la floraison), 2 applications avaient déjà été réalisées sur l'ensemble des arbres traités. Un éventuel effet des traitements pourrait donc être envisagé pour expliquer cette différence de nombre de baies, qu'un essai en randomisation totale arbre par arbre, avec un nombre élevé de répétitions et de baies, rend statistiquement peu probable. L'analyse de ce nombre de baies à la 1ère observation, n'a pas permis d'obtenir un classement des traitements comparable à celui de leur efficacité calculée avec les pourcentages de pertes.

Le seul dénombrement instantané des populations de baies ne permet pas de distinguer les pertes dues à l'anthracnose d'éventuelles pertes dues à d'autres causes (régulation physiologique, etc...). Le simple comptage hebdomadaire du nombre de baies saines et malades apparait insuffisant pour caractériser précisément l'action des fongicides.

La comparaison de l'évolution du nombre de baies tombées et de baies malades, sur les arbres témoins et traités (fig.1 et 2), met en évidence la nécessité d'affiner nos observations.

Evolution de l'infection sur les arbres témoins:

Sur l'ensemble des 500 rameaux mis en observation sur les arbres témoins, pour un effectif initial de 28 000 baies environ, nous avons obtenu un pourcentage élevé de chute de l'ordre de 82 % (tab.2). Ces pertes importantes sont comparables à celles que nous avions obtenues sur le même site l'année précédente, en 1989.

L'examen de l'évolution des pertes au cours du temps (fig.1) montre que dès le début des observations, des chutes de baies sont observables. Les pertes les plus importantes se situent entre la 4ème semaine et la 12ème semaine d'observation (soit entre la 12ème semaine et la 20ème semaine après la floraison); ensuite, celles-ci sont de moindre importance.

L'étude de l'évolution du nombre de baies malades montre qu'un petit nombre de baies atteintes est visible dès la 2ème semaine d'observation (10ème semaine après la floraison). C'est pendant les 6 semaines suivantes que ce nombre de baies malades va augmenter pour atteindre un maximun à la 8ème semaine d'observation (16ème semaine après la floraison); ensuite, ce nombre va rester relativement constant.

A partir de la seconde moitié de la période d'observation (baies agées d'environ 4 mois), il est vraisemblable que les chutes sont dues majoritairement à des baies malades; seul le développement de nouvelles lésions peut expliquer la relative stabilité du nombre de baies malades observées à chaque comptage.

Tab.2: Moyenne des pourcentages de perte de baies par objet. (calculs sur des effectifs de 25.10^3à 32.10^3baies).

Objets	Pertes
Pennfluid (PEN)	82.63
Témoin non traité (TNT)	81.75
Dacobre 500 WP (DAC)	75.37
Kocide 500 (KOC)	74.93
Nordonil (NOR)	74.79
Octave (OCT)	66.22
Arabicobre (ARA)	65.90
Dacobre WDG (WDG)	65.44
Anilox 350 (ANI)	58.16
Nordyrène (NYR)	53.32
Exp 02314 A (EXP)	41.06

Tab.3: Analyse de variance du pourcentage de perte de baies après tranformation des données en Arcsinus (P=0,05).

	DDL	S.C.E.	CARRES MOYENS	F	PROB
TOTALE	1093	207482.53	189.83		
TRAITEMENTS	10	67017.20	6701.72	51.67	0.0000
RESIDUELLE	1083	140465.33	129.70		

C.V.= 20.2%

Tab.4: Comparaison de l'efficacité des produits mis en essai. (ordre croissant de gauche à droite; les produits présents sous une même barre ne sont pas différents significativement entre eux). (NEWMAN-KEULS; P=0,05).

PEN	TEM	DAC	KOC	NOR	OCT	ARA	WDG	ANI	NYR	EXP
A										
		B								
					C					
									D	
										E

Il est interessant de noter qu'au cours des semaines d'observation 1 à 7, le nombre de baies tombées apparaît toujours supérieur au nombre de baies malades observées les semaines précédentes. Nos observations ne permettant pas de distinguer les chutes dues à la maladie, d'éventuelles chutes physiologiques, il ne nous est pas possible de donner une signification précise à ces pertes enregistrées lors des premières semaines d'observation.

Evolution de l'infection sur les arbres traités à l'"Exp 02314 A":

Afin de caractériser l'effet d'applications de substances fongicides sur l'évolution de l'infection, nous examinerons le produit ayant montré la meilleure efficacité pour la réduction du nombre de baies tombées. Il s'agit du traitement, en 5 applications, à l'aide de l'"EXP 02314 A" qui a réduit la chute des baies d'environ 50% (tab.2) par rapport au témoin non traité.

Le calendrier d'intervention pour la protection chimique contre l'anthracnose des baies a été défini à partir des données obtenues lors de travaux antérieurs (Muller, 1978). C'est ainsi que pour notre essai, le premier des 5 traitements ayant été effectué 2 semaines après la floraison principale, les arbres avaient déjà reçu 2 applications lors de la première observation ($8^{ème}$ semaine après la floraison). Les 3 autres applications ont été réalisées durant les semaines d'observation 3, 7 et 10.

Pour ces arbres traités, l'évolution des pertes au cours du temps (fig.2) présente une différence importante avec celle obtenue sur le témoin (fig.1). Les chutes enregistrées sur les arbres de ce traitement ont été régulières, sans présenter de pic comme nous l'avions enregistré sur les arbres témoins entre la $4^{ème}$ et la $11^{ème}$ semaine d'observation. Une légère diminution des pertes est enregistrée à la fin de ces observations.

L'évolution de la population de baies malades des arbres traités (fig.2) présente une augmentation régulière, de la $2^{ème}$ jusqu'à la $10^{ème}$ semaine d'observation, pour ensuite diminuer progressivement jusqu'au dernier comptage, au cours duquel un peu plus de 2000 baies malades ont été dénombrées. Cette régularité de l'évolution du nombre de baies malades enregistrée sur les arbres traités, en comparaison avec les arbres témoins, traduit vraisemblablement un ralentissement du développement de la maladie par le traitement (arrêt de l'apparition de nouvelles baies malades).

Le traitement à l'aide de ce produit a eu pour effet de réduire fortement les chutes de baies. La majeure partie de cette diminution des pertes s'est produite lors des premières semaines d'observation, lorsque le nombre de baies malades observées sur le témoin était le plus faible.

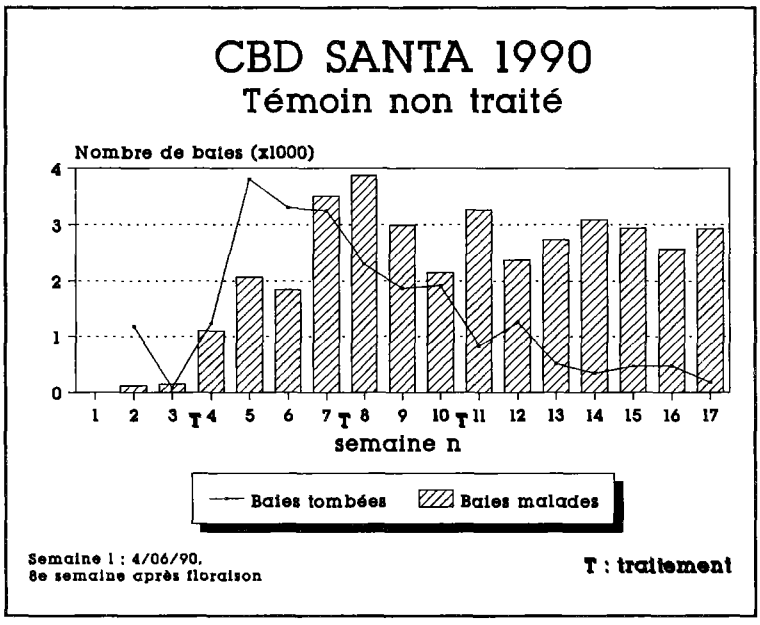

Fig.1: Evolution du nombre de baies tombées et de baies malades sur les arbres témoins.

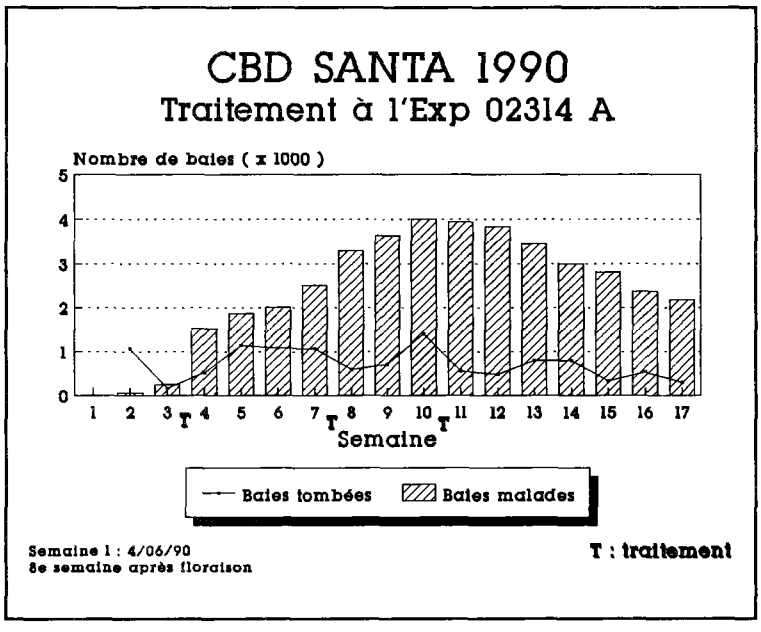

Fig.2: Evolution du nombre de baies tombées et de baies malades sur les arbres traités avec l"EXP 02314 A".

Si une action de ce produit sur la physiologie de la plante, permettant une diminution d'éventuelles chutes physiologiques, n'est pas à exclure, toutefois, il apparaît probable que, lors de nos observations, nous n'avons pas été en mesure de relever la totalité des baies malades. Ceci pourrait s'expliquer par un délai de chute de ces baies inférieur à une semaine et/ou des attaques discrètes difficilement observables, comme par exemple au niveau du pédoncule des fruits.

CONCLUSIONS:

La disparition des grandes exploitations ne nous permettant pas de trouver des champs productifs suffisamment vastes, nous sommes régulièrement contraints de mettre en place des essais de comparaison d'efficacité de formulations fongicides sur de petites surfaces en milieu paysan. Dans ce cas, la méthode d'expérimentation en **randomisation totale arbre par arbre**, qui nous a permis de mettre en comparaison 11 traitements avec 100 répétitions par traitement (1100 caféiers en expérimentation) dans une plantation paysanne d'environ un hectare, apparaît satisfaisante.

Avec le pourcentage de perte de baies, calculé à partir du nombre total de fruits enregistrés entre le premier et le dernier comptage, nous avons obtenu une bonne séparation statistique des différents produits mis en comparaison. Pour mesurer l'efficacité de traitements phytosanitaires, la prise en compte d'autres critères comme le poids final de récolte ou le pourcentage maximum de baies malades est envisageable. La recherche de la méthode la plus discriminante reste à entreprendre.

Un nombre très élevé de pertes est toujours enregistré dans ces essais, notamment sur les arbres témoins. Plus particulièrement sur des baies agées de 2 à 3 mois 1/2, se produisent des chutes dont le nombre est toujours supérieur à celui des baies malades observées lors des semaines précédentes.

En plus des chutes dues à la forte pression infectieuse, il est possible que des chutes physiologiques soient également comptabilisées dans les pertes, nos observations ne permettant pas de les distinguer des pertes dues à la maladie. Une sous estimation du nombre de baies malades enregistrées, due à des symptômes difficilement observables, comme par exemple des attaques discrètes au niveau du pédoncule des fruits, ou à un délai de chute inférieur à 1 semaine, pourrait être à l'origine de ces pertes non explicables par nos observations.

Afin de tenter de comprendre ces chutes non expliquables par nos observations actuelles, et, d'apporter des précisions sur le délai de chute des baies malades, nous avons mis en place, au cours de la campagne 1991, des essais dans lesquels les baies atteintes de C.B.D. sont repérées individuellement. Ces comptages

hebdomadaires, sur 5 branches repérées au hasard sur 100 caféiers, de la floraison jusqu'à la maturation, devraient nous permettre de progresser dans la connaissance de l'évolution de la maladie et d'orienter nos choix pour une méthodologie pratique et précise d'évaluation au champ de l'efficacité de formulations fongicides. La même méthodologie pourrait être utilisée pour évaluer au champ le degré de sensibilité à l'anthracnose de nos collections de clones et d'hybrides.

La lutte chimique contre le CBD est rendue nécessaire, dans cette caféière camerounaise d'Arabica sensibles de haute altitude, par la forte pression infectieuse régulièrement rencontrée. Toutefois, une transformation du système de culture doit impérativement être envisagée afin d'améliorer la productivité pour tenter d'amortir le coût des traitements fongicides qui s'avèrent, dans le contexte actuel, difficilement rentables.

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Summary : coffee berry disease (C.B.D.) in Cameroun : epidemiology and chemical control

Coffee berry disease (CBD) due to *Colletotrichum coffeanum* Noack *sensu* Hindorf, a strictly African disease which can destroy up to 80% of the crop, occurs only at high altitudes where the climate is cool. Chemical control is necessary in Cameroon because practically all high-altitude coffee plants are very sensitive varieties. Following earlier work in which a treatment calendar was drawn up, based on epidemiological studies and climatic conditions, fungicidal molecules supplied by the crop-protection industry are tested in the field each year.
Because there are no large homogeneous plantations, a test method applicable to small plots had to be found. Total randomisation tree by tree proved to be usable and accurate, which is the major original feature of the work done in the past few seasons.The following criteria were adopted for assessing efficacy : the peak of the instant percentage of diseased berries; the percentage of berries dropping between the second and fourth months after flowering; the weight of berries picked.

These tests were also used to obtain more detailed epidemiological data.

LES PSEUDOCOCCIDAE DÉPRÉDATRICES DU CAFÉIER AU GUATEMALA

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Introduction

Le café est l'activité économique la plus importante au Guatemala. L'exportation de cette production constitue la principale source de rentrée de devises pour ce pays et représente environ 30 % des exportations totales.

Les ravageurs des racines constituent l'un des facteurs limitants dans la production. Actuellement les cochenilles racinaires (Homoptera, Pseudococcidae), entraînent de sérieux dégâts dans certaines zones caféières du pays. Malgré leur importance, peu de travaux leur ont été consacrées.

En raison du peu d'information que l'on possède sur la présence possible de diverses espèces de Pseudococcidae ainsi que sur leur biocoenose, et avant d'entreprendre toute étude qui pourrait être centrée sur les plus dangereuses, nous avons donc procedé à un recencement le plus complet possible des espèces appartenant à ce groupe ainsi qu'à des observations de terrain et de laboratoire permettant de dégager quelques caractéristiques de ces ravageurs dans leur milieu écologique.

Dégâts

Les dégâts provoqués par ces insectes, se traduisent par une modification de l'aspect des organes aériens. Il se produit une décoloration des feuilles, donnant à la plante envahie un espect panaché, chlorotique caractéristique. La chute partielle ou totale du feuillage peut s'en suivre. Le déssèchement des rameaux, des grosses branches s'observe également dans le cas d'infestation virulente.

Au niveau du système racinaire, lors des infestations chroniques, les Pseudococcides sont souvant cachées sous une couche brunâtre autour du pivot et des racines principales ; celle-ci est formée par un champignon (en cours de détermination) qui d'aprés certains auteurs pourrait être : Bornetina sp (LAVABRE, 1964 ; COSTE, 1955 ; NAKANO et- al 1981) ; Polyporus coffeae (BAUN, 1968) ; Diacanthodes sp (SEKHAR, 1964 ; CHACKO et SREEDHARAN, 1981 et VENKATARAMAIAH, 1988). Sous cette croûte on découvre les cochenilles enfermées dans de petites cavités. Ce manchon provoque alors la destruction de la racine et la mort de la plante.

1. Les Pseudococcidae au Guatemala : Inventaire et écologie

Matériel et méthode d'étude

Pour cette étude on a effectué un certain nombre d'observations de terrain dans diverses parcelles de Coffea arabica (98 exploitations) distribuées dans toutes les zones productrices de café. Ces observations concernent les caféiers mais aussi les arbres d'ombrage et les mauvaises herbes présentes dans la caféière.

Les insectes prélevés (cochenilles et fourmis associées vivant au niveau des racines), ont été envoyés pour leur détermination au Museum National d'Histoire Naturelle de Paris, au British Museum de Londres. Les fourmis sont en cours de détermination à l'Université Paul Sabatier de Toulouse.

Lors de notre échantillonnage, nous avons noté les différentes situations écologiques dans lesquelles les prélèvements ont été effectués : les conditions culturales : variétés de caféier, âge de la plantation, altitude, température ; les conditions édaphiques : structure physique et chimique du sol, etc.

L'infestation des caféiers par les cochenilles a été évaluée selon 6 niveaux de population, choisis arbitrairement de la manière suivante :

niveau	effectifs de cochenilles (dans les 15 premiers cm de pivot)
0 = aucune cochenille	0
1 = quantité très faible	1 - 6
2 = quantité faible	7 - 40
3 = quantité moyenne	41 - 250
4 = quantité forte	251 - 1600
5 = quantité très forte	1601 - 10000

Les niveaux 2 à 5 ont été observés sur le terrain.

L'étude de l'influence des données édaphiques sur le niveau de population des cochenilles (CO1 = niveau bas ; CO3 = niveau élevé) a été établie à partir d'une analyse des Correspondances Multiples.

Une première analyse a été réalisée en retenant 5 paramètres : le pH du sol, l'humidité du sol, la quantité de matière organique, le type de sol et l'altitude des plantations. Dans la deuxième analyse nous avons étudié l'aspect topographique ainsi que la profondeur des colonies dans le sol, tout en retenant le pH, l'humidité et le type de sol.

Nous avons également tenté de préciser la répartition verticale et horizontale des Pseudococcidae rencontrées sur les racines, de 50 caféiers âgés de 4 ans : sur chaque pivot racinaire les populations ont été dénombrées sur des portions succesives de 5 cm de long, allant du collet (niveau 0) à 20 cm de profondeur. Sur les racines secondaires, la même opération a été effectuée mais de façon horizontale pour des portions succesives de 5 cm depuis le pivot jusqu'à l'extrémité de ces racines.

Résultats

Dix espèces ont été relevées dont neuf Pseudococcidae et une Coccidae.

Dans toutes les régions productrices de café échantillonnées, au moins une espèce de cochenille des racines du caféier a pu être observée : il s'agit de Dysmicoccus cryptus, espèce dont l'effectif est toujours le plus

élevé.

Geococcus coffeae est également bien répresentée ; mais bien qu'elle soit très répandue, ses populations ont présenté de bas niveaux dans la plupart des cas.

Les espèces Planococcus citri, Paraputo sp. Pseudococcus elisae et Dysmicoccus brevipes sont moyennement répresentées.

Les autres espèces (Puto antioquensis, Pseudococcus longispinus, Planococcus halli et Toumeyella liriodendri), n'ont été trouvées que très rarement.

Dans nos relevés, toutes les variétés de C. arabica que nous avons rencontrées (Catuai, Caturra, Pacas, Pache, Bourbon et Catimor) sont succeptibles d'héberger des cochenilles racinaires.

Les Pseudococcidae colonisent également les plantes de couverture du sol ainsi que les arbres d'ombrage. Six des dix espèces de cochenilles concernées ont été observées sur diverses mauvaises herbes (10 espèces), dans certains cas trés fréquentes comme celui de la cochenille P. elisae sur Ipomoea tiliacea et sur I. trifida et celui de D. cryptus sur Solanum nigrum.

Concernant les arbres d'ombrage, les Inga spp (Mimosaceae) sont les plus fréquentés : 4 espèces de cochenilles ont été rencontrées sur ces arbres : D. cryptus ; P. citri ; Paraputo sp et D. brevipes ; avec une fréquence plus ou moins grande.

D'après les analyses des échantillons de sol pratiquées, on peut noter que la relation entre les différents paramètres analysés n'est pas très marquée. Cependant, nous pouvons définir les conditions les plus favorables à la présence de l'insecte (fig. 1) : les plantation sur sol sableux, au pH acide à neutre (4,25 à 7,5), avec une humidité comprise entre 25 et 48 % présentent les plus forts taux d'infestations. Dans ces conditions de sol, les colonies se concentrent de préférence dans les 15 premiers cm du sol.

Concernant la répartition des Pseudococcides sur les racines (pivot et racines secondaires confondues, tableau 1), on peut remarquer que c'est à une profondeur comprise entre 5 et 10 cm que l'on observe la plus grande quantité d'individus (37%) et que plus de 80% de la colonie se rencontrent dans les 15 premiers cm de profondeur.

Il faut toutefois noter que nos observations ont été effectuées pendant la saison des pluies, à une époque où les cochenilles sont concentrées vers la surface du sol.

2. Bioecologie de Dysmicoccus cryptus

D. cryptus est l'espèce dominante dans les caféières guatémaltèques. et occasione les dégâts les plus grâves. Cette espèce neotropicale decrite par HEMPEL, 1918, est déjà recencée au Brésil (NAKANO, 1972) et au Paraguay. Polyphage, elle se développe sur C. arabica, Citrus sp et Saccharum officinarun (WILLIAMS, 1970 ; WILLIAMS ET WATSON, 1988) .

2.1. Biologie

Matériel et méthodes d'étude

Dans un premier temps nous avons cherché à définir au laboratoire les potentialités biotiques de cette espèce. Pour des raisons de facilité nous avons utilisé des tubercules de pomme de terre comme support nutritif. 4 températures ont été testées : 15, 20, 25 et 30° C et respectivement 55, 65, 70, et 80 % d'humidité relative. Un total de 40 individus a été suivi au cours 2 générations successives.

Nous avons évalué la capacité de ponte en dénombrant au moment de leur sortie, toutes les larves mobiles (espèce ovovivipare) apparaissant pendant toute la période de ponte et d'éclosion. Nous avons pu également observer la mortalité enrégistrée en fonction de la température.

Résultats

Le développement de D. cryptus se fait au cours de 3 stades larvaires successifs avant l'apparition de la femelle. La durée du cycle vital est définie par la durée du développement larvaire et de la maturation sexuelle.

Les données du tableau 2 montrent qu'à 20^0 C, le cycle vital de l'insecte dure environ 64 jours. Au cours du développement le stade le plus long est le premier stade larvaire (environ 16 jours) et l'adulte est obtenu en moins de 40 jours. Toutefois les femelles obtenues ne pondent qu'après une période de maturation de 28 jours, et leur durée de vie totale avoisine les 100 jours.

A 25^0 C le développement s'accelére et l'adulte est obtenu au bout d'un mois. La période de maturation sexuelle n'est plus que de 23 jours, et la longévité des adultes est de l'ordre de 91 jours.

A 30^0 C le développement est encore plus rapide puisque les femelles apparaissent en moins d'un mois. De même la maturation est plus courte (20 jours) et la durée de vie plus brève (78 jours).

Concernant la fécondité, il apparait (tableau 3) que la température de 25^0 C semble être la condition thermique la plus favorable à la ponte. En effet à cette température on obtient une ponte totale qui est proche du double de celle observée à 20^0 C (respectivement 192 et 104) et supérieure du double à celle revelée à 30^0 C (79).

Manifestement quelle que soit la température c'est au stade L1 que l'on enregistre la plus forte mortalité, celle ci étant plus faible au stade L2 et pratiquement nulle lors du passage au stade adulte. Ainsi à 20 et 25^0 C moins de 4% des larves néonates sont éliminées tandis qu'à 30^0 C la mortalité dépasse 25% (tableau 3).

Si l'on considère la mortalité totale enregistré on peut remarquer que si à 20 et 25^0 C les chances de survie sont supérieures à 70% elles sont nulles à 15^0 et n'atteignent pas 60% à 30^0 C. Donc les températures de 20 et 25^0 C semblent le mieux convenir au développement de D. cryptus.

Quoi qu'il en soit les capacités de multiplication de cette cochenille dans une gamme de températures allant de 20 à 25^0 C sont particulièrement importantes. Bien que trés variable d'un individu à l'autre,

Fig. 1 : Analyse des correspondances multiples.
(Disposition des paramètres sur le plan 1-2)

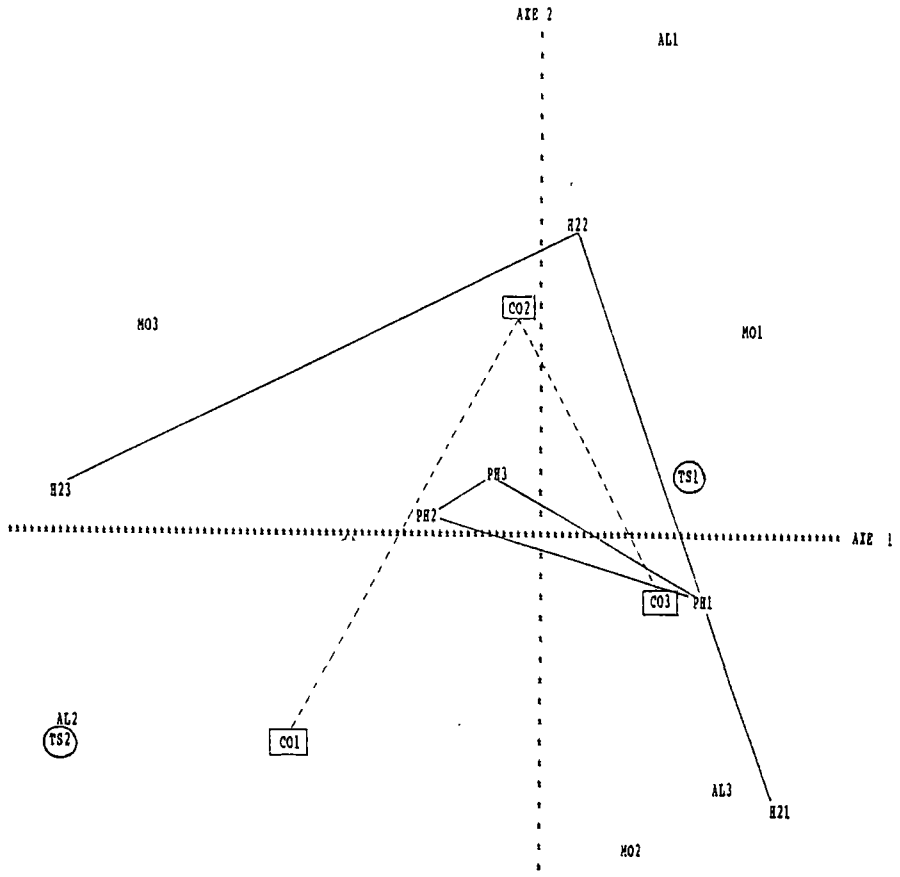


Tableau 1 : Répartition moyenne de la population de cochenilles sur la racine des caféiers.

Répartition verticale (cm)	Répartition Horizontale (cm) (portion de disque dont le centre est le pivot racinaire)				TOTAL
	0-5	5-10	10-15	15-20	
0-5	P = 559,8	-	-	-	559,8 (23 %)
5-10	P = 597,9 RS = 156,6 T = 751,5	- RS 90,6	RS 35,4	RS 12,6	890,1 (37 %)
10-15	P = 478,4 RS = 24,8 T = 503,2	RS = 24,0	RS = 15,0	-	542,2 (23 %)
15-20	P = 407 RS = 12,6 T = 419,6	-	-	-	419,6 (17 %)

P = Pivot RS = Racine secondaire T = Total

la fécondité des femelles est assez élevée (environ 200 oeufs à 25° C).

Si l'on admet qu'au cours de développement seul le stade L1 présente une mortalité spontanée relativement importante, la descendance d'une femelle au bout d'un an est :

- à 20° C, soit à la 5ème génération, de 12 x 10^9 individus.
- à 25° C, soit à la 5ème génération, de 26 x 10^9 individus.

Tableau 2 : Durée moyenne du développement des différents stades de D. cryptus (jours). Rapport avec la température. (effectifs, 140 individus).

Stades	Durée moyenne du développement (jours)		
	20° C effectifs 80	25° C effectifs 80	30° C effectifs 80
L1	15,9 ± 1,0	12,6 ± 0,3	10,1 ± 1,1
L2	10,5 ± 1,3	8,9 ± 0,9	8,3 ± 0,9
L3	10,8 ± 1,5	9,1 ± 1,3	8,9 ± 1,4
Durée du développement larvaire	37,2 ± 1,4	30,6 ± 1,1	27,3 ± 1,2
Maturation sexuelle	27,6 ± 1,9	22,9 ± 1,7	20,1 ± 2,1
Cycle vital	64,8 ± 2,1	53,5 ± 1,5	47,4 ± 1,9
Longévité des femelles	94,8 ± 5,1	90,5 ± 3,1	77,5 ± 2,3

Ecologie

Matériel et méthodes d'étude

Dans un deuxième temps nous avons tenté d'observer dans quelle mesure les potentialités pouvaient s'exprimer dans la nature, dans les caféières guatémaltèques.

Nous avons donc tenté (inspirés des travaux de FABRES, (1979 ; 1981) ; LE RU, (1984), d'estimer l'abondance des populations de ce ravageur à partir des données recueillies pendant la saison des pluies (mai - octobre) dans deux plantations (0,7 hectare chacune), situées dans deux conditions climatiques différentes (ferme "La concha" : 1300 m d'altitude, 1800 mm de pluie et une température moyenne de 22° C ; ferme "Panorama" : 1250 m d'altitude, 3000 mm de pluie et une température moyenne de 20° C). Dans les deux cas, les caféiers sont âgés de 4 ans, appartiennent à la variété "Pacas" et sont plantés à 2 m en inter-ligne et à 1 m sur la ligne, avec des arbres d'ombrage (Inga sp), sur

un sol sableux, profond, développé sur matériel volcanique.

Nos comptages, sur 50 plantes, toutes les 3 semaines, portent sur le morceau du pivot compris entre le collet et 10,0 cm de profondeur sur une aire constante de 100 cm^2 environ.

Tableau 3 : Evolution numerique des populations des femelles de D. cryptus au cours du développement en fonction de la température. Effectifs obtenus par femelle (mesurés sur 20 femelles).

Condition d'élevage	20° C	65 % H.R	25° C	70 % H.R	30° C	80 % H.R
Stade	Effectifs	% de mortalité	Effectifs	% de mortalité	Effectifs	% de mortalité
L1	104,6 ± 45,8	3,8	192,0 ± 61,3	2,5	79,9 ± 31,8	25,9
L2	100,6	1,8	187,3	0,2	58,8	3,2
Mâles L3	22,6 76,6	0,4	45,7 141,2	0	11,6 45,4	0
Mâles Femelles	22,6 76,2		45,7 141,2		11,6 45,4	
Mortalité totale	6,1	5,8	5,6	2,7	22, 4	28,2
Coefficient de survie des ♀	0,733		0,736		0,572	
pouvoir de reproduction*	76,6		141,3		45,4	

* N. d'individus à la génération suivante

Résultats

Dans les deux zones étudiées on a observé un accroissement de la population aprés la reprise des pluies, lorsque l'humidité du sol a dépassé 30 % .

Dans la ferme "La concha" (figure 2), la population atteint un pic maximun aprés 3 mois environ. Vers la fin septembre s'observe une chute assez brutale de la population qui résulte probablement d'un excés d'humidité qui peut constituer un facteur directement défavorable au développement de la cochenille ou qui favorise l'action entomopathogène de certains organismes à l'égard de ce ravageur.

Dans la deuxième plantation ("Panorama"), (figure 3) l'effectif de cochenilles augmente rapidement pour atteindre vers la mi-juilliet un pic

élevé. Cette fois, on trouve à la mi-mai une population constituée par des L3 et un nombre assez important de femelles. Ceci supposse que le point de départ de la gradation se situe un mois plus tôt (mi avril), époque qui correspond à la reprise des pluies dans cette région. Ici nous n'avons pas enregistré de chute de la population jusqu'au 11 octobre.

Les résultats obtenus laissent supposer que l'humidité du sol intervient dans le développement de l'insecte et il semble qu'un excés d'humidité (supérieure à 50 %) soit un facteur limitant.

Nous avons suivi les relations existant entre la fourmi Solenopsis sp (détermination à l'UPS, Toulouse, 1990) et la cochenille D. cryptus, cette association ayant été souvent observée dans les plantations.

Ces fourmis développent une activité importante dirigée vers les cochenilles. Les colonies de D. cryptus sont visitées par Solenopsis et certains individus sont même déplacés sur des distances allant de 1 à 3 m pour être installés sur d'autres caféiers, sur des arbres d'ombrage ou amenés vers un nouveau nid en phase de développement. Ces individus sont alors à l'origine de nouvelles colonies qui se développeront ultérieurement.

Fig. 2 : Evolution de la population de D. cryptus. Ferme "La concha"

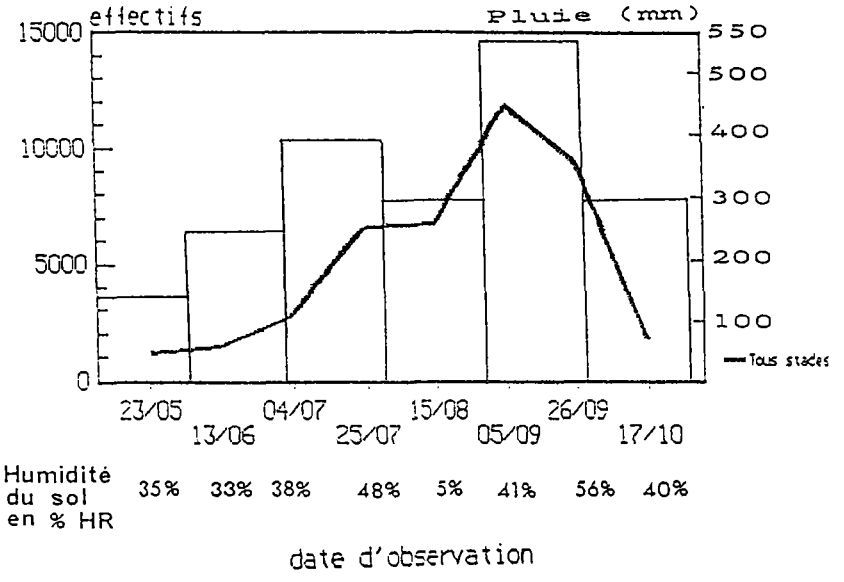
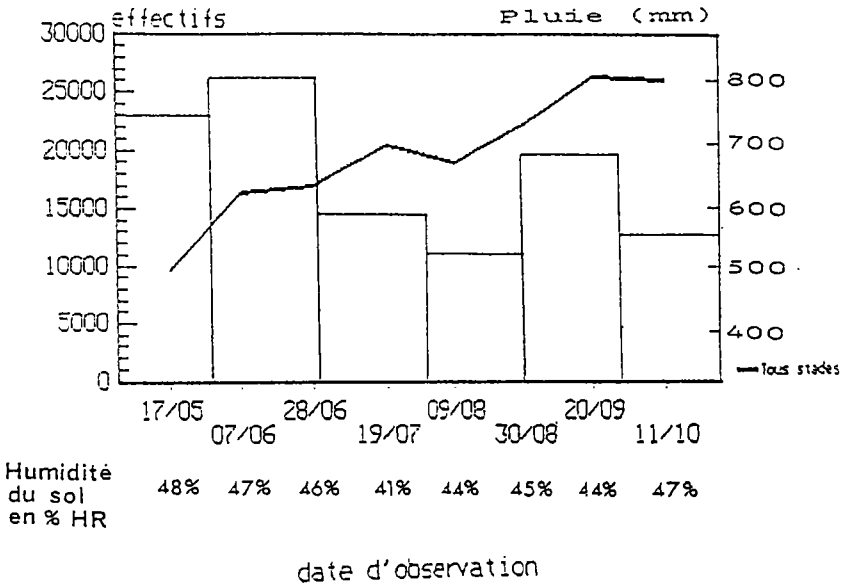


Fig. 3 : Evolution de la population de D. cryptus. Ferme "Panorama"



Conclusion

Dix espèces de cochenilles racinaires ont été rélevées, parmi lesquelles D. cryptus présente la répartition géographique la plus large.

Insectes trés polyphages, elles peuvent être hébergées par diverses variétés de C. arabica mais aussi par les mauvaises herbes et les arbres d'ombrage présents dans la caféière. Pour certaines espèces un seul type d'hôte semble possible mais pour d'autres comme D. cryptus par exemple, toutes les espèces végétales rencontrées dans la plantation semblent sucseptibles d'être attaquées. Cette remarque est importante car les stratégies futures de lutte contre ces ravageurs devront prendre en compte cette caractéristique.

Sur le caféier elles se fixent sur le pivot (concentrées dans les 15 premiers cm à partir du collet) mais elles peuvent aussi atteindre les racines secondaires.

La relation entre les paramètres du sol et les niveaux de population de cochenilles n'est pas très marquée, cependant ces insectes sont plus fréquents et abondants dans des sols sableux avec une humidité moyenne, pH allant de l'acide au neutre.

Cette étude a permis de préciser d'une part le rôle déterminant de la température dans l'évolution de D. cryptus et d'autre part a permis de connaître l'évolution de leurs populations au cours de la saison des pluies. Les résultats obtenus montrent que l'humidité du sol intervient dans le développement de l'insecte. Dans les deux zones étudiées on observe un accroissement de la population aprés la reprise des pluies lorsque l'humidité du sol a dépassée 30 % . De façon pratique, la lutte chimique contre ces insectes necessitera donc des interventions à ce moment-là de la progradation de ces ravageurs.

Les associations D. cryptus-Solenopsis sp apparaissent comme les plus fréquentes. Ces fourmis sont capables de transporter les cochenilles et de les installer sur des caféiers sains permetant alors le développement de novelles colonies. Dans une stratégie de lutte contre D. cryptus on devra donc tenir compte de ce phénomène et effectuer d'abord un controle sur ces Hymènoptères.

En définitive,après cette première phase d'étude exploratoire, le maintien des populations de cochenilles dans les caféières à des niveaux inférieurs au seuil agronomique de dégâts, doit faire l'objet de toute une série d'expèrimentations portant sur la lutte chimique (époque d'intervention), la lutte biologique (utilisation d'entomopathogènes) et la lutte génétique (selection, création de variétés résistantes).

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RESUME

Le but de cette étude est de proposer quelques éléments de base sur les Pseudococcidae infeondées aux racines du caféier au Guatemala. Tout d'abord on a procédé en 1989 à un recensement le plus complet possible des espèces appartenant à ce groupe, ainsi qu'à celui des fourmis qui leurs sont associées.

Des observations de terrain permettant de dégager quelques caractéristiques de ces ravageurs dans leur milieu écologique ont été effectués.

Parmi les dix espèces relevées, Dysmicoccus cryptus présente la répartition géographique la plus large. On a donc tenté d'étudier plus particulièrement les potentialités biotiques et d'estimer l'abondance de leurs populations pendant la saison des pluies (mai-octobre) dans deux conditions climatiques différentes. Les associations avec des fourmis (Solenopsis sp) ont été aussi étudiées.

UNA APLICACIÓN DE LA BIOTECNOLOGÍA PARA EL CONTROL DE LA BROCA DEL CAFÉ

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INTRODUCCION

El hongo entomopatógeno Beauveria bassiana ataca la broca del café Hypothenemus hampei, penetrando su cutícula por acción enzimática e invadiendo tejidos internos y hemolinfa de los cuales se nutre. El hongo se desarrolla internamente en el insecto hasta que sus hifas vuelven al exterior y sus propágulos están en capacidad de colonizar nuevos insectos.

Diversas cepas de este hongo presentan diferente grado de patogenicidad sobre diversos hospederos.[7]. Por esta razón es posible y necesario llevar acabo una selección permanente de cepas para afinar su espectro de acción, centrándolo sobre la broca y haciéndolo menos importante sobre insectos benéficos.

Las mayores dificultades para el empleo de este hongos entomopatógenos como insecticidas de uso corriente han sido: encontrar métodos de producción eficientes y rentables de grandes volúmenes de propágulos poder proteger este material contra el stress ambiental y contra antagonistas químicos y biológicos, y poder obtener un producto final que permita un manejo como el de cualquier insecticida comercial.[2],[3].

En el Laboratorio de Investigaciones L.I.Q.C.de la Federación Nacional de Cafeteros de Colombia, se han desarrollado varios métodos de producción masiva de los diversos tipos de propágulos del hongo y se han obtenido formulaciones de alta eficacia en pruebas experimentales de campo que permiten un manejo sencillo del producto y una alta estabilidad durante su almacenamiento.

PRODUCCION DE PROPAGULOS

El hongo B. bassiana presenta diferentes tipos de propágulos dependiendo del ambiente en que se desarrolle, principalmente de la relación Carbono/Nitrógeno del medio nutritivo, del pH y del Oxígeno disponible. [1],[8]. El desarrollo de los cuerpos fructíferos es afectado radicalmente por el tipo de cultivo que se lleva a cabo. Los diferentes propágulos tienen caracteristicas morfológicas y fisiológicas muy diferentes.

En cultivo sobre la superficie de un medio el hongo produce conidios de forma redonda, de trés a cuatro micrones de diámetro y de doble pared. El cultivo en inmersión en un medio líquido, conduce al hongo a la formación de propágulos de origen ártrico o (Septación micelial), de forma elipsoidal, con tamaños variables, en promedio de seis micrones de longitud y 2 micrones de ancho y de pared sencilla, conocidos con el nombre de blastosporas. También se obtienen en cultivo en inmersión propágulos de origen similar a los conidios obtenidos en superficie, pero con pared más delgada que la de estos y mayor tamaño, conocidos en literatura como conidios de inmersión.

Por naturaleza los conidios obtenidos por cultivos en superficie están dotados de mejores condiciones para sobrevivir en ambientes más hostiles y por mayor tiempo, en ausencia de alimento, que los demás propágulos.

En el L.I.Q.C. se han desarrollado medios de cultivo y se han obtenido parámetros fisico-químicos óptimos para la obtención de cada uno de estos propágulos a escala laboratorio y escala piloto . En cultivo en superficie se obtienen entre 7.5x10^9 y 10^10 conidios/cm2, dependiendo de la cepa que se elija. Si asumimos basados por la literatura 10^13 conidios/hectárea como dosis indicativa para el control adecuado de una plaga con B. bassiana, el costo del medio de cultivo desarrollado para obtener, con alta reproducibilidad, este material asciende a $ 32 pesos colombianos, cerca de US$ 0.05, el área necesaria es cerca de 0.1 m2 y el tiempo es de 10 a 12 días. Las condiciones de cultivo logradas para alcanzar alta productividad no requieren de bombeo de Oxígeno ni de cuartos asépticos. El hongo se obtiene libre de contaminaciones y de fácil separación del medio de cultivo.

El cultivo en inmersión se lleva acabo en un reactores que permiten el seguimiento, medida y control del pH, del Oxígeno disuelto, la temperatura, la agitación mecánica, la entrada continua de nutrientes y la salida continua de producto. Se facilita así el proceso de optimización de parámetros de cultivo y de medio nutritivo. En este sistema de cultivo se obtienen entre 2x10^9 y 5x10^9 blastosporas/ml y entre 5x10^8 y 10^9 conidios/ml en un tiempo de 72 horas . Los medios desarrollados para la obtención de estos propágulos son de costos y reproducibilidad similares a las del medio mencionado para conidios de superficie. Con las mejores productividades obtenidas en inmersión, serían necesarios 2 litros de volumen de trabajo para obtener en 72 horas blastosporas suficientes para asperjar una hectárea. En un reactor de 100 litros de volumen de trabajo se obtendría material suficiente para 50 hectáreas en tres días.

Otro material que se logró obtener por cultivo en reactor fué el de partículas miceliales de tamaño pequeño (de 200 a 300 micrones de diámetro).

El número de partículas por mililitro de medio de cultivo osciló entre 38.000 y 60.000 con peso seco de 6.5 a 7.0 mg/ml. Este material tiene la capacidad de producir conidios cuando es expuesto a condiciones ambientales sin requerir un medio nutritivo adicional. Se obtuvieron entre 2x10^8 y 5x10^8 conidios/mg de peso seco, lo que corresponderia a 3x10^9 conidios/ml de medio de cultivo.

FORMULACIONES, SECADO Y PRUEBAS PRELIMINARES DE CAMPO

El material obtenido por los diferentes sistemas de cultivo se protege con coadyuvantes apropiados contra desecación, cambios bruscos de presión osmótica y radiación ultravioleta y se diluye en un vehiculo inerte que facilite la estabilidad del hongo en el campo y permita por parte del usuario un manejo muy similar al de los productos insecticidas de uso común.

En pruebas de laboratorio se ha demostrado que el hongo formulado resiste sin pérdida de viabilidad un proceso de secado hasta el 5% de humedad residual. Igualmente se ha comprobado la conservación de la capacidad germinativa y del grado de patogenicidad del producto en polvo seco, después de varios meses de almacenamiento. El producto seco sin formular pierde el 50% de viabilidad en aproximadamente un mes de almacenamiento en identicas condiciones.Las pruebas preliminares de campo se llevaron a cabo con material formulado.

El material de conidios obtenidos en cultivo de superficie ha demostrado hasta el momento ser el de mayor eficacia en el control de la broca en el campo. Se ha logrado hasta un 70% de control de población de broca con tres asperciones espaciadas 15 días. Los productos de reactor están en proceso de prueba. Los ensayos de laboratorio sobre H. hampei muestran un alto grado de patogenicidad tanto de las blastosporas como de los conidios obtenidos en inmersión y los producidos por las partículas miceliales en contacto con el ambiente. Esto sugiere que es posible con una adecuada protección lograr con los diferentes materiales un alto control de la población de broca en el campo. Mejorar las formulaciones de estos materiales es uno de los objetivos actuales de investigación.

PRODUCCION A ESCALA PILOTO DE FORMULACIONES DE Beauveria bassiana

Con base en los resultados de laboratorio y los resultados preliminares de campo, se tomó la decisión de producir material de prueba suficiente para llevar a cabo ensayos de eficacia en campo a mayor escala y por lo tanto estadisticamente más significativos. El escalamiento piloto de la producción de formulaciones de B. bassiana, debe contemplar el escalamiento de la producción del hongo, el del proceso de separación y concentración del material obtenido, el del proceso de formulado y finalmente el de secado de la formulación. Además de permitir la producción de material suficiente para pruebas semicomerciales y comerciales de campo, el escalamiento piloto debe ser la base de la evaluación técnico-económica de un futuro producto industrial que compita económicamente con los insecticidas químicos.

Asumiendo que la productividad por mililitro para reactores de grandes volúmenes es la misma que la obtenida en reactores de dos litros, que la eficacia en campo de los diferentes tipos de material fúngico es similar y

que son necesarios 10^13 propágulos/Hectárea/aspersión, se estimaron volúmenes y áreas de cultivo necesarios para producir material para 1.000 Hectáreas por mes, es decir se estimaron los requerimientos para obtener 10^16 propágulos por mes.

Para las blastosporas, con una productividad de 5x10^9 blastosporas/ml/3 días, equivalente a 5x10^13 blastosporas/l/mes, para obtener 10^16 propágulos por mes sería necesario un reactor de 200 l de volumen de trabajo, con sistema de producción de inóculo, sistema para trabajo continuo y control de: pH, Oxígeno disuelto, rpm, nivel, espuma y bombeo continuo de medio y de producto.

Para conidios de inmersión, dado que la productividad promedio lograda hasta ahora con estos propágulos es de 5x10^8 conidios/ml de medio, es decir 10 veces menor que en blastosporas, el reactor para producir material para 1.000 hectáreas cada mes debe tener un volumen de trabajo de 2.000 litros y estar dotado como se mencionó para el caso de blastosporas.

Respecto a las partículas de micelio, estas pueden producir conidios una vez hayan sido asperjados en el campo. La cantidad de conidios que producen depende de la masa micelial. Así se estableció y se comprobó una relación entre conidios producidos y cantidad de masa micelial por mililitro de cultivo: 3x10^9 conidios/ml/3 días = 3x10^13 conidios/l/mes; lo que requeriría de un reactor de 330 litros de volumen de trabajo para 1.000 hectáreas/mes.

Para conidios de superficie, tomando la menor productividad: 7.5X10^9 conidios/cm2/12 días = 1.87x10^10 conidios/cm2/mes, la obtención de material para 1.000 hectáres/mes, requeriría 5.3x10^5 cm2 de área de cultivo. Estos 53 m2 de área de cultivo se pueden disponer en unidades de cultivo de cerca de 0.1 m2, sobre estanterías debidamente diseñadas. En un cuarto de 2,3 m de altura, son necesarias 10 m2 para la ubicación de estas estanterías.

El proceso de separación y concentración de material fúngico y el de formulación y secado tienen esencialmente los mismos requerimientos y costos independientemente del proceso de producción que se siga. El estimativo de costos diferenciales se puede llevar a cabo entonces con base en los costos de producción de biomasa. El sistema de cultivo en superficie, es de alta productividad y bajo costo.Sinembargo es un sistema que demanda mayor mano de obra que el de reactor.Este factor debe ser evaluado en función de la escala de producción. Por otra parte desarrollar un sistema continuo o semicontinuo de producción del hongo en cultivo en superficie es técnicamente factible.

La planta piloto que se instaló para cultivo en superficie tiene un área total de 40 m2 distribuidos así: 14.5 m2 del cuarto de producción de biomasa; 6 m2 de área de inoculación; 8 m2 de área de elaboración de medios y 9 m2 de área para la separación, concentrado, formulado y secado del producto. La capacidad instalada es para 2.000 hectáreas/mes y el costo total incluyendo la adecuación del área fué de US$.70.000.oo.

CONCLUSIONES

1.El escalamiento de la producción de hongos entomopatógenos con miras a la obtención de un producto comercialmente competitivo con los insecticidas químicos, es visto usualmente en literatura como un tema de cultivo de medios líquidos en reactores.[4],[5],[6]. Sinembargo con el objetivo de costos iguales para productos de igual eficacia, no puede ser descartada la alternativa del sistema de producción en superficie.La introducción de mejoras y automatizaciones en el proceso de producción en superficie puede llegar a hacer de este sistema el de mejores resultados técnicos y económicos a cualquier escala de producción.

2. A escala piloto (2.000 hectáreas/mes.), la producción del material de reactor tendría un costo cuatro veces mayor, segun las productividades logradas para cada tipo de propágulo.

3. Para el control de la broca del café no parece aventurado afirmar que el hongo entomopatógeno Beauveria bassiana, ofrece una alternativa como insecticida de uso corriente; sin que las investigaciones tanto en la parte microbiológica como en la de ingeniería y en la de campo hayan terminado y aún con innumerables conocimientos, innovaciones y optimizaciones por incluir, se dispone de un producto de la línea "Safe Alternatives" de relativa alta eficacia y costo comparable con el de otros productos comerciales.

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SUMMARY

AN APPLICATION OF BIOTECHNOLOGY TO CONTROL THE COFFEE BERRY BORER

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There is a growing interest on methods for the integrated pest control because of ecological and in last resort economical benefits.

In our laboratory during the last years methods have been developed that allows the production of infective forms and preparations of Beauveria bassiana at the pilot plant level to the handling and applications of the control products on the field are presented.

Results obtained in the field, production costs and other aspects of the utilization of this bioinsecticide are discussed.

DYNAMIQUE DES POPULATIONS DE *PRATYLENCHUS COFFEAE* SUR CAFÉIER DANS LE SUD-OUEST DU GUATEMALA

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Introduction

Les nématodes représentent actuellement un grave problème pour la caféiculture en Amérique Centrale. Parmis ceux ayant la plus large répartition et causant le plus de dommages aux plantations de café, Pratylenchus coffeae est signale au Salvador (Abrego & Holdeman, 1961; Vasquez,1980), au Nicaragua (Calderon & Marban, 1988), au Costa Rica (Figueroa, 1987) et au Guatemala (Schieber, 1966; Fernandez,1968). Les plantes infestées par P. coffeae présentent, des symptômes de déficience minérale, une défoliation générale, ceci pouvant aller jusqu'à la mort progressive de la plante dans des délais variables (Monteiro & Lordello, 1974; Schieber,1966). Au niveau du système racinaire, on constate la disparition d'une grande partie du chevelu racinaire et une nécrose généralisée du tissu cortical des racines secondaires, lequel se détache facilement du cylindre central des racines. Peu d'études ont été réalisées sur la dynamique de populations de P. coffeae. L'objectif de ce travail était donc d'étudier les fluctuations de populations de ce nématode dans une région caféicole très importante au niveau économique pour le Guatémala, en relation avec le régime des précipitations, facteur très important dans les conditions écologiques de la région où a été réalisée l'étude.

Matériels et méthodes

Ce travail a été réalisé sur la station expérimentale d'ANACAFE (Asociacion Nacional de Café) "Buena Vista", San Felipe, Retalhuleu, située sur le versant sud de la "Sierra Madre" constituée par une chaîne de volcans. Le site d'étude se trouve à une altitude de 450 mètres et sur des andosols dont les caractéristiques apparaissent dans le tableau 1. Les données de température ont été prises sur une station météorologique proche du lieu d'étude et à une altitude très voisine ("Los Brillantes") et sont représentées sur la figure 1. Les données de précipitations ont été relevées quotidiennement sur le site et sont exprimées sur la figure 2.A. L'étude a été menée sur une caféière plantée avec du Coffea arabica Variété Catuai Rouge sur pied franc. Cette caféière fut plantée en juin de l'année 1983 et recépée en décembre de l'année 1987.
Les populations de P. coffeae ont été suivies mensuellement depuis mai 1989.
Le prélèvement effectué sur 20 plantes est constitué de 4 échantillons , correspondant chacun à 5 plantes. L'échantillonnage au niveau de chaque plant était réalisé sur un site à mi-distance du rayon de la projection de la canopée du caféier et sur une profondeur de 20 cm., d'après l'étude réalisée au Costa Rica sur la distribution verticale et horizontale des nématodes sur caféier Arabica (Bolivar, Salazar & Echeverri, 1984). Le choix des 20 plantes échantillonnées s'est effectué à chaque fois de manière systématique sur un plan représentant la parcelle de façon qu'elles couvrent uniformément toute l'aire étudiée. La parcelle ayant au total 400 plantes, il a pu être possible de choisir la localisation des prélevats de façon à ne pas renouveler un échantillonnage sur les mêmes plantes avant un délai d'un an. Les 4 échantillons 'ont été chaque fois traités séparément sur une partie aliquote de 25 grammes de radicelles . Après broyage au mixeur, on a procédé au tamisage des racines sur la série de tamis suivante: 850 um., 150 um.,45 um., 38 um. et 5 um., en récupérant le refus des 3 derniers tamis. Les

nématodes sont séparés des débris végétaux par la méthode de centrifugationflottation avec solution de saccharose (Coolen,1979).

Résultats

Les fluctuations des populations totales de pratylenchus coffeae (larves et adultes) sont caractérisées par deux pics qui se reproduisent à la même époque d'une année sur l'autre (Fig. 2.B.). Les populations de nématodes au moment de ces pics dépassent les 250 par gramme de racines pour atteindre dans un cas environ 360 nématodes par gramme de racines. Le premier pic de population intervient durant la saison sèche, pendant les mois de janvier et février. Le deuxième pic, lui se produit durant la saison des pluies pendant les mois de juin et juillet, correspondant aussi à une époque désignée localement comme "cannicule" durant laquelle on assiste à une diminution de l'intensité des pluies comme le montre la figure 2. Durant la deuxième moitié de la saison des pluies, de août à octobre, les populations de P. coffeae, au contraire diminuent pour atteindre les niveaux les plus bas de l'année, à peine supérieurs à 50 nématodes par gramme de racines. Entre les deux pics de populations, durant la période d'installation de la saison des pluies, on observe des niveaux de population intermédiaires proches de 150 nématodes par gramme de racines.
Si l'on compare les courbes de fluctuations de populations d'adultes et de larves (Fig. 3.A.), on s'aperçoit qu'il y a coïncidence des pics de populations pour les deux stades et que la structure de la population de P. coffeae reste assez stable. Les stades larvaires représentent en moyenne au cours du temps 50,4 % de la population totale avec un écart-type de 8,4 % .
Les populations d'oeufs (Fig. 3.B.) fluctuent selon les mêmes tendances que celles des adultes et des larves avec quelques exceptions pouvant être attribuées à la difficulté de récupération des oeufs et surtout à la confusion possible avec les oeufs d'autres espèces de nématodes bien que présents en quantités beaucoup plus faibles.
Quant au sex ratio, comme le montre la figure 4, il varie beaucoup au cours du temps sans que l'on puisse trouver une évolution logique de celui-ci. La moyenne au cours du temps du sex ratio est de 0,74 mâles pour une femelle avec un écart-type de 0,25 .

Discussion

Cette étude montre des variatios cycliques des populations de P. coffeae pouvant être expliquées principalement par la climatologie et en particulier le régime des précipitations. On peut penser que cette influence de la climatologie n'est qu'indirecte, les nématodes endoparasites étant d'abord sous la dépendance de la dynamique racinaire, en relation avec les cycles physiologiques du caféier. San Juan & ad. (1981) ont mis en évidence dans la même zone écologique que celle étudiée et à une altitude voisine, l'existence de deux pics de croissance végétative du caféier, l'un se produisant en avril-mai et l'autre en juillet-août. Par ailleurs, Huxley et Tuker (1976) rapportent que les périodes de plus grande croissance des racines précèdent les périodes de plus grande élongation des rameaux orthotropes . Les deux pics de populations de P. coffeae de janvier-février et de juin-juillet pourraient donc résulter de deux pics de croissance racinaire. De plus Arias (1987) mentionne que les périodes courtes de sécheresse semblent importantes pour la croissance du système racinaire. Or le premier pic de population du nématode intervient durant la saison sèche et le deuxième durant la période de "canicule" correspondant à une forte diminution de la quantité de précipitations pendant la saison des pluies. Huxley et Tuker (1976) ont observé également que les métabolites produits par photosynthèse étaient transférés, lors de ces périodes de croissance racinaire, vers le système tronc-racines. On peut donc penser que la qualité chimique des racines est plus favorable au développement des nématodes lors de ces périodes. Quénéhervé (1989) de la même manière observe que les popuplations de P. coffeae et de Radopholus similis, sur bananier augmentent durant la saison sèche, période correspondant à l'émergence de nouvelles racines.
Gil (1981) observe également, dans une étude de dynamique de populations de P. coffeae au Salvador, la présence d'un pic de populations en juin mais n'observe aucun pic durant la saison sèche, ce qui peut être attribué comme le mentionne l'auteur, aux basses températures qui sont enregistrées durant cette période et à l'altitude (1200 m.) à laquelle a été réalisé ce travail. Alors que comme le montre la figure 1, le facteur température varie peu au cours de l'année dans les conditions de l'étude et les températures minimales restent toujours relativement élevées.
Pour les niveaux de populations très bas d'août à octobre, au moment de la recolte des fruits mais aussi au moment de plus forte pluviosité dans l'année, les facteurs explicatifs possibles seraient alors 1) un fort ralentissement de la dynamique racinaire pendant la maturation des cerises, 2) un problème d'excès d'eau dans le sol du fait de la forte capacité de rétention en eau des andosols présents sur le site d'étude avec comme conséquence des problèmes d'asphixie racinaire et de développement de pathogènes, bactéries et champignons, accélérant le processus de dégradation des racines, supprimant par la-même le support d'alimentation du nématode. De plus le nématode trouverait alors dans le sol des conditions hostiles. Kumar et D'Souza (1971) ont observé que la capacité d'oviposition des femelles de P. coffeae était réduite lorsque celles ci étaient placées dans l'eau. Le sol ne constituerait donc pas un milieu favorable pour le renouvellement des popûlations du nématode.

Conclusions

Il s'avère nécessaire de réaliser d'autres suivis de dynamiques de populations de P. coffeae dans d'autres zones écologiques et en particulier à différentes altitudes en prenant en compte

le facteur température. Il se peut en effet que le pic de population de la saison sèche soit limité par ce facteur à partir d'une certaine altitude.
Au niveau pratique il faudra essayer de définir en augmentant la quantité de données observées un calendrier de prélèvements pour la surveillance sanitaire. Un échantillonnage en avril-mai pourrait se révéler utile pour prévoir le pic de juin-juillet.
Du point de vue des applications de nématicides, ce travail met en évidence la nécessité d'agir très rapidement, quand cela est nécessaire, dès le début de la saison des pluies pour pouvoir contrôler le pic de juin-juillet. Il apparait nécessaire également selon cette étude et dans les conditions de celle ci d'
assurer une protection à la plante durant la saison sèche pour éviter l'infestation massive des nouvelles racines qui sont en formation durant cette période et qui auront un rôle important à jouer dans l'alimentation de la plante à l'entrée de la saison des pluies et donc pour la préparation de la récolte à venir. On peut penser que dans les conditions de l'étude, une application après récolte en fin de saison des pluies pourrait contrôler ce pic de populations de P. coffeae de janvier-février.
Par contre, selon l'étude réalisée et dans les conditions de celle ci, il ne paraît pas justifié de faire des applications de nématicides durant la période d'août à octobre, comme cela est parfois pratiqué, du fait de la forte diminution des populations de P. coffeae naturellement durant cette époque de l'année.

Tableau 1. Caractéristiques physico-chimiques du sol de la parcelle étudiée.

GRANULOMETRIE		
Argiles	(0 - 2 um.)	4,5 %
Limons	(2 - 50 um.)	17,3 %
Sables	(50 um. ...)	78,2 %
MATIERE ORGANIQUE		2,9 %
pH		6,1

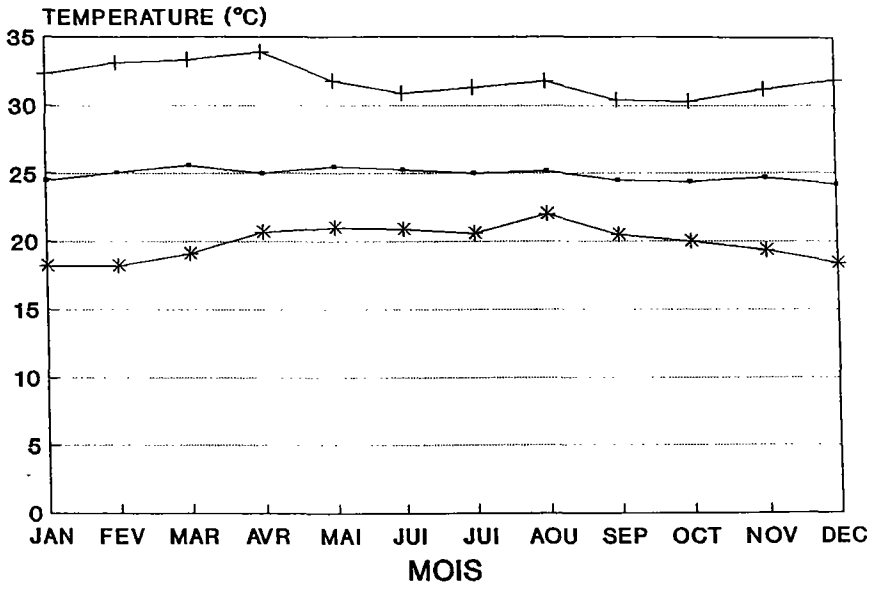

Fig. 1. Données de température de la station "Los Brillantes"; ▪ : Moyennes mensuelles des températures; + : Moyennes mensuelles des températures maximales; ✳ : Moyennes mensuelles des températures minimales.

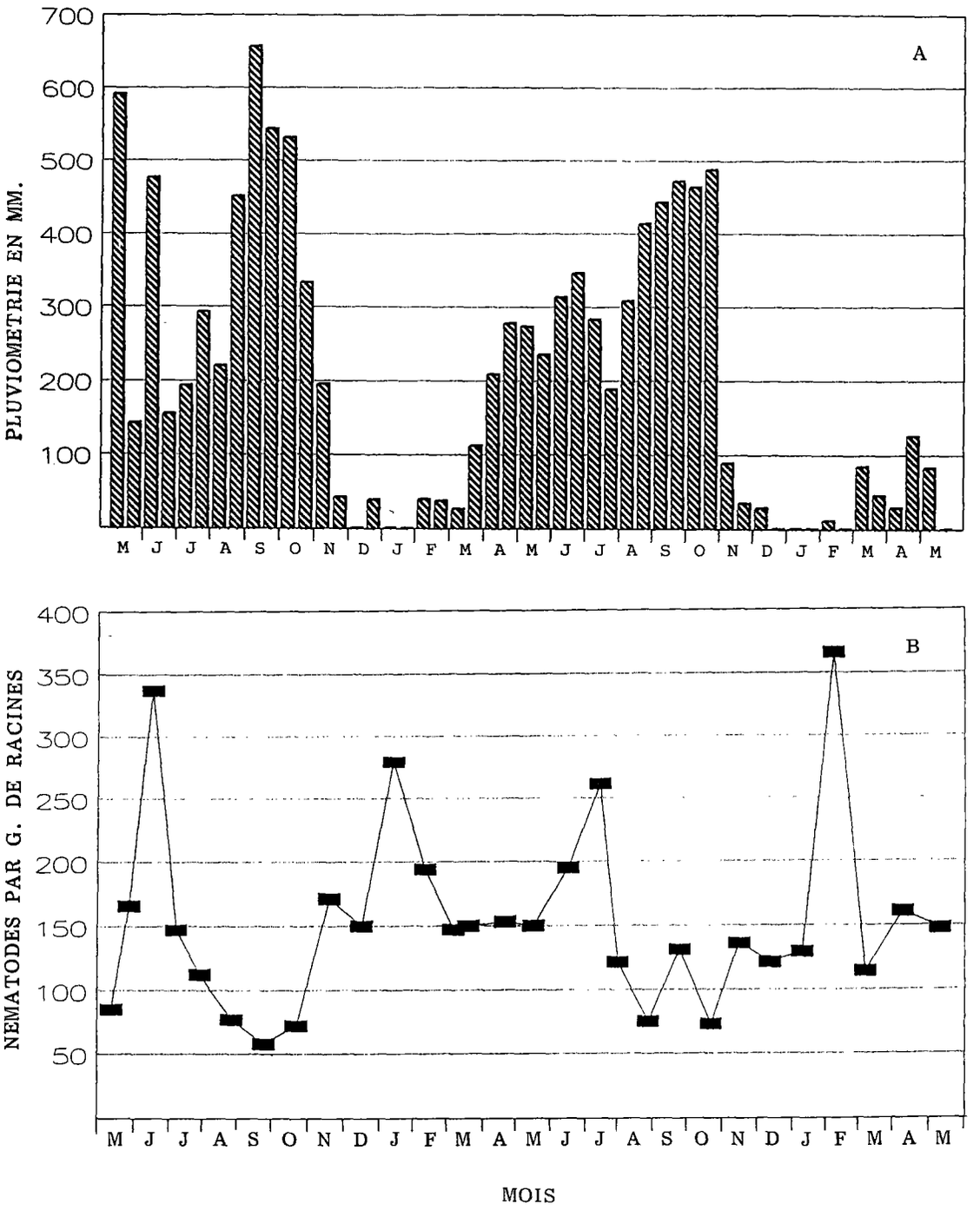

Fig. 2. Dynamique de populations de Pratylenchus coffeae sur la station de Buena Vista (Mai 1989 à mai 1991) : A :Pluviométrie par quinzaine.B :Fluctuations saisonnières des populations de P. coffeae dans les racines de caféier (Adultes et larves).

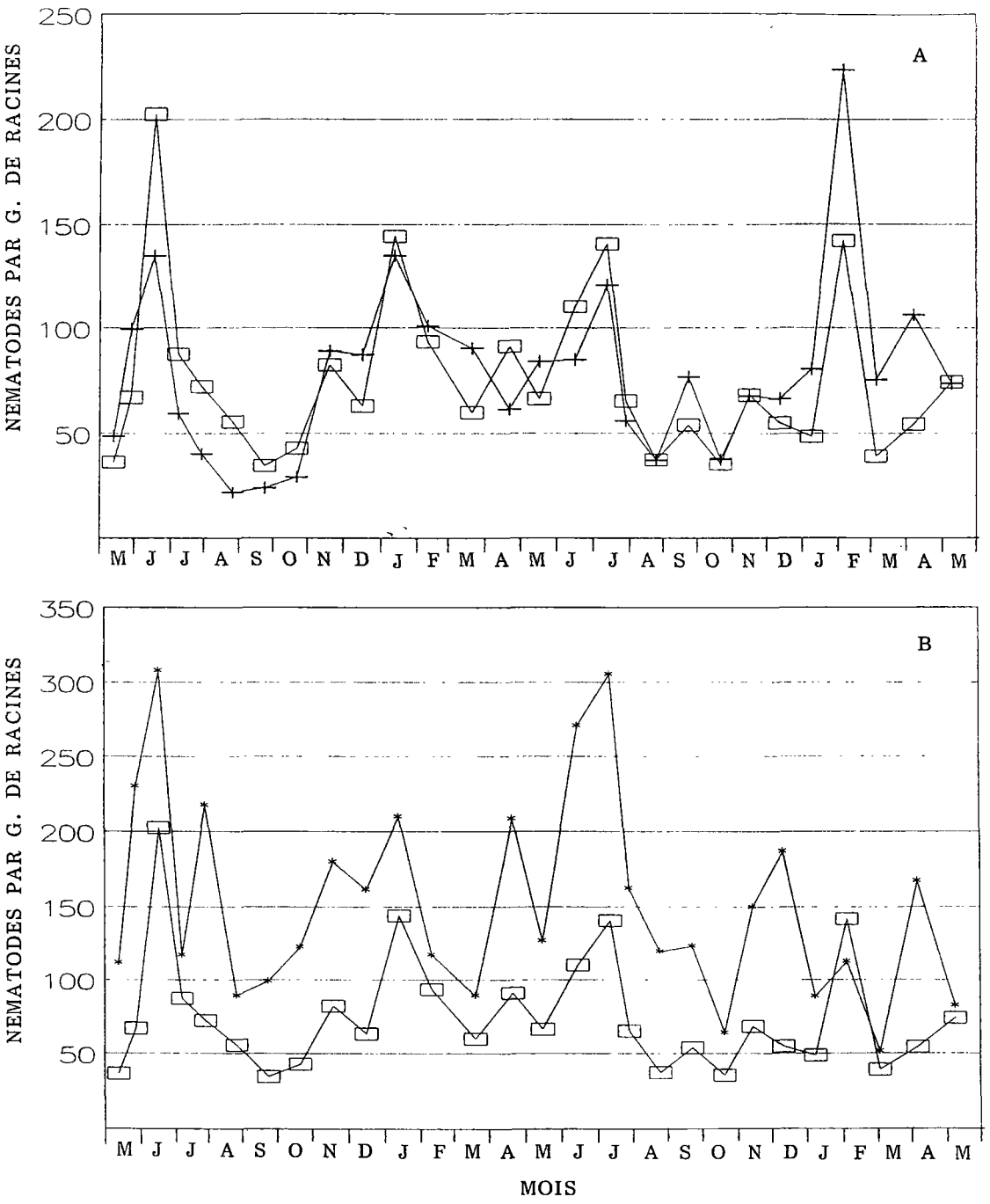

Fig. 3. Fluctuations saisonnières de population des différents stades de Pratylenchus coffeae dans les racines de caféiers sur la station Buena Vista de mai 1989 à mai 1991. A : □ : Adultes; ⊣ : Larves. B : □ : Adultes; ✳ : Oeufs.

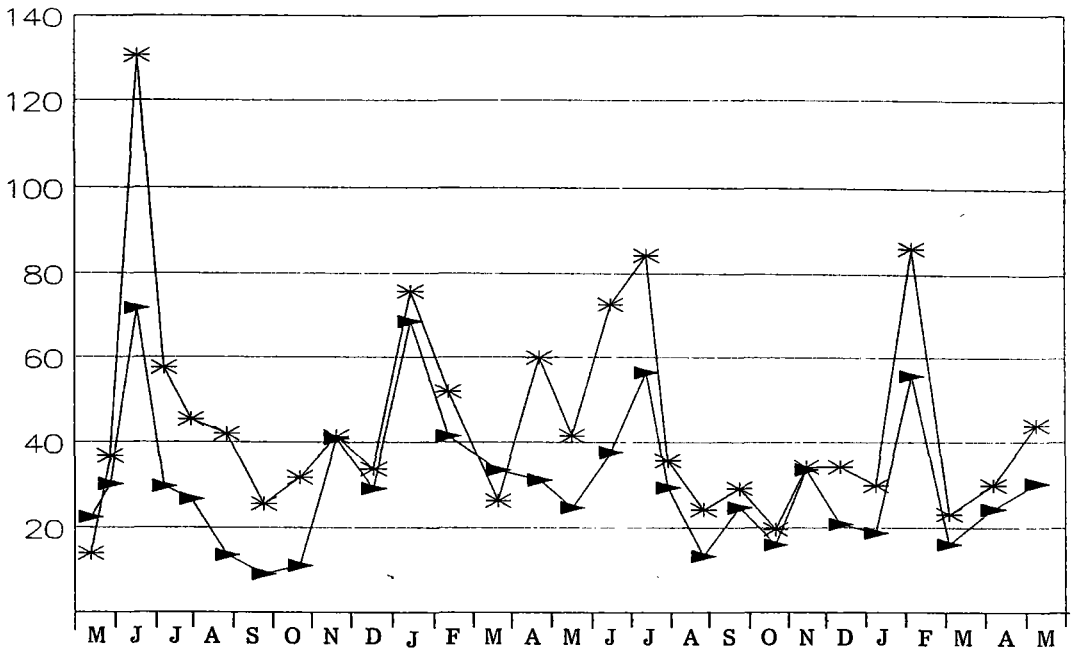

Fig. 4. Fluctuations saisonnières des populations de mâles : ►, et de femelles : ✳, de Pratylenchus coffeae dans les racines de caféier sur la station de Buena Vista (Mai 1989 à mai 1991).

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Résumé :

Les nématodes représentent, de manière générale, un problème très grave pour la caféiculture d'Amérique Centrale, et plus particulièrement P. coffeae, qui est l'espèce ayant l'impact économique le plus important sur le café au Guatemala.

La dynamique de population de ce nématode a été suivie durant deux ans dans une plantation adulte de caféiers arabica. Au niveau des racines, la dynamique de population de cette espèce est régulée dans les conditions de l'étude par la pluviométrie et la physiologie de renouvellement du système racinaire. On observe deux pics de populations au cours de l'année. Le premier a lieu durant la saison sèche, époque correspondant au maximum de renouvellement des racines. La deuxième intervient au début de la saison des pluies, une fois celle-ci bien établie, alors que durant l'époque de maximum de pluviosité (août-septembre), les populations diminuent fortement. Au cours de l'année, la structure des populations de ce nématode reste relativement stable et les pics des différents stades coïncident.

Summary : The dynamics of populations of Pratylenchus coffeae Zimm on coffee plants in south west Guatemala

Nematodes in general are a very serious problem for coffee growers in Central America. In particular, P. coffeae is the species with the greatest economic impact on coffee in Guatemala.

The dynamics of populations of this nematode were observed for two years in an adult arabica plantation. At root level, the dynamics of populations of this species are regulated, in the conditions of study, by rainfall and the physiology of renewal of the root system. Two population peaks occur during the year. The first is during the dry season, when root renewal is at a maximum. The second comes at the start of the rainy season, as soon as the latter is well established, whereas in August and September, the period of maximum rainfall, populations drop significantly. Over the year the population structure of this nematode remains relatively stable, and the peaks of the different stages coincide.

RECHERCHE DE LA RÉSISTANCE À *MELOIDOGYNE* SP. DANS UNE COLLECTION DE *COFFEA ARABICA*

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1. INTRODUCTION

Le problème des nématodes chez le caféier a été signalé depuis longtemps (Jobert, 1878; Goeldi 1887; Zimmermann, 1898). Mais le problème est resté presque oublié pendant plusieurs années. Ceci peut s'expliquer par le manque de spécialistes et la carence d'information technique et surtout parce que les dégâts qu'ils provoquent, qui sont de caractère insidieux, ont été attribués à d'autres facteurs, comme par exemple les problèmes de fertilité des sols.

Avec la pérennisation de la monoculture du caféier le problème est devenu plus important. Plusieurs chercheurs ont signalé que le transport des plants à partir de pépinière infestée par les nématodes est vraisemblablement la cause principale de leur dissémination (Schieber, 1968; Gonçalves et al. 1978; Arango et al., 1982).

Les nématodes les plus importants, affectant le caféier sont : Meloidogyne spp et Pratylenchus spp (notamment Pratylenchus coffeae). On trouve communément le genre Meloidogyne sur racines de Coffea arabica. Sasser (1979) signale une diminution de 10 p. cent de la production en Amérique Centrale, au Mexique et dans les Caraïbes, à cause de Meloidogyne exigua.

Actuellement, la méthode de lutte la plus employée contre ces déprédateurs est l'utilisation des nématicides. Toutefois, si les traitements chimiques sont efficaces, ceux-ci posent certains problèmes; ils sont chers et leur application, relativement dangereuse, n'est pas toujours à la portée des petits producteurs, qui sont très nombreux dans les pays d'Amérique Centrale.

La lutte génétique par utilisation de variétés ou porte-greffe résistants, apparaît comme une des possibilités particulièrement intéressante de la lutte intégrée contre les nématodes du caféier.

Le présent document expose et discute les travaux réalisés au Laboratoire de Nématologie du CIRAD, Montpellier, dans le but de rechercher des sources de résistance.

2. MATERIEL ET METHODES

2.1 LE MATERIEL VEGETAL

Les lignées expérimentales en évaluation, sont issues de graines récoltées sur de plantes de C. arabica dans la collection de l'Institut de Recherches Agronomiques du Cameroun (IRA) à Foumbot. Pour la plupart, il s'agit de lignées d'origine semi-spontanée d'Ethiopie, collectées par une mission ORSTOM en 1966 (Charrier, 1978).

Ces origines ont montré des degrés differents de résistance à Hemileia vastatrix (Muller, 1978) et au CBD, Colletotricum coffeanum (Charrier, 1985).

2.1 LES NEMATODES (Meloidogyne sp)

La population de Meloidogyne sp. utilisée provient de racines de C. arabica originaires du Guatemala. Dans un premier temps on a inoculé des petits caféiers en serre avec des nématodes extrait de ces racines. L'inoculum a été par la suite maintenu sur des plants de tomate (variété "Nainemor").

Pour la identification de l'espece, l'étude des isoestérases a été realisé sur des extraits des femelles. On a obtenu zymmograme différent (Figure 1) des zymmogrames des espèces Meloidogyne incognita et M. exigua (Esbenshade et Triantaphyllou, 1990). Des études morphologiques et des nouvelles caractérisations biochimiques sont en cours.

Le criblage variétal a été fait avec une culture pure de Meloidogyne sp. (provenant d'une seule masse d'oeufs).

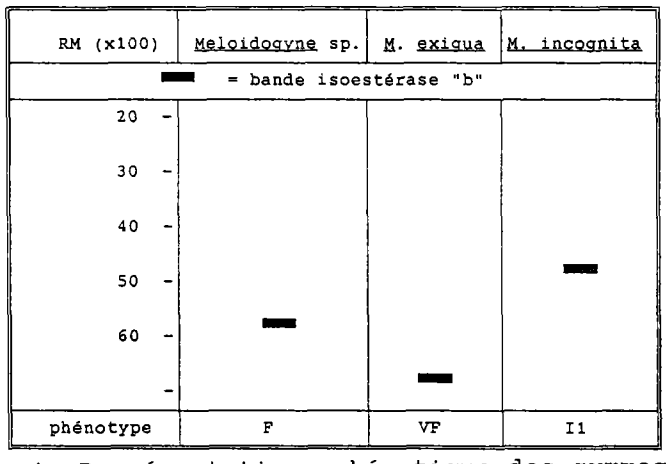

Fig. 1. Représentation schématique des zymmogrames de Meloidogyne spp.

2.3 CONDUITE DES PLANTS

Lorsque les plants de caféiers issus de semis ont atteint le stade "papillon", ils ont été repiqué dans des pots en matière plastique, de 500 cm^3 pour le premier essai, et de 750 cm^3 pour le deuxième, et remplis avec une mélange de terreau et de sable (proportion 2:1 - pH : 5.5 -) stérilisés (four Pasteur pendant 1 heure à 180 °C).

Les plantes repiquées sont restées deux mois en serre, où la température varie entre 20 et 30 °C et où l'humidité relative oscille entre 50 et 90 %. Ensuite, les plantules ont été placées dans une cellule climatisée à température constante (25°C), dont l'humidité relative oscille entre 75 et 85%.

2.4 OBTENTION DE L'INOCULUM

L'inoculum utilisé est constitué de juvéniles du 2ème stade (J2). Des racines de tomate infestées sont broyées (au mixeur) et passées sur une colonne de tamis, et ensuite les larves de la suspension sont récupérés par une technique de macération aérobie (fig. 2). Au bout de 18 heures on récupère l'eau de trempage. La suspension de larves obtenue est placée dans un tube puis dénombrée au microscope pour ajuster la dose à inoculer. La suspension acqueuse préalablement dosée est déposée autour de chaque plante de caféier, à l'aide d'une micropipette (60 jours après le repiquage des plantules).

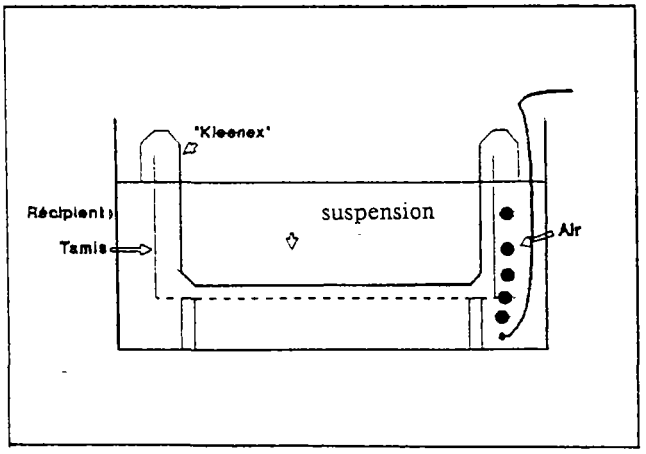

Fig. 2. Dispositif pour la récupération de l'inoculum d'après Sarah (1991).

3. ETUDES REALISEES

3.1 ESSAI n° 1

3.1.1 DEROULEMENT

On a évalué 38 lignées de C. arabica, dont 33 appartenant aux origines éthiopiens de la prospection ORSTOM de 1966 (on a utilisé une mélange de semences de quelques plantes de chaque descendance), comme témoins sensibles on a utilisé la variété Catuai et 4 autres variétés (Mulungu, Coorg et 2 lignées de Catimor). On a utilisé un dispositif simple en randomisation totale à quatre répétitions (une plante par pot) avec une dose d'inoculum de 100 juvéniles (J2)/caféier.

Les observations ont été réalisés quatre vingt dix jours après l'inoculation. L'extraction des nématodes (oeufs et juvéniles), dans la totalité des racines, est réalisée après broyage et tamisage, par la technique de centrifugation-flottaison. La suspension de nématodes est ramenée à un volume constant; puis leurs nombres (oeufs + juvéniles) sont déterminés par observation au microscope et rapportés au gramme de racine.

Pour l'étude de la réaction des plantes vis-à-vis de Meloidogyne sp., on a calculé le taux de reproduction relative du nématode (rapport entre la densité de population des nématodes chez les plantes testées et la densité de population moyenne du témoin Catuai). Chaque plante est classée selon un indice de reproduction relative (IRR) correspondant à l'échelle suivante :

indice (IRR)	taux de reproduction relative $\frac{\text{densité pop. plante}}{\text{densité pop. } \bar{X} \text{ témoin}} \times 100$
0	0
1	1 - 10%
2	11 - 25%
3	26 - 50%
4	> 51%

On a estimé aussi pour chaque génotype, l'indice pondéré d'infestation (IPI) en utilisant la formule (Arango et al, 1982):

$$IPI = \frac{(\text{Nb plantes } i = 1) \times 1 + \ldots (\text{Nb plantes } i = 4) \times 4}{\text{Nb total plantes} \times 4}$$

Chaque plant individuel est considéré comme résistant, s'il présente un indice égal ou inférieur à 2 (Gonçalves et Ferraz, 1987). Les lignées sont considérés comme sensibles si elles présentent des IPI supérieurs à 0.50 (Arango et al., 1982; Fassuliotis, 1985).

3.1.2 RESULTATS

Le Tableau 1 montre pour chaque lignée évaluée l'IPI, ainsi que le nombre de plants se plaçant dans chacune des classes (0,1,2,3 et 4) de l'IRR.

Les variétés Coorg et Mulungu (Mu 1) et les Catimors (T 12870 et T 8667) montrent tous des IPI égaux ou similaires a celui du témoin Catuai, ceci qui est en accord avec ce que l'on prévoyait pour ces variétés, réputées hautement sensibles.

Les résultats montrent une grande variabilité entre les différentes origines éthiopiennes étudiés. On observe également une variation importante à l'intérieur de certaines lignées.

Dans ce grand groupe on rencontre ;

- des lignées présentant des IPI similaires à celui du témoin Catuai : ET 19, ET 51.

- des lignées presentant des IPI assez proches du témoin, mais avec quelques plantes classées comme résistantes : ET 11b, ET 21 et ET 53.

- des lignées dont 75 p. cent des plantes sont résistantes et dont l'IPI, inférieur à 0.50, permettre de les considérer comme résistantes : ET 45 C7, ET 36bc2, ET 31, ET 60, ET 37C4 et ET 11c.

TABLEAU 1.

Meloidogyne sp. en génotypes de caféier (essai n° 1) : indice de reproduction relative (IRR) et indice pondéré d'infestation (IPI).

génotype	nombre de plants d'après leur indice de reprod. relative (IRR)					indice pondéré d'infes. (IPI)
	0	1	2	3	4 (**)	
MU 1					4	1.00
CATUAI (*)					4	1.00
COORG					4	1.00
T 12870			1		3	0.88
ET 19				2	2	0.88
ET 51		1			3	0.81
T 8667		1		1	2	0.75
ET 11b		2			2	0.69
ET 21		2			2	0.63
ET 53		2		1	1	0.56
ET 45C7		2	1		1	0.50
ET 45C1			4			0.50
ET 36bc2		3			1	0.44
ET 31		3			1	0.44
ET 60		3			1	0.44
ET 37C4		3			1	0.44
ET 11c		3		1		0.38
ET 24		2	2			0.38
ET 43		3	1			0.31
ET 2		3	1			0.31
ET 14		3	1			0.31
ET 35		3	1			0.31
ET 34		3	1			0.31
ET 35dc6		3	1			0.31
ET 28		4				0.25
ET 26		4				0.25
ET 13		4				0.25
ET 4		4				0.25
ET 17		4				0.25
ET 29bc1		4				0.25
ET 33		4				0.25
ET 18		4				0.25
ET 27		4				0.25
ET 44		4				0.25
ET 50		4				0.25
ET 47		4				0.25
ET 16		4				0.25
ET 40		4				0.25

nombre total de plants par lignée = 4

(*) Variété témoin $\bar{X}$ = 373.52 individus (oeufs + larves)/g

(**) 0 = 0, 1 = 3.73-37, 2 = 38-93, 3 = 94-186 et 4 > 187 ind/g
(0%) (1-10%) (11-25%) (26-50%) (>50%)

0, 1 et 2 = résistante

36bc2, ET 31, ET 60, ET 37C4 et ET 11c.

- enfin, des lignées qui présentent des IPI entre 0.25 et 0.38, et dont toutes les plantes sont classées comme résistantes : ET 40, ET 16, ET 47, ET 50, ET 44, ET 27, ET 18, ET 33, ET 29bc1, ET 17, ET 4, ET 13, ET 26, ET 28, ET 35dc6, ET 34, ET 35, ET 14, ET 2, ET 43, et ET 24.

3.2 **ESSAI n° 2**

3.2.1 DEROULEMENT

Les résultats du premier essai, nous ont conduit à mettre en place un deuxième essai, en augmentant le nombre de répétitions pour pouvoir faire des interprétations plus valables sur la nature de la résistance. 34 origines éthiopiennes (issus de semences de plants individuels de chaque descendance) ont été évaluées, dont :

- 17 génotypes évalués lors du premier essai.
- 17 génotypes nouveaux.

Comme témoin on a utilisé la variété Catuai. On a travaillé avec un dispositif en randomisation totale à 12 répétitions (12 plants regroupées à 4 par pot) la dose de d'inoculation étant de 180 juvéniles (J2) par plante.

L'évaluation de la résistance a été faite quatre-vingt-dix jours après l'inoculation; elle a porté sur la notation du nombre de masses d'oeufs extérieures. Ceci a permis de classer les génotypes en différents niveaux de résistance ou de sensibilité d'après l'échelle de Taylor et Sasser (1978) :

Indice (IMO)	nombre de masses d'oeufs/plant
0	0
1	1 - 2
2	3 - 10
3	11 - 30
4	31 - 100
5	>100

Chaque plant individuel est considéré comme résistant s'il présente un indice de masses d'oeufs (IMO) égal ou inférieur à 2. Les lignées sont considérés comme sensibles s'ils présentent des IPI supérieurs à 2.

3.2.2 RESULTATS

Le tableau 2 montre pour chacun des génotypes évalués, le nombre de plantes classées selon leur IMO et l'IPI. On retrouve ici pour les origines éthiopiennes, la même tendance que dans le premier essai, cet-à-dire, l'existence d'une grande variabilité entre les lignées en évaluation.

Il est possible de différencier quelques sous-groupes comme dans le premier essai ;

- des origines présentant des IPI similaires à celui du témoin avec un pourcentage de plantes sensibles variant entre 70 et 100 p. cent : ET 61, ET 49, ET 19 et ET 53.

- des origines présentant un pourcentage de plants résistants d'environ 75 p. cent : ET 10, ET 21, ET 5, ET 24, ET 11b et ET 30.

TABLEAU 2.

Meloidogyne sp. en génotypes de caféier (essai n° 2): indice de masses d'oeufs (IMO) et indice pondéré d'infestation (IPI).

génotype	nombre de plants d'après leur indice de masses d'oeufs (IMO).						indice pondéré d'infest. (IPI)
	0	1	2	3	4	5 (*)	
CATUAI					7	5	4.4
ET 61					12		4.0
ET 49	1			1	8	2	3.8
ET 19			1	1	10		3.8
ET 53	2		2	1	6	1	3.0
ET 1	2		3	2	2	1	2.5
ET 10	7			1	4		1.6
ET 21	8		2	2			1.2
ET 5	8		1	1	2		1.1
ET 24	9		1		1	1	0.9
ET 11b	9		1	1	1		0.7
ET 30	10				2		0.7
ET 6	8	1	3				0.6
ET 3	10		1	1			0.4
ET 55	11				1		0.3
ET 31	10	1		1			0.3
ET 15	11		1				0.2
ET 41	11	1					0.1
ET 35	11	1					0.1
ET 2	12						0.0
ET 4	12						0.0
ET 8	12						0.0
ET 9	12						0.0
ET 11c	12						0.0
ET 14	12						0.0
ET 16	12						0.0
ET 17	12						0.0
ET 18	12						0.0
ET 20	12						0.0
ET 26	12						0.0
ET 27	12						0.0
ET 28	12						0.0
ET 34	12						0.0
ET 52	12						0.0
ET 54	12						0.0

nombre total de plants par lignée = 12

(*)

0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = > 100 masses d'oeufs/pl

0, 1 et 2 = résistante

- trois origines présentant seulement une plante sensible sur douze (8 p. cent) : ET 3, ET 55 et ET 31.

- enfin, des génotypes dont toutes les plantes sont classées comme résistantes : ET 54, ET 52, ET 34, ET 28, ET 27, ET 26, ET 20, ET 18, ET 17, ET 16, ET 14, ET 11c, ET 9, ET 8, ET 4, ET 2, ET 35, ET 41, ET 15 et ET 6.

4. DISCUSSION

En ce qui concerne les dix-sept génotypes communs aux deux essais, les résultats obtenus sont très semblables d'un essai à l'outre, notamment pour les origines qui presentent de bas indices de reproduction des nématodes : ET 16, ET 27, ET 18, ET 17, ET 4, ET 26, ET 28, ET 34, ET 35, ET 14 et et 2. De même les génotypes ET 19 et ET 53 ont montré des indices de sensibilité assez proches de celui du témoin Catuai.

Dans le premier essai on retrouve un groupe de lignées qui montrent une importante variation intra-lignées, les lignées ET 11b et ET 21 en font partie. Cette grande variabilité pourrait être dûe au fait que l'on a évalué du matériel issu d'un mélange de semences de quelques plantes de chaque descendance. Cependant, dans le deuxième essai on rencontre la même tendance, cette fois-ci, en utilisant des semences collectées sur des plantes individuels. De façon générale, à l'intérieur de ces lignées on peut rencontrer des pourcentages de plantes résistantes variant entre 58 et 84 p. cent. Ceci fait penser à une possible hétérozygotie des plantes mères.

Les deux méthodes utilisées pour les études de la résistance : - quantification des masses d'oeufs extérieures, et - détermination des densités de population après extraction, ont permis toutes le deux, de faire une discrimination semblable des lignées testées, bien qu'elles aient été testées dans deux essais différents. Certaines chercheurs ont déjà signalé qu'il y a une bonne corrélation entre les deux méthodes (Golçalves et Ferraz, 1987).

5. CONCLUSION ET PERSPECTIVES

Les résultats obtenus suggèrent qu'au sein des origines sylvestres de C. arabiça il doit exister un important réservoir de gènes pour la résistance aux espèces du genre Meloidogyne.

Ces résultats laissent penser qu'il s'agit d'une résistance du type monogénique ou oligogénique dominante vis-à-vis de Meloidogyne sp., et la variabilité observée à l'interieur d'une partie des lignées évaluées, peut être expliquée par l'hétérozygotie des plantes mères o par des pollinisations croisées entre lignées résistantes et sensibles.

On peut envisager l'utilisation des génotypes résistants dans les programmes d'amélioration génétique pour la lutte contre les nématodes du caféier. Ce matériel pourrait être utilisé comme source de gènes de résistance dans un programme d'hybridation intervariétale. Dans le cas de gènes de résistance dominants, les hybrides F1, avec des variétés locales, pourraient être utilisés directement.

Cette utilisation devra être précédée d'études complementaires de l'héritabilité de la résistance, et de l'évaluation des meilleures lignées vis-à-vis des autres souches et espèces de Meloidogyne (notamment M. incognita) et de l'espèce Pratylenchus coffeae. Ces travaux sont en cours.

On peut également envisager l'utilisation directe des génotypes éthiopiennes résistants, comme des porte-greffes des variétés cultivées, comme cela se fait depuis plusieurs années avec la variété Robusta de Coffea canephora (Reyna, 1966).

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RESUME

Les nématodes appartenant au genre Meloidogyne sont d'importants ravageurs des caféiers, spécialement de Coffea arabica. Un étude a été entreprise afin d'évaluer la résistance au Meloidogyne sp. de 50 lignées originaires d'Ethiopie (Prospection ORSTOM de 1966).

Les résultats ont montré une grande variabilité pour la résistance à Meloidogyne sp. Les génotypes ET 16, ET 27, ET 18, ET 17, ET 4, ET 26, ET 28, ET 34, ET 35, ET 14 et ET 2 ont présenté de bas indices de reproduction des nématodes.

On peut envisager l'utilisation des génotypes résistantes dans les programmes d'amélioration génétique de C. arabica dans les pays où les nématodes de Meloidogyne spp posent des problèmes pour la caféiculture.

SUMMARY

The nematodes belonging to the genus Meloidogyne constitute important pests of coffee cultivation, specially of Coffea arabica. The objective of the present research was to evaluate resistance to Meloidogyne sp. of 50 introduction of C. arabica from Ethiopia (ORSTOM's prospection of 1966).

The results have shown great variation in resistance to Meloidogyne sp. among the Ethiopian introductions. The genotypes ET 16, ET 27, ET 18, ET17, ET 4, ET 26, ET 28, ET 34, ET 35, ET 14, et ET 2 have shown a light degree of reproduction of the nematode.

The main conclusion of the present study is the possibility to use the selected resistant coffee types in coffee breeding programmes in countries where Meloidogyne nematodes constitute a problem of coffee cultivation.

INNOVATION TECHNOLOGIQUE ET QUALITÉ
Un exemple d'amélioration spectaculaire : le café Arabica au Burundi

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INTRODUCTION

Le monde économique est en pleine mutation. "Il ne s'agit pas de constater le déclin d'activités traditionnelles au profit de technologies et d'industries nouvelles, mais de comprendre la transformation subtile et profonde du rapport existant entre le coût de la main-d'oeuvre, du capital et de l'énergie.

Le rapport information/masse propre à chaque bien est sur le point de s'inverser."(1). Paul HAWKEN, auteur de ce texte poursuit:"Nous entrons dans une phase de transition entre une structure économique de masse et une structure économique d'information et les difficultés auxquelles nous heurtons actuellement traduisent cette transition. De grandes sociétés sont menacées, des banques sont au bord de la faillite, des gouvernements se succèdent au fur et à mesure que leurs échecs économiques ruinent la confiance des électeurs. Tandis que les gouvernements et les hommes d'Etat hésitent sur la marche à suivre, les consommateurs, les ménages, et les chefs d'entreprise sont déjà en train d'évoluer"...

"L'un des meilleurs moyens pour s'adapter aux conditions nouvelles est de renforcer le contrôle de qualité (2).Pendant un siècle, l'économie s'est développée à l'aide d'énergie de moins en moins chère. Ce mécanisme favorisait la vitesse de production, la quantité et la taille comme base du gain, de la croissance, de la puissance. Aujourd'hui, au contraire, il convient de mettre l'accent sur le soin apporté à la production, l'unité des produits et leur qualité. La qualité c'est le contraire du gaspillage, c'est ce qu'implique le terme même d'économie.

Si notre économie est en contraction, ce n'est pas comme en 1930 ou à d'autres périodes de crise. Nous sommes dans une phase d'adaptation et

de sélection naturelle où tous les aspects de notre économie, de la production à la consommation vont subir une révolution: le passage de la masse à l'application accrue d'intelligence et de coopération. Dans l'économie d'information, les rapports entre industrie, main-d'oeuvre et consommateurs seront tout autres qu'ils n'ont été; la qualité n'est pas seulement une technique, c'est une attitude qui exige la communication et l'entente."

La qualité, selon la norme française, c'est l'aptitude d'un produit ou d'un service, à répondre aux besoins des utilisateurs. Cette définition permet de passer de la notion de qualité du produit à celle de la qualité de l'entreprise ou structure qui le génère; la première étant garantie par la seconde. Cette démarche, qui consiste à remonter de l'effet aux causes, est fondamentale dans toute la démarche qualité car elle associe bien l'entreprise à la gestion de la qualité:

- définition d'une politique
- mise en oeuvre des moyens
- mesure des écarts
- correction des écarts

UN EXEMPLE D'AMELIORATION DE LA QUALITE DU CAFE:LE BURUNDI

L'exemple pris dans cet exposé pour illustrer mon propos sur la défense de la qualité vise un petit pays producteur, le Burundi, qui a consenti un gros effort en peu d'années par ses investissements et son changement radical dans ses approches techniques, commerciales et économiques.

Bien lui en a pris, car dans une conjoncture mondiale désastreuse pour les matières premières, notamment le café, il a pu résister au choc économique sans trop de dommages et a acquis une réputation de sérieux auprès des acteurs financiers. En une bonne dizaine d'années, les structures ont été bouleversées avec un résultat positif mais aussi les mentalités d'"ilien" se sont ouvertes pour se tourner vers le monde extérieur illustrant bien le propos antérieur que la qualité est une question d'état d'esprit.

Lorsque je visitai ce petit pays producteur enclavé au sein du centre de l'Afrique, je connus ce sentiment de l'économie de qualité nécessaire à sa subsistance, la vente du café intervenant pour plus de 90% dans ses revenus à l'exportation. Tous les défauts se cumulaient malgré les potentialités naturelles:

- faible technicité agronomique
- cueillette grossière des fruits
- traitement post-récolte hétérogène et mal conduit alors que toutes les précautions sont à prendre durant cette phase cruciale pour la qualité finale
- achat sans prise en compte de la qualité et toujours aux dépens de l'agriculteur (poids)
- transport dans des véhicules non étanches et utilisés à de multiples emplois qui favorisent par contact, des souillures et risques de communication d'odeurs étrangères

- stockage du café en parche en des lieux ou des locaux inadaptés
- manque de contrôle de qualité à l'entrée des usines de conditionnement
- équipement insuffisant et obsolète de ces usines
- stockage prolongé du café vert dans des conditions climatiques et matérielles peu favorables
- transport lent vers le port d'exportation (Dar es Salaam) avec nombreuses ruptures de charge
- services commerciaux inadaptés chargés des ventes à l'exportation
- manque de moyens de communication modernes et fiables permettant de traiter les actions commerciales avec rapidité
- manque de relations commerciales avec d'autres acteurs les acheteurs traditionnels
- ventes par un comité qui se réunissait une fois par semaine en visant la spéculation alors que les moyens d'information étaient insuffisants
- transport du café vert "prêt export" uniquement par la voie ferroviaire et lacustre peu fiable (en temps et en sécurité:"freinte moyenne de 2%)
- absence de recherches systématiques sur le café alors que ce produit est essentiel à l'économie nationale

C'est donc à tous les niveaux qu'il convenait d'intervenir: technique, commercial, logistique et économique. Si nous considérons le côté purement technique, là aussi il était nécessaire d'intervenir tout le long de la filière et non pas seulement au niveau des usines de conditionnement comme on aurait tendance à le croire: "...il faut être averti du fait, qu'aussi soignée que soit la préparation, elle ne peut améliorer les qualités du café, mais qu'il est très aisé d'abîmer un café par une préparation mal conduite. Les qualités intrinsèques de la fève dépendent de la variété cultivée, du sol, du climat, de l'état de maturité, des attaques éventuelles d'insectes, des pratiques culturales"(3).

Il ne fallait donc pas se borner à améliorer un point de la filière café, à savoir le conditionnement, mais travailler la qualité à tous les niveaux et notamment en amont; directement à la récolte.

Parallèlement à la construction des usines de conditionnement qui disposent d'un matériel moderne et efficace, des unités de traitement par voie humide ont été multipliées dans les zones de production et ont facilité le contrôle de la qualité des fruits amenés par les nombreux petits producteurs. C'est la condition essentielle pour atteindre de hauts standards de qualité; nous connaissons tous des exemples comparatifs de structures caféières dans lesquelles il n'a pas été tenu compte du traitement post-récolte direct et du contrôle indispensable: situation confortable à court terme mais désastreuse à long échéance.

ANALYSE DU SYSTEME TRADITIONNEL

1)Méthode de traitement traditionnelle (section humide)

Le traitement post-récolte consistait à dépulper le café dans de petites unités de dépulpage munies d'un dépulpeur à disque manuel. Ces petits centres étaient équipés de bacs de lavage des cerises et de bacs permettant d'éliminer le mucilage et lavé les fèves en parche. Ces centres multiples distribués à travers le pays (on en compte plus d'un millier) présentaient de nombreux inconvénients préjudiciables à la qualité finale du produit:

- difficulté d'éliminer le mucilage directement après dépulpage sans procéder à une phase de fermentation permettent une hydrolyse complète du mucilage.

- mauvais entretien mécanique général du matériel, réglages non effectués, remplacement des pièces défectueuses non suivi, prélèvement des pièces du dépulpeur pouvant servir à d'autres fins,...

- matériel rarement nettoyé après utilisation d'où prolifération des microorganismes sur la pulpe ou les grains laissés dans l'appareil et production de goûts indésirables tels que "foul", "puant", "putride" dans la boisson finale si par mégarde ces grains contaminés passent dans un lot destiné à l'exportation.

- amenée d'eau colmatée, bacs de réception souillés, aires de travail mal entretenues favorisant toutes les pollutions imaginables.

- utilisation d'eaux chargées en matières organiques et minérales par manque de filtration physique élémentaire.

Se débarrasser du mucilage sans pratiquer la fermentation est difficilement concevable sans disposer de moyens mécaniques, chimiques ou enzymatiques. Or, les paysans amenant leur café dans ces centres éprouvaient de grande difficultés à se débarrasser totalement du mucilage.

En fait, la plupart des grains en parche étaient séchés alors que des fragments de mucilage restaient adhérents.Lors du séchage solaire, ce mucilage devenait le siège de fermentations indésirables qui communiquaient des arômes désagréables évoqués ci-dessus dans des termes bien sentis.

Devant les difficultés de la démucilagination, les fermiers rentraient chez eux avec leur café dépulpé et le laissaient dans les paniers afin qu'une fermentation naturelle s'installe et hydrolyse les pectines. Ces durées de fermentation étaient laissées à l'appréciation du paysan et tous les cas de figure étaient envisageables : la couleur des parches permettaient de juger des mauvaises pratiques en usage.

Les conditions de séchage étaient loin d'être satisfaisantes: durée d'exposition au soleil insuffisante, aire de séchage peu soignée, incorporation de matières étrangères si l'opération se déroule sur la terre battue. Le café en parche préparé dans de telles conditions

présentait une parche grisâtre, terne peu attrayante et comprenait pierres,poussières,branchettes, feuilles,...

2)Unités de conditionnement

La totalité du café en parche descendait des zones de production pour être traité dans quatre unités de conditionnement localisées à Bujumbura.

Capitale mais aussi et surtout port d'embarquement sur le lac Tanganyika, le café "export" est acheminé vers Kigoma (Tanzanie) par voie lacustre où il est transféré sur wagon et acheminé par rail vers Dar es Salaam.

Le choix de Bujumbura comme site de conditionnement du café était certes justifié puisq'il s'agit d'une voie obligatoire de transit avant embarquement. Par contre, situé à une altitude de 700 mètres, Bujumbura n'est peut-être pas le meilleur site de stockage pour une bonne conservation du produit, d'autant plus que le voyage ferroviaire pour traverser la Tanzanie relève parfois de l'aventure puisque les statistiques de transport révèle une durée de transfert variant de 4 jours à 4 mois!! Et à Dar es Salaam, port situé en zone particulièrement chaude et humide, les conditions de stockage ne sont certes pas les meilleures (Tmoy= 23°C ; HRmoy= 73%).

Le stockage pouvant se prolonger dans de telles conditions, il était illusoire d'envisager l'exportation de lots de qualité.

De toute manière, les cafés arrivant dans les usines de traitement de Bujumbura ne pouvaient qu'être de piètre qualité et ils ne pouvaient qu'être déparchés et triés tant bien que mal dans des unités de conditionnement complètement obsolètes à l'exception d'une des unités dont le matériel dépassait à peine 20 ans d'âge.

Les chaînes de traitement étaient élémentaires et peu propices à l'élaboration d'une qualité correcte susceptible de se défendre sur le marché international:

- réception manuelle avec pesée sac par sac de lots hétérogènes qui n'étaient pas séparés en fonction de leur qualité
- contrôle du degré de siccité" sous la dent"
- prise d'échantillon à l'aide de sonde qui ne peut prendre en compte les grosses particules de matières étrangères
- stockage du café en parche en sacs (ceux-ci n'étaient pas vidés sur une grille à larges mailles pour vérifier si des pierres ou autres grosses particules de matières étrangères n'avaient pas été ajoutées. On ne pouvait s'en apercevoir qu'au cours de l'usinage. Les sacs étaient empilés manuellement dans des hangars ou à l'extérieur (en espérant qu'il ne pleuve pas) en pyramides énormes qu'il fallait ensuite désempiler dans des conditions souvent périlleuses.
- nettoyage grossier dans des tarares vieux et inefficaces
- déparchage dans des appareils maintes fois ressoudés et usés ayant subis de nombreuses recharges métalliques faute de pièces de rechange.
- énergie de déparchage importante de l'ordre de 15 à 18 kWh/tonne de café vert
- mauvaise pureté de classement des trieuses granulométriques dont les perforations ne correspondaient plus aux normes par usure des

ouvertures; colmatage fréquent des trous des tamis par des brosses de débourrage usées et mal adaptées d'où pertes de rendement

- les lots de café subissaient un triage granulométrique permettant une séparation des catégories selon la taille des grains, étaient séparés densimétriquement dans des catadors pour éliminer uniquement les particules les plus légères et la poussière, mais étaient ensuite remélangés car il n'existait plus aucun marché en fonction du calibre.
- le dépoussièrage était une opération quasi inexistante et les conditions de travail se révélaient souvent effroyables
- les organes de manutention étaient réparés avec des moyens de fortune; notamment les vis étaient rechargées par soudure d'un fer à béton autour des spires!
- l'usinage saisonnier étalé sur quelques mois de l'année exige une puissance installée importante alors que le transport et le stockage du café vert duraient de nombreux mois dans des conditions peu favorables à une bonne conservation
- l'usinage saisonnier nécessite parallèlement une concentration importante de main-d'oeuvre temporaire qualifiée ou non; on accumule ainsi les paramètres influençant les prix de revient d'usinage
- le triage colorimétrique manuel exigeait une main-d'oeuvre abondante, pas toujours vigilante vu la pénibilité de la tâche.
- le café marchand était conditionné dans des sacs trop grands et les marques imprimées des deux côtés; ces motivations paraissent mesquines mais quand on connaît le prix élevé de la sacherie gagner près de 20% sur ce poste se justifie pleinement
- les conteneurs étaient remplis avec des lots de 250 sacs de 60 kg (15 tonnes) alors qu'ils peuvent en contenir 275 soit un supplément de 10% !

C'est donc à l'ensemble de la filière qu'il fallait s'attaquer pour apporter des améliorations techniques, économiques, financières, commerciales et logistiques et provoquer un élan dans la formation du personnel si on voulait fixer les mentalités de tous les acteurs sur l'objectif "qualité".

AMELIORATION DES PRATIQUES AGRONOMIQUES

Il n'est pas de notre ressort d'exposer ici les améliorations apportées dans les pratiques agronomiques mais nous voudrions cependant citer quelques faits mettant en évidence les coordinations nécessaires entre les diverses disciplines scientifiques afin de résoudre les problèmes posés qui sont souvent d'ordre complexe:

1) un des défauts caractéristiques du café de l'Afrique de l'Est réside dans l'existence de grains à "goût de patate", encore appelé "peasy" ou "erbsig" selon les acheteurs. Des études anciennes conduites par Stolp (4) avaient montré que cet "off flavour" était dû à l'introduction d'une bactérie du genre Xanthomonas dont l'agent vecteur était une mouche banale, Ceratitis ("fruit fly") mais le mécanisme était mal connu. Gibson (5), au Kénya, avait également mis en évidence ce phénomène très préjudiciable à la qualité finale de la boisson car quelques grains suffisent pour développer ce goût désagréable dans un tambour de torréfaction.

Il s'avère que les fruits sur l'arbre manifestent déjà cette odeur forte mais non systématique; seuls quelques rares fruits sont atteints d'où la difficulté de détection, soit sur l'arbre, soit dans la suite des opérations. Vitzthum (6) a démontré que ce goût de "peasy" était dû à la présence de la 2 isopropyl 3 méthoxy pyrazine, ce que nous confirmons en y ajoutant la 2 isobutyl 3 méthoxy pyrazine moins caratéristique mais présentant le même défaut d'arôme. Nous avons trouvé récemment que des entérobactéries du genre Erwinia peuvent être responsables de l'élaboration du goût de pois, cette bactérie isolée et mise en culture a développé les composés cités ci-dessus avec une très forte intensité.

Il est fort probable que plusieurs types de bactéries peuvent synthétiser ces arômes responsables du goût de pois et qu'elles soient introduites par des agents vecteurs tels que les mouches du fruit mais il reste à expliquer pourquoi un plus grand nombre de fruits ne présentent pas les mêmes caractéristiques car le pourcentage de fruits attaqués est relativement faible.

Parallèlement à ces études entreprises par une équipe comprenant entomologiste, bactériologiste et chimiste, au plan pratique, la technologie peut apporter une solution car les grains provenant des fruits attaqués répondent à la lumière ultra-violette et il s'avère donc possible d'éliminer ces grains défectueux, qui ne présentent aucune anomalie à l'oeil nu ni en présentation physique, ni en couleur.

Le planteur pourrait également traiter à l'aide d'insecticide mais il ne perçoit pas très bien la nécessité de ce traitement car les fruits ne font pas l'objet de dégâts qui entraîneraient des pertes de rendement.

2) La couleur des grains de café du Burundi est généralement pâle et ce phénomène peut être dû à une carence en Zn ainsi que nous avons pu le vérifier sur des échantillons issus de parcelles traitées. Le Zn peut intervenir dans la synthèse d'acide aminé tel que la phénylalanine qui combinée avec les acides chlorogéniques et les acides phénols peut intervenir sur le blanchîment des grains (7). Les sols du Burundi, souvent carencés en cet élément, peuvent influer sur la couleur des

grains et aussi éventuellement l'arôme par abaissement du taux d'acide aminé tel que la phénylalanine dont on connaît le rôle en tant que précurseur.

AMELIORATION DU TRAITEMENT PAR VOIE HUMIDE

Comme signalé dans l'introduction, les fruits étaient dépulpés dans de petits centres villageois par les producteurs eux-mêmes qui devaient ensuite assurer l'élimination du mucilage par frottement (opération bien difficile quand on la pratique mécaniquement), le lavage, le transport du café parche humide et le séchage solaire sur les lieux de la plantation.

La nouvelle structure a permis d'implanter des centres de traitement des fruits comprenant:

- réception et contrôle de qualité des fruits

Avant réception définitive, l'état de maturité et de fraîcheur des fruits est contrôlé; ces deux facteurs sont des plus importants sur la qualité finale de liqueur: seuls les fruits murs développent le plein arôme (présence des sucres et A.A.nécessaires et en bon équilibre) mais il est indispensable qu'ils n'aient pas séjourné en tas durant une période trop longue après la cueillette car une fermentation alcoolique s'installe, la masse s'échauffe et de nombreuses réactions enzymatiques s'installent aboutissant à la formation de composés de types aldéhydes, cétones et esters notamment le méthyle 2 butanoate d'éthyle et l'acétate d'isoamyle qui caractérisent les lots contaminés par les fèves puantes. Cette phase de réception est en conséquence cruciale pour la qualité du produit.

En cas d'échauffement accidentel des fruits, les lots sont refusés. En ce qui concerne l'état de maturité, les fruits rouges sont seuls acceptés; en cas de négligence, les apporteurs sont priés de trier les cerises sur des tables mises à disposition sur une aire proche de l'atelier. Une telle pratique décourage rapidement toute tentative de fraude.

Deux types de bacs de réception existent: réception sous eau avec bac muni de siphon de manière à séparer les drupes légères, feuilles et branchettes éventuelles ainsi que les pierres et bac de réception à sec avec alimentation par écluse (rotary feeder).

- dépulpage dans un appareil à disques en fonte qui donne satisfaction sur le plan technique bien que basé sur un procédé très ancien. On peut reprocher à cet appareil une consommation excessive en eau nécessaire à l'entraînement des pulpes et au dépulpage proprement dit.

- séparation des grains en parche selon la densité dans un pregraader Aagaard en sortie de dépulpage. Cette pratique permet de séparer les fermentations de grains lourds de qualité supérieure des grains légers qui sont généralement moins appréciés. Cet appareil a surtout comme avantage de pouvoir récupérer des grains sains issus de cerises flottantes. Effectivement, dans un pourcentage élevé de cas, un des grains de ces cerises est dense et peut parfaitement être récupéré dans la fraction lourde (8,9).

- fermentation mixte à sec et ensuite sous eau de manière à accélérer la première phase de l'opération et la prolonger au maximum. Cette opération de fermentation assure l'hydrolyse du mucilage mais il faut également y voir un affinage car parallèlement se produit une extraction de matières solubles telles que acides phénols, acides chlorogéniques, diterpènes, caféine,etc.

- trempage du café après élimination complète du mucilage afin de compléter cette extraction des matières solubles dans l'eau.

- complément de lavage et séparation densimétrique dans des drains laveurs. On pourrait croire que cette opération est inutile puisque une flottation a déjà été exercée dans le pregraader mais il ne faut cependant pas oublié que:

a) la flottaison avant fermentation n'est pas suffisante, les temps de passage sont très brefs dans cet appareil

b) après fermentation, les fèves sont débarrassées de leur mucilage, et n'ont donc plus le même volume ni la même masse spécifique. Après fermentation, le volume des fèves diminuent et la masse volumique augmente ce qui tend à rendre la vitesse de chute des particules plus constante. Si la démucilagination est incomplète pour certaines fèves, il sera possible de les séparer plus facilement des fèves bien débarrassées de tout fragment de mucilage étant donné que la vitesse de chute des particules dans un fluide tel que l'eau est donnée par la relation suivante:

$$V=k\ [\ D(\rho-1)\ ]^{1/2}.$$

Si D et ρ sont le diamètre et la masse volumique de la fève avant fermentation; D' et ρ' après fermentation ; le rapport des deux vitesses aboutit à obtenir une vitesse de chute identique sous la condition que le rapport qui suit soit respecter:

$$D/D' = (\rho' - 1)/(\rho - 1)$$

- égouttage du café lavé sur tables revêtues d'un treillis à larges mailles.

- séchage solaire sur tables de séchage avec fond grillagé recouvert d'une toile en fibre plastique laissant passer facilement l'air et imputrescible ce qui n'était pas le cas de la toile jute. Durant le séchage, le café est fréquemment remué.

Il ne fait aucun doute qu'une telle méthode de traitement soigneusement observée conduise à une excellente qualité de café par rapport à la méthode artisanale encore largement répandue mais qui tend à régresser face à la multiplication des usines de lavage (actuellement au nombre de 100 environ). L'objectif est de doubler le nombre de ces installations dans les prochaines années. Cette nouvelle structure libère du temps au producteur qui peut se consacrer à d'autres tâches notamment dans le domaine vivrier, soigner la cueillette, et être certain de recevoir le juste prix pour le produit de sa récolte ce qui n'était pas toujours le cas en vendant sur les marchés.

Par ailleurs, l'amélioration de la qualité est spectaculaire et à présent le Burundi offre sur le marché ces cafés "Fully Washed" selon l'appellation locale par opposition au café "Washed" appellation pouvant

prêter à confusion pour un café qui n'est en fait pas fermenté. A l'heure actuelle, la production de ce café haut de gamme représente 30% de la production totale et on peut considérer que ce changement a été obtenu en 10 ans mais que l'accélération est particulièrement nette depuis la campagne 1986/87.

PERSPECTIVES

Si de gros progrès ont été réalisés dans l'amélioration de la qualité grâce à l'introduction de ces ateliers de traitement par voie humide et le soin rigoureux apporté à chaque opération, il reste des progrès à accomplir à différents niveaux:

- résolution des problèmes de file d'attente devant un guichet (cf. chaîne de Markov)
- diminution du coût des investissements par simplification du process et du matériel tout en maintenant les qualités finales du produit; en bref optimisation des opérations
- diminution des consommations d'eau au dépulpage, lavage et classement densimétrique dans les drains laveurs
- conception de dépulpeurs nouveaux moins exigeants en eau
- utilisation rationnelle des pulpes et épuration des eaux usées actuellement rejetées dans les rivières (problème mondial)
- amélioration du système de séparation densimétrique en se basant par exemple sur l'hydrocyclone afin de réaliser des séparations systématiques et parfaitement reproductibles alors qu'à présent elles dépendent de l'opérateur humain.L'étude des transferts de quantité de mouvement en analyse dimensionnelle devrait permettre de déterminer à partir des nombres de Re, Ne et Ar (Ar=Ne.Re^2) les vitesses limites de chute des fèves dans l'eau et déterminer les caractéristiques du matériel de séparation densimétrique
- diminuer les surfaces des tables de séchage par récupération de l'énergie solaire et insufflation de cet air légèrement réchauffé par ventilation forcée de faible puissance. Le séchage artificiel est difficilement envisageable vu le coût élevé du fuel au Burundi pays très enclavé; la combustion de la parche n'est pas possible dans la structure actuelle puisque le café est amené tel quel aux usines de conditionnement et il convient de conserver et transporter préférentiellement le café sous cette forme. La production de méthane à partir des eaux usées peut être une solution partielle.

En résumé, des études sur modèles mathématiques dans un premier temps et sur du matériel de conception nouvelle faisant appel aux lois du génie industriel permettraient d'optimiser les conditions de préparation dans les centres de traitement post-récolte. Quant à la préparation des cafés "Washed", l'accent devrait être porté indubitablement sur la formation des producteurs afin de se montrer soigneux dans l'exécution des tâches et l'entretien du matériel: c'est une épreuve de longue haleine mais à long terme c'est l'action la plus payante pour la nation.

AMELIORATION DES USINES DE CONDITIONNEMENT

Nous avons décrit précédemment les conditions de fonctionnement des unités de déparchage et triage qui existaient à Bujumbura. Ces unités ont été remplacées par deux usines de conditionnement, l'une à Bujumbura,

l'autre située à l'intérieur du pays à Gitega en zone de haute altitude. Compte tenu de la forte augmentation des cafés "Fully Washed" (FW) , la première unité est à présent dévolue au conditionnement du café W et celle de Gitega sera de plus en plus consacrée au traitement du FW pour des raisons évidentes :

- localisée à l'intérieur même de la zone de production

- meilleure conservation des cafés de haut de gamme en haute altitude (éviter le blanchîment caractéristique des zones trop humides).

Les deux usines sont conçues selon les bases suivantes:

Réception

- Pont bascule entrée et idem pour la sortie
- Parking des véhicules où sont prélevés les échantillons. En cas de contestation, les véhicules n'encombrent pas les quais de réception
- Laboratoire d'analyse permettant d'apprécier les qualités physiques et dans l'avenir les qualités organoleptiques
- Quai de déchargement par sacs avec grille séparatrice permettant d'éliminer les grosses matières étrangères.

Le café en parche est réceptionné à une teneur en eau de 10,5% et un barème de réfaction est appliqué en fonction de ce paramètre.Au-delà de 12,5% les lots sont refusés. Des campagnes d'information actives ont permis d'atteindre cette valeur sans trop de difficultés de la part des producteurs et tous les opérateurs de la chaîne sont à présent satisfaits et ne contestent plus le bien-fondé de cette notion fondamentale de qualité.

Le café réceptionné passe dans un nettoyeur séparateur permettant d'éliminer les matières étrangères légères ou lourdes ainsi que les parches folles ou grains nécrosés. Le café parche ainsi débarrassé de la plupart de ses impuretés est transféré dans un silo peseur équipé de jauges de contrainte. C'est sur la base de ce poids que l'apporteur sera rémunéré. En cas de contestation, il lui est loisible de reprendre son lot de café en ayant toutefois acquitter les frais de triage.

Après nettoyage et pesage, le café parche est envoyé vers la section séchage ou le stockage en fonction du taux d'humidité.

Le séchage est appliqué aux lots de café excédant 10,5% (jusqu'à la limite de 12,5%) dans des séchoirs verticaux et cette opération entraîne une réfaction sur le prix d'achat. Cette règle se révèle efficace car un faible pourcentage des lots est reséché durant la campagne, il s'agit d'un moyen dissuasif qui a permis de lever un gros problème de qualité qui existait autrefois.

Stockage

Le café parche sec (10,5%) est stocké en vrac dans un magasin ou dans des cellules cylindriques verticales ventilées. L'utilisation de ces cellules permet de différencier les lots en fonction de leur qualité. En cas de nécessité une ventilation est prévue afin d'éliminer les reprises d'eau éventuelle. Ces cellules offrent donc plus de souplesse pour sérier les lots, les transférer vers le séchoir en cas de nécessité ce qui n'est pas le cas du stockage en vrac dans le magasin.

Le café FW est usiné et conservé dans l'usine de Gitega, en haute altitude, afin de lui préserver toutes ses qualités. L'unité de Bujumbura est vouée au traitement de l'Arabica washed et de la faible production de Robusta limitrophe de l'usine mais plutôt en voie de disparition.

Déparchage

Le café parche est repris par voie pneumatique et après passage sur un nettoyeur séparateur est expédié sur un déparcheur Jackson dont le principe est basé sur l'impact contre une paroi métallique. Cet impact est obtenu par la force centrifuge résultant du rotor entraîné par un moteur de 4 KW à une vitesse de rotation de 1450 tours/mn.

Les avantages de ce type de déparcheur sont énormes par rapport aux systèmes de type Engelberg traditionnel:

- faible consommation d'énergie 1 KWh/tonne de café vert au lieu de 18 KWh/tonne pour un smout

- la force acquise par une fève en parche est de l'ordre de 3N avec une vitesse d'environ 70 m/s (accélération supérieure à 1000 g) ce qui correspondrait en appliquant le théorème des impulsions liées aux quantités de mouvement à une durée de déparchage de 6/1000 s. Durant ce très court temps, le grain n'a pas le temps de subir des transferts de chaleur important d'autant plus qu'il est constamment ventilé par la turbine qui introduit simultanément de l'air frais dans l'enceinte de déparchage.

- s'agissant d'un simple impact, le grain ne subit pas de dégradation physique de la couche cireuse et est donc mieux protégé de la pénétration de l'oxygène de l'air (d'où meilleure conservation du grain au cours du stockage) que des fèves ayant subi un déparchage par frottement. Vu les différences de consommation énergétique, il faut bien que celles-ci se transforment en chaleur qui a le temps de temps de se transmettre au produit au cours de l'opération.

- possibilité de stocker du café vert au lieu du café parche (d'où un gain en volume de l'ordre de 20% ce qui n'est pas négligeable dans le coût de construction des magasins)

- la puissance installée est faible et le débit élevé pour un encombrement de machine très réduit (4 KW ; 5 T/h CP ; d rotor= 0,46m).

Les inconvénients de ce matériel:

- la totalité des fèves ne sont pas déparchées en un seul passage vu les forces d'impact qui sont fonction de la masse(2N pour un grain de 2/10 gramme et 3N pour un grain de 3/10 gramme) et des teneurs en eau qui modifie l'élasticité de la parche. Cependant, il est nécessaire de disposer de parche pour assurer le dépelliculage et le polissage éventuel.

- nécessité d'utiliser des alliages résistant à l'abrasion pour les entretoises qui relient les deux plateaux du rotor. Une usure rapide déséquilibre le rotor.

Dépelliculage

Cette opération est un mal nécessaire car elle est actuellement basée sur le frottement des grains et des parches pour éliminer la pellicule argentée d'où frottement et en conséquence échauffement plus ou moins important du grain. Mais il est indispensable d'enlever la pellicule pour permettre le tri colorimétrique dans les trieuses électroniques qui doivent visualiser la couleur du grain de café et aussi pour des raisons de présentation physique lors de la commercialisation.

La seule solution éprouvée jusqu'à présent et la plus satisfaisante réside dans l'utilisation du smout mais il convient de remarquer que malgré toutes les précautions prises, en particulier dans la forme sophistiquée du lining du rotor, on n'échappe pas au frottement et donc à l'échauffement même si celui-ci est fortement limité dans ce type d'appareil.Un rapide et simple calcul montre que le temps de séjour moyen est de l'ordre de 1,7s dans l'appareil ce qui est infiniment plus long que le séjour dans un déparcheur rotatif et permet des échanges de chaleur au niveau des grains. De réels progrès sont à faire dans ce domaine par application d'autres techniques telles que les brosses épointeuses utilisées pour d'autres produits agricoles. C'est sans doute le point faible de cette installation.

Séparation granulométrique

Rien de bien original n'a été introduit à ce niveau si ce n'est l'attention apportée à la pureté de classement réalisées dans des trommels rotatifs étagés peu gourmand en énergie et de grande simplicité mécanique et surtout munis de bobines en bois fortement excentrées par rapport à l'axe qui permettent un décolmatage des plus efficaces. De meilleures tamis de classement granulométrique sont à présent disponibles grâce à la technique du laser qui permet des perforations de tôle sans bavure

Classement densimétrique

Le classement densimétrique est totalement dépendant du classement granulométrique puisque la méthode des similitudes appliquée aux transferts des quantités de mouvement nous enseigne que la vitesse d'équilibre de chute des particules dans un fluide est fonction du diamètre de la particule et de la masse volumique réelle. Il est donc essentiel, avant d'effectuer des classements granulométriques, de calibrer les grains de café afin de fixer le paramètre diamètre pour ensuite obtenir de bonnes séparations densimétriques.

La connaissance du nombre d'Archimède (Ar) obtenu par simple calcul permet de déterminer la valeur du nombre de Re par lecture sur graphique et d'en déduire la vitesse limite pour chaque particule. Trop souvent il n'est pas tenu compte de ces calculs ni d'ailleurs du paramètre altitude qui modifie les débits optimaux à donner aux appareils de séparation densimétrique (catadors ou tables densimétriques).

Au Burundi, des tables densimétriques travaillant en aspiration à travers un diffuseur provoquant la fluidisation des grains sur une surface inclinée vibrante (double inclinaison) grâce à un moteur à balourd. L'investissement de ce matériel est élevé mais le travail qui en résulte est d'une grande efficacité à condition de respecter les vitesses

d'air, d'alimenter régulièrement la surface de tri, de contrôler les inclinaisons pas toujours faciles à maîtriser vu que la table s'incline selon deux axes. Régler autant de paramètres n'est pas aisé et une simulation devrait permettre de faciliter la tâche à partir du moment où on doit régler un appareil en influant sur plus de 3 paramètres. L'intervention de l'informatique serait certainement d'un grand secours.

Triage colorimétrique

Les deux usines sont équipées de trieuses colorimétriques bichromatiques indispensables au tri du café Arabica mais en outre, l'usine de Gitega est également capable de traiter les lots de FW par un tri colorimétrique supplémentaire sous lumière ultra-violette. Certaines fèves paraissent de couleur normale mais peuvent toutefois présenter à la tasse des goûts désagréables: fèves puantes (stinker beans) et aussi les fèves à goût de pois (ou patate) dont nous avons déjà parlé au début de cet exposé.

Ces fèves, à condition qu'elles proviennent de la campagne en cours, peuvent être détectées sous UV et rejetées par les trieuses électroniques. L'investissement d'un tel matériel se justifie car le problème des fèves à goût de pois est particulièrement insidieux: peu de grains sont atteints; par contre ils peuvent communiquer aisément leur défaut aux fèves environnantes lors de la torréfaction.

Toutefois, vu le coût des traitements, il serait préférable d'éliminer au maximum les défauts par des moyens mécaniques moins onéreux pour alléger au maximum le travail des trieuses électroniques et en diminuer le nombre. Toutes les possibilités de réglage existent sur les machines modernes mais il ne faut pas perdre de vue la rentabilité de l'opération. Une élimination plus poussée doit être compensée par une plus-value suffisante du produit trié.

Si sur 100 kg de café brut, on élimine x kg de déchets, il s'agit d'envisager le triage colorimétrique sous la condition que:

$$(100 - x) (P + p) + xP' > 100 (P + F)$$

L'optimisation financière ne doit jamais être négligée dans la recherche de la qualité.

PERSPECTIVES

Malgré les améliorations techniques spectaculaires, quelques difficultés doivent encore être soulevées pour satisfaire au mieux l'objectif de qualité et d'optimisation de la production:

- amélioration du contrôle de qualité notamment les détections précoces de fèves à mauvaise odeur
- accélération des opérations de contrôle par une automatisation plus poussée (analyse d'images et des couleurs par exemple)
- nouvelle conception en matière de dépelliculage
- élimination plus importante des poussières toujours abondante dans le café qui ne cesse de subir une desquamation des cellules au cours des manipulations mécaniques
- introduction d'un épierrage complémentaire à la réception en plus des nettoyeurs séparateurs

- récupération des sous-produits (parches) qui sont actuellement transportées pour être brûlées sur un terrain vague (environ 6 000 tonnes peuvent être transformées en charbon pour utilisation ménagère ou brûlées dans des foyers industriels (briquetterie, séchoirs, ...)
- optimisation mécanique des divers appareils en créant des simulations en fonction des paramètres: inclinaison adéquate des tamis de triage densimétrique et granulométrique, vitesse de rotation du déparcheur en fonction de la teneur en eau et de la masse moyenne des fèves en parche, vitesse d'air pour le classement pneumatique en relation avec l'altitude, l'humidité relative de l'air et sa température
- contrôle plus fréquent du bon fonctionnement des machines en cours d'usinage par des prises d'échantillons systématiques toutes les heures et examen en laboratoire
- optimisation économique du coût des opérations et du prix de revient de l'usinage afin d'améliorer la compétitivité: on ne peut plus opposer les concepts de qualité et de réduction de coût surtout en cette période de surproduction du café. Les usines doivent satisfaire les besoins du client mais elles doivent aussi se montrer soucieuses du coût global qui englobe le coût d'acquisition, le coût de fonctionnement ainsi que le coût de la maintenance.

CONSEQUENCES DE LA MODERNISATION DES INSTALLATIONS

Nous venons de voir les conséquences techniques apportée par la modernisation des centres de traitement par voie humide et des usines de conditionnement. Mais il faut ajouter les conséquences dans divers domaines que les modifications ont entraînées:

1) Domaine économique

- disponibilité d'un grand nombre de journées pour le producteur libéré des tâches de traitement post-récolte qui peut se consacrer à d'autres travaux tels que les cultures vivrières

- meilleure rémunération du planteur apportant son café dans les centres de dépulpage

- meilleure garantie du poids de sa récolte

- meilleure qualité et en conséquence meilleur prix de vente à l'exportation et surtout garantie de vente en cette période où l'offre est nettement supérieure à la demande

2) Domaine commercial

- contacts plus nombreux avec les acheteurs suite à une meilleure information notamment sur la réputation de qualité

- suivi informatisé du trajet suivi par le café

- amélioration des techniques de vente (avec dans le futur proche une vente aux enchères selon le système des "auctions" pratiqué au Kénya par ex.)

- meilleur suivi des cours mondiaux grâce au branchement sur le réseau de communication international

3) Domaine logistique

- expédition rapide des lots de qualité par voie routière (camions porte conteneurs scellés) plus rapide et plus sûr et non plus seulement par voie du rail

- diversification des ports d'expédition et non plus seulement Dar es Salaam

- transport exceptionnel par voie aérienne pour les lots de haut de gamme

- accélération des formalités administratives (autrefois les connaissements arrivaient régulièrement après le lot de café)

4) Domaine de la formation

- nécessité de former des techniciens en mécanique, électricité, informatique, techniques commerciales et financières et surtout possibilités d'emploi fortement limités par le passé

- adaptation des mentalités aux techniques modernes industrielles et formation d'une classe ouvrière et non plus seulement agricole

Le taux de rentabilité ne suffit pas pour juger de l'opportunité de réalisation d'un projet mais il faut tenir compte aussi de (10):

- la politique de vente sur le marché international
- de l'impact psychologique sur la population dû à la construction d'usines modernes dans le pays
- de l'amélioration des conditions de travail et de l'élévation du degré de qualification des emplois
- du développement de centres secondaires auprès des ateliers de dépulpage et des usines de conditionnement.

En une dizaine d'années, l'objectif "qualité" que le Burundi s'était fixé, a permis d'accomplir un bond spectaculaire dans les différents domaines de la filière. L'effort se poursuit mais l'état d'esprit d'une politique volontariste d'amélioration de la qualité existe et il s'agit à du principal catalyseur de la réussite.

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Rapport CCCE 1988

TECHNOLOGICAL INNOVATION AND QUALITY - AN EXAMPLE OF SPECTACULAR IMPROVEMENT : ARABICA COFFEE IN BURUNDI.

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FRANCE.

To obtain a merchantable coffee of quality requires careful treatment at all levels of intervention, from harvest through to storage.
It is the attention to detail, scrupulously applied to all operations throughout the chain of preparation, that ensures the elaboration of a product of consistent quality possessing the organoleptic characteristics demanded by the market.
Knowledge of the constituents of the product, and of the effect of the individual operations on the constituents that are aroma precursors, thus becomes fundamental to the management of post-harvest treatment.
Burundi, a small producer in world terms, set it's strategy on producing quality coffee. In order to guarantee this high quality, innovative techniques were applied to post-harvest treatment.
The improvement in quality was spectacular and this country now exports top quality products, much appreciated on the world market.

INNOVATION TECHNOLOGIQUE ET QUALITE - UN EXEMPLE D'AMELIORATION SPECTACULAIRE : LE CAFE ARABICA AU BURUNDI.

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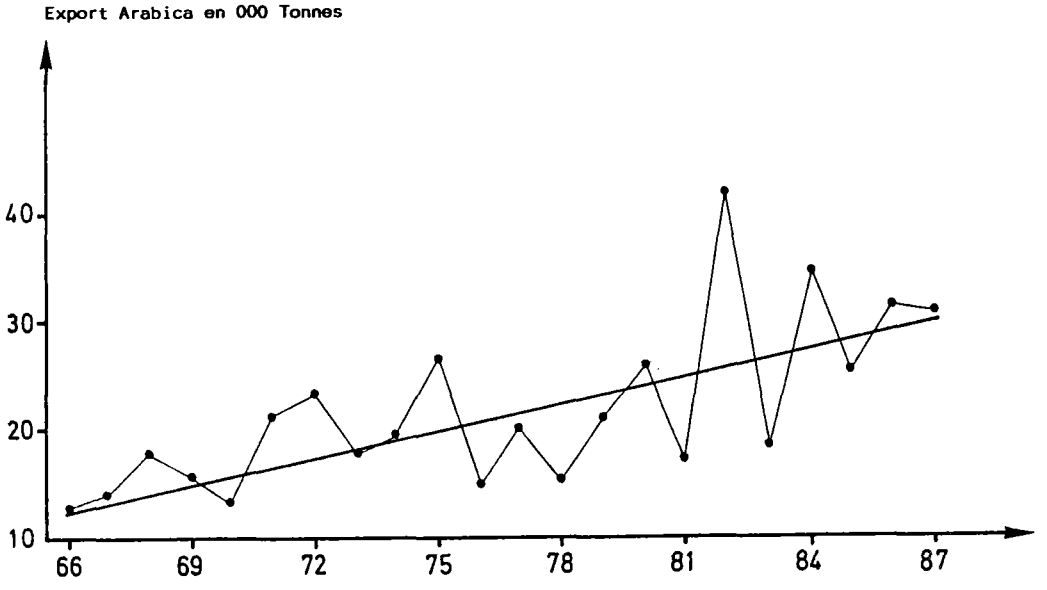
FRANCE

L'obtention d'un café marchand de qualité nécessite des soins attentifs à tous les niveaux d'intervention allant de la récolte au Stockage.
C'est l'addition des détails opératoires scrupuleusement suivis le long de la chaîne de préparation qui assurera l'élaboration d'un produit homogène et aux caractéristiques organoleptiques exigées par les marchés.
La connaissance des constituants du produit et de l'impact des opérations unitaires sur ces constituants précurseurs d'arôme devient fondamentale pour la conduite du traitement post-récolte.
Le Burundi, petit producteur au niveau mondial, s'est fixé pour stratégie de produire un café de qualité. Pour garantir cette haute qualité, les techniques les plus innovantes ont été appliquées au traitement post-récolte (déparchage, stockage, triage). L'amélioration qualitative a été spectaculaire et le pays exporte à présent des produits "haut de gamme" bien appréciés sur le marché mondial.

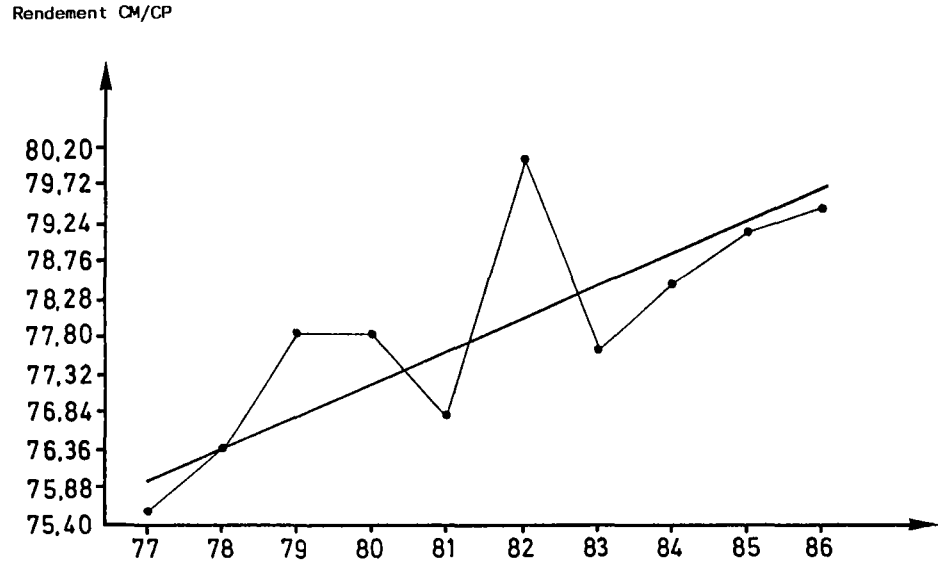
ANNEXES



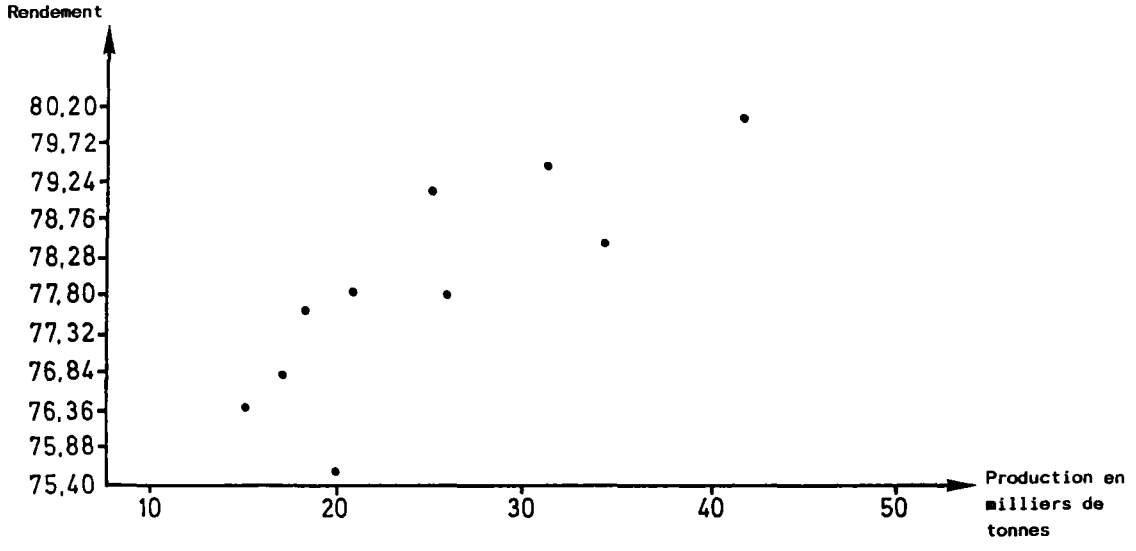
Campagne 65/66 à 86/87

EVOLUTION DES QUALITES DE CAFE AU BURUNDI

Type café	80/81	81/82	82/83	85/86	86/87	87/88	88/89
FW AAA	0	0	0	3,6	1,2	1,7	5,1
FW AA	0	0	1	1,9	6,8	11,1	10,7
FW A	5,2	5,6	8,3	4	5,8	9,4	10,1
TOTAL FW	**5,2**	**5,6**	**9,3**	**9,5**	**13,8**	**22,2**	**25,9**
OCIRU 2	0	0	0	29,8	38,5	28,4	33,6
OCIRU 3A	42,1	45,8	40,9	27,8	22,7	9,6	16,3
OCIRU 3B	44,5	42,3	41,3	25,3	15,8	18,4	14,8
DIVERS	8,2	6	8,4	7,6	9,1	10,2	9,4
TOTAL W	94,8	94,1	90,6	90,5	86,1	66,6	74,1



Evolution des rendements café marchand/café parche
en fonction des années



Evolution du rendement en fonction de la production

$$V = k\sqrt{D(\rho - 1)}$$

$$\frac{D}{D'} \simeq \frac{\rho' - 1}{\rho - 1}$$

$$\vec{f} = m.\omega^2 \vec{R}$$

$$\vec{v} = \omega \vec{R}$$

$$\int_o^t f \, dt = m \int_v^o dv$$

$$Re = \frac{V.D}{\nu}$$

$$Ne = \frac{\frac{1}{6} \pi D^3 (\rho_2 - \rho_1) g}{\frac{V_\ell^2}{2} \cdot \frac{\pi D^2}{4} \cdot \rho_1}$$

$$Ar = Ne \cdot Re^2 = \frac{4}{3} \frac{D^3 . \rho_1 (\rho_2 - \rho_1) g}{\eta^2}$$

$$Ar \longrightarrow Re \longrightarrow V_\ell$$

Rentabilité du triage électronique

$(100 - X)(P + p) + x P' > 100 (P + F)$

X : pourcentage de déchets éliminés

F : frais de triage /kg de café brut

P : prix ex usine en kg de café brut

p : plus-value/kg due au triage

P': valeur commerciale des déchets

LA GESTION INDIVIDUELLE AU SERVICE DE L'INNOVATION TECHNIQUE DANS UN BASSIN CAFÉIER DU MEXIQUE

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Le projet de Recherche-Développement des marges de l'aire Caféière Xalapa-Coatepec (Ver.), comporte deux volets essentiels qui sont représentés par deux départements du CIRAD:

- l'IRCC, pour l'amélioration de la filière café,
- le DSA pour implanter une méthode de conseil de gestion intégré.

1.- INTRODUCTION:

Comme tous les pays lourdement endettés, le Mexique subit les conséquences de la politique d'ajustements structurels dictée par les organismes financiers internationaux.

Devant le manque d'alternatives et la rapidité à laquelle se réalisent toutes ces mesures draconiennes, c'est bien la capacité des exploitations agricoles à se "moderniser" qui est interpellée.

D'autre part la dérégulation qui secoue l'économie nationale se traduit aussi par le renchérissement des moyens de production (intrants, matériel, équipement etc.) et par la réduction du crédit bonifié pour les producteurs de faibles ressources ("Productores de Bajos Ingresos",P.B.I.).

2.- METHODOLOGIE DU SYSTEME DE GESTION:

La méthode utilisée donne la priorité à l'implantation d'un système de gestion simple et efficace afin d'améliorer les procédés de prise de décision au sein de l'exploitation.

FIGURE 1

CYCLE D'APPLICATION DE LA METHODE DE CONSEIL DE GESTION

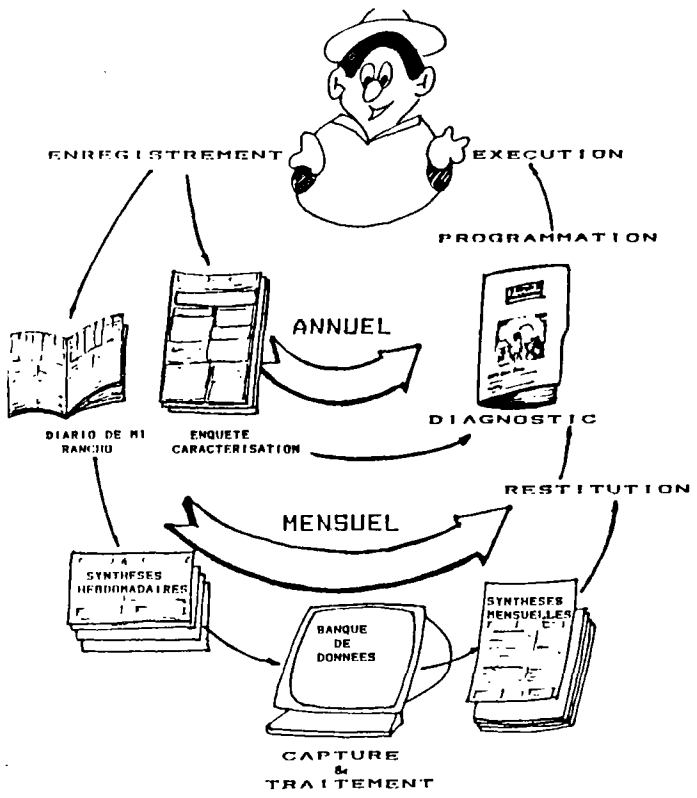


FIGURE 3: DEPENSES DOMESTIQUES ET DE PRODUCTION: MOYENNES MENSUELLES

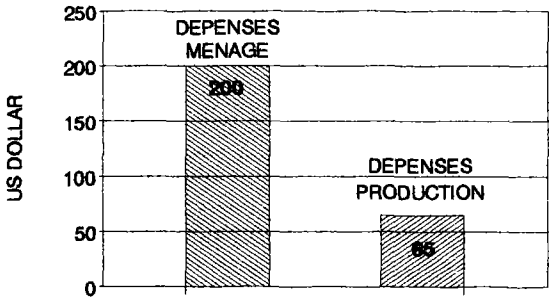
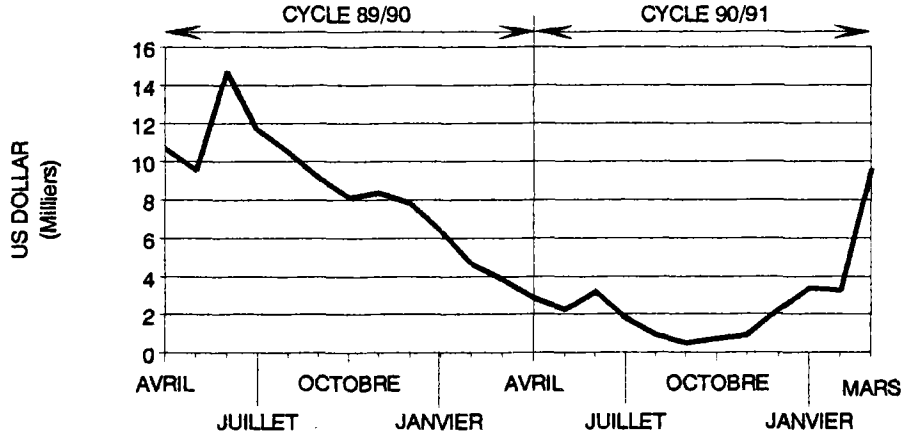


Figure 2 : COURBE DES FLUX DE TRESORERIE DES CYCLES 89/90 ET 90/91. TOTAL GROUPE TUZAMAPAN.



2.1.- L'enregistrement des résultats technico-économiques:

Le système repose sur un suivi au jour le jour des différents flux de l'exploitation. Les informations sont enregistrées par le producteur dans un petit cahier, qui fait office à la fois, de "journal de caisse" et de carnet de bord de l'exploitation.

Bien qu'à priori une telle procédure parut peu réaliste étant donné le manque d'habitude des agriculteurs et la réticence des techniciens, une minorité de producteurs volontaires de l'ordre de 10 à 50% de l'effectif total s'astreint à cette discipline quitte, parfois à "embaucher" leurs propres enfants en cas d'analphabétisme.

Une "enquête diagnostic" est levée pour tout le groupement au début du processus et permet d'élaborer une typologie. Les résultats alors obtenus avec l'échantillon de volontaires s'extrapolent au total de la communauté.

Ce système est actuellement implanté dans une dizaine de groupes du bassin de Xalapa-Coatepec et depuis un cycle dans le bassin caféier voisin de Tlapacoyan.

D'autres instruments plus spécifiques permettent soit de caractériser les systèmes de cultures, soit d'élaborer le plan de masse de l'exploitation, ou encore d'actualiser les stocks et d'élaborer à chaque fin de cycle le bilan ou le compte d'exploitation de l'exercice.

2.2.- Le conseil de gestion:

Les principaux bénéficiaires du système sont les producteurs eux-mêmes, qui peuvent, dès lors et en pleine connaissance de leurs possibilités, élaborer avec l'aide des techniciens un programme d'innovations techniques et économiques, en vue "de choisir un système de production permettant d'obtenir, d'une façon durable, un profit élevé, compte tenu du milieu et de la conjoncture" (CHOMBART DE LAUWE, 1963).

De manière à ce que le producteur se convainque de la méthode et que s'établisse le niveau de confiance nécessaire au bon déroulement du système, le technicien n'attend pas la fin du premier cycle (figure 1), pour restituer les informations aux producteurs. Bien au contraire il profite des bilans mensuels pour les réunir et organiser des "analyses de groupe". Il démarre en même temps un conseil rapproché afin de maintenir l'attention des bénéficiaires avec des résultats intermédiaires patents.

Le cycle comporte deux parties :

1. Annuelle, qui débute par l'enquête de caractérisation et conclut l'exercice avec le diagnostic qui débouche sur l'élaboration du plan de production pour l'année suivante,

2. Mensuelle, avec la capture et les restitutions des informations enregistrées par les producteurs.

La méthode de conseil de gestion repose sur la réalisation de plusieurs cycles successifs afin de compléter ses trois étapes (IGER, mars 1980) :

- La PREPARATION des décisions,
- Les DECISIONS,
- Le CONTROLE.

Sur la base des résultats obtenus au terme de trois cycles agricoles au sein du groupe de TUZAMAPAN, nous allons présenter deux exemples qui reflètent cette démarche:

3.- L'ANALYSE DE LA TRESORERIE ET LE COUT DES INTRANTS,

3.1.- Le flux de trésorerie :

L'analyse de la trésorerie sert fondamentalement à détecter les problèmes de liquidité, d'évaluer les ressources propres et de diagnostiquer l'accumulation ou la décapitalisation.

3.1.1- Le système famille/exploitation

Les soldes mensuels de caisse permettent d'élaborer chaque année une courbe de trésorerie (figure 2). Elle est la synthèse de l'intime imbrication des comptes du ménage et des entrées ou dépenses agricoles et non agricoles. En effet tout passe par la même caisse. Nous parlons ainsi de système famille/exploitation (BROSSIER et al. 1988).

Dans la plupart des cas les dépenses domestiques sont beaucoup plus importantes que les dépenses productives (figure 3). Cependant de grands sacrifices sont consentis par la famille en période de semis (Mai,Juin) ou de soudure (septembre). Phénomène que CHOMBART DE LAUWE appelle "la gestion du ventre creux" (1963).

3.1.2.- Une trésorerie ne permettant pas d'investir

Dans le cas des groupements fortement caféiers comme TUZAMAPAN, le cycle 1989-1990 (d'avril 89 à mars 90) a été catastrophique (figure 4). Le résultat de l'exercice a été négatif dans 75% des cas et la marge brute a été en moyenne de 77 dollars US par hectare contre 760 dollars l'année suivante.

Ce sont les "entrées de canne à sucre" qui ont permis de payer les dettes et de réaliser, tardivement les maigres préparatifs pour le nouveau cycle.

En 89-90 à peine un tiers du groupe a fertilisé une ou deux fois selon les parcelles.

La trésorerie de 90-91 bien que se rétablissant en fin de cycle (figure 2) grâce à des revenus de café nettement améliorés (figure 5), n'a pas non plus permis cette année la réalisation des travaux préparatoires du cycle suivant.

3.1.3.- Des stratégies et des situations variées

Seuls les producteurs possédant des revenus conséquents en canne à sucre ou provenant d'autres activités agricoles ou non (figure 6), sont en mesure de réaliser les investissements nécessaires à la mise en place du cycle suivant.

En effet les dépenses de canne à sucre sont toutes préfinancées par l'usine et déduites du montant des ventes lors de la ristourne finale. Les revenus du café permettent, eux, de rembourser les emprunts et les dépenses extraordinaires du ménage.

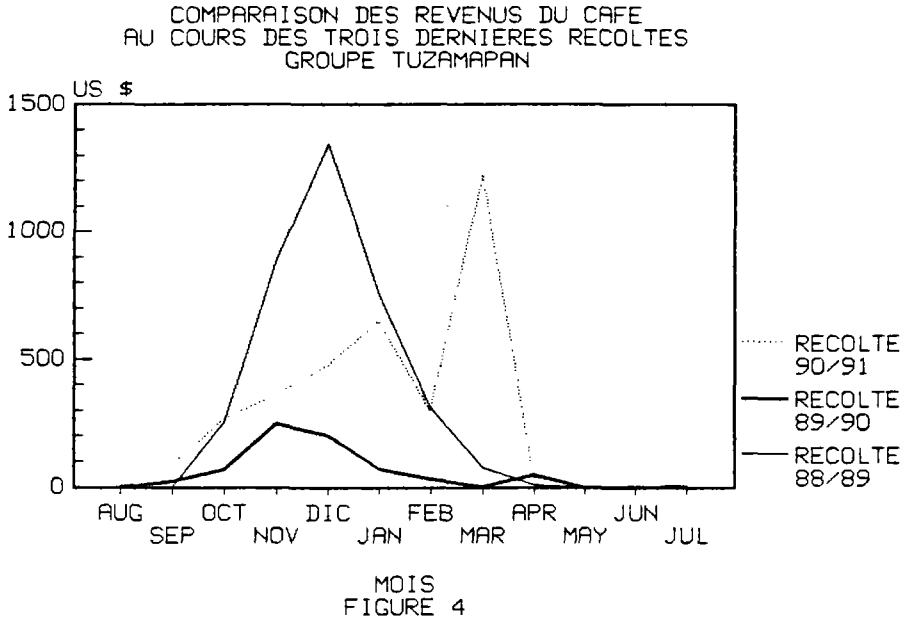
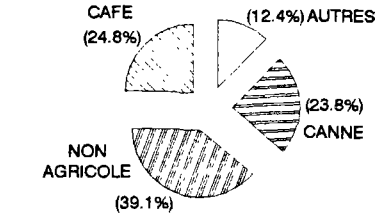
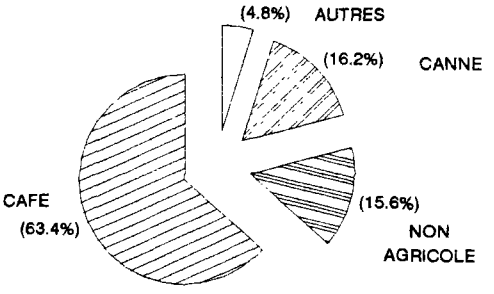


FIGURE 4

Figure 5 : PRODUITS PAR ACTIVITES

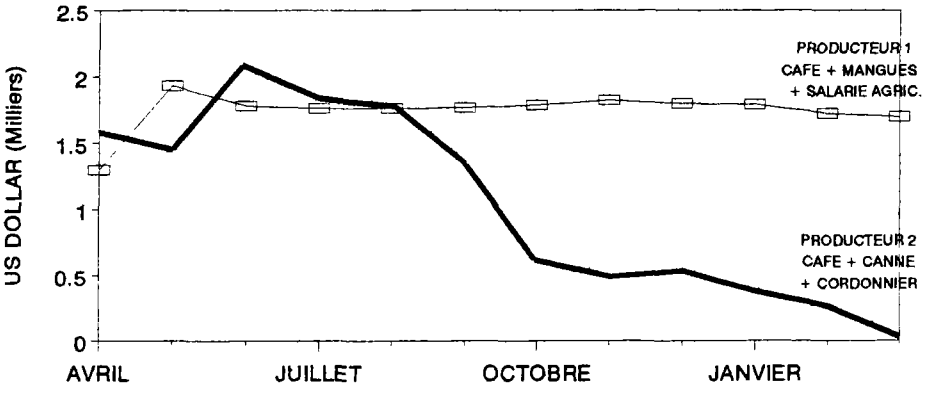


CYCLE 89/90
PRODUIT MOYEN ANNUEL: US $ 2,720



CYCLE 90/91
PRODUIT MOYEN ANNUEL: US $ 5,350

Figure 6 : COURBES DE TRESORERIE EN FONCTION DE DEUX STRATEGIES. CYCLE 89/90.



3.1.4.- Un foncier bloqué... seule solution: l'intensification

Cependant canne et café sont en concurrence directe au niveau du parcellaire. L'amélioration du niveau de vie des producteurs par l'augmentation du volume de café produit (paramètre discriminant principal de la typologie) ne peut se faire que par l'intensification des systèmes de culture et par des investissements relativement lourds.

Si les systèmes actuels arrivent à se maintenir grâce à l'alternance de bons et de mauvais cycles de café, par contre l'adoption d'innovations nécessite un effort financier supplémentaire.

3.1.5. Une solution de long terme: l'industrialisation et la qualité

Beaucoup de groupements ont espéré améliorer leur profit et ainsi pouvoir investir dans leur appareil productif par le biais de la transformation de la cerise en café parche avant que ne disparaissent les accords de la OIC. Malheureusement, le groupe de TUZAMAPAN (tableau 1), pour lequel bien que la dernière récolte soit similaire à celle de 1988-1989 et bien que 85% du café soit maintenant transformé, ne libère pas encore de disponibilités financières pour investir.

Tableau 1: EVOLUTION DES PARAMETRES DE RECOLTE DANS UNE STRATEGIE D'INDUSTRIALISATION

CYCLE	PROPORTION CAFE PARCHE / VENTES TOTALES	TOTAL RECOLTE TONNES CERISES	PRODUIT DES VENTES $ US
88/89	0 %	119	29,634
89/90	60 %	42	6,546
90/91	85 %	122	30,528

Il reste cependant l'espoir que contrôlant leur propre industrialisation les groupements puissent améliorer la qualité de leur produit et obtenir une meilleure valeur ajoutée.

Pour l'instant seul un programme de crédit préférentiel, subventionné et opportun serait capable de soutenir l'effort financier que suppose l'amélioration des systèmes de culture caféiers.

3.2.- Le coût des intrants :

La médiocrité de la productivité et la faiblesse des ressources propres, nous amènent à analyser de près les coûts de production. Que ce soit pour l'implantation de nouveaux systèmes intensifs, pour l'amélioration modérée de certains paramètres du système traditionnel, il est prioritaire d'essayer de diminuer les coûts de production.

3.2.1.- Premier poste des dépenses: le coût de main d'oeuvre

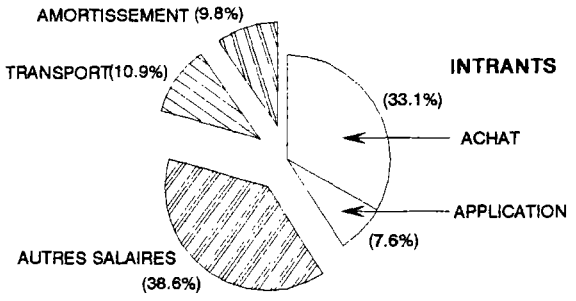
Le travail est de loin le poste le plus important dans les coûts de production du café (tableau 2). La récolte en constitue l'essentiel. Dans ce calcul, nous ne prenons pas en compte la main d'oeuvre familiale, en effet il

Tableau 2: PROPORTION DES COUTS DE MAIN D'OEUVRE ET DE RECOLTE DANS LE COUT DE PRODUCTION TOTAL

CYCLE	PROPORTION MAIN D'OEUVRE / COUT TOTAL	PROPORTION RECOLTE / MAIN D' OEUVRE TOTALE	/ COUT PROD. TOTAL
MAUVAIS CYCLE 89/90 2 TONNES/HA *	70 %	50 % SOIT 61 US $/Ha	35 %
BON CYCLE 90/91 5 TONNES/HA *	85 %	90 % SOIT 342 US $/Ha	77 %

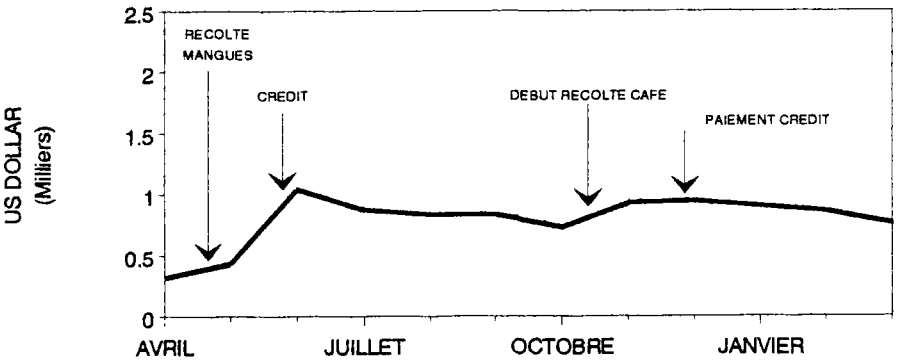
* TONNES DE CERISES

Figure 7 : COUT DE PRODUCTION DE CAFE HORS RECOLTE.



COUT MOYEN PAR HA: 102 US $
(SUR 2 CYCLES ET 25 HAS)

Figure 8 : IMPACT DU CREDIT SUR LA TRESORERIE



est très arbitraire de donner au travail familial le prix du "jornal" payé au "péon". Dans les petites exploitations familiales la main d'oeuvre est en fait rétribuée par les dépenses du foyer.

Le coût de production total peut passer du simple (173 US $/ha) à plus du double (446 US $/ha) selon le volume récolté. Cependant ce poste ainsi que le transport ne posent pas de problèmes d'avance de trésorerie.

3.2.2.- La principale avance de trésorerie: les intrants

Sans les frais de récolte le coût de production par hectare devient stable et descend à 108 US $. La fertilisation est le premier poste après la récolte et représente de 30% à 40% du coût sans récolte, devant les désherbages qui oscillent autour de 25% (figure 7).

L'achat des engrais, leur transport et leur application constituent presque 50% des avances de trésorerie à la culture.

3.2.3.- La mauvaise trésorerie accentue l'irrégularité des cycles

Cependant les dépenses de fertilisation ont baissé très sensiblement de 45 US $/ha à 30 US $/ha entre le cycle de 89-90 et de 90-91. Ce phénomène s'explique simplement par le manque de liquidité au moment où doivent se réaliser les achats des intrants.

Ce déphasage entre la trésorerie et le cycle productif, ne pourra être résolu qu'avec un crédit de campagne opportun (figure 8).

4.- CONCLUSION:

Les premiers résultats obtenus par cette méthode mettent bien en évidence la variabilité des situations et des stratégies paysannes. Cela a l'énorme avantage de nous sortir du domaine des moyennes et des simulations pour nous projeter dans la complexité du réel, sans pour autant nous faire perdre de vue les tendances fortes qui constituent le principal défi à la modernisation des exploitations agricoles. D'autre part, en termes opérationnels, l'accent est de nouveau mis sur la nécessité du financement de l'innovation.

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RESUME

LA GESTION INDIVIDUELLE AU SERVICE DE L'INNOVATION TECHNIQUE DANS UN BASSIN CAFEIER DU MEXIQUE

La modernisation du secteur agricole qui est proposée dans le cadre de la politique d'ajustements structurels menée par le gouvernement mexicain lance un défi essentiel aux petites exploitations agricoles "ejidales". La professionnalisation de l'agriculture en est l'enjeu principal. Dans ce contexte, le projet de Développement Intégré des Marges de l'Aire Caféière de Xalapa-Coatepec (DIMAC) a pour objectif essentiel d'augmenter la productivité des systèmes de production grâce à une série d'innovations technico-économiques. Les choix technologiques que réalisent ensemble techniciens et paysans se basent sur une meilleure connaissance du fonctionnement des systèmes de culture et de l'entreprise en général. Nous présentons ici la méthode et les outils utilisés pour ces prises de décisions dans le cadre de l'activité conseil de gestion ainsi que quelques résultats qui mettent bien en évidence les difficultés financières des petites exploitations caféières comme facteur limitant essentiel à l'intensification des systèmes en place. L'irrégularité provoquée par l'alternance entre bons et mauvais cycles de café est accentuée par la faiblesse de la trésorerie qui ne permet pas d'acheter les intrants nécessaires. Les stratégies paysannes anti-risques mises en place reposent sur une gamme très variées de combinaisons de pluriactivités agricoles ou non et sur l'augmentation de la productivité des moyens de production. Cependant cette gestion de court terme n'est pas suffisante et seule une politique de crédits opportuns et individualisés pourra permettre le déblocage de cette situation.

SUMMARY

PERSONAL ECONOMIC MANAGEMENT LIKE SUPPORT FOR TECHNICAL INNOVATION IN A MEXICAN COFFEE AREA

The agricultural modernization that suggests the political framework of structural adjustments carried out by Mexican Government is an essential challenge for "ejidales" small farms. The professionalization of the agricultural sector is the main stake of it. In this context the Integrated Developement project of the fringes of the Xalapa-Coatepec Coffee Areas (DIMAC) has the increasing productivity of farming systems as objective, thanks to technical-economic innovations. Technological choices made by both technicians and peasants are based on the better knowledge of cropping systems and of whole entreprise. As follows we present the method and instruments used for decision making as part of the agricultural administration activities as well as some results that demostrate financial difficulties of small coffee farms like major limitant factors for traditional cropping systems. The irregularity brought out by good and bad coffee cycles alternance is accentuated by the restricted funds that dont

permit the buying of necessary inputs. The anti-risk strategies used by peasants, are based on a very varied range of agricultural and not pluri-activity combinations and on the production means increased efficiency. However this short term management is not sufficient and only a policy of timely and individualized credits is able to solve this situation.

RESUMEN

LA ADMINISTRACION AGROPECUARIA AL SERVICIO DE LA INOVACION TECNICA EN UNA CUENCA CAFETALERA MEXICANA

La modernización del sector agropecuario, propuesta en el marco de la politica de ajustes estructurales realizada por el gobierno mexicano representa un desafío esencial para las pequeñas unidades ejidales de producción agropecuaria. El desarrollo empresarial del sector representa el reto principal. En este contexto, el proyecto de Desarrollo Integral de los Màrgenes del Area Cafetalera de Xalapa-Coatepec (DIMAC) tiene como objetivo principal aumentar la productividad de los sistemas de producción gracias a una serie de innovaciones técnico-económicas. Las elecciones tecnológicas que conjuntamente realizan técnicos y campesinos, se fundamentan en el conocimiento cabal del funcionamiento de los sistemas de cultivo y de la empresa en general. A continuación se presentan el método y las herramientas para dichas tomas de decisión dentro de las actividades de administración agropecuaria asi como algunos resultados que ponen en evidencia las dificultades financieras de las pequeñas explotaciones cafetaleras, como limitación esencial a la intensificación de los sistemas existentes. La iregularidad provocada por la alternancia entre buenos y malos ciclos de cafe esta acentuada por el limitado flujo de efectivo que no permite la adquisición de insumos necesarios. Las estrategias anti-riesgos establecidas por los campesinos se basan en una serie muy diversa de combinaciones de pluriactividades agropecuarias o no y sobre el aumento de la eficacia en los medios de producción. Sin embargo este manejo de corto plazo no es suficiente y solamente una política de crédito oportuno e individualizado podría permitir alguna solución.

EVALUACIÓN DEL EFECTO DE LA TECNOLOGÍA TRANSFERIDA A TRAVÉS DE LOS « GRUPOS DE AMISTAD Y TRABAJO » A PEQUEÑOS CAFICULTORES EN GUATEMALA

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INTRODUCCION

Durante la primera parte de la decada de los 80's, la Asociación Nacional del Café de Guatemala -ANACAFE- implementó como metodología de trabajo en asistencia técnica los Grupos de Amistad y Trabajo, bien conocida ahora como "GAT". La metodología pretendió entre otras, hacer mas efeciente el uso de los escasos recursos en asistir técnicamente a pequeños y medianos caficultores, lograr mayor cobertura y mediante el trabajo entre grupos de productores amigos lograr mayor adopción de mejores y más modernas formas de producir café.

La metodología GAT como tal ha sido ya evaluada en Guatemala. En 1986 con el apoyo del Programa Cooperativo para la Protección y Modernización de la Caficultura en la Región Centro Americana -PROMECAFE- se realizó una evaluación que uso como referencia la situación de la producción antes y después de que los productores se integraran a los GAT. Tanto la evaluación como los resultados en la práctica demostraron las bondades de la metodología de transferencia. Sobre esa base, PROMECAFE transfirió la experiencia horizontalmente a otros países de la región como Honduras, El Salvador y mas recientemente en Nicaragua y Panamá.

El propósito de esta investigación, más que confirmar los resultados de evaluaciones previas, fue considerar una metodología de análisis que permita evaluar el efecto causado en la producción cafetalera por la tecnología transferida, a través de los GAT, usando como referencia lo que acontece dentro de productores GAT y no GAT.

Por el tipo de análisis realizado, algunas congeturas sobre la racionalidad de los diferentes tipos de productores se pueden enunciar. Finalmente, un afinamientos de esta metodología de análisis, así como una extensión de la misma hacia estudios de caso de los diferentes grupos de productores tipificados podría constituir la base para un programa de asistencia técnica más orientado a productores objetivo.

Un programa de esta naturaleza, permitiría el uso más eficiente de los escasos recursos humanos y de capital con que los países de la región cuentan para asistir técnicamente a los

pequeños y medianos productores, que dicho sea de paso conforman cerca del 85 % del total de productores en Centro América.

OBJETIVOS

GENERAL

Evaluar el efecto causado en la producción de café de los pequeños caficultores por la tecnología transferida, a través de los Grupos de Amistad y Trabajo.

ESPECIFICOS

- Diferenciar al grupo de productores GAT de los no GAT por el nivel de tecnología empleada en la producción de café.

- Medir el efecto de la tecnología adoptada en la producción de café.

METODOLOGIA

En términos generales para evaluar el efecto, en función de la producción, de la tecnología empleada por productores GAT; hubiera sido posible simplemente comparar los rendimientos promedio de los productores GAT y los no GAT y mediante una prueba de t concluir. Sin embargo, en atención al objetivo específico: "Diferenciar a los productores por nivel de tecnología", fue necesario efectuar un análisis más elaborado.

Esta diferenciación, se logró utilizando la técnica de análisis de agrupamiento (Cluster Analysis), para lo cual se utilizaron 16 variables continuas que identifican el nivel tecnológico de los productores.

LOCALIDAD

La investigación se realizó en el municipio de Nueva Santa Rosa, en el departamento de Santa Rosa, zona cafetalera de Guatemala que se identifica como una región propia de medianos y pequeños caficultores. Se localiza a 70 kms. aproximadamente de la ciudad capital hacia el sur este por la Carretera Interamericana, tiene una altitud de 1000 msnm. Por sus condiciones de suelo, precipitación de 1200 mm. anuales y un largo período seco, se le considera no ideal para el cultivo del café, sin embargo por tradición y relativos buenos precios, la caficultura en el municipio se ha incrementado en los últimos años.

ENCUESTA

Esta se efectuó entre pequeños caficultores de Nueva Santa Rosa. Se consideraron dos muestras: una para los productores GAT y otra para los no GAT. Se asume que estas pertenecen a poblaciones de un mismo universo socio-culturalmente hablando. Un sondeo previo permitió asumir lo anterior en base a la homogeneidad étnica, escolaridad y principal actividad económica.

El marco de referencia estadístico para la encuesta dentro de los productores GAT, lo constituyó el marco de lista. Se aplicó un muestreo simple aleatorio, determinándose un tamaño de muestra de 50 para un valor estadístico de "student" de 2.5% y una proporción a estimar de 0.5 para la obtención de la máxima varianza en la distribución binomial.

Por la dificultad de obtener un marco de lista para los productores no GAT, se optó por encuestar un número igual de ellos. Es decir, 50 pequeños productores no GAT.

BOLETA

La boleta de encuesta fue diseñada para que la información permita estructurar el paquete tecnológico empleado en la producción de café por los productores encuestados en los diferentes estadios de su plantación, tales como semilleros, víveros y plantación definitiva. Además se incluyeron algunas variables de tipo socioeconómico que permiten valorar mejor el entorno en que se desarrolla su actividad productiva.

ANALISIS DE AGRUPAMIENTO

Este análisis permite agrupar aquellos productores que por su forma de producción presentan cierta similitud. El grado de similitud se mide aprovechando la variabilidad que

presentan los valores incluídos en la boleta de encuesta. En un nivel cero de similitud, que matemáticamente se le conoce como "distancia euclidiana", cada productor es un grupo diferente.

Con forme la distancia se aleja de cero se empiezan a agrupar aquellos productores con ciertas similitudes hasta llegar a una distancia en que todos los productores forman un solo grupo.

El criterio empleado en el estudio para determinar el nivel de agrupamiento a analizar fue considerar como adecuado el grado de similitud anterior a la conformación de un grupo con más del 50% de los productores encuestados.

TIPIFICACION

Cada uno de los grupos de productores diferenciados fueron tipificados utilizando para el efecto los valores modales de las 37 variables analizadas en la encuesta. Esto permite establecer los distintos niveles de tecnología empleados en la producción de café.

COMPARACION DE RENDIMIENTOS

Diferentes comparaciones fueron realizadas entre las medias de los rendimientos por unidad de área de los diferentes grupos formados, así como la comparación del grupo total de productores GAT y no GAT mediante pruebas de "t".

ANALISIS ECONOMICO

Una primera aproximación del análisis económico fue realizada sobre el costo adicional del nuevo paquete tecnológico empleado por los productores GAT y el retorno verificado a nivel de ingresos adicionales por concepto de mayores rendimientos en la producción de café. A este respecto, cabe indicar que ninguna consideracion fue hecha sobre los sistemas de producción de finca, en donde el café desde luego era el rubro más importante.

RESULTADOS Y DISCUSION

AGRUPAMIENTO

Se identificarón cinco grupos de productores a una distancia euclidiana de 0.75. La gráfica de la siguiente página, presenta el "Dendrograma" del análisis de agrupamiento el cual indica un primer grupo de 39 productores, uno de 13, dos de 8 y dos de 7. Los restantes 25 productores no se agruparon a una distancia euclidiana aceptable, por lo que se consideraron como productores notoriamente distintos a los integrados en los cinco grupos arriba indicados.

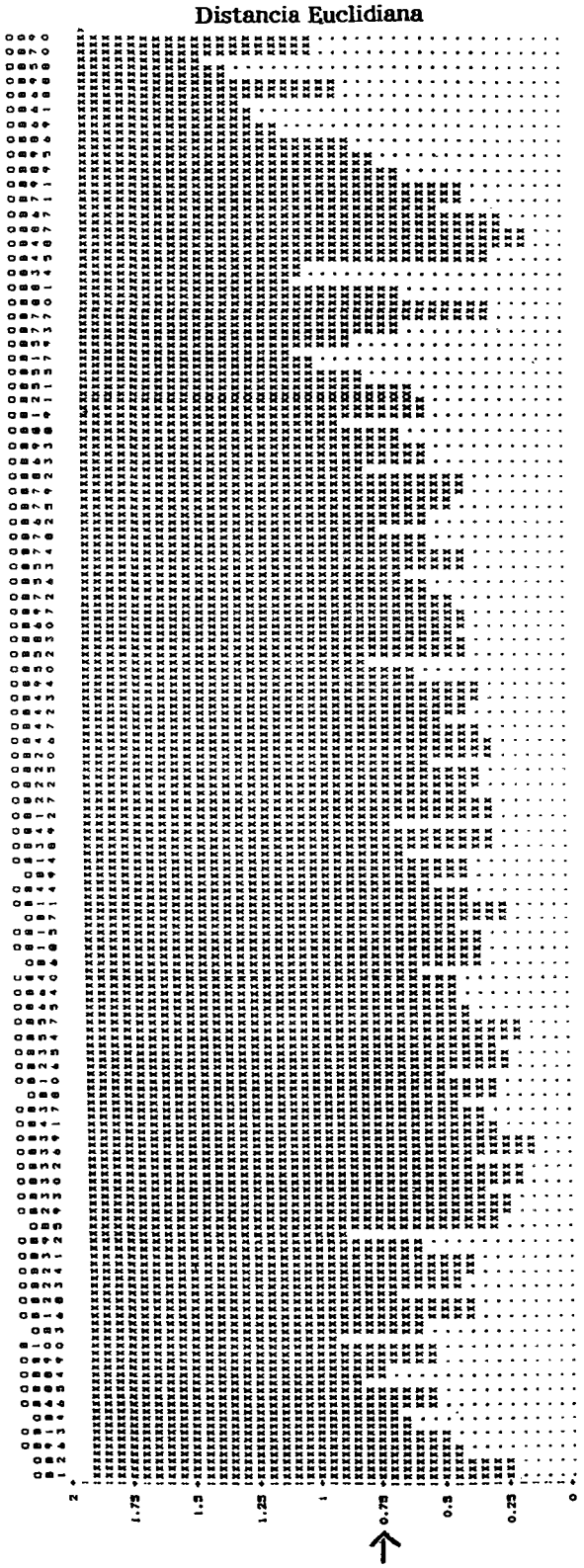
En el cuadro 1, se presenta la conformación de los grupos claramente diferenciados por el análisis de agrupamiento. Esta conformación permite claramente indicar que los grupos 1 y 2 identifican con bastante precisión a los grupos NO-GAT y GAT respectivamente.

Cuadro 1. Analisis de Agrupamiento de los Pequeños Productores de Nueva Santa Rosa, Santa Rosa, Guatemala.

GRUPO	INTEGRANTES		
	GAT	NO-GAT	TOTAL
1	5	34	39
2	13	0	13
3	4	4	8
4	3	5	8
5	2	5	7
TOTAL	27	48	75

Muchas consideraciones se pueden hacer en relación a la conformación de los otros grupos, así como al hecho de que 23 productores GAT y 2 NO-GAT quedaron por fuera del agrupamiento, sin embargo para los fines de este estudio, la discusión de los resultados se centro sobre las diferencias entre los grupos típicos (Grupos 1 y 2).

Gráfica . Dendograma del Análisis de Agrupamiento para Pequeños Productores de Café en Nueva Santa Rosa, Santa Rosa, Guatemala, 1990.



Cuadro 2. RESUMEN DE LAS CARACTERISTICAS MODALES DE LOS GRUPOS IDENTIFICADOS EN EL ANALISIS DE AGRUPAMIENTO PARA PEQUEÑOS CAFICULTORES DE NUEVO SANTA ROSA. SANTA ROSA. GUATEMALA 1990.

GRUPO	ALDEA	RANGO EDAD (AÑOS)	EDUCACION FORMAL(%)	RANGO AREA DEDICADA A CAFE (Ha)	AREA MODAL DEDICADA A CAFE (Ha)	PROPORCION AREA CULTIV. DEDICADA A CAFE (%)	INDIVIDUOS CULTIV CAFE (%)	DURACION DE LA ETAPA DE SEMILLEROS (DIAS)	VIVERO							PLANTACION DEFINITIVA			
									DISTAN. PLANTAS.	CAFIC. APLIC. FERT.(%)	FORMULA UTILI-ZADA	No. DE APLIC.	COMBATE ENFERM.	COMBATE INSEC-TOS	No. LIM-PIAS	DURA-CION VIVERO	TAMAÑO TRAS-PLAN-TE	VARIE-DAD MAS CULTIV	DIST. DE PLANT.	RANGO EDAD PLANTA-CION
1.	El Portezuelo	23-64	46	0.27-2.8	1.4	67-100	28	60	30 Cm.	97	15-15-15	1	No	No	3	2 años	60cms.	Catuaí	2x1m.	1-40 años
2.	El Anillo, Ojo de Agua	33-70	54	1.3-9.1	2.1	60-100	31	60	20 Cm.	100	20-20-0	2	Si	Si	3	1 año	60cms.	Catuaí	2x1m.	1-35 años

CONTINUACION DEL RESUMEN DE LAS CARACTERISTICAS MODALES DE LOS GRUPOS IDENTIFICADOS EN EL ANALISIS DE AGRUPAMIENTO PARA PEQUEÑOS CAFICULTORES DE NUEVO SANTA ROSA. SANTA ROSA. GUATEMALA 1990.

GRUPO	ALDEA	PLANTACION DEFINITIVA										SOMBRA			RENDIMIENTO		ASISTEN. TECNICA
		APLICA FERTIL.	FORMULA FERTIL. UTILI-ZADA	APLICA CION AL AÑO	EPOCA DE APLICA CION	CANTI-DAD POR PLANTA	COMBATE ENFERM.	COMBATE INSEC-TOS	No. LIM-PIAS/ AÑO	EPOCA DE LIMPIA	PRACTI-CA PODA	EPOCA PODA	ESPE-CIES UTILIZA-DAS	DISTAN-CIA PLANTAS	EPOCA PODA	RANGO (qq/mz)	MODA (qq/mz)	
1.	El Portezuelo	Si	15-15-15 y 46-0-0	2	Jul-Oct	4 oz.	No	No	2	May.Ago Oct.	Si	Marzo/ Abril	Cuje Banano	6x6 m.	Marzo/ Abril	48-150	80 y 96	No
2.	El Anillo, Ojo de Agua	Si	20-20-0 y 46-0-0	2	May-Sep	3 oz.	No	Si	3	May.Ago Oct.	Si	Marzo/ Abril	Cuje	8x8 m.	Abril	33-144	96	Si

FUENTE: Encuesta calificada

TIPIFICACION DE LOS GRUPOS DE CAFICULTORES

El cuadro 2, resume las características modales de los grupos típicos identificados en el análisis. Como ya se indicó el grupo 1, corresponde a los productores NO-GAT y el grupo 2 a los productores GAT.

Como se puede ver en el cuadro los productores de ambos grupos pertenecen a diferentes aldeas del municipio de Nueva Santa Rosa. Entre las diferencias mas relevantes en las variables no agronómicas se puede notar que existe un mayor porcentaje de educación formal en el grupo 2. Hecho significativo es que el área en hectáreas dedicada a cafe es mayor dentro de los productores GAT (2), el rango para estos va de 1.3 a 9.1 Ha., mientras que para los NO-GAT va de 0.27 a 2.8 ha. Si a esto agregamos que el porcentaje de productores que cultivan exclusivamente cafe es mayor también para los del grupo 2., podemos concluir que el café representa una actividad productiva mas importante para ellos.

Es importante aclarar que a pesar de que las diferencias numéricas en las variables analizadas parecieran mínimas, estas son significativas cuando se trata de pequeños productores que en los extremos no van más alla de las 10 ha. por finca.

Para facilitar la discusión de los resultados de la tipificación con respecto a las variables agronómicas el cuadro 3, presenta un resumen de las diferencias más significativas que se encontraron al comparar los grupos 1. y 2.

Cuadro 3. Características Diferenciales en el Nivel de Tecnología entre los Grupos de Productores NO-GAT y GAT.

CARACTERISTICA	N-GAT	GAT
VIVERO:		
- DISTANCIAS	30 cm.	20 cm.
- FERTILIZANTE	15-15-15	20-20-0
- APLICACIONES	1	2
- CONTROL FITOSANITARIO	no	si
- DURACION	2 años	1 año
PLANTACION:		
- FERTILIZANTE	15-15-15 urea	20-20-0 urea
- EPOCAS DE APLICACION	jul. y oct.	mayo y sept.
- CONTROL DE INSECTOS	no	si
- CONTROL DE MALEZAS	2 veces	3 veces
- EPOCAS DE CONTROL	jul. y oct.	mayo, agos. y oct.
- TIPO DE SOMBRA	ingas y musaceas	ingas
- DISTANCIAS	6 x 6 m.	8 x 8 m.

En la fase de vívero, existe una diferencia fundamental en cuanto a la duración del mismo, siendo esta de 2 años para el grupo de los NO-GAT, lo que agronómicamente es indeseable por los problemas de desarrollo radícular de la plantía en el campo definitivo. El distanciamiento de 30 cm. en parte es explicado por la larga duración en el vívero, lo que además de constituir un problema agronómico como ya se dijo resulta en costos adicionales para los productores.

En cuanto al tipo de fertilizante empleado, la fórmula 20-20-0 utilizada por los productores GAT resulta más adecuada de acuerdo a la recomendación para los suelos de origen volcánico de la región. Sin embargo, las dos aplicaciones efectuadas son insuficientes para producir vívero de óptima calidad (Se recomiendan 5 aplicaciones). Más problemático aún será el caso de los NO-GAT quienes únicamente hacen una aplicación.

Por último en el vívero, el control fitosanitario es es una práctica comun para los productores GAT, lo que contribuye a producir vívero de mejor calidad.

En la plantación definitiva, las diferencias se notan en el tipo de fertilizante, al igual que en vivero los productores GAT utilizan una fórmula completa más adecuada para el tipo de suelo de la región. Pero tal vez lo más importante en el aspecto de fertilización sea la época de aplicación.

En el caso de los productores GAT la pimera aplicación (mayo) coincide con el crecimiento rápido de la planta al inicio de la época lluviosa y la segunda aplicación (septiembre), coincide con la fase de maduración de la cosecha. Como se ve el período crítico es cubierto por ambas epocas de aplicación, no pudiéndose decir lo mismo en el caso de los productores NO-GAT, quienes hacen coincidir su primera aplicación (julio) con la época de crecimiento y formación del fruto y la segunda aplicación en octubre cuando ya inicio la cosecha.

El control de plagas insectiles es práctica común para los productores GAT, siendo la principal plaga en la región la broca del fruto del café (Hypotenemus hampei). Los productores NO-GAT no efectúan el control de la plaga, lo que al final no afecta seriamente los rendimientos pues la venta del café producido se realiza en cereza. Este no sería el caso si los pequeños productores pergaminizaran sus cosechas.

Los productores NO-GAT efectuan únicamente dos limpias (control de malezas), estas se realizan a mano en ambos grupos. La primera limpia la hacen hasta en julio cuando ya se ha establecido competencia entre las malezas y el cultivo. Esto no ocurre en los cafetales de los productores GAT quienes inician las limpias en mayo.

Ambos grupos utilizan como sombra del cafetal Ingas, pero en el caso de los productores NO-GAT también usan diferentes especies de Musaceas. Los distanciamientos usados por estos últimos productores (6 x 6 m.) resultan en una sombra muy cerrada para el cafetal, lo que desfavorece la actividad productiva del mismo, a la vez que favorece el desarrollo de plagas como la broca.

Esta diferenciación en el nivel tecnológico de la producción de café por parte de los pequeños productores, incide en el nivel de rendimientos como se discute a continuación.

RENDIMIENTOS

El cuadro 4, presenta la comparación de rendimientos promedio, primero entre el total de productores GAT y NO-GAT encuestados y luego entre los grupos identificados por el análisis de agrupamiento.

Cuadro 4. Comparación de Rendimientos Promedio de Café Cereza en qq. por manzana.

GRUPOS	RENDIMIENTOS	PROBABILIDAD > t
GAT	94.8	
NO-GAT	83.3	0.12
GRUPO 1. (NO-GAT)	84.9	
GRUPO 2. (GAT)	94.1	0.23

Como lo muestran las comparaciones en ambos casos, el redimiento promedio de los productores GAT es mayor que el de los NO-GAT al 12 y al 23 % de significancia respectivamente.

ANALISIS ECONOMICO

El análisis económico consideró el diferencial en costos como producto del diferente nivel tecnológico practicado por los grupos que resultarón del análisis de agrupamiento, así como el diferencial de ingresos resultado del mayor rendimiento que presentaron los

productores GAT. Este análisis considero precios nominales de 1990.

* Diferencial en Costos: Q 420.00 / manzana.
* Diferencial en Ingresos: Q 805.00 / manzana.
* Tasa Marginal de Retorno: 192 %
* Tasa Minima de Retorno: 67 %
* Balance a favor: 125 %

La Tasa Mínima de Retorno esta conformada por 27 % que es el interes bancario más 40 % que representa el riesgo que implica el manejo de la nueva tecnología.

El nivel de tecnología empleado por los productores GAT representa una mejor alternativa económica para los pequeños productores de la regíon ya que existe un balance positivo del 125%. Esto significa que por cada Quetzal invertido en la nueva tecnología, no sólo se está recuperando la inversión, sino están retornando Q 1.25 más por manzana, después de considerar el costo del capital y el riesgo implicito en el cambio de tecnología.

La anterior consideración si se asume que la racionalidad de los productores es maximizar los ingresos netos, lo que es cierto únicamente para aquellos productores que especializan la producción de cafe en su finca.

CONCLUSIONES

La tecnología transferida a traves de los Grupos de Amistad y Trabajo -GAT- produce efectos positivos en la producción de café de los pequeños productores de Nueva Santa Rosa. A pesar de que la metodología empleada en esta evaluación carece de elementos objetivos que permitan concluir sobre el fenómeno de irradiación de tecnología de productores GAT a NO-GAT, los autores del trabajo se permiten indicar que en la práctica existen evidencias de que este fenómeno se esta produciendo en la región. Una muestra de ello es el rendimiento promedio de la zona que en términos generales es superior al rendimiento promedio nacional.

La metodología de transferencia ha contribuido a diferenciar el nivel tecnologico empleado en la producción de cafe, permitiendo mayores rendimientos a los integrantes de los GAT.

El más alto nivel de tecnología empleado por los productores GAT representa una posición económica más ventajosa cuando se compara con el nivel de tecnología de los productores NO-GAT.

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EVALUATION OF TECHNOLOGY TRANSFER EFFECT THROUGH THE "FRIENDSHIP AND WORK GROUPS" TO SMALL COFFEE PRODUCERS IN GUATEMALA

Juan Carlos Méndez

SUMMARY

The objective of this research was to determine if there is differece in the technological level of coffee production between producers working into Friendship and Work Groups "F & W G" as a technology transfer methodology and producers with the same social-cultural level working independently in the Nueva Santa Rosa Village. One of the parameters evaluated to stablish that difference was the yield per unit area. The sample studied was of 50 cases each, an the analytical framework was conducted under "Cluster Analysis Technique". 5 groups were obtained, 2 out of those identificate clearly to F&WG producers one, and no F&WG producers the other. The F&WG producers present a higher level of technology on weeds control, insects control and fertilization practices. Finally, a difference on yiel crop was found, which is related to the differences found on technology level.

EVALUACION DEL EFECTO DE LA TECNOLOGIA TRANSFERIDA A TRAVES DE LOS "GRUPOS DE AMISTAD Y TRABAJO" A PEQUENOS CAFICULTORES EN GUATEMALA

Juan Carlos Méndez

RESUMEN

El objetivo de esta investigación fue determinar si hay diferencia en el nivel de tecnología en la producción de café entre productores trabajando en Grupos de Amistad y Trabajo "GAT" como metodología de transferencia y productores con el mismo nivel socio-cultural trabajando independientemente en el municipio de Nueva Santa Rosa. Uno de los parametros evaluados para establecer la diferencia fue el rendimiento por unidad de área. La muestra estudiada fue de 50 para cada grupo, y la metodología fue una "Técnica de Análisis de Agrupamiento". Se obtuvieron 5 grupos, 2 de los cuales identifican claramente a los productores GAT uno y los no GAT el otro. Los GAT presentan un nivel más alto de tecnología en control de malezas, insectos y prácticas de fertilización. Finalmente, se encontró una diferencia en rendimiento, la cual esta relacionada con las diferencias en el nivel de tecnología de producción.

RAPID ESTIMATION OF COFFEE YIELD

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INTRODUCTION

Analysis of the components of yield provides information on a number of horticulturally important processes such as partitioning of resources within the plant material. This may be useful in evaluating breeding materials and selecting appropriate parents (Hancock et al., 1984, Hardwick and Andrews, 1980, and Hoover et al., 1988). Such analysis may also be useful in predicting the effects of cultural modification such as pruning (Siefker and Hancock, 1986) and fertilization (Ljones and Sakshaug, 1967). More importantly, the analysis of the interrelationship of the components of the yield can be used to predict the yield potential of the crop.

Coffee is a very labor intensive crop. Harvesting coffee, unlike, many other crops is not a one time activity because of the lack of uniform ripening of the fruits. As many as 7 harvests may be required per season over 3 to 5 months. This makes it very time consuming, and in the places that are extremely short of agricultural labor, very expensive. From an experimental perspective, repetitive harvesting and lack of resources and labor at the time of harvesting, create a serious problem. A yield estimation procedure is needed to screen coffee genotypes for their yield potential grown over a wide range of environments in Hawaii. Such predictive technique has been used successfully in other crops of similar nature (Daubeny et al., 1986). This procedure should exploit the functional relationship between the yield and the components of yield and be used to predict the standing crop yield of the coffee cultivars before actual harvesting takes place. The apparent advantages of a yield estimation procedure are;

- It enables researchers to screen the yield potential of many genotypes grown in different environments.
- It drastically reduces the total labor and time involved in harvesting the coffee trees and can be used to evaluate the effects of cultural practices.

- It is independent of the time sensitivity of coffee harvesting and can be used to predict yield 3-4 months in advance.
- The early yield prediction may help a country or farm to plan and create marketing for their products.

The relationship between yield and the components of the yield can be described as, yield = number of fruits /tree * weight /fruit, where, number of fruits/tree = number of fruiting nodes/ tree * number of fruits/node. This relationship can mathematically be expressed as Y = f (N * W), where, Y = Yield, N = Number of fruits/tree, and W = Weight/fruit. An empirical regression model can describe this relationship and estimate the yield of an individual coffee tree. Y = a + b (x), where, Y = yield predicted, a = intercept, b = slope, X = yield estimated = fruiting nodes/tree * fruits/node * weight/fruit

A procedure was developed based on estimating the fruiting nodes/tree, the average number of fruits/node and average fruit size (components of yield) in one sector or quadrant (vertical 90° sector of canopy from top to the bottom) of a tree.

MATERIALS AND METHODS

Two cultivars, 'Guatemalan' and 'Catuai' were selected in August, 1990 at the Hawaiian Sugar Planters' Association's (HSPA) reserved farm at Kunia, Oahu, Hawaii. These were part of the multilocation Hawaii State Coffee Trial (HSCT). The cultivar 'Guatemalan' (*C. arabica typica*) is tall, vigorous, and high yielding. This cultivar has been under cultivation in the Kona region of Hawaii for more than a century. The cultivar 'Catuai' (*C. arabica typica*) is a hybrid of the cultivars, Caturra and Mundo Novo. This cultivar is semi-dwarf, compact, heavily branched, high yielding and well adapted to coastal conditions.

A preliminary study indicated that a sample size of 12 quadrants is required to detect 13 to 15 % differences among trees within and between the cultivars (Table 1). Fruits per node were less variable on the middle fruitful node position than on the basal and distal node of the lateral (Table 2). Twelve representative trees of each cultivar, four in each replication were tagged. Total fruiting nodes and fruits/node for each tree were estimated by dividing trees into vertical quadrants according to the estimation protocol presented below. Fruiting nodes that fell into the selected quadrant were counted. Fruits/node were counted on approximately the middle node position on every lateral branch from the bottom to the top of the quadrant as the fruits/node on the middle node position were closer to the average fruits/node on the lateral (Table 2). Total number of the actual fruits/tree were counted to determine the accuracy of sampling.

Table 1. Determination of sample size(tree) for a desired level of difference

Marginn of error (%)	d	d^2	$N = (Z^2S^2)/d^2$
3%	55	3021	275
6%	110	12082	69
9%	165	27185	31
12%	220	48330	17
15%	275	75515	11
18%	330	108742	8

Z = Standardized normal variate at 5 % significance level d = desired level of difference to be detected = number of fruits Sample Mean=1832 (fruits) and S^2 = Variance = 216532

Table 2. Correlation between fruits/node at different node position and the average fruits/node on the lateral.

	Fruits per node		
Variable	Basal node	Middle node	Distal node
Fruits/basal node			
Fruits/middle node	.47		
Fruits/distal node	.49	.73	
Lateral average	.58	.89	.72

Basal node = The second fruitful node position from the basal end of the lateral.
Middle node = Middle fruitful node position on the lateral.
Distal node = The next to last fruitful node on the lateral.

To determine if changes in fruit and green bean size occur during harvest season, red ripe fruits were harvested at 15 day intervals. Fruit size (g/fruit) at each harvest was determined by taking a random sub-sample of 300 to 400 fruits from the pool of the harvested fruits. Finally, weighted average of the fruit size were determined for the season. The data on both the cultivars were combined and the yield prediction equations developed.

Regression and correlation analysis were employed to study the nature of relationship between yield components and the yield. Estimated yield based on the components of yield for each harvest season were regressed against the actual harvested yield. Two yield prediction equations were developed. The first equation was based on the correction factor (CF) obtained by the ratio of the harvested yield to estimated yield based on the early ripe fruit size. Hence, predicted yield = (fruiting nodes/tree * fruits/node * first harvest fruit weight) * CF. The second equation was based on the regression equation obtained by regressing estimated yield from first harvest fruit weight against the actual harvested yield. Hence, the predicted yield, $Y = a + bx$, where a = intercept, b = slope (regression coefficient), and x = estimated yield based on the first harvest fruit weight, which is fruiting nodes/tree * fruits/node * first harvest fruit weight.

Data for the validation of the model prediction equations were obtained from a separate experiment on 'Guatemalan' conducted at the Waimanalo Experiment Station of the University of Hawaii, Oahu. Observations on components of yield were made exactly the same as was done for the development of the yield prediction model

Estimation protocol

- Select at least 12 normal appearing trees per cultivar or treatment avoiding trees at the end of rows.
- Stand in the alley in front of a tree, hold pole A about 60 cm from the stand and visually divide the canopy into two equal halves from top through the canopy to the bottom (Fig 1). Adjust the position of the pole taking into consideration of the whole canopy as well as the trunk of the tree.

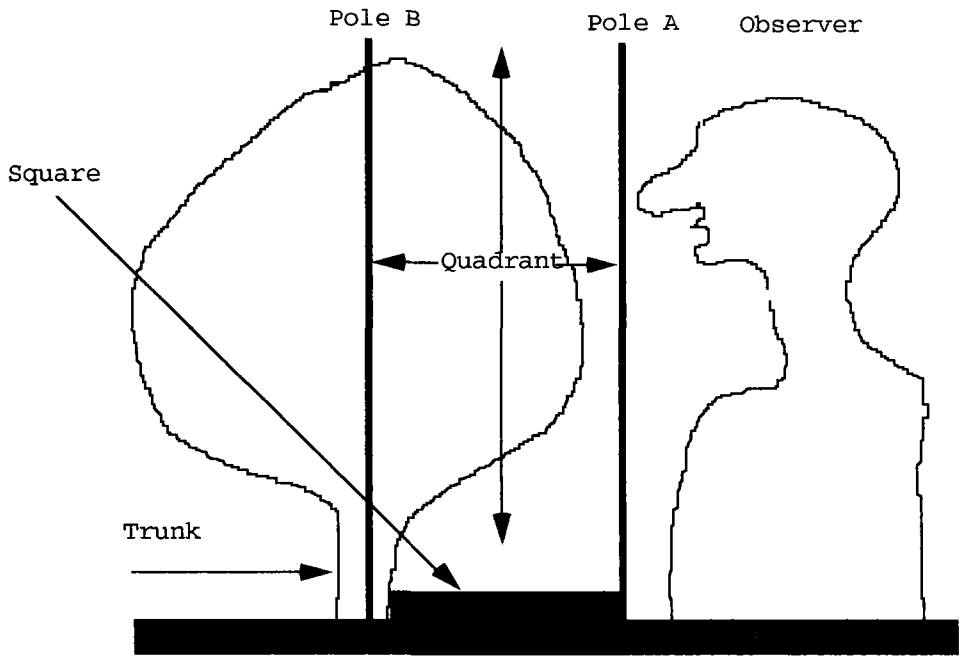

Fig 1: Side view of a tree quadrant

- Position a metal carpenter's square so that one arm points to pole A and the other makes a right angle at the base of the trunk. Place pole B in the row at the other end of square. Compensate for trunk thickness by shifting pole B one half of the trunk diameter in the row (Fig 2). The canopy structure from the top to the bottom of the tree falling in the area demarcated by the intersection of these two poles, constitutes a 90° section of the tree circumference or quadrant.

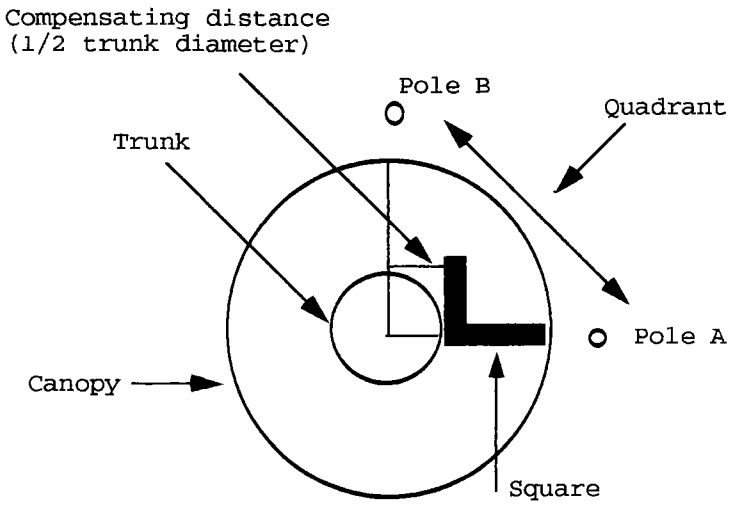

Fig 2: Top view of a quadrant

- After establishing quadrant boundaries, count all the fruiting nodes on the laterals in the quadrant.
- Select the lateral at the bottom of the quadrant. Record this as lateral number 1. Find approximately the middle fruiting node on the lateral and count the number of fruits at that node. Repeat the procedure until

reaching the top fruitful lateral in the quadrant. Calculate average fruits/node

- Select next tree and establish a different quadrant. Repeat the procedure. Select the quadrant to represent all four orientations. Because the underlying assumption that the distribution of fruiting nodes and the fruit per nodes in one quadrant represents the entire tree may not be true, all four quadrants representing must be sampled
- Calculate total number of fruits per quadrant and per tree by multiplying the total number of fruiting nodes per quadrant by the average number of fruits/node in the quadrant, and fruits/tree by 4
- Prior to first harvest, randomly select and pick 200-300 red ripe fruits from all over the tree canopy and determine fruit size.
- Calculate the total yield of the fruits in each tree.

RESULTS AND DISCUSSION

The study indicated that the most important variables, contributing to the yield, were the fruiting nodes per tree and the fruits per node. Fruiting nodes/tree and fruits/node were highly positively correlated with estimated and harvested yield, whereas fruit size was negatively correlated with harvested yield and all the components of the yield (Table 3). These two variables explained respectively 73 % and 66 % variability in the harvested yield (Fig 3 and 4).

Table 3. Correlation matrix for yields and the components of yield

	Variables	1	2	3	4	5	6
1	Fruiting node						
2	Fruits/node	.499					
3	Estimated fruits	.896	.818				
4	Actual fruits	.877	.809	.985			
5	Harvested yield	.855	.816	.98	.983		
6	Estimated yield	.872	.83	.993	.967	.977	
7	Average fruit size	-.25	.047	-.12	-.22	-.11	.006

Fruits per tree were estimated by the functional relationship between fruiting nodes/tree and the fruits/node as follows: Estimated Fruits (EF) = fruiting nodes/tree * fruits/node.

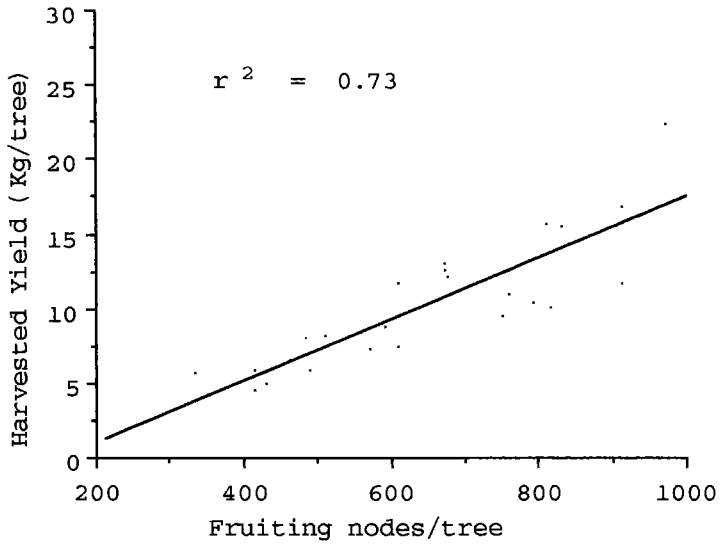

Fig 3. Relationship between fruiting nodes and the harvested yield/tree

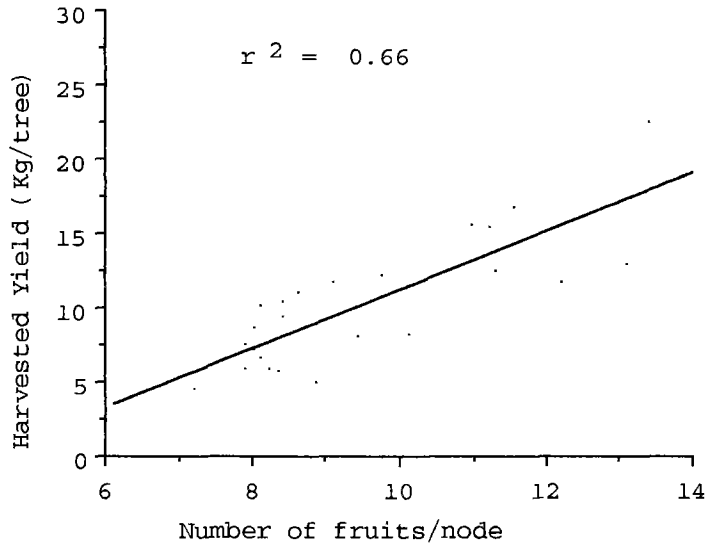

Fig 4. Relationship between fruits/node and the harvested yield

The estimated fruits regressed against the actual fruits explained 97 % of the variation inherent in the actual fruits. The high r^2 value and the random distribution of the residual indicated that the model fitted the data very well (Fig 5).

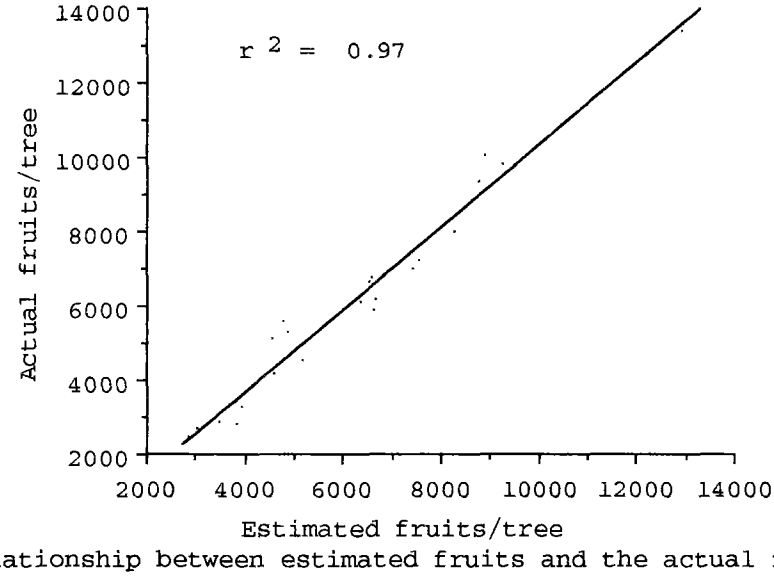

Fig 5. Relationship between estimated fruits and the actual fruits/tree

The next step in the development of the model was to estimate the weight per fruit. It was necessary to determine whether or not the fruit weight varied throughout the harvest season.

There was a gradual decrease in fruit size from the first to the last harvest period (Table 4). Early ripe fruits were the largest (1.9 g) and the last harvest fruits the smallest (1.64 g).

Table 4. Variation of fruits size (g/fruit) within season

Harvest	Size (g/fruit)	S	CV	% difference from season average
First	1.91	.22	11	10
Second	1.82	.26	14	6
Third	1.72	.10	6	1
Fourth	1.71	.14	8	0
Fifth	1.64	.13	8	4
Season average	1.71	.09	5	

S = Standard deviation, and CV = Coefficient of Variation

The average fruit size was very close to 3rd, 4th, and 5th harvest fruit size, indicating that the fruit size stabilized after second harvest.
It seems quite likely that the fruits that were set earlier had the distinct advantage of drawing more photosynthates from the source.

Estimated yields (EY) based on the season average and 1st, 2nd, 3rd, 4th, and 5th harvest fruit size were computed. These estimated yields based on season average fruit size, and the 1st, 2nd, 3rd, 4th, 5th harvest fruit sizes were regressed against the harvested yield. All regression models explained over 91 % of the variability in the harvested yield, season average fruit size had better predictability than the different harvest period fruit size. This is expected because the average fruit size represents the true fruit size. Computing the average fruit size requires harvesting the tree, however, a sub-sample of early ripe fruits can be used to estimate yield even before the first harvest actually starts. Hence, the model based on the early ripe fruit size can be most conveniently used for the rapid estimation of the yield of a standing coffee tree without actually harvesting the tree. Estimated yield based on the early ripe fruit weight explained up to 92 % variation in the actual harvested yield (Fig 6).

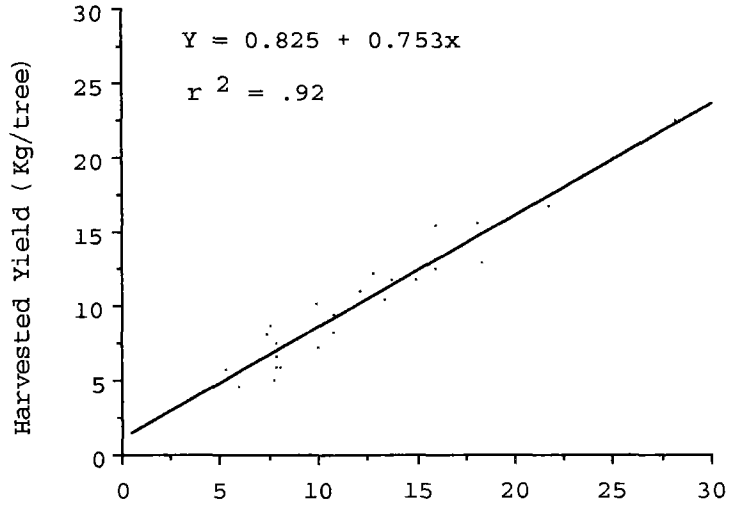

Fig 6. Relationship between estimated yield and the harvested yield

The ratio of harvested yield to the estimated yield (.837) based on the early ripe fruit size overestimated the harvested yield by 16.3 %. This can be attributed to two factors: the larger weight of the early ripe fruits and the error associated with the model. This ratio of harvested yield to the estimated yield (.837) was incorporated as a correction factor in the yield prediction equation.

Two yield prediction equations were developed. The first based on the correction factor and the second based on the regression equation obtained by regressing estimated yield from first harvest fruit weight against the actual harvested yield (Fig 6).

- A. Predicted Yield (PY_A) = (Fruiting nodes/tree*fruit/node* first ripe fruit weight)*.837
- B. Predicted Yield (PY_B) = .753(x) + .825, where .753 = slope (b), .825 = intercept and x = estimated yield based on first harvest fruit size (fruiting nodes/tree * fruit/node * first ripe fruit weight).

Table 5. Validation of fruit yield prediction equations

Model	Mean (Kg/tree)	Pred-HY	t-value	% Error
Harvested Yield (HY)	7.75			
Estimated Yield (EY_1)	9.4	1.65	10.14^{**}	21
Predicted Yield $PY_A = EY_1*.837$	7.86	.11	$.743^{NS}$	1.5
Predicted Yield $PY_B = .825+.753(EY_1)$	7.89	.14	$.842^{NS}$	1.8

Estimated Yield (EY_1)=(Fruiting node/tree*Fruit/node*1st harvest fruit size)

** = Significant at P = .01, NS = Not Significant

These equations were validated on the data from a separate experiment. The first equation predicted yield with 1.5 % error. The second equation predicted with less than 2 % error compared to the actual harvested yield (Table 5). Predicted yields from both equations were not significantly different from the harvested yield. Hence, both equation can be used to predict yield in coffee.

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SUMMARY

The quadrant based sampling procedure was designed to estimate the two most important components of yield, fruiting nodes/tree and fruits/node. The accuracy of the yield estimated is directly dependent upon the accuracy with which these components are estimated. The estimation procedure demonstrated that it can accurately estimate the components of the yield since, the yield estimated based on these components explained 92 % variability in the harvested yield. Two yield prediction equations were developed. The first equation was based on the correction factor obtained by the ratio of the harvested to estimated yield based on the early ripe fruit size. The second was based on the regression equation derived by regressing the estimated yield from first harvest fruit weight against the harvested yield. The regression model explained 92 % of the variability in the harvested yield. These equations were validated on the data obtained from a separate experiment. Both these equations predicted with less than 2 % error with respect to the harvested yield, however, the equation with correction factor was more accurate than the regression equation. Predicted yield from both the equations were not significantly different from the harvested yield and, therefore, both the equations can be used effectively for yield prediction in coffee.

PURINE ALKALOID FORMATION DURING SOMATIC EMBRYO DEVELOPMENT OF *COFFEA ARABICA*

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INTRODUCTION

The question, how the purine alkaloid (PA) formation of somatic embryos of *C. arabica* can be determined non-destructively gave rise to this investigation. In suspension-cultured cells, the PAs caffeine and theobromine disregard the cellular barriers and are dispersed equally to the ratio of volume tissue to volume medium (Baumann and Frischknecht, 1988). Based on this observation we investigated the formation and excretion of purine alkaloids in somatic embryos at four different developmental stages: (1) mature, but still kept in darkness (stage B), (2) mature and green, exposed to light (stage C), (3) germinating (stage D), and (4) with primary root and expanded cotyledons (stage E). The results show that mature somatic embryos already dispose of a complete biosynthetic capacity to produce PA comparable to the adult coffee plant.

MATERIALS AND METHODS

Developmental stages. See Introduction; additionally, PA formation of embryonic tissue (stage A) and of young plantlets with four leaf pairs (stage F) were measured.

Somatic embryo formation. Somatic embryos of *C. arabica* were induced basically according to Söndahl and Sharp (1977) but in suspension yielding masses of well-shaped mature somatic embryos by Self Controlled Somatic Embryogenesis (SCSE) (Neuenschwander and Baumann, 1991).

Incubation. Somatic embryos or plantlets were put on a piece (ca. 2 cm^2) of polyester (Plantex, CCP-102, Pharmacare, Brügg, Switzerland) in a Petri dish (ø 4 cm) with 2 ml embryo induction medium without hormones (Neuenschwander and Baumann, 1991) and incubated at a low radiation of 2 $\mu mole.m^{-2}.s^{-1}$ (fluorescent lamp, Philips) during 7 days.

Extraction of caffeine and theobromine. Medium (100 µl) was filtered (0.2µm, ACRO LC 13, Gelman Sciences Inc., Ann Arbor, MI, USA) and used directly for HPLC analysis. The tissue was suspended in water (4.4 ml/ g fr.wt) and acidified (240 µl HCl conc/ g fr.wt) and thereafter sonicated at 30° C and centrifuged. An aliquot of 25 µl of the supernatant was analysed by HPLC.

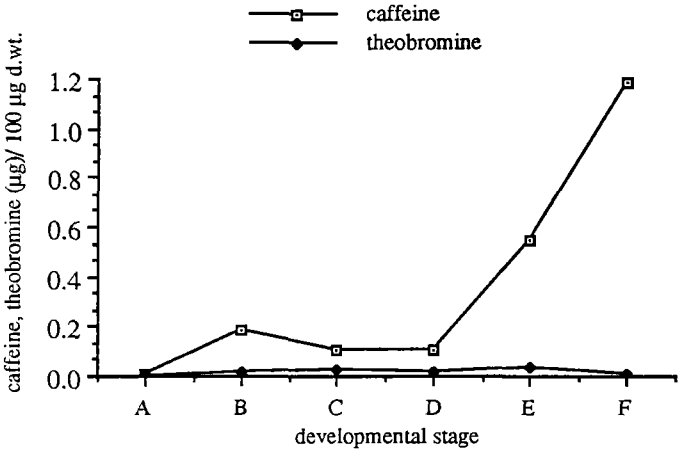
HPLC analysis was carried out according to Baumann and Röhrig (1988).

Dry weight was determined after drying to constant weight at 60° C.

RESULTS AND DISCUSSION

Fig. 4 PA content of tissue. The caffeine and theobromine content of embryos or plantlets was determined with HPLC after a one-week period of incubation. Each point corresponds to a developmental stage (Fig. 2). The values of stage E and F are single values; the rest are means of 4 (B), 3 (C) and 2 (D) induviduals. **A**: PA content (µg) was converted to 100 µg d.wt; **B**: PA content (µg) per embryo or plantlet.

A



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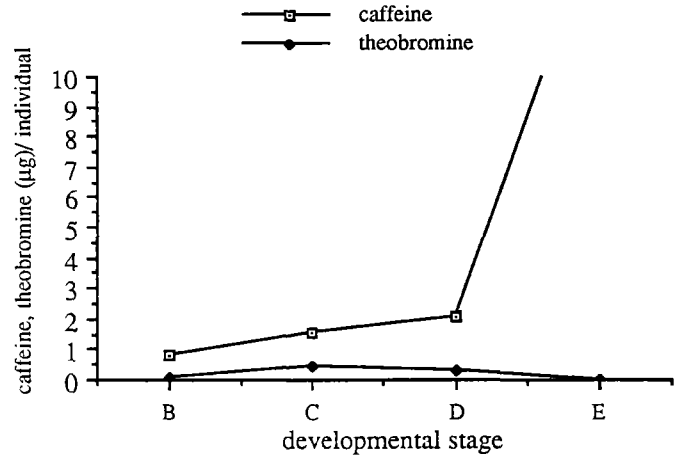
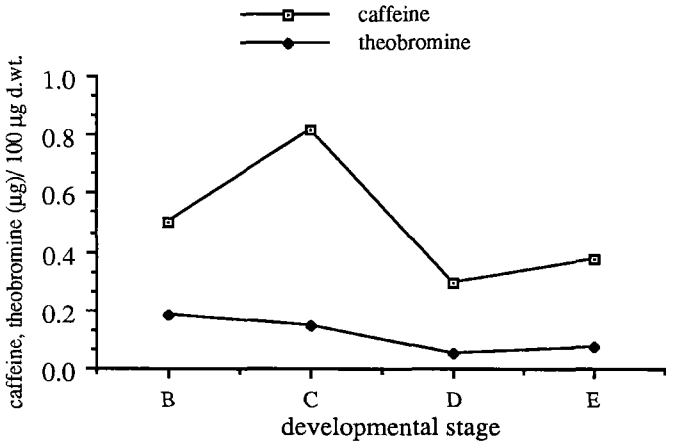


Fig. 5 Excretion of PA into the medium. The caffeine and theobromine concentration in liquid embryo induction medium without hormones was determined with HPLC. **A**: Excretion of caffeine and theobromine (µg) into themedium, converted to 100 µg d.wt; **B**: Excretion of caffeine and theobromine (µg) per embryo or plantlet into the medium.

A



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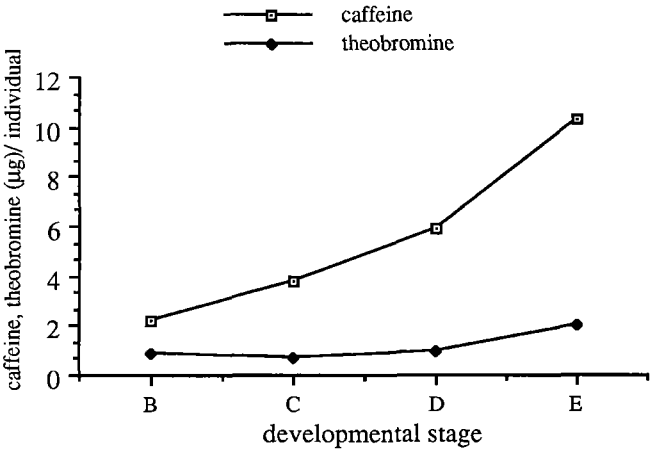

Fig. 4 A and B show that the relative (d.wt) and absolute caffeine content of somatic embryos is low (< 0.2%) up to stage D. Later it increases reaching 1.2% (d.wt) and 15 µg, respectively. The relative value corresponds to the content of coffee beans. The content of theobromine was comparingly low in all developmental stages and is in contrast to the high theobromine content (25 to 50% of total PA) found in young leaves of one-year-old coffee plants which have a high rate of caffeine synthesis (Frischknecht et al., 1986). PA synthesis of somatic embryos is not substantially changed while greening (stage C).

After a one-week incubation period 70% of the total caffeine and about 75% of the total theobromine content is found in the incubation solution. The relatively high PA concentration in the medium allowed us to screen efficiently a large number of embryos in microtest plates (Fig. 3).

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SUMMARY

The formation and excretion of purine alkaloids (PA) during somatic embryo development was studied by analyzing, after a one-week incubation period, the tissue and the aqueous incubation solution of four different developmental stages: mature embryos, embryos exposed to light, germinating embryos and plantlets with cotyledons. The study shows that mature somatic embryos already dispose of a complete biosynthetic capacity to produce PA comparable to the adult coffee plant. While the caffeine content is increasing during embryo germination and reaches 1.2% (d.wt) the theobromine content of all stages is low. Somatic embryos of all four developmental stages readily excrete PA into the incubation solution. After the one-week incubation period 70% of the total caffeine and about 75% of the total theobromine content can be found in the solution. Therefore it is possible to determine the PA content of somatic embryos non-destructively by analyzing the incubation solution.

THE EFFECT OF ETHEPHON AND ADENINE ON PURINE ALKALOID SYNTHESIS IN COFFEE CELL SUSPENSION CULTURES

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Introduction

Coffee cell suspension cultures are an ideal system to investigate secondary metabolism. Purine alkaloids are produced readily and reproducibly in concentrations comparable to the plant [1]. When cells are cultivated in permanent darkness caffeine as well as its precursors theobromine and 7-methylxanthine equilibrate rapidly between cells and medium. Therefore, the compounds are quantified easily by analysing the medium.
However, when cells are kept under photoperiod, caffeine biosynthesis is stimulated [2] and considerable amounts of chlorogenic acid (5-CQA) are formed. Increasing intracellular concentrations of chlorogenic acid are associated with increasing cellular compartmentation of caffeine presumably due to the formation of the caffeine-chlorogenic acid complex [3].

The present studies were initiated in order to answer the following questions: (A) Will caffeine biosynthesis in suspension-cultured cells be stimulated by

ethylene, a plant hormone known to increase secondary metabolite levels in such systems [4]?
(B) In case of a stimulation, will it be possible to augment the ethylene effect by the application of other purine alkaloid-promoting factors such as light or adenine, a rapidly transformed substrate in caffeine biosynthesis [5-8]?

Instead of the gaseous ethylene we used ethephon, which is added to the medium and decomposes to ethylene according to the following reaction:

$$Cl\text{-}CH_2\text{-}CH_2\text{-}PO(OH)_2 + H_2O \rightarrow CH_2{=}CH_2 + H_3PO_4 + HCl$$

ethephon **ethylene**

Results and discussion

Fig. 1(**a**;**b**) shows the effect of ethephon on purine alkaloid production of cell suspension cultures of *Coffea arabica* cultivated either in the dark or under photoperiod. In darkness (**a**) formation of caffeine is significantly, and that of the caffeine precursors 7-methylxanthine and theobromine preferentially stimulated by increased ethephon concentrations. Cells grown under photoperiod (**b**) exhibit a quite different response to the ethephon concentrations used: 0.1 mM sligthly stimulates caffeine synthesis, whereas higher concentrations have an inhibitory effect on caffeine formation but favor the precursor accumulation already observed under dark conditions. However, light-induced stimulation of caffeine synthesis (see controls grown under photoperiod and in darkness, respectively, Fig. 1) is not additionally enhanced by ethylene.

On the other hand, the combination of ethephon with adenine (Fig. 2 and 3) leads to a drastic increase in the production of caffeine (twofold), theobromine (tenfold), and 7-methylxanthine (twentyfold).

A very surprising effect of ethephon treatment is illustrated in Fig. 4: The light-induced synthesis of chlorogenic acid is completely inhibited at ethephon concentrations of 1.0 and 10 mM. This phenomenon may also explain why the presence of ethylene prevents light-stimulated caffeine production. Obviously the latter is correlated to intracellular chlorogenic acid accumultion and in consequence to complex formation.

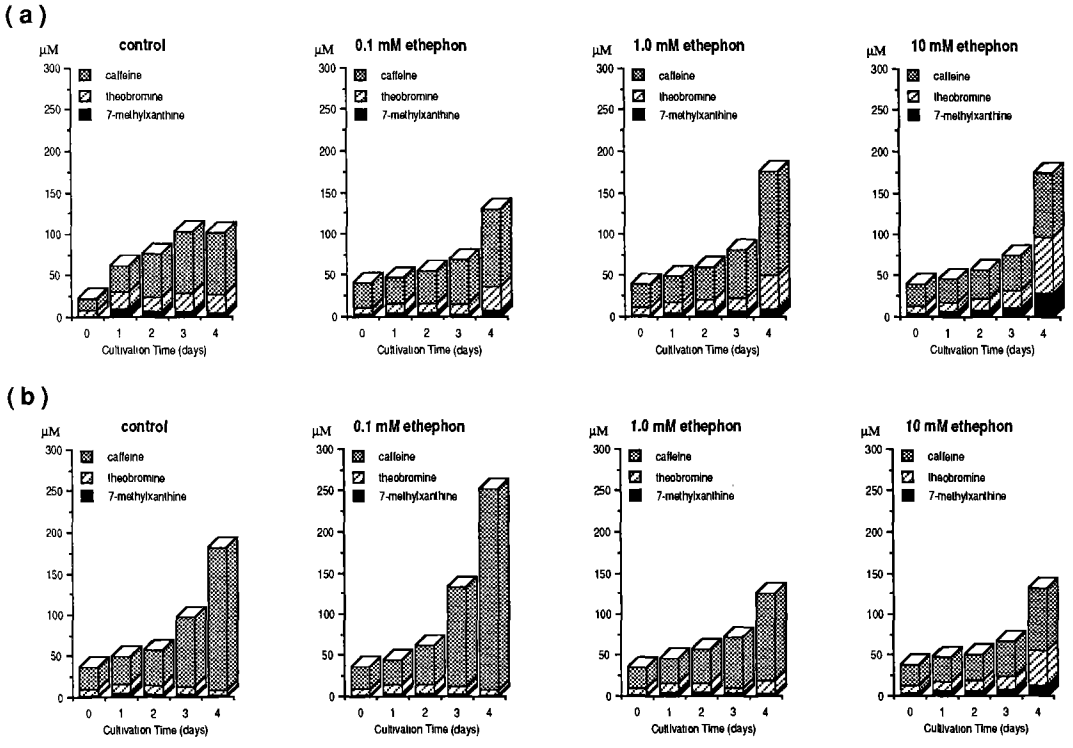

Fig. 1
Effect of ethephon and light on purine alkaloid production (concentrations in the medium) of coffee cell suspension cultures. Cells were cultivated either in the dark **(a)** or under photoperiod **(b)**.

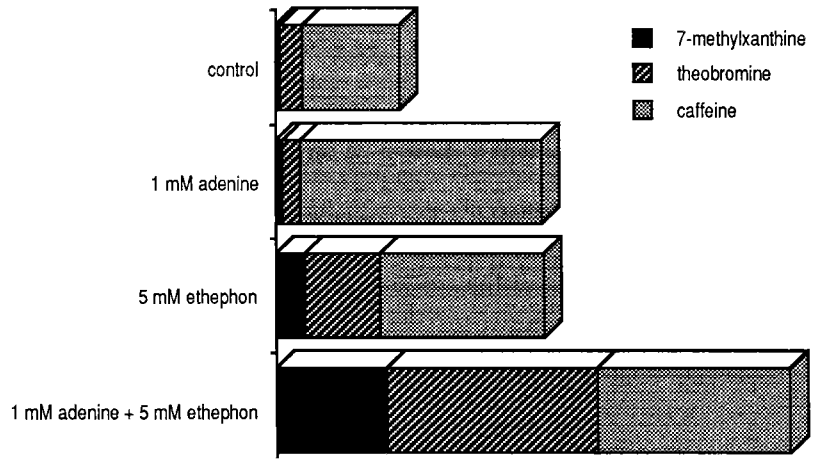

Fig. 2
Effect of ethephon and adenine on purine alkaloid production of coffee cell suspension cultures: alkaloid concentration in the medium after 7 days of cultivation in the dark.

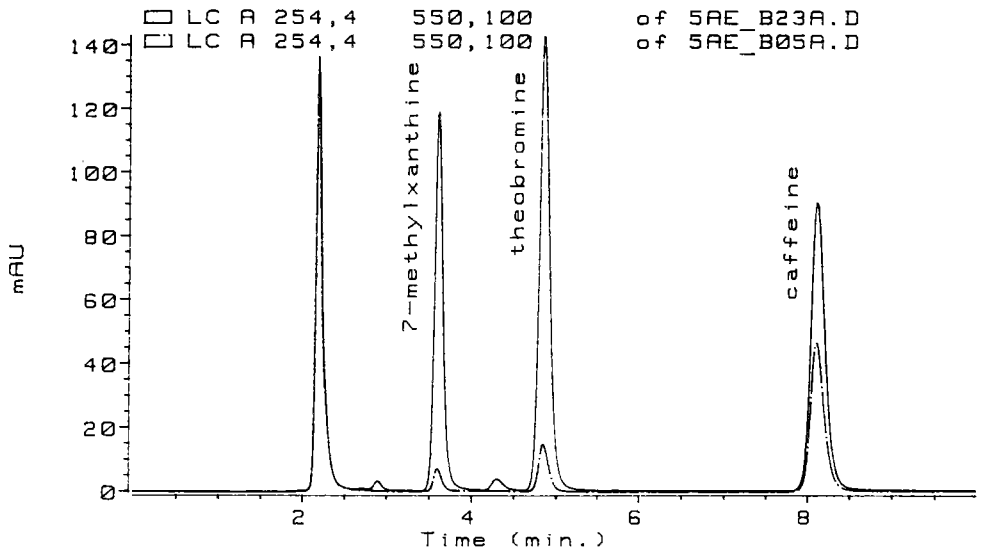

Fig. 3
HPLC-chromatogram of the medium of coffee cell suspension cultures after 7 days of cultivation in the dark: Control (dashed line); 1 mM adenine + 5 mM ethephon (solid line).

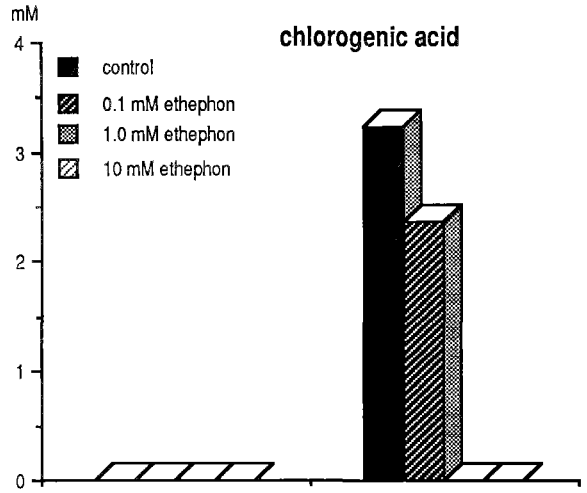

Fig. 4
Effect of ethephon and light on intracellular chlorogenic acid content of coffee cell suspension cultures.

Experimental

Cell line

The cell line used had been established according to [9] from primary callus cultures of *Coffea arabica.*

The suspension cultures were cultivated in a commercially available Murashige-Skoog medium (Flow Laboratories, Irvine, U.K.) supplemented with 30 g/l sucrose, 10 mg/l L-cysteine, 1 mg/l thiamine, 1 mg/l 2,4-D, 0.2 mg/l kinetin, and subcultured (10 g fresh wt / 60 ml medium) every 2 weeks. Cultures in 250 ml Erlenmeyer flasks were kept in the dark at 27°C on a gyratory shaker with 90 rpm (standard conditions).

Experimental conditions

200 µl of a solution of ethephon (1M ; 0.5M ; 0.1M ; 0.01M) and adenine (0.1M), respectively, was added to 10 ml medium in a 50 ml Erlenmeyer flask. Then 10 g cells (fresh wt) were added and the flasks were covered by aluminium foil. They

were either kept in the dark, i.e. standard conditions (see above) or under a photoperiod of 13 hr light and 11 hr darkness in a growth chamber.

Quantification of purines and chlorogenic acid (5-CQA)

150 µl of the medium was filtered through a 0.45 µm filter (MILLEX-FH 13, Millipore, USA) and used directly for HPLC analysis of caffeine, theobromine, and 7-methylxanthine.

For the determination of 5-CQA approximately 150 mg cells (fresh wt) were frozen in a Eppendorf tube (liquid N_2), thawed for 10 min, sonicated for 10 min and centrifuged (3 min, 12'000 g). The supernatant was then centrifuged through a protein filter (Ultrafree-MC, 10'000 NMWL, Millipore, USA) and analysed by HPLC.

HPLC was carried out using a C-8 Lichrospher RP-Select B column (250 x 4 mm, 5 µm particle size). Diode array detector signals were set at 254 and 323 nm for quantification of caffeine and 5-CQA, respectively. The injection volume was 20 µl and the flow rate 1 ml per min. Authentic standards and on-line UV spectra between 220 and 400 nm were used for identification. The eluents were 50 mM ammonium phospate, pH 5.6 (A) and methanol/acetonitrile, 1:1 (B). The gradients employed were either 15-30 % B in A over 10 min (medium) or 0-25 % B in A over 25 min (cell extracts).

Summary

The effect of ethephon, an ethylene-releasing compound, was studied in cell suspension cultures of *C. arabica* cultivated either under photoperiod or in the dark. In darkness purine alkaloid production, especially the formation of the caffeine precursors 7-methylxanthine and theobromine is increased. Cells cultivated under photoperiod are very sensitive to ethephon: low concentrations stimulate alkaloid formation whereas high amounts inhibit purine alkaloid synthesis as well as the production of chlorogenic acid, both usually stimulated

by light. When ethephon is added in combination with adenine, which is a widely accepted early precursor of purine alkaloid biosynthesis, a drastic stimulation of 7-methylxanthine and theobromine synthesis is observed in the dark.

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BIOGENESIS OF RIO FLAVOUR IMPACT COMPOUND : 2,4,6-TRICHLOROANISOLE

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BACKGROUND

In recent studies, we identified 2,4,6-trichloroanisole (TCA) as the impact compound of Rio flavour in green and roast coffee (Spadone and Liardon, 1987; Spadone et al., 1989; 1990). The presence of TCA in Rio coffee appeared to be linked to severe mould infection (Dentan et al., 1987). Furthermore, the geographical localization of Rio flavour and TCA occurrence observed for Brazilian coffees, seemed to be due to specific mould species, as well as to the extent of the microbiological contamination (Liardon et al, 1989).

TCA is a powerful odourant responsible for a number of off-flavour cases in foods and beverages (e.g. wine cork taint) (Maarse et al., 1987). In coffee, a flavour threshold of 0.001 ppb was found and the characters imparted were described as musty, earthy, phenolic, woody, burnt, rubbery (Liardon et al., 1989).

AIM OF THE STUDY

In most off-flavour cases involving TCA, the presence of this compound could be related to residual chlorophenols, a widely used class of biocides, which are converted into anisoles by various moulds (biomethylation). In the case of cork taint, however, Maujean (1985) established that TCA was biosynthesized *de novo* by *Penicillium spp.*. In Rio coffee, several observations led us to postulate that TCA occurrence had also a natural origin (Liardon et al., 1989): 1) the unique occurrence of TCA and its immediate precursor, 2,4,6-trichlorophenol (in typical contamination cases, homologs or isomers have always been found), 2) several moulds species isolated from coffee beans found to possess chloroperoxidase activity (Table 1), or 3) to be capable of methylating chlorophenols to form chloroanisoles. On the basis of these observations and by analogy to the results of Maujean, TCA formation mechanism was hypothesized as shown in Figure 1.

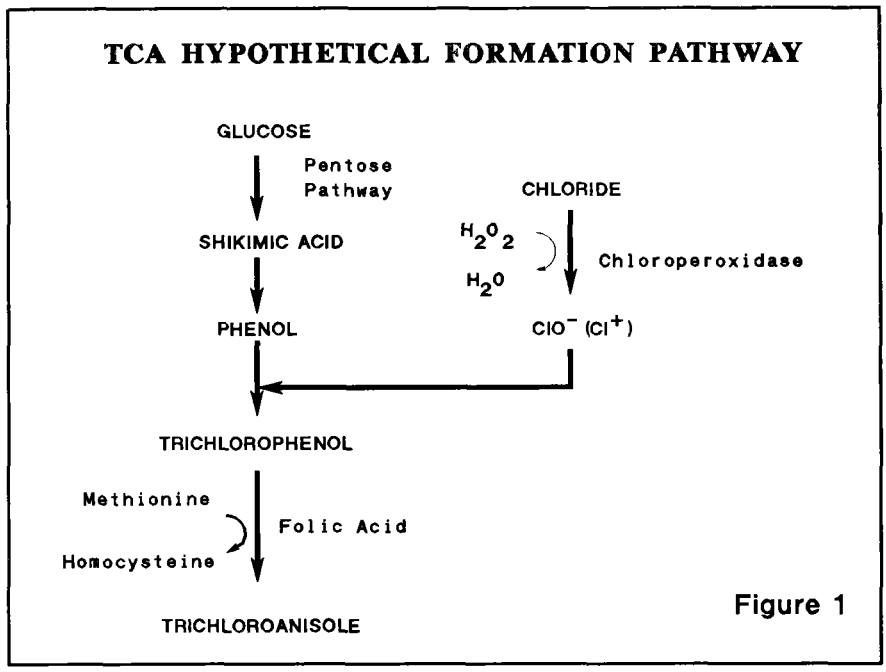


Figure 1

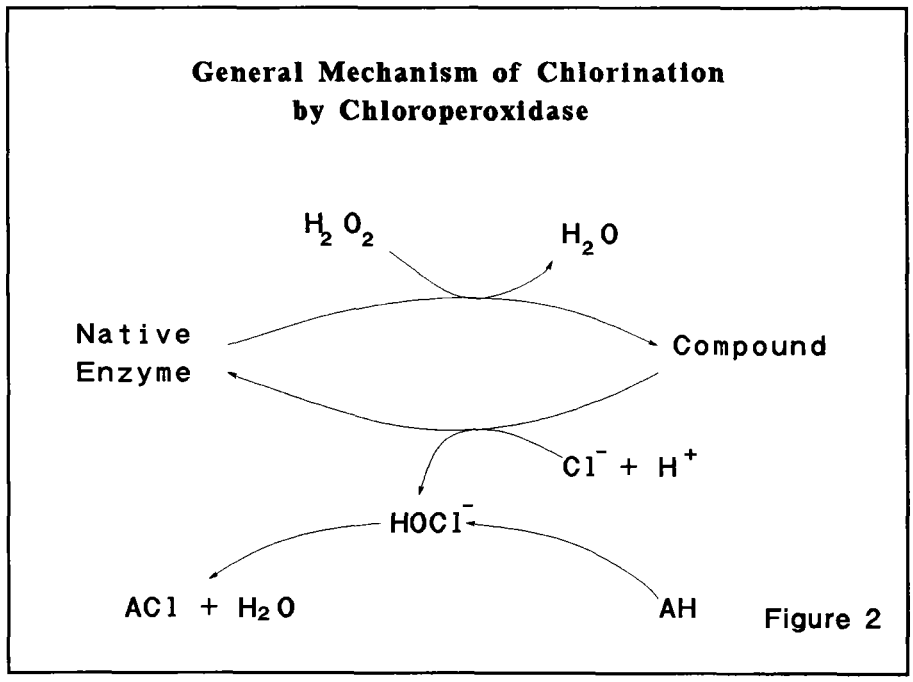


Figure 2

Table 1. Occurrence of Chloroperoxidase Activity in Green Coffee Moulds.

Aspergillus niger	R
Aspergillus ochraceus	R
Aspergillus versicolor	R
Penicillium hirsutum	R
Penicillium crustosum	R
Fusarium graminearum	R
Wallemia sebi	R
Verticillium lateritium	R
Humicola grisea	R
Drechselera sp.	NR
Phoma sp.	NR

NR,R: Isolated from Brazilian coffee from (Non) Rio area

Chloroperoxidase activity test: Hunter et al., 1987

This mechanism involves the formation of phenol via the shikimic acid pathway and its chlorination through the action of chloroperoxidases. These enzymes, which have been found in numerous moulds and bacteria, form a particular class of peroxidases capable of oxidizing chloride into Cl^+ ("active chlorine") which will then react with organic substrates (Neidleman and Geigert, 1986). This mechanism is illustrated in Figure 2. It should be noticed that the actual chlorination step is probably not controlled by the enzyme. As far as hydrogen peroxide is concerned, it is a metabolite of most microorganisms and is usually destroyed by peroxidases and catalases.

Our first approach to verify the hypothesis of TCA biogenesis consisted in testing the capacity of various green coffee moulds to generate this compound in the presence of added active chlorine (main stream of the mechanism shown in Fig. 1). To this effect, moulds isolated from Rio coffee beans were grown in culture broths of varying composition or in green coffee suspensions and the resulting samples were analyzed for the presence of TCA.

EXPERIMENTAL CONDITIONS

Incubation: Culture media (broths or green coffee suspension) were inoculated with 10^7-10^8 conidia and incubated at 26°C for 2 to 10 days without agitation. Following the incubation, the samples were frozen until being analyzed. The composition of the different media used in this study is given below:

Broth A

Glucose	20.0 g/l (200 g/l for W. sebi)
Bacto-peptone (DIFCO, 0118-01)	1.0 g/l
Malt broth extract (DIFCO, 0113-01)	20.0 g/l
Sterilisation 15 min at 121°C	pH: 6.1

Broth B	
Bacto yeast nitrogen base (DIFCO, 0919-15)	6.7 g/l
Shikimic acid (Fluka, 85091)	5.0 g/l
L-Methionine (Aldrich, 15, 169-6)	0.20 g/l
Folic acid (Aldrich, 23, 587-3)	2.0 ug/l
Sterilisation by filtration	pH: 5.4
Broth C	
Glucose	20.0 g/l (200g/l for W. sebi)
Bacto-peptone (DIFCO, 0118-01)	1.0 g/l
Bacto malt extract (DIFCO, 0186-01)	19.0 g/l
Bacto yeast extract (DIFCO, 0127-01)	1.0 g/l
Sterilisation 15 min at 121°C	pH: 5.5
Green coffee	
Whole coffee beans (Santos) in in distilled water	75 g/225 ml
Sterilisation 1 min at 121°C	pH: 5.8

Analysis: The isolation and quantification of TCA in the incubated samples was achieved using the procedure developed for the determination of TCA in Rio coffee (Spadone and Liardon, 1987). Volatiles were isolated from complete samples (medium and biomass) by simultaneous distillation-extraction in a Nickerson-Likens apparatus using methylene chloride. The extracts were concentrated to a small volume and analysed by GC/MS tuned for the specific detection of TCA (Selected Ion Monitoring).

RESULTS AND DISCUSSION

Seven mould species isolated from green coffee beans were incubated for 2 to 5 days in two media (broth A and B) of similar composition to those described by Maujean (1985). In particular, the composition of B was specifically designed to verify the role of the shikimic acid pathway in the eventual formation of TCA. Active chlorine was added in the form of sodium hypochlorite. Non inoculated samples were also included as control in each series. All samples were then analysed for their content in TCA.

As can be seen in Table 2, significant amounts of TCA were detected in five of the tested moulds. The results, however, were found to depend on the incubation medium, possibly reflecting differences in the metabolism of the various moulds. The formation of TCA by *A. niger* and *F. graminearum* was observed only in medium B, probably due to a low prevailance of the shikimic acid pathway in these two species. On the other hand, the incapacity of *A. fumigatus* and *P. granulatum* to produce TCA in medium B could be explained by a limiting effect of shikimic acid, primarily used by the microorganisms as energy source. Alternatively, these two moulds might possess a different metabolic route for the formation of phenol. In any case, further investigation should be done before making any definite conclusion.

A. versicolor was the exception among the tested moulds, as being capable of generating TCA in both media. In addition, it was the most efficient TCA producer. Interestingly, in a previous study this microorganism was specifically identified on Brazilian coffee beans from a region with prevailing Rio flavour occurrence (Liardon et al., 1989). In view of the possible significance of the present result, a second test was carried out. In this test, *A. versicolor* was incubated in two media of slightly different compositions, A and C, and for two different periods, 3 and 7 days. Active chlorine was also added at two different levels, 169 and 390 mg/l. However, the analysis of Cl^+ concentration after 1 hour incubation showed a considerable reduction of the active chlorine concentration, due to reaction with components of the broths. For the higher initial level, the remaining concentration was 24 mg/l in medium A and 17 mg/l in medium C, while for the lower initial

level, the active chlorine had disappeared. Logically, TCA was found only in the samples starting at 390 mg/l active chlorine. These results are presented in Table 3. As can be seen, the formation of TCA took place during the first 3 days, longer incubation time leading to a decline of TCA concentration. It was assumed that this decline was due to losses by volatilization and entrainment by air circulation in the incubator.

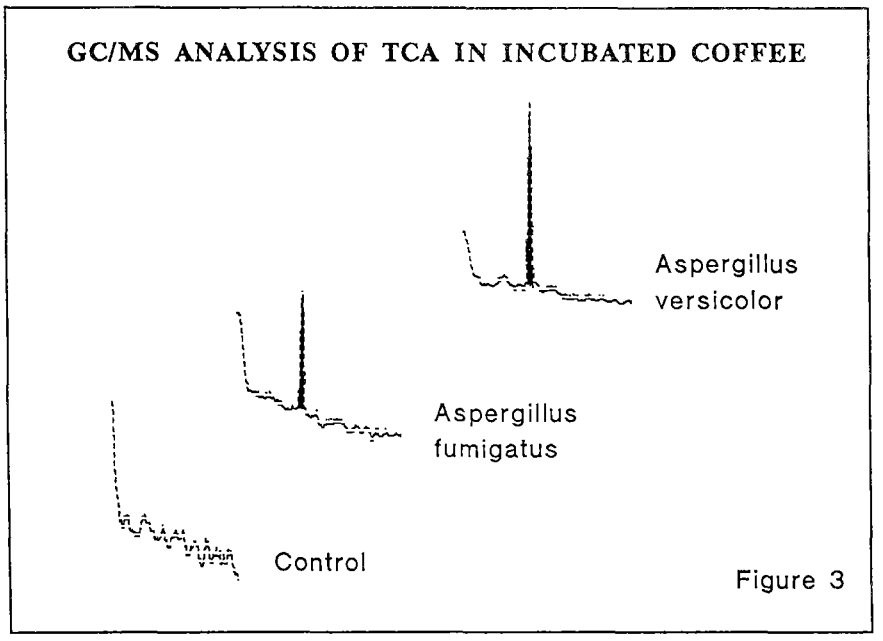
Table 2 TCA Formation by Green Coffee Moulds in Incubation Media.

	TCA Content (ng/l)	
Medium: Initial Cl^+:	Broth A 576 mg/l	Broth B 288 mg/l
Aspergillus niger	n.d.	0.36
Aspergillus ochraceus	n.d.	n.d.
Aspergillus versicolor	2.07	0.45
Aspergillus fumigatus	1.28	n.d.
Penicillium granulatum	0.96	n.d.
Fusarium graminearum	n.d.	0.38
Wallemia sebi	n.d.	n.d.
Control	n.d.	n.d.

n.d.: not detected

Table 3 Influence of Incubation Time on TCA Formation by *A. versicolor*.

	TCA Content (ng/l)	
Incubation time:	3 days	7 days
Broth A	1.65	1.23
Broth C	0.93	0.45



In the next step, the two most active species were inoculated in a suspension of green coffee beans with no other additive than sodium hypochlorite. Control samples, in which either the addition of sodium hypochlorite or the inoculation had been omitted, were also incubated. After 10 day incubation all these samples were analysed for the presence of TCA. The measured concentrations are reported in Table 4. The corresponding mass chromatograms are reproduced in Fig. 3. As can be seen, TCA formation was observed with both species in the presence of active chlorine and the measured concentrations were of the same order of magnitude as found in Rio coffee samples.

Table 4 TCA Formation by Two *Aspergillus* Species Incubated on Green Coffee Beans.

Initial active chlorine:	2.6 ng/g coffee	650 mg/l
TCA Concentration:	ng/g coffee	ng/ml
A. fumigatus	8.8	2
A. versicolor	13.4	3
Control	n.d.	n.d.

n.d.: not detected

CONCLUSION

The generation of 2,4,6-trichloroanisole (TCA) by green coffee moulds in the presence of active chlorine (Cl^+) is an important step in the verification of TCA natural origin in Rio coffee. Cl^+ occurrence in the natural process is supported by experimental evidence of

chloroperoxidase activity in a number of green coffee moulds. Nevertheless, this part of the mechanism still remains to be demonstrated. At this stage, however, some unsuccessful preliminary trials have shown that the main difficulty will be to find the optimum working conditions for coffee mould chloroperoxydases.

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Summary

Previous studies have established that Rio flavour was mainly due to the presence of 2,4,6-trichloroanisole (TCA) in green coffee beans at ppb levels and that the appearance of this contaminant was always linked to heavy fungal and bacterial infections of the beans. More recent investigations have focused at finding the origin of TCA in green coffee. To this effect, various moulds isolated from green coffee beans were incubated in culture broths containing sodium hypochlorite. TCA formation was observed for several of these moulds. Similarly, TCA was also produced by incubating *A. versicolor* and *A. fumigatus*, in a suspension of green coffee beans in the presence of hypochlorite. These results are an important step in the verification of TCA biogenesis by green coffee moulds.

Résumé

Des études précédentes ont permis d'établir que le goût rioté est essentiellement dû à la présence du 2,4,6-trichloroanisole (TCA) dans les grains de cafés verts. Par ailleurs, la présence de ce contaminant est toujours associée à une importante infection fongique et bactérienne. Depuis lors, les efforts ont porté sur l'élicidation de l'origine du TCA dans le café vert. A cet effet, des moisissures isolées sur des grains de café ont été incubées dans différents bouillons de culture contenant de l'hypochlorite de sodium. Plusieurs de ces moisissures ont donné lieu à la formation de TCA. De la même manière, l'incubation de *A. versicolor* et *A. fumigatus* dans une suspension de grains de café verts et en présence d'hypochlorite a conduit à la formation de TCA. Ce résultat est un premier pas important pour la vérification de la biogénèse du TCA dans le café vert.

A NEW ROASTING COMPONENT IN COFFEE

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The roasting process of coffee produces a lot of new compounds. Fig. 1 shows the HPLC-chromatogram of a sample of crude Robusta coffee prepared for the determination of diterpenes by the method of Speer (1). Fig. 2 demonstrates the HPLC-chromatogram of the corresponding roasted coffee.
Both chromatograms differ in that Fig. 2 shows apart from the well known substances cafestol and 16-O-methylcafestol the detection of a further, unknown substance.
Fig. 3 demonstrates the HPLC-chromatogram of a sample of crude Arabica coffee. Here roasting results in two additional compounds apart from the well known diterpenes kahweol and cafestol (Fig. 4).
From several experiments we already know that the concentrations of cafestol and especially kahweol are reduced by the roasting process.

Therefore our aim was to prove whether these new compounds could be identified as decomposition products of the two above mentioned diterpenes.
After pyrolisation of cafestol we analysed the solution by HPLC. The chromatogram in Fig. 5 displays the unidentified peak.
To identify the structure of this substance, a sample of Robusta roasted coffee was repeatedly chromatographed using semipreparative HPLC. The elution range of the unknown substance was fractionated. After removal of the solvent the remaining white crystalline substance was analysed by capillary gaschromatography/mass spectrometry.

The EI-mass spectrum of the silylized compound is shown in Fig. 6. Fig. 7 demonstrates the CI-mass spectrum.
The molecular weight of the new substance is 298. It differs from weight of cafestol by 18 masses. Therefore the new compound originates from cafestol by dissociation of water.

Fig. 8 presents the probable structure of the compound, that still is to be verified.
Meanwhile, we also confirmed another compound resulting by water dissociation of kahweol.

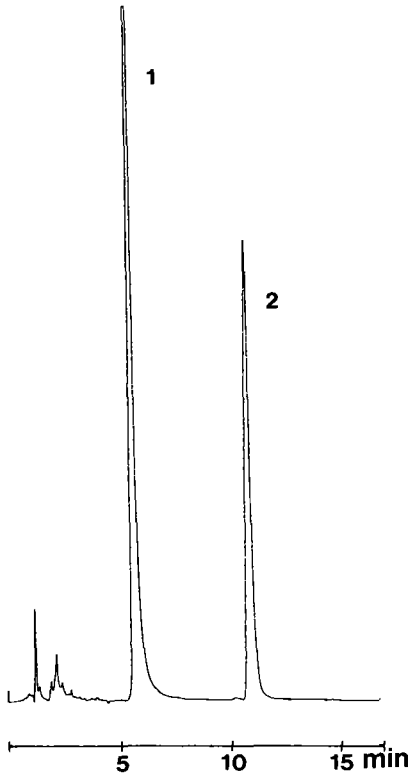

Fig. 1 Guinea crude Robusta coffee
1 = Cafestol 2 = 16-O-Methylcafestol

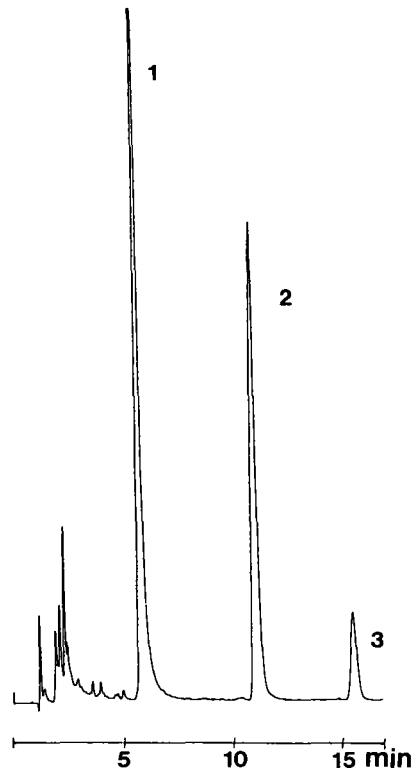

Fig. 2 Guinea Robusta coffee roasted at 285C, 3 min.
1 = Cafestol 2 = 16-O-Methylcafestol
3 = Unknown

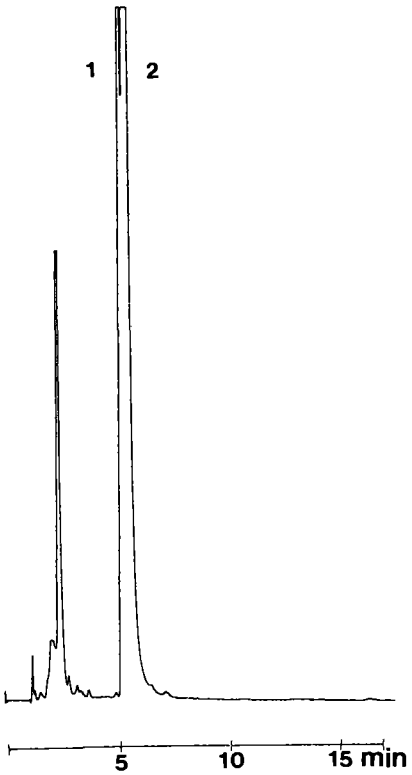

Fig. 3 Colombia crude Arabica coffee
1 = Kahweol 2 = Cafestol

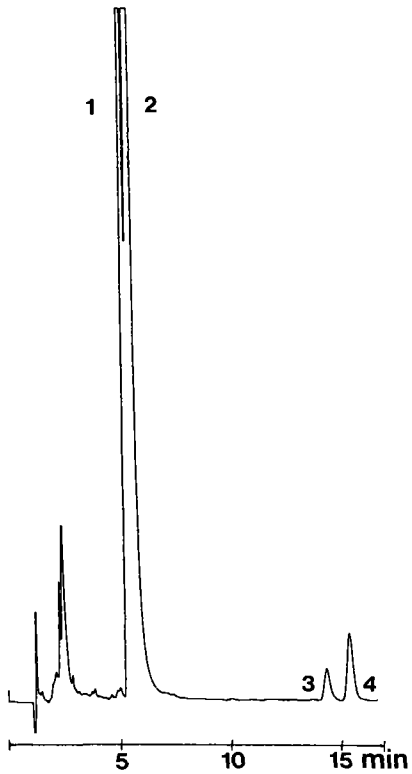

Fig. 4 Colombia Arabica coffee roasted at 270C, 3 min.
1 = Kahweol 2 = Cafestol
3 and 4 = Unknown

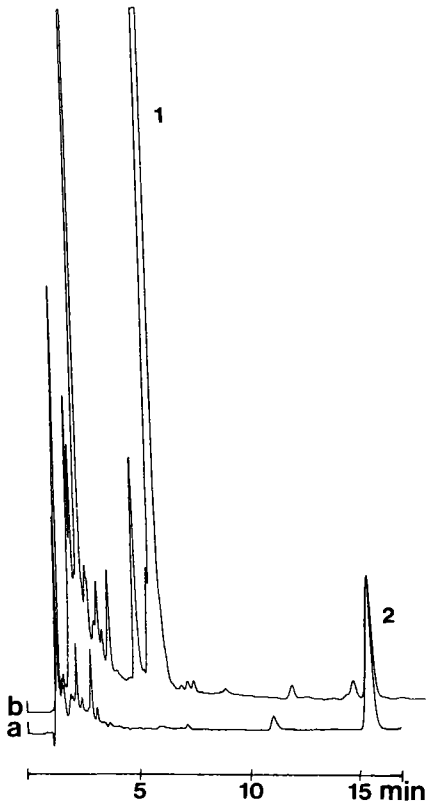

Fig. 5 a) The unknown substance fractionated by semipreparative HPLC
b) The unknown substance after pyrolysis of cafestol
1 = Cafestol 2 = unknown substance

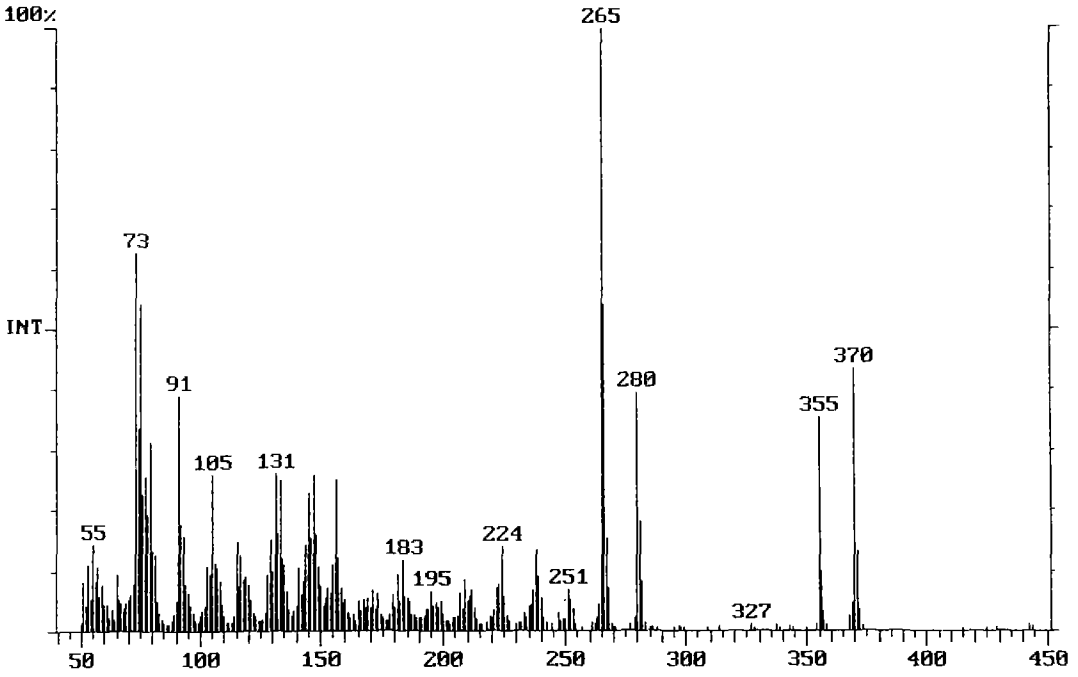

Fig. 6 EI-mass-spectrum of the unknown substance

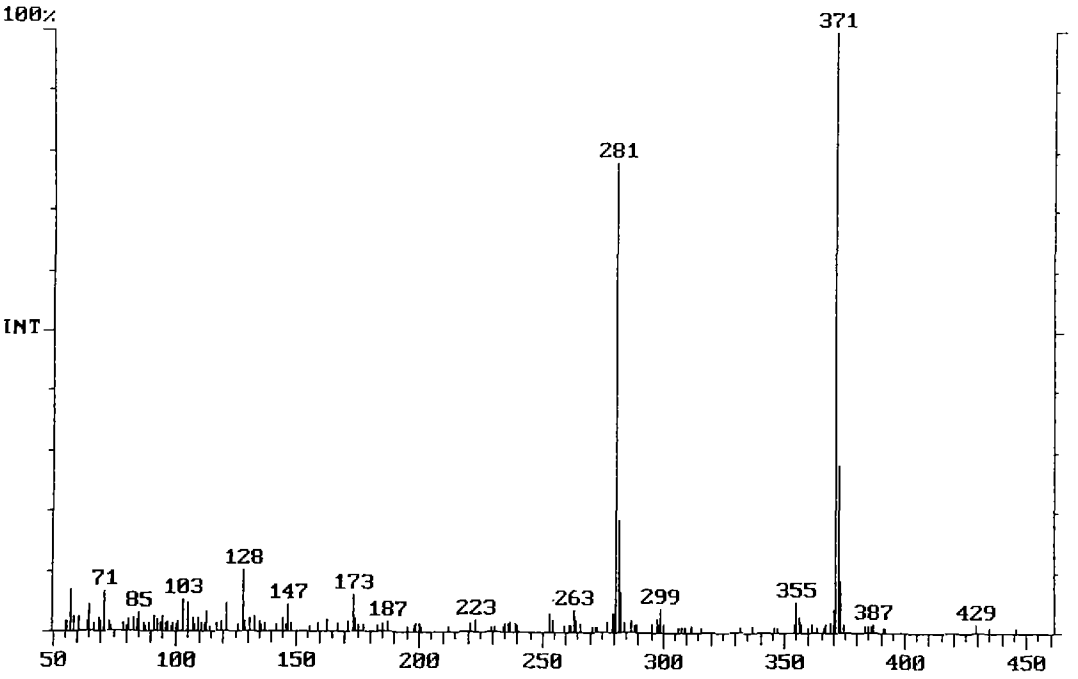

Fig. 7 CI-mass-spectrum of the unknown substance

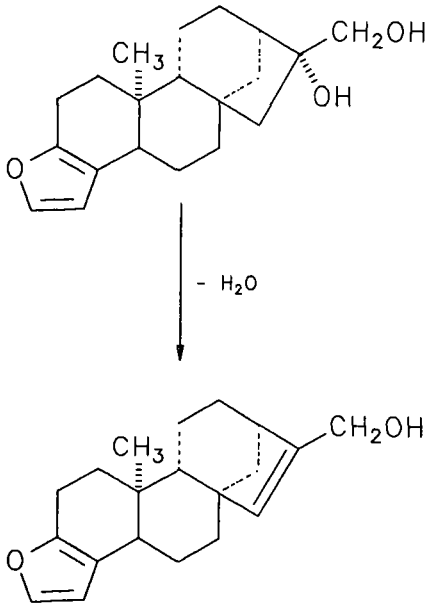

Fig. 8 Probable structure of the new compound

Methods

1.PYROLYSIS OF CAFESTOL

7,50 mg cafestol are pyrolised in a sealed vial at 275°C for 10 minutes. The pyrolyzate is solved in 5 ml dichloromethane and 1 ml of this solution is dried in nitrogen stream and prepared by adsorption chromatography on a disposable extraction column according to SPEER (1) and analysed by HPLC.

2. HPLC

Column:	250/8/4
Packing material:	Hypersil ODS, 5 µm
Solvent:	Acetonitrile/water 60:40 (V:V)
Flow rate:	1,0 ml/min
Detection:	220 nm
Injection:	20 µl

3. SEMIPREPARATIVE HPLC

Column:	250-10
Packing material:	Lichrosorb RP-18, 7 µm
Solvent:	Acetonitrile/water 60:40 (V:V)
Flow rate:	4,00 ml/min
Detection:	220 nm
Injection:	300 µl

4. SILYLATION

Some crystals of the isolated substance are silylated with 0.1 ml TMCS (trimethylchlorosilane) and 0.2 ml MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide) for four hours at 85°C. After cooling down 0.7 ml iso-octane are added.

5.GC/MS

Column:	DB-5, 30 m, i.ϕ 0,32 mm, 0,25 µm
Injection:	1 µl (PTV)
Temperature program:	60°C-1,5 min-10°C/min ad 280°C
Xfer line:	260°C
Manifold heater:	230°C
Reactant gas:	Methanol

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Summary

The roasting process causes chemical changes of many coffee components, one of these - a decomposition-product of cafestol - is presented in this paper.

Resumen

El proceso del tostado causa cambios quimicos en muchos componentes del café, uno de estos - un producto de descomposición del cafestol - es presentado en este trabajo.

CORRELATION BETWEEN SENSORY EVALUATION DATA (TASTE AND MOUTHFEEL) AND NEAR INFRARED SPECTROSCOPY ANALYSES

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INTRODUCTION

Sensory evaluation of coffee has been practised since coffee was discovered. One of the legends about its discovery is that a goat keeper called Kaldi, who was living in the part of Africa known at that time as Abyssinia, discovered the cherries and introduced them to monks from a nearby monastery. The monks found that after drinking the beverage prepared from the berries helped them to keep awake during prayers (ICO 1988). Be that as it may, coffee has been appreciated for its unique flavour characteristics for more than a thousand years, and in the twentieth century its value is still directly proportionate to its quality in terms of its flavour. The concept of quality in coffee is, however, a topic that generates controversy and definitions of coffee quality vary widely. P. Pochet gave one of the most complete definitions: "The quality of coffee in the accepted sense of the term includes the physical, chemical and organoleptic properties mainly sought after by the consumer" (Pochet, 1990).

The physical characteristics of green and roasted coffee are very important when assessing quality. Nevertheless, sensory evaluation of coffee, or cup tasting as it is called among coffee producers, exporters and traders, is essential for the final grading of a coffee batch.

Cup tasting still remains the ultimate method of determining the quality of coffee as a beverage but, like any other sensory methodology which relies on human judgement, it is considered to be a subjective and sometimes doubtful method.

As with many food products, the alternative would be to resort to analysis based on the chemistry of coffee as an "objective" method which permits a complete assessment of coffee quality. However, even the volatile constituents of coffee have been and still are the subject of numerous investigations. Whether it be the identification of coffee aroma volatiles (Van Straten et al. 1986, and Vitzthum 1975), the use of a newly adapted analytical technique to identify new coffee volatiles (Cros et al. 1979, Jennings et al. 1985, Liardon and Spadone, 1985 and Shibamoto, 1981), the relationship between coffee volatiles and some sensory characteristics (Holscher et al. 1990, and Liardon et al. 1987), or the study of the characteristics and the impact of some of the already identified coffee volatiles on the overall coffee aroma (Clark 1990), the fact remains that measuring volatiles, either known or unknown, is a very expensive and impractical procedure for routine quality control purposes.

Attempts have also been made to utilize the analysis of the non-volatile constituents of coffee to determine its quality. Amorin and his co-workers (1974) investigated the chemistry of Brazilian green coffee and its relationship to the coffee grades established by cup tasters.

It should be borne in mind that establishing a valid correlation between a sensory measurement, which is subjective, and any selected objective measurement requires that the physical, physicochemical or chemical parameters should closely resemble the sensory parameter, as evaluated by cup tasters. In the case of coffee it is important to realize that there is no analysis or instrument capable of simulating cup tasting or the sensory evaluation of coffee by coffee experts, trained tasters, or even regular coffee drinkers. Many correlations between sensory and chemical data are intended only as a close approximation or as a predictor of sensory judgements and not as a full replacement of the cup tasting procedure.

Williams and his collaborators (1989) explored the possible correlations between the sensory data produced by a trained coffee panel and by analysis of the volatile and non-volatile constituents of coffee samples, with particular reference to bean maturity, but no definitive pattern could be established in respect of this important factor which greatly affects the quality of green coffee.

It is difficult to establish significant correlations between sensory and analytical data in the case of coffee mainly because of the complexity of overall coffee flavour (aroma/taste/mouthfeel) and the sophistication of available techniques such as gas chromatography, mass spectrometry, high performance liquid chromatography, etc., which does not permit this type of analysis to be implemented as a routine quality control tool given the length of time required for the preparation and analysis of samples and the high costs involved.

An analytical technique known as Near Infrared Spectroscopy (NIR) has been applied successfully to several food products (Hall et al., 1986). This technique has the advantage of requiring minimum sample preparation. Spray and his co-workers (1990) tested the NIR-method for determining types of meat and established successful discrimination among four meat types. Scotter and his co-workers (1990) used NIR for the analysis of strawberry jam, and reported high multiple regression coefficients in fructose and fruit content determinations. Martens and Martens (1986) investigated the use of NIR for predicting the chemical and sensory quality of peas. They found that using a relative ability of prediction (RAP) index developed by Martens and Naes (1987), a high RAP could be obtained for the sensory characteristics only when the NIR, physical and chemical data were combined. (RAP = 0.86 for texture characteristics and 0.72 for flavour).

The present study was designed to compare two descriptive approaches to the sensory evaluation of coffee and to investigate potential correlations between the NIR and sensory data.

MATERIALS AND METHODS

Coffee samples

Twelve Arabica coffees from the stocks of the Promotion Fund of the International Coffee Organization were selected for this study. Table 1 lists these samples, indicating the code given to each, the processing method used in preparing the coffee, and the commercial description.

TABLE 1 - COFFEE SAMPLES DESCRIPTION

CODE	ORIGIN	PROCESSING METHOD	TYPE
S1	ZAIRE	WET	KIVU - 3
S2	PAPUA NEW GUINEA	WET	HIGH GROWN KORFENA
S3	ETHIOPIA	WET	KAFFA LIMU G2
S4	INDONESIA	WET	G1 - BLAWAN
S5	COSTA RICA	WET	SHB
S6	BRAZIL	DRY	IPANEMA - HAND PICKED
S7	BOLIVIA	WET	FINE WASHED
S8	MALAWI	WET	FQ1
S9	MEXICO	WET	ALTURA
S10	BURUNDI	WET	FULLY WASHED "SUPER"
S11	UGANDA	WET	HIGH GROWN BUGISU - AA
S12	ZIMBABWE	WET	CODE - 52

Roasting and brewing

The twelve coffees were roasted in a Wolverine Jet Roaster at 260°C for 125 seconds. Samples were then ground in a Bizerba grinder to a medium-fine degree (setting 5.5), vacuum packed in bags of 55 grams and stored at a temperature of 4°C until required for sensory evaluation.

The brews were prepared in four electric filter Technivorm Clubline machines. Each of the four machines was used to prepare each of the four samples served in fifteen sensory sessions. Brews were prepared using 55 grams of ground coffee per 1.0 litre of purified water (reverse osmosis). Hotplates were kept at a setting of 3/4 after the coffee had been brewed according to the manufacturer's instructions.

Physical and physicochemical analyses

The colour of the roasted, ground and brewed coffees was determined, using a Dr. Lange Colorimeter LF90 calibrated to 100 percent reflectance with the standard Kaffe-A (Dr Lange UME 3 1984). The pH of the brewed coffee was measured with an Alpha 500 pH meter placed directly in the beverage at room temperature (22°C). Finally, the soluble solids content was analyzed using an oven method (Nordic Coffee Brewing Centre 1974).

Sensory evaluation

Six sensory characteristics were evaluated, namely: Acidity, Bitterness, Sweetness, Body, Astringency and Overall Aroma. A quantitative descriptive profiling test was conducted following a complete balanced block design for the twelve coffee samples with five replicates, serving only four samples at one time. A total of fifteen sensory evaluation sessions were required to complete the experimental design.

Using this experimental design, two approaches were tested during sensory evaluation. For the first (Approach One) the four samples were served one at a time for full evaluation of the six flavour characteristics. For the second (Approach Two) the four samples were served simultaneously and each flavour characteristic was evaluated by comparing it with this characteristic in the other samples.

Coffees were served black (no added sugar or milk) in specially designed tasting cups (approximately 50 ml. volume, black lined inside) labelled with three random digit numbers. Serving portions were approximately 40 ml. Tasters were asked to taste directly from the cup, spoons were not provided. Tasters were served the coffee samples at temperatures between 60°C and 65°C.

A fully computerized French manufactured sensory data capture system (ACTIS) was used to collect and process the data obtained in the sensory tests. Instructions for the test appeared on a small screen monitor, and sensory ratings were given using small analytical keyboards with a structured nine-point scale. Panellists were also requested to complete a traditional scoresheet with their sensory ratings for the six selected flavour characteristics, in order to analyse the sensory data using alternative statistical programmes. The panel consisted of eleven members for Approach One and six members for Approach Two. All panellists had experience in the sensory evaluation of coffee and had been involved in the development of the consumer-oriented descriptive vocabulary for coffee used in the investigation.

NIR analysis

Following the same experimental design as the one used for the sensory evaluation the coffee samples were analyzed by the Near Infrared Spectrometer. Spectra of the green and roasted and ground coffees were obtained. Samples were prepared according to specified instructions (NIR-Systems 1989). An NIR Spectrometer model 6250 was used for all NIR analyses.

Statistical analysis

Analysis of variance, correlation analysis, stepwise regression analysis, cluster analysis and principal component analysis were applied to the sensory data as well as to the chemical data obtained from the NIR predictions on green coffee. Partial Linear Square Regression was used to develop the equations for the prediction of sensory characteristics based on the spectra of green and roasted and ground coffees.

Four statistical programmes were used to conduct these analyses: SPSS/PC+ version 4.0 (Norusis/SPSS Inc. 1990); SENSTAT-2; ACTIS (from the French manufactured sensory data capture system); and the statistical programmes of the computerized Near Infrared Spectrometer (Westerhaus and Shenk 1989).

RESULTS AND DISCUSSION

Physical and physicochemical analyses

The colour of the twelve roasted coffees was measured to provide an indicator of the degree of roast of each coffee sample. No attempt was made to match the colour of roast for all twelve coffees since the use of fast roasting made it reasonably easy to keep roasting conditions constant for all samples.

In accordance with the experimental design fifteen sensory evaluation sessions were held. Four samples were brewed for each session and a total of sixty brews was prepared for each of the two approaches followed. Colour, percentage of soluble solids, and pH were analyzed for each of the sixty brews in the two sensory tests. The results of these analyses are shown in Table 2.

TABLE 2 - AVERAGE VALUES OF PHYSICAL AND PHYSICOCHEMICAL MEASUREMENTS MADE ON THE TWELVE COFFEE SAMPLES[1]

ORIGIN	PHYSICAL AND PHYSICOCHEMICAL ANALYSES						
	Colour (roasted coffee)	Approach One (brew)			Approach Two (brew)		
		Colour	% Soluble Solids	pH	Colour	% Soluble Solids	pH
Bolivia	89.30	17.48	0.1419	4.40	18.04	0.1410	4.51
Brazil	105.08	16.24	0.1377	4.65	15.82	0.1395	4.67
Burundi	88.46	15.46	0.1378	4.32	16.84	0.1378	4.38
Costa Rica	95.16	14.44	0.1468	4.26	15.16	0.1489	4.42
Ethiopia	98.04	15.20	0.1287	4.34	15.72	0.1412	4.56
Indonesia	102.96	18.80	0.1491	4.54	17.58	0.1578	4.57
Malawi	105.46	16.84	0.1271	4.35	17.04	0.1417	4.45
Mexico	100.48	17.14	0.1524	4.42	19.20	0.1727	4.53
Papua New Guinea	100.68	15.06	0.1329	4.33	15.80	0.1406	4.40
Uganda	105.54	18.82	0.1470	4.32	21.10	0.1641	4.49
Zaire	96.30	14.64	0.1551	4.25	16.90	0.1557	4.33
Zimbabwe	109.36	16.50	0.1238	4.26	16.54	0.1370	4.36

1 Values are the average of five replicates.

The results indicated that the range of variation between the highest and lowest roast colour value among the twelve samples was only 20.9 reflectance units. These differences were very difficult to detect with the eye. No significant correlations among these variables was found.

Sensory evaluation

Of the two approaches tested during the investigation, the one in which samples were evaluated individually one at a time (Approach One) provided greater differentiation of the twelve samples and showed consistency among tasters.

Table 3 shows the results of the analysis of variance and the level of statistical significance of each source of variation for each approach.

TABLE 3 - STATISTICAL SIGNIFICANCE FOR EACH SOURCE OF VARIATION FROM THE ANALYSIS OF VARIANCE

Attribute	Approach One			Approach Two		
	T	S	R	T	S	R
Acidity	***	***	ns	***	***	ns
Bitterness	***	***	ns	***	ns	ns
Sweetness	***	ns	ns	***	ns	ns
Body	***	***	**	***	ns	ns
Astringency	***	***	ns	***	ns	ns
Aroma	***	***	**	***	ns	*

T = Tasters; S = Samples; R = Replicates

The results indicate that tasters seemed to perform better when presented with only one sample to assess the six sensory characteristics selected than when presented with four samples to assess each of the sensory characteristics on a comparative basis.

It should be noted that when a set of different samples is presented at one time, tasters may create a different relative scale by using the samples with the highest and lowest intensity values as reference points when assessing each sensory characteristic. Responses to the samples of the same coffee would thus vary from session to session.

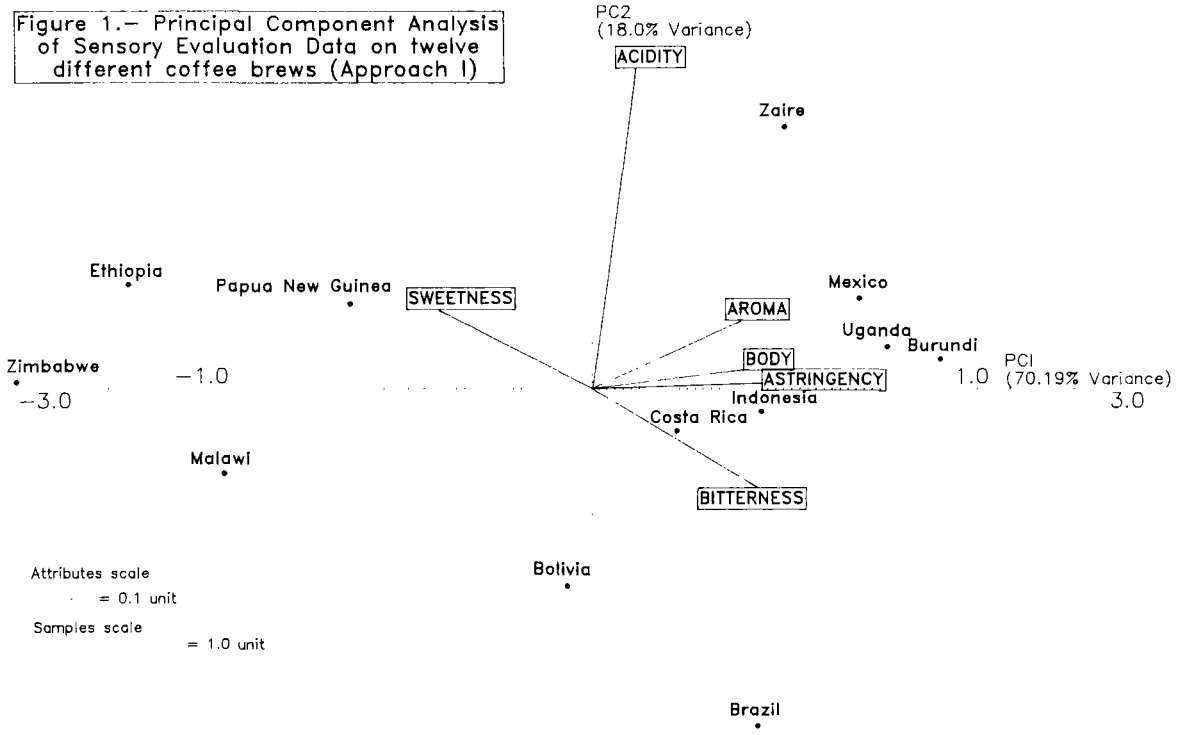
After establishing that Approach One provided better results for the sensory evaluation, additional statistical analyses were conducted on the data. Using the intensity range given to each of the sixty coffee brews served, for Acidity, Bitterness, Body and Aroma, a cluster analysis was carried out in order to group the sixty samples into three categories:

(1) Coffees with high Acidity, Body and Aroma but with low Bitterness
(2) Coffees with medium Acidity, Body, Aroma and Bitterness; and
(3) Coffees with low Acidity, Body and Aroma but with medium Bitterness.

The cluster analysis established clear-cut groups. The coffees from Ethiopia, Papua New Guinea and Zaire were consistently grouped into category (1); the coffees from Burundi, Indonesia, Mexico and Uganda into category (2); and the coffees from Bolivia, Brazil, Costa Rica, Malawi and Zimbabwe into category (3).

The principal component analysis of the sensory data collected for Approach One confirmed the grouping established by the cluster analysis (see Figure 1).

Figure 1.– Principal Component Analysis of Sensory Evaluation Data on twelve different coffee brews (Approach I)



NIR analysis

Spectra of green and roasted coffee samples were produced. In the case of the green coffee samples the chemical composition was predicted using the calibration equations (these are included in the software of the NIR Spectrometer, model 6250) for nine constituents, namely: protein, extractable solids, lipids, total carbohydrates, sucrose, caffeine, trigonelline, and moisture.

The Brazilian coffee showed the highest protein content (15.67%) and the coffee from Zaire the lowest (12.62%). This result tallied with the grouping of the Brazilian coffee in category (3), for coffees with the highest degree of bitterness (the high

content of some aminoacids has been associated with a high degree of Bitterness) while the coffee from Zaire was placed in category (1), for coffees with low Bitterness.

The content of lipids and extractable solids, which is sometimes related to the Body of brewed coffee, ranged from 11.95 percent to 15.15 percent for lipids and from 31.00 percent to 33.44 percent for extractable solids. Once more, these values tallied with the results obtained from the cluster analysis, with the lowest values for coffees in category (3) and the highest values for coffees in category (1).

Similar results were obtained for the other constituents analyzed by NIR (see Table 4).

TABLE 4 - CHEMICAL COMPOSITION OF GREEN COFFEE SAMPLES BY NIR ANALYSIS

ORIGIN	CONSTITUENTS (%)							
	PROTEIN	EX.SOL	LIPIDS	CHO	SUCRO	CHLO	CAFF	TRIG
Bolivia	14.06	32.78	15.01	7.00	4.99	5.77	1.47	1.05
Brazil	15.67	33.08	12.70	6.94	5.56	5.70	1.78	1.06
Burundi	13.56	33.41	14.69	8.01	6.41	6.01	1.44	1.15
Costa Rica	14.84	32.40	13.55	7.54	5.99	5.93	1.51	1.00
Ethiopia	13.39	32.33	15.15	7.20	5.41	6.22	1.51	1.00
Indonesia	15.19	32.75	13.52	7.12	5.43	5.66	1.48	1.33
Malawi	14.71	31.00	11.95	7.58	6.29	6.00	1.45	1.25
Mexico	15.00	32.55	14.39	7.13	5.41	6.20	1.52	1.35
Papua New Guinea	14.58	32.52	14.16	7.51	6.08	5.66	1.48	1.01
Uganda	13.24	31.76	13.83	7.31	5.65	5.86	1.36	1.13
Zaire	12.61	33.44	14.92	8.40	6.51	5.55	1.24	0.95
Zimbabwe	12.99	32.24	14.66	7.85	5.98	5.84	1.26	1.02

EX.SOL = Extractable Solids; CHO = Total Carbohydrates; SUCRO = Sucrose; CHLO = Chlorogenic Acids; CAFF = Caffeine; TRIG = Trigonelline.

Correlations between and within sets of data

When the correlation matrix was produced for the three sets of data (sensorial, physicochemical, and NIR) very few significant correlations were found. For instance, Acidity correlated positively with Lipid content ($r = 0.365$; $n = 60$) and with total carbohydrate content ($r = 0.416$; $n = 60$) and negatively with protein content ($r = 0.500$; $n = 60$), caffeine content ($r = 482$; $n = 60$) and pH ($r = -0.379$; $n = 60$).

Within sets of data, statistically significant correlations were found in the case of the NIR data between protein and lipid content ($r = -0.507$; $n = 60$), protein and total carbohydrate contents ($r = -0.454$; $n = 60$), protein and caffeine contents ($r = 0.776$; $n = 6$) and protein and trigonelline contents ($r = 0.511$; $n = 60$).

Predictions of sensory scores from NIR analysis

Stepwise regression analysis was applied to find equations which may predict sensory scores for the six sensory characteristics evaluated in this investigation (see Table 5).

TABLE 5 - RESULTS OF THE STEPWISE REGRESSION ANALYSIS ON SENSORIAL, PHYSICOCHEMICAL AND NIR DATA

Sensory Characteristic	Variables in the equation	Correlation Coefficient (R)	Significance of F-Ratio
Acidity	Protein (NIR)	0.500	***
	Soluble Solids (%)	0.574	***
Bitterness	Soluble Solids (%)	0.412	***
Sweetness	Soluble Solids (%)	0.430	***
	Millivolts (pH-meter)	0.510	***
	Total Carbohydrates (NIR)	0.568	***
Body	None	-----	---
Astringency	Extractable Solids (NIR)	0.305	*
Aroma (overall)	None	-----	---

*, *** = Statistical significance at $p < 0.05$ and $p < 0.001$ respectively.

Partial linear square (PLS) regression analysis was used to develop equations based on the correlations between NIR spectra and sensory data. Two equations were developed, one using the spectra of green coffee and the other using the spectra of roasted coffee.

Tables 6 and 7 show the correlation coefficients and the standard error of calibration and validation from the developed equations for green and roasted coffee respectively.

TABLE 6 - GREEN COFFEE BEAN CALIBRATION FOR SENSORY CHARACTERISTICS

Sensory Characteristic	Number of samples used	Range	R	SEC	SEV
Acidity	58	5.12 - 6.78	0.85	0.24	0.32
Bitterness	59	4.45 - 5.43	0.45	0.45	0.49
Sweetness	60	3.14 - 3.78	0.59	0.23	0.27
Body	59	5.20 - 6.05	0.24	0.38	0.43
Astringency	60	3.83 - 4.72	0.39	0.43	0.46
Aroma (overall)	60	5.32 - 5.98	0.39	0.41	0.44

R = Correlation coefficient
SEC = Standard error of calibration
SEV = Standard error of validation

TABLE 7 - ROASTED COFFEE BEAN CALIBRATION FOR SENSORY CHARACTERISTICS

Sensory Characteristic	Number of samples used	Range	R	SEC	SEV
Acidity	59	5.12 - 6.78	0.78	0.28	0.32
Bitterness	59	4.45 - 5.43	0.76	0.33	0.48
Sweetness	60	3.14 - 3.78	0.36	0.31	0.31
Body	60	5.20 - 6.05	0.37	0.40	0.44
Astringency	59	3.83 - 4.72	0.79	0.28	0.38
Aroma (overall)	60	5.32 - 5.98	0.22	0.40	0.43

R = Correlation coefficient;
SEC = Standard error of calibration;
SEV = Standard error of validation

CONCLUSION

Quantitative descriptive assessment of the taste, mouthfeel and overall aroma of coffee brews proved to be more efficient when panellists were served one sample at a time than when they compared four samples served simultaneously.

Even though all the coffees used in this experiment were Arabicas of good quality, significant differences were detected by the panel of trained tasters. This result confirmed that the origin of the coffee and the climatic conditions under which it is grown influence the development of flavour characteristics which are unique to each coffee. The coffees were chosen at random in order to identify real correlations between the sets of data. These showed that it is difficult to reproduce the results of the sensory responses of a trained panel by using chemical or analytical techniques. Acidity was the one sensory characteristic of coffee for which acceptable correlations were found with the chemical data predicted by NIR. The equations developed with the NIR spectra for green and roasted coffee showed the highest correlation coefficients for Acidity, particularly the equation for green coffee. The equation for roasted coffee also showed significant correlation coefficients for Astringency and Bitterness. However, it is important to highlight the fact that because of the characteristics of the coffees studied in this investigation (a small range of variation for all six sensory characteristics) it was more difficult to obtain highly significant correlations. Further studies, focussing on only one coffee origin and different quality grades, are being conducted at the ICO and results should be available by the end of the year.

It should be noted that ideal correlations between NIR spectra and sensory data could be artificially obtained if the coffees analyzed by NIR were selected on the basis of their sensory characteristics. The calibration obtained in this manner would, however, apply only to the selected coffees and would be useless for analyzing any other type of coffee.

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SUMMARY

Twelve Arabica coffees were analyzed by sensory descriptive techniques using a trained Panel which concentrated on describing the taste and mouthfeel of each sample. At the same time, the twelve green coffees and their roasted counterparts were analyzed by Near Infrared Spectroscopy. In addition, colour, pH and the percentage of soluble solids were measured in the coffee brew. Statistical techniques were applied to establish whether there were significant correlations between the two sets of data as well as within each set. Principal Component Analysis was also used to examine sensory differences among the twelve Arabica coffees.

RESUMEN

Doce cafés de la especie Arabica fueron evaluados usando técnicas descriptivas de evaluación sensorial y un grupo de catadores entrenado, los cuales se concentraron en evaluar el gusto y la sensación oral que deja en la boca cada una de las tazas de café despues de evaluarlas. Simultaneamente las mismas doce muestras de café verde y tostado fueron analizadas con un Spectrofotometro del Cercano Infrarojo. Además a cada taza de café preparada se le midió el color, el pH y el porcentage de solidos solubles. Técnicas estadísticas fueron aplicadas para establecer correlaciones significativas entre los dos grupos de datos así como dentro de cada grupo de datos. La técnica estadistica del Componente Principal se utilizó para hacer notar las diferencias sensoriales entre las doce muestras de café Arabica.

PROCESS FOR THE EXPLOSIVE COMMINUTION OF ROAST COFFEE

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Background: This method relates to a process for the explosive comminution of cellular material of animal or plant origin, in which the material is introduced into a pressure chamber, subjected to compressed gas therein, and then discharged from the chamber with explosive pressure release against an impact surface.

The basis for such a process goes back to the early forties of this century, when German researchers like Prof. Rammler et al tried to crush lignite with compressed air and steam when discharging the material. The first German Patent in food application was filed in 1976 as DE 2632045 C2 titled: "Vorrichtung zur Explosionszerkleinerung von Samen, Früchten oder Pflanzen". The German company "KWD Kohlensäure-Quelle Deutschland" in Bad Hönningen continued developing the process and filed patents using carbon dioxide as gas.

Successful applications were found by KWD when pulverizing spices and also pretreating peaces of fruit and wine grapes before extracting the juices via pressing. The method has two important advantages:

- Carbon dioxide disintegration protects the cells of food material against oxidation and undesired microbiological reactions.
- The comminution via explosive pressure release leads to open cell structures which allow better use and extraction of valuable components. The compression of particles often appearing in conventional grinding equipment, is thus circumvented.

KWD created the word combinations "cell cracking" and "aroma safing" for the treatment of the mentioned food products. Where wine is concerned the process works as follows: the grapes are first separated from their stalks in a conventional way and then put into a cylindric autoclave injected with pressurized carbon dioxide (3 bar). Carbon dioxide of natural source is to be preferred. The contents of the vessel will then very quickly be released via a decompression valve into a conveying system leading to a press where the juice is obtained. The juice is then transferred to a storage tank after passing a micro filter which also works with help of natural carbon dioxide.

The fresh juice from red wine grapes has a significantly darker colour. Both white and red wines pretreated by this method are preferred in blind tests on account of their more intensive and attractive taste compared to conventional preparations of the same origin. Also fruit juices and jellies can be treated with this method giving better taste and colour.

Attempts at "Cell Cracking" of coffee were performed using this simple method, but were unsuccessful. In a series of test runs with different materials, a KWD research group found that the disintegration depends on how the material which is to be disintegrated hits an impact surface upon pressure release.

The results of this development led to new findings which are filed in patent applications in 23 different countries: In Germany on June 3rd, 1988 (3818915.1), in Europe on May 27th, 1989 (89109583.8) and in the USA on June 2nd, 1989 (360978).

In the following chapters the application of "cell cracking" on the advanced explosive comminution of roast coffee will be discussed in detail. In many cases the explanatory text is directly cited from the patent applications as the author is the same.

According to the findings on which the process is based, the impact against the impact surface imparts a mechanical impetus to the particles. Often this alone starts the bursting process causing the comminution. At the beginning of the discharge step, the particles hit the free hard wall and actually absorb the mechanical impetus.

In the course of the pressure release or discharge step, however, a layer of comminuted particles forms on the impact surface.

This causes the subsequent particles to hit this comparatively softer layer on the impact surface, so that they no longer receive the mechanical impetus which triggers the bursting process.

Moreover, the subsequent particles no longer have the kinetic energy of the initially impacting particles due to continuing discharge of the pressure chamber and the consequent decrease of the pressure difference. As a result thereof, the bursting of the material which impacts consecutively does not take place uniformly, and a material is obtained which contains coarse as well as fine fractions.

In the case of ground coffee a material with too great a variation of particle size is not wanted. Therefore in the above mentioned processing the coarse material was separated from the fine material, e.g., by sieving, and was recycled to explosive comminution.

This causes increased expenses and may involve losses of valuable components. Even with repeated recycling of the coarse fraction, complete comminution of the material cannot be achieved. This is explained by the fact that the recycled material is structurally damaged, and upon pressure release the pressure compensation takes place without a bursting and hence without the desired comminution effect. If attempts are made to reduce the proportion of coarse material by increasing the pressure, however, such fine material results that further processing may be made more difficult due to the formation of fine dust, clogging of filters etc.

The advantage of the described new method is achieved in that the material undergoes pressure release in small portions against the grinding mechanism of a mill as an impact surface. Any coarse particles still occurring despite explosive comminution are immediately comminuted by the mill. In this respect separation or recycling can be dispensed with.

Furthermore, the small portions and the moving mechanism of the mill ensure that the coffee beans continuously meet free hard impact surfaces.

Detailed description: As used herein, the term "small portions" is understood to refer to portions which can be carried through the mill within the period of time required by a particle which has not been burst by itself. This period differs from material to material,

but is generally rather short. Suitable mills which have a large throughput-rate are preferred. Disc mills and toothed disc mills in particular have proved most suitable.

It is desirable that roast coffee beans which are to be disintegrated should not come into contact with oxygen and/or moisture during and after comminution. Therefore the process makes provision for the material to be held and discharged completely in an inert gas atmosphere. Carbon dioxide is used mainly as compressed gas, preferably of natural source.

The coffee beans which are to be comminuted can additionally be cooled to prevent the loss of aroma constituents during comminution. This may take place indirectly in cooling devices during storage. Furthermore provisions may be made for mill cooling. It preferably takes place by direct contact of the material with an inert cooling medium, preferably cold carbon dioxide in liquified or solid form (carbon dioxide pellets).

It is for many reasons necessary to recycle the gas; optionally after separation of volatile constituents which it has acquired. The manner in which the volatile separation takes place is common knowledge. For instance, the compressed recycled gas may be separated by passing suitable absorption agents or by means of changes in pressure and/or temperature.

The compressed volatile free gas is conveyed to a gas storage vessel for re-use as a compressed gas or as a cooling medium in the described process. It also serves to displace air or oxygen from the material which is to be disintegrated, from pipes and other parts of the equipment up to the packing machines thereby allowing oxygen and moisture free packs. When comminuting coffee beans, external carbon dioxide is only needed for the start-up period, as roasted coffee creates its own gas during roasting.

The subjection of cellular material to compressed gas lasts over a certain period of time, depending on the structure and composition of the cellular material. Soft materials, having a larger proportion of liquid, require shorter periods, e.g. wine grapes. Harder materials such as roasted coffee beans require somewhat longer periods. Holding times of a few minutes are sufficient for cellular materials such as whole roasted coffee beans.

The roasted coffee beans to be comminuted are subjected to carbon dioxide in a lock chamber, and transferred into one or more pressure loading chambers while the pressure is maintained. They are then transferred cyclically therefrom into a plurality of pressure chambers and are discharged from the respective chambers in succession into the mill's grinding mechanism. This permits a high throughput of material with a particularly short mill-idling time.

If the material flow supplied to the pressure loading chambers via the lock chamber corresponds with the above mentioned pressure chambers, it is possible for the process to be performed continuously permitting optimal use of equipment and gas.

The pressure range in which the process operates is mainly dependent on the cellular matarial and the desired degree of comminution. The most favourable conditions in each case must be determined by tests. When using carbon dioxide as a compressed gas and roast coffee as the material to be comminuted, the process is preferably carried out at a pressure of about 25 to 35 bar.

In the process a "plurality" of pressure loading chambers or pressure chambers is understood to mean 3,4 or more chambers depending, inter alia: on the desired throughput rate of the beans, on the capacity of the chambers, on the type and capacity of the mill used, and on the magnitude of the pressure difference upon pressure release etc.

The portion size of the material to be comminuted and discharged against the mill's grinding mechanism has also to be determined by taking into account the given limiting conditions. The decisive factor is that the time-span between the emergence from the pressure chamber and the impacting and entry into the grinding attachment is, for a substantial part of the material, not longer than the time required for pressure compensation by unbroken cells, e.g. a few seconds for coffee beans.

Common Process applications: Preferably cellular material is to be used which contains pharmaceutically and/or cosmetically active components. In particular, parts of known plants which contain active components may be utilized as cellular material to be comminuted such as fennel, hawthorn, senna, gentian, poppy or velerian.

Suitable cell materials which contain aromas and which, after appropriate preparation, may be used as spices, flavorings, foods or beverages include: tarragon, coriander, caraway, marjoram, nutmeg, mace, pepper, pimento, vanilla, and cinnamon etc. Ideally, the process is used to comminute roasted coffee.

Process advantages: The forces released upon explosive comminution are surprisingly better utilized than in conventional processes due to the portional discharge of the material to be comminuted. Where a toothed disc mill is used, in addition to its large capacity, it is possible to control the upper limit of the particle size of the comminuted material as needed, by suitable mill adjustment.

A further advantage is that when using carbon dioxide as a compressed gas, the material can be processed with the exclusion of oxygen. This inert gas atmosphere can be produced - particularly economically by using recycled waste gas - in optional prior treatment stages, and can be maintained during the comminution operation until packing. As a consequence of such processing there are the following

Advantages for roast and ground coffee:

- prolonged shelf life of the products
- lower bulk density
- increased in-cup yield
- better brew acceptance compared to standard products.

Illustration of the process: Figure 1 illustrates a detailed example of the comminution of roasted coffee in a pilot plant. All vessels, pipes and valves are marked with short symbols. The layout of the plant can so be followed using the below-mentioned nomenclature:

- "P1" to "P4" = connecting pipes
- "V1" to "V7" = valves
- "RCS" = roast coffee supply reservoir
- "LC" = lock chamber
- "FGS" = fresh gas supply
- "PLV" = pressure loading vessel
- "PRCs" = pressure release chambers
- "DM" = disc mill
- "AC" = aroma condensor
- "GCP" = gas compressing pump
- "GT" = gas tank

Process description: RCS works as a supply reservoir to LC via the V1 opening at atmosperic pressure. Also rinsing of the fresh beans with carbon dioxide via P4 can be done in RCS. LC works as a lock between RCS and PLV. In PLV the beans are subjected to compressed gas either from GT via V3 or FGS via V4 when V1 and V2 are closed.

When the desired pressure is reached, the respective filling valve V3 or V4 can be closed and V2 opened to allow a connection to PLV which is under the same 30 bar pressure. For new filling the gas in LC has to be taken back to GT with help of GCP via a pipe (not shown) and LC can receive fresh beans via V1.

Beans flow from LC into PLV, before going down to four small PRCs which work alternatively as discharge slots feeding DM under explosive pressure release. The empty PRCs will quickly be recompressed with gas to 30 bar via V6 and filled with coffee via V5. The decompressed gas from DM passes AC and then either goes via P4 for rinsing RCS or it is compressed in GCP to fill GT.

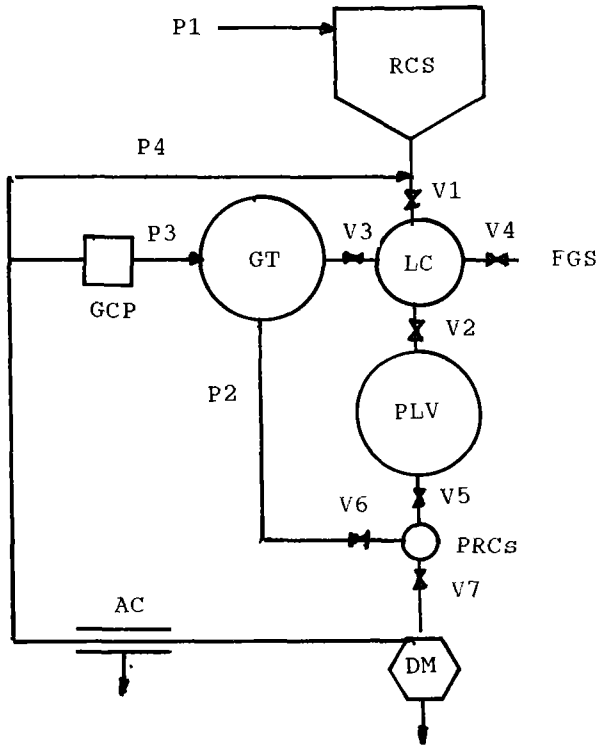

Figure 1 explains the setup of the "cell cracking" equipment.

The process is described in different steps in more detail:
- the start up - the production - and the stop -

For the start up care must be taken to rinse the entire equipment with carbon dioxide, mainly passing V4 to rinse LC and RCS. When starting a new plant it is necessary to add fresh gas also: to PLV, the PRCs, DM, the packaging line and all other Ps. These gas connections are not shown, as in an operating plant they would still be filled with gas from former use.

Fresh roast coffee at atmospheric pressure, has to be filled into PLV holding about 25 kg, into LC holding 12.5 kg and into RCS holding about 100 kg. For this step V1 and V2 are open.

V1 is then closed, and LC and PLV filled with compressed gas of 30 bar; via V3 if GT is full, otherwise via V4 with fresh gas.

The comminution can start 3 minutes later when this pressure has been reached by filling and discharging the 4 PRCs cyclically in succession by appropriate opening and closing of the respective valves. Each PRC containes 0.25 kg coffee beans and has a cycle time of 15 seconds.

The explosive comminution is performed in a pulsing stream of 16 "shots" or 4 kg per minute. The DM must be of sufficient size to accommodate this amount. It is not difficult to reduce the tempo of the plant if desired.

Longer stops can be performed in running the RCS and all following vessels up until the PRCs are empty. In this way, no coffee beans have to remain in the equipment.

For shorter stops only the PRCs need to be empty, as there is no risk should the beans remain in a carbon dioxide atmosphere over night or weekend, thus permitting high flexibility.

Operation costs: As coffee allows a self supply of carbon dioxide mainly electrical energy for the gas compressor accounts for the operation costs. These are rather low as will be shown in the following calculation.

The amount of carbon dioxide per kg of roast coffee can be estimated when following Figure 1: the compression of gas to 30 bar will be needed first in LC when it is completely filled with beans.

Coffee beans have a bulk volume of about 3.2 l/kg and
a mass volume of 0.8 l/kg.
The difference of 2.4 l/kg is the volume
of the compressed gas which will fill LC together with the beans.

Carbon dioxide has a specific gravity of 62.3 kg/m³ (30 bar and 30°C).
So 2.4 l gas/kg coffee in LC weigh 0,15 kg.

The PRCs need also compressed carbon dioxide. It is more than in LC per kg of coffee, as the chambers must be filled with the compressed gas before the beans are loaded. So for each kg of coffee the whole volume of 3.2 l/kg must be filled, which is 0.2 kg gas. Both amounts add up to 0.35 kg gas per kg comminuted coffee.

How much does it cost to compress this? Commercial data from a unit compressing greater volumes of carbon dioxide from 1 to 30 bar calculate with an energy consumption in the range of 50 KWh/1000 kg CO_2. This calculates to 0.05 KWh/kg carbon dioxide or

0.018 KWh/kg comminuted coffee.

The monetary additional expenses for this unique technology with all the listed advantages for a high value product such as coffee are in the range of

0.2 cent per kg comminuted coffee.

There are good expectations that this method will successfully be used in the near future in many coffee producing companies, and

KWD Kohlensäurequelle Deutschland

will, with pleasure, give assistence in further development work.

Summary: A new process for the grinding of roast coffee is presented. A unique way of comminution is used which has product advantages in terms of prolonged shelf life, lower bulk density, increased in-cup yield and better brew acceptance compared to standard products.

In this process roasted beans are introduced into a system of pressure chambers, subjected to compressed carbon dioxide therein and then discharged in small portions with explosive pressure release against the grinding mechanism of a mill as an impact surface.

The process and its background is described in detail. Costs and prospects of the process are discussed, an overview of the patent situation is given.

Zusammenfassung: Ein neues Verfahren zum Mahlen von Röstkaffee wird vorgestellt. Eine besondere Art der Zerkleinerung wird benutzt, die Produktvorteile erbringt hinsichtlich velängerter Haltbarkeit, niedrigerem Schüttgewicht, erhöhter Ausbeute in der Tasse und besserer Akzeptanz des Getränks im Vergleich zu Standardprodukten.

Bei dem Verfahren werden geröstete Kaffeebohnen in ein System von Druckgefäßen gegeben, dort mit Kohlendioxid unter Druck gesetzt und in kleinen Portionen unter explosionsartigem Druckentspannen gegen das Mahlwerkzeug einer Mühle als Prallfläche entladen.

Es werden das Verfahren und seine Hintergründe genauer beschrieben, Kosten und Aussichten diskutiert und die Patentsituation erläutert.

INFLUENCE OF HEATING RATE ON SOME PHYSICAL AND PHYSICO-CHEMICAL PROPERTIES OF COFFEE BEANS DURING THE ROASTING PROCESS

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INTRODUCTION

Coffee roasting is a process carried out in different ways throughout the world. In Italy, where roasting is usually performed in a very intense way, in order to obtain "dark" or "ultra dark" coffee, the bean can reach very high temperatures (220°-240°C).
Roasting time varies greatly according to the equipment, the process conditions, the origin and the kind of blend of coffee, and the degree of roasting required for the final product.
Owing to the roasting process, coffee beans change chemical composition and physical characteristics and the final properties of coffee brew, for the most part, depend on changes developed during roasting (Da Porto et al. 1991, Nicoli et al. 1990).
One of the most evident physical changes which occurs during roasting is that of the colour, ranging from yellow-green of the crude bean to brown-black of the roasted one. Changes in colour take place simultaneously with weight loss and volume increase of the bean (Lerici et al. 1980); obviously the kinetics of these changes are different depending on heating rate of the coffee beans.
As it's known the weight reduction is due to water loss and to thermal decomposition of some compounds into volatile products(Sivetz et al, 1979, Clarke at al, 1987, Lerici et al, 1980). Furthermore, during the roasting a remarkable increase of the bean volume also takes place caused by the pressure of gases (mainly steam and CO2) released from the inside of the coffee bean. The volume increase together with the weight loss lead to a decrease in density (Dalla Rosa et al, 1980).
The colour measurement represents a convenient and rapid tool in roasting control. HunterLab meter color system is largely used in food processing and quality control (Mastrocola et al. 1990, Mastrocola et al. 1991) but very few data are reported regarding its use for coffee analysis (Da Porto et al. 1991).

The purpose of this research was to study the main physical changes of coffee samples roasted from a light to an ultra dark level, maintaining a constant heating rate. Other coffee samples were then roasted at the same roasting level ("medium"), but changing heating rate conditions.

MATERIAL AND METHODS

Green (crude) coffee beans (Coffea Arabica, cv Santos) were roasted in a hot air circulating, high performance laboratory roaster of 1 kg capacity. Maintaining constant heat transfer conditions, coffee samples were discharged from the roaster at increasing final temperature, from 155 ("light") to 245°C ("ultra dark")(table 1).

Samples	Roasting time	Temperature (°C)
1	3'17"	155
2	3'45"	170
3	3'58"	190
4	6'17"	205
5	7'12"	215
6	7'23"	220
7	8'04"	225
8	8'18"	235
9	9'00"	240
10	10'00"	245

Table 1: Conditions of roasting process.

In the second part of research, a pilot plant of 15 kg capacity was used to roast a blend of C. Arabica (80%) and C. Canephora var. Robusta (20%). Samples of 10 Kg of crude coffee were roasted in order to increase the amount of heat per unit of product. The equipment allowed a reduction of the hot air flow during the roasting by means of a cut-off valve. In this way four different roasted coffee samples were obtained :

A : use of the total heating power during all the roasting process (roasting time 2' and 5");
B : reduction of 50% of heating power after 1 min of roasting (roasting time 2' and 20");
C : reduction of 50% of heating power after 45" of roasting (roasting time 3' and 10");
D : use of only 50% of heating power until 1' and 30" of roasting than use of the total heating power until the end of the process (roasting time 3' and 30");

Analytical controls

- Moisture (%): by weight on ground coffee samples after heating in oven according to AOAC method.
- Weight Loss (%): by weight of coffee samples before and after roasting.
- Density (g/ml) : using a suitable picnometer according to the methodology described in a previous paper (Lerici et al. 1980).
- Color analysis : by a Tristimulus Colorimeter (Chromameter-2 Reflectance, Minolta, Japan), equipped with a CR 100 measuring head. The standard C.I.E. condition, with illuminant C (6774°K) (Clydesdale, 1978; Duran, 1978) was used. The instrument was standardized against a white tile (L*= 95.3, a*= -

1.0, b*= 0.8) before each measurement. Color is expressed in L* (lightness variable), a* and b* (chromaticity coordinates), θs (Hue angle) = tan-1 b* and b*, S (saturation index) = (a*2 + b*2)1/2.

RESULTS AND DISCUSSION

Figure 1 shows the moisture reduction and the density decrease as a function of roasting degree, expressed in terms of weight loss, for coffee samples roasted maintaining a constant heating rate. The observed changes of moisture and density were in agreement to the results reported in a previous resarch note (Da Porto et al 1991).

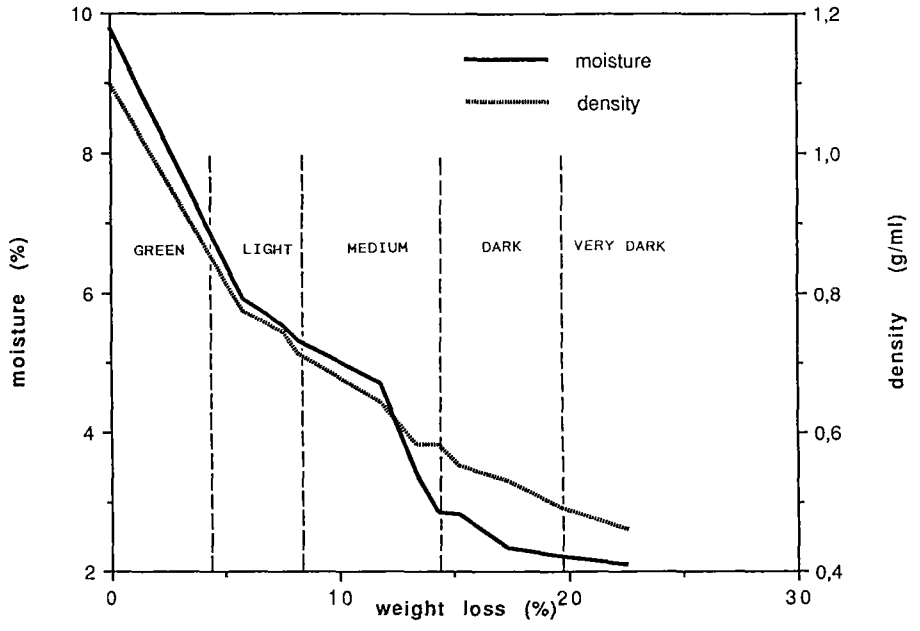

Figure 1: Changes of moisture and density versus weight loss in coffee beans at different level of roasting.

Figure 2 shows the changes of colour coordinates (L*, a*, b*) for coffee samples at different roasting levels for whole beans. As expected the lightness values decrease with the increasing of the weight loss.

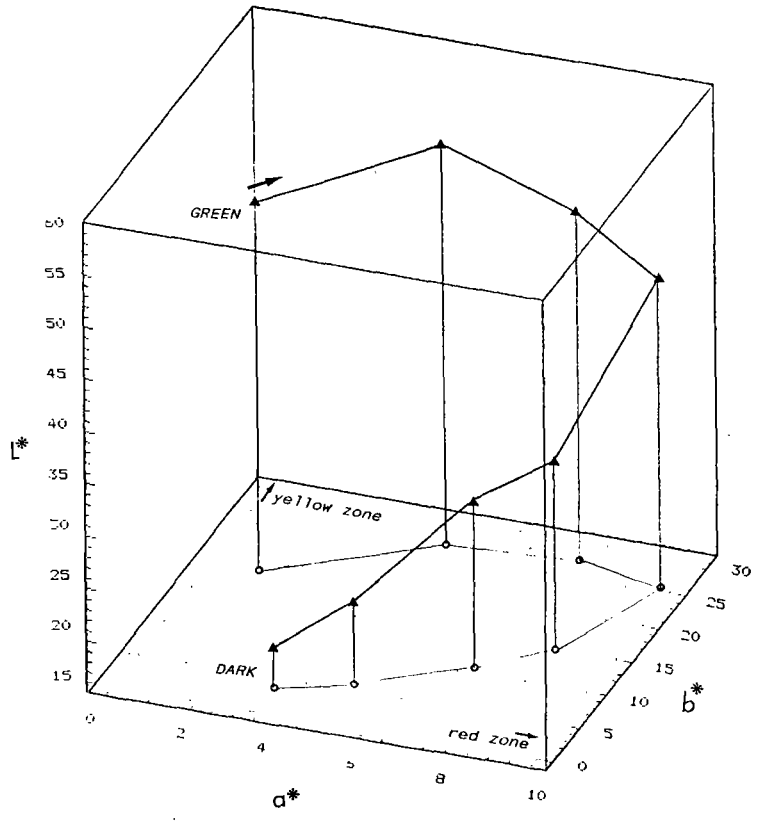

Figure 2: Changes in lightness (L*) and colour (a*, b*) in coffee beans at different level of roasting.

Considering parameters a* and b* it is interesting to note that the colour of the coffee moved from the green zone, of the crude coffee beans, to the red zone of medium roasted samples, than returned back to the green zone but in a lower yellow band. In addition, instrumental tristimulus values were reduced to color functions such as tan-1 b*/a* and (a*2 + b*2)1/2, and these

color functions were represented in a polar graph (fig. 3).

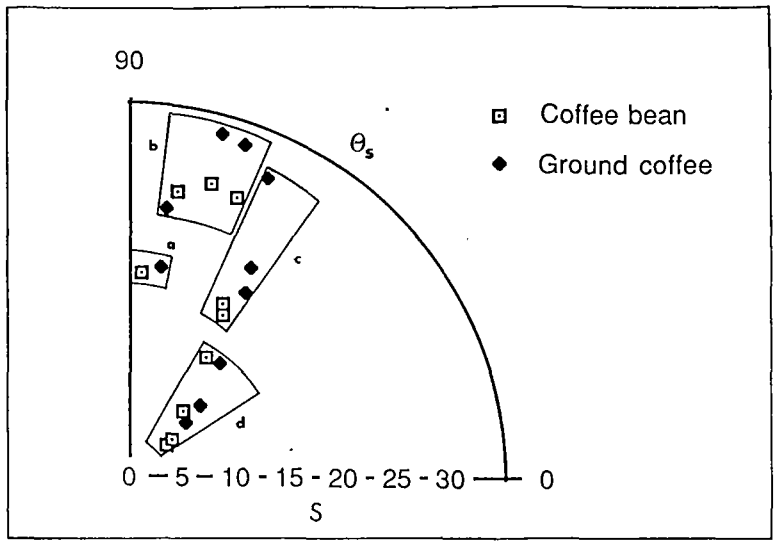

Figure 3: Colour polar coordinates (θs=tan-1 b*/a*, S=(a*2+b*2)1/2) for samples of coffee beans and ground coffee at different level of roasting: a=green, b=light, c=medium, d=dark.

When polar coordinates (Hue angle and Saturation index) were used, coffee beans and ground coffee, at every level of roasting, can be located in a well defined colour zone; these zones can be identified numerically as reported in table 2.

Degree of roasting	(a*2 + b*2)1/2		tan-1 b*/a*	
	min	max	min	max
GREEN	19	20	81°	90°
LIGHT	25	34	82°	69°
MEDIUM	15	31	57°	66°
DARK	4	14	41°	58°

Table 2: Minimum and maximum values of Saturation index (S=(a*2+b*2)1/2) and Hue angle (θs=tan-1 b*/a*) for samples of coffee beans and ground coffee at different level of roasting.

In the second part of the research, coffee samples were roasted at the same level ("medium": about 11% of weight loss), with a varied heating rate. In figure 4 and 5 changes of volume and density respectively are plotted versus roasting time for four different heating rates. Our trials show that by varying the heating rate it is possible to obtain samples of coffee at the same level of roasting but with a notable difference in term of volume

(fig.4) and density (fig.5) of the beans.

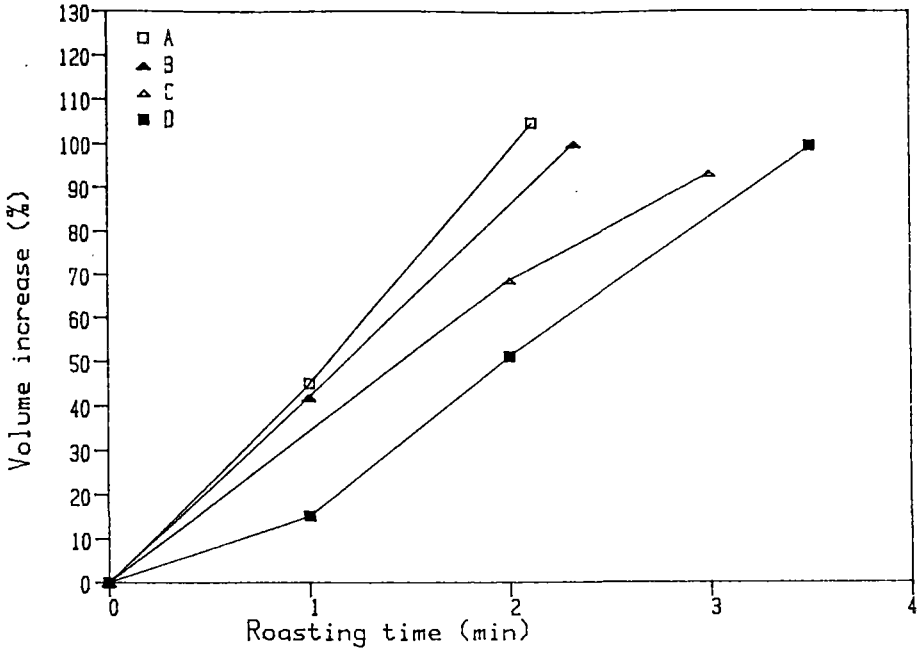

Figure 4: Volume increase in coffee beans during roasting for different heating rates.

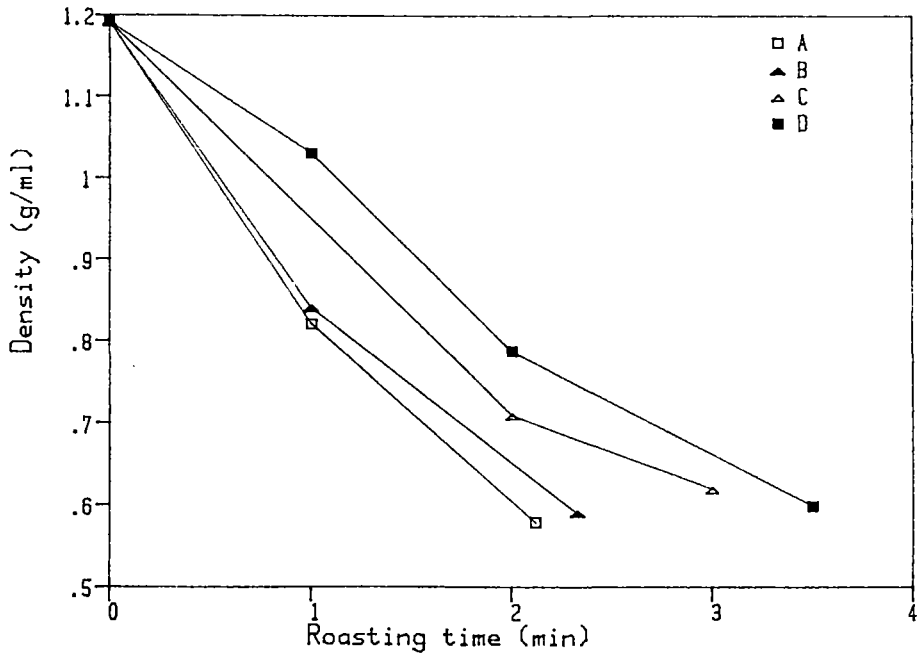

Figure 5: Density decrease in coffee beans during roasting for different heating rates.

SUMMARY

Some physical and physico-chemical properties such as weight loss (W.L.), density (d), volume increase (V.I.) and color (expressed in L*, a* b* parameters - C.I.E. 1976) of the beans were measured in coffee samples having different degrees (from light to very dark) of roasting. The trials were carried out in a roaster under well defined time/temperature conditions. The influence of the heating rate of the beans during the roasting process was evaluated in coffee samples at the same final roasting level. In our trials, with the increasing of the heating rate of the beans during the roasting process, interesting results in terms of V.I. and density were obtained.

RIASSUNTO

Alcune proprieta' fisiche e fisico-chimiche, come il calo peso (W.L.), la densita' (d), l' incremento di volume (V.I.) e il colore (espresso in L*, a*, b* - C.I.E., 1976) sono state studiate su campioni di caffe' a diverso grado di tostatura (da "light" a "very dark"). Le prove sono state effettuate in una tostatrice da laboratorio in condizioni di tempo/temperatura ben definite. E' stata valutata inoltre l' influenza della diversa velocita' di fornitura del calore durante il processo di torrefazione su campioni con lo stesso grado di tostatura finale. Nelle nostre prove, incrementando la velocita' di processo, sono stati ottenuti risultati interessanti in termini di V.I.e di densita' dei grani di caffe'.

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EFFECT OF SOME EXTRACTION CONDITIONS ON BREWING AND STABILITY OF COFFEE BEVERAGE

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Introduction

During the last few years there has been an increasing interest for large scale production of high quality coffee brew that can be used as a ready to drink beverage; in fact coffee brew can be drunk immediately just after its extraction or can be stored warm or refrigerated for a period of time varying from hours to some days. Nevertheless It is well known that the quality of coffee beverage decreases over a period of five hours of storage at room temperature due to the increase of acidity (Sivetz and Desrosier, 1979; Clarke, 1987). This quality loss, which seems more evident for the beverages obtained with dark roasted coffee (Dalla Rosa et al. 1989; Nicoli et al, 1990), could be caused by the hydrolysis of esters as reported by Maier et al., 1984.
Several authors studied the variables affecting the homemade extraction of coffee beverage: the degree of roasting and particle sizes (Clo and Voilley, 1983), the effect of high temperature of extractions (Diaz and Ruiz, 1980) and different methods of brewing (Pangborn, 1982, Voilley et al. 1981, Dalla Rosa et al. 1986). The kinetic and the mechanisms of extraction of methylxantines and volatile compounds from coffee during brewing were studied by Spiro and Selwood, 1984; Spiro and Page, 1984; Spiro and Hunter, 1985; Nicoli et al., 1987, Ndjnenkem et al., 1981 and Severini et al., 1987.
The purpose of the present investigation was to follow the extraction process of dark roasted coffee with easy detectable parameters such as electrical conductivity, pH and colour measurements. Acidity changes of the beverage were also studied under different storage conditions in order to correlate the shelf-life of the product with its storage temperature.

Materials and methods

All the experiments were carried out using dark roasted coffee (Coffea canephora var. Robusta). The moisture content was 98 g kg^{-1} for green coffee and 22 g kg^{-1} for roasted coffee with a total weight loss of 174 g kg^{-1}. After roasting the coffee samples were ground in the mill. The particle size distribution of the grind ranged from 0.3 to 0.6 mm.
The beverage was obtained by solid-liquid extraction carried out in a screw capped flasks

placed in a stirrer water bath (Dubnoff, Milan).The ratio between coffee powder and water was 1:10 (100 g litre^{-1}).
Different times (from 1 to 30 minutes) and temperatures (25, 32, 50, 80 and 100 °C) were used. At the end of each extraction the beverage was filtered using Whatman filter paper n.1.
The samples were subjected to the following analytical determinations:
- solid content % : using AOAC Method (1980)
- solid yield % : it is defined in percentages; the ratio of total solid in the beverage to the weight of ground coffee.
- electrical conductivity: measured with a conductivimeter (Hanna mod.8333) with a range from 0 to 199 mSiemens and manual compensation of the temperature. Measurament sensitivity was 1%.
- pH: measured by potenziometric method (Beckman 3560 pHmeter)
- Optical density: as an index of colour intensity, measured at 420 nm (Varian DMS 80 uv-visible spectrophotometer) on the diluted beverage (1:10 v/v).
Coffee beverages were pasteurized at 70 °C and stored at -20, +4, +20, +30, and +40 °C.
Acidity changes of the beverages were followed by pH measuraments during storage and expressed as hydrogen ion molar concentration. At each storage temperature the rate costant (k) of the reaction was calculated from the slope due to the increasing of hydrogen ion concentration versus storage time (Nicoli et al., 1989 and Dalla Rosa et al., 1990).
The shelf-life of the beverage was evaluated according to the following equation (Labuza, 1982)

$$SL = |l - i|/k_a \qquad 1)$$

where SL is the shelf-life value expressed in days, l is the limit threshold of acceptance in terms of pH, which was chosen 4.80, as reported by Dalla Rosa et al., 1990, i is the pH value of the beverage just after brewing and k is the reaction rate costant.
Data of extraction parameters (conductivity, $[H^+]$, solid yield %, optical density) and $[H^+]$ during storage were analysed by least squares fit of a responce surface using a quadratic model as proposed by Gacula and Singh, 1984:

$$\hat{Y} = \beta_0 + \beta_1 St + \beta_2 ST + \beta_{11} St^2 + \beta_{22} ST^2 + \beta_{12} St*ST \qquad 2)$$

where, $\hat{Y}$ = estimated value of selected parameter; St = extraction or storage time, minutes or days; ST = extraction or storage temperature, °C; St^2 = power effect of time; ST^2 = power effect of temperature; St*ST = interaction effect between time and temperature; β_{ij} are parameters to be estimated by multiple regression routine.

Results and discussion

Extraction process

The extraction process was followed by measuraments of electrical conductivity. The relationship between solid content % and electrical conductivity of the beverage is shown in figure 1.
The good correlation between the two parameters (y=0.1347+ 1.5643x; r=0.9997) allowed us to follow the extraction process throught simple and rapid conductivity measurements.
Figure 2 shows the changes in electrical conductivity as a function of time and temperature of the extraction process.
It can be observed that when the extractions were carried out at high temperatures, the contact time did not significantly affect the solid content of the beverage; in fact no great differences in conductivity can be noticed between 1 and 30 minute extraction beverages obtained at 80 and 100 °C.
On the contrary, at low temperatures of extraction, the maximum conductivity value is reached after at least 20 minutes of process.
In figure 3 and 4 changes of optical density of the beverage and of solid yield % respectively, as a function of process time and temperatureare reported. It is possible to note that, even for high temperatures of extraction, the colour of the beverage appears more affected by the contact time than that observed for conductivity. For both, optical density

Figure 1

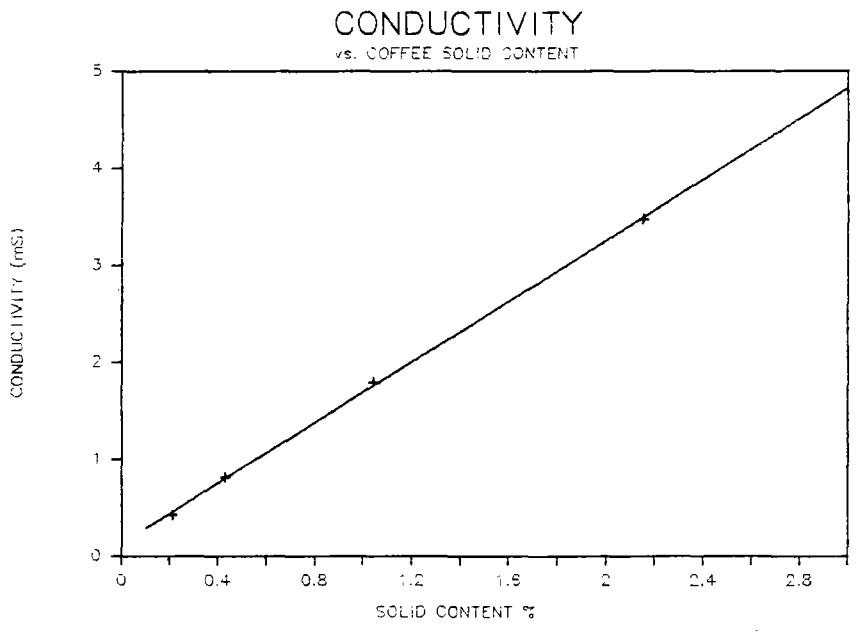

Figure 1: Relationship between electrical conductivity and solid content % of coffee beverage ($r=0.9997$; $p<0.0001$).

Figure 2

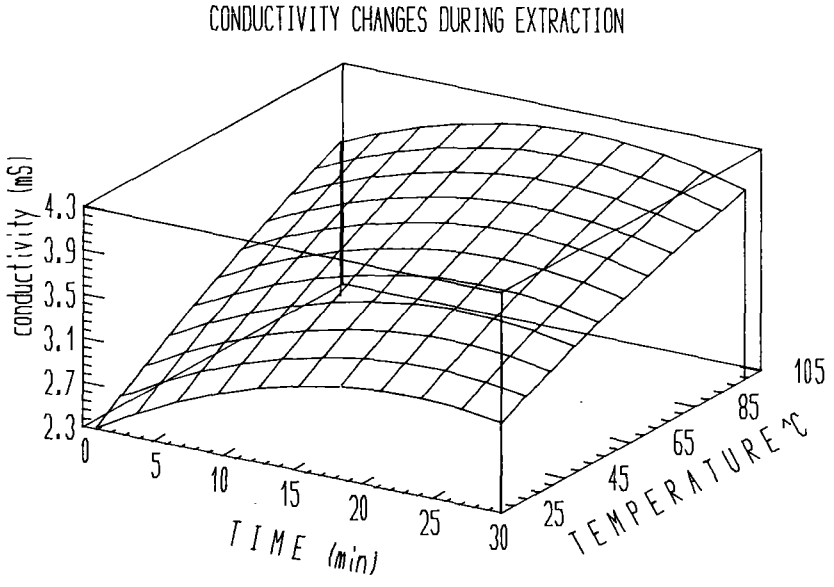

Figure 2: Response surface showing the change of conductivity as a function of extraction time and temperature (adj.R^2=0.9326; $p<0.0001$)

and solid yield %, the optimum combination of process conditions was found to be between 17 to 22 minutes and above 90 °C. In this range, in our experimental conditions, the process yield reaches a maximum around 30 %.

Figure 3

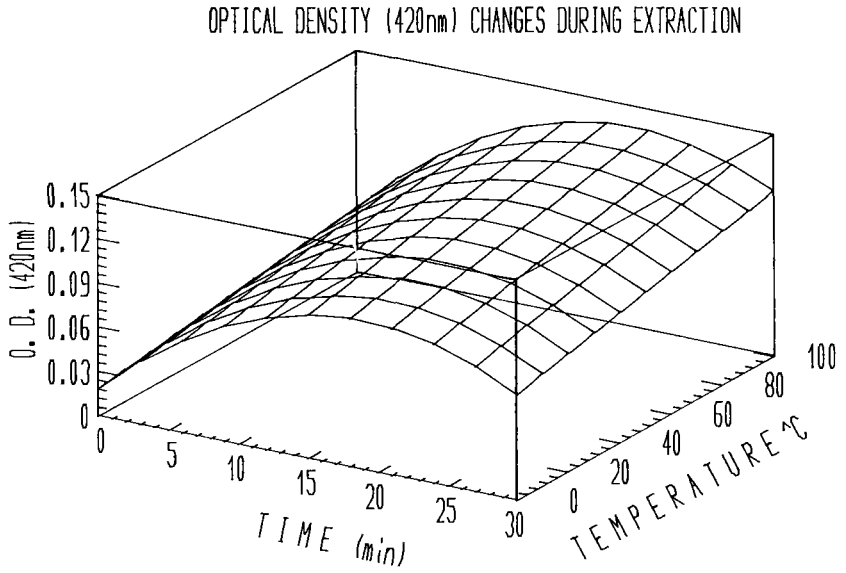

Figure 3: Response surface showing the change of optical density (420 nm) as a function of extraction time and temperature (adj.R^2=0.8764; p<0.0001)

Figure 4

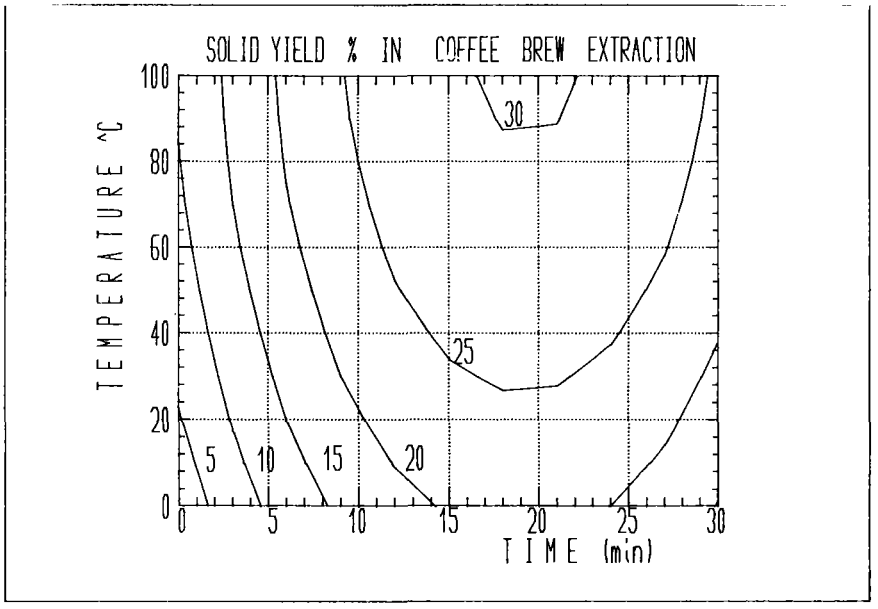

Figure 4: Response contour plot showing the change of the extraction yield as a function of process time and temperature (adj.R^2=0.9318; p<0.0001)

Figure 5 shows pH changes of the coffee beverages as a function of time and temperature of the extraction process. It easy to observe that, increasing the temperature above 40°C the pH falls down from 5.8 to 5.65 even for 1 minute extraction; a significant pH decrease was also found as a function of time for extractions carried out at high temperatures .

Figure 5

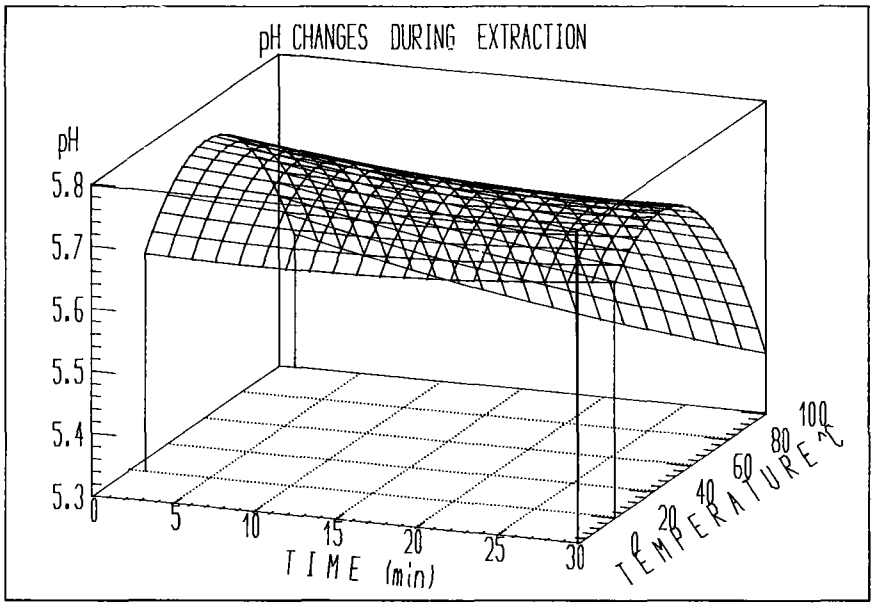

Figure 5: Response surface plot showing the change of the coffee beverage pH as a function of extraction time and temperature (adj.R^2=0.9114; p<0.0001)

Beverage storage

Figure 6 shows hydrogen ion concentration changes as a function of storage time and temperature. It can be observed that the acidity of the beverage increases as a function of storage time; this effect was enhanced significantly by storage temperature. In fact acidity was found to be the most important factor in limiting the acceptability of coffee beverage, as reported by several authors (Sivetz et al, 1979; Pangborn, 1982; Feria Morales, 1989; Dalla Rosa et al., 1990).
In table 1 changes in pH values of coffee brew stored at frozen state (-20°C) are reported.

Table 1: changes in pH values of coffee beverage stored at -20 °C.

Storage time (days)	pH	[H^+]
0	5.43	$3.71\ 10^{-6}$
5	5.42	$3.80\ 10^{-6}$
19	5.39	$4.07\ 10^{-6}$
25	5.35	$4.46\ 10^{-6}$
35	5.32	$4.78\ 10^{-6}$
62	5.22	$6.02\ 10^{-6}$

Figure 6

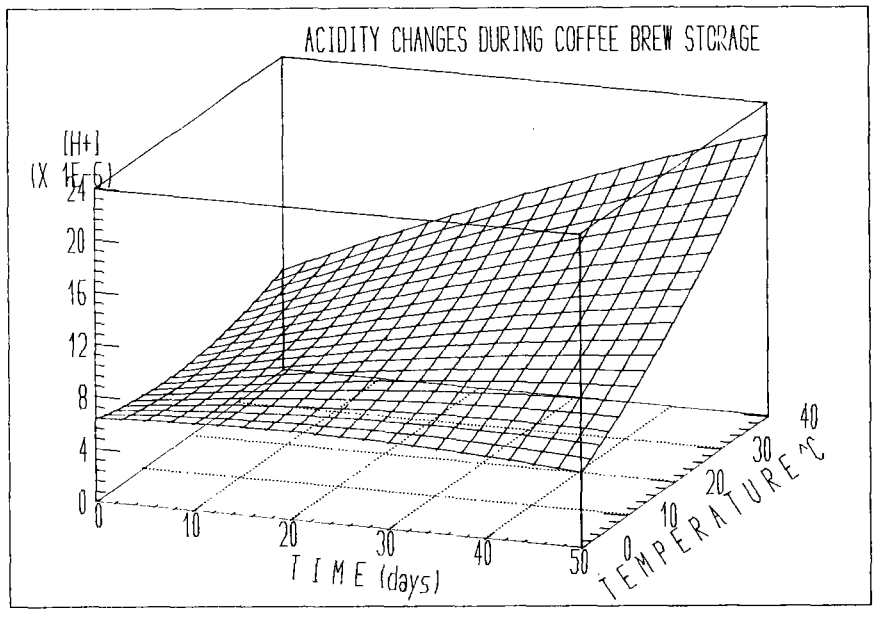

Figure 6: Response surface plot showing the change of the coffee beverage pH as a function of storage time and temperature (adj.R^2=0.9746; p<0.0001)

It is interesting to observe that the pH showed a slow but significant decrease even at this low temperature. This result does not seem in agreement with those reported in figure 6 where, at very low storage tempeatures, no acidity changes are detectable. Probably, in the product stored at -20 °C, the presence of high concentrated unfrozen solutions could lead to a reaction rate higher than that expected.
Table 2 reports the zero order rate constant (k) of the acidity increasing and the end point shelf-life of the coffee brew for different storage temperatures.

Table 2: zero order rate costants of the increase of acidity of the coffee beverage and shelf-life values as a function of storage temperature.

Storage Temperature °C	k (H^+ day^{-1})	Shelf-Life (days)
-20	3.4 10-8	366
+ 4	3.2 10-7	251
+20	11.7 10-7	100
+30	18.6 10-7	50
+40	30.3 10-7	31

As expected the shelf life of the product increases significantly with the decrease of the storage temperature. Nevertheless the refrigerated or frozen conditions are only able to slow the increase of acidity of the beverage during storage time.

Conclusions

From the results presented in this paper a good relationship was found between solid content % and electrical conductivity of the coffee beverage. Conductivity measurements carried out

during the extraction process showed that the contact time does not affect the solid content of the beverage if the extraction is carried out at high temperatures. In our experimental conditions the optimum conditions to obtain the maximum extraction yield were above 90 °C with an extraction time ranging between 15-20 minutes.
Acidity of coffee beverage can be affected by time and temperature of extraction and storage,but freezing temperatures did not stop the acidity increase.

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Summary

A solid liquid extraction of dark roasted coffee at different processing conditions was followed using some easy detectable parameters such as electrical conductivity, pH and colour.
The changes in pH of the beverages, subjected at different storage conditions, were also measured in order to evaluate the shelf-life of the product in relation of its storage temperature.

Riassunto

Alcuni parametri strumentali (conducibilità elettrica, pH, colore) sono stati utilizzati per seguire l'andamento di un processo di estrazione solido-liquido di caffè. E' stato così possibile valutare l'effetto dei fattori tempo e temperatura di estrazione, in modo tale da stimare, attraverso un modello matematico, le condizioni ottimali per ottenere la maggiore resa di processo.
Sulla bevanda di caffè ottenuta è stato valutato lo scadimento qualitativo nel tempo (in termini di aumento dell'acidità) in funzione della temperatura di conservazione. Sulla base di un limite di accettabilità posto arbitrariamente, sono stati calcolati i tempi limite di conservazione (shelf-life) ad ogni temperatura considerata.

A TOOL FOR THE CLASSIFICATION OF GREEN COFFEE SAMPLES

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INTRODUCTION

One of the major problems encountered by industries operating in the sector of natural raw materials is the intrinsic variability of the characteristics of these materials. This renders it difficult to maintain the quality of the final product within a constant range. In attempting to establish precise product characteristics, difficulty arises in defining the features of the raw materials and the mode of measurement. At illycaffe', investment in quality has always been considered a strategic element and the careful attention devoted to quality control of raw material has lead to the development and consolidation of illycaffe's own methodology in controlling green coffee lots entering the production process.
The control methodology for raw material currently consists of three steps: geometric, dimensional and colorimetric classification, olfactory classification of defective beans and tasting of the beverage prepared from the roasted, ground and brewed sample. The objective of the latter step is to assign a grade to the taste, aroma and tactile characteristics (body and astringency) of the sample of the lot under examination.
This paper intends to present an automatic tool that solves and consolidates within a methodology the technique of this method's first step and that has the function of: 1) automatically recognizing colorimetric and dimensional characteristics of single green coffee beans, 2) automatically sorting out defective beans in classes, 3) automatically classifying coffee lots according to an original criterion or according to international standards mainly applied on the basis of characteristics listed in point number 2), 4) storing the information related to analyzed samples into a database for further elaborations. Though not yet covering all modes of classification exhaustively, this tool permits a reduction of time and costs, and an improvement in reliability and reproducibility of measurements. Further, by consolidating a method into the form of an instrument, this tool is easy to operate and does not require highly specialized personnel.

TECHNICAL-SCIENTIFIC BACKGROUND

The international coffee market applies various standards of product classification set by trade organizations in both producing countries and consumer countries; in many cases, such standards have been partially embodied in national legislation. Factors determining product characteristics, and consequently product price, are: the country of origin, processing method, crop, presence of defects, shape, grading, bean color, flavor, aroma and body.
Methods of classifying coffee characteristics differ from country to country. Suffice it to mention the New York Coffee and Sugar Exchange system which is accepted in Brazil; it sets seven standard types on the basis of the quantity of imperfections found in a one-pound sample. A similar system developed by French legislation (Decree 65-763 September 3th 1965) is accepted in French-speaking producing countries.
Since 1964, the International Standards Organization (ISO) has endeavored to establish precise norms on quality definition:
ISO 3509-1984 Glossary of terms relating to coffee and its products
ISO 4072-1982 Method of sampling green coffee in bags
ISO 4150-1980 Green coffee : size analysis by manual sieving
ISO 4149-1980 Green coffee : olfactory , visual examination and determination of foreign matter and defects
ISO 6667 Green coffee : determination of proportion of insect-damaged beans
Nevertheless, these norms are the result of an attempt of standardization and stem from trade agreements and heuristic rules. Moreover, though setting precise modes of operation for the classification of defects, these standards envisage an operator who, for all his experience, may be subject to variations and imprecision in his personal perception in detecting defects. The idea that led to the development of the present tool originates from a series of problems encountered by illycaffe' in recent years in attempting to define a sole, objective and reproducible method capable of assigning the lots a precise classification, which also takes into account the actual influence of various defects on the organoleptic qualities of the product. This has spurred research and formulation of proposals as the one in [4], for a method based on the assignment of a quality rating based on gravimetric values of beans, or in [3] which underlines the relationship between the colorimetric/morphologic and organoleptic characteristics of classes of the main defects of green coffee.
These proposals have brought a windfall in know-how both on classification methodology and on selectioning technology of green coffee on a colorimetric base; sorting has become a necessity as a consequence of the quality deterioration of the raw material. In the field of sorting, in particular, improvements have been obtained in the modes of use and in the technology of these machines through a joint illycaffe'-Sortex effort which has led to an increase of patents in this domain. The following patents have proved to be most useful references:
USP 4,807,762 (Gunson's Sortex Ltd, illycaffe' 1989)"Procedure for Sorting a Granular Material and a Machine for Executing the Procedure" which claims a system of colorimetric sorting of coffee by means of computer assisted mapping techniques, and USP 4,699,273 (Gunson's Sortex Ltd, illycaffe' 1987) "Sorting machine" using l.e.d. in which the optical system employs lighting units and solid-state backgrounds that perform the recognition of the size of the bean under examination.

TECHNICAL PROBLEMS AND THEIR SOLUTIONS

The objective followed in developing the present tool was to achieve automation in the phase of color measurement of the lots under examination, in the phase of their grading measurement and in that of detection of the various defects, and to possibly produce a measurement value assessing quality. The functioning of this instrument had to be guaranteed by a constant and reproducible color and size reference in order to eliminate problems linked with personal judgements and assessment errors by

technicians in charge of this phase. In addition, given the large quantity of lots that are analyzed daily (consider that it takes an expert operator an average of 30 minutes to classify an 80 gr. green coffee sample), the intention was to considerably reduce operational time in order to enable a larger amount of samples to be classified.
By starting off from these general objectives, the following design characteristics for the automatic system were defined:
1) a compact system equipped with a graphic workstation and control software, memorization and processing of acquired data;
2) automatic acquisition of samples composed of a maximum of 3000 beans, with a rate of presentation of 20 beans per second;
3) minimization of errors due to presentation geometry (positioning, rotation);
4) minimization of errors due to bean size and its natural asymmetry;
5) color measurement in relation to a constant color reference comparable to spectrophotometric standards;
6) measurement of bean size;
7) development of techniques of pattern recognition that permits the automatic sorting out of defects and of types of coffees and their classification.
8) automatic generation of report and statistical information on the acquired data also with graphical visualization.

The system's architecture (fig.1) mainly consists of four parts: the sample's system of presentation, the optical box, the acquisition and control hardware and software, processing software.

- System of Presentation

Problems in reading color information of a solid opaque object are solved in basic spectrophotometry by measuring the object's reflectivity with the use of a spectrophotometer fitted with an integrating sphere to minimize problems of geometry and scatter due to the object's surface. But when a rapid reading of a falling object presented to a measurement system is required, problems concerning geometry and scatter are compounded by problems concerning size, fall trajectory and, in the particular case of coffee beans, asymmetry of the bean and lack of uniformity of color shades.
The main objective pursued in designing the optical box was minimizing the disturbance factors that would have increased measurement errors to an unacceptable degree. The problem of bean presentation has been solved by a feeding system consisting in a tank containing the beans; this system is equipped with an opening from which a small quantity of material is discharged at a constant rate on a disk rotating at a constant speed. The disk is fitted with a containment spiral having the function of separating and spacing the beans. Once the bean is spaced from the successive ones by means of the spiral, it is conveyed to the rim of the disk from which it falls through a short tube into the optical box.

- Optical Box

The optical box consists of three elements: lighting unit, background and sensor. The lighting unit was obtained from high intensity LEDs in 670 nm. and in 565 nm. color bands; the l.e.d.s were fitted radially on a support according to different angles so as to illuminate the central area of the optical box in a uniform and isotropic way. Such configuration was chosen by a simulation program which operated within the bounds of a low number of emitting units on one hand, and the necessity to minimize the reflection effect of the object's surface on the other.
Backgrounds are composed of 670 nm. LEDs linearly spaced on a plate of a semitransparent material and are opposed to their respective optical sensors.
The system of observation consists of three optical sensors placed at 120 degrees on an ideal circumference perpendicular to the trajectory of the fall of the bean. The sensors of the optical systems are constituted by fast silicon photodiodes type PIN.

- Acquisition and Control

An electronic control circuit lights up the backgrounds and the lighting unit according to a 180 degree lag pattern with a frequency of a couple of Kilohertz. Thus, when the background is lit up, the lighting unit is turned off and sensors gather a light signal which is proportional to the degree of background coverage. When the lighting unit is on, instead, only the light diffused by the examined bean is gathered. Signals coming from the sensors are then amplified and demodulated synchronously with the lighting unit/background control system so that at the end three red color signals, three green color signals and three size signals are produced. In addition, a data acquisition system composed of a AST Premium 386 personal computer with a 25Mhz clock equipped with a mathematical 387 coprocessor and an National Instruments AD MIO analog to digital acquisition board with 16 analog inputs, capable of a speed of 15 microseconds per sample, digitalizes signals so they can be processed by the software. The next step consists in transforming the color information of the coffee bean in the optical box into two luminance/crominance coordinates obtained from the color signals. This transformation entails an initial calculation of the reflectivity which is obtained by dividing the gathered color signal value by the respective size signal value. Hence, the acquired color value is independent of the size of the object. Then, the average color of the object is calculated in each of the selected color bands. Finally, color information is transformed in luminance and crominance coordinates to upgrade its manageableness. This procedure is explained from an algorithm point of view in appendix A.

- Software

The software has been subdivided in different programs according to the functions it has to perform, instead of in a sole procedure. The reason of this decision was that compact programs are easier to maintain and that the Windows 3.0 user interface permits an effective integration of different programs into one sole group of uniform functional capacity. Given the complexity of the problem, it was necessary to resort to various systems of development in the generation of the software according to the specific problem faced in each case: a macro assembler was employed for data acquisition and hardware control, and C6.0 Microsoft compiler and National Instruments LabWindows system of development for the other parts. One program controls the functioning capacity of the hardware and permits the acquisition of a predetermined number of coffee beans, calculates the color and size values of each bean and memorizes the results on a file. Another program processes the files containing the values of homogeneous bean groups belonging to coffee types and characteristic defects acquired previously, and generates reference maps.

Still another program reads the file containing the data of a sample previously acquired and calculates the characteristic statistical parameters of the sample by using reference maps; moreover, it permits their graphic visualization. Also a series of programs (tools) were developed to permit the set up and verification of the machine's functioning capacity.

Thus summarizing, the logical steps that are performed are:

1) the drawing up of reference maps by acquiring homogeneous groups of defective beans;
2) acquisition of a representative sample of the lot under examination and calculation of the characteristic parameters (color and dimensions);
3) rejection of defective beans on the basis of reference maps, and the counting of defective beans.
4) sample classification according to parameters calculated in points 2) and 3) by employing the NY tables etc. or tables taking into consideration the defect's organoleptic effect on the quality that have been defined and used at illycaffe'.

DISCUSSION AND RESULTS

As can be seen on fig.2, measurement error of the same bean is +- 4, mainly due to the bean's position when falling into the optical box; moreover, objects with the same color shades but with different dimensions show a measurement error comparable to the preceding one, thus confirming the validity of the compensation method adopted (fig.3).
It is possible to improve this measurement error by optimizing the presentation system and the optical box. In designing the classificator, the application of traditional statistical criteria did not yield good results and so a "fuzzy" classification technique was employed that treats concave/convex profiles also in multidimensional spaces.
Bean classification by this method is extremely effective and, from a computational point of view, extremely efficient.
The calculation of characteristic parameters (fig.8) - as average luminance, average crominance and average dimension - produces information related to the color of the sample and which consequently is easy to convert in any of the standards used (fig.4). Moreover, the dimension parameter is directly related to the sieve (fig.5).

UTILIZATION OF THE TOOL

Possible utilizations of the tool are the following:
1) recognizing and classifying a coffee sample according to a set of types based on a fixed pattern.
2) recognizing and counting characteristic defects (fig.6) in a sample, and for forecasting statistically the frequency of defects in a sample coming from a specific coffee lot.
3) measuring characteristic parameters as color and dimension; other possible uses of the instrument are being studied. One example could be estimating the average specific gravity obtained by dividing the acquired beans' weight by their volume calculated on the basis of the measured dimensional values. Another example could be verifying the congruency between a sample of a lot that is offered for purchase and a sample of a lot that has been purchased. Another example still is the selectioning of small subgroups of beans with certain colorimetric and/or dimensional characteristics to be used in chemical, physical and organoleptic studies. Moreover in certain cases, it is possible to determine by the graphic representation whether a sample is the result of a mix of different lots. Finally, it is possible to verify the validity of the setup of the industrial bichromatic sorting machines by calculating the percentages of good beans and defective beans that are found in the group of rejected or accepted beans (fig.7).

CONCLUSIONS

Work performed to date enables us to foresee new technological possibilities, for it could be possible to extend the color bands within the visible range and to connect the series with an optical system that observes the beans under UV fluorescence, thus increasing the amount of information and improving the defect recognition capacity in order to extend the possibilities of classification.
A feasibility study on an information system for assisting the purchase of raw material is currently under way in illycaffe's laboratories. The tool described in the present paper constitutes one of the data generators that will be integrated in this system.

ACKNOWLEDGEMENTS

The author is very grateful for their contribution to: L.Mari (software developing), S.Cossetto (hardware developing) and to all the the illycaffe's chemical laboratory staff.

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USP 4,807,762 (Gunson's Sortex Ltd, illycaffe' 1989) "Procedure for Sorting a Granular Material and a Machine for Executing the Procedure", E. Illy, W. S. Maughan.

APPENDIX A

L1 value of color signal at a determined wavelength acquired from sensor
S1 percentage of background coverage
W1 value of colur signal of a white reference (having 90% reflectivity)
n number of samples for each bean , k constant value

R1 reflectivity of object @ L1
R2 " " @ L2
L pseudo luminance
C pseudo crominance

$$R1 = \frac{\sum_{i=1}^{n} (L1i/S1i)/W1*k}{n}$$

$$L = (R1 + R2) / 2$$

$$C = R1 - R2 + 50$$

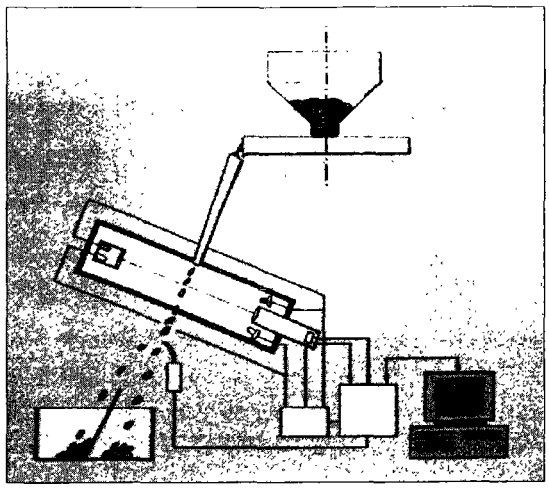


Fig.1 System's architecture

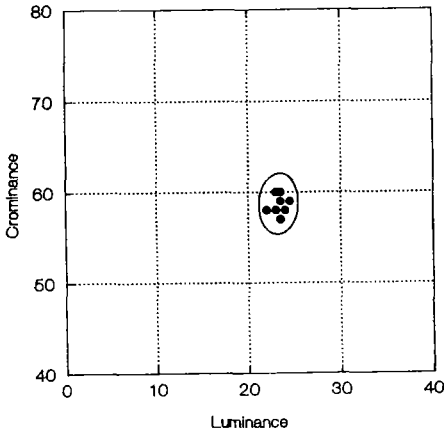


Fig.2 Error of measurement

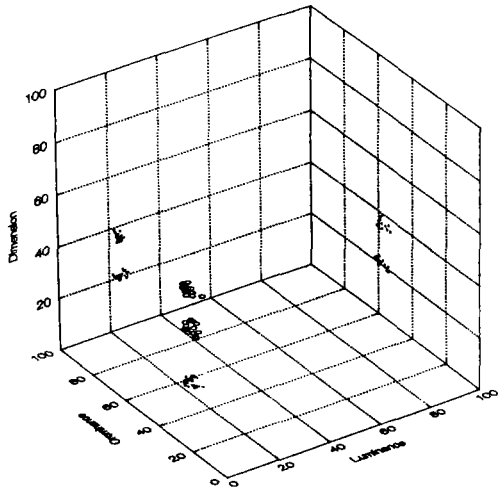


Fig.3 Result of dimensional compensation

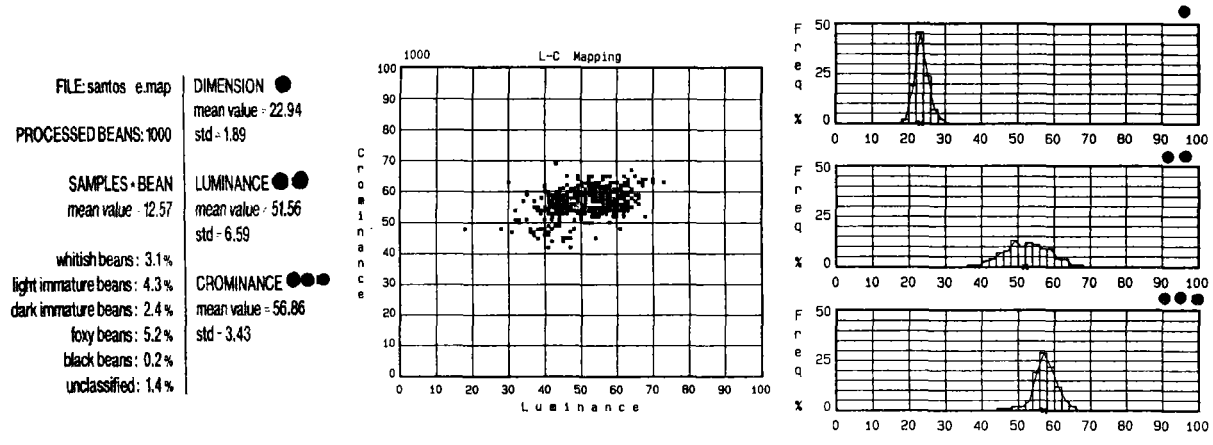


Fig.4 Characteristic statistical parameters and defect forecast

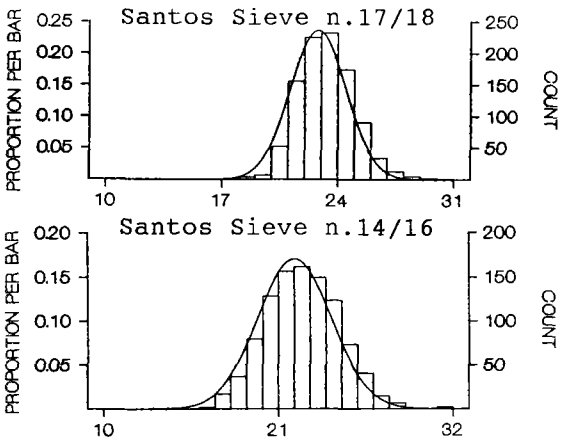


Fig.5 Dimensional classification

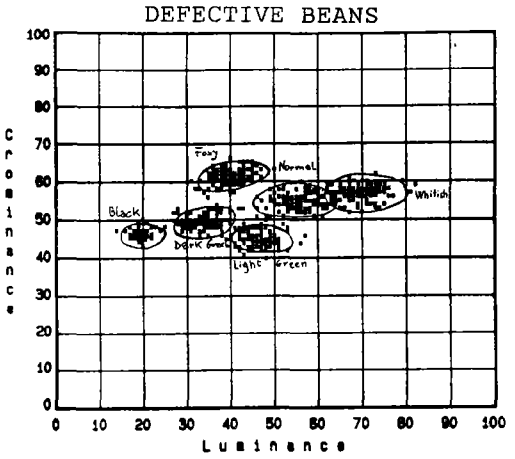
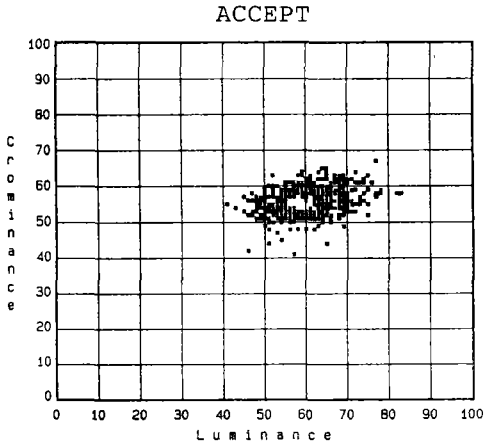


Fig.6 Pattern of defective beans



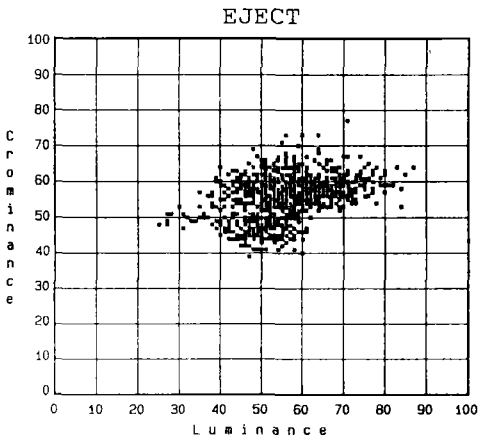


Fig.7 Check of the setup of industrial bicromatic sorting

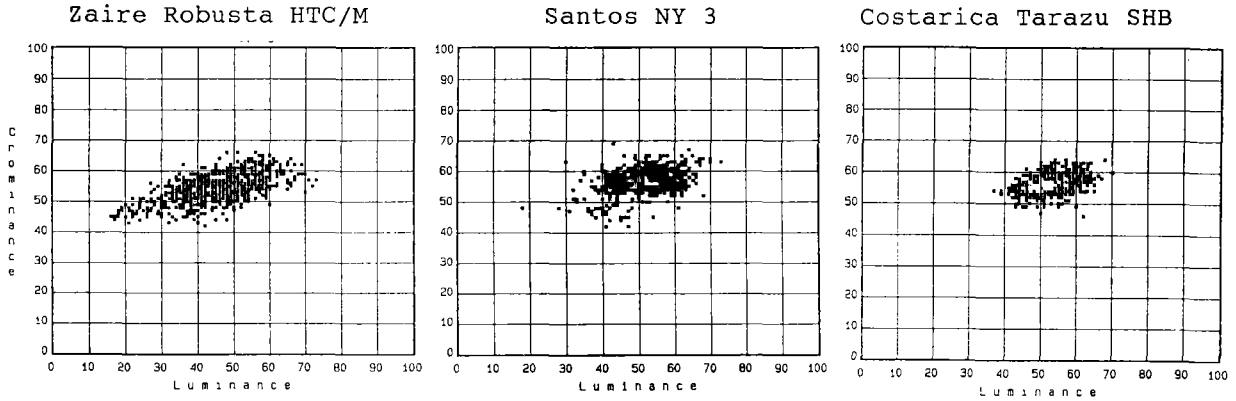


Fig.8 Pattern of different types of green coffee lots

A TOOL FOR THE AUTOMATIC CLASSIFICATION OF GREEN COFFEE SAMPLES

Tool for the automatic measurement of the colorimetric and dimensional characteristics of green coffee.
On the basis of the characteristics of a plurality of beans gathered from samples of lots of green coffee, it is possible to automatically generate a classification of the lots according to an original criterion or to existing international standards.
This instrument allows to reduce the time, costs and errors present in the methods normally used in the classification of green coffees.

UN INSTRUMENT POUR LA CLASSIFICATION AUTOMATIQUE DE ECHANTILLONS DE CAFE' VERT

Un instrument pour mesurer automatiquement les caractéristiques colorimétriques et dimensionnelles du café vert.
Sur la base des caractéristiques d'une pluralité de grains provenants de échantillons de parties de café vert, il est possible délivrer automatiquement une classification des parties qui se base sur un critérium original ou sur les standards internationaux.
Cet instrument permet de réduire temps et charges et d'éviter les erreurs présents avec les méthodes utilisées pour la classification du café vert.

EIN INSTRUMENT FÜR DIE AUTOMATISCHE KLASSIFIZIERUNG VON ROHKAFFEEMUSTERN

Ein Instrument für die automatische Messung der Farb-und dimensionalen Charakteristiken von Rohkaffee.
Auf der Grundlage der Charakteristiken einer Vielzahl von Bohnen, ausgewählt aus Mustern von Rohkaffeepartien, ist es möglich, automatisch eine auf Originalkriterien oder internationalen Standards basierende Klassifizierung der Partien zu erhalten.
Dieses Instrument erlaubt eine Ersparnis von Zeit und Kosten und die Vermeidung von Fehlern, die häufig bei den für die Klassifizierung von Rohkaffee angewandten Methoden auftreten.

EVALUATING PERFORMANCE OF COFFEE CULTIVARS IN HAWAII USING STABILITY ANALYSIS

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INTRODUCTION

Rapid expansion of Hawaii's coffee industry beyond its historic home in Kona (Hawaii island) is occurring. It will increase 300% to 3000 ha from 1987 to 1992 (Cavaletto et al., 1991). Minimizing the economic risk demands evaluation of cultivars and production schemes across a vast range of potential production environments as quickly as possible. On-farm experiments are essential if evaluation is to occur rapidly. These types of experiments have several advantages:

- cultivars and technologies are exposed to "real life" conditions,
- cultivars and technologies can be compared in many environments,
- scientist's costs are greatly reduced by sharing them with the farmer,
- farmers observe performance as often as they like,
- technology transfer process is greatly accelerated.

Farmer-managed, on-farm experiments also have disadvantages. The precise control and data collection desired by scientists is extremely difficult. The mortality rate of on-farm experiments is high. Even the ones that survive are often compromised with regard to standard statistical design and analysis.

Stability analysis was developed almost thirty years ago for multilocation testing of cultivars by Finlay and Wilkinson (1963). Its assumption is that stability of performance is one of the most desirable properties of a cultivar. The exact environmental factors at a given location or season that influence yield and quality often cannot be measured easily. The mean performance of all cultivars at a location is used as a measure of environmental quality. This very simple yet powerful analytical technique was adapted by Hildebrand (1984) to evaluate production technologies in farmer-managed on-farm experiments. Its application and comparison with other methods for evaluating cultivars of fruit and beverage crops is more recent (Pritts and Luby, 1990).

METHODS

Individual cultivar performance is regressed at each location with the location mean. Cultivar mean, regression coefficient or slope (b), and coefficient of determination (r^2) are calculated. Cultivar means > the grand mean (all cultivars) indicate a high level of performance. A slope ≤ 1 and $r^2 > 0.5$ indicates stability of performance as good as the average cultivar. By definition, the slope of the regression equation of the cultivar mean across location means should be 1. Each cultivar evaluated in the stability analysis should be at every location or at least at the best and poorest locations for equal comparisons.

In the first harvest season, cherry yield was the mean of the total fresh weight of cherry from 2 or more trees per row. Green bean yield and quality were determined from composited subsamples (Cavaletto et al., 1991). General field design was paired 10 m rows per cultivar in three replications in a randomized, complete block design; however this was not possible at all sites. In the second season most cooperators used an yield estimation procedure (Upreti et al.,1991) in lieu of harvesting cherry throughout the season.

RESULTS AND DISCUSSION

Stability parameters (Table 1) are calculated from the regression of each cultivar's yield with the location mean. Ideally, all cultivars are at all locations, alternatively at worst and best sites. When this is not possible interpretation must be made very carefully. Note the location means of 'Guatemalan'- a *typica* land race introduced from Guatemala in the 19th century and standard of Kona coffee. Catuai is always superior to the location mean but Guatemalan has a higher mean than Catuai.

Table 1. Cherry yield (1989-90) and stability parameters

Cultivar		Location					Stability parameters		
		Kukui	Keaau	Eleele	Kona	Kunia	mean	slope	r^2
				(kg/tree)					
Blue Mountain	- BM	-	-	2.0	3.0	3.5	2.86	0.53	0.99
Bourbon, Pink	- PB	1.0	3.1	3.1	-	5.8	3.25	0.9	0.83
Catuai	- CT	2.5	-	3.2	-	5.6	3.78	0.76	0.99
Caturra, Red	- RC	-	-	2.6	3.8	7.4	4.58	1.64	0.83
Caturra,Yellow	- YC	2.4	1.9	2.6	6.1	7.8	4.16	1.34	0.94
Guadalupe	- GD	0.8	-	4.0	-	5.9	3.57	1.13	0.89
Guatemalan	- GT	-	-	2.7	4.6	5.0	4.10	0.85	0.97
Kent	- KT	-	2.8	3.1	-	4.1	3.32	0.32	0.99
Pretoria	- PT	0.2	-	0.4	3.8	-	1.11	1.11	0.85
Progeny 502	- PG	-	0.5	4.1	6.1	5.2	3.99	1.17	0.77
S.L. 28	- SL	1.1	0.5	2.9	-	-	1.50	1.47	0.89
location mean		1.34	1.47	2.82	4.55	5.58	3.29	-	-

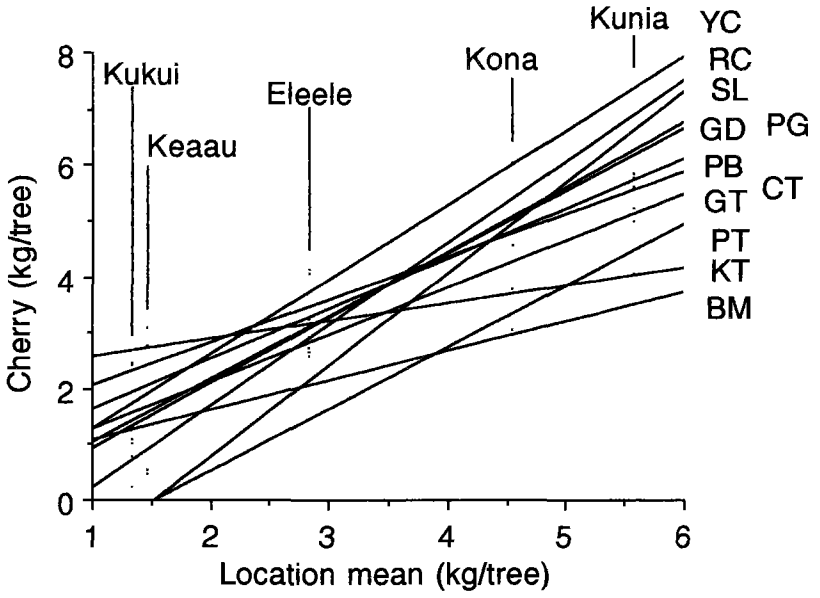

Fig.1. Cherry yield across locations

The relationships in Table 1 and Fig. 1 are represented in Fig. 2. Cherry yields of cultivars are plotted in one of four quadrants. The quadrant in the lower right contains cultivars with yields ≥ the grand mean for all cultivars and with slopes (b) ≤ 1. These are Guatemalan, Catuai, Kent and Pink Bourbon. These are considered higher yielding than average with performance more stable than average. Cultivars in the upper right quadrant have means ≥ the grand mean but slopes ≥ 1 are less stable. Cultivars in the lower left quadrant have means ≤ the grand mean and b ≤ 1 are considered low yielding but stable. Cultivars in the upper left quadrant have yields ≤ grand mean and b ≥ 1. These are low yielding, having even poorer adaptation in poor locations than average. In this first harvest season Pretoria and S.L. 28 are in this quadrant.

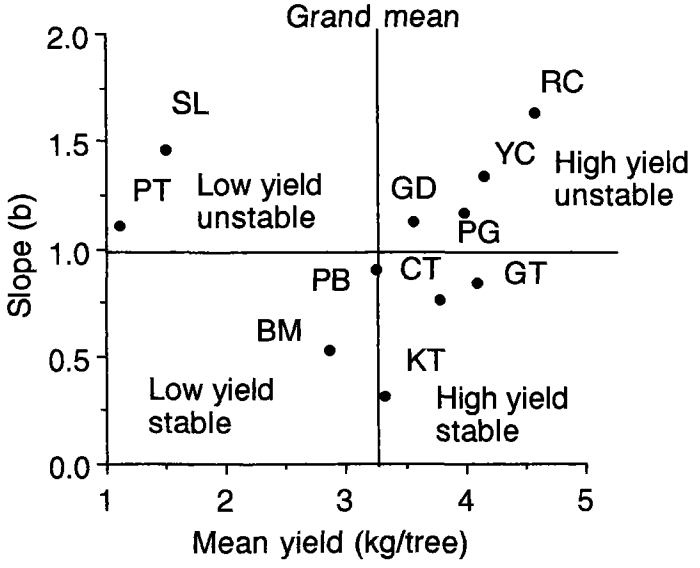

Fig. 2. Cherry yield and stability by cultivar.

Cherry yield of Red Caturra (Fig. 2) is highest but it is in the upper right quadrant. It is important to determine if the performance is due to lower than average yields at poor locations or much higher than average yields at the best sites. From Table 1 it appears to have slightly below average performance at the average locations, very high yield at the best. Unfortunately, adequate interpretation is impossible because, like Guatemalan it was not at the two poorest sites.

Yield of green bean (percent recovery times cherry yield) shows Guatemalan to be superior in yield and stability (Fig. 3). Cultivars fall in two groups- low stability and yield and stable, high yields. This pattern is quite different from cherry yield (Fig. 2) suggesting that recovery is impacted by poor sites.

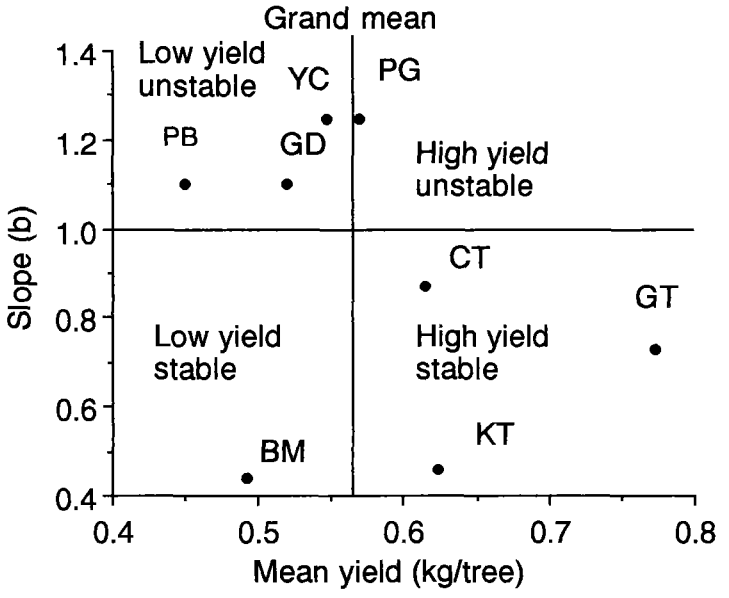

Fig. 3. Green bean yield for 1989-90 season.

We attempt to describe the commercial impact of location on bean size via grading (Fig. 4). In Hawaii green beans grades are partially based on size and prices vary by grades. Price by grade is used to reduce the cultivar response to location for bean size to a single number. Green bean samples of each cultivar from each location are graded and each grade fraction weighed. The price per grade and sample portion are multiplied to determine the green bean value ($/kg). Prices (US$/kg) are averages for different grades of the 1989-90 Kona crop: extra fancy $13.60, fancy $13, No.1 $12.65, prime $11.88, No. 3 $11 and off grade $5.50.

This is obviously an overly simplistic analysis as other factors used in grading such as defects and cupping were not used. However it is still useful. The range of bean value was very small. This is primarily due to small difference in price between the top three grades which span 4 mm in screen size. Guatemalan, Blue Mountain, Progeny 502, and S.L.28 had large bean size. Kent's value ($/kg) was quite unstable indicating a strong response of bean size to locational differences.

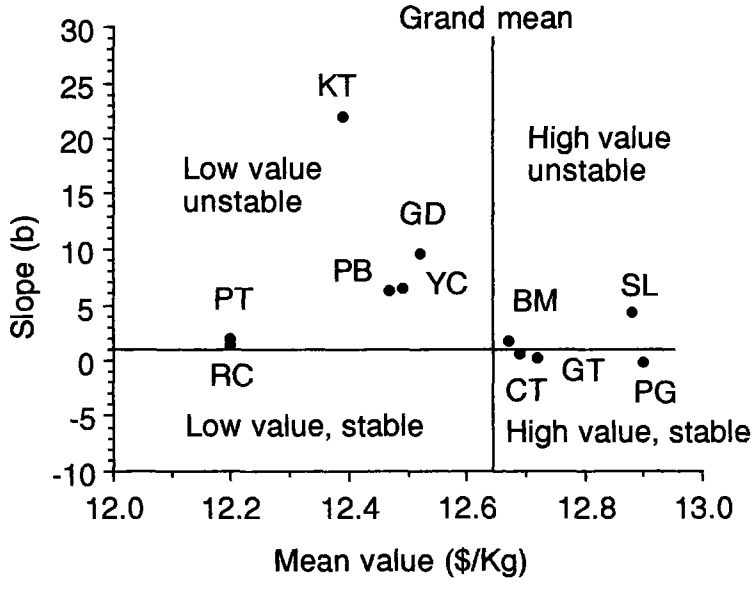

Fig. 4. Green bean value ($/kg) for the 1989-90 season.

Value per tree (Fig. 5) was calculated by multiplying bean value ($/kg) and yield of green bean (kg/tree). Catuai and Kent had high, stable value per tree. Guatemalan the Kona coffee standard had highest value ($9 per tree) but was less stable.

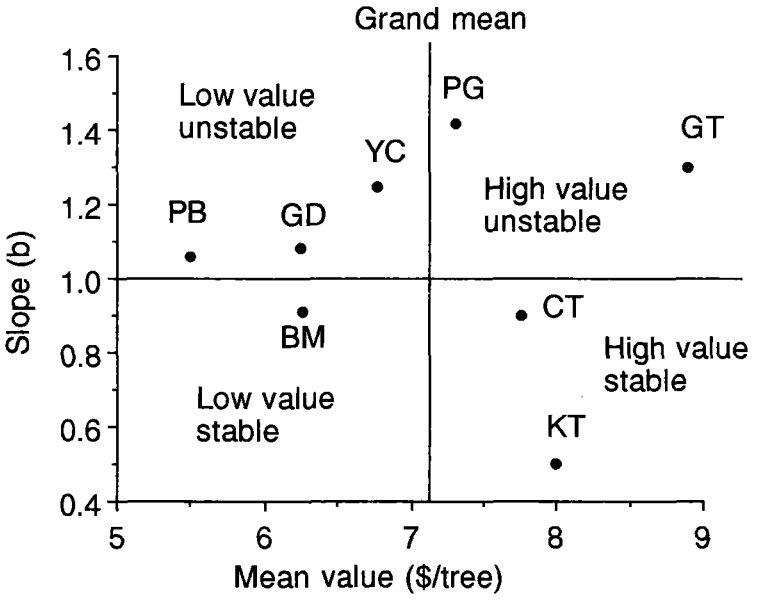

Fig. 5. Crop value ($/tree) for the 1989-90 season.

Cherry yields from the first and second harvests, fourteen location by year combinations (1989-90, 1990-91) are represented in Fig. 6. Yields of Catuai, Guatemalan and Kents were high and stable. Red Bourbon and S.L. 28 had higher yields in a few locations but quite low yields elsewhere.

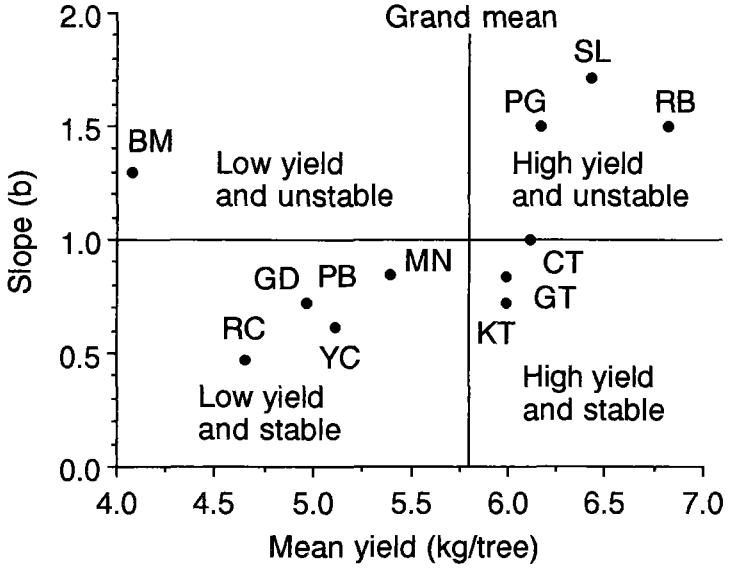

Fig. 6. Cherry yield per tree for the 1989-90 and 1990-91 seasons.

The result of harvesting and quality determination for three years will be evaluated and graphically presented to cooperators, current and potential coffee growers using stability analysis. As demonstrated it can greatly simplify interpretation of large data sets.

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SUMMARY

Hawaii's coffee industry will increase 300% to 3000 ha from 1987 to 1992. Hawaii's historic Kona coffee district is planted to a *typica* land race,'Guatemalan', introduced one hundred years ago.

Eight on-farm cultivar evaluation experiments were established on former sugar cane fields designated for coffee beyond Kona. Results of cultivar response to different environments are reported as cherry yields over two years (first and second year harvested), green bean yield, green bean value ($/kg), and value per tree. Stability analysis was employed to classify cultivars into four groups. These were high yield or value (above the experiment grand mean and stable response across environments, regression coefficient or slope, $b \leq 1$), above the mean but less stable ($b > 1$), less than the mean and stable response and lastly less than the mean and less stable.

The cultivars 'Guatemalan', 'Red Catuai', and 'Kent' had the highest stable cherry yield. Cultivar differences for green bean yield and per tree value were large in the first year. Bean value based on 1989 prices for Kona coffee grades showed little cultivar or location response.

YIELD, SIZE AND CUP QUALITY OF COFFEES GROWN IN THE HAWAII STATE COFFEE TRIAL

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INTRODUCTION

A century ago, coffee was produced commercially throughout the State of Hawaii. Thrum (1898) reported a total of 5579 ha planted in 1898 on Hawaii, Maui, Oahu, and Kauai. Gradually, that production was reduced and commercial production was concentrated in the Kona District on the Island of Hawaii, where there are currently approximately 810 ha in cultivation (State of Hawaii, 1989).

Two situations have prompted interest in the expansion of coffee production in Hawaii. First, is the decline in profitability of the sugar industry which presently occupies 70,000 ha (State of Hawaii, 1989). Sugar companies have sought to diversify their agricultural base with alternative crops, among them, coffee. Secondly, the expansion of the premium/gourmet coffee market in the United States suggests the possible development of a larger niche market for Hawaiian coffee.

The selection of suitable cultivars for expansion requires information on adaptability of potential cultivars to locations with widely differing conditions of temperature, rainfall and elevation. Yield and quality are important considerations in site and cultivar selection. Furthermore, due to high labor costs in Hawaii, the suitability of cultivars for mechanical harvesting is also important.

Accordingly, the Hawaii State Coffee Trial was initiated in 1985 to plan for a major expansion of the industry. The trial is an ongoing cooperative effort between the University of Hawaii and private industry. This paper reports a portion of the early results of that trial.

That genetic, environmental, and physiological factors affect green coffee

quality has been well established (de Gialluly, 1959; Wellman, 1961; Cannell, 1974). Yet, little is known about the performance of currently available cultivars in Hawaii outside of the Kona district. The objective of this study was to determine the effects of cultivar and location on the quality of coffee grown in Hawaii. A long-range objective of the study is to determine quality stability for each cultivar.

MATERIALS

Seedlings of twenty-one cultivars were started in 1986 and were transplanted in eighteen locations in the State of Hawaii in 1987. The sites to be reported in this paper are shown in Figure 1 and described in Table 1. The first major harvest which occurred in 1989, included Pink Bourbon, Yellow Caturra, Guadalupe, Kent, Progeny 502, S.L. 28, and 6661.

Figure 1. Hawaii State Coffee Trial Locations - 1989

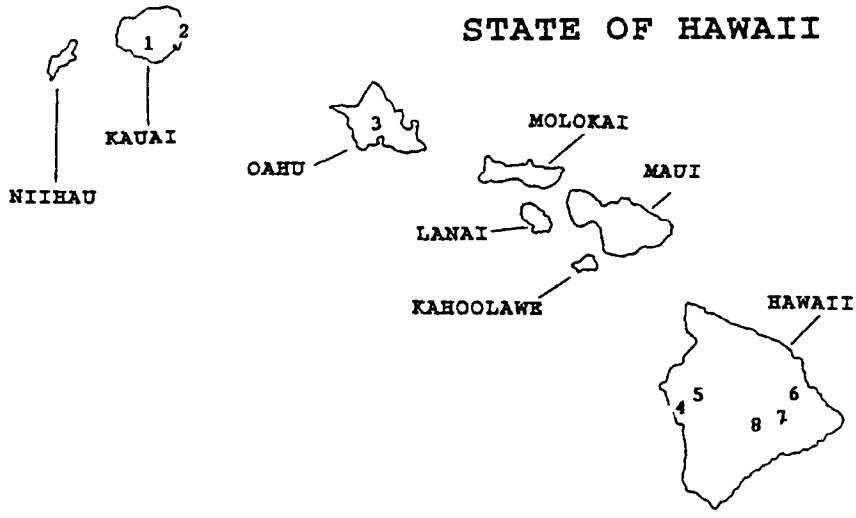

Table 1. Site descriptions for the Hawaii State Coffee Trial - 1989

Map no.	Location	Elevation (m)	Average rainfall[z] (mm)	Average temperature[x] (°C)
1	Kauai - Eleele	90	1170	23.4
2	Kauai - Lihue	25	1440	22.6
3	Oahu - Kunia	70	510	23.1[w]
4	Hawaii - Kona	395	1260	21.6
5	Hawaii - Kona	700	1260	21.6
6	Hawaii - Keaau	90	3920	23.6
7	Hawaii - Kukui	410	4000[y]	21.7
8	Hawaii - Pszyk	480	5049	21.1

[z] National Oceanic and Atmospheric Administration (1989)

[y] Rainfall Atlas of Hawaii (1986)

[x] Atlas of Hawaii (1986)

[w] Site data

METHODS

Sample preparation. Coffee cherries were harvested at peak season and flown to Honolulu for processing and analysis. Cherries were mechanically pulped and wet-processed (fermented). Parchment was dried in a mechanical oven until the moisture content was 10.5 to 13.0%, after which it was mechanically hulled and winnowed. Samples used to determine percentage of green bean recovery were pulped and hulled by hand to ensure no loss by mechanical means. Samples that were to be cupped were roasted to standard color on the day prior to evaluation. An infusion was prepared by the addition of approximately 120 ml of water just below the boiling point to 7.5 g of roasted, ground beans.

Quality evaluation. Green bean recovery was determined as percentage of cherry weight after adjusting green bean moisture to 10.5%. Green bean size grade was determined by using standard coffee bean screens to determine the percentage of beans by weight in each category.

Body and acidity of the beverage were evaluated by a group of six trained panelists. Panelists were asked to rate the coffees on a line scale that ranged from thin body to heavy body or from low acid to high acid. Their ratings were then converted to numerical scores based on a 10-point scale.

RESULTS

Green Bean Recovery. Table 2 indicates that green bean recovery is affected by both cultivar and location. The cultivar, Kent, had the highest recovery percentage across locations, followed by Progeny 502 and 6661. The locations with the largest average recovery occurred in the two Kona locations (395 m and 700 m) with 19.8% and 21.5% recovery respectively. No clear-cut elevation, rainfall or temperature effect was observed.

Table 2. Green bean recovery (yield)[z]. Percentage green bean recovered based on cherry weights.

	Location and elevation								
Cultivar	Kauai Eleele 90 m	Kauai Lihue 25 m	Oahu Kunia 70 m	Hawaii Kona 395 m	Hawaii Kona 700 m	Hawaii Keaau 90 m	Hawaii Kukui 410 m	Hawaii Pszyk 480 m	Average
Bourbon - Pink	18.4	-	14.7	-	-	15.8	15.4	14.6	15.8
Caturra - Yellow	15.5	13.2	13.8	-	21.5	15.3	15.0	14.7	15.6
Guadalupe	14.9	-	13.8	19.6	-	-	14.9	-	15.8
Kent	18.0	-	19.7	21.2	-	19.0	15.4	-	18.7
Progeny 502	18.9	-	16.0	-	-	17.2	-	18.0	17.5
S.L. 28	18.0	-	13.6	19.6	-	15.5	-	-	16.7
6661	17.2	-	16.3	18.8	-	-	16.4	-	17.2
Average	17.3	13.2	15.4	19.8	21.5	16.6	15.4	15.8	

[z]Based on 10.5% green coffee bean moisture

Green Coffee Bean Size Grade. Table 3 reports the percentages of beans that fell into the largest size category (Hawaii Extra Fancy). These data are thus an indicator of bean size in a given lot. The data indicate that there were differences in bean size between cultivars and between locations. Progeny 502 had the highest percentage (45.6) of Hawaii Extra Fancy green beans across the locations. Highest percentages of Extra Fancy beans came from Keaau, Kukui and Pszyk sites (41.1, 35.2, 33.1 respectively). These locations are all in the same district and in high rainfall areas. Cannell (1974) has reported that bean size was determined by the amount of rainfall during the fruit expansion stage of development. Elevation did not appear to be a size-determining factor.

Table 3. Green bean size grade. Percentage of Hawaii Extra Fancy green beans[z].

	Location and elevation								
Cultivar	Kauai Eleele 90 m	Kauai Lihue 25 m	Oahu Kunia 70 m	Hawaii Kona 395 m	Hawaii Kona 700 m	Hawaii Keaau 90 m	Hawaii Kukui 410 m	Hawaii Pszyk 480 m	Average
Bourbon - Pink	2.2	-	0.0	-	-	5.1	25.3	31.1	12.7
Caturra - Yellow	10.8	2.7	0.0	-	13.6	6.3	19.3	22.8	10.8
Guadalupe	6.0	-	1.9	25.6	-	-	48.3	-	20.4
Kent	19.0	-	15.7	35.8	-	59.1	51.0	-	36.1
Progeny 502	40.0	-	43.9	-	-	52.9	-	45.3	45.5
S.L. 28	19.6	-	7.3	32.7	-	55.7	-	-	28.8
6661	3.6	-	9.3	7.8	-	-	32.3	-	13.2
Average	14.5	2.7	11.2	25.5	13.6	35.8	35.2	33.1	

[z]Hawaii Extra Fancy bean size - screen 19 and larger.

Cupping Acidity. Acidity scores shown in Table 4 indicate that acidity was positively correlated with elevation. Locations at higher elevations (Kona 700 m and 395 m; Pszyk 500 m) scored higher in acidity than lower elevation locations. Some differences due to cultivar were observed but were not consistent across sites.

Table 4: Average cupping acidity scores.

	Location and elevation								
Cultivar	Kauai Eleele 90 m	Kauai Lihue 25 m	Oahu Kunia 70 m	Hawaii Kona 395 m	Hawaii Kona 700 m	Hawaii Keaau 90 m	Hawaii Kukui 410 m	Hawaii Pszyk 480 m	Average
Bourbon - Pink	1.4	-	2.1	-	-	2.0	1.9	3.0	2.1
Caturra - Yellow	1.1	1.0	1.4	-	4.5	1.2	2.4	4.0	2.2
Guadalupe	2.2	-	2.9	2.5	-	-	3.1	-	2.7
Kent	1.5	-	2.5	4.4	-	1.4	2.1	-	2.4
Progeny 502	1.4	-	1.4	-	-	3.1	-	4.0	2.5
S.L. 28	1.9	-	1.6	2.7	-	3.9	-	-	2.5
6661	2.9	-	2.7	3.1	-	-	4.0	-	3.2
Average	1.8	1.0	2.1	3.2	4.5	2.3	2.7	3.7	

Scale: 1 (low acid) - 10 (high acid)

Cupping Body. Cupping scores for body shown in Table 5 indicated that differences due to cultivar and location were slight.

Table 5: Average cupping body scores.

	Location and elevation								
Cultivar	Kauai Eleele 90 m	Kauai Lihue 25 m	Oahu Kunia 70 m	Hawaii Kona 395 m	Hawaii Kona 700 m	Hawaii Keaau 90 m	Hawaii Kukui 410 m	Hawaii Pszyk 500 m	Average
Bourbon - Pink	3.4	-	3.0	-	-	2.3	3.5	2.8	3.0
Caturra - Yellow	3.4	2.9	3.1	-	1.8	2.8	2.6	2.0	2.7
Guadalupe	3.1	-	2.5	3.9	-	-	2.4	-	3.0
Kent	3.9	-	3.3	4.4	-	2.8	3.1	-	3.5
Progeny 502	3.2	-	3.0	-	-	2.8	-	2.4	2.9
S.L. 28	2.0	-	2.4	2.4	-	3.1	-	-	2.5
6661	1.9	-	2.5	2.6	-	-	2.6	-	2.4
Average	3.0	2.9	2.8	3.3	1.8	2.8	2.8	2.4	

Scale: 1 (thin body) - 10 (heavy body)

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SUMMARY

Twenty-one coffee cultivars are being grown in 18 locations in the Hawaii State Coffee Trial to determine the effect of cultivar and location on coffee yield (green bean recovery), bean size and cup quality. Only a limited number of cultivars and locations fruited in the first harvest year and those results are reported.

Green bean recovery was affected by both cultivar and location, but no clear-cut elevation effect was observed. Green bean size was related to rainfall but not to elevation. Large differences between cultivars and between locations were observed. Cup tests showed a distinct effect of elevation on acidity with acidity positively correlated with elevation. Acidity differences due to cultivar were only slight. Body differed slightly between cultivars, but no clear location effects were seen.

EVALUACIONES DE CAMPO CON EL HONGO *BEAUVERIA BASSIANA* PARA EL CONTROL DE LA BROCA DEL CAFÉ, *HYPOTHENEMUS HAMPEI* EN COLOMBIA[1]

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INTRODUCCION

La aparición de la broca del café *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) en Colombia en 1988, determinó que CENICAFE iniciara investigaciones para implementar un programa de manejo integrado de esta plaga cuyo componente principal fueran agentes de control biológico.

El hongo *Beauveria bassiana* (Balsamo) Vuillemin, se considera que puede jugar un papel importante en la regulación de poblaciones de la broca bajo las condiciones en que se desarrollan las plantaciones de café en Colombia.Este patógeno se ha encontrado infectando la broca en forma natural practicamente en todos los paises en donde ha llegado esta plaga.

A pesar de lo anterior un análisis de la literatura indica que son muy pocos los estudios de campo que se han llevado a cabo para explorar la potencialidad de *B. bassiana* en la represión de la broca. La falta de un producto formulado en polvo efectivo contra la broca, ha sido la mayor limitante para desarrollar estos estudios.

En este informe se presentan resultados preliminares de evaluaciones de campo con formulaciones de *B. bassiana* cepa 069 aislada de la broca del café en Ancuya, Colombia. Durante 1990 los experimentos se llevaron a cabo con una formulación líquida refrigerada y durante 1991 con una formulación más estable en polvo.

REVISION DE LITERATURA

La broca del café bajo condiciones de confinamiento se ha observado que puede ser atacada por varios hongos como *Metarhizium anisopliae, Paecilomyces tenuipes, Numuraea rileyi y B. bassiana*, sin embargo esta última especie se ha encontrado asociada con las poblaciones de campo de *H. hampei* por lo que ha merecido que se estudie más detalladamente su efecto sobre esta plaga.

B. bassiana se ha encontrado infectando poblaciones de broca en Java, Sri Lanka, Uganda, Camerún, Jamaica, Méjico, Brasil y Ecuador (Prior, 1987) y recientemente en Colombia. La incidencia del hongo varía bastante de un país a otro; por ejemplo en Kenya las infecciones aparentemente son raras pero se registran altos niveles en Camerún, Java, Brasil, Méjico y Ecuador (Moore y Prior, 1988). Aunque estas diferencias pueden deberse a factores climáticos, es tambien posible suponer que la broca esta mejor adaptada al hongo en su tierra nativa en Africa, pero sucumbe a razas exóticas que encuentra cuando llega a un nuevo sitio.

Recientemente Jiménez (1989) comprobó que existen diferencias en virulencia entre razas de *B. bassiana* hacia la broca del cafeto. El evaluó bajo condiciones de laboratorio, 46 aislamientos del hongo originados en 13 paises y encontró que el 35% de ellos causó mortalidades mínimas del 50% y cinco de estos aislamientos alcanzaron altos niveles de infección. Falta determinar la eficiencia de estos aislamientos bajo condiciones de campo.

Los intentos de control biológico de la broca del café usando *B. bassiana* han sido muy pocos y se han caracterizado por la poca continuidad después de ensayos iniciales. Carneiro (1984) en el brasil después de aplicar el hongo en dosis de 10^8 - 10^{12} esporas/ha. encontró un bajo porcentaje de control. Lo anterior, indica, se debió a condiciones ambientales no propicias. Fernandez et al. (1985) aplicó *B. bassiana* a un cafeto que tenía una infestación del 10% de broca, usando una dosis de 300 ml de una suspensión de 1×10^9 conidias/ml. Al cabo de 10 dias observó una mortalidad de 91%. Tronconi et al. (1986) en Honduras, usó un aislamiento de este hongo obtenido directamente de broca el cual aplicó en el campo a una concentración de 2×10^7 conidias/ml. y después de 36 días encontró mortalidades hasta del 66.2%.

La anterior revisión de literatura permite concluir que el hongo *B. bassiana* puede ser una herramienta útil para el control de *H. hampei* ya que indica que este insecto es más severo bajo condiciones humedas que son precisamente las condiciones bajo las cuales el hongo *B. bassiana* podría ser más efectivo, sín embargo, se desconocen muchos aspectos básicos como son: la determinación de las razas más virulentas contra la broca; una tecnología fácil y económica para producirlo a escala comercial; formulaciones del hongo especialmente con base en aceites para obtener una mejor dispersión y permanencia del hongo en el campo; información sobre equipos y metodología de aspersiones que garanticen un buen cubrimiento y penetración del producto en los cafetales; y por último evaluacione de campo con adecuado seguimientos para determinar la acción del patógenos en diversos ecosistemas cafeteros.

Tambien es importante estudiar el efecto de otros plaguicidas de uso común en cafetales, especialmente fungicidas sobre el hongo. Se dice que los fungicidas cúpricos para el control de la roya del cafeto pueden inhibir la acción de *B. bassiaba* (Prior, 1987). Asi mismo se ha encontrado que insecticidas como metilparation, carbosulfan, azinphosethyl, inhiben la germinación de esporas de *B. bassiana* in vitro (Aguda et al., 1984).

METODOLOGIA

El hongo *B. bassiana* utilizado en los diferentes ensayos correspondió a la Cepa 069 de CENICAFE, aislada inicialmente de la broca del café, *H. hampei* en Ancuya, Nariño (Vélez y Benavides 1990). El hongo se produce formulado en cantidades experimentales en los laboratorios de la Federación Nacional de Cafeteros de Cenicafé y el LIQC. El proceso es similar al desarrollado por Samsinakova (1966) en el cual la producción se hace en dos fases, la primera bajo fermentación para la producción de blastosporas y luego en bandejas en superficie utilizando sustratos definidos en base a fuentes de nitrógeno y carbohidratos.

Durante mayo-septiembre de 1990 se realizaron tres estudios con una formulación líquida en las localidades de Ansermanuevo, Valle del Cauca y Garzón, Huila, con el fin de establecer si se podía inducir la enfermedad por *B. bassiana* (Bb) en la broca y observar la infección después de 1, 3 y 6 aspersiones del hongo.

Para el estudio 1, se escogió un lote de 35 árboles, los que se asperjaron con una formulación líquida de Bb en la dosis de $0.76x10^{10}$ conidias/árbol. La evaluación se hizo en cinco árboles escogidos al azar y en cada uno se seleccionaron cinco ramas en las que se contó el número total de frutos brocados y de éstos los infectados por Bb, 45 días después de una sóla aspersión.

El estudio 2 fue igual al anterior pero se hicieron tres aspersiones de Bb a los 0, 25 y 42 días usando una dosis de $2.37x10^{11}$ conidias/árbol.

El estudio 3 se llevó a cabo en Garzón, Huila y la variación consistió en evaluar el efecto de seis aspersiones espaciadas cada 15 días usando una dosis de $1.6 - 7.7x10^{10}$ conidias/árbol. En esta finca las evaluaciones se pudieron realizar por un espacio de 119 días.

Durante mayo - junio de 1991, se iniciaron evaluaciones de Bb utilizando formulaciones en polvo en una finca en Ansermanuevo, Valle, en un cafetal de variedad Colombia sembrada a 1x1m e infestada con broca, la cual fue arrendada por Cenicafé para tener control sobre todas las actividades y poder organizar los experimentos en una mejor forma.

El hongo en polvo, antes de asperjarlo, se le adicionó un aceite vegetal en igual proporción y luego se diluyó en la cantidad de agua requerida. Las aspersiones se hicieron después de que se ocultó el sol, con aspersoras manuales de presión previa retenida, las cuales fueron calibradas con anterioridad.

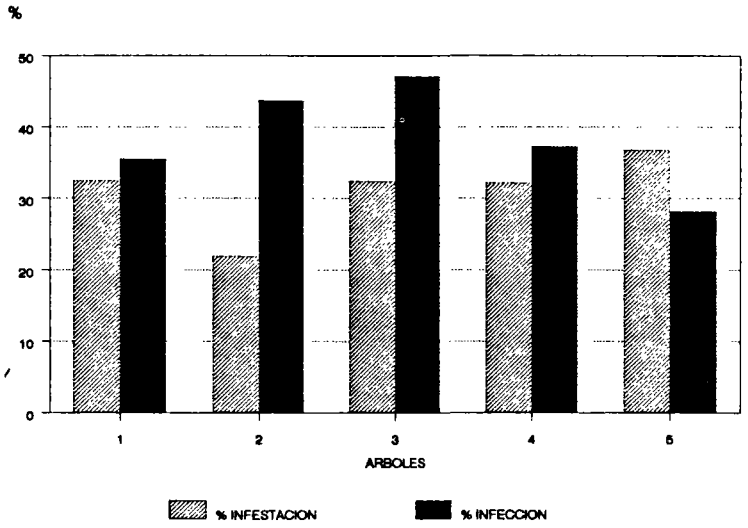
El estudio 4 consistió en evaluar la mortalidad de la Broca, utilizando diferentes fracciones del hongo Bb que se obtienen durante los procesos de fermentación (blastosporas, conidias sumergidas, cuerpos hifales) y de superficie (conidias áereas) usando una dosis de $1x10^{8}$ fracciones del hongo/árbol, asperjados a los 0 y 17 días de iniciado el experimento. El ensayo se arregló en un diseño completamente al azar, usando una rama/árbol/ parcela y cinco repeticiones. Las ramas se marcaron adecuadamente para su fácil localización y se infestaron con 100 brocas usando un cilindro de anjeo recubierto con una manga de tul. A las 24 horas se evaluó el porcentaje de infestación por broca y se asperjaron los tratamientos. Para la evaluación se registró el porcentaje de infestación y de infección del hongo por parcela a los 17 y 35 días después de iniciado el ensayo.

El estudio 5, se desarrolló en la misma finca y tuvo como objetivo comparar siete formulaciones diferentes del hongo, basadas en la cepa 069, producidas por diferentes laboratorios, usando la dosis de $1x10^{8}$ conidias/árbol. La metodología usada fue igual a la del ensayo anterior, con la excepción que sólo se asperjó el hongo al iniciar el experimento y se hizo una evaluación a los 18 días.

RESULTADOS Y DISCUSION

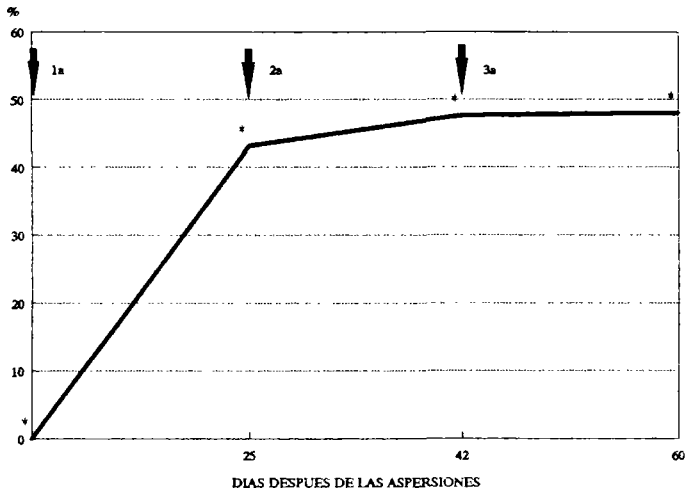
Los resultados se presentan en las Figuras 1 a 5. En el estudio 1 (Figura 1) se logró un 36% de infección por Bb en promedio, con una sóla aspersión. En el estudio 2 (Figura 2) la infección por Bb fue más alta (48.1 % en promedio) después de tres aspersiones. En el estudio 3 (Figura 3) se muestra el efecto del hongo sobre la broca durante un periodo de 119 días de evaluaciones, después de realizar seis aspersiones. La infección por Bb se incrementó hasta alcanzar un promedio de 69.0%. Las condiciones de humedad en la zona de Ansermanuevo son más bajas que en Garzón en donde se realizó el estudio 3, lo cual podría explicar en parte la diferencia de los resultados. En términos generales los tres estudios muestran que se puede inducir infección por Bb y que los niveles son más altos a medida que se hacen más aspersiones.

FIGURA 1- MORTALIDAD DE LA BROCA DEL CAFE POR *B. bassiana* 48 DIAS DESPUES DE UNA ASPERSION CON FORMULACION LIQUIDA 0.76 X 10^{10} CONIDIAS/ARBOL



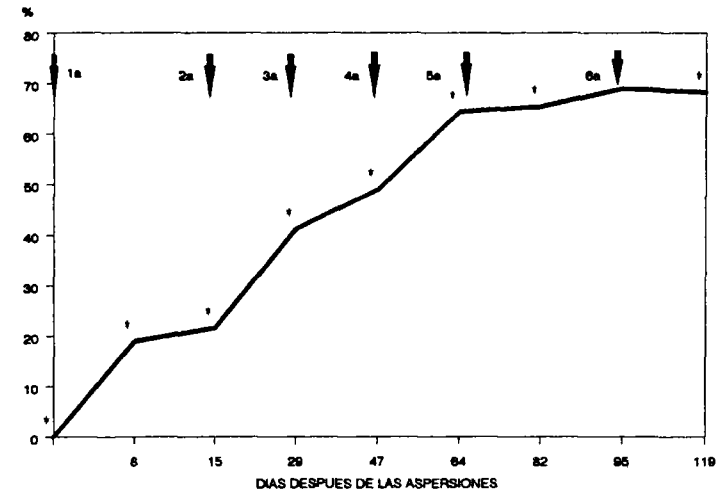
FINCA EL RUBI, ANSERMANUEVO, COLOMBIA
MAYO-JUNIO DE 1990.

FIGURA 2- MORTALIDAD DE LA BROCA DEL CAFE POR *B. bassiana* DESPUES DE TRES ASPERSIONES USANDO UNA FORMULACION LIQUIDA EN DOSIS DE 2.33 X 10^{11} CONIDIAS/ARBOL



FINCA EL RUBI, ANSERMANUEVO, COLOMBIA.
JUNIO-AGOSTO DE 1990.

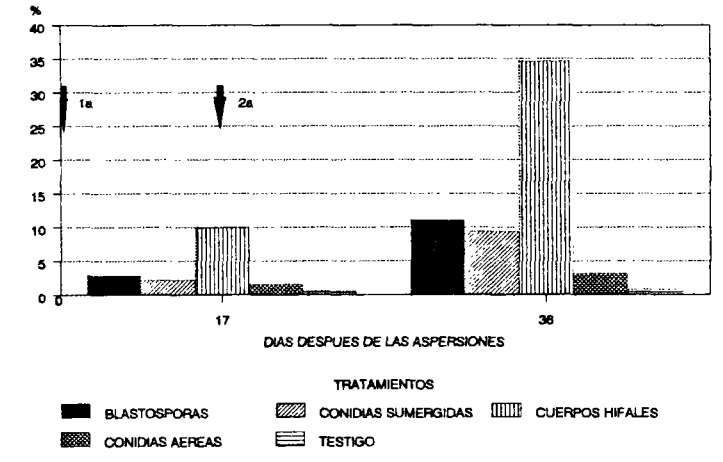
FIGURA 3- MORTALIDAD DE LA BROCA DEL CAFE POR *B. bassiana* DESPUES DE SEIS ASPERSIONES USANDO UNA FORMULACION LIQUIDA EN DOSIS DE 1.6-7.7 X 10^{10} CONIDIAS/ARBOL



FINCA LA ULAMA, GARZON, COLOMBIA,
JUNIO - SEPTIEMBRE DE 1990.

En el estudio 4 (Figura 4) se observan diferencias en la infección del Bb de acuerdo a la fracción del hongo que se utilice. Se puede ver como los cuerpos hifales, formados por un aglomerado de hifas, son capaces de inducir una mayor infección. Esto prodría deberse a a que de acuerdo a lo anterior, ésta fracción del hongo es más concentrada que las otras que están cuantificadas individualmente, mientras que los cuerpos hifales forman un grupo de hifas del que se desconoce su cantidad.

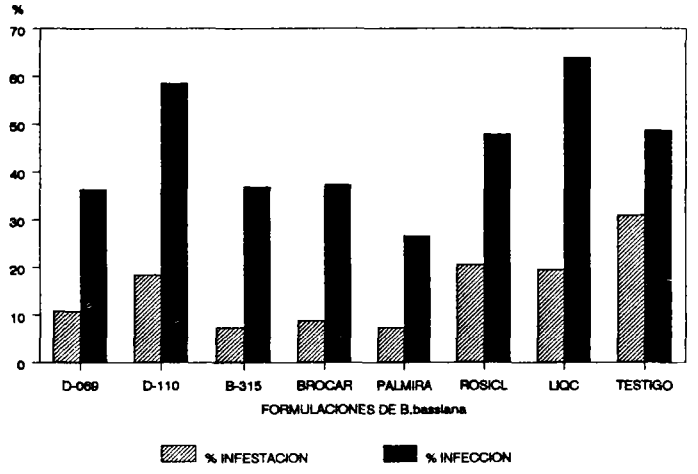
FIGURA 4- MORTALIDAD DE LA BROCA DEL CAFE POR *B. bassiana* DESPUES DE DOS ASPERSIONES A LOS 0 Y 17 DIAS USANDO DIFERENTES FRACCIONES DEL HONGO



FINCA LA PEDRERA, ANSERMANUEVO, COLOMBIA
MAYO-JUNIO DE 1991

Los resultados del estudio 5 (Figura 5) muestran que todas las formulaciones evaluadas causan mortalidad a la broca siendo la denominada LIQC la que alcanzó el mayor porcentaje. Sin embargo los resultados no se muestran claros debido a la infección causada en el testigo, el cual fué superior a cuatro formulaciones. Es posible que la causa de estos resultados se deba a contaminación o a que las parcelas se deban separar más unas de otras.

FIGURA 5- MORTALIDAD DE LA BROCA DEL CAFE POR *B. bassiana* 18 DIAS DESPUES DE ASPERJADOS LOS TRATAMIENTO



FINCA LA PEDRERA, ANSERMANUEVO,COLOMBIA, MAYO-JUNIO DE 1991.

CONCLUSIONES

Este estudio presenta resultados preliminares sobre los cuales todavía no se puede concluir definitivamente en algunos aspectos, pero si se puede establecer que los niveles de infección de Bb sobre la broca del café se pueden incrementar mediante aspersiones del hongo. Estos niveles son más altos a medida que se deposita más hongo en los cafetales.

Para recomendar este biopesticida falta estudiar muchos aspectos importantaes entre los cuales se puede citar: épocas y dosis de aspersión, selección de razas más virulentas, optimizacioón de las formulaciones, supervivencia del hongo en el campo, compatibilidad con plaguicidas y con otros metodos de control.

RESUMEN

La broca del café, *Hypothenemus hampei* (Ferrari) fué introducida a Colombia en 1988, inmediatamente Cenicafé inició un programa de investigación para desarrollar un plan de manejo integrado de la broca basado en el control biológico como un componente principal.

El entomopátogeno *Beauveria bassiana* (Balsamo) Vuillemin es uno de los factores de mortalidad de la broca que se encuentra frecuentemente en todos los paises donde este insecto es introducido. La patogenicidad

mostrada en laboratorio y por el aislamiento local denominado Cenicafé 069 ha motivado que se reproduzca en cantidades suficientes para desarrollar experimentos de campo.

Los estudios preliminares realizados durante 1990 usando una formulación líquida de *B. bassiana* en dosis cercanas a 1 X 10^{10} conidias/árbol mostraron que es factible inducir la enfermedad en una población de broca. Los niveles de infección variaron desde un 36% cuando se realizó una sola aspersión del hongo hasta 69.0% cuando se hicieron seis aspersiones.

Con el desarrollo de formulaciones en polvo y la facilidad para arrendar fincas cafeteras infestadas con broca para experimentación se iniciaron estudios en mayo de 1991 para observar en mayor detalle el efecto de *B. bassiana* sobre la broca.

SUMMARY

The coffee berry borer, *Hypothenemus hampei* (Ferrari) was introduced to Colombia in 1988 and inmediately CENICAFE started a research plan to develop a pest management strategy against the borer based in biological control as a main component.

The entomapathogen *Beauveria bassiana* (Balsamo) Vuillemin es one of the key mortality factors found associated with the borer in all the countries where it is introduced. The pathogenicity shown under laboratory conditions of a local isolated (CENICAFE 069) has prompted us to reproduce this fungus in enough amount for field trials.

Preliminary studies carried out during 1990 using a liquid formulation of *B. bassiana* in dosages about 1 x 10^{10} conidia/tree, have shown that it is possible to induce the disease in the borer population. Infection levels varied from 36% with only one fungus spray to 69.0% with six sprays.

With the development of powder formulations and the facility to rent coffe farms infested with the borer field trials were started in May 1991 to observe closely the effect of *B. bassiana* on the coffee berry borer.

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PRELIMINARY RESULTS ON COMPARATIVE GC ANALYSES OF VOLATILES PRODUCED BY THE COFFEE BERRIES

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Recent evidence for allelochemical relationships between the coffee borer : *Hypothenemus hampei* (Ferr.) and the coffee : *Coffea sp.* (Giordanengo et al., in press) led us to investigate by chemical analyses (GC, GCMS) the volatiles produced by Robusta coffee berries.

GC analyses of either pentanic or acetonic washes of green and red coffee berries showed quantitative differences in resolved peaks. These solvents were choosen to extract repectively aliphatic and cyclic hydrocarbons for the pentane and alcohols, aldehydes and acids for acetone.

Pentanic washes contained more volatile compounds than acetonic washes; however, differences between washes of fresh green berries and fresh red berries.were more noticeable with acetonic washes. Fresh red berry have been found by biological tests to be more attractive than fresh green berry and thus the differences observed in the chemical composition of the washes could account for the preferential selection of the red berry by the scolytes. All these compounds are now being identified using GC-MS and structures such as sesquiterpenes, acids, aldehydes, and esters have been determined.

Further behavioural tests will be conducted to determine whether all the differences shown by GC have biological significance.

The Identification of volatile attractants could aid in the management of the coffee berry borer by providing lures for traps which could be used to monitor pest populations.

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INTRODUCTION

Recent studies on the coffee berry borer have provided evidence that female borers use volatile chemicals from the host to locate feeding and oviposition sites. Pentane and acetone extracts of mature and immature berries were tested for biological activity and then were analysed using GC and GC-MS.

MATERIAL AND METHODS

- Volatile extractions

Either red or mature green berries were extracted within 2 or 4 hours after field collection. Solvents used were pentane or acetone. Twenty berries were individualy washed in 1 ml of solvent during 30 s. A nitrogen airstream was used to condense to 100 μl the resulting extract and to reduce further oxidation. Analyses of red berry extracts with either pentane or acetone and of green berry extracts in acetone were then performed without any purification.

- Analyses

GC analyses were performed on a Girdel 30 equipped with a non polar column : Fused silica CPSil 8CB. 25m., 0.32 id (Chrompack, The Netherlands). Temperature of the oven was programmed from 40°C to 60°C at the rate of 20°C per min. followed by a step of 4 min. at 60°C and then 60°C to 280°C at the rate of 8°C per min. Injector was used in splitless mode (25 s., 190°C). The GC apparatus is linked to a Ribermag R10-10C mass spectrometer used in electronic impact (70 EV, 30-500 m.a.u.).One μl of each extracts was injected for analyses.

RESULTS

Results account for a putative specifity of peaks 37 and 39 in red berries. These compounds have now to be tested as candidate attractants towards the coffee berry borer females as well as compounds 68, 69, 72, 73. Chemical structures of most of these compounds were identified or tentative identifications have been undertaken but regarding the lack of behavioural signification, the structures will not be given in this poster.Comparison of Fig. a and Fig. b showed that pentanic extracts were more complex than acetonic extracts, especially concerning low molecular weight compounds. Acetonic extracts of green berries (Fig. c) were very different from acetonic extracts of red berries.

CONCLUSION

Further behavioural tests will be conducted to determine whether all the differences shown by GC have biological significancThe Identification of volatile attractants could aid in the management of the coffee berry borer by providing lures for traps which could be used to monitor pest populations.

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RAPPORT DE SYNTHÈSE / SUMMARY REPORT

Rapport de synthèse

Le XIV[e] Colloque de l'Association Scientifique Internationale du Café (ASIC) s'est déroulé du 14 au 19 juillet 1991 à l'hôtel Ritz Carlton de San Francisco, sur invitation de la National Coffee Association des Etats-Unis.

Il a réuni deux cent cinquante participants provenant d'un peu plus de trente pays. Le Dr J. R. Coughlin, Président de l'ASIC, doit être félicité pour l'organisation et le grand succès de cette manifestation scientifique.

Effets physiologiques

M. V. Arnaud a présenté un exposé sur la formation de paraxanthine au cours du métabolisme de la caféine chez l'animal et chez l'homme. On a montré chez l'homme que 80 % de la caféine sont déméthylés en paraxanthine. La paraxanthine est excrétée sous forme de métabolite acétylé et est utilisée pour la détermination du phénotype d'acétylation dans le métabolisme des drogues. De grandes variations entre individus ont été observées en ce qui concerne l'élimination de la caféine et les concentrations inverses du plasma en paraxanthine.

Les effets de la caféine sur les performances ont été passés en revue par K. Bättig selon quatre approches. Au cours d'expériences classiques multitâches, la caféine combattait les effets de la fatigue, surtout pour les tâches longues et monotones. Le temps de réaction était notamment réduit et on a montré que la conséquence la plus liée à l'absorption de caféine était la résistance à l'endormissement. Des effets sur l'éveil et la mémoire n'ont pas été constatés ou l'ont été de manière non significative. D'autres directions de recherche en cette matière, telles que les mécanismes de récompense ou les corrélations entre effets sensoriels et comportementaux ont été tracées.

L'exploitation de vingt-quatre études qui se recoupent par D. Thelle a révélé que le café filtré avait moins tendance à augmenter la teneur en cholestérol du plasma des buveurs de café que le café non filtré (bouilli). Des expérimentations croisées planifiées ont confirmé cette action et des recherches supplémentaires ont montré que l'agent (ou les agents) actif(s) pour l'augmentation du cholestérol était(ent) contenu(s) dans la fraction lipidique du café. Cependant, la nature de cet agent naturel et son mécanisme d'action ne sont pas encore connus.

D. Grobbee a passé en revue plusieurs études en prenant en compte plusieurs facteurs de risque tels que lipides sériques, tension artérielle, facteurs hémostatiques, rythme cardiaque. En général, les données mettant en relation la consommation de café et ces facteurs de risque n'étaient pas concluantes, à l'exception des lipides sériques. Mais la consommation de café filtré n'a aucune incidence sur les lipides sériques.

Une étude de A. Peters a montré que les modes de préparation variés des boissons de café entraînaient des différences dans leur composition. Filtration goutte à goutte, moka (style italien), percolation, cafetière, café instantané ont été comparés quant à la durée de préparation, le rapport café/eau, la dimension des particules, la teneur en caféine et l'acidité. Comme on s'y attendait, on a pu observer de grandes variations pour certains des paramètres choisis.

A. Sivak a exploité des études épidémiologiques relatives au cancer par rapport à la consommation de café. Les données relatives à quatre organes : sein, colon, ovaire et pancréas, ont été passées en revue et les ordres de grandeur du risque relatif spécifique ont été présentés. Selon de multiples études, il n'y a pas d'accroissement du risque de cancer pour le sein et le colon. En raison d'importantes variations, il n'a pas été trouvé d'augmentation cohérente des risques relatifs pour l'ovaire et le pancréas.

M. Lachs a utilisé des études de contrôle de cas analysant le risque de cancer de la vessie chez les consommateurs de café. Parmi trente-deux études, seules huit répondaient aux critères de qualité selon trois chercheurs indépendants. Cette exploitation n'a pas fait apparaître de risque accru de développement de cancer de la vessie chez l'homme ou la femme.

En utilisant l'abajoue du hamster pour le développement de tumeurs orales induites par voie chimique, E. G. Miller a observé une inhibition de la formation de tumeur lorsque du café vert ou du caféstol et du kahwéol extraits du café vert étaient introduits dans l'alimentation. Les résultats indiquent qu'à côté de ces deux diterpènes, d'autres agents chimiopréventifs non identifiés doivent être présents dans les grains de café vert.

La formation de N-nitrosamines, N-nitrosomorpholine *in vitro* et N-nitrosoproline *in vivo,* chez l'homme a été étudiée par C. D. Leaf pour plusieurs concentrations de café soluble décaféiné commercial. Les résultats indiquent clairement que le café n'accroît pas et même, aux concentrations élevées, pourrait potentiellement abaisser la nitrosation gastrique chez l'homme.

Ch. Schlatter a procédé à une revue critique des données analytiques en matière d'aflatoxines et d'ochratoxine A dans les graines de café et les a confrontées au risque de développement d'un cancer au cours d'une vie $1:10^6$. L'exposition à ces mycotoxines par le café semble faible par rapport à celle due à d'autres denrées alimentaires. La torréfaction détruit partiellement l'ochratoxine résiduelle. Le transfert éventuel à partir des grains contaminés vers la boisson a été peu étudié.

A. Leviton a passé en revue vingt-six rapports sur une liaison éventuelle entre consommation de café et risques pour la reproduction chez la femme tels que faible poids à la naissance, accouchement prématuré, avortement spontané et anomalies congénitales. La problématique des erreurs de sélection, des confusions et de l'estimation

de l'exposition est illustrée. Il semble que la consommation de café est plutôt un marqueur d'autres expositions et que le lien entre café/consommation de caféine et effets sur la reproduction diminue quand la qualité de l'étude satisfait aux critères essentiels.

Dr H. P. Würzner
Conseiller de l'ASIC
(traduction)

Chimie

Dans son exposé magistral sur la chimie de la flaveur du café, T. Shibamoto a montré que l'organe olfactif humain avait un pouvoir de perception trois à six fois plus élevé que le meilleur détecteur par chromatographie en phase gazeuse. En vue de mieux appréhender les différences organoleptiques entre les cafés, les chimistes, à l'avenir, devraient accorder plus d'importance à la détermination des voies chimiques de l'arôme en identifiant les précurseurs individuels des notes clés de la flaveur. Des exemples sur la formation des composés hétérocycliques ont été fournis.

J. Blank a présenté la nouvelle technique d'analyse par dilution de la flaveur utilisée pour identifier les composés marquants de l'arôme dans le café. Il a insisté sur les composés volatils caractéristiques prédominants des cafés Arabica.

W. Holscher et H. Steinhart ont également appliqué la technique de dilution. Ils ont décrit l'isolement et l'identification de nouveaux composés soufrés à pouvoir aromatique élevé, présentant des séries d'odeur de l'ordre de la partie par quadrillion.

Tentant d'imiter le système olfactif humain avec les neurones primaires et secondaires, T. Aishima a utilisé six différents capteurs de gaz semi-conducteurs en SnO pour analyser l'espace de tête de l'arôme du café. Les différences de résistance par rapport aux capteurs de référence ont été enregistrées et informatisées pour l'analyse modèle d'identification. Il a pu clairement distinguer entre différentes variétés de grains et différents degrés de torréfaction.

C. Macku et T. Shibamoto ont étudié les propriétés antioxydantes des matières volatiles du café. Les études préliminaires indiquent que les composés actifs sont des mono et dialcylpyrroles.

Au cours de leur travail sur la stabilité de l'arôme des grains de café fraîchement torréfiés, H. Steinhart et W. Holscher ont caractérisé un groupe de composés sulfurés et carbonylés à bas point d'ébullition qui pourraient être utilisés comme indicateurs. L'analyse des différences permettrait de distinguer clairement les grains d'Arabica et de Robusta fraîchement torréfiés de ceux vieux de dix jours.

Un modèle empirique pour une meilleure compréhension des réactions qui donnent au café torréfié moulu le caractère « éventé » a été proposé par D. C. Hinman. Un moyen de calcul de l'action des différents types de traitement et de conditionnement sur la durée de conservation du produit a été discuté.

J. McCarthy *et al.* ont présenté une enquête relative aux résidus de pesticides organochlorés et organophosphorés dans des boissons de café. Aucun résidu n'a été détecté. Des échantillons surchargés, torréfiés et préparés en boisson ont présenté des pertes supérieures à 88 %. Ce travail montre que l'exposition des consommateurs aux pesticides recherchés est négligeable.

C. Brera *et al.* ont étudié le taux de persistance de contaminants spécifiques dans le café. Des échantillons surchargés en aflatoxine B1 ont montré une destruction de 97 % à 100 % après torréfaction et préparation de la boisson.

M. Quijano-Rico *et al.* ont concentré leur travail sur une compréhension meilleure et plus nuancée des effets de divers ions métalliques au cours des transformations biochimiques dans le caféier.

La contamination du café soluble par des pellicules présentes dans le café torréfié peut être détectée en déterminant les teneurs en xylose, mannitol et sucres libres selon R. M. Noyes.

J. Prodolliet *et al.* ont fait part de leurs expériences avec la chromatographie d'échange d'anions haute performance et détection ampérométrique pulsée pour l'analyse des glucides des aliments. La meilleure séparation des mono et disaccharides du café soluble peut être obtenue avec l'eau prise comme éluant.

B. M. Scholz-Böttcher et H. G. Maier ont présenté un travail sur de nouveaux isomères de l'acide quinique présents dans le café. Le degré d'isomérisation a été proposé comme nouvelle méthode de détermination du degré de torréfaction du café.

M. N. Clifford *et al.* ont présenté leur important travail sur les tannins de la pulpe de café. Des méthodes d'isolement des tannins à l'échelle préparative ont été données ; elles peuvent aboutir à l'avenir à des techniques de caractérisation plus précises.

K. Speer *et al.* ont suggéré que le 16-O-méthylcafestol pouvait être un indicateur de la présence de Robusta dans des mélanges de cafés torréfiés. Ce composé peut également être détecté dans les cafés solubles.

H. K. Cammenga *et al.* ont présenté un rapport sur le comportement physico-chimique des méthylxanthines, caféine, théophylline et théobromine, en solutions aqueuses.

G. R. Waller *et al.* ont identifié un triterpénoïde, l'acide ursolique, dans les feuilles de *Coffea arabica ;* ce composé présenterait certaines actions antitumorales. Dans un second exposé, G. R. Waller, H. Ashihara et T. Suzuki ont présenté leurs recherches sur le devenir des méthylxanthines dans le caféier et le théier.

J. Jentsch a fait état d'études relatives aux effets de l'acide férulique, de l'acide chlorogénique et de la caféine sur la pepsine.

C. A. Barboza et J. R. Ramirez-Martinez ont réussi l'analyse de la couleur rouge de la pulpe de café. Les anthocyanes identifiées sont la 3-cyanidine-glycoside et la 3-cyanidine-arabinoglycoside.

J. B. Rothfos et S. H. Oestreich-Janzen au cours de leur revue détaillée relative aux mesures de couleur ont montré que les mesures de « transflectance » sur la boisson de café doivent faciliter un meilleur contrôle du procédé. Le système Hunter Lab est proposé. Les modifications de la couleur d'infusions de café stockées à différentes températures ont été citées en exemple.

Technologie

Dans son exposé magistral, constituant une revue précise et détaillée, J. T. Rotruck a insisté sur les défis,

pour l'industrie alimentaire des années 90, du traitement des déchets solides. Une politique intégrée de maîtrise des déchets est nécessaire pour traiter les résultats de la réduction des sources, les possibilités de recyclage et la teneur des produits recyclés. Les déchets solides doivent être considérés comme d'autres attributs clés des aliments tels que la sapidité, la commodité et la nutrition. Les résultats environnementaux doivent constituer une part importante des stratégies industrielles.

Au cours de son exposé impressionnant, E. Dentan a montré des images au microscope électronique de fèves défectueuses présentant des flaveurs herbeuses, terreuses, éventées et moisies.

M. Sivetz a décrit l'expérience pratique qu'il avait des torréfacteurs disponibles dans le commerce, utilisant la torréfaction automatique en lit fluidisé.

M. Petracco et G. Marega, dans une nouvelle présentation technique, ont montré la possibilité d'une meilleure compréhension de la dynamique de la mouture du café.

R. Clarke a insisté sur la nécessité d'une meilleure connaissance des paramètres physiques de l'arôme du café, par exemple : volatilité relative, coefficient de diffusion liquide, etc. Ceci permettrait une meilleure compréhension du comportement de l'arôme du café au cours de la transformation et de la conservation.

E. Stahl et E. Turek ont étudié l'utilisation des glucides des cafés moulus épuisés ; ils ont décrit la transformation des mannanes en mannitol : un additif alimentaire éventuel.

W. Heilmann a décrit le procédé Secoffex, technique de décaféination des grains de café vert par l'eau ou plus précisément un extrait liquide de café vert. Ce procédé est commercialisé en Allemagne.

D. A. Linning a présenté une installation de décaféination par le CO_2. La caféine est éliminée du CO_2 en circulation en abaissant la pression et la température.

Dr O. G. Vitzthum
Secrétaire scientifique de l'ASIC
(traduction)

Biotechnologie

La session sur les biotechnologies a permis d'apprécier les progrès réalisés dans les voies nouvelles de la multiplication végétative et des manipulations génétiques grâce à la culture *in vitro*. Les travaux plus traditionnels de sélection par les hybridations intra et interspécifiques n'ont pas pour autant été négligés, car plus directement applicables.

Cette session a débuté par deux exposés magistraux complémentaires :

Le premier, du Dr P. Dublin (IRCC/CIRAD), sur les techniques modernes de multiplication *in vitro* des caféiers Arabica sélectionnés. Après avoir rappelé les schémas d'amélioration actuellement développés, P. Dublin a situé respectivement l'impact de la micropropagation et de l'embryogenèse somatique pour la multiplication conforme des individus d'élite, en vue de la création de plantations clonales.

Le second, du Dr M. Sondahl (DNA Plant Technology), centré sur les premiers résultats obtenus par variation somaclonale. Environ 18 000 vitroplants issus de neuf cultivars Arabica ont été pris en observation au champ. Environ 10 % présentent une variation de caractéristiques pouvant avoir un intérêt agronomique et servir pour l'amélioration des variétés.

Le développement de la multiplication du matériel végétal sélectionné par l'embryogenèse somatique en milieu liquide a été présenté par A. Zamarripa (IRCC/FRANCERECO) : les conditions nécessaires à cette multiplication en masse sont maintenant bien établies pour l'Arabica.

Outre l'exposé du Dr Sondahl, plusieurs communications concernant la création de variabilité et la transformation génétique selon différentes approches ont été proposées :

— Les recherches du Dr C. A. Ocampo (LIQC, Colombie) présentées par le Dr Quijano-Rico portent sur la réussite de l'infection d'hypocotyles du cultivar Caturra par des souches sauvages d'*Agrobacterium tumefaciens*.

— La maîtrise de l'isolement et de la régénération de protoplastes de plusieurs espèces de caféiers est désormais acquise, selon le Dr J. Spiral (FRANCERECO). De plus, l'obtention de plantes transgéniques a été tentée par coculture de ces protoplastes avec *Agrobacterium tumefaciens*.

— Le Dr Barton (ESCAGENETICS) a rapporté la première expérience d'incorporation d'ADN étranger pour l'électroporation de protoplastes de caféiers.

Par ailleurs, ces recherches au niveau cellulaire ouvrent la voie à des études fines sur la physiologie et la biosynthèse chez les caféiers :

— Le Dr W. E. Goldstein (ESCAGENETICS) a exposé les potentialités de la culture de cellules végétales pour la production de métabolites secondaires variés.

— Le Dr T. W. Baumann (Université de Zurich) a étudié la compartimentation sub-cellulaire de la caféine et des acides chlorogéniques grâce à l'utilisation de protoplastes foliaires.

— Ms G. M. Nazario (Université de Californie) a proposé deux voies de biosynthèse de la caféine et de la théobromine, suivies par des précurseurs radio-actifs.

Enfin, les recherches sur l'amélioration génétique des caféiers Arabica en Amérique et Robusta en Afrique continuent à progresser par les voies traditionnelles de l'hybridation de géniteurs conservés dans les collections de matériel génétique.

— Le Dr J. J. Osorto (IICA/PROMECAFÉ) a fait le bilan du programme de sélection réalisé par PROMECAFÉ pour les pays d'Amérique centrale. Il a en particulier montré l'intérêt de trois variétés très productives, résistantes aux maladies et parfaitement adaptées aux conditions de la caféiculture centre américaine.

— L'évaluation des performances agronomiques des cultivars Arabica cultivés à Hawaï et la qualité du café produit ont été présentées sous forme d'affiches par le Dr Bittenbender et le Dr Cavaletto (Université de Hawaï).

— C. Montagnon (IRCC/CIRAD) a complété l'analyse de la diversité génétique dans les collections de *C. canephora* rassemblées en Côte d'Ivoire : des populations originales de caféiers sauvages et cultivés ont été identifiées pour intervenir en sélection.

— T. Leroy (IRCC/CIRAD) a montré l'efficacité du schéma de sélection récurrente mis en place dans les

années 80 en Côte d'Ivoire pour la production de clones haut producteurs, aux caractéristiques agronomiques améliorées. Dans une seconde communication, T. Leroy a indiqué les progrès réalisés pour une sélection sur la qualité à la tasse des souches de Robusta.

— A. Yapo (IDEFOR/IRCC) a présenté les résultats de la sélection dans la deuxième génération de croisements interspécifiques de *C. canephora* par *C. liberica.*

Enfin, P. Charmetant (IRCC/CIRAD) a établi une synthèse des expérimentations réalisées en Côte d'Ivoire pour améliorer la fertilité de l'hybride Arabusta F1. La qualité du café produit et l'intérêt génétique de cet hybride interspécifique sont certains, même si son utilisation en plantation n'est pas actuellement envisagée.

Phytopathologie

En phytopathologie, nous ne pouvons que regretter l'absence de nos amis portugais, dont on sait qu'ils ont apporté une contribution extrêmement importante à la connaissance de la rouille orangée et qui s'engagent maintenant dans l'étude de l'anthracnose des baies, ou CBD, due à *Colletotrichum coffeanum* Noack *sensu* Hindorf.

Cette maladie, très importante dans les zones de culture de l'Arabica de haute altitude en Afrique, mais qui pourrait faire des ravages considérables sur le continent américain si elle y était introduite, ce qui explique l'inquiétude des producteurs de ce continent, a fait l'objet de deux communications :

— D. Berry a montré qu'au Cameroun la lutte chimique contre cette maladie était nécessaire et qu'il convenait d'améliorer nos connaissances sur l'épidémiologie. Le principal intérêt de cette communication réside cependant dans la méthodologie employée pour l'expérimentation destinée à la comparaison de l'efficacité des fongicides en champ : la randomisation totale arbre par arbre et le repérage individuel des baies malades afin de ne pas confondre les pertes dues à cette maladie avec les pertes de fruits d'origine physiologique.

— La communication du Dr G. Moreno, de CENICAFÉ, montre que la Colombie, qui a su, avec la variété Colombia, se prémunir contre la rouille par la voie génétique, se prépare à associer la résistance à l'*Hemileia* à la résistance contre le CBD, avec déjà des résultats très prometteurs, par l'utilisation de l'Hybride de Timor.

Entomologie

Les études sur les déprédateurs du caféier ont fait l'objet de communications qui font ressortir la diversité des problèmes posés à la caféiculture.

Les travaux présentés font état de recherches portant sur l'action des odeurs dans le comportement des insectes, sur l'utilisation d'organismes entomopathogènes, ainsi que sur la possibilité de sélectionner des caféiers peu sensibles aux attaques des déprédateurs. On peut regretter que W. Kafka (du Max-Plank Institut für Verhaltensphysiologie) n'ait pu présenter son travail fondamental sur le rôle des cellules réceptrices d'odeurs, dans l'orientation et le vol des insectes.

Mais L. Brun (ORSTOM, Nelle Calédonie), démontrant ces propriétés olfactives, a présenté une affiche attestant que le scolyte des baies est attiré préférentiellement par l'odeur des cerises rouges. Il a mis en évidence des différences dans la teneur en substances volatiles entre les cerises rouges et les cerises vertes.

Les chercheurs de Colombie, Mlle E. Morales (du LIQC à Bogotá) et A. Bustillo de CENICAFÉ, envisagent le contrôle des populations de scolytes des baies par l'utilisation d'un champignon entomopathogène, *Beauveria bassiana,* dont l'efficacité au champ a été démontrée.

La multiplication, à l'échelle semi-industrielle, de ce champignon a été entreprise et l'intérêt économique de son application en caféière colombienne a été évalué.

A. Garcia (ANACAFÉ/IRCC) et L. Villain (IRCC/PROMECAFÉ/ANACAFÉ) ont présenté des travaux portant sur le complexe parasitaire des racines : cochenilles et nématodes. Ont été évaluées leurs caractéristiques biotiques ainsi que leur écologie, dont la connaissance précise permettra de proposer rapidement un mode de lutte efficace. Parmi ces modes de contrôle, il faut citer la voie génétique de la résistance variétale, dont nous a entretenu F. Anzueto (ANACAFÉ/IRCC), qui a mis en évidence des différences très importantes de sensibilité aux nématodes, notamment parmi les caféiers d'origine éthiopienne, qui ont été réunis grâce à la prospection effectuée en Ethiopie par l'ORSTOM et l'IRCC en 1966, ce qui souligne la richesse génétique de ce matériel dans lequel on a également trouvé des résistances incomplètes à la rouille et à l'anthracnose des baies.

Agronomie

En Agronomie, les deux communications de C. Lambot et D. Snoeck, qui travaillent à l'Institut des Sciences Agronomiques du Burundi, ont mis en relief la nécessité d'un choix judicieux du schéma expérimental dans les essais agronomiques, qu'ils visent la comparaison variétale ou les recherches sur la fertilisation minérale.

Nous devons souligner l'intérêt de la communication de Ph. Vaast de l'IRCC, qui a relaté ses premiers travaux, conduits à l'Université de Davis aux Etats-Unis, sur les mycorhizes du caféier. Cette communication ouvre en effet des perspectives nouvelles, du fait qu'elle souligne le rôle positif de ces organismes, qui permettent une meilleure utilisation des ressources minérales mises à la disposition de la plante et l'exploitation de l'azote atmosphérique, toutes choses qui sont de nature à réduire les coûts d'exploitation de la caféiculture et à en augmenter la productivité.

Relatant les travaux conduits conjointement au Mexique par deux départements du CIRAD, l'IRCC et le département Systèmes Agraires, B. Sallée, de l'IRCC, a fait ressortir, à travers l'étude des comptes d'exploitation des petits producteurs, les difficultés que ces paysans rencontrent pour améliorer leurs méthodes de culture et moderniser leurs plantations.

Cette approche scientifique en vue de mieux connaître le monde rural, et qui a aussi pour but de former les paysans pour les rendre plus aptes à faire des choix techniques judicieux, a montré en outre qu'ils étaient

capables d'adhérer à des projets de modernisation, en particulier en ce qui concerne la fertilisation minérale, fondée sur l'application en grandeur réelle du diagnostic sol, qui conduit à l'adoption de formules d'engrais rationnelles, adaptées aux divers types de sols de la région, ce qui s'oppose aux habitudes du pays où jusqu'ici une seule formulation, inadaptée à la plupart des cas, était offerte. La connaissance du monde rural et de ses contraintes, en vue de mieux adapter les propositions techniques capables de conduire à une modernisation des exploitations, est aussi la préoccupation majeure de PROMECAFÉ et de ses partenaires nationaux dans les travaux conduits dans le cadre des groupes d'amitié et de travail au Guatemala. La communication de Juan Carlos Mendes sur ce sujet permet, comme celles de B. Sallée et R. G. Pasquis, de rappeler, s'il en était besoin, que les travaux des chercheurs n'ont de sens que si le transfert des résultats de la recherche dans le monde paysan peut être fait dans de bonnes conditions. Ce transfert ne sera possible que si on dispose d'une formule d'intégration des paysans eux-mêmes aux opérations de diffusion des techniques de culture améliorées, en les groupant en communautés dynamiques.

Après un préambule sur la nécessité d'améliorer la qualité des cafés, J.-C. Vincent cite l'exemple du Burundi, petit pays d'altitude enclavé dans le centre de l'Afrique, producteur d'Arabica.

Il décrit les conditions antérieures très mauvaises de préparation des récoltes nécessitant impérieusement une révision complète du processus de préparation. Celle-ci fut confiée à l'IRCC. J.-C. Vincent décrit les points sur lesquels il a dû intervenir : dépulpage, tri des récoltes, déparchage, etc. Cette amélioration permit une revalorisation importante des cafés et, par ricochet, eut une incidence sur la formation des techniciens (électriciens, mécaniciens, etc.).

Enfin G. Upreti, de l'Université de Hawaï, a initié l'auditoire aux techniques employées pour la prévision des récoltes de café.

R. Coste adresse finalement à tous ses collègues ses vifs remerciements pour le concours de haute qualité apporté à ce XIV[e] colloque et pour les soins donnés à la présentation de leurs communications ou à leurs affiches.

Il leur fixe rendez-vous dans deux ans, en France, à Montpellier avec de nouveaux résultats dans les recherches et l'espoir d'une nouvelle avancée dans le domaine de la connaissance des caféiers et des cafés, avec pour objectif l'amélioration des conditions de production et de la qualité des récoltes.

A. Charrier, R. A. Muller *et al.*
Lecture donnée par R. Coste
Secrétaire administratif permanent de l'ASIC

Summary report

The XIVth Colloquium of the International Coffee Scientific Association (ASIC) took place from July 14th to July 19th 1991 at the Ritz Carlton hotel in San Francisco, at the invitation of the National Coffee Association of the United-States.

It brought together two hundred and fifty participants coming from more than thirty countries. The congratulations for the organization and the great success of this scientific meeting should go to Dr J. R. Coughlin, President of ASIC.

Physiology

The relationship of paraxanthine formation in caffeine animal and human metabolism was presented by M. J. Arnaud. It was shown that in man, 80 % of caffeine is demethylated to paraxanthine. Paraxanthine is excreted as an acetylated metabolite and is used in determining the acetylation phenotype in drug metabolism. Large inter individual variations in caffeine elimination and the inverse paraxanthine plasma concentrations are observed.

Four different approaches to investigate the effects of caffeine on performance were reviewed by K. Bättig. In classical multitask experiments, caffeine improved effects of fatigue especially in long lasting and monotonous tasks. Reaction time was consistently shortened and antagonism to sleepiness has been shown to be the most reliable consequence of caffeine exposure. Effects on arousal and memory are inconsistent or absent. New directions of research in this area, such as reward mechanisms, correlations between sensory and behavioral effects were indicated.

Meta-analysis of twenty-four cross-sectional studies by D. Thelle revealed that filter coffee was less likely to raise cholesterol plasma levels in comparison to unfiltered (boiled) coffee in coffee drinkers. Cross over designed experiments confirmed this effect and further investigations showed that the cholesterol raising agent(s) are contained in the coffee lipid fraction. The mechanism and the nature of this natural agent is however at present unknown.

D. Grobbee reviewed several studies taking into account several risk factors such as serum lipids, blood pressure, hemostatic factors, heart rate. In general, the data linking coffee drinking to these risk factors were inconclusive with the exception of serum lipids. But drinking filter coffee has no effect on serum lipids.

In his study, « Brewing makes the difference », A. Peters shows that coffee brewing techniques result in differences of coffee brew composition. Drip filter, Mocha (Italian Style), percolator, cafetière, instant coffee were compared in relation to brewing time, coffee-water ratio, particle size, caffeine content and acidity. As expected, large variation in some of the determined parameters were encountered.

A. Sivak reviewed the epidemiological data on four organ sites : breast, colon, ovary and pancreas and presented the specific relative risk ranges. Breast and colon showed no increased cancer risk in multiple studies. For ovary and pancreas, due to large variations, no consistent increases of relative risks were found.

In his paper : « A Metanalysis of Case Control Studies Investigating the Risk of Bladder Cancer in Coffee Drinkers », M. Lachs states that eight out of thirty-two studies were found to meet the quality criteria by three independant researchers. No increased risk for the development of bladder cancer in men or women was detected in this metanalysis.

Using the hamster cheek pouch for chemical induced oral tumor development, E. G. Miller noticed an inhibition of tumor formation when green coffee beans, or cafestol and kahweol extracted from coffee beans were administered in the diet. The data indicate that besides these two diterpenes, other unidentifiable chemopreventive agents must be present in green coffee beans.

N-Nitrosomorpholine *in vitro* and N-Nitrosoproline formation *in vivo* were investigated by C. D. Leaf using several concentrations of commercial decaffeinated instant coffee. The results indicate coffee does clearly not increase, and at higher concentrations potentially could decrease, gastric nitrosation in humans.

Ch. Schlatter critically reviewed analytical data on aflatoxin and ochratoxin A in coffee beans and confronted them to the lifetime risk of $1:10^6$ of cancer development. In comparison to other food items exposure to these mycotoxins from coffee appears low. Roasting destroys partially residual ochratoxin. Possible transfer from contaminated beans to the brew has not been extensively determined.

Twenty-six reports on possible relationship of coffee drinking and reproductive hazards, such as low birth weight, prematurity, spontaneous abortion and congenital abnormalities were reviewed by A. Leviton. The problematic of selection bias, confounders and exposure estimation were illustrated. It appears that coffee drinking is more a marker of other exposures and that the relation of coffee/caffeine consumption to reproductive effects decreases when the quality of the study satisfies essential criteria.

Dr H.P. Würzner
ASIC Adviser

Chemistry

In his plenary paper on the flavour chemistry of coffee, T. Shibamoto stated that the human olfactory organ still has a 3-6 orders of magnitude higher perception capability than the best gas chromatographic detector. In order to understand the sensory differences between coffees, chemists in the future should put more emphasis on determining the chemical pathways of the aroma by identifying the individual precursors of key flavour notes. Examples for the formation of heterocyclic compounds were given.

The new technique of flavour dilution analysis used to identify aroma impact compounds in coffee was presented by J. Blank. He stressed the predominant characteristic volatiles in Arabica coffees.

W. Holscher and H. Steinhart also used the dilution technique. They described the isolation and identification of new sulfur compounds with high aroma potency having odour thresholds at the parts per quadrillion level.

In trying to imitate the human olfactory system with primary and secondary neurons, T. Aishima used six different semi-conductor gas sensors made of SnO, to analyse headspace coffee aroma. The resistance differences towards reference sensors were recorded and computerised for pattern recognition analysis. He could clearly distinguish between different bean varieties and degrees of roast.

C. Macku and T. Shibamoto investigated the antioxidant properties of coffee volatiles. Preliminary results indicate the active compounds being mono- and dialkylpyrroles.

In their work on the stability of aroma from freshly roasted whole beans, H. Steinhart and W. Holscher characterised a group of low boiling sulfur and carbonyl components that could be used as indicators. By discriminant analysis freshly roasted beans and ten day old Arabicas and Robustas could be clearly distinguished.

An empirical model for better understanding the staling reactions of roast and ground coffee was proposed by D. C. Hinman. A means of calculating the effect of different processing and packaging options on the expected shelf life of the product was discussed.

J. McCarthy *et al.* reported on a NCA survey of coffee brews for organochloride and organophosphate pesticide residues, none were detected. Spiked samples, roasted and brewed, showed losses greater than 88 %. This work showed that consumer exposure to the tested pesticide is negligible.

C. Brera *et al.* studied the survival rate of specific contaminants in coffee. Samples spiked with aflatoxin B1 showed a 97 % to 100 % destruction after roasting and brewing.

M. Quijano-Rico *et al.* focused their work on a better and more complex understanding of the effects of various metal ions on biochemical processes in the coffee plant.

Contamination of soluble coffee by husks, present in the processed roast coffee, can be detected by measuring the levels of xylose, mannitol and free sugars according to R. M. Noyes.

J. Prodolliet *et al.* reported on their experience with high performance anion-exchange chromatography using pulsed amperometric detection for the analysis of carbohydrates in foods. The best separation of mono- and disaccharides in soluble coffee could be achieved with a pure water eluent.

Work on new quinic acid and quinide isomers present in coffee was reported by B. M. Scholz-Böttcher and H.-G. Maier. The degree of isomerisation was proposed as a new method for determining the roast degree of coffee.

M. N. Clifford *et al.* reported on their comprehensive work on tannins in the coffee pulp. Methods for the isolation of the tannins on a preparative scale were given ; they may result in more precise characterisation techniques in the future.

16-O-Methylcafestol was suggested by K. Speer *et al.* as an indicator for Robustas present in roast coffee blends. This compound could also be detected in soluble coffees.

H. K. Cammenga *et al.* reported on the physicochemical behaviour of the methylxanthines caffeine, theophylline and theobromine in aqueous solutions.

G. R. Waller *et al.* reported on the identification of the triterpenoid ursolic acid in coffee Arabica leaves ; this

compound is claimed to have certain antitumor activities. In a second paper G. R. Waller, H. Ashihara and T. Suzuki reported on investigations of the pathways of methylxanthine compounds in coffee and tea plants.

J. Jentsch reported on studies, examining the effects of ferulic acid, chlorogenic acid and caffeine on pepsin.

C. A. Barboza and J. R. Ramirez-Martinez succeeded in the analysis of the red colour of the coffee pulp. The anthocyanins were identified as 3-cyanidin glucoside and 3-cyanidin arabinoglucoside.

« Transflectance » measurements on the coffee brew will facilitate a better process control, J. B. Rothfos and S. H. Oestreich-Janzen reported in their detailed overview on colour measurements. The Hunter Lab system is proposed. Colour changes of coffee infusions stored at different temperatures were used as examples.

Technology

In a precise and detailed overview, J. T. Rotruck in his plenary paper stressed the challenges for the food industry in the nineties in the handling of solid waste. An integrated waste management policy is necessary to handle the issues of source reduction, recyclability and recycle content. Solid waste will have to be considered along with other key food attributes like taste, convenience and nutrition. Environmental issues must be an important part of business strategies.

E. Dentan showed in her impressive paper, photomicrographs of defective coffee beans with grassy, earthy, musty and mouldy flavours.

M. Sivetz described the practical experience with commercially available roasters using automated fluid bed roasting.

By a novel presentation technique, M. Petracco and G. Marega demonstrated a new approach for the better understanding of coffee grinding dynamics.

R. Clarke stressed the need for a better knowledge of the physical parameters of coffee aroma, e.g. relative volatility, liquid diffusion coefficient, etc. This will give a better understanding of the coffee aroma behaviour during processing and shelf life.

H. Stahl and E. Turek investigated the utilisation of carbohydrates from spent coffee grounds ; they described the conversion of mannans to mannitol, a speciality chemical.

W. Heilmann described the Secoffex process, a technique for decaffeinating green coffee beans with water or more precisely a green coffee liquid extract. The process is being commercialised in Germany.

D. A. Linning described a decaffeination plant based on the CO_2 decaffeination process. Caffeine is removed from the circulating CO_2 by lowering the process pressure and temperature.

Dr O. G. Vitzthum
Scientific secretary of ASIC

Biotechnology

The biotechnology session enabled us to appreciate the progress achieved in vegetative propagation and genetic manipulation as a result of *in vitro* culture. The more traditional breeding methods based on intra and interspecific hybridisation were not neglected, however, being of more direct application.

The session began with two complementary addresses :

The first was by Dr P. Dublin (IRCC/CIRAD) and dealt with modern *in vitro* techniques for the propagation of selected Arabica coffee plants. After describing the improvement programmes currently under development, P. Dublin went on to specify the impact of micropropagation and somatic embryogenesis on the multiplication of elite individuals for the creation of clonal plantations.

The second address, by Dr M. Sondahl (DNA Plant Technology) was focused on initial results obtained by somaclonal variation. About 18 000 vitroplants produced from nine Arabica cultivars were kept under field observation. Approximately 10 % exhibited character variation that could be of agronomic interest and of use in varietal improvement.

The propagation of selected plant material by somatic embryogenesis in liquid medium was presented by A. Zamarripa (IRCC/FRANCERECO) : the conditions required for the mass propagation of Arabica have now been determined.

In addition to the paper by Dr Sondahl, several other communications were concerned with the creation of variability and different approaches to genetic transformation :

— The research by Dr C. A. Ocampo (LIQC, Colombia), on the successful infection of Caturra cultivar hypocotyls with wild strains of *Agrobacterium tumefaciens* was presented by Dr Quijano-Rico.

— The isolation and regeneration of protoplasts from several coffee species has now been mastered according to Dr J. Spiral (FRANCERECO). An attempt has been made to produce transgenic plants by culturing such protoplasts with *Agrobacterium tumefaciens*.

— Dr Barton (ESCAGENETICS) reported his first experiment on the incorporation of foreign DNA for the electroporation of coffee protoplasts.

This research at the cellular level gave way to finer studies of coffee physiology and biosynthesis :

— Dr W. E. Goldstein (ESCAGENETICS) described the potential of plant cell culture for the production of various secondary metabolites.

— Dr T. W. Baumann (Zurich University) used foliar protoplasts to examine the subcellular compartmentation of caffeine and chlorogenic acids.

— Ms G. M. Nazario (California University) proposed two routes of caffeine and theobromine biosynthesis based on the use of radioactive precursors.

Finally, research into the genetic improvement of Arabica coffee in America and Robusta coffee in Africa continues to progress by the traditional hybridization of parent material from the germplasm collections :

— Dr J. J. Osorto (IICA/PROMECAFÉ) summarized the breeding programme being carried out by PROMECAFÉ in Central American countries. He demonstrated the particular interest of three highly productive varieties, that are disease-resistant and perfectly adapted to the coffee-growing conditions of central America.

— Evaluations, in the form of posters, of the agronomic performance of Arabica cultivars grown in Hawaii

and the quality of the resulting coffee were presented by Drs Bittenbender and Cavaletto (University of Hawaii).

— C. Montagnon (IRCC/CIRAD) has now completed his analysis of the genetic diversity of the Côte d'Ivoire collections of *C. canephora* and new populations of wild and cultivated coffees have been identified for breeding purposes.

— T. Leroy (IRCC/CIRAD) demonstrated the efficacy of the recurrent selection programme set up during the 1980's on the Côte d'Ivoire for the production of high-yielding clones with improved agronomic characteristics. In a second paper T. Leroy indicated the progress achieved in selecting for drinking quality in Robusta strains.

A. Yapo (IDEFOR/IRCC) described the results of selection within a second generation of interspecific crosses between *C. canephora* and *C. liberica*.

Finally, P. Charmetant (IRCC/CIRAD) reviewed the experimental work being carried out on the Côte d'Ivoire to improve the fertility of the Arabusta F1 hybrid. The quality of the coffee and the genetic interest of this interspecific hybrid are certain even though its plantation is not currently envisaged.

Phytopathology

On account of their important and widely acknowledged contribution to research on orange rust and their current investigations on berry anthracnosis or CBD due to *Colletotrichum coffeanum* Noack *sensu* Hindorf, the absence of our Portuguese friends was greatly regretted.

CBD is of considerable importance in Africa in the high altitude Arabica production zones, and could cause considerable damage on the American continent if introduced. It was the subject of two papers :

— D. Berry showed the need for chemical control of this disease in Cameroon and indicated that our understanding of disease epidemiology must be improved. The principal interest of this paper concerned the procedure used in field experiments for the comparison of fungicide efficacy : total tree by tree randomisation and the identification of individual diseased berries so as not to confuse losses resulting from the disease with fruit losses of physiological origin.

— The paper by Dr G. Moreno, of CENICAFÉ, described how Colombia protects itself genetically against rust attack by planting the variety Colombia, and is now preparing to combine *Hemileia* resistance with resistance to CBD, with already promising results, by using the Timor Hybrid.

Entomology

Studies of coffee depredators were described in several papers which brought out the diversity of the problems confronting coffee culture.

Research concerning the effect of odour on insect behaviour, the use of entomopathogenic microorganisms, as well as the possibility of selecting for coffee plants with reduced sensitivity to pest attack was reviewed. It was regretted that W. Kafka (of the Max-Plank Institut für Verhaltensphysiologie) was unable to present his fundamental research into the role of odour receptor cells in insect orientation and flight.

L. Brun (ORSTOM, New Caledonia) demonstrated such olfactory properties, however, using a poster to show how the coffee berry borer is preferentially attracted by the smell of the red cherries. Differences between the red and green cherries were demonstrated with regard to the content of volatile substances.

The Colombian research workers, Miss E. Morales (LIQC at Bogotá) and A. Bustillo of CENICAFÉ, envisage the use of an entomopathogenic fungus, *Beauveria bassiana*, the field efficacy of which has been demonstrated, to control berry borer populations.

This fungus has already been multiplied on a semi-industrial scale and the economic interest of its application in Colombian coffee production has been assessed.

A. Garcia (ANACAFÉ/IRCC) and L. Villain (IRCC/PROMECAFÉ/ANACAFÉ) presented their work on the root parasite complex that consists of mealy bugs and nematodes. Biotic and ecological characteristics were investigated, and a precise evaluation should enable an effective method of control to be proposed rapidly. Amongst such control methods the genetic route, involving the varietal resistance described by F. Anzueto (ANACAFÉ/IRCC), who demonstrated the existence of considerable differences in sensitivity to nematodes, should be cited. This is especially true of the Ethiopian coffees which have been assembled as a result of the prospection carried out by ORSTOM and IRCC in Ethiopia during 1966. The considerable genetic resources of this material, which also include incomplete resistance to rust and to CBD, are stressed.

Agronomy

In the Agronomy section, the two papers by C. Lambot and D. Snoeck, who work at the Burundi Institute of Agronomic Sciences emphasized the need for a suitable experimental design in agronomy trials whether for varietal comparison or research into mineral fertilization.

The great interest of the communication by Ph. Vaast of IRCC describing the author's preliminary investigations at the University of Davis, in the United States, on coffee mycorrhizae should be underlined. This communication opens up new horizons by emphasizing the positive role of such organisms in permitting better utilization of the mineral resources available to the plant and of the atmospheric nitrogen, all of which should reduce coffee production costs and increase productivity.

Describing the joint investigations being carried out in Mexico by two CIRAD departments, i.e. the IRCC and Agrarian Systems, B. Sallée of the IRCC showed how a study of management costs on small plantations, has brought to notice the difficulties encountered by the farmers in improving farming methods and modernizing their plantations.

The aim of this scientific approach was to provide a better understanding of the rural environment, and also to train farmers to make wiser technical decisions. It revealed that the farmers were able to stick to modernization projects, particularly mineral fertilization sche-

mes. These were based on the application of soil diagnosis to real-life situations, leading to the adoption of rational fertilizer formulae suitably adapted to the different types of soils in the region, in contrast to past local custom in which a single formulation, poorly adapted to the majority of cases, had been offered. An understanding of the rural environment and its constraints, with a view to better adapting technical propositions to culminate in farm modernisation is also a major preoccupation of PROMECAFÉ and its national partners in work being carried out through friendship groups and workgroups in Guatemala. The paper by J. C. Mendes on this subject, like the ones by B. Sallée and R. G. Pasquis, remind us, if this is still necessary, that agronomy research is only worthwhile if the transfer of the results to the farming world can be made under good conditions. This transfer will only be possible if a means of involving the actual farmers in the diffusion of improved cultural techniques is developed, by grouping them in dynamic communities.

After mentioning the need to improve coffee quality, J.-C. Vincent cited the example of Burundi, a small high altitude country, enclaved in central Africa and producer of Arabica coffee.

A description was given of the former very poor conditions which had necessitated a complete revision of processing operations. This had been entrusted to the IRCC. J.-C. Vincent went on to indicate the various points at which intervention had been necessary: pulping, sorting, hulling, etc. The consequence of these improvements was a considerable revalorization of coffee production. This reform has had repercussions on the training of technicians (electricians, mechanicians, etc.).

Finally G. Upreti, of the University of Hawaii, introduced us to methods of predicting coffee yields.

R. Coste warmly thanked his colleagues for the high quality of this 14th Colloquium and for the care and consideration given to the presentation of papers and posters.

A rendez-vous was fixed in two years time at Montpellier, France, in the anticipation of new research results and further knowledge of the coffee plant and coffee drink, as well as means of improving production conditions and harvest quality.

A. Charrier, R. A. Muller *et al.*
Lecture given by R. Coste
Permanent Administrative Secretary of ASIC
(translation)

SOMACLONAL VARIATION AS A BREEDING TOOL FOR COFFEE IMPROVEMENT

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INTRODUCTION

1. Importance of Coffee

Coffee is a beverage prepared from roasted seeds (beans) of Coffea spp. after grinding. Coffee can be prepared by infusion in boiling water, or percolating, or by hot water under pressure (espresso). The history of coffee begins on the 13th Century when seeds (Coffea arabica) were taken from SW Ethiopia to Yemen which was the first country to establish commercial coffee plantations (Carvalho 1988). Today, coffee beverage is prepared from two species C. arabica and C. canephora (Robusta) which account for 75% and 25% of total consumption, respectively. Arabica trees produce an aromatic beverage that is more appreciated in the world market. Coffee consumption during the period of 1985-90 averaged 5.5 million Mt (92 million bags) which contributed to an average trade of $12 billion dollars a year in the commodity market (Filgueiras 1991).

2. Coffee Breeding and Genetics

Coffee improvement through breeding methods is difficult in both cultivated species. Arabica is the only tetraploid species in the genus and so it is isolated from other sources of natural variability. Robusta has self-incompatible genes which hold this species in a highly heterozygous state. Nonetheless, many commercial cultivars have been released through breeding which have provided yield increases and new sources of disease/pest resistance.

Genetic analysis in C. arabica has identified ca. 40 mutants. Almost all of these mutants are controlled by single genes with expression of dominance, recessiveness, or partial-dominance in relation to the alleles in the 'Arabica' variety (Carvalho 1958; Carvalho et al. 1969). The appearance of mutants with monogenic inheritance reflects the partially diploidized allotetraploid nature of arabica.

3. Somatic Cell Genetics

With the advancement of in vitro cell culture, many techniques have been developed to assist plant breeding: micropropagation, embryo rescue, haploid production, cell selection, somaclonal variation, somatic hybridization, and transformation. Somaclone variation is derived from pre-existing and in vitro variability of somatic cells and it can be attributed to chromosome alterations (polyploidy, aneuploidy, deletions, inversions), gene amplification, mitotic crossing-over, and point mutation (Evans & Sharp 1986; Larkin & Scowcroft 1981). Somaclonal variation has been used to recover at high frequency natural genetic variability from existing commercial varieties (Evans & Sharp 1983).

This present research program was established to evaluate the extent of variability recovered from coffee somatic cells and its potential for assisting breeding programs for C. arabica improvement.

MATERIALS AND METHODS

1. Plant Material

Different agronomically important cultivars or selections were utilized for this study:

Tall varieties: Yellow Bourbon (YB), Mundo Novo (MN), and Icatu (IC)

Short varieties: Red and Yellow Catuai (CTR and CTY), Caturra (CA), Catimor (CR), Aramosa (AR), and mixed genotypes (MG).

Donor plants were established in greenhouse conditions from seeds provided by the Dept. Genetics, Institute of Agronomy, Campinas, SP Brazil.

2. Cell Culture Techniques

Mature leaves were surface sterilized with 1.6% sodium hypochlorite for 30 min and rinsed three times in sterile deionized water. Leaf explants of ca. 7 mm^2 were cut from the leaf blade, excluding the midvein, margins, and apical and basal portions of the leaf. The leaf explants were incubated in 10 x 100 mm disposable petri dishes charged with 15 ml of the following induction medium (IM): MS inorganic salts (Murashige & Skoog 1962), nicotinic acid (15 μM), Pyridoxine (15 μM), thiamine (30 μM), myo-inositol (550 μM), L-cysteine (210 μM), sucrose (117 μM), kinetin (20 μM), 2,4-D (5 μM), and Difco agar (8 g/l). The cultures were incubated in the dark at 25 $\pm$ 2°C for 42-50 days. After this period, the tissues

were subcultured to a "conditioning medium" (CM) containing 0.5 X MS salts (except KNO_3, 2X), nicotinic acid (15 μM), Pyridoxine (15 μM), thiamine (30 μM), myo-inositol (550 μM), L-cysteine (210 μM), sucrose (58.4 mM), kinetin (2.5 μM), NAA (0.5 μM), and Difco agar (8 g/l). All cultures were kept under light (1200 lux) with a 12h day/12h night regime at 25 $\pm$ 2°C. This process led preferentially to the pathway of High Frequency Somatic Embryogenesis (HFSE; Söndahl & Sharp 1977; Söndahl et al. 1989). Using a modified conditioning medium containing a cytokinin (10-20 μM) and 2,4-D (0.1 - 0.5 μM), the Low Frequency Somatic Embryogenesis (LFSE) pathway is enhanced. More complete details of coffee tissue culture protocols has been provided by Söndahl et al. (1983).

Isolated embryos were transferred to 20 x 150 mm test tubes containing a germination medium consisting of 0.5 x MS salts, coffee vitamins (see IM), myo-inositol (550 μM), sucrose (58.4 mM), L-cysteine (210 μM), PVP-40 (10 g/l), and Difco agar (7 g/l). Culture tubes were kept under light conditions (1200 lux) under a 12h day/12h night regime at 25 $\pm$ 2°C. All culture media were adjusted to pH 5.5 before autoclaving.

Germinated embryos were transferred to an artificial soil mix containing 0.5 x MS inorganic salts. Plantlets were allowed to grow inside Magenta™ boxes under growth chamber conditions (16h light, 24-28°C) for ca. 6 weeks before transferring to special pots (45 x 200 mm nursery cones). At the time of transferring to cones, each plantlet received a code number. The origin of each plantlet (HFSE or LFSE) was maintained throughout the various developmental phases. Hardening was accomplished within 6-8 weeks under a fog bench covered with a 80% shade net and then plantlets were allowed to grow under normal greenhouse conditions.

3. Nursery and Field Plots

Plants with 6-8 pairs of leaves were shipped in the growing cones to a field nursery. After 4-8 weeks, coffee plantlets were transferred to 60 x 150 plastic bags filled with enriched soil mixture. Following 6-9 months of vegetative growth, young coffee plants were transplanted to the field. Regenerated plants of each donor variety were transplanted together. Control plants of donor varieties were placed at the beginning and at the end of each row and additional control plants were used as border rows. Several experimental fields (EC-1 through EC-18) were established according to the availability of somaclones at the nursery. The experimental fields were located in a typical coffee farm located at ca. 20° latitude south at 1,040 m altitude with min. and max. average temperatures of 20-26.4°C. The soil is a Red Latossol with 4-6% declivity. The yearly average rainfall is 1,600 mm with a predominance of rain during the months of Sept-March and minimal or no precipitation during April-August. A plant density of 3.5 X 2.0 m was adopted throughout the fields. The first fields were established in 1986 and the last planting was in November 1990. Recommended fertilization practices and five applications of copper fungicides (control for leaf rust) were made during every growing season. Weeds were controlled mechanically to avoid the use of herbicides.

4. Evaluations

Observations were taken on R_0 somaclones at the time of first harvest. Morphological characteristics were all compared with control plants from the donor genotype. All lab and field data from each individual somaclone is being stored in a customized DataBase Program. Progeny tests of the most interesting mutations (or classes of mutations) have been initiated.

RESULTS

1. Laboratory

A total of 185,000 somatic embryos were isolated from nine genotypes used for this program from which 54,000 (29%) successfully completed the maturation and germination steps. A total of 21,000 plantlets were transferred to growing cones under greenhouse conditions. Hence, a recovery rate of 39% was observed for the hardening and soil adaptation phases. The survival frequency under greenhouse conditions was 95%, which allowed the shipment of ca. 20,000 somaclones to the field nursery. The relative contribution of each coffee genotype utilized in this somaclonal program is illustratated in Figure 1.

Some genotypes had a tendency to preferentially follow the HFSE pathway while other genotypes yielded embryos equally from both pathways. The genotypes Caturra, Red Catuai, Yellow Catuai, Yellow Bourbon, and Aramosa produced most of the embryos through HFSE. On the other hand, Icatu, Catimor, and Mundo Novo yielded similar number of embryos from both HFSE and LFSE. There was a tendency of LFSE embryos to germinate better (45% germination rate) in contrast with HFSE embryos (24% germination rate). However, great variability in germination rates was observed within each genotype, for instance: Mundo Novo embryos had 93% (LFSE) and 92% (HFSE) germination as opposed to Caturra which had 36% (LFSE) and 12%

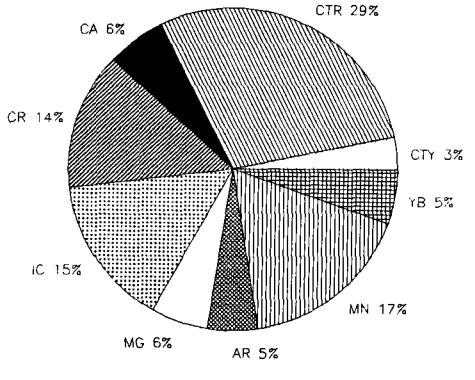

Figure 1.

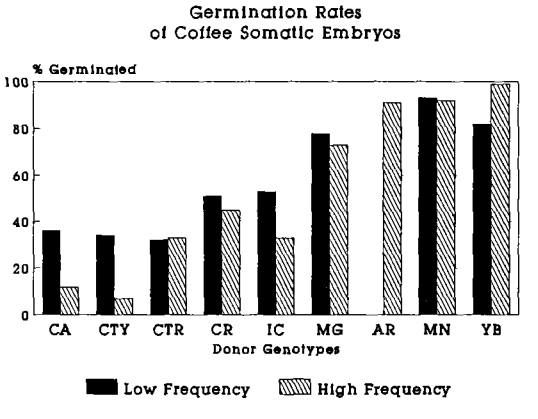

Figure 2.

(HFSE). An illustration of the germination rates observed among the genotypes studied with respect to the origin of the embryos is provided in Figure 2. The highest germination rates were observed for the varieties Yellow Bourbon, Mundo Novo, and Aramosa.

2. Somaclone Fields

A total of 16,350 somaclones and controls were established under field conditions. These were transplanted to 18 experimental fields established during the period of March 1986 to November 1990. Periodically, plantlets were shipped from the greenhouse to the field nursery and as they reached the proper vegetative growth stage they were transplanted to the field. The recovery rate due to shipment and nursery conditions was 73% and the survival in the field after transplanting was 82% (Table 1). At one instance, unusually high plant losses were recorded among young plants in fields EC-5 and EC-6 (total 1141 out of 3237) due to unusual early cold weather during the Fall of 1988. Replants were made in subsequent months.

Table 1. Accumulated data on coffee somaclones under nursery and field conditions.

Genotypes	Nursery			Field		
	Total No. Shipped	Total No. Somaclones Planted	Recovery (%)	Total No. Planted	Total No. Somaclones Lost	Recovery (%)
CA	920	879	95.54	879	26	97.04
CTY	544	493	90.63	493	16	96.75
CTR	5475	4328	79.05	4328	828	80.87
CR	3920	2061	52.58	2061	857	58.42
IC	3315	2244	67.69	2244	490	78.16
MG	1195	841	70.38	841	59	92.98
MN	3114	2554	82.02	2554	194	92.40
YB	1072	804	75.00	804	105	86.94
AR	869	744	85.62	744	63	91.53
Total	20424	14948	73.19	14948	2638	82.35

3. Variability for Agronomic Characteristics

Presently, a total of 12,176 somaclones have been evaluated in the field (78% of total; Fig. 3). The remaining somaclones will be screened for variability as they reach maturity. Any deviation from the normal phenotype of the control variety was tabulated as variation. A total of 1,196 mutated somaclones,

or a total variability frequency of ca. 10% (Table 2) was observed. The variability rate greatly differed from genotype to genotype. Low frequency of variants was found in Red Catuai (3.3%) and Aramosa (3.1%) as opposed to Yellow Bourbon (30.6%), Caturra (22%) and Yellow Catuai (22.3%). The frequency of total variation observed among the somaclones evaluated so far is illustrated in Figure 4.

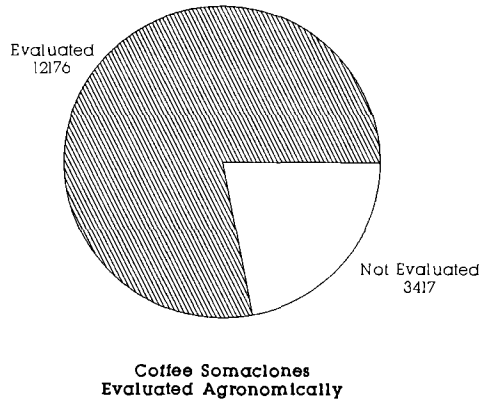

Figure 3.

Table 2. Coffee Somaclones. Number of Mutations by Genotype

Genotype	Total Mutations	Total Evaluated	% Variability
CA	193	879	22.0
CTY	99	444	22.3
CTR	140	4275	3.3
MG	172	441	39.0
CR	197	2061	9.6
IC	197	2243	8.8
MN	110	1253	8.8
AR	10	325	3.1
YB	78	255	30.6
Totals	1196	12176	9.8

Among the several classes of variability observed (Table 3), the highest frequencies were found for Red to Yellow cherry color (42.3%), and Tall to Short plant stature (3.8%). All these mutated traits are controlled by well characterized single dominant genes. This result clearly illustrates the tendency to detect changes from recessive the

Table 3. Variability of coffee somaclones: morphological and single gene traits.

Classes of Variation	Total No. Somaclones Evaluated	Total No. Mutated Somaclones	Presumed Genotype	Variation Frequency (%)
Tall to Short	3724	140	Ct/--	3.76
Short to Tall	8416	107	ct ct	1.27
Arabica to Laurina	11726	12	lr lr	0.10
Leaf Morphology	12167	160		1.32
Purpuracens	12167	32	pr pr	0.26
Cherry Morphology	12167	20		0.16
Red to Yellow	11468	134	xc xc	1.17
Yellow to Red	699	296	Xc/--	42.35
Maragogype	12167	1	Mg/--	0.01
Polyploids	12167	9	8x	0.07
Aneuploids	12167	77		0.63
Sectorial Chimera	12167	1		0.01
Plant Architecture	12167	185		1.52
Lethal (Dead)	12167	1686		13.86

to the dominant state. However, traits described as dominant or semi-dominant also changed to a recessive expression, for example: short to tall stature (1.3%), red to yellow cherry color (1.2%) and normal to purpuracen leaves (0.3%). Besides 1,141 plants eliminated due to cold weather, there has been an additional 1,686 somaclones that did not survive under field conditions. They were considered as carrying mutations detrimental for field survival (13.9% of total population). It is interesting to note the presence of one Maragogype mutation (Mg/-) from Yellow Catuai and one sectorial chimera from Catimor.

Another group of somaclones are possibly expressing physiological and/or quantitative characteristics (Table 4). Some variability has been observed for early maturing (0.05%), late maturing (0.3%), uniform maturing (0.03%), susceptibility to ants (0.2%), susceptibility to diseases (0.7%), and resistance to leaf rust (0.07%). Somaclone plants were evaluated for bean size and the majority of them were classified as sieve #16 (same as controls). However, a small group of 89 plants had a predominance (greater than 50%) of large beans of sieve #18 (1.2%) and another group of 201 somaclones had a tendency to produce small beans of sieve #14 (2.7%).

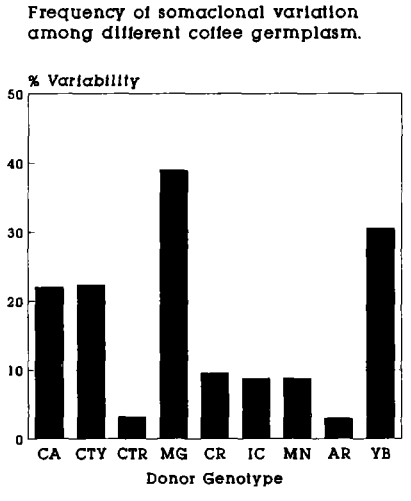

Figure 4.

Table 4. Variability of Coffee Somaclones: physiological and quantitative characters.

Classes of Variation	Total No. Somaclones Evaluated	Total No. Mutated Somaclones	Variation Frequency (%)
Early Maturing	12167	6	0.05
Late Maturing	12167	33	0.27
Uniform Maturing	12167	4	0.03
Semperflores	12167	27	0.22
Ant Susceptible	12167	28	0.23
Disease Susceptible	12167	88	0.72
Disease Resistant	12167	9	0.07
Large Beans (#18)	7504	89	1.19
Small Beans (#14)	7504	201	2.68

An assessment was made to identify if there was any tendency for larger or smaller rates of variability in relation to the original regeneration pathway. Based on 7,772 somaclones evaluated, 452 (12%) variants were derived from HFSE and 415 (10.4%) from LFSE pathway. Hence, there is no evidence to date of increased variability depending on the origin of the somatic embryos, i.e. direct vs. indirect somatic embryogenesis process.

DISCUSSION

The overall conversion rate of regenerated embryos to germinated plants was only 29%. Attempts to obtain higher conversion rates could be made by improving maturation/germination conditions or by utilizing a liquid phase in parts of the process. Somatic embryos of interior spruce accumulated more storage proteins and did not follow precocious germination when treated with 30 μM ABA (Roberts et al. 1990). However, the average frequency of root emergence in spruce was still low (25%) for a micropropagation process. It is interesting to note that somatic embryos derived from LFSE had a tendency to higher germination rates than embryos from HFSE (45% vs. 24%). Also a very strong genotypic effect was observed in germination rates of coffee somatic embryos: Mundo Novo (93% LFSE) as opposed to Caturra (36% LFSE). In somaclonal variation programs the culture conditions are optimized to increase variability in contrast to micropropagation in which the aim is to achieve the highest conversion rate and minimal variability. For coffee, is has not been detected a correlation between conversion rate and frequency of variation at the R_o plant level.

The overall rate for the hardening process of coffee plantlets was low (39%) but with improvements of the process the average recovery rate by the end of the program was 75%. The survival rates of coffee somaclones under nursery conditions and field conditions were 73% and 82%, respectively. These rates are lower than the recovery rates observed with coffee seedlings which demonstrates that the somaclones either must be handled with greater care or that some somaclones have reduced viability.

Somaclonal variation has been described in many cultivated species (sugar-cane, potato, geranium, rapeseed, celery, etc.), but still there are only a few cases in which detailed genetic analysis of the variants have been made (Evans 1988). Among seed propagated crops, the stability of selected mutant types can be established in subsequent generations (R_1 and R_2 tests). Several of the coffee somaclone mutants are currently being analyzed in the R_1 generation.

This present work with Coffee trees represents one of the first examples of somaclonal variation with a perennial species. Among the types of variation observed so far, a few have been identified that are agronomically desirable. The availability of short stature variants from high yielding tall varieties like Mundo Novo, Icatu, and Yellow Bourbon is attractive since harvesting short varieties provides a cost saving benefit to coffee farmers. Larger bean size captures higher prices in the market, but the stability of this character within a high yielding genotype needs to be demonstrated. Several individual plants displayed a high level of resistance to coffee leaf rust. Fungicide applications were provided to keep this disease under control, but low level of incidence of leaf rust was always present on the plants. The presence of a few somaclones completely free from any symptom of leaf rust throughout successive years could offer new sources of resistance. Differences in maturation cycle (early and late types) from existing high yielding materials would be beneficial for farmers (harvesting schedule) and coffee processors (improved quality).

Similar benefits could be expected from uniform maturing somaclones. However, these traits are greatly affected by environmental and nutritional conditions and so there has been no clear evidence as yet of the real benefit of these variant types for commercial use.

SUMMARY

Somaclonal variation is a cell genetics technique that permits the isolation of genetically stable mutants from commercial varieties. It explores the natural and in vitro variability of somatic cells following plant regeneration (somaclones). This variability has been attributed to chromosome alterations (breakage, translocations, deletions, polyploidy, aneuploidy), gene amplification, transposon activation, somatic crossing-over, and point mutations.

Commercial production of coffee has been greatly improved by plant breeding. Coffee is a perennial tree that requires a minimum of 20-25 years to release new varieties. Somaclonal variation is an excellent method to shorten coffee breeding programs, since it provides access to new mutant forms in high yielding genotypes within a short-period of time (4-8 years).

Somaclones of several commercial arabica varieties and a few non-commercial genotypes have been produced and are now in the fourth year of field evaluation. An overall variability of 10% has been recorded among 12,176 plants evaluated to date. Among the variants, we have observed alterations in leaf and branch morphology (leaf size and shape; angle of lateral branches), plant stature (tall to short; short to tall), cherry color (red to yellow, yellow to red), cherry size and shape (including one Maragogipe mutant), increased yield over donor control varieties, variability in maturation (early and late types), and susceptibility/tolerance to diseases (leaf rust and cercospora). Selections will continue throughout several consecutive years to permit the isolation of new stable varieties among the tall and the short stature cultivars. The use of somaclonal variation is being studied as an alternative method for coffee breeding programs.

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