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List of participants

Improving Water Productivity in Arabica Coffee (mini review)

H. A. M. VAN DER VOSSEN

Consultant Coffee Breeding and Agronomy, 1606CA, 18, Venhuizen, the Netherlands

SUMMARY

This review paper discusses the various genetic and agronomic opportunities for substantially increasing water productivity (WP) in arabica coffee, in the face of diminishing water resources due to climate change. Retention and greening of coffee leaves can be enhanced by twice-yearly "tonic" sprays of broad-spectrum fungicides. In Kenya, this resulted in yield increases of 40-85%, cumulatively over five years, for arabica coffee genotypes resistant to coffee berry and leaf rust diseases. There were highly significant varietal differences in leaf retention responses to stress conditions, independent of tonic sprays. It is evident that selection for leaf longevity together with tonic sprays of fungicide (on disease-resistant cultivars only) can significantly increase WP in arabica coffee. Other options for further enhansing WP in arabica coffee are: host resistances to important diseases and pests, the use of short-stature cultivars, F1 cultivars to exploit hybrid vigour for yield, high-density planting, disease and pest control (chemical, biological) of susceptible cultivars, adequate supply of plant nutrients (N in particular) and shade trees in areas marginal to coffee cultivation.

INTRODUCTION

One of the anticipated effects of climate change is diminishing global freshwater resources available for agricultural production. There is, therefore, an urgent need to increase productivity per unit of water – "more crop per drop" – to meet the demands for food and other agricultural commodities by an ever-increasing world population (Smith *et al*, 2001; Bennett, 2003).

The amount of yield per unit of water used is referred to as water productivity (WP). Most selection work for improved drought tolerance targets survival and reduction in water use, not improved photosynthesis under a given supply of water (Morison *et al*., 2010). Blum (2005) has also pointed out that there is usually a negative relation between yield potential and drought tolerance. Improving WP, rather than the search for drought tolerance, is now seen as the way forward (Passioura, 2004).

The 10.4 million ha of planted coffee represents a mere 0.8% of the global area of arable and perennial crops (1.3 billion ha) and some 1.5% of total annual crop water use (6.7×10^{12} m^3). Coffee crop water use is mostly green, i.e. stemming from precipitation, and less than 5% is blue water, i.e. from irrigation (Siebert and Döll, 2010). The WP for coffee is estimated at about 0.1 kg/m^3 transpired water averaged over world total production, which is rather low against the 2 kg/m^3 for wheat (Passioura, 2004). Comparing these two very different crops may be arguable, considering the large difference in harvest index (HI = rate of useful product over total plant biomass) and in value of the product. Nevertheless, there appears to be considerable room for improving WP in arabica coffee (*Coffea arabica* L.). It should contribute to arresting the downward trend in productivity and quality predicted for important arabica coffee regions in the face of climate change (Oberthür *et al.*, 2012).

This review paper discusses the various genetic and agronomic opportunities for substantially enhancing WP in arabica coffee.

THE EFFECT OF DROUGHT STRESS ON COFFEE PHYSIOLOGY

The following paragraphs are extracted from three authoritative review papers on this subject by DaMatta (2004a, 2004b, 2006).

Drought is an environmental factor that produces water deficits in plants, which is initiated when low water potential develops and cell turgor begins to fall below its maximum value. It develops slowly and increases in intensity the longer it lasts. The time factor plays a crucial role in survival and also maintenance of productivity under drought conditions.

Coffee leaves show, irrespective of water supply, a high relative water content at the turgor loss point, usually close to 90%, a phenomenon largely associated with low cell wall elasticity. The high relative water content maintained by coffee leaves under dehydrating conditions appears to be a means of avoiding rather than tolerating desiccation. The physiological mechanisms behind this are related to the strong sensitivity of coffee stomata to both soil and atmospheric water deficits.

Drought-tolerant coffee genotypes are better able to maintain leaf water potential and relative water content under conditions of water stress. Dehydration is postponed by low stomatal conductance and this would partly explain their delayed wilting and leaf shedding compared to drought-sensitive genotypes. However, productivity may suffer as a consequence of reduced rate of transpiration and photosynthesis.

In some other plant species the accumulation of osmotically active solutes, like proline and ammonium compounds, can be used as indicator of adaptation to water shortage, but in coffee no satisfactory relation has been found with drought tolerance.

Drought tolerance in coffee is a complex character and selection progress for improved drought tolerance in combination with stable yields has been low. Traits of importance to productivity under water stress in coffee are for example a vigorous and deep root system, effective stomatal control of transpiration and capacity to retain sufficient leaf area. These are important mature-plant characters, which cannot be selected in experiments with potted seedlings.

Short-stature cultivars (e.g. yellow caturra) with their dense crowns were found to have a lower (25%) transpiration rate than the tall *typica* cvs with their open crown architecture. In coffee regions with a high evaporative demand, like in N-E Brazil, short-stature cvs have been producing well, while *typica* cvs have resulted in crop failure even with supplementary irrigation.

High-density coffee planting reduces evapo-transpiration – complete ground cover leading to lower soil temperatures and less weed growth – and stimulates deeper rooting to reach water from lower soil horizons. There is less overbearing and biennial cropping as a result of mutual leaf shading, which tempers flower bud initiation. For plant densities up to 5000 tr/ha, plant nutrient demand per ha is not increasing, i.e. fertilizer-use efficiency is better than for coffee planted at conventional spacings.

Application of N-fertilizers was found to promote stomatal conductance and rate of transpiration in coffee. Adequate N-supply promotes leaf longevity and photosynthetic capacity, probably by triggering mechanisms of chloroplast protection and of qualitative membrane changes.

The main effects of shade trees are decreased wind speeds and temperature fluctuations, increased relative humidity and lowering leave-to-air vapour pressure deficits. This stimulates better stomatal conductance, thus allowing better CO_2 assimilation, without proportional increase in transpiration. The drier and hotter the area, the greater is the benefit of shading, especially in combination with short-stature cultivars. Shade trees, particularly those with deep rooting, do not seem to adversely affect the water balance of the coffee crop.

THE "STAY-GREEN" TRAIT AND IMPROVED WATER PRODUCTIVITY IN SORGHUM

Drought tolerant sorghum hybrid cultivars with the "stay-green" trait maintain green stems and upper leaves longer when water is becoming limited during grain filling. The retention of photosynthetically active leaves under post-anthesis drought does increase yield significantly in "stay-green" compared with hybrids not possessing this character. Grain yield was positively correlated ($r = 0.75^{□□}$) with green leaf area at maturity and increased by ≈ 0.35 t/ha for every day that onset of leaf senescence was delayed (Borrell *et al*., 1999, 2000a).

The stay-green trait in sorghum (and other crops) is probably a consequence of the balance between N demand by the grain and N supply translocated from the vegetative parts of the plant and from uptake of the roots during grain filling (Borrell *et al*, 2000b; Kassahun *et al*, 2010). This seems to arise from positive feedback in N-uptake. Plants that maintain N in their leaves during grain filling, and hence stay green, fix more C, which in turn enables roots to continue extracting soil nitrogen, so that the system is self-reinforcing (Borrell *et al*, 2001; Passioura, 2004).

The delayed leaf senescence allows, therefore, for longer uptake of soil water and plant nutrients, uninterrupted photosynthesis and more carbohydrates becoming available for grain filling. A similar physiological mechanism appears to operate in the increased leaf retention and productivity observed in arabica coffee effected by "tonic" sprays of broad-spectrum fungicides.

LEAF RETENTION AND PRODUCTIVITY IN ARABICA COFFEE

In the 1930s arabica coffee growers in Kenya and Tanzania had started applying twice-yearly "tonic" sprays with copper fungicides to increase production, not to control coffee leaf rust (CLR) or berry (CBD) diseases which were unimportant in most coffee growing areas at that time This caused the leaves to turn dark green, without the so-called "weak spots" common on unsprayed coffee leaves, and natural leaf abscission to be delayed by 2-3 months. As a result, yields increased spectacularly (> 50%) in subsequent years. This tonic effect was not restricted to copper fungicides, but purely organic fungicides, such as captan and captafol, produced equally large yield increases. Fungicides are assumed to reduce the saprophytic microflora on the leaf surfaces, which induce the leaves to senesce and drop prematurely, especially when the coffee trees are exposed to water stress (Rayner, 1957). That fungicides, particularly those possessing a wide spectrum of activity, are retarding leaf senescence due to a marked reduction in the phylloplane microflora, was demonstrated for captan in apple (Hislop and Cox, 1969), for zineb in barley (Dickinson, 1973), for captafol in potato

(Brainbridge and Dickinson, 1972) and for captafol and other fungicides in arabica coffee (Waller and Masaba, 2006).

However, when CLR and in particular CBD became major problems in the 1960s, intensive spray programmes with fungicides were necessary to prevent heavy crop losses, to CBD in particular. Mulinge and Griffiths (1974) concluded that the occasional tonic sprays with fungicides, usually at half the concentration required for disease control, had actually aggravated the incidence of both CBD and CLR. The tonic effect of fungicides became confounded with disease control and was no longer relevant to the coffee growers.

The development of new hybrid cultivars of arabica coffee with host resistance to CBD and CLR in Kenya in the1970s stimulated renewed interest in the tonic effect. Would such tonic sprays of fungicides also increase yields in the disease resistant cultivars, without running the risk of loosing part of the crop due to insufficient host resistance? A field experiment (B3b, 1972-1978) with a range of 14 highly resistant to susceptible genotypes, each with or without twice-yearly tonic spray applications (aqueous mixture of 0.5% copper oxychloride and 0.2% captafol) gave the following results (Van der Vossen, 1982):

- Cumulative yields over five years were 42 – 87% higher in the tonic-sprayed subplots of disease resistant genotypes, but 15% lower in the susceptible cv. SL34 due to heavy crop losses caused by CBD and CLR (Table. 1).
- The tonic-sprayed subplots of resistant genotypes remained free from CBD and CLR incidences during all the five years, while infection was dramatically increased in those of susceptible genotypes.
- Average leaf longevity was significantly higher in all tonic-sprayed subplots, and so were the two most important components of coffee yield, % bearing nodes and number of berries per node. Much of this gain in potential yield induced by tonic sprays was lost only in susceptible genotypes due to CBD and CLR infection.
- Bean size was not much different on average, but cup quality was significantly improved by tonic sprays.

Table 1. The effect of tonic sprays (T) of fungicide on cumulative yields (1974-78) in B3b.

Genotype	**Total clean coffee t/ha (1974-78)**		
	No T	**T**	***% Increase***
SL34	5.9	5.0	*-15*
P2	7.1	10.1	*42*
P3	4.5	8.4	*87*
P4	7.2	13.4	*86*

(Significant difference noT – T per genotype, at P < 0.05 = 1.5 t/ha).

There is considerable genotypic variation in leaf retention and responses to tonic sprays in arabica coffee, but the differences are small at any one time and cumulative measurements over long periods on tagged branches are required for reliable detection. A number of studies were, therefore, undertaken to develop a rapid and relatively simple test to enable effective selection for better leaf retention (Van der Vossen and Browning, 1978). Ethylene plays an important role in the control of leaf abscission and it is a common metabolic product of fungi and diseased plant tissue. In coffee, as in many other crops, leaf abscission is finally mediated

by ethylene and endogenous ethylene levels are likely to be higher in unsprayed leaves due to its production by the phylloplane microflora, as well as the injured tissue of the weak spots (Browning, 1975)

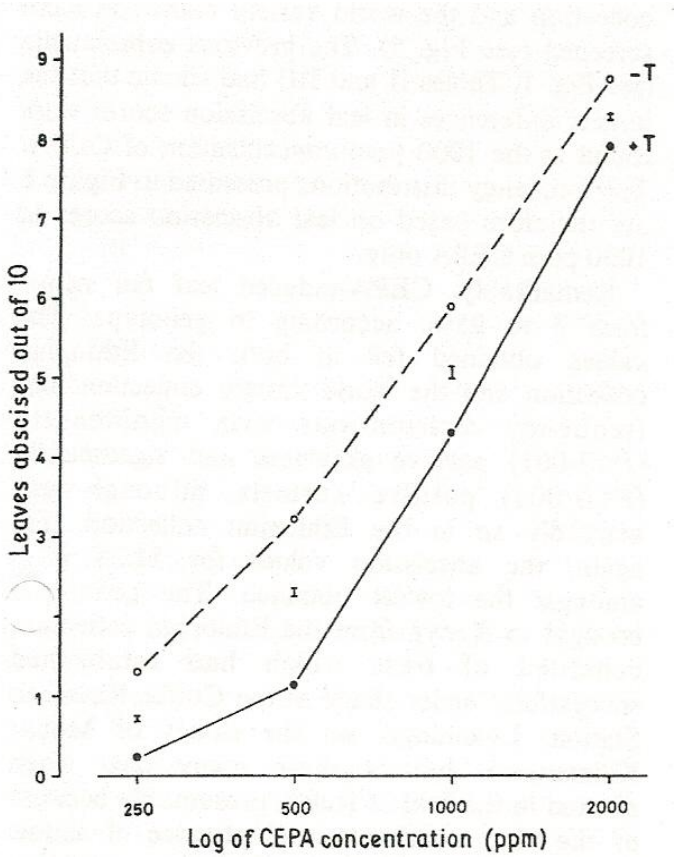

Figure 1. Leaf abscission induced by CEPA sprays on trees receiving tonic (+T) or no sprays (–T) with fungicides in trial B3b. Vertical bars are SEs of each treatment mean.

The experiments were carried out by applications of aqueous solutions of CEPA (Ethrel), which releases ethylene in vivo and readily causes leaf abscission in arabica coffee (Browning and Cannell, 1970). The number of leaves remaining on the treated branches (5 undamaged leaf pairs) were counted six days after spray applications.

Tonic-sprayed trees in field experiment B3b showed significantly lower leaf abscission responses to CEPA compared to those in unsprayed subplots (Fig. 1). Unsprayed cv. SL28 gave considerably lower leaf abscission responses to various doses of CEPA applications than the semi-wild accession Rume Sudan, also not sprayed with fungicide (Fig. 2).

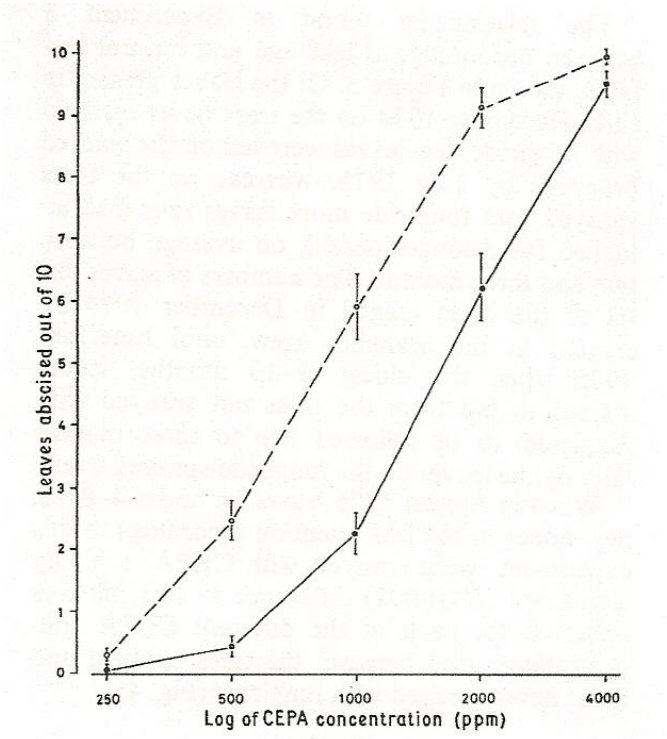

Figure 2. Leaf abscission induced by CEPA sprays on 21 trees of cv. SL28 (lower graph) and 21 trees of accession Rume Sudan (upper graph). Vertical bars are SEs of the means.

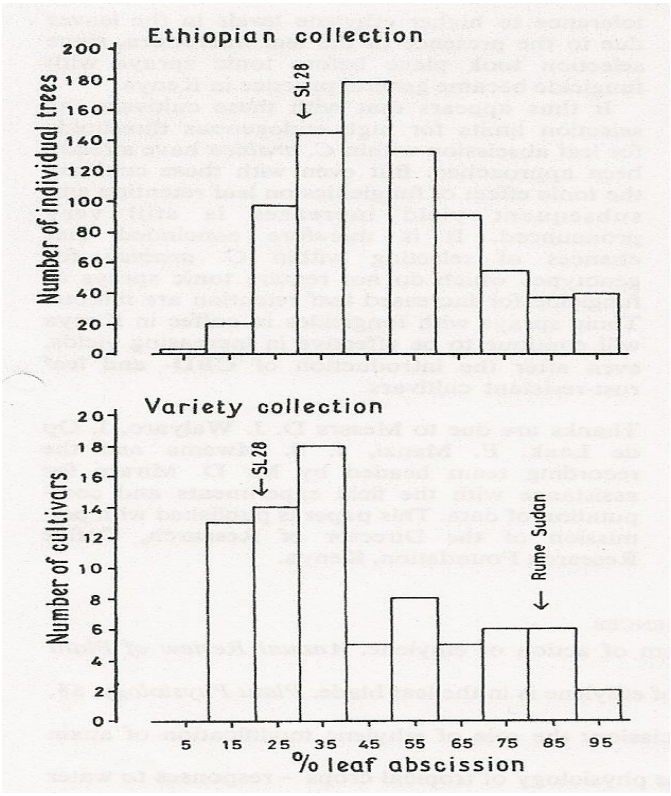

Figure 3. Frequency distributions of variation in leaf abscission responses to CEPA sprays at 1000 ppm concentration. Upper histogram includes 795 trees of the ET collection. Lower histogram includes 77 entries of the variety collection (4 trees per var.).

A large-scale screening in germplasm collections of *Coffea arabica* (never sprayed with fungicides), present at the Coffee Research Station in Kenya, gave large genotypic differences in leaf abscission responses to CEPA spray applications (Fig. 3). Another trial with monthly CEPA spray applications on 10 (unsprayed) SL28 trees over one full year showed considerable seasonal variation, with leaf abscission responses becoming higher during periods of water stress. In Kenya, coffee trees become water-stressed in the dry season, even when irrigated, due to the severe evaporative demand of the atmosphere.

The results of the studies indicate that the activity of the leaf microflora, as well as water stress, contribute to accelerated senescence and abscission through increasing the endogenous ethylene levels in the leaves. SL28 and most of the other East African cultivars, including the new disease resistant hybrids, are obviously already at the top end of tolerance for high endogenous ethylene levels, but nevertheless respond significantly to fungicide sprays by delayed leaf abscission and considerably higher yields.

CONCLUSIONS

Drought-tolerance in coffee is usually associated with early stomatal closure in response to water stress, but that reduces CO_2 assimilation and consequently lowers yield. Molecular-genetic research projects, mainly on young coffee plants, have identified genes that control responses to dehydration stress, but their effects on yield are still unknown (Freire *et al*., 2010; Stein *et al*., 2010). It seems unlikely that this approach will make a substantial contribution to breeding for improved WP of arabica coffee, at least in the short term.

Table 2. Summary of opportunities for increasing WP in arabica coffee.

Breeding	* disease resistances (CLR, CBD, ……)
	* pest resistances (GM ?)
	*** leaf retention/longevity**
	* short-stature (Ct) cvs.
	* hybrid vigour for yield (F1's)
Agronomy	*** tonic sprays**
	* close spacing
	* disease and pest control
	* nutrient supply (especially N)
	* shade trees (marginal areas)

However, there exist already various opportunities for enhancing WP in arabica coffee (see Table 2), which may more than double this when applied in combination. First of all, host resistances to important diseases and pests prevent heavy crop losses and are as such major factors of sustaining productivity without wasting available water. Besides, diseases resistances to CBD and CLR enable the application of tonic sprays of fungicides as another, very cost-effective, means of substantially increasing yields.

The dose-responses of leaf abscission to CEPA sprays offer a rapid and relatively simple test to select for genotypes with superior leaf retention. This appears to be related to a vigorous and deeper root system, more effective stomatal conductance and improved photosynthesis

under limited water resources. It is evident that selection for leaf longevity plus tonic sprays of fungicide (on disease resistant cultivars only) can substantially increase WP.

Complementary options for still further enhancing WP in arabica coffee are:

- Short-stature, compact cultivars (Ct-gene from Caturra): these transpire some 25% less water than tall *typica* or *bourbon* coffee cultivars with a more open crown architecture (DaMatta, 2006).
- F1 cultivars to exploit hybrid vigour for growth and yield (Van der Vossen, 2001; Bertrand *et al*., 2011).
- High-density planting: complete ground cover results in less soil water lost by evaporation and transpiration from weeds; a denser and deeper root system leading to more efficient uptake of water and plant nutrients (DaMatta, 2004b).
- Disease and pest control (chemical, biological) to prevent heavy crop losses in susceptible cultivars.
- Adequate supply of plant nutrients: leaf longevity and photosynthetic capacity are related to leaf-nutrient status, N in particular (Borrel and Hammer, 2000b).
- Shade trees in areas marginal to coffee cultivation (adverse climatic and/or edaphic conditions): to reduce soil and atmospheric water deficits, temperature extremes and wind speeds (DaMatta, 2004b, 2006).

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Modelling Vegetative Growth and Architecture of Coffea arabica Cultivars under Water Stress

J. DAUZAT[1], S. GRIFFON[1], P. MARRACCINI[2], G. C. RODRIGUES[3]

[1]CIRAD, UMR AMAP, Montpellier, France
[2]CIRAD UMR DAP, Montpellier, France
[3]Embrapa-CNPTIA, Campinas, Brazil

SUMMARY

Two *Coffea arabica* cultivars, IAPAR59 (drought tolerant) and RUBI (drought susceptible), were grown for two years with/without irrigation during the dry seasons. In addition to eco-physiological, anatomical and molecular biology observations, complete descriptions of plant architecture were performed. All architectural data were analysed owing to the AMAPstudio-XPlo software. The methodology for analysing data and the main results are presented here.

A first general effect of drought was a decrease of the growth rate (i.e. an increase of the phyllochron) during the dry season. This effect, higher for Rubi than for Iapar59, concerned similarly the main stem and the branches of different ramification orders. Another important response concerned the setting of secondary and tertiary branches on the second year. Globally the treatments had effects on all studied variables but no architectural trait appeared as specifically responsive to temporary droughts.

Aside the analyses of cultivars plasticity, the data collected in this study provided matchless information that is currently used for building a functional structural coffee model coupling architectural rules and ecophysiological processes such as the carbon acquisition. The outlines of this so-called Functional Structural Plant Modelling approach are presented as well as its interest for deriving the very primary mechanisms of phenotypic plasticity.

INTRODUCTION

Drought and unfavourable temperatures associated to global climate changes are expected to seriously hamper the cultivation of coffee in marginal lands in the future.

Several studies addressed the effects of drought on coffee physiology. They pointed out that the physiological mechanisms underlying coffee tolerance to drought are largely related to the strong sensitivity of coffee stomata. Alternatively, the morphological plasticity of coffee tree to drought remains poorly explored. The purpose of the present paper is therefore to fill that gap.

Studies of phenotypic plasticity in plants are commonly confined to some global variables such as plant height, leaf area per plant, number of branches or biomass whereas the parameters of the plant structure are ignored. It follows that the specific organogenetic responses (e.g. the branching process) resulting from genes activation can't be identified. The emphasis is therefore put here on the growth parameters that build the plant structure and, namely, the growth process in terms of number of nodes and the ramification process.

MATERIALS AND METHODS

The trial was set in the experimental station of Embrapa-CPAC, Brazil (15°35' S, 45°43' W). The experimental design was composed of 17 plots including 39 plants of each cultivar. Six months old plantlets were transferred from nursery to the field in Dec. 2007 and followed for 2 years. Three irrigation treatments were applied as shown in figure 1, with "i" and "n" coding irrigated and non-irrigated treatments respectively. Three times a year (before, during and after the dry season) 7 to 21 plants of each cultivar were taken off from the field to the laboratory for the description of their aerial architecture as well as for the description of their root system. Observations included: the basal diameter of the trunk and each branch, the length of each internode and each leaf (plus the total leaf area per branch for the first three dates), the dry mass of the trunk and branches and the dry mass of leaves for each branch. Additionally, the length of leaves and internodes were measured every week on 2 branches in 5 plants per treatment x genotype in order to monitor the kinetic of organ expansion.

Architectural data were coded in the Multiscale-Tree-Graph format and loaded in the AMAPstudio-XPlo software for visual checking and data extraction in an interactive mode or by scripting. Data were exported to R for further analyses.

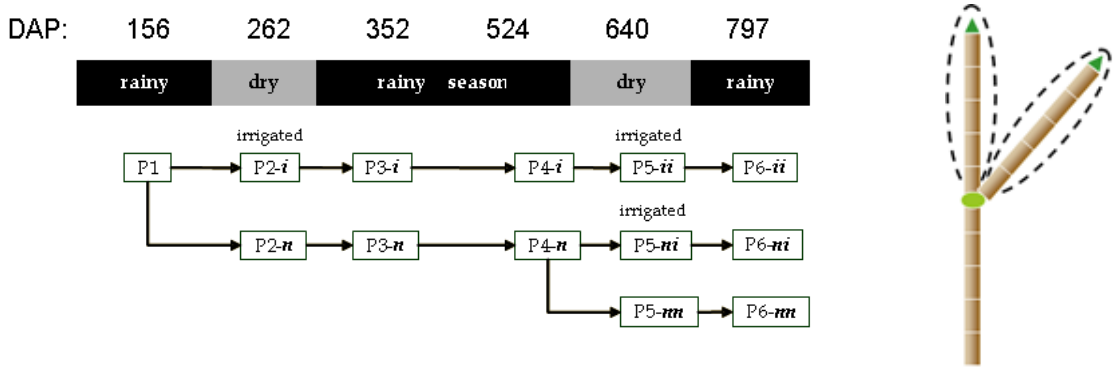

Figure 1. Left: treatments applied during the experiment, with "i" and "n" coding for "irrigated" and "non-irrigated" treatments respectively. Right: Comparison of the number of nodes on a branch with the number of nodes on the distal portion of its bearer.

RESULTS

Following analyses focus on the evolution of plant structure and its plasticity to water stress in terms of number of main stem nodes, number of 1st order and 2nd order branches, size of 1st order and 2nd order branches and leaf area.

The number of trunk nodes linearly increases with time for irrigated plants, indicating a constant phyllochron of about 20 days (Figure 2). The growth rate is decreased by water stress events, particularly for the Rubi cultivar. Given that all trunk nodes bear two branches at the exception of the very basal node, the number of 1st order branches is about twice the number of trunk nodes.

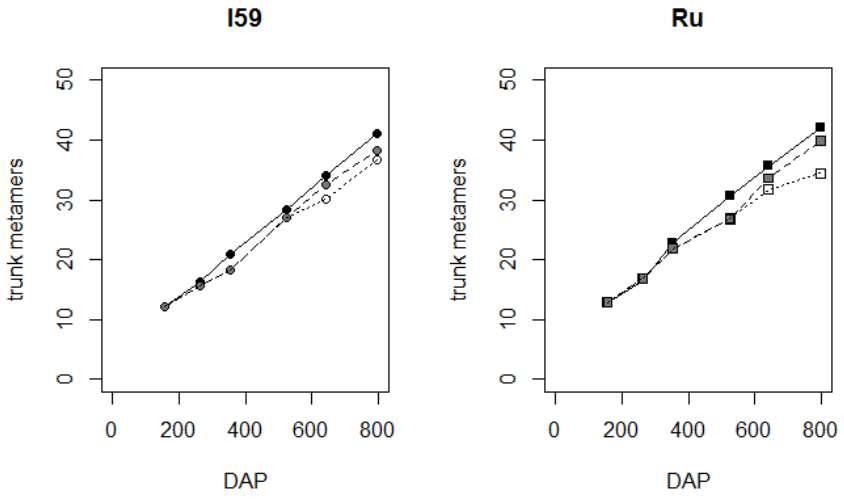

Figure 2. Evolution over time (Days After Planting) of the number of trunk nodes. The "ii", "ni" and "nn" treatments are represented by black symbols, grey symbols and open symbols respectively.

Unlike 1st order branches that systematically develop on trunk nodes with no delay, 2nd and 3rd order branches are unevenly distributed on their bearer and are set up with quite variable delays. Rapidly the number of 2nd order branches exceeds the number of 1st order branches whereas the number of 3rd order branches is still quite low at the end of the experiment (Figure 3, left). The setting of 2nd order branches is quite sensitive to a temporary drought. Rubi is slightly more depressed by drought than Iapar59 but is able to set a large number of ramifications when irrigated during the second dry season (Figure 3, middle and right). An unexpectedly high number of 2nd order branches was observed in the dry-irrigated treatment at DAP 640. These newly appeared shoots were often thinned later on.

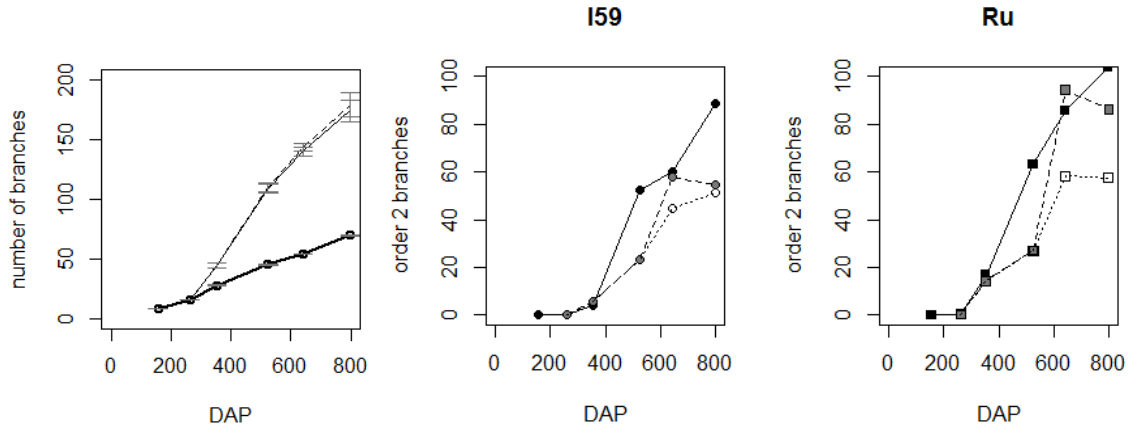

Figure 3. Number of branches per plant. Left: number of 1st order branches (thick line), 1st+2nd order branches (simple line) and 1st+2nd+3rd order branches (dotted line). Middle and right: number of 2nd order branches in the different treatments (see figure 2 for symbols explanation).

As detailed below (Figure 7), 2nd order branches are much shorter on average than 1st order branches that are themselves shorter than the trunk. Therefore the number of metamers (i.e.

the number of nodes) is a more integrative indicator of the vegetative growth than the number of branches. Figure 4 shows that most metamers at plant level belong to 1st order branches. Interestingly, water stresses have a smoother effect on the number of metamers than on the number of ramifications.

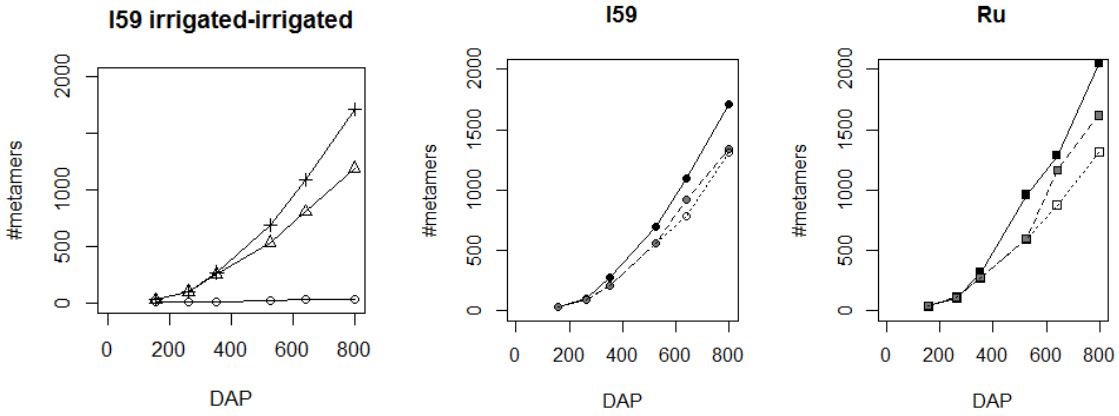

Figure 4. Number of metamers per plant. Left: number of metamers on trunk (o), trunk+1st order branches (△) and trunk+1st and 2nd order branches (+). Middle and right: total number of metamers in the different treatments. See figure 2 for symbols explanation.

The leaf area depends on the number and size of plant axes (trunk and branches of all branching order) and the lifespan of the leaves. Water stresses have a strong effect on the leaf area (Figure 5). Oddly the leaf area of irrigated Rubi plant remains stagnant from P4 to P5 (DAP 524 to 797). Further observations would be necessary to check if this trend is temporary or not.

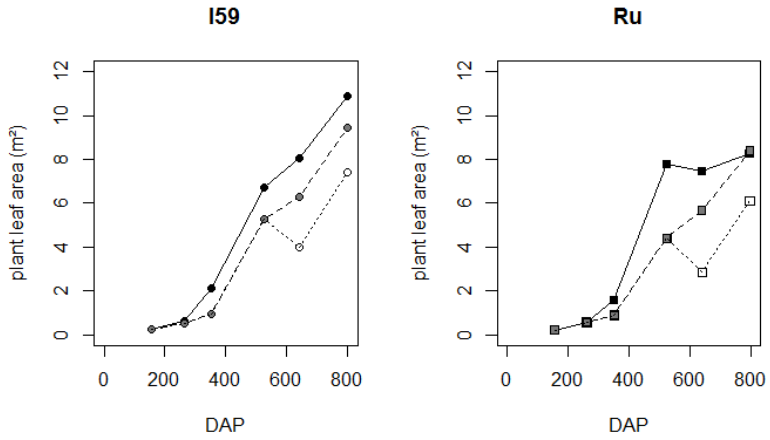

Figure 5. Total leaf area per plant (m²). See figure 2 for symbols explanation.

Primary branches appear with no delay when a new metamer is formed by the main stem apical bud. Moreover when comparing the number of nodes on these branches to the "distal" number of nodes on the trunk (see Figure 1) one can see that the primary branches have the same growth rate as the trunk, i.e. they share the same phyllocrhon. This is the case for all treatments and all growth stages for branches having up to 20 nodes (Figure 6). Alternatively

the growth rate of lowest branches progressively decreases as they get older. This trend is similar for the two cultivars whatever the treatment.

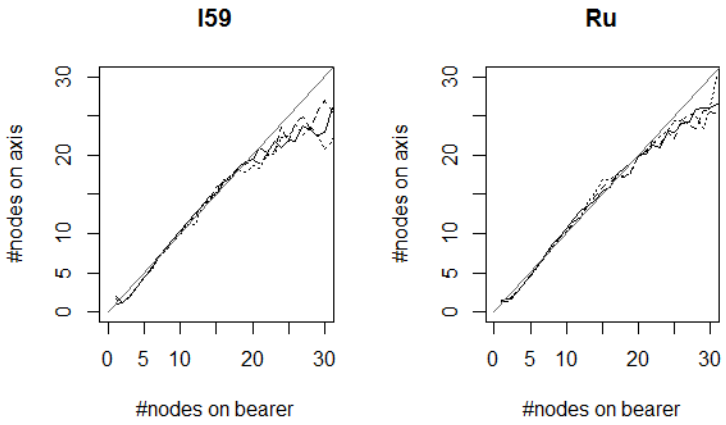

Figure 6. Number of nodes on 1st order branches vs. the number of nodes in the distal portion of the trunk (see Figure 1 right for definition). Treatments "ii", "ni" and "nn" are represented by solid, dashed and dotted lines respectively.

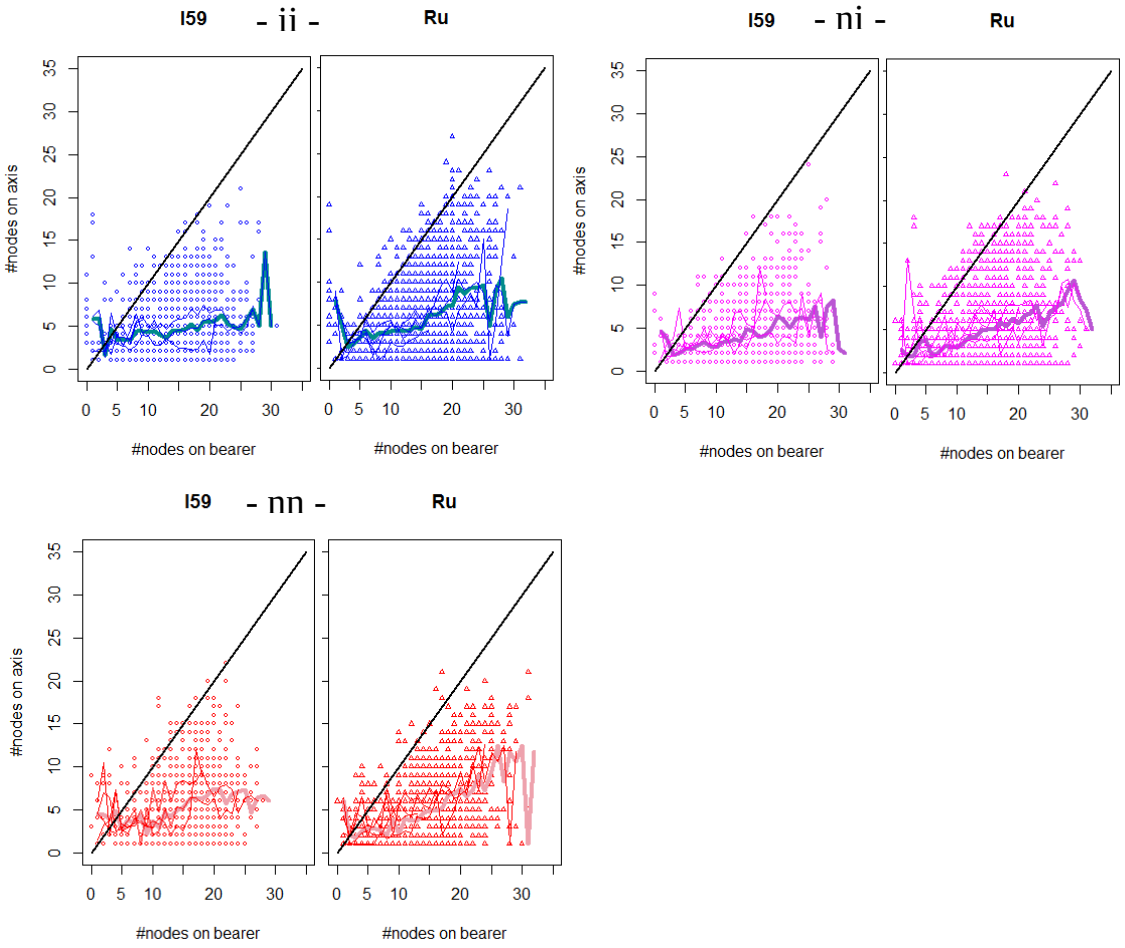

Figure 7. Number of nodes on 2nd order branches vs. the number of nodes on the distal portion of their (1st order branch) bearer (see Figure 1 for definitions).

The comparison of the number of nodes on 2nd order branches with the number of nodes in the distal portion of their bearer (1st order branches) shows that some 2nd order branches can have as much nodes as their bearer (Figure 7). The working assumptions are that these 1st order branches develop with no delay when the bearing metamer is formed and have the same phyllochron as their bearer. However most of 2nd order branches are delayed, leading to branches of all sizes along the bearer branch. Despite the quite large variability of the size of these ramifications, a clear linear trend appears when plotting their average nodes number vs. the nodes number of their bearer. The same trend is similar for the two cultivars in all treatments with a very slight tendency of higher slope for Rubi, especially in the dry-dry treatment.

Young primary branches having less than 15 nodes are seldom ramified. The ramification rate of older branches then increases roughly linearly with the number of nodes on the bearer branch i.e. with time. These two features are shared by the two cultivars in all treatments as shown in figure 8.

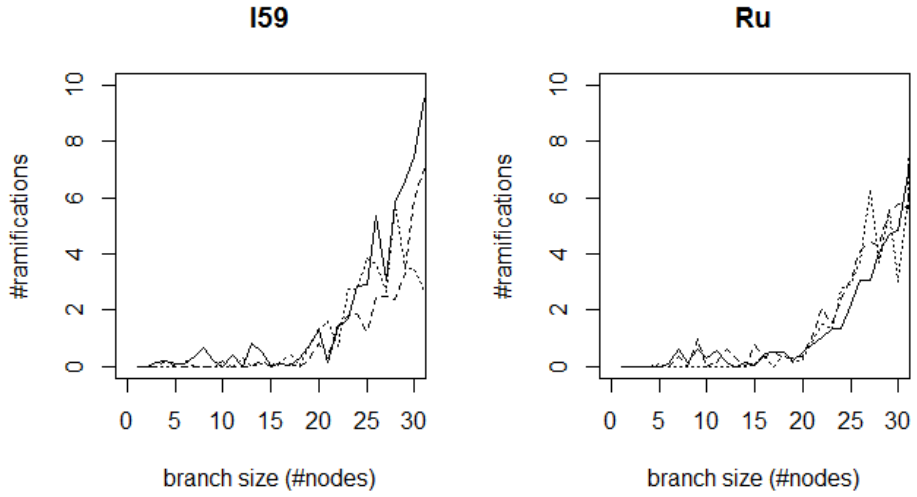

Figure 8. Number ramifications on 1st order branches as a function of their number of metamers.

DISCUSSION

Coffee trees develop a simple architecture in early stages with a single stem bearing systematically two branches on each node. Moreover the main stem and 1st order branches have about the same growth rate. Subsequently, the 1st order branches progressively branch out 2nd order ramifications and reduce their growth rate after reaching the size of 20 metamers.

Water stresses have an effect on the growth rate of the main stem (increase of its phyllochron). Although this effect is not drastic, it has important impacts on the plant structure since it results in less 1st order branches that have themselves a decreased growth rate. The overall effects at plant scale are a reduction of both the number and the size of vegetative axes (stem and branches). Plants irrigated only on the second year did not make up from their first year growth loss but they nevertheless recovered their regular growth pattern with apparently no delayed effects other than the ones induced by a reduced plant structure.

The effects of water stresses are more marked for Rubi, considered as drought susceptible, than for Iapar 59, considered as drought tolerant. The main physiological difference observed between the two cultivars concern their stomatal regulation: Rubi exhibited a more

anisohydric behaviour than Iapar 59. Consequently Rubi plants keep their stomata open as long as soil water resources are available and then collapse whereas Iapar 59 plants avoid severe water shortage by reducing their transpiration. This observation is consistent with the fact pointed out by DaMatta and Ramalho that the physiological mechanisms underlying coffee tolerance to drought are largely related to the sensitivity of coffee stomata to water deficits. Differences in water use affect the carbon acquisition in different ways but there is no evidence that the biomass allocation is changed.

Figure 9. Example of 3D plant model generated using the AMAPstudio software suite.

In order to further address the functional conditions that rule the plant development, we are currently developing a functional-structural approach. The first step is to build 3D plant models (Figure 9) by integrating the organogenetic rules analysed above. The second step consists of simulating the plants carbon acquisition Then functional hypotheses relating the branching pattern with the plant trophic status can be evaluated.

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Shade has Antagonistic Effects on Coffee Berry Borer

E. SANCHEZ[1,2], B. P. DUFOUR[3], A. OLIVAS[2], E. DE M. VIRGINIO-FILHO[2], S. VILCHEZ[2], J. AVELINO[2,3,4]

[1]IDIAP, Panama
[2]CATIE, Turrialba, Costa Rica
[3]CIRAD, UPR 106, Montpellier, France
[4]IICA-PROMECAFE, Guatemala

SUMMARY

This work was addressed to clarify shade effects on the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae). The research was carried out in 2010, in Turrialba, Costa Rica, at 600 m of elevation, in a trial established by CATIE where different shade trees and coffee managements are compared. We studied several combinations of three levels of exposure to light (full sun, medium shade of *Erythrina poeppigiana*, dense shade of *E. poeppigiana* and *Chloroleucon eurycyclum*) and three coffee managements (organic with applications of the entomopathogen fungus *Beauveria bassiana*, conventional with insecticide sprays, and conventional insecticide-free). The response variables were: (i) populations of *H. hampei* in green, ripe and dry berries, and (ii) number of bored berries. We also monitored the microclimate in full sun exposure and dense shade conditions. Shade tended to increase *H. hampei* populations into coffee berries. With the conventional management, with insecticide or with no pest control, the number of CBB females, into the ripe and dry berries from the branch, was 70 % higher on average under shade than at full sun exposure. When analyzing the percentage of bored berries, we observed almost the same trend. With the conventional management, with insecticide, shade significantly increased the proportion of berries bored by the insect: 6.4 % and 2.4 % of bored green and ripening berries at the beginning of the harvest under dense shade and at full sun exposure respectively. However, shade effect was reversed when *B. bassiana* was applied. The proportion of bored berries was significantly higher under the moderate shade (4.9 %) as compared with the dense shade (4.0 %). Shade has therefore antagonistic effects on *H. hampei*. In one side, shade increases CBB populations when no *B. bassiana* is applied, but in the other side, it decreases the number of bored berries when the entomopathogen fungus is sprayed. This can be explained by the microclimatic conditions which were more favorable to the insect and to the entomopathogen fungus under shade. Under dense shade, temperatures were buffered (particularly high temperatures) and relative humidity and plant organs wetness were higher as compared to full sun exposure.

INTRODUCTION

Shade tree effects on coffee berry borer (CBB) are poorly understood. Different effects are mentioned in the literature. Shade has often been reported to favour CBB infestations. However, different shade effects have been described according to the shade cover that trees provide. For instance, abundant CBB populations were observed under dense shade (60-70 % of shade cover), whereas similar low infestations were observed at full sun exposure and under moderate shade (40-50 %). In other circumstances, no shade effect was detected. Shade has even been recommended for fighting CBB within the framework of an increase of temperatures related to climate change.

Shade effects on coffee pests and diseases are usually not clear, because shade may stimulate several pathways at the same time with opposed effects, some favouring the pest and others hampering it. The balance of these effects is therefore unsure. The case of CBB seems to support that view. For instance, shade trees can provide propitious conditions for some species that are directly involved in CBB biocontrol, as birds, ants, and the entomopathogen fungus *Beauveria bassiana*. However, full sun exposure seems favourable to other natural enemies. According to Hargreaves, *Prorops nasuta* Waterston and *Heterospilus coffeicola* Schmiedkenecht, natural CBB parasitoids in Uganda, seem more active on CBB populations colonizing berries exposed to sun than on those under shade. In addition, shade may directly affect CBB survival. Some shade trees are alternative hosts for CBB. Microclimatic conditions under shade may also have an effect on CBB. It is frequently admitted that CBB survives for longer and reproduces better in humid and shady conditions. Moreover, shade tends to buffer temperatures and may affect CBB through that way. The direction of the effect would depend on if these buffered temperatures are closer or not to CBB temperature optima. Shade may also influence CBB populations through its effect on the coffee plant, on its phenology, particularly on its flowering pattern, which is a key factor explaining the growth of CBB populations.

This work was addressed to clarify shade effects on the coffee berry borer.

MATERIALS AND METHODS

A field experiment was carried out from February to August 2010 at the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) experimental station (Turrialba, Costa Rica; 9° 53’ N, 83° 38’ W). This location has climatic characteristics conducive to CBB development. Rainfall is almost evenly distributed throughout the year and abundant, 2700 mm 69-year average annual rainfall. The CATIE experimental station is located at 600 m above sea level, close to the lowest altitude at which coffee is cultivated in Costa Rica. The mean annual temperature is 22 °C (53-year average) with very little variation. The absence of marked dry season has consequences on the coffee flowering pattern. Flowerings are multiple, spreading normally from December to May, and of low intensity, resulting in multiple fruit cohorts and harvest rounds from July to December.

The study was performed on a 6 ha long term trial established in 2000 by CATIE where several coffee production systems are compare. Coffee production systems differ with respect to shade trees and input regimes for nutrient, pest and weed management. Main plots are different shade trees used alone or in combination two by two. A full sun treatment was also included in the trial. Main plots are subdivided in four subplots, as a maximum, corresponding to two crop management strategies, conventional and organic, with two intensity levels of input application each. In our study, we only used four of the 20 combinations of shade conditions and input regimes present in the trial: (i) full sun and (ii) shade provided by *Erythrina poeppigiana* and *Chloroleucon eurycyclum*, both in combination with the least intensive conventional input regime, including insecticide applications against CBB, (iii) shade provided by *E. poeppigiana* only and (iv) shade with *E. poeppigiana* and *C. eurycyclum*, both in combination with the most intensive organic input regime, including applications of the entomopathogen fungus *B. bassiana* to control CBB. We incorporated in the study two additional combinations, by using plots contiguous to the trial and established at the same time. These plots were managed under the same least intensive conventional input regime as in the trial, but with no use of any CBB control method. One of these treatments was at full sun exposure, and the second one with shade provided by *E. poeppigiana*. Each combination is replicated three times in a randomized block design.

The shade percentage was checked twice in March and May 2010 in four places of each plot. We did this using a spherical densiometer employed to measure forest overstory density. We obtained an average shade cover ranging from 23 to 41% in the *E. poeppigiana* plots, and from 48 to 69 % in the *E. Poeppigiana* with *C. eurycyclum* plots.

Two main CBB population assessments were performed: (i) the number of individuals at different development stages (particularly live females) and times, found in bored coffee berries (dry, green, ripe) coming from the ground and from the tree (ii) the progress over time of the number of bored berries on the coffee tree.

Air temperature, wetness (free water) and relative humidity were also monitored. Two Hobo H21-001 weather stations were positioned at the center of two close plots with contrasted light exposure conditions: (i) full sun exposure with low intensive conventional management and no insecticide applications and (ii) dense shade provided by *E. poeppigiana* and *C. eurycyclum* with high intensive organic management. Each weather station had nine sensors: four rigid wetness sensors, two air temperature sensors and three air temperature relative humidity sensors.

We used a general linear mixed model to analyse the data, where the combinations of shade and input regimes were considered as fixed factor and the assessment dates as random factor. When significant differences were highlighted, contrast analyses were performed to compare different shade conditions under the same input regimes.

Microclimate data were analysed by comparing intra-day variations for the two plot conditions. We plotted (i) the means of five sensors for air temperature (ii) the means of three sensors for relative humidity, and (iii) wetness frequency, which was deduced from the four wetness sensors. Data were processed separately according to the daily rainfall amount: no rain, 0-5 mm, >5 mm.

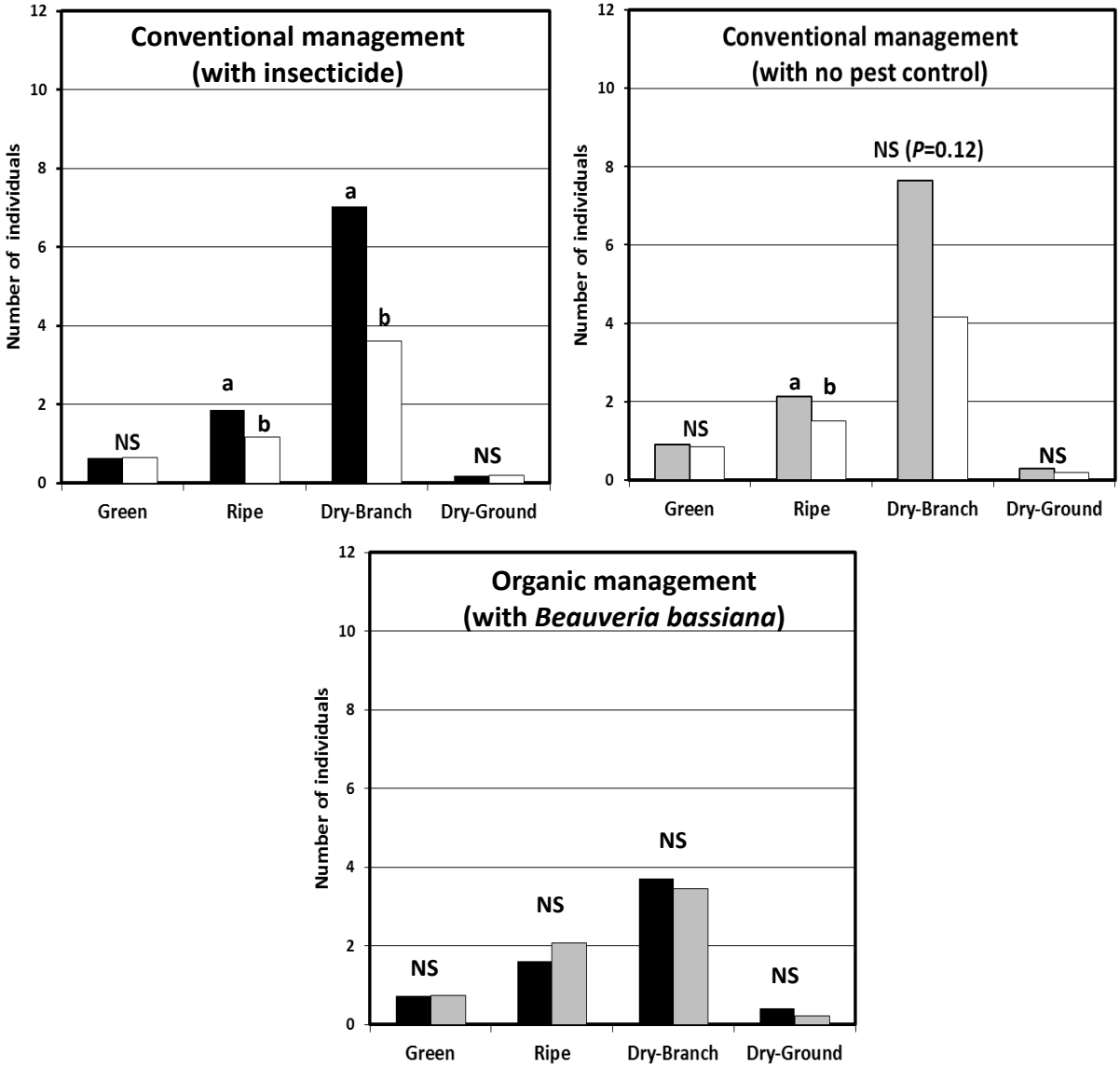

Figure 1. Shade effects on the number of live females into different kinds of berries (green, ripe, dry from the branch and from the ground). Average of five assessments from February to June 2010. Black: dense shade provided by *Erythrina poeppigiana* and *Chloroleucon eurycylum*. Gray: regular shade provided by *E. poeppigiana.* White: full sun exposure. Data with different letters are significantly different according to the contrast analysis (*P*<0.05). NS: not significant *P*>0.05).

RESULTS AND DISCUSSION

With the conventional management, with insecticide and with no pest control, shade tended to increase the number of CBB females into the ripe and dry berries from the branch (Figure 1). The number of females was almost the double in each kind of berry. However, no differences were found into the green berries and dry berries from the ground, possibly because the

number of females was very reduced. The green berries were recently colonized and the dry berries from the ground were mostly abandoned. With the organic management, we did not see any difference between the two studied conditions of shade. The number of females was low in both conditions, suggesting a favourable effect of shade on CBB control under the organic management (Figure 1).

When analyzing the percentage of bored berries, we observed almost the same trend (Figure 2). With the conventional management, with insecticide, shade increased the proportion of berries bored by the insect. This effect was not found with the conventional management with no pest control, possibly because the shade cover was lower. Shade effect was completely reversed when B. bassiana was applied. The proportion of bored berries was higher under the moderate shade as compared with the dense shade (Figure 2).

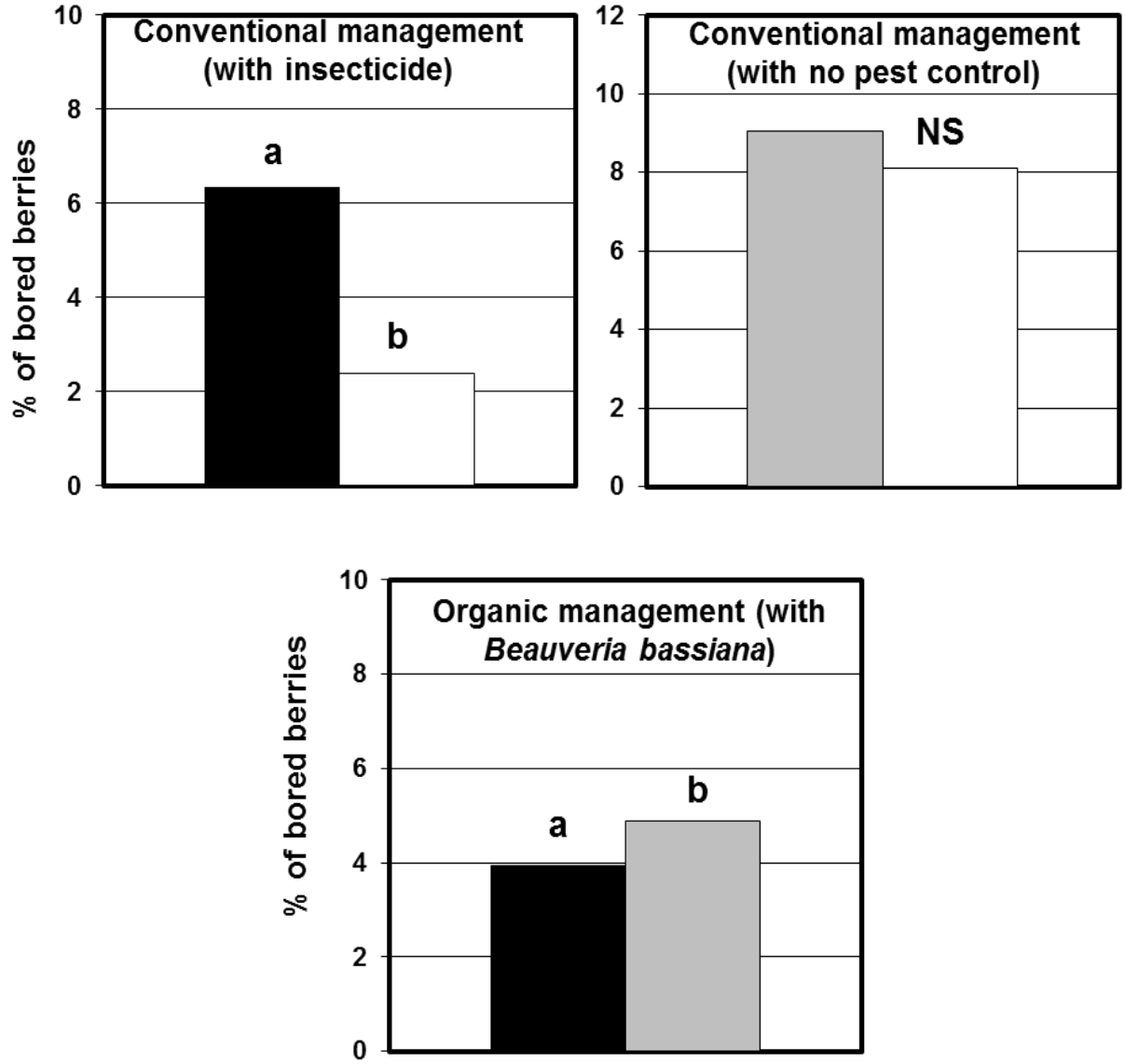

Figure 2. Shade effects on the proportion of bored berries (green and ripening berries). August assessment (at the beginning of the harvest). Black: dense shade provided by *Erythrina poeppigiana* and *Chloroleucon eurycylum*. Gray: regular shade provided by *E. poeppigiana.* White: full sun exposure. Data with different letters are significantly different according to the contrast analysis (*P*<0.05). NS: not significant (*P*>0.05).

This behavior can be probably explained by the microclimatic conditions which were more favorable to the insect and to the entomopathogen fungus under shade. Under dense shade, light was reduced, temperatures were buffered (particularly high temperatures) and relative humidity and plant organs wetness were higher as compared to full sun exposure (Figure 3). It is noticeable that in dry days, microclimate in the plantation under shade is equivalent to

microclimate at full sun exposure on low rainfall days. Similarly, microclimate under shade on low rainfall days is equivalent to microclimate at full sun exposure on high rainfall days.

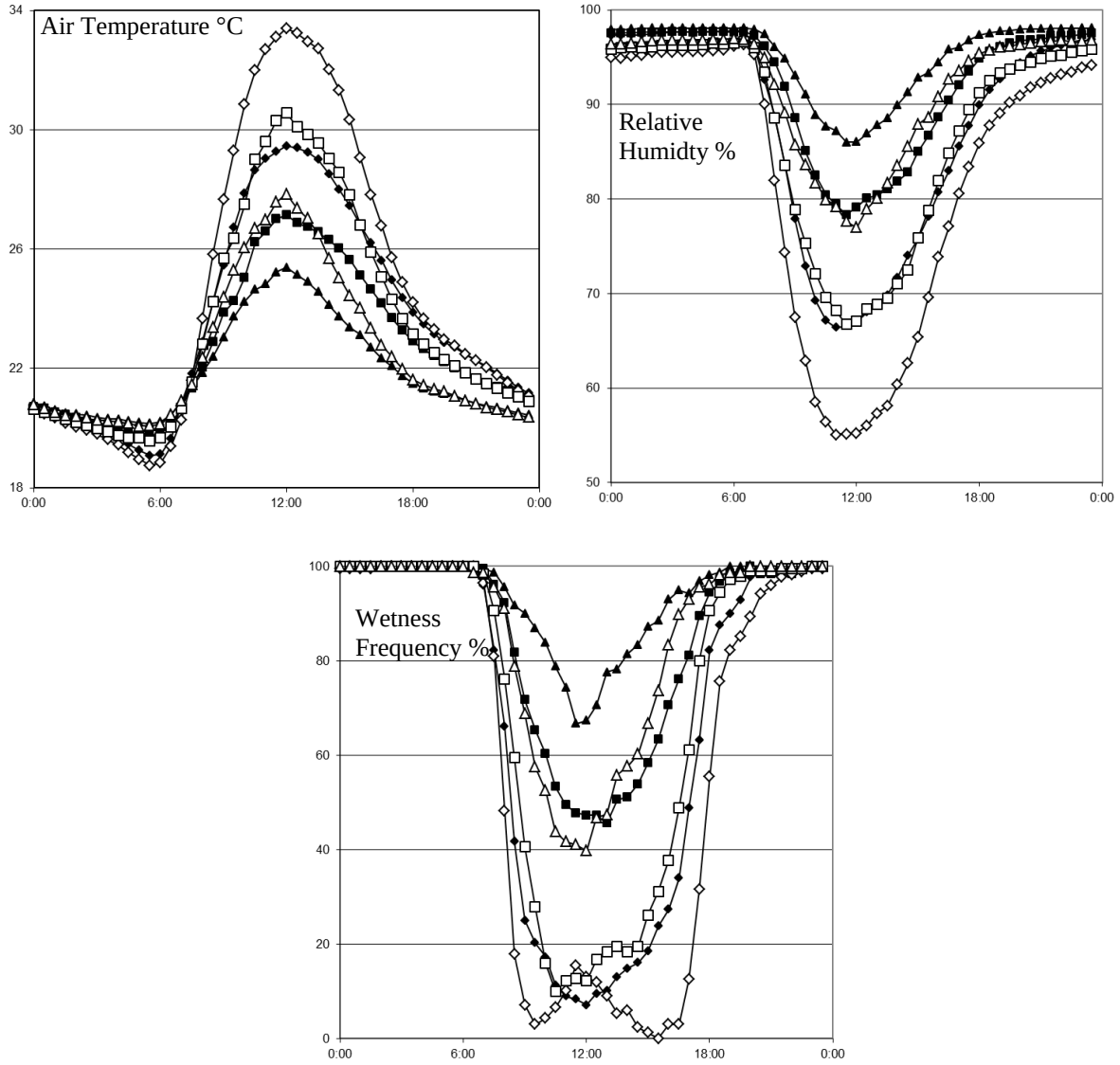

Figure 3. Intra-day variations in air temperature, wetness, relative humidity, under different daily rainfall conditions (no rainfall: rhombus, 0-5 mm: square, >5 mm: triangle) with full sun exposure (white) and under dense shade (*Erythrina poeppigiana* + *Chloroleucon eurycylum*, black).

On dry days and on low rainfall days, average temperatures exceeded 30°C at full sun exposure around noon , whereas under shade this temperature was not reached. In general, the proportion of surviving *H. hampei* colonizing females decreases considerably above 30°C. The development is also strongly restricted. Concerning humidity, Baker mentioned that the borer development and survival were improved between 90-95% of relative humidity. Under shade, the relative humidity was always at least 10 % higher than at full sun exposure, with a minimum of 65 % on average in dry days around noon.

Our results indicate that shade has antagonistic effects on *H. hampei*. In one side, shade increases CBB populations when no *B. bassiana* is applied, but in the other side, it decreases the number of bored berries when the entomopathogen fungus is sprayed. It can be concluded

that with no applications of *B. bassiana*, it is better to reduce shade cover to control CBB. On the contrary, when *B. bassiana* is applied, it is better to provide higher shade cover to enhance the entomogenous fungus activity. However, there is probably not a single response for shade effects on CBB, and variations can be expected in diverse environments. A site-specific shade management is necessary.

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Shade is Conducive to Coffee Rust as Compared to Full Sun Exposure under Standardized Fruit Load Conditions in a Sub-Optimal Zone for Coffee in Costa Rica

D. F. LOPEZ-BRAVO[1], E. DE M. VIRGINIO-FILHO[1], J. AVELINO[1,2,3]

[1]CATIE, Turrialba, Costa Rica
[2]IICA-PROMECAFE, Guatemala, Guatemala
[3]CIRAD, UPR 106, Montpellier, France

SUMMARY

This work was addressed to clarify shade effects on coffee rust (*Hemileia vastatrix*). We dissociated direct effects of shade on the fungus through microclimate modifications from its indirect effects through fruit load reduction. The trial was set up in Turrialba, Costa Rica at 600 m of elevation, in a coffee plot initially under shade provided by *Erythrina poeppigiana.* The plot was subdivided into two subplots: one was maintained under shade, whereas shade was eliminated in the second subplot. In each subplot, we removed fruiting nodes from 40 coffee plants in order to obtain the following four levels: none, 150, 250, and 500 fruiting nodes per coffee plant. With the homogenised fruit load, the intensity of the coffee rust epidemic was greater in the shaded subplot, with a 21.5% increase in incidence and a 22.4% increase in severity. Two mechanisms were suggested. Firstly, we highlighted a dilution effect due to host growth which was 25.2% and 37.5% greater in full sunlight when considering new leaves or new leaf area respectively. Secondly, the microclimate was more conducive to coffee rust under shade, with lower intra-day temperature variations, due to lower maxima, and a higher leaf wetness frequency.

INTRODUCTION

Shade effects on coffee pests and diseases are usually not clear, as shade may be propitious to a given process in the life cycle of a noxious organism and hamper another process at the same time. The balance of these antagonistic effects is variable and often controversial, as in the case of coffee rust.

A high shade percentage may reduce coffee rust attacks by regulating yields, which could partly explain the results obtained by Soto-Pinto *et al.* in Mexico. For reasons not well understood, epidemics are more intense when fruit load is high. This condition is often reached with full sun exposure. As a consequence, a high degree of shade may negatively affect the development of this disease through the effects of shade on fruit load. However, shade also buffers temperatures, intercepts light and probably increases moisture in the plantation. These effects are all conducive to the infection process, which may explain the opposite results observed in Central America.

These two probable antagonistic pathways are combined under natural conditions. In order to clarify their individual effects, we dissociated the two factors by manually homogenising fruit loads under two light exposure situations, under shade and in full sunlight.

MATERIALS AND METHODS

A field experiment was carried out in 2008 and 2009 at the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) experimental station (Turrialba, Costa Rica; 9° 53' N, 83° 38' W). This location has climatic characteristics conducive to coffee rust development. Rainfall is almost evenly distributed throughout the year and abundant, 2700 mm 69-year average annual rainfall. The CATIE experimental station is located at 600 m above sea level, close to the lowest altitude at which coffee is cultivated in Costa Rica. The mean annual temperature is 22 °C (53-year average) with very little variation.

The trial was set up in a coffee plot initially under shade provided by the tree legume Erythrina poeppigiana. The plot was subdivided into two subplots: one was maintained under shade, whereas shade was eliminated in the second subplot. The shade percentage was assessed each month, or each month and a half, during the rainy season. We did this using a spherical densiometer used to measure forest overstorey density.We obtained an average shade cover of 23% and 57% in the last five months of 2008 and 2009 respectively.

In each subplot, we removed fruiting nodes (FN) from 40 coffee plants in order to obtain the following four levels: none, 150, 250, and 500 fruiting nodes per coffee plant. We obtained 10 coffee plants with 0 FN, 11 with 150 FN and 250 FN, and only eight with 500 FN, as coffee plants with more than 500 FN were rare in the plot.

On each of the selected coffee plants, four branches were identified in two different storeys (two in the middle of the coffee plant, and two in the upper storey). Coffee rust progress was monitored by inspecting young leaves on these branches. For the inspections, we followed the methodology proposed by Kushalappa and Ludwig. Each leaf was first individually mapped. On each inspection, we recorded the presence or absence of the leaf and its area, when present, using a diagrammatic scale. We also scored the presence or absence of coffee rust and the proportion of diseased leaf area using the scale proposed by Kushalappa and Chaves.

Two kinds of variables were calculated from the collected data for each coffee plant:

- The cumulative percentage of diseased leaves or leaf area on each assessment date and the Area Under these Disease Progress Curves (AUDPC, in days.%), as a measurement of the incidence or of severity respectively.
- The percentage of new leaves or new area produced during the year with respect to the number of leaves or the leaf area of the first assessment date, as a descriptor of host growth.

Air and leaf temperatures, leaf wetness and relative humidity were also monitored. A Hobo H21-001 weather station with nine sensors was positioned at the centre of each subplot: five rigid leaf wetness sensors, three air temperature sensors and one air temperature relative humidity sensor were located at different heights and places in the same way in both subplots. These microclimate variables were monitored from August 2008 to December 2009. Leaf temperature was monitored from August to October 2009 only. For that purpose, in each subplot, we used a Campbell CR23X station with four thermocouples sensors (copper-constantan) located close to the air temperature sensors.

To test the effects of the number of fruiting nodes and plot conditions (mainly light exposure conditions) on coffee rust and host growth, we performed analyses of variance using the mixed model. Each coffee plant was considered as a replicate.

Microclimate data were analysed by comparing intra-day variations for the two plot conditions. We plotted (i) the means of four sensors for air temperature (ii) leaf wetness frequency, which was deduced from the five leaf wetness sensors (iii) relative humidity and (iv) the means of four thermocouples for leaf temperature. Data were processed separately according to the daily rainfall amount: no rain, 0-5 mm, >5 mm.

RESULTS AND DISCUSSION

No interaction was found between plot conditions and the number of fruiting nodes per coffee plant.

As expected, the intensity of the coffee rust epidemic increased in line with fruit load. We quantified a 28.9% increase in coffee rust incidence and a 129.2% increase in severity on plants with 500 fruiting nodes as compared to plants with no fruits. Intermediate severity levels were found with 150 FN and 250 FN (Figure 1). Fruit load effects were logically clearer when considering severity. Fruit load mostly affected coffee rust after the fungus penetrated the leaf, through plant physiology. However, our results also indicate that pre-infectious events, probably at the penetration stage, could be affected by the fruit load, as lower incidences were found on coffee plants with no fruits.

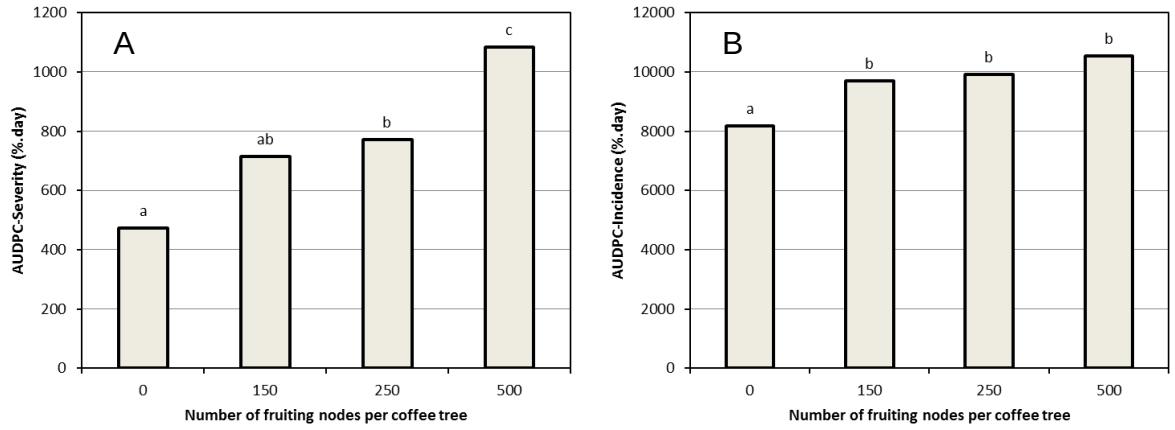

Figure 1. Fruit load effects on coffee rust. Area Under the Disease Progress Curve (AUDPC) expressed as cumulative percentage of infected leaf area (A) or of infected leaves (B). Data with different letters are significantly different according to the LSD test (P<0.05).

With the homogenised fruit load, the intensity of the coffee rust epidemic was greater in the shaded subplot, with a 21.5% increase in incidence and a 22.4% increase in severity (Figure 2). However, severity under shade was not significantly different from that at full sun exposure at P< 0.05 but at P<0.15. Shade effects on coffee rust progress were more obvious when considering incidence because shade probably favoured pre-infection processes, and not so much colonization, on the contrary of fruit load.

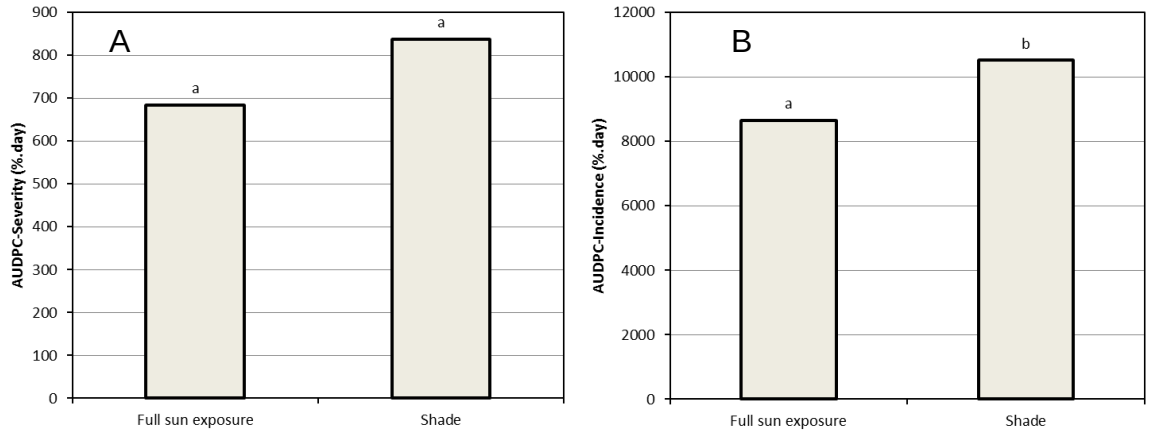

Figure 2. Shade effects on coffee rust. Area Under the Disease Progress Curve (AUDPC) expressed as cumulative percentage of infected leaf area (A) or of infected leaves (B). Data with different letters are significantly different according to the LSD test (*P*<0.05).

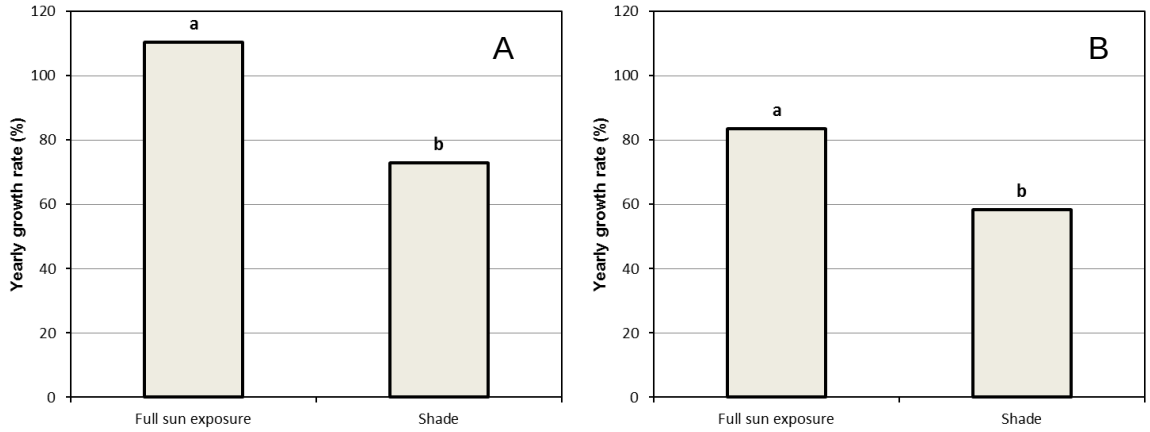

Figure 3. Shade effects on coffee growth. Coffee growth is expressed as the percentage of new area (A) or new leaves (B) produced during the year with respect to the leaf area or the number of leaves of the first assessment date. Data with different letters are significantly different according to the LSD test (P< 0.05).

Two mechanisms were suggested. Firstly, we highlighted a dilution effect due to host growth which was 25.2% and 37.5% greater in full sunlight when considering new leaves or new leaf area respectively (Figure 3). This dilution effect is due to the introduction of new healthy leaves or leaf area over time, which reduces the apparent infection rate.
Secondly, the microclimate was more conducive to coffee rust germination and colonization under shade, with lower intra-day temperature variations (air temperature and leaf temperature), due to lower maxima, and a higher leaf wetness frequency (Figures 4 and 5). This was especially true in 2009 when greater shade cover was maintained. However, lower levels of relative humidity were obtained with full sun exposure which is conducive to uredospores dispersal by wind (Figure 4). Considering the long distances over which uredospores can be dispersed and as the study plots were adjacent, differences in the dispersal rates were probably not relevant in our trial.

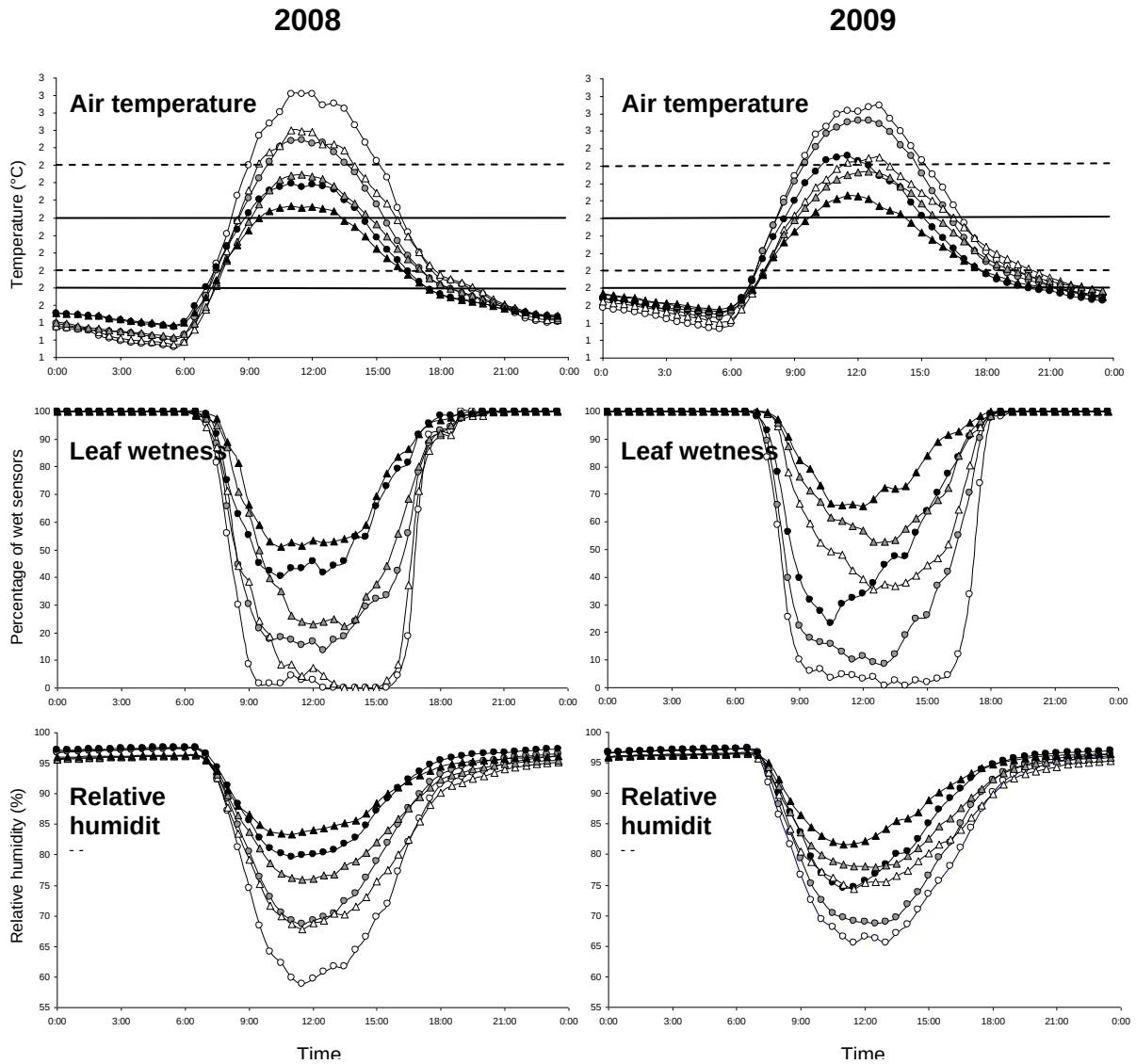

Figure 4. Intra-day variations of air temperature, leaf wetness, relative humidity during the rainy season, under different daily rainfall conditions (no rainfall: white, ≤5 mm: grey, >5 mm: black) with full sun exposure (○) and under shaded conditions (∆) over two consecutive years. Optimum temperature range for germination and infection (continuous lines) and for the latent period (dotted lines) according to Waller.

We concluded that shade has antagonistic effects on coffee rust. Coffee rust is reduced by shade because shade reduces the number of fruiting nodes and the number of fruits per node. It is possible to find higher coffee rust severities and incidences with full sun exposure when the fruit load is heavy, than under shade when the fruit load is lower. However, with an equivalent number of fruiting nodes, coffee rust incidence and, to a lesser extent, severity were greater under shade. The service provided by shade in controlling coffee rust is necessarily associated with a disservice that consists in reducing yield in the short term.

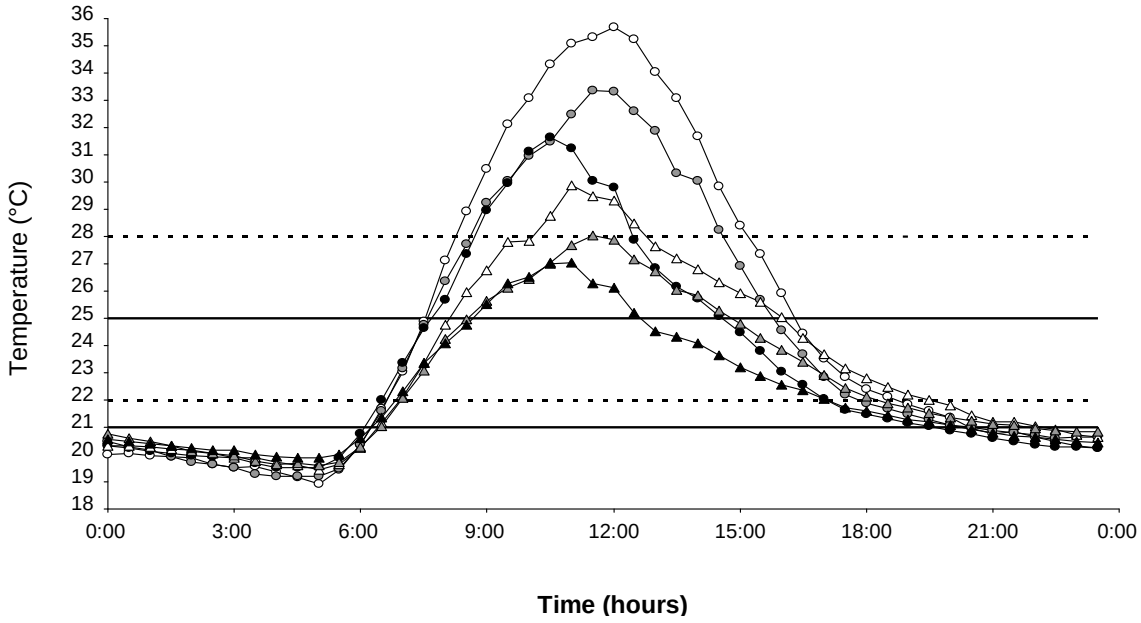

Figure 5. Intra-day variations of leaf temperature during the 2009 rainy season, under different daily rainfall conditions (no rainfall: white, ≤5 mm: grey, >5 mm: black) with full sun exposure (○) and under shaded conditions (∆). Optimum temperature range for germination and infection (continuous lines) and for the latent period (dotted lines) according to Waller.

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Successful Case Studies of Adopting Improved Coffee Varieties in Tanzania

D. L. KILAMBO, D. L. MTENGA, N. M. NG'HOMA, R. NGOMUO, J. M. TERI

Tanzania Coffee Research Institute (TaCRI), P. O. BOX 3004, Moshi, Tanzania

SUMMARY

TaCRI recommended 15 improved Arabica hybrids which are resistant to strains of *Colletotrichum kahawae* and *Hemileia vastatrix*, and four (4) Robusta varieties resistant to coffee wilt disease (CWD) caused by *Gibberella xylarioides*. The improved varieties are also of high yielding and excellent beverage quality. At the beginning of their official release, rumors spread among coffee stakeholders' that; improved varieties may break disease resistance, may produce poor yields, and have poor root system and taste therefore disturbing coffee market in Tanzania. This prompted close follow up of the established coffee varieties across regions. Observations made since their establishment in coffee farms for more than eight years show that the varieties continued to hold disease resistance, maintaining productivity and acceptable cup taste. Three case studies are being highlighted on continued good characteristics of the varieties; two for Arabica, and one in Robusta coffee growing areas in Western Tanzania. Assessments of disease resistance for Arabica varieties were effected using a rating scale of 1-6; 1, implies resistant and 6, susceptibility. For Robusta varieties the scale was 1 and 2; 1, resistant and 2, dead. Records on productivity and cup taste were also collected. Varieties considered for data collection were; Arabica hybrids N39-3 (SC8) and KP423-1 (SC10), and Maruku 1 (MR10) and Maruku2 (13/61) for Robusta. One compact breeding line in pipe line for official release was part of the evaluation. Commercial varieties N39 and KP423 for Arabica and MS1 for Robusta were used as checks. Improved coffee varieties continued to show disease resistance, yield advantage of more than 2 tons per hectare over traditional disease susceptible varieties, and outstanding cup taste described as of specialty coffee.

INTRODUCTION

Tanzania's stagnated coffee production is largely the result of declining yields. One of the main causes of this problem is continuing cultivation of traditional varieties for both Arabica and Robusta. Arabica varieties are highly susceptible to Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* (Waller & Bridge), and leaf rust by *Hemileia vastatrix* Berk et. Br. Commercial Robusta cultivars succumbed to coffee wilt disease also referred to as Fusarium wilt. These disease problem leads to yield losses, inferior quality and therefore low economic returns to coffee growers (Chimilila *et. al.*, 2008).

Several approaches have been implemented for effective control of these diseases to include routine application of fungicides for the control of CBD and CLR (Ngulu *et. al.*, 1998), and eradication of diseased Robusta plants (Kilambo *et. al.*, 2009). But none of these approaches have been offering effective management of disease prevails. Uses of fungicides are expensive and sometimes have environmental effects such as soil toxicity upon continuous use of copper based fungicides (Kullaya, 1984). Eradication of diseased trees is cumbersome, expensive, and not effective means of eliminating parts of inoculum. Use of disease resistant

coffee plants proved to be effective means of disease management in many countries (van der Vossen, 2005).

Searching for coffee varieties with strong base of disease resistance has been strongly highlighted by Ndondi and Nyange (1990). By 2012 Tanzania accomplished important milestone by having official release of fifteen (15) improved Arabica hybrids and four (4) Robusta varieties. The varieties have stronger base of disease resistance, of higher productivity and excellent cup taste. Despite this important milestone, rumors spread among coffee stakeholders' that; improved varieties may break disease resistance, may produce poor yields, and have poor root system and taste therefore disturbing coffee market in Tanzania. This prompted close follow up of the established coffee varieties across regions.

The objective of this study is to confirm the performance of improved coffee varieties in farmers' fields 3 to 8 years after official release.

MATERIALS AND METHODS

Data were collected from already established coffee plants in Kilimanjaro, Mbeya and Bukoba. Varieties under on – farms included Arabica clones N39-3 and KP423-1, and Robusta clones Maruku1 and Maruku2. One compact breeding line CVT6 was also included in the evaluation. Commercial cultivars N39 and KP423 for Arabica and MS1 for Robusta were used as controls. Ten coffee trees replicated twice were considered in data collection for each of the coffee variety. As shown in Figure 1, sites location falls under diverse coffee ecosystems; Northern Zone coffee growing areas experiencing bi – modal type of rainfall, contrasting Southern Highlands and Lake Zone receiving uni – modal rainfall. Weather data representing these areas were also collected to measure the performance of improved coffee varieties.

Figure 1. Map of Tanzania showing sites locations of the farms.
Key: Location of the farms.

Records for disease resistance for CBD and CLR were measured using a scale of 1 to 6; 1, implies resistant and 6, susceptibility. For Robusta varieties scale 1 and 2; 1, resistant and 2,

dead. Data on yields were collected from individual trees, eventually converted into Kg/ha. Samples from red ripe cherries were also processed for cup taste analysis. Arabica samples were wet processed (Robinson, 1964), and Robusta dry processed (Lingle, 1984). Descriptive of the cup commonly used worldwide was adopted (ICO, 2004; Lingle, 1986).

RESULTS

Disease resistance performance

Data on CBD and CLR resistance performance were collected for two seasons; 2011 and 2012 from sites located at Kibosho – Kombo, Moshi District Northern coffee growing area, and Khanji, Mbozi District Southern Highlands coffee growing area. Figures 2 to 3 summarizes disease resistance performance of the varieties per specific location.

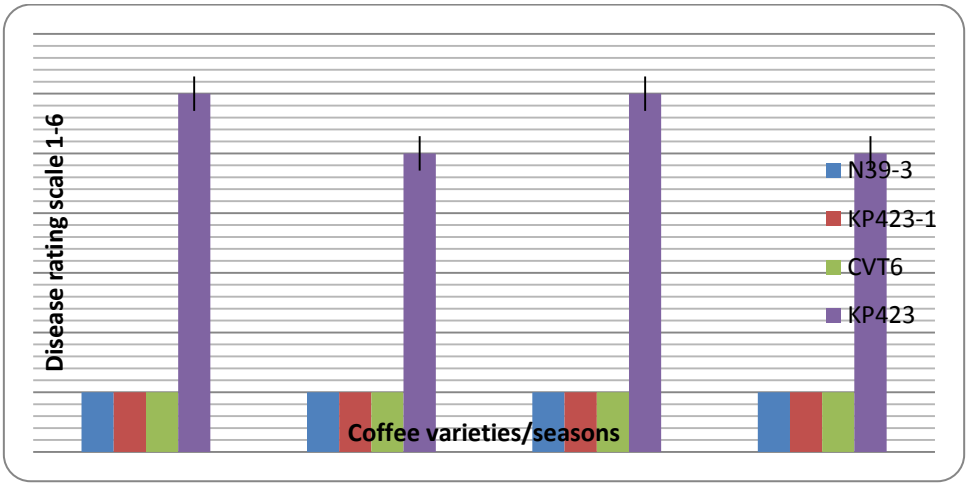

Figure 2. Disease resistance of Arabica hybrids at Kibosho – Kombo 2010 and 2011.

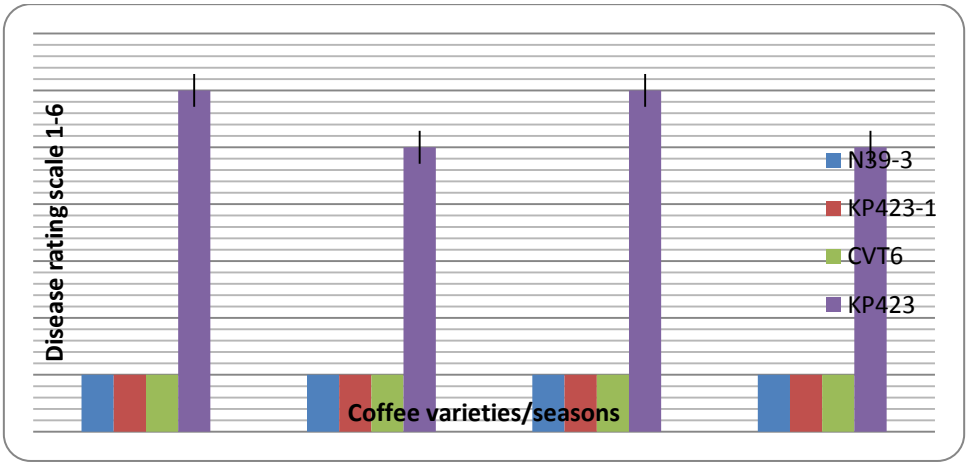

Figure 3. Disease resistance of Arabica hybrids at Khanji 2010 and 2011.

Results from both sites show that commercial variety KP423 had a score of 5 to 6 an indication of susceptibility, but improved varieties showed resistance to both CBD and CLR.
Data on CWD resistance were also collected for two (2) seasons; 2010 and 2011. Figure 4 summarizes resistance performance of Robusta varieties.

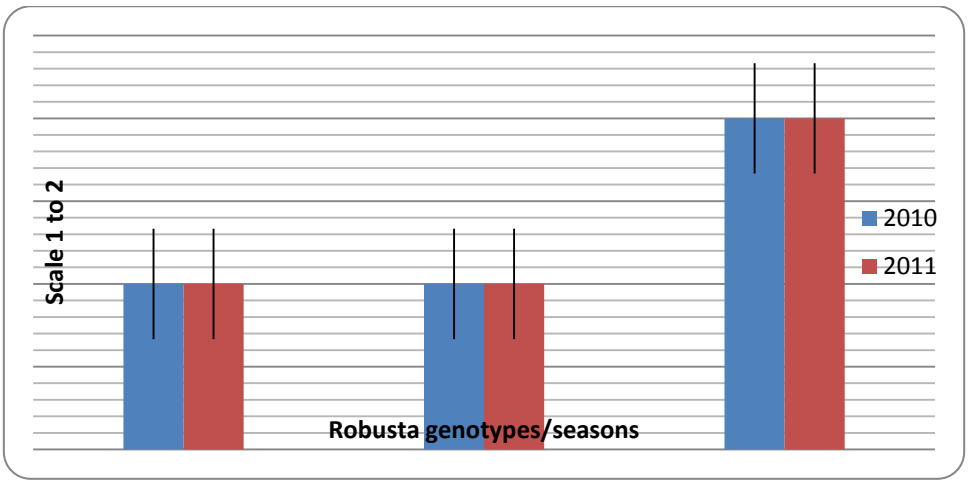

Figure 4. CWD resistance of Robusta clones in Kagera 2010 and 2011.

While dead trees were recorded from commercial variety MS1, the two Robusta varieties established continue to show resistance.

Yield records

Records on yield were considered for two seasons 2010 and 2011. Figures 5 and 6 summarize yield data for Kombo and Khanji sites.

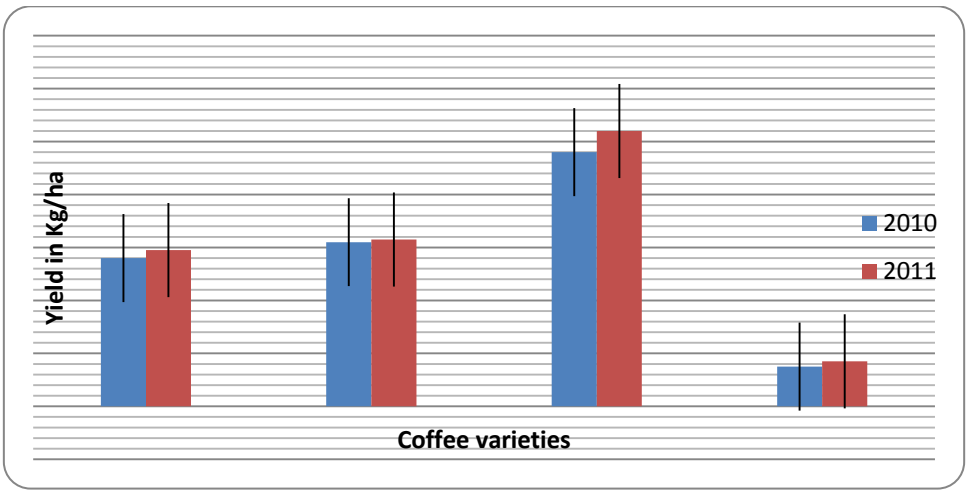

Figure 5. Yields records of improved Arabica hybrids compared to KP423 at Kombo.

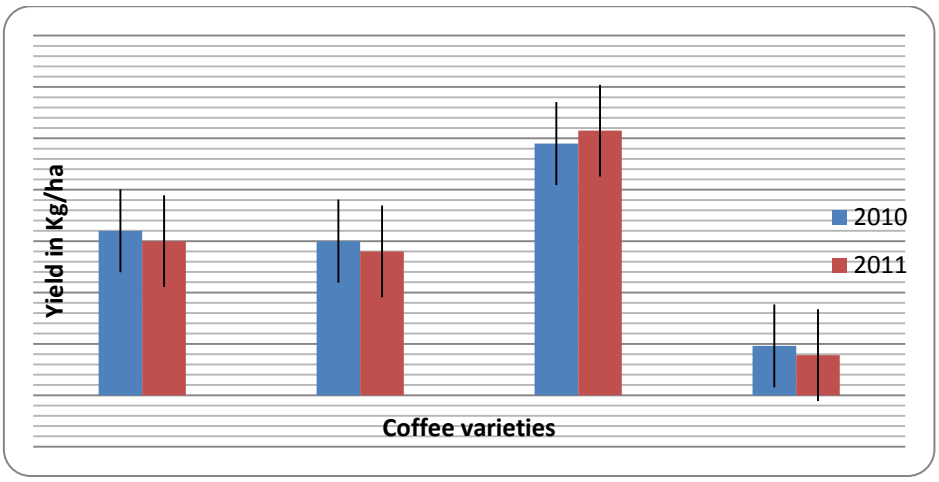

Figure 6. Yield records of improved Arabica hybrids compared to KP423 at Khanji.

Figures 5 and 6 confirmed that yield realized from commercial variety KP423 is significantly lower ($P < 0.05$) than N39-3, KP423-1 and breeding line CVT6.
Yield records for Robusta varieties are summarized in figure 7.

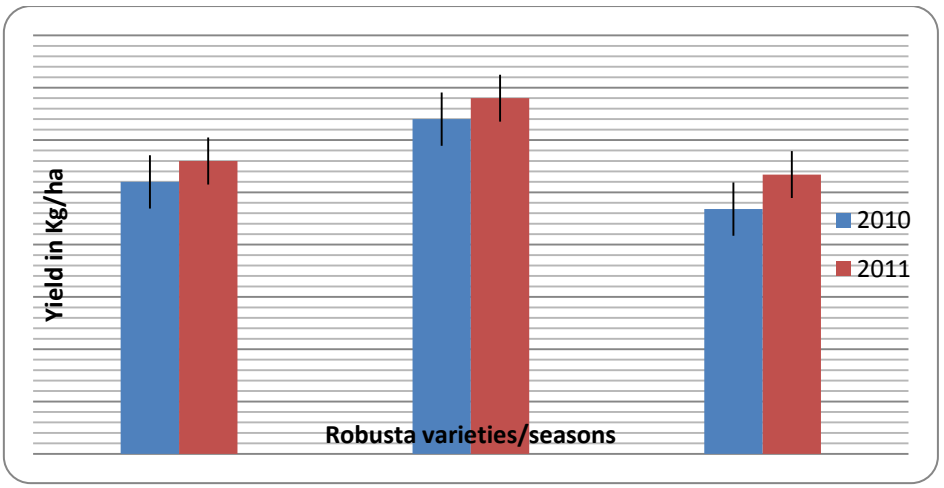

Figure 7. Yield data of Robusta clones compared to MS1 CWD susceptible variety at Bushasha

Descriptive of the cup taste

Prepared samples were tasted to describe the aroma/sweetness of the cup. Results per variety summarized across sites are presented in Table 1.

Table 1. Descriptive of the cup taste of the coffee varieties

Variety	Description
N39-3	Pleasant aroma, balanced in flavour
KP423-1	Pleasant aroma, balanced in flavour
CVT6	Citric acid and sweet
KP423	Sweet aroma
Maruku1	Typical natural Robusta
Maruku2	Nice aroma like mild Arabica
MS1	Typical natural Robusta

DISCUSSION

Disease resistance shown by Lyamungu Arabica hybrids have been deeply discussed (Kilambo *et. al.,* 2008). Yields as well as the quality of these varieties have been consistently superior over traditional varieties (Teri *et. al.,* 2004). Characteristics of Robusta selections in Tanzania have also been discussed (Kilambo *et. al.,* 2010). In these three case studies improved coffee varieties continue to exhibit good attributes of disease resistance, higher yields and cup taste. Despite being grown under different agro – ecological zones, the selections portrayed potentials for disease resistance, higher yields and cup taste (Figure 2 to 7, Table 1).

Inferior performance shown by Arabica traditional varieties especially on susceptibility may be a result of narrow genetic base of disease resistance. In central America Bertrand (2000) reported that while traditional varieties of Bourbon, Typical, Caturra and Catuai were susceptible to leaf rust because of their narrow genetic base, hybrids coffee varieties were resistant as they inherent wide genetic base. To maintain production, the use of pesticides especially fungicides is a must in Arabica coffee because it is more prone to CBD and CLR pressure. This possibly adds to the cost of production. Considering the nature of infection of coffee wilt disease of blocking the conductive tissues (Hakiza, 2004), resistance of the selected Robusta varieties implies that possibly host plants produces chemicals that inhibits the growth of pathogenic fungus. Braga *et. al.* (1998), reported that species of tropical Rubiaceae to have polysaccharides composition which may have phytoalexins to inhibit fungal infection.

CONCLUSIONS

Despite being grown under diverse agro-ecological zones, improved coffee clones continued to hold stability of disease resistance and in production, and beverage quality compares well with results used for official release of the varieties. This demonstrates that the varieties are the spring board for the coffee green revolution in Tanzania.

ACKNOWLEDGEMENTS

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A Current Perspective on Climate Variations and their Effects on Coffee Disease Management in Colombia

C. RIVILLAS, M. A. CRISTANCHO, A. L. GAITÁN

Plant Pathology Department, National Coffee Research Center, CENICAFE, Manizales, Colombia.

SUMMARY

During the 2008 to 2011 growing seasons, an unusual incidence raise of several coffee diseases was recorded around Colombian coffee fields, that comprised uncommon Coffee Leaf Rust (CLR) epidemics above 1,600 m, outbreaks of Pink disease and *Phoma* Die Back, and numerous reports of American Leaf Disease occurring in plantations under full sun exposure. Differences in environmental and agronomic conditions during the period 2008-2011 were considered as direct causes of these diseases high incidence, including deficient fertilizations due to international oil price increases, but mostly the larger and more frequent rainfalls resulting from recurring La Niña events in that period. In the Colombian central coffee-growing region, annual precipitation exceeded in 600 mm the historical average of 2,400 mm. A similar pattern was observed in most of the climatic stations located all over the coffee growing areas where also solar irradiation was on average 11% below the regular 1,775 hours per year. Together with sunlight reductions due to cloudy skies, a narrower range between maximum and minimum daily temperatures, a deficient nutrient uptake in water-saturated soils that slowed shoot growth, preventing plant recovery, and the absence of dry periods, completed the conditions that favoured blooming of pathogen populations. Molecular and phenotypic characterizations of thirty coffee rust isolates taken before and during the current epidemics indicated that the CLR outbrake was caused by isolates belonging to race II, the prevalent race of *Hemileia vastatrix* in Colombia since 1983, with no evidence of increments in race aggressiveness. During the last four years, field experiments of chemical control demonstrated de efficiency of cyproconazol at concentrations of 250 cc/Ha, applied in a preventive and opportune fashion according to the flowering periods and the onset of the rainy seasons, demonstrating the absence of fungicide resistance among *H. vastatrix* field inocula. In addition, a sales records survey of fungicides used in the control CLR indicated that less than 10% of the areas planted with susceptible varieties did receive any degree of chemical protection. Our experimental results and observations corroborated the strong influence of climatic conditions in the widespread activity of CLR and other diseases, and oriented the decision making process for a national campaign to control the disease, that ultimately decreased average incidence levels from 40% to 12%. Field trips and evaluations inside high CLR incidence zones confirmed the durable resistance of multi-line cultivars Colombia and Castillo derived from crosses between Caturra and Timor Hybrid 1343. Towards the future, proper planting densities, weed management, shadowing practices and fertilization schedules should be carefully observed in order to limit the impact of diseases during high precipitation periods. In the long term, the replacement of susceptible varieties for resistant ones is the most economical and environmentally effective measure to be taken. The combined use of historical meteorological data, weather recording stations, field disease evaluations, and epidemiological analysis are now the basis for the implementation of an early warning system, essential for the design of mitigation and adaptation strategies that ensure coffee production in the potential events of climate change in the years to come.

INTRODUCTION

Since 2008, a series of un common disease outbreaks or incidence increases have been observed in the Colombian coffee growing region, mostly related to the Coffee Leaf Rust (CLR), showing up above 1600m, but also involving severe attacks of Pink disease (*Erythricium salmoniclor*) reaching the trunk of the tree, and Dieback (*Phoma spp*.). There have also been cases of diseases associated to traditional coffee cultivation under shade conditions, that nor re-emergence in modern sun exposed plots, such as American Leaf spot (*Mycena citricolor*) and Black Rot (*Corticium koleroga*).

Moreover, a reduction of the number of coffee bags produced was significant by 2008, when the monthly production never went above one million, for a total of 8 million bags, 30% short of the average from the previous 6 years. Concerns on the effect of these disease on coffee production and general plantation health led to a national survey carried out on November 2010 in 1394 plots between 3 and 5 years old, indicated that CLR infection percentages were on average above 40%, a level that is considered over the limits were control measures are effective. It is well known the effect of severe attacks of CRL on production, either by increasing the cherry to parchment bean conversion ratio, from 5:1 to 8:1, but also reducing in at least 23% de total yield of the plantation.

To elaborate on the causes of the high CLR incidence, an integral analysis was accomplished, including the four major components of disease onset: the host, the pathogen, the human activities and the environment.

MATERIALS AND METHODS

Tests were performed on susceptible plants using 30 isolates of *Hemileia vastatrix* collected from around the coffee producing departments of Antioquia, Caldas, Tolima, Cauca, Risaralda, Santander, Cesar and Cundinamarca, to determine differences in uredospore germination, aggressiveness under greenhouse conditions, sporulation density, and also to characterize genetic diversity using AFLP molecular markers. Reference DNA for races II, VII. VIII, XIV and XXII was provided by the CIFC (Coffee Rust Research Center, Portugal).

Field experiments on chemical control using the recommended fungicide molecule (Cyproconazole) formulated as ALTO 100 (Syngenta) applying 3 sprays of 0.25l/Ha were carried out for three years in Chinchina, Caldas, as well as in Quimbaya, Quindío, to determine the effectiveness of the treatments as well as the possible presence of resistance isolates on those areas. Data on the total sales in the country of these fungicides was obtained from the national Institute of Agriculture (ICA).

Multiple farm assessments by the Plant Pathology Department at Cenicafé were complemented by data from additional disease surveys (eight between 2011 to 2012) coordinated by Cenicafe´s Biometry department, that were completed nationwide with the help of the Extension Service of the Colombian National Coffee Growing Federation (FNC), involving up to 4600 plots distributed in the whole country, in coffee plantations selected randomly from the FNC´s SICA database (Coffee Information System) that at the time contained over 1 millions entries corresponding to individual coffee plots. Climatic data from the Coffee Weather Network was provided by the Agroclimatology Department at Cenicafé

RESULTS AND DISCUSSION

For the host component, the first nationwide survey indicated that 70% of the plots with susceptible varieties (mostly C. arabica cv. Caturra), had disease incidences of 30% and higher, while 94% of the plots with resistant varieties (mostly Coffea arabica cv. Colombia, but also Castillo, all derived from crosses between Caturra and Timor Hybrid 1343) presented infection values of 20% or less. This indicated that the resistant varieties were holding the attack of the pathogen, and that at those levels chemical control was not necessary on them. The data confirmed that plots of susceptible varieties located above 1,600m were presenting high level of CLR.

In the pathogen component, molecular characterizations with 48 polymorphic AFLP fragments (out of 349) indicated that Colombian isolates clustered together and apart from CIFC reference samples, suggesting that the genetic variability present in the country was restricted to the variations of the original race II present in the country since 1983, and that no new genotypes were responsible for the epidemics observed. These isolates showed uniformity in their uredospore germination profiles, with 55 to 75 germination percentages between 16 an 28°C, falling rapidly afterwards to reach zero germination at 32°C. Latency periods were statistically the same (19 days on average) for the isolates tested. Sporulation density was the same for isolates collected below 1,600m, and significantly reduced for those above that altitude, therefore indicating that the current epidemics is not caused by new or better fit isolates or races of H. vastatrix. Triazol effectiveness was demonstrated for the two locations evaluated, where the production periods ended with less than 30% incidence for the cyproconazole treatment, while the controls reached 70% before presenting high defoliation due to CLR attack (Figure 1).

The analysis on the human activities indicated poor chemical control use, supported by data from fungicide sales for 2010 that confirmed a volume of product acquired by the growers enough to protect only 10% of the country's susceptible plantation area, if applied properly. Field observations repeatedly included deficient plantation management, including insufficient fertilization, lack of weeding practices in young plantations and over proliferation of orthotropic shoots in stumped coffee trees. These conditions were the result of a revaluation of the Colombian peso, that reduced the profits of goods exported in US dollars, and also by the boost experienced by oil prices during the first half of 2008, when they doubled, reaching a historic high, affecting the costs of fertilizers in a very significant way. Both phenomena occurred in the middle of a rising trend in coffee prices since 2002.

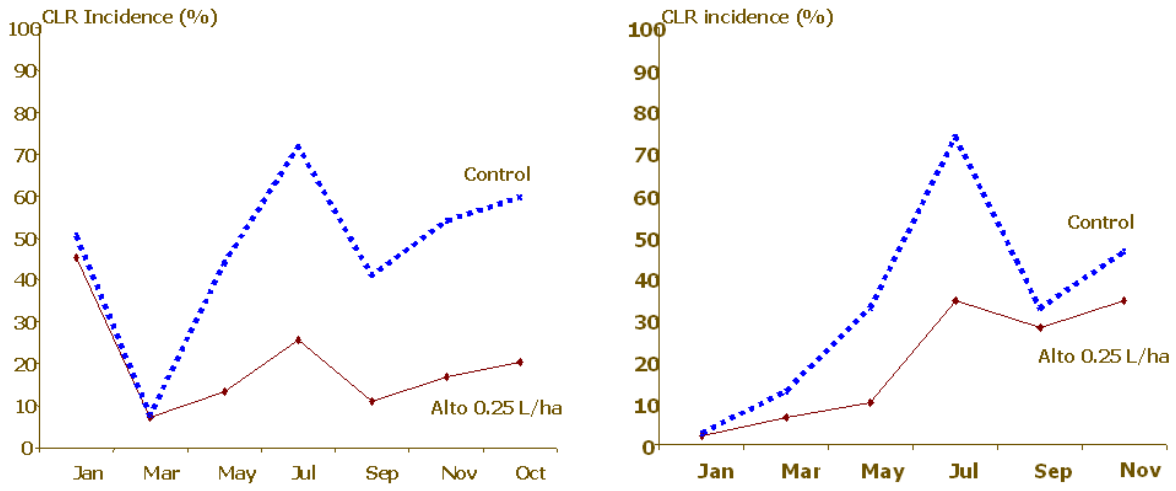

Figure 1. CLR incidence in *C. arabica* var. Caturra in plots located in Chinchiná, Caldas (left) and Quimbaya, Quindío (Right) during 2010, under 3 cyproconazol sprays, compared to the control.

Finally, the environment component was reviewed for the climate factors. Although the weather behavior can not be generalized for the multiple geographic conditions that are present in the Colombian coffee growing areas, for several places the effect of La Niña events occurring since 2008 resulted in increased rainfall (exceeding 3,000 mm/year), reduction in of sunlight (below 1,775 hours/year), and a narrower thermal amplitude (difference between the daily minimum an maximum). The overall change was the absence of dry periods, frequent splashing of rain drops, low luminosity and presence of a constant liquid layer on the leaves under non-extreme temperatures, all determinant factor for dispersion and germination of *H. vastatrix* uredospores. Excess water also implies nutrient wash off from soils, as well as poor absorption activity in the roots. Together with the deficient fertilizations, the situation prevented plat recovery after rust attack, in an opposed condition to high attacks related to large yields.

This analysis was the basis for the FNC to deploy a country wide campaign ("Rust-free Colombia, a national goal"), focused on direct help with fungicide and fertilizer to 180,000 coffee growers with CLR susceptible plots with plantations within productive ages (2 to 5 years), in addition to radio an television announcements on CLR integrated management, that gave priority to correct agronomical practices and to apply in an opportune manner the recommended fungicides. At the same time, support was provided to susceptible coffee plantations above 6 years old to renovate with resistant varieties. As a result, the infection level was reduced from 40% to 12% (Figure 2).

The perspective towards the future of coffee cultivation in Colombia invariably involves a closer consideration of the weather events, including not only El Niño/La Niña, but also larger phenomena such as the Pacific Decadal and North Atlantic Oscillations, together with the influence of the Inter Tropical Confluence Zone, and the geographic variation associated to a place characterized for the abundance of mountain chains, in order to improve forecasting in both location and time.

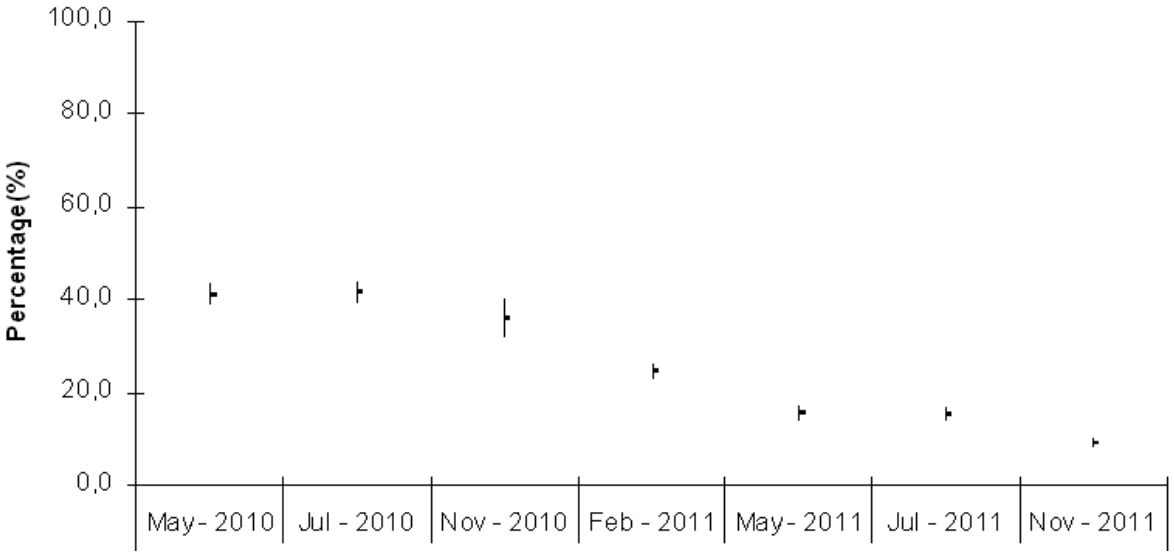

Figure 2. CLR infection percentage as measured nationwide from 2010 to 2011 showing a continuous reduction on incidence.

This view of the FNC regarding Climate Variations has been summarized in a strategy denominated "Climate-smart Coffee Cultivation", that includes the replacement of susceptible varieties by resistant ones, the continuation of national disease surveys, the upgrade and increase of the weather station network, the development of climate change indexes for coffee and the modeling of agroclimatological data to customize weather models to the several regions involved in coffee production.

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Coffee Breeding in Kenya: Achievements, Challenges and Current Focus

B. M. GICHIMU

Coffee Research Foundation, P.O. Box 4 – 00232, Ruiru, Kenya

SUMMARY

Coffee production in Kenya is seriously constrained by two fungal diseases namely Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* (Waller & Bridge) and Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix* (Berk. and Br.). Growing resistant varieties is believed to be the most cost effective and sustainable means of managing plant diseases. In Kenya considerable success has been made in coffee breeding to improve yields, quality and to manage the two economically important diseases. However, emerging issues such as climate change have brought up new challenges which require to be addressed to ensure sustainability in coffee production. This paper reviews some of the achievements, challenges and future prospects/approaches to develop cost effective and sustainable coffee varieties that enhance yield and quality.

INTRODUCTION

Coffee belongs to the genus *Coffea* in the Rubiaceae family which contains some 640 genera and 10000 species (Bremer, 1996) and is mostly grown in tropical and sub-tropical regions (Berthaud and Charrier, 1988). The genus *Coffea* consist of approximately 105 taxa (Kumar *et al.*, 2008) and has been reorganized into two subgenera: *Coffea* and *Paracoffea* (Bridson, 1987). Particular attention has been paid to the subgenus *Coffea* which includes two cultivated species of economic importance, *Coffea arabica* L. and *Coffea canephora* Pierre (Anthony *et al*., 2002; Kumar *et al.*, 2008). *C. arabica* is tetraploid (2n = 4x = 44) and is self-fertile while other *Coffea* species are diploid 2n = 2x = 22) and generally self-incompatible (Masumbuko *et al.*, 2003). *C. arabica* has two distinct botanical varieties *C. arabica var. arabica* (usually called Typica) and *C. arabica var. bourbon* (usually called Bourbon) (Krug and Carvalho 1951 as cited in Hue, 2005). World production of arabica coffee is still largely based on cultivars developed long ago by line selection within the *typica* and *bourbon* varieties, or in offspring of crosses between these two (Van der Vossen, 2009).

In Kenya, coffee was introduced as a cash crop in 1900's by Europeans colonialists, and has remained one of the most important products of the country's agriculture. Over 90% of the total Kenya coffee acreage is under Arabica coffee, while the rest is occupied by Robusta coffee (Omondi *et al*., 2001; Gichimu *et al*., 2010). Production of *C. arabica* is seriously constrained by diseases (Omondi *et al*., 2001; Gichuru *et al*., 2008). The major diseases are Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae*, Coffee Leaf Rust (CLR) caused by *Hemileia vastatrix* and Bacterial Blight of Coffee (BBC) caused by *Pseudomonas syringae* pv. garcae (Omondi *et al*., 2001). Introduction of resistance genes in *C. arabica* involves crossing with donor varieties, followed by backcrossing to restore desirable traits, especially yields and quality (Gichuru *et al*., 2008). However, breeding of Arabica coffee is largely constrained by its narrow genetic diversity resulting from its narrow geographic origin, its self-fertilising nature and the historical or selective bottlenecks in its agricultural adoption (Chaparro *et al.*, 2004). Over the years, coffee breeders have tried to widen the

genetic base of Arabica coffee by having more introductions and undertaking hybridisation programmes to create variability (Lashermes *et al*., 1999).

Coffee breeding in Kenya started in 1920s (Melville 1946; Thorold, 1947). Emphasis in selection was primarily for high yields, better bean size and liquor quality (Walyaro, 1983). This saw the selection and subsequent release of the first Kenyan coffee varieties (SL28, SL34 and K7) in 1930s. These cultivars produce high yields of fine quality coffee but are susceptible to CBD, CLR and BBC although K7 has resistance to some races of CLR as well as partial resistance to CBD. A breeding programme for disease resistance started in 1971 after the outbreak of CBD and CLR in the late 1960s. The main breeding goal has been to develop cultivars that combine resistance to diseases with improved yields and quality (Van der Vossen, 1973; Walyaro, 1983). In 1985, the first disease resistant hybrid cultivar, Ruiru 11, that is also high yielding, of fine quality and compact growth was released (Omondi *et al*., 2001). Further research and development culminated to the release of other disease resistant cultivars namely Batian 1, Batian2 and Batian 3. Their unique features include tall stature, true breeding and resistance CBD and CLR. They are also high yielding with good bean and liquor quality (Gichimu *et al*., 2010).

COFFEE BREEDING PROGRAMME IN KENYA

Initial coffee breeding mainly depended on the selection of elite varieties/cultivars from germplasm collections from Food Agricultural Organization (FAO) and ORSTOM (now IRD) French missions, along with accessions obtained through exchange with other coffee research institution across the world. Due to the nature of coffee tree, the qualities desirable for crop production were found to be mostly antagonistic (Van der vossen and Walyaro, 1981), for example, high yielding and good quality varieties were most of the time found to be highly susceptible to pests and diseases and vice versa. Although the original "French Mission" coffee plant in Kenya were observed to be generally less susceptible to CBD than later selections such as SL28 and SL34 (Van der Vossen and Walyaro, 1981), selection of true resistance within this material was considered unrewarding because of apparent lack of genetic variation. A number of accessions in the variety showed a high degree of field resistance to CBD and some certain CLR races. However, none of these possessed all desirable traits such as disease resistance, high yield, good quality and desirable growth habits. It therefore required a well planned breeding program to combine all or most of the desirable traits in a single variety.

The success of coffee breeding program depended on effective methods of early selection for disease resistance, the stability of disease resistance, full restoration of yield and quality and practical methods of large scale multiplication of the new disease resistant varieties. Initially, little knowledge was available on the above topics and it therefore necessitated ignition of a number of research projects on them to support the main breeding programme. Much of this research work resulted in the application of new and efficient methods of selection that led to an acceleration of the breeding programme. This resulted in the development of new disease resistant varieties of Arabica coffee much earlier than originally scheduled (Njoroge *et al*., 1981; Van der Vossen and Walyaro, 1981). Using this programme, it was possible to breed not only for resistance to CBD and CLR but also to select for high yields, good quality (both bean and cup) and desirable growth habit (either compact or tall).

The breeding programme was carried out along the following stages:

- Identification of genetic variability (e.g. resistance to diseases) from germplasm collections (parental genotypes).

- Selection within the parental genotypes followed by single crosses between disease resistant varieties and the best (high yielding and good quality) local cultivars.
- On the basis of information obtained, a number of F1 hybrids are selected for further improvement.
- Selected F1's representing different groups are involved in further crosses (with other elite cultivars or backcrossed to the elite parent) to incorporate other characters to improve the single hybrids
- Multiple crosses to assemble in one plant the desired traits of more than two varieties.
- Backcrosses of selected plants from the multiple crosses to the best local cultivars to improve on yields and quality.
- Selfing to fix the genes. For hybrid cultivars, steps 5 and 6 are omitted and selfed progenies are finally crossed for hybrid seed production.
- Field evaluation in different agro-ecological zones (adaptation trials).
- For true breeding cultivars, superior individual trees from superior lines are then selected for commercial seed production

The crossing was initiated right at the start of the programme which resulted in progenies of a large number of single crosses made between supposedly disease resistant varieties and susceptible commercial varieties. With this breeding programme, it is possible to develop hybrids as well as true breeding cultivars.

BREEDING FOR RESISTANCE TO CBD

CBD was first reported in Kenya in 1922 in newly established coffee plantations on the slope of Mt Elgon in Western Kenya (Mc'Donald, 1926). The effect of the disease then spread out in the succeeding years reaching east of Rift Valley by 1939. By 1951, it had spread through all the main coffee growing areas in the country (Rayner, 1952). From Kenya the disease spread to Angola in 1930, Zaire in 1937, Cameroon between 1955 and 1957, Uganda in 1959, Tanzania in 1964, Ethiopia in 1971 and Malawi in 1985 (Hindorf, 1975; Firman and Waller, 1977). Until now the disease has been restricted to East, Central and South African coffee-growing regions (Hindorf and Omondi, 2010) but strict precautions have always been taken to prevent the introduction of this disease to other parts of the world. In Kenya the effect of CBD was severely felt during the cropping years of 1962-1963 and 1967-1968 where coffee production loss rose to 80% (Griffiths *et al*., 1971). The disease infects all stages of the crop from flowers to ripe fruits and occasionally leaves, but maximum crop losses occur following infection of green berries with the formation of dark sunken lesions with sporulation, causing their premature dropping and mummification.

CBD resistance in Arabica coffee is believed to be horizontal/quantitative in nature (Robinson, 1974; Van der Graaf, 1981; 1985) and appears to be controlled by major genes on three different loci (Van der Vossen and Walyaro, 1980). The three genes have been identified in the varieties Rume Sudan *(R* and *k* genes), Hibrido de Timor *(T* gene) and K7 *(k* gene) (Van der Vossen & Walyaro, 1980). Similarly, the Catimor variety has also been shown to possess the *T* gene of resistance present in Hibrido de Timor (Agwanda et al., 1997). The moderately resistance variety K7 carries only the recessive k-gene (Vossen and Walyaro, 1980). The variety Pretoria also has *k*-genes (Omondi et al., 2001). The three genes of resistance have since been exploited in the Kenyan breeding programme either in pursuit of pure line varieties or for production of hybrid cultivars (Agwanda et al., 1997). The hybrid variety, Ruiru 11 and three pureline varieties, Batian 1, 2 and 3, are products of these strategies. Ruiru 11 was released to farmers in 1985 while the Batians were released in 2010.

The four varieties combines the superior quality attributes of the elite breeding lines as well as CBD and CLR resistance genes originating from Hibrido de Timor, Rume Sudan and K7.

Success in breeding for resistance to CBD was enhanced by availability of an efficient method for early screening for resistance through hypocotyl inoculation on 6-week old seedlings developed by Van der Vossen *et al*., 1976. For many years, selection for resistance to the disease has either been based solely on the seedling inoculation method or both on seedling inoculation and field expression of resistance on mature trees (Van der Graaff, 1981; Agwanda *et al*., 1997). The seedling inoculation method has contributed significantly by shortening the time required to identify resistant progenies from crosses involving resistant and susceptible donors. However, its efficiency becomes limited when a breeder is interested in accumulating a number of resistance genes into an improved cultivar, since this would require test crossing. Given the long generation cycle (five years) characteristic of Arabica coffee, the test cross approach is highly time-consuming and thus represents a real bottleneck to rapid development of varieties resistant to CBD. In view of this, Coffee Research Foundation embarked on the use of molecular markers which not only facilitate the pyramiding of resistance genes through marker-assisted selection, but are also useful in selecting against the genetic background of the donor varieties (Agwanda *et al*., 1997). This approach remains among the current focus at CRF and some candidate markers have been identified (Agwanda *et al*., 1997; Gichuru 2007) with search for more continuing.

BREEDING FOR RESISTANCE TO CLR

Coffee Leaf Rust (CLR) caused by the obligate parasitic fungus *Hemileia vastatrix* is a major disease which greatly limits Arabica coffee (*Coffea arabica* L) production in almost all growing countries around the world (Prakash *et al*., 2004, Hindorf and Omondi, 2010; Gichimu, 2012). Although it is rather difficult to estimate precisely the global impact of this disease, the economic damage to world Arabica coffee production has been estimated to be between 1 and 2 billion US Dollars per year (Van der Vossen, 2001) due to crop losses of 20–25% (Prakash *et al*., 2004). Chemical control of CLR by use of fungicides is expensive leading to high production costs and is not safe to humans and environment (Gichuru *et al*., 2008; Gichimu, 2012). In view of the economics and to minimise the chemical input for disease management, the development and cultivation of tolerant cultivars is the most effective and viable option (Gichimu, 2012). Therefore, the development of coffee varieties resistant to CLR has been one of the major breeding objectives in many countries including Kenya ((Prakash *et al*., 2004; Gichimu, 2012; Gichuru *et al*., In Press). The symptoms of the disease are characterized by a dusty or powdery coating of yellow uredosori covering the underside of the coffee leaves (Silva *et al*., 2006).

Breeding for resistance to CLR took into consideration the worldwide distribution of the disease and the multiple races of the pathogen. In 1955, the governments of the United States of America (USA) and Portugal established the Coffee Rust Research Centre (CIFC) in Oeiras, Portugal to coordinate CLR research without the risk of spreading new rust races to producing countries. Resistance to CLR is inferred from Flor's Gene-for-Gene concept, which states that for every major gene-conditioning resistance in the plant, there is a corresponding gene-conditioning virulence in the pathogen (Flor, 1971). The resistance genes in the host are designated 'SH' while the virulence genes in the pathogen are designated 'v' (Hindorf and Omondi, 2010). The CLR resistance is conditioned by at least 9 resistance genes designated as SH1–SH9 (and others not yet identified), either singly or in combination, while the corresponding virulence genes have been indicated as V1–V9 (Bettencourt and Rodrigues 1988; Hindorf and Omondi, 2010). Of the 9 resistance factors, SH1, SH2, SH4 and SH5 have been found in *C. arabica*. The other genes, SH6, SH7, SH8 and SH9, have been introgressed

from the diploid species *C. canephora*, while SH3 probably originates from another diploid species, *C. liberica* (Gichimu 2012). So far, there are 49 races of the pathogen that have been characterized the world over (Gichuru *et al*., In Press).

The resistance genes identified in *C. arabica*, used either singly or in combination, have not provided durable resistance to most of the races of rust fungus. In contrast, the SH3 gene from *C. liberica* as well as certain genes from *C. canephora* has provided long-lived protection under field conditions (Van der Vossen, 2005). In a collaborative effort between CIFC and Arabica coffee-producing countries around the world, several varieties resistant to rust were developed. The most notable variety that was introduced in most countries was the Colombian Catimor, combining CLR and CBD resistance and compact growth (Castillo and Moreno, 1988; Hindorf and Omondi, 2010). In Kenya breeding for rust resistance has been combined strategically with breeding for CBD and present effort is to simultaneously work on these two diseases so that all varieties developed will be resistant to the two fungal diseases. The breeding programme discussed earlier in this paper was effectively used to breed for resistance to both diseases. The release of Ruiru 11 hybrid variety and three pure line varieties, Batian 1, 2 and 3, all of which combines resistance to both diseases (Gichimu *et al*., 2010) is an output of this strategy. The adoption of these varieties by farmers has led to drastic reduction in use of fungicide sprays and eventual high returns to farmers.

SELECTION FOR HIGH YIELDS AND QUALITY

The duration of a breeding programme in arabica coffee *(Coffee arabica* L.) to produce new cultivars resistant to important diseases, largely depends upon the efficiency of selection for yield since methods of early selection for disease resistance are already available (Walyaro and Van der Vossen, 1979; Agwanda *et al.*, 1997). In the early past, higher productivity in Arabica coffee was achieved by straight selection for yield taken over considerable number of years (Carvalho and Monaco, 1969). Later studies demonstrated that there exists a high correlation between some growth characters and yield as well as plant vigour and yield (Walyaro and Van der Vossen, 1979). Indirect selection for yield potential is now possible when the first two years' data for growth, plant vigour and yield components are considered (Gichimu and Omondi, 2010). For quality, Van der Vossen (1973) observed that a minimum of 2 years of production is required to assess the bean size and cup quality factors in coffee.

The breeding programme in Kenya was strategically designed to enable development of varieties that combines resistance to the major diseases of economic importance with high yields and quality. Several varieties including Hibrido de Timor, Bourbon, K7, Rume Sudan, N39, SL4, SL34 and SL28 were used as progenitors to fully utilize the available genetic variability. A number of promising trees combining CBD and CLR resistance with plant vigour, high yield and good quality were selected from progenies of the multiple crosses. These were used in a programme of backcrosses to the best local cultivars (SL28 and SL34) and subsequent selfing to fix the genes. Currently, marker assisted backcrossing using available markers for diseases resistance efficiently enables rapid restoration of yield and quality after the target gene has been successfully transferred.

EMERGING CHALLENGES AND CURRENT FOCUS

The increase of greenhouse gas emissions (carbon dioxide and methane) in the atmosphere is causing wide changes in atmospheric events, influencing climate change and variability with critical impacts on coffee production. These include, shifting of optimal growing zones, changes in rainfall (amount and distribution), changes in dynamics of crop diseases and pests, changes in crop yields and quality, loss of agricultural land due to either rising sea levels

and/or desertification (Kimemia, 2010). Presently, coffee breeding in Kenya focuses on development of varieties with tolerance to abiotic stresses such as draught, salinity and high temperatures. Methods for early selection for tolerance to such stresses are already being explored targeting a variety that would combine this with other desirable traits such as disease resistance, yield and quality.

Apart from CBD and CLR, Bacterial Blight of Coffee (BBC) caused by *Pseudomonas syringae* pv *garcae* is another and the only bacterial disease of economic importance in Kenyan coffee. The spread of the disease is highly restricted, being present mainly in Brazil and Kenya (Silva *et al*., 2006). For a long time, BBC was restricted to the west of the Great Rift Valley in Kenya (Kairu 1985). The disease is however, gaining importance since it is endemic in areas with great potential for coffee expansion as land becomes scarce in the traditional coffee growing areas in Kenya. In addition, with the current shifts in weather pattern caused by climate change, the disease is becoming more widespread. Although copper based fungicides are recommended for BBC control, these sprays become less effective as infection pressure increases (Mugiira *et al*., 2011). Other challenges associated with chemical control approaches include high costs, phytotoxicity and residual effects of the fungicides (Abera *et al*., 2011). More so, as the hectareage of coffee covered by CBD resistant cultivars increases, BBC epidemiology may be expected to change as the use of fungicides drastically reduces. Previous studies have identified some Arabica coffee genotypes with resistance to *P. syringae* pv *garcae*. They include Catuaí x Icatu derivative IPR 102, Catucaí, Icatu and Hibrido de Timor (Ito *et al*., 2008). Breeding against the disease forms part of the current and future prospects of coffee breeding in Kenya and these introductions could therefore form the basis of this objective.

Efforts to improve the genetic base of resistance are applied during and after the development of disease resistant varieties but this is being challenged by the narrow genetic base of Arabica coffee and diverse variation within the pathogen. Isolates from the same or different geographic origins have been found to vary in aggressiveness and or pathogenicity (Firman and Waller, 1977; Masaba and Van der Vossen, 1978; Van der Vossen, 1985; Omondi *et al*. 2000). This shows the importance of re-evaluating existing varieties from time to time with an aim of identifying lines with broad based resistance. It has long been recognized that more durable forms of disease resistance can be devised if there is better knowledge of both the dynamics of the pathogen populations and the factors that determine host resistance or susceptibility. However, with climate change phenomenon, these factors are constantly and drastically changing and are becoming increasingly difficult to understand or to cope with.

When studying *C. kahawae* isolates from Kenyan cultivars, Omondi *et al*. 2000 observed some variation in aggressiveness among isolates but no differential pathogenicity was observed and hence no physiological races for *C. kahawae* were detected. The absence of races could be as a result of the pathogen co-evolving with genetically narrow based *C. arabica* species forming the bulk of the varieties grown in Kenya (Omondi *et al*. 2000). There are recent cases of CBD in infection on varieties hitherto considered resistant thus showing some weakened resistance probably caused by changes in climate, increased variation in pathogen virulence and/or pathogenicity. For *Hemileia vastatrix* recent work by Gichuru *et al*. (In Press) using Kenyan isolates have found that there are six (6) new races (III, XVII, XXIII, XXXVI, XLI and XLII) carrying three new virulence genes (v_1, v_7, $v_{8)}$ and possibly v_9. This represents a serious threat to CLR resistant varieties including Hibrido de Timor and as well as resistant commercial varieties in the country. This calls for identification of new sources of resistance and application of gene pyramiding to ensure durable resistance.

Apart from the challenges related to the actual breeding, other drawbacks are related to multiplication of new varieties for distribution to growers. Despite their wide acceptance by the farmers, full adoption of the new varieties has been limited by lack of sufficient planting materials to meet the national demand. This challenge is bigger in Ruiru 11 that in Batian because the former is an F1 hybrid hence the limitation of seed production which is the preferred method of propagation. Efforts to supplement seed production with vegetative propagation using clonal cuttings and mass propagation through tissue culture have not matched the high demand. The highest demand for planting materials was observed in 2010/2011 propelled by good coffee prices experienced during this period.

CONCLUSIONS

In Kenya considerable success has been made in coffee breeding to improve yields, quality and to manage the two economically important fungal diseases, CBD and CLR. However, emerging issues such as climate change have brought up new challenges which require to be addressed to ensure sustainability in coffee production. The challenges faced forms the basis for future prospects/approaches to develop cost effective and sustainable coffee varieties that enhance yield and quality.

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Cultivation of Perennial Herbaceous Legumes in Weed Management in Coffee Plantation on the Cerrado

J. C. F. SANTOS[1], A. J. DA CUNHA[2], F. A. FERREIRA[3], R. H. S. SANTOS[3], N. S. SAKIYAMA[3], P. C. DE LIMA[4]

[1]EMBRAPA Café, Sede EMBRAPA Final, Av. W/3 Norte, 70.770-901, Brasília, DF
[2]UNICERP Univ.Cerrado Patrocínio, Av. Líria Lassi S/N, 38.740-000, Patrocínio, MG
[3]UFV Universidade Federal de Viçosa, Depart. de Fitotecnia, 36.570-000,Viçosa, MG
[4]EPAMIG Empresa de Pesq. Agro. MG, UFV-Vila Gianetti, 36.571-000, Viçosa, MG

SUMMARY

The intercropping of legumes with coffee plants is an alternative practice for soil cover and weed management. The objective of this work was to evaluate herbaceous legumes cultivation on the weeds in coffee crops in the Cerrado (Brazilian savannah). The experiment was set up in a Catuai coffee crop at eight years of age and with a3.80 x 0.70 m spacing. It was used a random block experimental design four replicates and ten treatments in a 4 x 2 + 2 factorial scheme, which was as follows: four legumes (forage peanut (*Arachis pintoi*); java hybrid (*Macrotyloma axillare*); perennial soybean (*Neonotonia wightii*) and wild ground nut (*Calopogonium mucunoides*)); two planting forms in the interrows of the coffee plantation with two and three rows of legumes spaced by 0.50 and 0.25 m, respectively. The two additional treatments consisted of hand weeding with a hoe and chemical control with glyphosate. Wild ground nut and forage increased soil coverage in the first and second year, respectively. Java hybrid maintained the greatest biomass yield in the two years, with the wild ground nut being the highest in the first year. The legumes decreased density and biomass of the weeds when compared with the additional treatments. Java hybrid, wild ground nut and forage peanut in the first year and java hybrid and perennial soybean, followed by forage peanut in the second year, decreased density and biomass of the weeds. Cultivation of two or three rows of legumes did not differ from each other in the evaluations. The cultivation of perennial herbaceous legume reduces the weeds of coffee plantation on the Cerrado.

INTRODUCTION

The soil cover with legumes provides improvements of the soil conditions and weed control with reduction of the costs for decrease of the use of fertilizers and of herbicides. The perennial herbaceous legumes contrary the annual ones present the advantage of good capacity have grown out after the pruning and to maintain the permanent vegetable of the soil cover. That practice requests determination of the best of coexistence system of those species with the coffee plant and the weeds, whose research was to evaluate the influence of perennial herbaceous legumes in the weed control and in the coffee plantation of the savannah.

MATERIALS AND METHODS

The experiment consisted of ten treatments, implanted only time in the factorial outline of 4 x 2 + 2. The first factor was composed by the perennial herbaceous legumes forage peanut (*Arachis pintoi*); java hybrid (*Macrotyloma axillare*); perennial soybean (*Neonotonia wightii*) and wild ground nut (*Calopogonium mucunoides*). The second factor was formed by two and three rows of legumes spaced by 0,50 m and 0,25 m respectively. The additional treatments

were the hand weeding (hoe) and the chemical control (glifosato). It was used a random block experimental design with four replicates in 40 plots. The treatments were applied in the two interrows of the plot of three lines of seven coffee plants with spacing of 3,80 x 0,70 m, being useful the five central plants. The zone of 0,80 m on each side of the coffee plants row was maintained cleans through of hand weeding, being conserved the distance of 20 cm of this weeding zone for the legume. The soil cover of the legumes were evaluated in the rainy period in november and february for the method of intersections of strings in a picture, forming a net of same squares, told for, adapted for a plastic net of (2 x 5) m, formed by a drained group of 200 squares, disposed in the center of each interrow of the plot. The soil cover is the sum of the numbers of squares on the vegetation of the legume. The biomass of the legumes were evaluated in the rainy period in november and february by the sampling of the study of the plants population, extracted of the sample of 0,5 m² of the plot, resulting from the picture of wood of 0,25 m² thrown in the two interrows. The density and biomass of the weeds were evaluated for two years, in the rainy period in november and february by the same sampling of the study of the plants population.

RESULTS AND DISCUSSION

In the soil cover, the legume wild ground nut was superior in the first year and the forage peanut had the higher covering in the second year (Table 1). The wild ground nut in spite of the fast establishment was shown little tolerant to the drought period and with difficult have grown out after the mechanic control. The forage peanut, although of the slower establishment is tolerant to the drought, creeping plant and of the vegetative propagation, it presents higher capacity have grown out after the pruning. The java hybrid and the perennial soybean stayed with tax would intermediate of covering of the soil, being both aggressives, could cause competition with the coffee and limitations in the crop. The cultivation of two or three rows of legumes did not influence the soil cover, the weed infestation and the coffee plantation.

Table 1. Soil cover with perennial legumes in the coffee plantation.

Treatments	**Soil cover (%)**	
Factors	**2007/2008**	**2008/2009**
Legume		
Forage peanut	60,50 b	92,25 a
Java hybrid	71,00 b	72,50 b
Perennial soybean	62,25 b	69,75 b
Wild ground nut	90,25 a	44,75 c
DMS	14,88	16,65
Row		
Two	72,85 a	67,80 a
Three	69,15 a	71,83 a
DMS	13,09	14,33
CV (%)	28,71	30,48

Means followed by different letters inside of each factor differ amongst themselves for the tukey test 5%.

Java hybrid maintained the greatest biomass yield in the two years (Table 2) due his fast growth, habit of plant creeper and good capacity have grown out after the pruning. The perennial legumes presented the greatest biomass yield in the second year.

Table 2. Biomass of the perennial legumes in the coffee plantation.

Treatments	**Biomass legumes (kg/ha)**	
Factors	**2007/2008**	**2008/2009**
Legume		
Java hybrid	1560 a	4460 a
Perennial soybean	1020 b	3430 b
Wild ground nut	1670 a	2320 c
Forage peanut	630 c	1110 d
DMS	339	583
Row		
Two	1095 a	3030 a
Three	1345 a	2630 a
DMS	295	513
CV (%)	43,81	35,26

Means followed by different letters inside of each factor differ amongst themselves for the tukey test 5%.

The legumes decreased density of the weeds when compared with the additional treatments (Table 3), reinforcing the potential of those species of they be used as soil cover in the weed control. The java hybrid stayed superior in the two years with higher weed control, in reason of the constant soil cover and highest biomass yield. The forage peanut together with the java hybrid in the first year and in second place in the second year, it promoted suppression of weed, although on this second year, have shown highest tax of soil cover and smaller biomass yield than the java hybrid. The wild ground nut, that had low infestation of the weed in the first year, allowed a superior infestation in the second year, maybe for her decrease of the soil cover and smaller biomass yield.

Table 3. Weed density in two years of intercropping of the bearing coffee crop with perennial legumes.

Treatments	**Weed Density (plants/m²)**	
Contrasts(1)	**2007/2008**	**2008/2009**
Additional	8,88	13,29
Legume	5,50^{A}	6,91^{A}
Hand weeding	8,75^{ns}	15,68
Chemical control	9,00	10,90^{A}
Factors(2)		
Legume		
Java hybrid	3,25 a	3,57 a
Perennial soybean	10,00 b	4,52 a
Forage peanut	5,00 a	7,98 b
Wild ground nut	3,75 a	11,55 c
DMS	2,29	2,76
Row		
Two	5,65 a	6,80 a
Three	5,35 a	7,02 a
DMS	2,03	2,45
CV (%)	25,28	22,97

Análise of contrast: ^{A}= significant; and ns = no significant, for the test F to 5% of probability. Means followed by different letters inside of each factor differ amongst themselves for the tukey test 5%.

The legumes decreased of the weed biomass when compared with the additional treatments (Table 4), reinforcing the capacity of those species of soil cover in inhibiting the development and growth of the weeds. It was observed that the java hybrid was shown superior in inhibiting in the two years, the biomass yield of the weeds.

Table 4. Weed biomass in two years of intercropping of the bearing coffee crop with perennial legumes.

Treatments	**Weed biomass (g/m²)**	
Contrasts [(1)]	**2007/2008**	**2008/2009**
Additional	18,35	22,71
Legume	11,79[A]	15,18[1]
Hand weeding	18,89	25,06
Chemical control	17,80	20,36[A]
Legume		
Java hybrid	6,72 a	9,00 a
Forage peanut	11,82 b	14,46 b
Perennial soybean	20,15 c	10,96 a
Wild ground nut	8,44 a	22,24 c
DMS	2,14	2,31
Row		
Two	11,80 a	14,22 a
Three	11,77 a	14,10 a
DMS	1,13	1,23
CV (%)	11,93	10,64

Análise of contrast: [A]= significant; and ns = no significant, for the test F to 5% of probability.

The forage peanut stands out for maintaining constant as second legume with potential of inhibition of the weeds density and biomass during the two years. The productivity of the coffee was not affected by the legumes.

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Herbaceous Legumes Intercropping in Weed Management of the Bearing Coffee Crop

J. C. F. SANTOS[1], A. J. DA CUNHA[2], F. A. FERREIRA[3], R. H. S. SANTOS[3], N. S. SAKIYAMA[3], P. C. DE LIMA[4]

[1]EMBRAPA Café, Sede EMBRAPA Final, Av. W/3 Norte, 70.770-901, Brasília, DF
[2]UNICERP Univ.Cerrado Patrocínio, Av. Líria Lassi S/N, 38.740-000, Patrocínio, MG
[3]UFV Universidade Federal de Viçosa, Depart. de Fitotecnia, 36.570-000,Viçosa, MG
[4]EPAMIG Empresa de Pesq. Agro. de MG,UFV-Vila Gianetti, 36.571-000, Viçosa,MG

SUMMARY

Weed control in coffee crops demands alternative practices which contribute towards the sustainability of coffee business. The objective of this work was to determine the influence of herbaceous legumes on weeds and on coffee culture. The experiment was set in Viçosa, MG, in a Catuaí coffee crop at 19 years of age and 10 years of pruning, with 3 x 1 m spacing.It was used a random block experimental design with four replicates, consisting of eight treatments in a 3 x 2 + 2 factorial scheme, with three legume species: forage peanut (*Arachis pintoi*), siratro (*Macroptilium atropurpureum*) and lablabe (*Dolichos lablab*) and two different planting forms in the crop interrows with two and three rows of legumes spaced by 0.50 and 0.25 m apart, respectively. The two additional treatments consisted of hand weeding using a hoe and chemical control with glyphosate. It was found that the legume lablabe at 90 and 120 DAP provided the greatest soil cover, the greatest predominance of the vegetation on the weeds and the least weed infestation. Lablabe and forage peanut presented the highest biomass yield in the first year and in the second year, respectively. The greatest reduction of the density and biomass of the weeds was promoted by lablabe and siratro in the dry season and with no differences between them in the rainy period in the first year and by forage peanut in the second year. Cultivation of two or three rows of legumes did not differ among each other for soil cover, weeds and coffee crop. The legumes increased soil moisture, reduced weed infestation in the first year and increased coffee yield in the last harvest when compared to the additional treatments. There were no differences in soil moisture and coffee cultivation among the legumes and among the additional treatments. Herbaceous legumes intercropping in bearing coffee crop reduces weeds, being an alternative for integrated weed management.

INTRODUCTION

The legumes consortium with perennial cultures consists of a alternative management that provides the soil cover and suppression of weeds, reducing impact, service and weeding cost. The vegetation or residues of covering plants interfere in the germination and in the growth of weeds, for the physical effect of competition that reduces the favorable environmental factors, or for the chemical effect of the alelopatic for influence of secondary compositions. That work of consortium of herbaceous legumes with the coffee crops aimed at to evaluate the effects in the covering of the soil, in the suppression of weeds and in the coffee crops.

MATERIALS AND METHODS

The experiment consisted of eight treatments, implanted only time in the factorial outline of 3 x 2 + 2. The first factor was composed by the herbaceous legumes forage peanut (*Arachis pintoi*), siratro (*Macroptilium atropurpureum*) and lablabe (*Lablab purpureus*). The second factor was formed by two and three rows of legumes spaced by 0,50 m and 0,25 m respectively. The additional treatments were the hand weeding (hoe) and the chemical control (glifosato). It was used a random block experimental design with four replicates in 32 plots. The treatments were applied in the two interrows of the plot of three lines of seven coffee plants with spacing of 3 x 1 m, being useful the five central plants. The soil cover, the predominance of the legumes and the infestation of the weeds were evaluate to the 90 and 120 DAP (Dias After Planting) for the method of intersections of strings in a picture, forming a net of same squares, told for, adapted for a plastic net of (1 x 6) m, formed by a drained group of 100 squares, disposed in the center of each interrow of the plot. The soil cover is the sum of the numbers of squares on the vegetation of the legume. The predominance of the legumes on the weeds is the sum of the numbers of squares of soil cover for the legume without the presence of the weeds. The infestation of the weeds is the sum of the numbers of squares on all the weeds. The biomass of the legumes was evaluated in may and december in the two years by the sampling of the study of the plants population, extracted of the sample of 0,5 m² of the plot, resulting from the picture of wood of 0,25 m² thrown in the two interrows. The density and biomass of the weeds were evaluated every two months, for two years, in the dry and rainy period for the same method of the study of the plants population, extracted of the sample of 0,5 m² of the plot, resulting from the picture of wood of 0,25 m² thrown in the two interrows.

RESULTS AND DISCUSSION

The forage peanut and the lablabe to the 90 or 120 DAP provided the greatest soil cover in the establishment phase. The forage peanut it allowed higher infestation of the weeds and the lablabe provided the higher predominance of the vegetation on those plants (Table 1). Those results resemble each other to of the evaluation of the species and time of legume management on the weed infestation of the coffee culture, whose lablabe to the four months, provided the greatest suppression.

Table 1. Soil cover and predominance of the legumes intercropping in weed management of thebearing coffee crop, march and april of 2008.

Treatments	Cover (%)		Predominance (%)		Infestation (%)	
	90 DAP	120 DAP	90 DAP	120 DAP	90 DAP	120 DAP
Legume						
Forage peanut	78,0 a	86,9 a	51,1 b	55,8 b	40,0 a	44,8 a
Siratro	64,1 b	72,4 b	54,0 b	67,6 b	23,3 b	14,8 b
Lablabe	85,0 a	92,5 a	81,8 a	90,9 a	6,9 c	3,8 c
DMS	13,4	14,1	13,5	15,1	9,9	9,7
Row						
Two	73,1 a	84,9 a	60,8 a	72,8 a	23,1 a	21,2 a
Three	78,3 a	82,9 a	63,8 a	70,0 a	23,7 a	21,0 a
DMS	9,0	9,5	9,1	10,2	6,7	6,6
CV(%)	16,41	15,42	19,66	18,95	30,98	30,40

Means followed by different letters inside of each factor in the column differ amongst themselves for the Tukey test 5%.

In the establishment phase the perennial legumes present slow growth taxes, compared with the annual legumes. In the first year the lablabe presented higher production of biomass and in the second year the forage peanut it was superior (Table 2). The higher production of biomass of the lablabe in the first year it is justified by treating of a legume of fast and inconstant growth. That legume had smaller resistance to the dry period and as plant creeper was stimulated by the shadow of the coffee and reducing the production of biomass the following year. The forage peanut and the siratro although initially slow, they possess uncertain growth and knocked down, with better distribution of their foliages and leaves closer of the soil. In the first year it is waited that the biomass of those species is low, meantime with greatest resistance to the dry period and greatest conservation of humidity of the soil, it increases capacity have grown out and the production of biomass, tending to be higher the following year.

Table 2 – Biomass (kg/ha) of the herbaceous legumes in the first and second year in intercropping with bearing coffee crop, Viçosa, MG, 2008 e 2009.

Treatments	**Year 2008 Biomass (kg/ha)**	**Year 2009 Biomass (kg/ha)**
Legume		
Forage peanut	456,64 b	1543,58 a
Siratro	598,46 b	1057,76 b
Lablabe	1250,04 a	270,63 c
DMS	352,48	289,88
Row		
Two	758,91 a	956,60 a
Three	777,85 a	958,05 a
DMS	236,04	194,12
C.V. (%)	35,30	23,30

Means followed by different letters inside of each factor in the column differ amongst themselves for the Tukey test 5%.

The forage peanut, in the first year, it allowed greatest density and biomass of the weeds, mainly in the dry period, while in the second year that legume was superior to the others in the reduction of that infestation and biomass in the two periods (Table 3). That reduction provided for the forage peanut in the second year it is reinforced by, whose legume, in two years of cultivation with coffee, it controlled the weeds in a satisfactory way, equal or better than the chemical and mechanical control.

Table 3 - Density and biomass of the weeds in the dry and rainy period in two years of intercropping of the bearing coffee crop with legumes, harvest 2008/2009 and 2009/2010.

Treatments	First Year 2008/2009		Second Year 2009/2010	
	Dry Period	Rainy Period	Dry Period	Rainy Period
Contrasts [1]			**Density (plants/m^2)**	
Additional	3,20	5,84	2,46^{ns}	3,05^{A}
Legumes	2,76^{ns}	4,29^{A}	3,99	4,23
Hand Weeding	3,54	6,45	3,97	3,76
Chemical Control	2,86^{ns}	5,24^{ns}	0,95^{A}	2,34^{ns}
Legume [2]				
Forage peanut	4,47a	4,66a	2,76b	2,51b
Siratro	2,55b	4,44a	4,51a	4,66a
Lablabe	1,27b	3,77a	4,70a	5,53a
DMS	1,37	1,36	1,69	1,34
Row [2]				
Two	3,01a	4,49a	4,01a	4,28a
Three	3,11a	4,12a	4,16a	4,55a
DMS	0,92	0,91	1,14	0,90
CV (%)	40,19	23,79	39,37	28,14
Contrasts [1]			**Biomass (g/m^2)**	
Additional	2,45^{ns}	8,49	2,70^{ns}	3,14^{A}
Legumes	3,00	5,76^{A}	4,71	4,83
Hand Weeding	3,31^{ns}	9,26^{ns}	4,33^{ns}	3,93^{ns}
Chemical Control	1,59	7,72	1,07	2,36
Legume [2]				
Forage peanut	5,21a	5,90a	3,25b	2,44b
Siratro	2,73b	6,02a	4,91ab	5,41a
Lablabe	1,07c	5,37a	5,97a	6,63a
DMS	1,63	2,18	2,16	1,73
Row [2]				
Two	3,31a	6,05a	4,88a	5,05a
Three	3,58a	5,48a	4,80a	5,23a
DMS	1,10	1,47	1,46	1,16
CV (%)	49,54	27,64	43,36	32,41

[1]*Análise of contrast:* [A]*= significant; and ns = no significant, for the test F to 5% of probability;*

[2]*Means followed by same letter in the column they don't differ amongst themselves in the Tukey test 5%.*

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Coffee Berry Borer (*Hypothenemus hampei*) Traps Assessment

M. ROJAS, H. ROJAS, G. GUERRERO, J. MORALES, C. DELGADO

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica. E-mail: mrojas@icafe.cr

SUMMARY

The use of traps to monitor and control the coffee berry borer in Costa Rica is a recommended practice of integrated pest management. This practice has been embraced and its technology has been validated with very good results in the field, in the countries of the region. Between 2006 and 2011, four trials were conducted to assess the capture efficiency of different trap types, their contribution to pest control and maintenance cost reduction.

A randomized complete block design with at least five replicates (traps) was used per treatment, installing each trap in an area of 500m^2 and using the methanol+ethanol attractant in a 3:1 relation. The trap methods assessed were funnel traps (traditionally used in Costa Rica), crafty bottle trap, two dry capture prototypes, glue traps and the malathion impregnated tube to kill the coffee berry borer *(tubo mata broca)*. The number of coffee berry borer insects captured per trap was calculated every two weeks in the water devices or at the end of every trial in the devices not using water as a trapping mean. Attack samplings at 90, 120 and 150 days after the main bloom, were conducted in the trials where the coffee berry borer killing tube was assessed, taking two samples of 100 fruits at random, per plot, from branches in similar age located at 5-6 m of the trap.

The results indicated that the captures from the disposable-bottle traps, the big funnel prototype without water and the glue trap were the same as those of the multiple funnel. However, the glue trap captured other types of insects, as well, such as Hymenoptera and Diptera, demonstrating that this is a not-specific trap. The trials assessing the attack in treatments with funnel traps and coffee-berry-borer killing tubes showed that none of the devices reduced pest damage significantly in relation to treatments without traps.

The bottle trap is a good option for small farms that can obtain the containers and give them the appropriate maintenance. The devices without water could be a good option for big farms, since they would be able to save in maintenance costs for big areas.

INTRODUCTION

Ethological control is a component of integrated management of the coffee berry borer in Costa Rica and consists of placement traps for females that survive to harvest, so is a complement to these practices. Its use allows monitoring the behavior of insect flight, defining the most appropriate times for spraying, identifying sites with the highest population and decreasing it to reduce damage to the next crop.

The development of the coffee berry borer trapping began in 1991 with the work of Mendoza in Brazil. After that investigations have been conducted in several countries to evaluate designs, colors, diffusers and alcohols release rates, location and height, effect of

environmental conditions and crop phenology, number of traps per unit area and trapping costs (Rodríguez et al. 2009).

In Costa Rica it was determined that multiple funnel trap white, with release of 240-300 mg/day of methanol: ethanol 3:1, placed at 1.5 m height in an area of 500 m^2, was very effective for monitoring and control of coffee berry borer, leading to reduce the levels of attack by 58% (Borbón 2007).

The aim of this work was to evaluate the capture efficiency of different types of traps, their contribution to pest control and reduce maintenance costs.

MATERIALS AND METHODS

Four trials were established in different regions of Costa Rica. In 2006 in Platanares of Pérez Zeledón, in 2007 in Barva, in 2011 in Pejibaye of Pérez Zeledón and Naranjo. All devices tested used the lure of methanol + ethanol in 3:1 ratio and placed at 1.5 m high covering an area of 500 m^2.

In Platanares used an unrestricted random design with 5 replicates (traps). Were compared multiple funnel trap with the disposable container of 2 L with two openings and a new prototype collector funnel with dry container. In Barva used an unrestricted random design with 10 replicates (traps). Were compared multiple funnel trap with dry collector and the plastic screen with adhesive (50 x 30 cm). In Pejibaye and Naranjo used a randomized block design with 10 replicates (traps). Multiple funnel was compared with a control without trapping and the "tubo mata broca" impregnated with malathion.

Was quantified the number of females captured per trap every two weeks on devices that used water, or at the end of each trial on devices that did not use water as a means of capture. In trials which evaluated the "tubo mata broca" was sampled CBB attack at 90, 120 and 150 days after main flowering in branches located in 5-6 m of the trap.

RESULTS AND DISCUSSION

In Platanares, the cumulative trial showed higher trap captures in disposable container and lower in the dry funnel screenless (Table 1). Under the study conditions, the traps would have captured between 140 000 and 180 000 females per hectare (Rojas et al. 2008). Barrera et al. (2008) indicate that handmade traps are widely used for monitoring and control of the coffee berry borer because of its low cost and capture efficiency. Add to PET traps contribute to recycling, are cheaper and have proven to be more efficient to capture CBB.

In Barva there were no statistical differences between the three models tested (Table 2), although each trap screen with rubber captured on average 671 diptera and 156 hymenoptera during the period, showing be little specific (Rojas et al. 2008). Borbón (2007) evaluated small and large screen traps, and reported that they were inefficient in capturing CBB compared with funnel traps.

The pest attack after flowering in Pejibaye showed no significant difference ($p < 0.05$) between treatments for any of the dates evaluated (Table 3). By contrast, Dufour (2007) using 17 traps BROCAP per hectare in 15 farms in El Salvador, obtained a reduction in the infestation of 84.6% in the first year and 87.1% in the second year, compared to the control lots. On this difference, the same author indicates that in full sunlight trapping the number of

females captured is very low, without much effect on the control and adds that to improve the results of trapping, you must associate the sanitary harvest, for ensure more effective control.

Table 1. Average females captured per trap between March and May 2006 in Platanares of Pérez Zeledón.

Trap type	Females captured
Multiple funnel	28914
Disposable container	35611
Dry funnel	23853
Dry funnel with strainer	29319
Lower limit (5% error)	25574
Upper limit (5% error)	33275

Table 2. Average females captured per trap between April and June 2007 in Barva. Kruskall-Wallis 1%.

Treatment	Females/trap	Effect
Multiple funnel	3269	ns
Dry funnel	3187	
Plastic screen with adhesive	4330	

In Naranjo was presented a gradual increase in the percentage of attack, which doubled from July to September (Table 4). From July to August the increase was lower in the "tubo mata broca", even statistically different ($p < 0.05$), but the effect was not sustained any longer and reached the other treatments in September. This may be related to the loss of effect of the insecticide impregnated cardboard tube.

The bottle trap is presented as a good choice for small farms, that they can get the containers and give proper maintenance to the traps. Meanwhile, the dry traps could be a good alternative for large farms without the cost of maintaining of large areas. Trapping for monitoring the pest will remain a strategy that will contribute to integrated pest.

Table 3. Percentage of CBB attack by treatment at 90, 120 and 150 days after flowering in Pejibaye, 2011.

Treatment	Attack (%)± s.d.		
	June 9	July 11	August 22
Control	4,8±2,2 ns	3,8±1,6 ns	4,7±2,7 ns
Multiple funnel	4,4±1,7	3,5±2,0	5,4±2,7
Tubo mata broca	3,3±3,1	4,1±2,7	5,0±3,4

Different letters in the same column indicate significant difference between treatments (Test LSD Fisher Alpha: 0.05). The percentage data were corrected to √ (x) +1 for statistical analysis.

Table 4. Percentage of CBB attack by treatment at 90, 120 and 150 days after flowering in Naranjo, 2011.

Treatment	Attack (%)± s.d.		
	July 5	August 4	September 2
Control	3,3±1,1 ns	6,9±2,4 a	7,9±6,6 ns
Multiple funnel	3,4±1,2	8,1±3,4 a	8,0±3,2
Tubo mata broca	3,0±1,8	4,8±2,8 b	8,4±5,2

Different letters in the same column indicate significant difference between treatments (Test LSD Fisher Alpha: 0.05). The percentage data were corrected to √ (x) +1 for statistical analysis.

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Efficiency of *Beauveria bassiana* as part of the Integrated Pest Management of Coffee Berry Borer in Costa Rica

M. ROJAS, G. GUERRERO, J. MORALES

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica. E-mail: mrojas@icafe.cr

SUMMARY

The entomopathogenic fungus *Beauveria bassiana* has proven to be one of the most important biological control agents of the coffee berry borer worldwide and has been incorporated as another element for integrated pest management in Costa Rica. Several trials for coffee berry borer management were conducted in different regions of the country between 2005 and 2011 using a preparation of *B. bassiana* native to Costa Rica with rice substrate and oil to evaluate its efficiency at the field.

Some of these experiments were located in Buenos Aires (2005), Orosi (2007), Barva (2008) and Santa Bárbara de Heredia (2011), at 630, 1000, 1180 and 1225 m.a.s.l., respectively. A randomized complete block design with five replicates and plots of 12 plants was used. For each trial, 400 L/ha were applied when most of the coffee berry borer was located in the penetration channel, with a dose of 1 X 10^{12} spores/ha. Mortality was assessed at 15, 30 and 45 days after application through dissection of samples of 25-30 fruits damaged by the coffee berry borer per Plot.

In Buenos Aires, mortality increased after application, reaching a maximum peak of 27 percent after 40 days. In Orosi, the mortality data, corrected according to the test plot, showed a peak of 27.4 percent a month after application. The experiment in Barva included the application of the fungus produced by liquid fermentation prepared with oil and it was concluded that under monitoring conditions, related to the insect's position in the fruit, both preparations controlled more than 55 percent of the insects. By increasing the dose to 5.5 x 10^{12} spores/ha, mortality reached 82 percent with the rice preparation and 67 percent with the oil preparation. In Santa Barbara, the coffee berry borer mortality caused by the fungus increased up to 20 percent 40 days after application, having the same control level with both preparations.

INTRODUCTION

The coffee berry borer (*Hypothenemus hampei*) is the most important insect pest on coffee plantations in Costa Rica and its management should be integrated and sustainable. It should include cultural control practices such as regulation of shade, coffee tree pruning and weed control, which hinder the multiplication of the pest and facilitate the harvest. These practices also complemented ethological control during the flight of the insect and biological control with the application of the fungus *Beauveria bassiana*.

The Instituto del Café de Costa Rica (ICAFE) assessed native isolates of *B. bassiana* have been found attacking the coffee berry borer in different parts of the country, characterizing genetically and subjecting them to tests of aggression against the CBB, resistance to ultraviolet light and reproducibility, among other (Echeverría et al. 2007). In this way it was

possible to identify the best strain of the fungus to reproduce in the CICAFE laboratory and offer it at no cost to all coffee producing country.

In order to evaluate the effectiveness of the fungus at the field level, between 2005 and 2011 were conducted several trials to control CBB applying *Beauveria bassiana* native of Costa Rica in substrate made of rice and oil in different regions.

MATERIALS AND METHODS

During the years 2005, 2007, 2008 and 2011 tests were conducted CBB control with *B. bassiana* native of Costa Rica made on rice substrate in different regions. The experiments were located in Buenos Aires, Orosi, Barva and Santa Bárbara de Heredia, at 630, 1000, 1180 and 1225 masl, respectively. The design was randomized blocks with five replications and 12 plants useful plots. For application in each trial used a volume of 400 L/ha and was conducted when most CBB was located on channel penetration, with a dose of 1×10^{12} spores/ha. In Barva evaluated the dose of 5.5×10^{12} spores/ha under normal field application and fruit marked.

In Buenos Aires the native strains A1-03 and Térraba were assessed. In Orosi was compared the effect of the number of applications. In Barva and Santa Bárbara the formulation in rice was compared with the oil formulation. In all trials, the mortality of CBB located in the penetration channel was assessed until 40-45 days after application, by dissecting samples of 25-30 affected fruits per parcel.

RESULTS AND DISCUSSION

In Buenos Aires increased mortality after the application to reach a peak of 27% after 40 days (Table 1). In Orosi mortality data corrected according to the control, showed a peak control 27% one month after the application (Figure 1). In Barva under normal field application control CBB was around 26% (Table 2) and under controlled position CBB in the fruit, mortality reached 82% in formulation with rice and 67% with the oil formulation (Table 3). In Santa Bárbara the CBB mortality at 40 days after the application was around 20%, showing no difference ($p < 0.05$) between the two formulations (Table 4).

In normal Rican coffee growing field, with use of regulated shadow and multiple blooms, the result of applying Beauveria bassiana native to 1×10^{12} spores/ha at 30-40 days later, usually exceeding 25% mortality of CBB located on channel penetration. Weather conditions and crop management for the proper development of the fungus can be changed for each situation, varying the level of control achieved by the entomopathogenic.

The fungus formulation in oil will improve the efficiency of the application and facilitate its preparation in the field, preserving the quality of the product and at least the same efficiency of control.

Table 1. CBB mortality at 12, 25 and 46 days after application (DAA) with B. bassiana formulated in rice. Buenos Aires, 2005. Rojas et al. 2006.

Treatment	Mortality (%)		
	12 DAA	*25 DAA*	*46 DAA*
Control	1,0 ns	0,0 c	4,0 b
Térraba	6,0	19,0 a	26,0 a
A1-03	12,0	11,0 ab	27,0 a

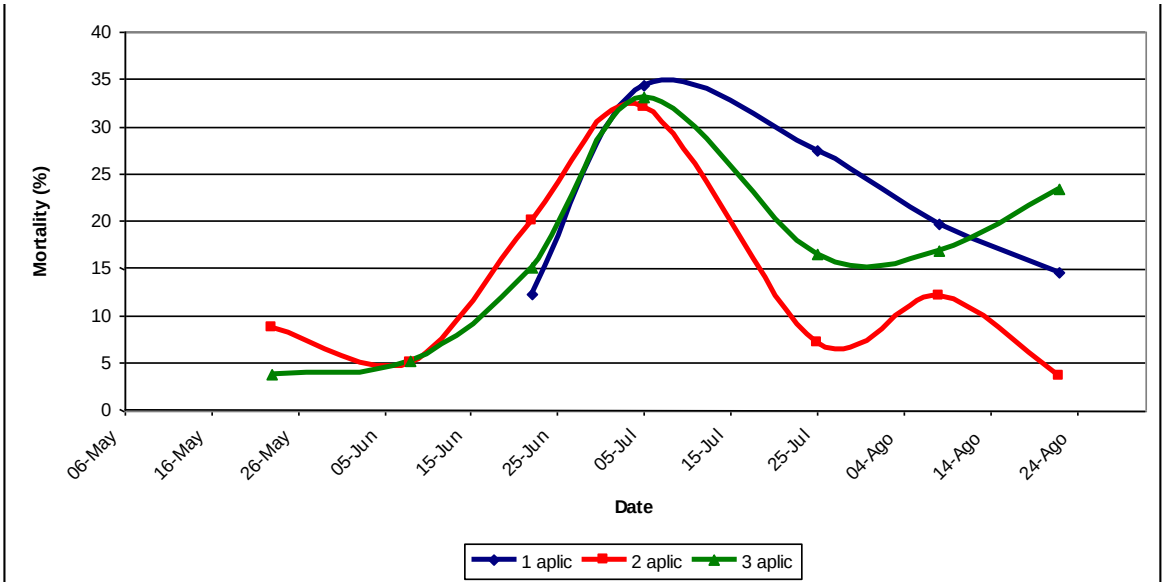

Figure 1. CBB mortality after applications with B. bassiana formulated in rice. Orosi, 2007. Data corrected according to the control (Schneider-Orelli). Rojas 2008.

Table 2. CBB mortality at 14, 27 and 41 DAA, in normal field conditions. Barva, 2008. Rojas 2010.

Treatment	Mortality (%)		
	14 DAA	*27 DAA*	*41 DAA*
Control	4,0 d	27,2 ns	17,0 c
Rice formulation	13,7 b	24,0	27,5 b
Oil formulation	8,7 c	16,0	26,3 b
Mycotrol 11 ES	19,4 a	20,0	36,0 a

Different letters in the same column indicate significant difference between treatments (LSD 5%).

Table 3. CBB mortality in fruits marked, 28 DAA. Barva, 2008. Rojas 2010.

Treatment	Mortality (%)	
	CBB spore	***Total***
Control	3,6	3,6
Rice 1,0 X 1012	44,4	55,6
Oil 1,0 X 1012	23,8	57,1
Mycotrol 1,0 X 1012	23,8	47,6
Rice 5,5 X 1012	63,6	81,8
Oil 5,5 X 1012	58,3	66,7
Mycotrol 5,5 X 1012	39,1	47,8

Table 4. CBB mortality at 20 and 40 DAA. Santa Bárbara, 2011.

Treatment	Mortality (%)	
	20 DAA	***40 DAA***
Control	5,3 b	8,4 b
Rice formulation	7,8 ab	21,0 a
Oil formulation	13,0 a	19,3 a

Different letters in the same column indicate significant difference between treatments (Test LSD Fisher Alfa: 0.05). The percentage data were corrected to √ (x) +1 for statistical analysis.

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Influence of Climate Changes on the Coffee Berry Borer (*Hypothenemus hampei*)

M. ROJAS

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica. E-mail: mrojas@icafe.cr

SUMMARY

The coffee berry borer is considered as the most economically serious pest in the cultivation of coffee, causing significant losses and increasing production and processing costs. Climate changes, mainly temperature and precipitation, affect the biological behavior of this insect. Temperatures raging from 20°C to 25°C (68°F to 77°F) contribute to the highest rate for insect growth and fecundity, while multiple blooms stimulated by slightly marked dry periods facilitate their survival and reproduction. Climate change projections indicate that temperature will increase and there will be changes in rainfall patterns, affecting coffee growing and changing pest behavior.

This paper includes information generated for several years on the different conditions of coffee growing in Costa Rica including dry matter accumulation in fruits and its association to the coffee berry borer attacks and the start of the insect's reproduction, duration of the insect's life cycle at different heights, population dynamics in remaining fruits and behavior of the insect's flight in different regions. Potential effects of climate change on coffee growing in Costa Rica are analyzed, and management recommendations to be implemented are provided. This study concludes that although environmental conditions change, the principle of pest control should be focused on keeping smallest population possible, and that may be achieved through the integrated pest management practices that have been recommended.

INTRODUCTION

The CBB has become the most economically important pest in coffee growing since its detection in Costa Rica in 2000. The climate has great influence on the biological behavior of the insect.

According to the IPCC macro climatic features are showing changes mainly in the last decade, excelling air temperature (Camargo and Marcelo 2008). Also due to the increase in temperature, the hydrological cycle is expected to be more intense, leading to very humid and very dry areas (Kumar 2010).

According to Porter (1991) and Watson (1997) cited by Villalobos and Retana (1999) some indirect effects of climate change would occur in populations of pests and diseases (migration, concentration, population flows, incidents, etc.). For example, Walyaro (2010) indicates that in East Africa the increase in temperature will increase the coffee berry borer infestation. Jaramillo et al (2009) based on its analysis of the climate in Ethiopia indicates that before 1984 was very cold for CBB complete one generation per year, but after that may complete one or two in that time period.

Guharay (2001) indicates that in years with lower rainfall multiplication of the CBB is greater, while Kumar (2010) reported that changes in the distribution pattern of the rains are causing problems in the spread and development of the pest primarily due to changes in crop phenology.

MATERIALS AND METHODS

Instituto del Café de Costa Rica has studied the development of the coffee fruit, on single and multiple flowering condition in 19 locations around the country, ranging in height from 550 to 1740 m. Regarding the duration of the life cycle of the coffee berry borer, trials have been conducted in nine localities, from 700-1740 m.

The behavior of insect flight has also been studied in different regions in order to identify periods of susceptibility of the pest in different environments and improve the integrated pest management. It also has monitored the population dynamics of the pest in the residual fruits of plant and ground, to study the impact of the population over the next harvest.

RESULTS AND DISCUSSION

The results indicate that under the conditions of Costa Rican coffee growing coffee berry borer can begin egg laying from 120 days after flowering (DAF) in the lowest zones and up to 172 DAF in the areas of greatest height (Figure 1A). For its part, in localities situated between 700 and 1150 m the cycle from egg to adult was completed between 40 and 45 days, while over 1200 m cycle took about 90 days to complete (Figure 1B).

The greater flight time of CBB is coincident in different growing regions of Costa Rica. In regions with prolonged dry period is concentrated the insect population and emerge in mass in a short period with the first rains. In areas that do not present a prolonged dry period, there is a more continuous emergence of females and a less marked peak flight (Figure 2).

In areas with Caribbean influence the largest populations of CBB in the fruits of the ground after harvest were presented between January and February. The maximum amount of biological stages ranged from 6 to 10 individuals per fruit. In drier areas the maximum population reached nearly 40 individuals per fruit and presented during the month of March (Figure 3). The early rains in these regions affect the multiplication of the pest in the residual fruits of ground, but stimulate blooms of coffee and insect flight in search of new fruits.

Under our conditions the coffee tree can reach a homogenous flowering with stimulation of a precipitation event greater than 10 mm, preceded by a dry period of about two months. If the rainfall pattern leans to short periods of drought and intermittent rain, the blooms will be scattered, shall fruit be in different stages of development and CBB will find ideal conditions for multiplication.

Considering the prospects of climate change, the temperature may increase several degrees over the next few years; so that today a coffee plantation is located at 1200 masl will experience the conditions of one which today stands at 1000 meters above sea level, if the temperature rises just 1 °C. Under this condition the pressure of CBB attack will increase considerably and control measures should be intensified.

Faced with the climate change and potential impacts they may have on the behavior of the coffee berry borer, we reiterate that the principle of pest control should be to keep the smallest population possible through integrated management practices that have been recommended. It

is impossible to control climatic variables of a site; therefore we must adapt management measures for the pest causes the least possible damage.

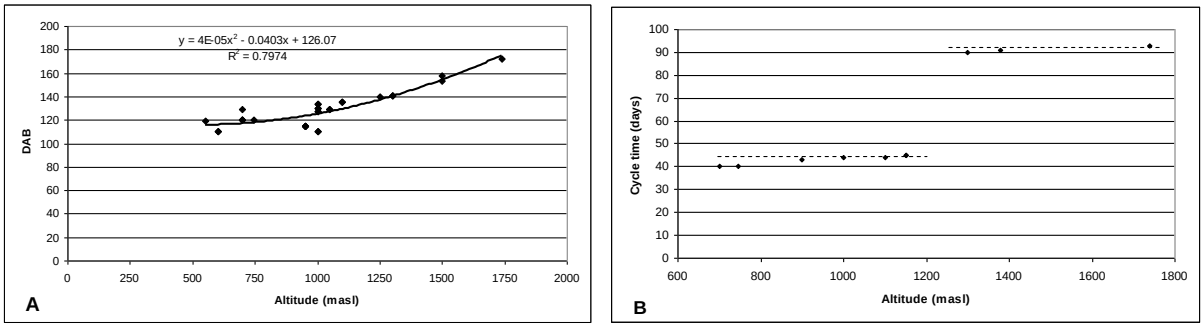

Figure 1. Influence of altitude on time for the accumulation of 20% of dry matter in the fruits of coffee (A) and the duration of the life cycle of the coffee berry borer (B) in Costa Rica.

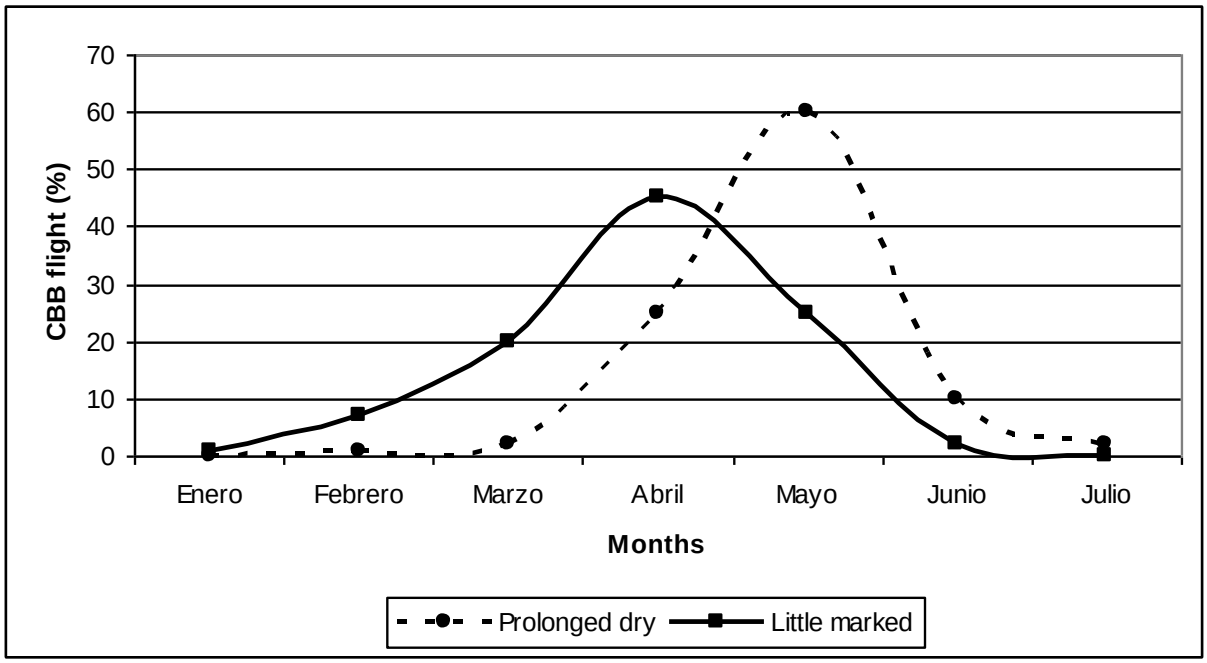

Figure 2. General behavior of the coffee berry borer flight under different weather conditions, prolonged dry period or little marked, after harvest in Costa Rica.

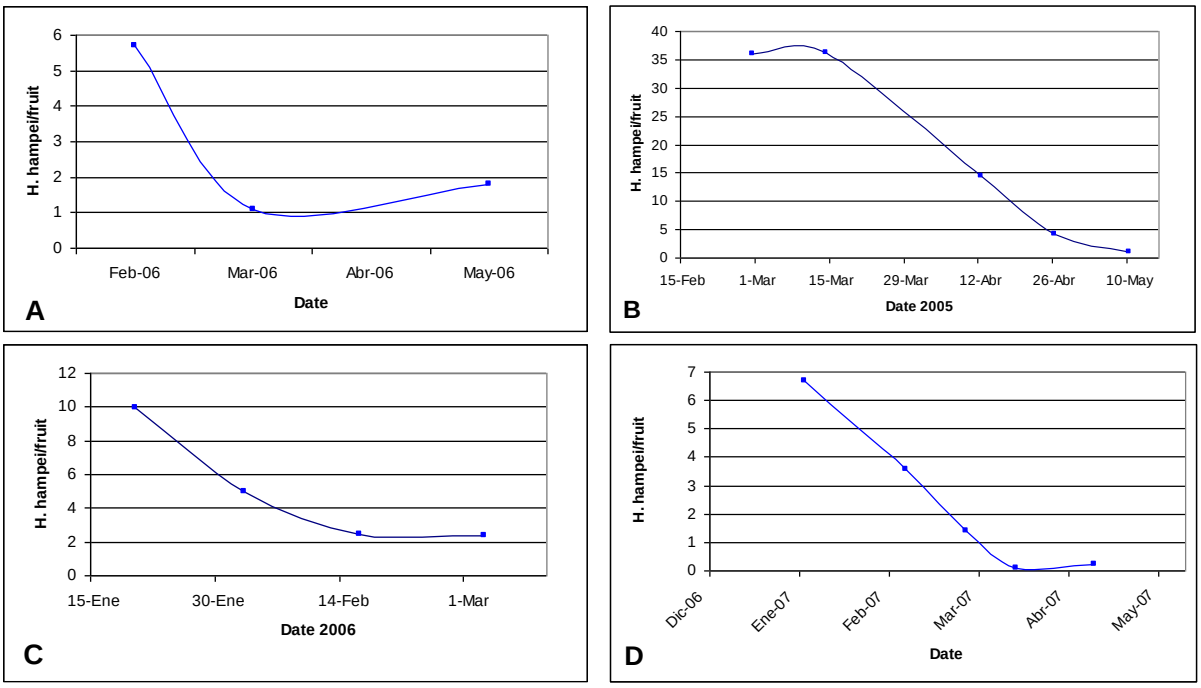

Figure 3. Coffee berry borer population per attacked fruit of ground, during the postharvest period in Turrialba (A and D) and Pérez Zeledón (B and C).

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Assessment of Alternative Insecticides to Control the Coffee Berry Borer (*Hypothenemus hampei*)

M. ROJAS, H. ROJAS, A. GAMBOA, J. OBANDO, G. GUERRERO, J. MORALES

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica

SUMMARY

The integrated management of the coffee berry borer includes the use of insecticides when it is strictly necessary; consequently, it is important to find environmentally friendly products for sustainable coffee growing. Under this premise, research has been conducted on chemical products that are a good economic, environmentally friendly and efficient option for pest control.

Between 2005 and 2011, more than 10 field trials have been carried out in different regions of Costa Rica to assess insecticides. Having endosulfan as reference, the biological efficiency of the following insecticides was evaluated chlorpyrifos, fipronil, profenofos+lufenuron, fenitrothion, chlorfenapyr, cypermethrin, imidacloprid, thiacloprid, spinosad, ethiprole, pirimiphos-methyl and imidacloprid+deltamethrin.

In the trials, there was used a randomized complete block design with five replicates, plots of at least 10 x 12m and applications with a motorized mist blower when the coffee berry borers are penetrating the coffee fruits. The assessments calculated the mortality of coffee berry borers located in the penetration channel, through dissection of 30-40 fruits infected with the coffee berry borer per plot, at least twice after the application.

Results from different trials have indicated control levels between 60 and 80 percent for chlorpyrifos, 40-67 percent for fenitrothion, 50-80 percent for fipronil, 20-69 percent for thiacloprid and 30-40 percent for pirimiphos-methyl. The control levels in the other insecticides assessed were very low and have not differed from the treatment without application.

INTRODUCTION

Integrated management of the coffee berry borer has been recommending the Coffee Institute in Costa Rica, includes the use of chemical control when strictly necessary, where it will present significant economic loss to the producer, but emphasizing the importance other practices to improve for the next crop. Under this guideline, and considering the ban of endosulfan is that research has been conducted on chemicals that constitute a good alternative economic, environmental and efficient control of the pest.

Several countries have investigated the chemical control of the coffee berry borer. Work in Colombia indicated that insecticides as pirimiphos methyl, fenitrothion, chlorpyrifos and fenthion are as effective as endosulfan, if the insect is penetrating the fruit (Villalba et al 1995). Honduras has reported good control of CBB with fenitrothion, fipronil, pirimiphos methyl and chlorpyrifos (Muñoz y Zelaya 1985; Muñoz y Trejo 1997). Brazil has reported the evaluation of a large number of insecticides to control the coffee berry borer, excelling

efficacy of fipronil, thiacloprid, imidacloprid and chlorpyrifos-ethyl as alternatives to endosulfan (Reis 2007).

The objective of the tests was to evaluate insecticides for control of the CBB under the conditions of Costa Rican coffee production.

MATERIALS AND METHODS

Between the years 2005 and 2011 were conducted more than 10 field trials to evaluate insecticides in different regions of Costa Rica. Referring to endosulfan, evaluated the efficacy of 12 biological insecticides to control the coffee berry borer (Table 1). In the design of trials used a randomized complete block with five replicates, plots of at least 10 x 12 m and power equipment applications where most of CBB was penetrating the fruit. Evaluations were performed by quantifying the dead CBB on channel penetration, to dissect samples of 30-40 fruits affected by plot, at least twice after the application.

RESULTS AND DISCUSSION

The results of the various investigations showed maximum levels of coffee berry borer mortality, 98% for endosulfan, 76% for chlorpyriphos, fenitrothion 75%, 84% for fipronil, thiacloprid 69% and 39% for pirimiphos methyl. Control levels of the other insecticides tested were very low and have not differed from control treatment (Table 1).

Table 1. CBB mortality achieved with different insecticides in Costa Rica.

Test Site	Year	Product	g.a.i./ha	Higher mortality (%)	Days after application
Buenos Aires	2005	Endosulfan	525	98	21
		Chlorpyriphos	907	69	7
		Fipronil	64	79	44
		Fipronil	166	84	44
San Pedro, P. Z.	2006	Endosulfan	525	88	15
		Chlorpyriphos	750	16	45
		Profenophos + Lufenurom	440	8	45
		Fenitrothion	750	22	15
		Clorfenapir	72	39	30
		Cypermethrin	75	35	30
Desamparados	2006	Endosulfan	525	92	7
		Clorpirifos	750	40	20
		Clorpirifos	960	76	7
		Profenofos + Lufenurom	440	12	7
		Fenitrothion	750	51	7
		Cypermethrin	75	18	20
CICAFE	2006	Endosulfan	525	80	7
		Profenophos + Lufenurom	220	3	45
		Profenophos + Lufenurom	330	4	45
		Profenophos + Lufenurom	440	6	45
		Profenophos + Lufenurom	550	6	7
CICAFE	2007	Endosulfan	525	88	7
		Chlorpyriphos	960	75	7
		Fenitrothion	750	75	7
Platanares, P. Z.	2008	Endosulfan	525	90	15
		Thiacloprid	100	69	15
		Imidacloprid	100	37	15
		Spinosad	50	37	15
Santa Bárbara	2010	Endosulfan	525	70	16
		Thiacloprid	96	26	16
		Ethiprole	200	8	16
		Pirimiphos methyl	750	28	16
Cajón, P. Z.	2010	Endosulfan	525	61	18
		Thiacloprid	192	52	18
		Ethiprole	200	10	18
		Pirimiphos methyl	750	39	18
Santa Bárbara	2011	Endosulfan	525	86	17
		Thiacloprid	96	17	17
		Fenitrotion	750	46	7
		Imidacloprid + Deltametrin	60+16	11	17
Cajón, P. Z.	2011	Endosulfan	525	86	14
		Thiacloprid	96	34	14
		Fenitrothion	750	43	7

Tracking infestation in branches showed marked residual effect of endosulfan, which is done very well until 45 DAA. Other insecticides such as fenitrothion, profenofos + lufenurom and ethiprole also residual effect achieved until 15 DAA (data not shown).

A study in Colombia found no significant difference in mortality when comparing endosulfan (98%) and chlorpyrifos (93%) 24 hours after the application, with the CBB in the penetration channel (Villalba et al 1995). In another study by Munoz and Trejo in Honduras was reported mortality of 85% with endosulfan (525 gai/ha) and 62% with fipronil (150 gai/ha) at 60 DAA. Villanueva (1984) did not report good control of CBB to use permethrin, decamethrin and cypermethrin.

So far it has evaluated a lot of insecticides in search of alternatives to the use of endosulfan in the country. Recently chlorpyrifos is registered for pest control and other companies are handling the registration of other products. We also study the product line with potential biological control of CBB and improved the method of evaluation of products, in order to take in the near term friendly alternatives with our coffee environment.

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Critical Density of *Meloidogyne exigua* in Adult Coffee Plants

M. ROJAS[1], L. SALAZAR[2], L. C. TORRES-MURILLO[1]

[1]Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica
[2]Universidad de Costa Rica, Laboratorio de Nematología

SUMMARY

The *Meloidogyne exigua* is the coffee root-knot nematode most commonly found in the Costa Rican coffee plantations, although its effect on the coffee yield is not clearly determined yet. This study aims at determining the critical density of *Meloidogyne exigua* in the adult coffee plants of the Caturra variety under semi-controlled field conditions.

The trial was conducted at the Center for Coffee Research (CICAFE), in Barva, province of Heredia, Costa Rica (24280 N and 521442 E Lambert Norte), at 1180 m.a.s.l., with a mean annual temperature of 21.5°C and a total annual rainfall of 2650 mm. Coffee plants of the Caturra variety formed in two axis, with a 2.0 x 1.0 m. planting distance, were planted in August 2009 in 100L pots with Dazomet-disinfected soil. A randomized complete block design with 10 replicates was used. The treatments were defined by the initial inoculum (Pi) of 125, 250, 500, 1000 and 2000 eggs +J_2 *M. exigua*/100 cm^3 soil, applied two months after planting. Adequate fertilization has been provided; in such a way that it would not be a limiting factor for development and production.

The plant development was evaluated in August 2010 and 2011 using height, stem diameter at the base, number of orthotropic nodes and branch length as indicators of each axis of the pot. Foliar samples were collected in September 2010, August and November 2011 for a complete chemical analysis of each plant. Soil and root samplings were collected in four blocks in September 2011, for the analysis of the *M. exigua* density. The 2011/2012 harvest was evaluated.

The evaluations of the four development variables showed no response to the initial inoculum of nematodes during the 2-year evaluation. The foliar analyses conducted in September 2010 and November 2011 indicated that the levels of elements were within the normal ranges and showed no response to initial inoculum of nematodes. The J_2 *M. exigua*/100 g root reported a strong relationship with the initial inoculum and regression clearly separated the highest Pi ($y = 0.0473x^2-36.776x+78087$; $R^2 = 0.9766$). This trial demonstrated a low density of *M. exigua* in the soil. There was no relationship observed between the first-harvest yield in kg/plant and the initial inoculum ($y = 8.7E-05x+7.06$; $R^2 = 0.0016$).

INTRODUCTION

Nematodes have been a significant obstacle to the production of coffee in Costa Rica. They are located in all growing regions and its spread has been mainly through the transfer of nursery plants to nematode unpopulated areas. The genera *Meloidogyne* and *Pratylenchus* are more spread out in the country (ICAFE, 1998).

The predominant species is *Meloidogyne exigua* in coffee plantations in Costa Rica (Villain et al. 1999; Alpízar & Alvarado 1999; Flores & López 1989 cited by Bertrand et al. 2000; Rojas 2008) and according Bertrand et al. (1998) its attack can lead to a decrease of 15% of the crop.

Barbosa et al. (2004) conducted a study in 125 coffee plantations with and without infestation of M. exigua, categorizing two ages of cultivation (less than 5 years and more than 5 years) and three levels of technology management according to the application of fertilizer and pest control. The field study indicated that M. exigua was not the major cause of decline in production in plantation with management level medium or low. In contrast, well-managed farms were intolerant populations as low as 3 J_2/100 cc of soil, with yield losses that reached 45%.

This study aims at determining the critical density of *Meloidogyne exigua* in the adult coffee plants of the Caturra variety under semi-controlled field conditions.

MATERIALS AND METHODS

The trial is set to the Centro de Investigaciones en Café (CICAFE), in Barva, Heredia (24280 N y 521442 E Lambert Norte), Costa Rica. The site is located at 1180 masl in an Andisoil, with average annual temperature of 21.5 °C and 2650 mm of total rainfall per year. The variety used is Caturra, with plants formed two axes, set to 2.0 x 1.0 m and full sunlight. It uses a design randomized complete block with 10 replications. The experimental unit comprises a potted plant. Treatments consisted of applying initial inocula of 125, 250, 500, 1000 and 2000 eggs+J_2 of *M. exigua*/100 cm^3 soil. The plants were established on August 25, 2009 in plastic barrels with approximate volume of 95 L and disinfected soil with Dazomet 97 MG (250 g/m^3). The pots were inoculated two months after transplantation.

During the first year (2009) the granular fertilization consisted of 30 g of 10-30-10 per plant at the time of planting, 30 g per plant of 18-5-15-6-0.2 in September and 30 g per plant of ammonium nitrate at the end of October. During the second year was fertilized with the formula 18-5-15-6-0.2 in May (40 g/plant) and August (60 g/plant) and 35 g of ammonium nitrate per plant in November. Fertilization of the third year (2011) was the application of 70 g/plant 18-5-15-6-0.2 in May and 100 g/plant in August, in addition to 53 g/plant ammonium nitrate in November.

In August 2010 and 2011 measured the height, stem diameter at the base, number of nodes in the orthotropic axis and along the branch number 8 from top to bottom in 2010 and 15th in 2011 on each axis of the pot. In September 2010, August and November 2011 were conducted chemical analyzes of leaves from each pot, taking 8 leaves of the third pair of productive branches of the middle third of each plant. In September 2011, soil and root sampling was performed in four blocks for density analysis of *M. exigua*. We assessed crop production 2011/2012 and was ranked the grain size.

RESULTS AND DISCUSSION

Development assessments conducted in 2010 and 2011 showed no response to the initial inoculum of *M. exigua* (data not shown). Foliar analysis September 2010 and November 2011 indicated that the elements were between normal ranges and showed no response to the initial inoculum (data not shown). Density analysis of *M. exigua* in root samples showed a curve responds to initial inoculum (Figure 1). It also showed the low density of *M. exigua* in soil (data not shown). The first crop production ranged between 5.97 and 8.39 Kg of fruit / plant

and the grain size percentage on sieve 16/64 between 68.33% and 77.50%, showing no relation with the initial inoculum ($p < 0.05$) (Table 1).

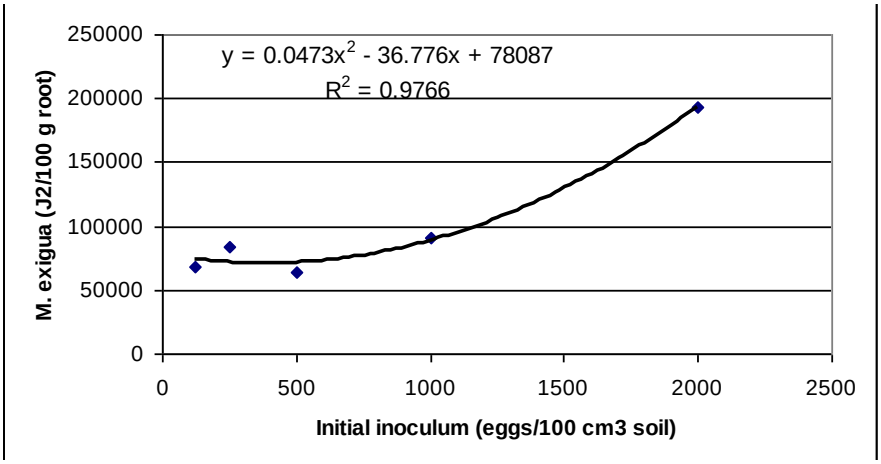

Figure 1. Response curve of the density of *M. exigua* in roots, relative to initial inoculum. September, 2011.

Table 1. Production of fruit at harvest 2011/2012 and percentage of grains above sieve 16/64 according to initial inoculum of *M. exigua*.

Eggs+J_2/ 100 cm^3 soil	Production (Kg/plant)	Above sieve 16/64 (%)
125	6,73	77,50
250	8,39	76,33
500	5,97	68,33
1000	6,81	74,83
2000	7,09	73,50
Regression	y = - 0,0003x + 7,3	y = - 0,0016x + 75,657
R^2	0,0518	0,03
p value	0,4745	0,3212

The initial inoculum of *Meloidogyne exigua* so far shows no clear effect on plant growth, nutrient content in leaves, production, and grain size. In a study about *M. exigua* critical density of nursery plants of Caturra, was found that nematode reduced height, stem diameter, number of orthotropic nodes and foliar weight, from initial populations of 2 eggs/substrate cm^3 (Rojas 2011). This indicates that the effect of the nematode on young plants is clear, but may be less important for adult plants. Baeza and Leguizamón (1977) indicate that the presence of Meloidogyne appears more critical during first months of development of the crop.

So far it is not clear the effect the pest population, so more time is warranted in order to relate the nematode population with potential losses it causes.

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Critical Density of *Meloidogyne exigua* in Coffee Nursery

M. ROJAS[1], L. SALAZAR[2]

[1]Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica
[2]Universidad de Costa Rica, Laboratorio de Nematología

SUMMARY

The purpose of this study was to evaluate the increasing density effect of *Meloidogyne exigua* on the development of nursery plants. In May 2009, coffee seedlings of the Caturra variety were transplanted in the cotyledon leaf stage of development, in two axes, in bags of 6" X 8" with 1335 cm^3 of substrate of soil + compost + rice chaff in a proportion 2: 1: 1 previously disinfected with dazomet to establish a trial with a randomized complete block design, with 11 treatments and 8 replicates. The treatments applied included initial populations (Pi) of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 eggs + J_2/cm^3 substrate applied one month after transplantation.

Height, stem diameter, number of orthotropic nodes and fresh weight were evaluated 234 days after inoculation. The model $y = m + (1-m)\ Z^{P-T}$ applied to the height, diameter, number of nodes and fresh weight indicated that the tolerance limit (T) was 0.66, 0.06, 0.13 and 0.88 eggs + J_2/cm^3 of substrate, respectively; the relative minimum yield (m) was 0.79, 0.79, 0.33 and 0.54 respectively, Pi equal to or higher than 64 eggs + J_2/cm^3 substrate.

The gall index, density of nematodes in the root and the reproduction rate were clearly higher than the plant control based on a Pi of 0.125 eggs + J_2/cm^3 substrate, while the density of nematodes in the soil was higher based on a Pi of 1 egg + J_2/cm^3 substrate. The gall index reached its maximum based on a Pi of 2; the maximum of nematodes in the soil and roots was reached with a Pi between 2 and 4; the maximum reproduction rate was 57 based on a Pi of 0.125. It was concluded that the nursery should be free of nematodes, and that even with a low Pi, the maximum population can be reached in a short time.

INTRODUCTION

The production of coffee nursery plants is carried out in all coffee growing regions of Costa Rica, where nematodes are also scattered, there is a risk of developing and carrying plants infested to field. Furthermore, the nursery is marketed under certain quality parameters that may be affected by *M. exigua*, resulting important to determine their effect on plant development.

In related studies, Ferreira and Crozzoli (1995) reported clear pathogenic effect of *M. exigua* on nursery plants of yellow Caturra, manifested by size reduction from initial populations of 16 eggs/cm^3 soil. They add that the air fresh weight and total fresh weight was reduced from 0.25 eggs/cm^3 soil.

The aim of the study was to evaluate the effect of increasing densities of *Meloidogyne exigua* on the growth of coffee nursery plants developed in pots outdoors.

MATERIALS AND METHODS

The trial was conducted outdoors at the Centro de Investigaciones en Café (CICAFE), Heredia, Costa Rica. The site is located at 1180 meters above sea level and has an average annual temperature of 21.5 °C and 2650 mm of total rainfall per year. Design was a randomized complete block with eight replications. The treatments consisted of initial populations of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 eggs+J_2/cm^3 substrate. The experimental unit consisted of a pot with two plants.

The Caturra variety plants were grown in polyethylene bags of 15 x 20 cm with 1335 cm^3 of substrate, containing soil+compost+rice husk ratio 2: 1: 1, previously disinfected with Dazomet 98% (250 g/m^3). Seedlings were transplanted with cotyledon leaves on May 2009 and a month later the inoculum was applied. The plants were fertilized with 6 g of slow release fertilizer 6-18-12 on June 4 and 2 grams of monoammonium phosphate on July 28, 2009.

The evaluation was performed 234 days after transplant, considering the height, diameter at the base of the stem, number of nodes in the orthotropic axis, air fresh weight, root fresh weight, gall index, population density of M. exigua in the roots and the substrate. The data were analyzed using the model $y = m + (1-m)\ Z^{P-T}$ (Seinhorst 1965) and response curves.

RESULTS AND DISCUSSION

The model $y = m + (1-m)\ Z^{P-T}$ applied to the variables height, diameter, number of nodes and air fresh weight indicated that the tolerance limit (T) was 0.66, 0.06, 0.13 and 0.88 eggs + J_2/cm^3 substrate, respectively, and performance relative minimum (m) was 0.79, 0.79, 0.33 and 0.54 respectively, with Pi greater than or equal to 64 eggs+J_2/cm^3 substrate (Figure 1).

The effect of *M. exigua* on height and diameter was very evident from initial populations close to 16 eggs/cm^3, agreeing with Ferreira and Crozzoli (1995) who worked on a similar test with yellow Caturra. Salas and Echandi since 1961 reported that M. exigua reduced the growth of nursery plants of 4 and 10 months at 34 and 45% respectively.

The variables of air fresh weight and number of nodes in the orthotropic were much more susceptible to nematode density, showing strong reductions in performance from initial populations of 2 eggs/cm^3 substrate. In this condition the air fresh weight was reduced more than 50% compared to uninoculated plants. Similar results were obtained by Ferreira and Crozzoli (1995), who reported a decrease in air fresh weight of nearly 60%.

In this study there were no signs of nutritional deficiencies or abnormalities in the color, even at the highest initial populations, which may be related to substrate quality and good nutrition. Other authors such as Figueroa (1974) and Gonçalves (1992) reported symptoms of malnutrition as chlorosis and dwarfism, related to the infestation of *M. exigua* in nursery plants.

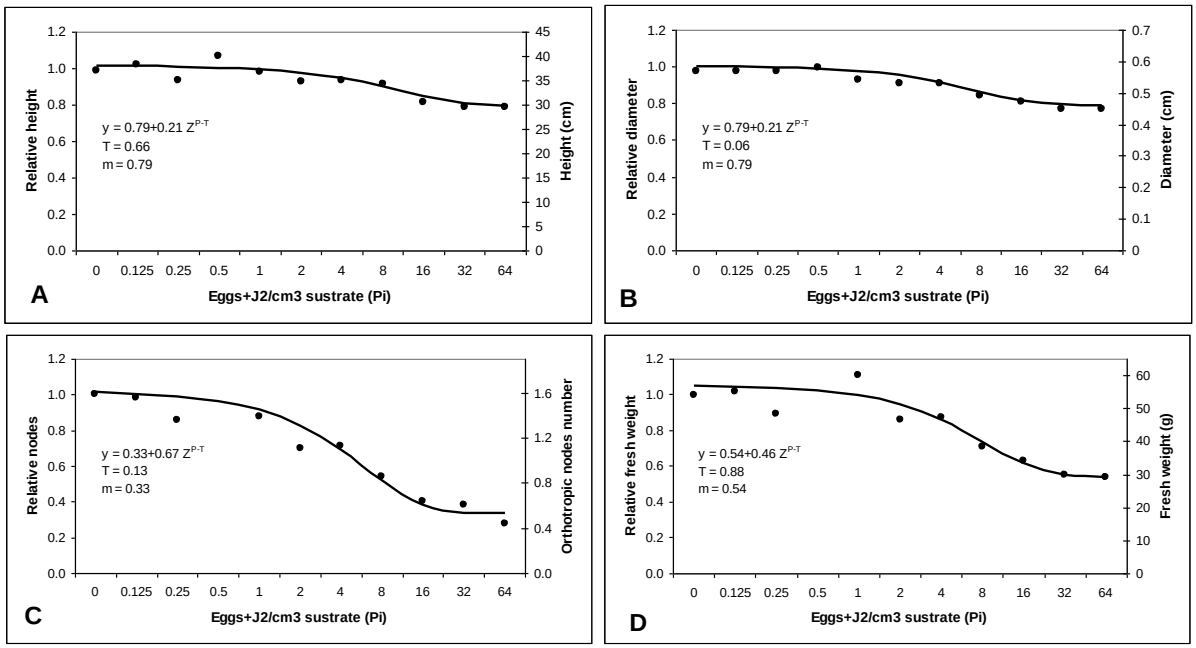

Figure 1. Effect of initial population (Pi) of M. exigua on the height (A), stem diameter (B), number of nodes in orthotropic axis (C) and the air fresh weight (D) of coffee nursery plants, 234 days after inoculation.

The root fresh weight was not clearly affected by the Pi (data not shown), but tended to be higher when there was greater density of nematodes in the root (Figure 2A) and soil (data not shown). The deformation caused by the nematode in the roots, evident in the high rate of galls (Figure 2B), may be related to this behavior.

The density of nematodes in the root and the reproduction rate were obviously higher than the control from a Pi of 0.125 eggs+J_2/cm^3 substrate (Figure 2C and 2D), consistent with Ferreira and Crozzoli (1995). The maximum population density of nematodes on the roots and the substrate was achieved with Pi between 2 and 4, while the maximum reproduction rate was 57, with Pi of 0.125 eggs+J_2/cm^3 substrate. The higher Pi had the lowest rates of reproduction. This indicates that the roots of nursery plants developed in a substrate with 2 eggs of *M. exigua*/cm^3 have the maximum galling when taking them to the field.

Thus, although the initial inoculum level is low in the soil where they develop a nursery, sooner or later the nematode population will reach the highest levels. These results have implications for the level of quality nursery plants sold, but the main problem is to bring plants infested field where the population will continue to grow in an uncontrolled way.

According to Gonçalves (1992), if the nursery plants they take to the field free of *M. exigua* and performs proper management of the plantation, the presence of the nematode in the field usually affects very little the production. Besides the use of nematicides in coffee farming is currently minimal, related with aspects of low effectiveness, restrictions by the certifying companies and high economic and environmental cost. Some aspects of prevention of infestation that may be mentioned are the seed and nursery establishment in areas free of the pathogen, prior sampling density of nematodes in the soil destined nursery development, chemical or thermal disinfection of the substrate, use of nematicides or biological control alternatives.

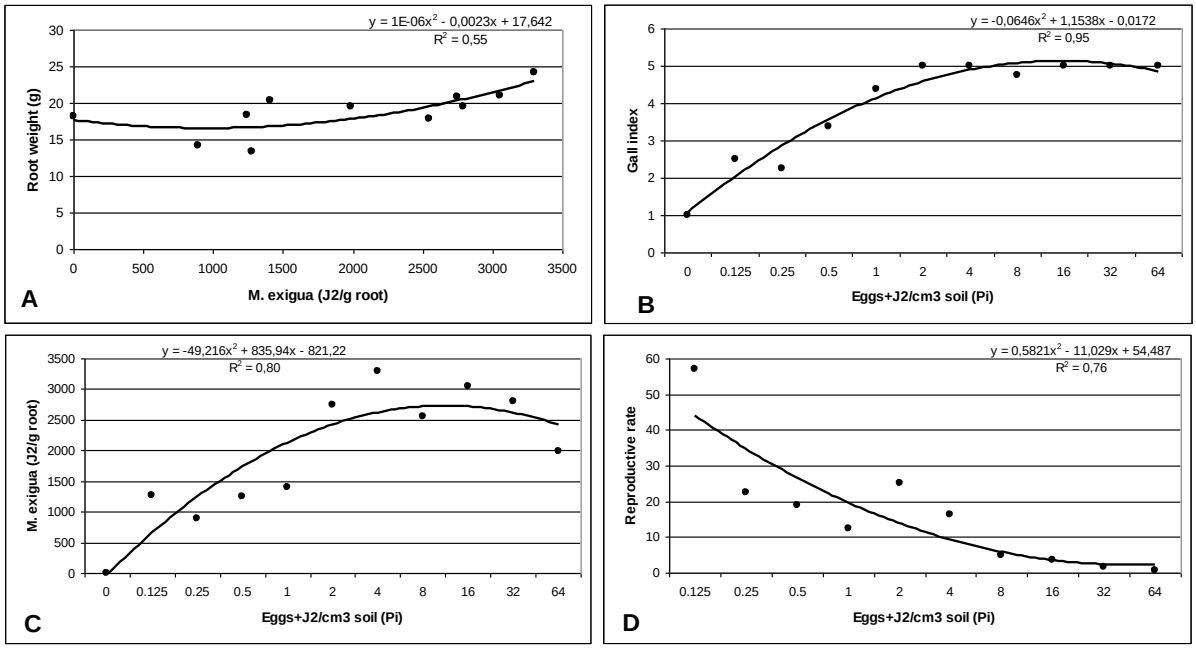

Figure 2. Effect of initial population (Pi) of *M. exigua*, on the fresh weight of root (A), gall index (B), density in the roots(C) and reproductive rate (D) in coffee nursery plants, 234 days after inoculation.

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The Coffee Berry Borer (*Hypothenemus hampei*) Control in Row Pruning

M. ROJAS, R. MESÉN, C. DELGADO

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica

SUMMARY

Assessments and a field trial were conducted with the purpose of studying the behavior of the coffee berry borer attack in plots with different pruning systems and validating the differentiated pest control in a row pruning system every three years. Samplings of the coffee berry borer attack were conducted during the rainy season in 2008 and 2009 in plots with different pruning systems in 29 sites of nine coffee growing locations of Costa Rica, including plots of 2-3 year total pruning and plots pruned per row every 3-4 years.

The field trial was conducted between May and August 2010 in a plot with row pruning every three years located in Pérez Zeledón. A paired t-test with seven replicates was used to compare the traditional insecticide application with the application only to the row with the oldest branches. Sampling of the initial attach was conducted per plot and age of row, before defining the insecticide application, assessing 100 fruits randomly taken per sample. After the differentiated application of the insecticide, assessments of the attack per plot and age of row were conducted at 30, 60 and 90 days after the application (DAA). At 90 DAA, 30 fruits infected with the coffee berry borer were dissected per plot and age of row to assess mortality.

The assessment of the coffee berry borer in sites where pruning is performed per plot every three years showed a reduction of 51 percent in the attacks to one-year plants in relation to the two-year plants. In plots where row pruning is performed every four years, attacks were 52 and 65 percent less in one and two-year plants than in three-year plants. A similar effect was observed in plots with row pruning every three years, where one-year plants had an average of 56 percent less attack than two-year plants.

In the field trial, the initial assessment showed that in average the three-year row was attacked by the coffee berry borer in 8.2 percent and the two-year row in only 2.2 percent, a significant difference of 73 percent. 90 DAA the percentage of fruits with coffee berry borer alive in the two and three-year row no indicated significant differences between the treatments. The differentiated control of the coffee berry borer in a pruning system per row performed every three years made it possible to save 30 percent of application time, 40 percent of insecticide and 36.5 percent of water used for aspersion, by making the application only to the 33 percent of the coffee plantation and without putting into risk the rest of the area cultivated.

It was concluded that pruning per plot and per row makes it possible to concentrate the coffee berry borer in small areas of the coffee plantation, which contributes to pest control management and investment reduction.

INTRODUCTION

The coffee berry borer attacks in the field can be distributed according to the age of the branches and the time of formation of the fruit, which in turn is influenced by the pruning system used. Few studies have been conducted to study the relationship between the coffee tree pruning system and CBB attack. The evaluation of the effect of pruning system on attack behavior provides valuable information to adapt management practices of the plague, as trapping and applications of *B. bassiana* or insecticide, seeking efficiency and profitability.

The aim of this work was to study the behavior of the coffee berry borer attack in plots of coffee with different pruning systems and validate differential control of the plague in a row pruning system every three years.

MATERIALS AND METHODS

Field sampling

Samplings of CBB attack were carried out during the rainy season of 2008 and 2009 in lots of varieties Caturra and Catuaí with different pruning systems, located in Poás, Juan Viñas, Orosi, Cachí, Turrialba, Pérez Zeledón, Barva, Heredia and Naranjo (Table 1).

The sampling method consisted in selecting 15 to 20 plants at random evenly distributed by lot, taking 100 fruits at random around and half height of each plant selected to calculate the percentage of fruits attacked by the plague. In the case of row-pruning, sampling was performed for each age branches independently.

Table 1. Assessment CBB attack carried out in batches with different pruning systems.

Evaluation	Method	Type of pruning	# sites
Branches 1 y 2 years	20 samples per batch	Lot with cycle every 3 to 4 years	6
Branches 1, 2 y 3 years	15 samples per age	Row with cycle every 4 years	8
Branches 1 y 2 years	15 samples per age	Row with cycle every 3 years	12

Assay of pruning per row

Was conducted during 2010 in Pérez Zeledón, at 615 meters above sea level, in an Ultisol with 3% slope, mean annual temperature of 24 ° C and 3000 mm of total rainfall per year. The variety used was Catuaí, with two axes formed plants, planting distance of 1.8 x 0.9 m, row-pruning every three years and regulated shade of *Erythrina poeppigiana*. Was used paired t test with seven replicates (Table 2). The total plot consisted of 162 plants (9 rows of 18 plants) and the useful by 20 plants.

Was evaluated initial attack CBB per plot and age of the row before defining the insecticide application, evaluating 100 fruits per plot at random. The insecticide (Endosulfan 35%, 525 g.a.i./ha) was applied according to treatment, using power equipment of backpack. Attack assessments were conducted per plot and age of the line at 30, 60 and 90 days after application (DAA). At 90 DAA 30 brocades fruits were checked per plot and age of the row to assess mortality. Data from attack and mortality percentage were transformed ($\sqrt{x}+1$) for statistical analysis.

Table 2. Treatments used to assess differential control of coffee berry borer with Endosulfan 35% (525 g.a.i./ha), in row-pruning system with cycle each three years, Pérez Zeledón, 2010.

Treatment	Application form	Applied plants/ha
1	Rows 1 and 2 years	4115
2	Row 2 years	2058

RESULTS AND DISCUSSION

Field sampling

The evaluation in sites with row pruning each three years, showed an average reduction of 56% of attack in the branches of one year compared to two years (Figure 1A). In plots with row pruning each four years, the branches of one and two years showed between 52 and 65% less attack than three years (Figure 1B). A similar effect was observed in batch pruning each three years, where the branches of one year had on average 48% less attack than branches of two years (data not shown).

Despite the increased attack that occurs in adjacent rows in a batch pruning (Castaño et al. 2005), systematic pruning offers the advantage of concentrating the coffee berry borer population in small areas. The branches of one and two years had low levels of attack that only need efficient harvest. Until the third year after pruning will experience greater attacks and then would apply stricter control measures after confirming its need through monitoring. In this way it allows direct control to a third or quarter of the coffee plantation instead of performing applications throughout the area and this contributes to the reduction of production costs and environmental pollution.

Under the conditions of Costa Rica and its concentrated harvest, at the time of pruning is performed has already concluded the harvest period. Therefore, the removal of the fruits of the plant at the end of the harvest is the work that must be done carefully to avoid major infestations in adjacent lots.

Assay of pruning per row

In the row of two years after pruning the CBB attack began around 8% and diminished to drop below 4.4% at 90 DAA in both treatments, no differences in any evaluation date (data not shown). In the row of one year the attack began between 1.6 and 2.7%, with a tendency to decrease in the treatment with application of insecticide in that row and increase where not was applied (Table 3).

The mortality caused by the insecticide at 90 DAA in the row of two years was around 30% in both treatments, while the percentage of fruits abandoned by the insect was maintained between 46 and 57%, amounting to between 78 and 86% and showing no significant differences ($p < 0.05$) between treatments (data not shown). The coffee berry borer mortality in row of one year, ranged between 11 and 27%, the abandonment from 45 to 58% and the sum of both variables between 70 and 73%, showing no differences between treatments (data not shown). The variable infestation did not exceed 1% in the different age row each treatment and did not differ between them (data not shown).

Assessments for 90 days after application, until shortly before the start of the harvest, showed that it is possible to apply insecticide only in 33% of the area of the plantation where the coffee berry borer attack exceeded 5%, without compromising the remaining cultivated area.

In addition to adequate control of the pest, with the differentiated application, in row pruning system each three years, was achieved a significant saving of time of application of the insecticide and the water used for aspersion (Table 4), in addition to the other associated costs (economic, environmental and health). Moreover, this system facilitates the harvesting work, recommended as part of integrated management.

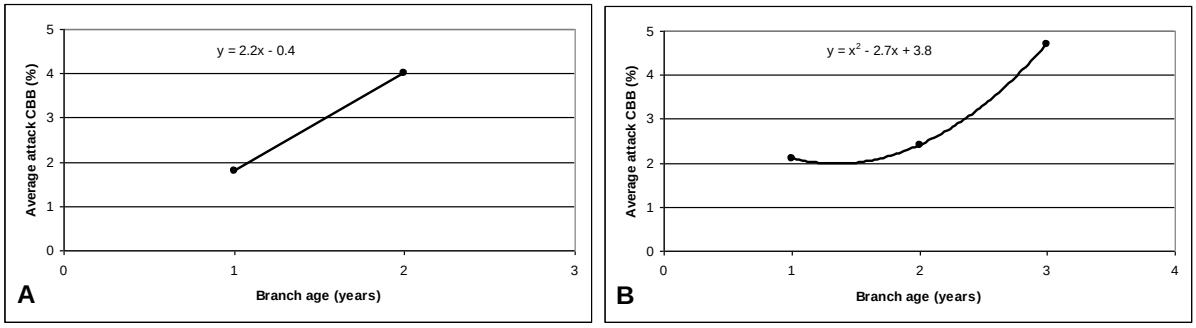

Figure 1. Percentage of CBB attack according to the age of the branches, in row pruning every three years (A) and four years (B).

Table 3. Percentage of CBB attack in row of one year according to insecticide treatment in row pruning every three years, Pérez Zeledón, 2010.

Treatment	Attack (%) days after application			
	0	***30***	***60***	***90***
Application rows 1 and 2 years	1,6 ns	2,7 ns	1,6 a	1,4 a
Application row 2 years	2,7	2,4	3,7 b	3,1 b

Different letters in the same column indicate significant difference between treatments (LSD Fisher Test Alpha: 0.05).

Table 4. Comparison of investment of time, insecticide and water per hectare according to the differentiated treatment of chemical control of CBB in row-pruning system every three years.

Treatment	Application time (hours/ha)	Dose (L c.p./ha)	Water volume (L/ha)
Application rows 1 and 2 years	1,43	1,0	315
Application row 2 years	1,00	0,6	200

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Study on Arabica Coffee Fruit Phenology and Bean Size in Relation to Agronomic Practice

Y. ALEMSEGED, S. G. TESFAYE

Ethiopian Institute of Agricultural Research, Jimma Research Center, P. O. Box 192, Jimma, Ethiopia

SUMMARY

Commercial coffee is processed and packed as green beans for various purposes, such as for processing, trade, and house hold use. Coffee bean size is primarily affected by its genetic characteristics, cultural practices applied and environmental factors.

Studies on coffee fruit phenology and bean size in relation to different agronomic practices were conducted at Jimma agricultural research centre using CBD resistant varieties for two cropping season.

Significant variation was observed between phenological stages for coffee fruit weight (fresh and dry) and estimated volume (length x breadth x Depth) during the growing weeks after anthesis. Generally, there exists two alternating and sinusoidal behaviors with very little growth (weeks 0-8), linear expansion (weeks 9-15), static (weeks 16-25), sigmoid stage (weeks 26- 32) and gradually decreasing growth and maturity stage (weeks 33-37).

On the other hand, open varieties had maximum values for both fruit and bean weight and volume. Bean size (length, breadth and depth) of the varieties was also affected by number of bearing heads per tree, fruit proximity on plagiothropic branches to words orthotropic main stem, branch age, shade level, population density and canopy nature.

It is speculated that genetic, seasonal climatic change and management practices dictate coffee fruit and bean size. Therefore, it is quite convincing that knowing the phenology of coffee fruit and factors affecting bean size would help to determine the optimum rate of nutrient application and irrigation scheme at time of stress and control berry drop, quality affecting factors and biennial bearing habit of the trees.

INTRODUCTION

Many physical properties of coffee beans are graded for various purposes in coffee biology, processing, trade and household use. Uneven roasting due to non uniform bean size is unique to Ethiopian coffee for which the country lacks dependable client in the international market. The non-uniformity of the coffee bean size is also the major problem even at the nursery level to get uniform germination and growth of coffee seedlings.

Coffee bean size is primarily affected by its genetic characteristics, cultural practices applied and environmental factors. In this regard, type of cultivar, age of the tree and the branch, fruit position on branch, fruit thinning, picking time, irrigation, pruning, number of bearing heads per tree, tree spacing, shade level, nitrogen fertilizer, copper fungicide spray, the amount of endosperm present and ratios between starch and crop level is the many attributes dictating coffee fruit and bean size.

Wormier and Njuguna demonstrated that coffee fruit comprised five growth phonologies: pin head, rapid fruit growth, endosperm growth, endosperm hardening and fruit ripening stages with approximately 0-7, 7-17, 12-19, 19-29 and 29-35 weeks after flowering, respectively. The period of rapid fruit growth is more dictating stage to bean size because at the end of this period the endocarp becomes lignified and physically restricts future internal expansion Dancer has shown that fruit size would benefit more from an ample moisture supply during the period of rapid growth stage.

As the rate of berry development determines the ultimate yield and crop quality, knowledge of coffee fruit phonology and critical stages when a stress could affect its normal growth would help optimize management and productivity of the crop.

This study was, therefore, conducted with the objective to take a systematic and quantitative measurement on coffee fruit at different developmental phases from early stage to maturity, so as to determine its growth pattern and the impact of prominent agronomic practices on bean size.

MATERIALS AND METHODS

The study was conducted at Jimma agricultural research center of the Ethiopian institute of agriculture. The center is found at an altitude of 1750 meter above sea level; with mean average rain fall of 1550 mm per annum and average maximum and minimum temperature are 25^0c and 11.2^0c, respectively.

Coffee fruit size (length, breadth and depth), volume and fresh and dry weights were measured at different fruit development stages for 37 consecutive weeks starting from anthesis (a week after flowering) until ripening. Besides fruit phenology, bean size was also studied in a randomized complete block design with four replications using different CBD resistant coffee cultivars grown under different management regimes including vertical number per tree, fruit position on branch nodes (from branch tip to orthotropic main stem), population density (trees per ha), branch type (primary, secondary and tertiary) and shade tree types.

A total of 120 fruits, 30 from each replicate of each cultivar, were collected and immediately taken to the laboratory to observe the cross sectional endosperm growth and fresh weight was taken using sensitive balance. The size of each fruit sample was measured as the longest side, length (L); the widest, breadth (B) and the cross sectional depth (D) using caliper. Fruit and bean dry weight was recorded after oven drying samples at 110 ^{0}C for 24 hours. Fruits and processed seeds from identified experimental fields were used to determine L, B and D and volume. Finally, the data were subjected to analysis of variance and Duncan Multiple Range Test (DMRT) to estimate the sensitivity of parameters to describe and define coffee fruit growth rates and bean size differences.

RESULTS AND DISCUSSION

Results of analysis of variance of phonological stages for coffee fruit weight (fresh and dry) and estimated volume (length x breadth x depth) during the growing weeks after anthesis showed highly significant variation during the growing weeks in all the coffee cultivar.

Accordingly, the rate of increase in coffee fruit size, fresh weight and volume followed a sigmoid type of growth with five distinct growth stages (Figure. 1 and 2) representing, Pin

head stage (weeks 1-8), a period with very little growth; expansion stage (weeks 9-15), a stage of rapid growth of future pulp and the integument for eventual parchment formation; endosperm growth (bean formation) stage (weeks 16-25); endosperm hardening stage (weeks 26-32), the period of accumulation of dry matter in the bean; and ripening or maturity stage (weeks 33-37), a stage of slight moisture increase with considerable decreasing expansion in size accompanied by change in color and subsequent moisture loses. This result agrees with the findings of Oybeda and Wormer and Nijuguna.

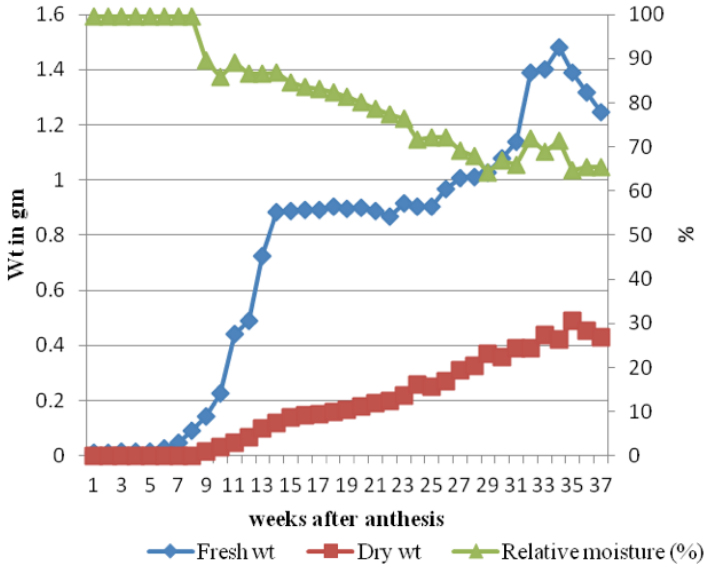

Figure 1. Coffee fruit weight change and relative moisture content.

Fruit dry weight increased linearly at a constant rate as time went on (Figure 1). This could be attributed to a constant rate of biomass accumulation in coffee fruits. Relative moisture content of the fruit decreased with increased fruit maturity (Figure 1). Furthermore, there was strong positive correlation (r = 0.89-0.99) between fruit growth parameters considered in this study.

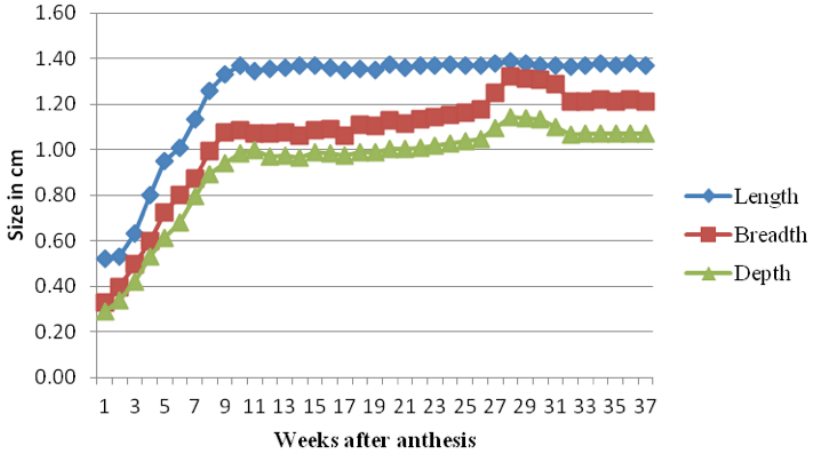

Figure 2. Coffee fruit size measurement.

Coffee bean length, breadth and depth were affected by number of verticals (bearing heads) per tree and fruit proximity on plagiothropic branch towards orthotropic main stem.

Accordingly, branch nodes nearer to the trunk (main stem) and trees with smaller number of bearing heads produced larger bean than those nodes at periphery of branch and trees with many verticals (Table 1). There were also significant differences in coffee bean parameters due to branch age and maximum values were recorded for beans grown on primary branches, followed by those on secondary tertiary branches. Branch types indicating differences in sink source relations on a tree (Table1) . In addition, differences in bean size among coffee lines were more associated with bean length and breadth than with depth as the respective mean values for these parameters were 0.900, 0.645 and 0.425 cm for compact type, 0.950, 0.695 and 0.435 cm for intermediate and 0.970, 0.625 and 0.430 cm for open cultivars. However, the effect of population density (Table 1), on bean parameters was inconsistent. On the other hand, coffee grown under moderate shade trees (Acacia, millettia and cordial trees) showed larger bean size than open and light shade. Different released coffee varieties also showed variation when screened on bean size screener (Table 2 &3).

Table 1. Coffee bean size as affected by number of bearing heads, fruit position on branch node, population density, and branch type and canopy class.

		Mean bean size (cm)			
Variant		Length (cm)	Breadth (cm)	Depth (cm)	Estimated volume (cm3)
Number of verticals (bearing heads)/tree	1	0.985	0.734	0.443	0.320a
	2	0.982	0.743	0.447	0.326a
	3	0.984	0.749	0.433	0.319a
	4	0.954	0.724	0.430	0.297b
	5	0.885	0.688	0.414	0.284b
	6	0.965	0.712	0.430	0.295b
	7	0.942	0.697	0.421	0.276c
Fruit position on branch nodes (from branch tip to the main stem)	1	0.88	0.69	0.40	0.243c
	2	0.89	0.68	0.40	0.236c
	3	0.91	0.68	0.41	0.254c
	4	0.94	0.67	0.42	0.265bc
	5	0.97	0.68	0.43	0.284ab
	6	0.99	0.69	0.43	0.294a
	7	0.99	0.70	0.41	0.284ab
Population density (trees/ha)	2500	0.945	0.685	0.424	0.274
	3265	0.938	0.683	0.428	0.274
	4444	0.948	0.670	0.424	0.269
	6400	0.938	0.678	0.425	0.270
	10 000	0.945	0.683	0.437	0.282
					NS
Branch type	Primary	0.97a	0.68a	0.43a	0.28a
	Secondary	0.93b	0.66a	0.42a	0.26a
	Tertiary	0.92c	0.60b	0.39b	0.20b
Coffee canopy classes	Compact	0.900	0.645	0.425	0.247b
	Intermediate	0.950	0.695	0.435	0.287a
	Open	0.970	0.695	0.430	0.290a

Means followed by the same letter(s) within a column are not significantly different at 0.05 probability level.

Table 2.Percent mean value of coffee bean screen size under different coffee shade trees.

Coffee screen size	Shade trees				
	Open field	***Calpurina subdecandrea***	***Acacia sp.***	***Milletia sp.***	***Cordia africana***
Us	9	8.33	1.67	3.67	1.67
14	46	36	13.33	19.67	16
4.0-4.5[1]	15	12.34	18	15.33	18
15	25	37	60.21	56	59.33
17	3.67	5.54	6.33	4.78	4.67
19	1	0.47	0.3	0.33	0.24

[1]*Pea berry*

Table 3. Percent mean value of coffee bean screen size under different coffee varieties.

Coffee screen size	Coffee varieties						
	75227	***7454***	***741***	***744***	***74158***	***74148***	***74165***
Us	2.67	2	5.33	0.37	4.67	2.33	1
14	13.12	12.33	19.67	1.6	24	17.33	16
4.0-4.5[1]	13.67	21.66	13	12.9	18.34	22.33	11.33
15	50	43.33	44.33	14	47	51	58.33
17	16	15.56	12.56	55	5.33	6.33	12.67
19	3.67	4.67	4.33	16	0.37	0.4	0.3

[1]*Pea berry*

In conclusion, a quantitative growth response of coffee fruit was expressed as change in fresh and dry weight, length, breadth, depth and estimated volume at different stages. Both fruit size and weight showed a similar sigmoid pattern of development. However, unlike increment in the breadth, depth and weight, the fruit attains its maximum length much earlier. Such information on the growth behavior of coffee fruits and beans would help to determine the optimum rate of nutrient application and irrigation requirement at times of stress and control berry drop, quality affecting factors and biennial bearing habits and thus improve productivity of the crop.

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Coffee Tree Productive Centre Potential as Affected by Different Training and Pruning Practices

Y. ALEMSEGED, S. G. TESFAYE, E. TAYE

Ethiopian Institute of Agricultural Research, Jimma Research Center, P. O. Box 192, Jimma, Ethiopia.

SUMMARY

Coffee pruning is essential to stimulate the production of new wood and maintain the tree at a manageable size. Pruning controls the shape and the height of the tree, which affects picking, while controlling the following year's crop. Selection of a pruning strategy requires information of how pruning will have an effect on the tree and give sustainable production. On contrary un-pruned trees produce bigger size of stem, more suckers, and large number of smaller fruiting branches.

An experiment was carried out with the objective of evaluating the coffee tree productive centre by applying different training and pruning practices. Four treatments, namely, single stem topped, two stem topped, two stem not topped and free growth were tested on open and compact coffee types using randomized complete block design with three replications at Jimma agricultural research center in Ethiopia. Topping was done at 2.20 meter height above the ground.

There was a marked difference between training and pruning treatments for productivity of productive centre. It was observed that 96% and 94% of the primary branches were out of production in single stem and two stem topped treatment followed by 64% and 57% in two stem not topped and free growth treatments respectively after six consecutive productions.

The bearing surface for free growth and two stem not topped were 21% and 14% and new (current) growth was 21% and 20%, respectively, on the 6^{th} potential cropping season. No new growth of primary branch in the productive centre was found for the topped single and two stem training treatments at this time. In general, all primary branches gave significantly higher crop only for three cropping years, while secondaries for two years during the study period.

Accordingly, free growths out yielded the other treatment and gave 2200 and 1800 kg/ha clean coffee for open and compact canopy classes, respectively. This is because of the contribution of newly growing bearing heads for subsequent potential crop of the trees.

Therefore, hard pruning during the first six harvests reduces the bearing surface and leads to proportional lose of productivity. Hence, free growth or no pruning with occasional control of weak suckers and removal of old, exhausted and unproductive lower dried primary branches seems to be a more economic benefit under conditions similar to Jimma.

INTRODUCTION

Pruning is an important cultural practice in management of modern coffee farms. It is a thinning process by which energy or vigor is concentrated in to certain parts of the plant by

cutting away others. The main aims of coffee pruning are to balance crop to leaf ratio, for high and sustainable yield, to facilitate harvesting and minimize the incidence of pest and disease.

Adaptation of pruning system is influenced by many factors, among which shade, coffee spacing, intercropping practice, mechanical cultivation; pest or disease occurrence, economics and the energy of people who carry out the operations are the major once. Besides, the agro-climatic factors should be also considered to practice pruning system.

In Ethiopia, the national average coffee yield is low. This could be attributed to several reasons, among which the predominant use of unimproved coffee cultivars and poor agronomic practices are the major factors. The use of improved coffee cultivars alone may not increase yields unless appropriate cultural practices such as training and pruning practices are applied. However, little research attempts have been made on coffee training and pruning practices. Hence, the existing practices to handle coffee trees largely depend on the experience of other coffee growing countries. It is therefore, imperative to develop suitable training and pruning systems for the different coffee types with the aims of to obtain sustainable high coffee yields by regulating the productive center of the trees.

MATERIALS AND METHODS

An experiment was conducted at Jimma agricultural research center in southwest Ethiopia with the objective of determining suitable training and pruning practices for different coffee cultivars. It was laid down using two morphologically distinct canopy classes (open and compact) coffee types planted on separate plots. Four training and pruning treatments, single stem topped at 2.20 meter height (Single stem topped), capped at 45cm height and grow two stem and topped at2.20 meter height (Two stem topped), capped at 45 cm height and grow two stem and grow free height (Two stem non topped), and free growth (no training and pruning) were applied. In free growth treatment only dried secondary or tertiary branch after harvesting were removed. The treatments were arranged in randomized complete block design with three replications of 20 experimental trees per plot. All the training and pruning practices were done three times a year (main pruning after harvesting and two times handling and de-suckering in between June and September. Coffee yield data has been recorded for six consecutive years, then tree productive center was evaluated based on dead, non bearing, bearing and current growth branch counts. The potential of primary and secondary branch nodes was also evaluated during the study period. All the parameters were analyzed using the standard statistical procedure and treatment mean separation was made according to Duncan multiple range test (DMRT) at 5% probability level.

RESULTS AND DISCUSSION

Yield variation among training and pruning treatments were non significant for both open and compact coffee types and in all cropping seasons (table1 and 2). Trees subjected to less intensive pruning (i.e. free growth) gave better mean clean coffee yields (2251 and 1855 kg/ha clean coffee for both open and compact type; respectively) than did the treated plots. Probably because of growth and maintain ace of more number of new secondary branch per primary and bearing head per tree as compared to the case with the pruned plots. In agreement with the results of this study, Barros et al. Endale et al., and Japiassu, L. B. et al., have reported that pruning does not enhance productivity of coffee trees after six harvests. As the vertical growth of orthotropic branches, commonly called suckers, was not controlled (no de-suckering applied), maintenance of more number of such potential bearing heads might

have contributed to the observed higher coffee yields of un-pruned or free grown trees in this experiment(table 3).

Table 1. Mean clean coffee yield(kg/ha) of open cultivar (75227) as affected by training and pruning practice.

Pruning practice	cropping year						Grand mean
	2006	2007	2008	2009	2010	2011	
Single stem topped	1787	1267	1755	4246	1794	1265	2019
Two stem topped	1722	1573	2497	3661	1590	1428	2079
Two stem un-topped	1565	1643	1906	4156	1618	1576	2077
Free growth	1954	1632	1903	4438	2112	1470	2251
S.E±	0.99	1.07	2.26	4.04	3.86	1.18	1.12
C.V %	9.58	12.16	19.46	16.98	37.62	14.3	9.25
LSD[1]	NS	NS	NS	NS	NS	NS	NS

[1] *NS= not significant at P=0.05*

Table 2. Mean clean coffee yield (kg/ha) of compact cultivar (74110) as affected by training and pruning practice.

Pruning practice	Cropping year						Grand mean
	2006	2007	2008	2009	2010	2011	
Single stem topped	1300	1695	1366	3361	485	1463	1612
Two stem topped	913	1535	1805	3345	773	1227	1600
Two stem un-topped	799	1470	2168	3064	865	1410	1629
Free growth	1524	1541	1927	3712	783	1642	1855
S.E±	2.01	1.28	2.58	1.61	1.7	2.08	0.88
C.V %	30.69	14.23	24.67	8.3	40.67	25.13	9.12
LSD[1]	NS	NS	NS	NS	NS	NS	NS

[1] *NS= not significant at P=0.05*

On the other hand, after six consecutive crop years harvesting on ten years old of coffee plants, the mean tree productive center specially the number of primary branches loses (non bearing plus dead branches count) declined by 96%, 94%, 64% and 57% for single stem topped, two stem topped, two stem un-topped and free growth respectively, in both canopy classes. In addition, no new branch growth was observed for the two stem topped treatment, rather 21% and 20% potential branch was counted for free growth and two stem un-topped treatments respectively. The bearing surface in free growth has six fold and three to four fold increment over single stem and two stem un-topped training and pruning systems; respectively.

Table 3. Productivity potential of compact coffee type (74110) after six cropping season.

Training and pruning practice	Primary branch (%)				
	Bearing	Non bearing	Dead	Current	Mean bearing head number
Single stem topped	3.58	78.89	17.53	0.00	1.00
Two stem topped	6.97	63.00	30.04	0.00	2.00
Two stem un-topped	14.60	43.56	18.62	23.22	2.00
Free growth	18.04	32.84	28.93	20.19	3.69

Table 4. Productivity potential of open coffee type (75227) after six cropping season.

Training and pruning practice	Primary branch (%)				
	Bearing	Non bearing	Dead	Current	Mean bearing head number
Single stem topped	3.79	71.80	24.40	0.00	1
Two stem topped	3.09	57.24	39.67	0.00	2
Two stem un-topped	15.11	43.41	23.92	17.56	2
Free growth	23.69	32.65	20.95	22.71	4

The maximum number of bearing node and berry count were recorded on the second growing year of the primary branch (figure 1). All primary branches gave significant crop only for three years, and then losses their productivity and leave the turn to the newly emerged secondary shoot. Similarly, secondary branches also bear potential crop only for two successive cropping seasons.

The node of a branch has coffee cherries only for one year. And next year, part of the branch that gave crop in the previous seasons bears no fruit. So, berries grow on the new wood, which is two years old and a branch node yields only for one year.

In a branch, the number of current and bearing node and berry count decrease through growing year, while the past crop zone of the branch increase (Fig 1). The maximum primary branch growth were up to 104 cm during the study period at the bottom canopy, which has 23.28, 49.72, 87.92, 96.33, 103.22 and 104cm length from first year to six year consecutively.

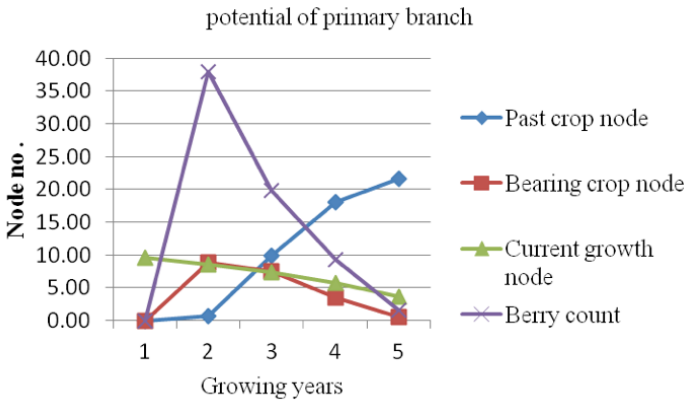

Figure 1.The coffee tree productive center performance.

In general, hard pruning during the first six harvests reduces the bearing surface and leads to proportionally decline in productivity of coffee trees. Therefore, a free growth adds bearing head annually per tree than the pruned plot and seems to have a more economic benefit besides the yield advantage under conditions similar to Jimma.

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Characterization of Fertility in Soils Devoted to Coffee Cultivation in the Perez Zeledon Region

V. M. CHAVES-ARIAS, L. PICADO QUIRÓS, J. GUZMÁN ÁLVAREZ

Instituto del Café de Costa Rica

SUMMARY

In order to characterize the coffee soil fertility in the Perez Zeledon region, between December and March 2008-2009, the coffee area of this region was systematically sampled. The following districts were included: Cajón, Daniel Flores, General, Pejibaye, Platanares, Páramo, Rivas, Río Nuevo, San Isidro del General and San Pedro (canton of Perez Zeledon, province of San José), and Boruca, Brunka, Buenos Aires, Colinas, Pilas, Volcán and part of Potrero Grande (canton of Buenos Aires, province of Puntarenas). Aerial photographs were used for selecting the collection sites. A grid with a density of one site every ten hectares was superimposed on the aerial photographs; every site was appropriated geo-referenced. A total of 1364 samples (equivalent to 13640 ha planted with coffee) were collected. Each sample was formed from six subsamples taken on the band of fertilization, at a depth of 0-20 cm, in a radius of approximately 10 m within the geo-referenced site. The water pH, interchangeable soil acidity (IA), Ca and Mg extracted with 1N KCl, and K, P, Cu, Zn, Mn and Fe with Modified Olsen were determined for each sample. According to the parameters established, at the regional level, of all samples 89 percent showed low pH, 69 percent showed high AI and the percentage of samples classified as low for each element was: Ca 73 percent, 72 percent Mg, K 38 percent, P 70 percent, Zn 39 percent, Mn 22 percent, Fe 0 percent and Cu 3 percent. In addition, this paper provides the results for each variable classified by district, fertility maps using the Arc-Gis 9.2 program and recommendations for fertilization programs.

INTRODUCTION

In Costa Rica there has been some work on collecting databases soil analysis, among which it should be noted that of Bertsch (1986) that groups results by canton 13,765 samples analyzed between 1978 and 1980 by the Ministry of Agriculture and Livestock. However in most cases the information obtained from the databases is not published, and is therefore very restricted use.

Aware of the importance for the competitiveness of the coffee industry, has the date information, the ICAFE has decided to conduct a soil sampling project nationally, allowing characterize soil fertility who grow coffee in Costa Rica, this report covering the coffee area of Perez Zeledon region comprising the canton of Perez Zeledon in the province of San José and the canton of Buenos Aires in the province of Puntarenas.

MATERIALS AND METHODS

In order to characterize the coffee soil fertility in the Perez Zeledon region, between December and March 2008-2009, the coffee area of this region was systematically sampled. The following districts were included: Cajón, Daniel Flores, General, Pejibaye, Platanares, Páramo, Rivas, Río Nuevo, San Isidro del General and San Pedro (canton of Perez Zeledon, province of San José), and Boruca, Brunka, Buenos Aires, Colinas, Pilas, Volcán and part of

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Table 1. Ranges used for classifying the different soil property evaluated.

Property		Variation
pH		Low (≤ 5,00) Middle-Low (5,01-5,50) Middle-High (5,51-6,00) High (>6,00)
A.I.	cmol(+)/L	Low (≤ 0,50) Middle-Low (0,51-0,99) Middle-High (1,00-1,50) High (>1,50)
% S.A.		Low (≤ 20) Middle-Low (21-30) Middle-High (31-40) High (>40)
K	cmol(+)/L	Low (≤ 0,20) Middle-Low (0,21-0,30) Middle-High (0,31-0,40) High (>0,40)
Mg	cmol(+)/L	Low (≤ 0,80) Middle-Low (0,81-1,50) Middle-High (1,51-2,00) High (>2,00)
Ca	cmol(+)/L	Low (≤ 3,00) Middle-Low (3,01-5,99) Middle-High (6,00-8,00) High (>8,00)
P	mg/kg	Low (≤ 10,00) Middle-Low (10,01-15,00) Middle-High (15,01-20,00) High (>20,00)
CICE	cmol(+)/L	Low (≤ 5,0) Medio (5,1-25) High (>25)

Property		Variation
Mn	mg/kg	Low (≤ 5,0) Middle (5,1-50,0) High (>50,0)
Zn	mg/kg	Low (≤ 2,0) Middle (2,1-10) High (>10)
Fe	mg/kg	Low (≤ 10) Middle (11-100) High (>100)
Cu	mg/kg	Low (≤ 2,0) Middle (2,1-20,0) High (>20,0)
Ca/Mg		Low (≤ 2,0) Middle (2,1-5,0) High (>5,0)
Ca/K		Low (≤ 5,0) Middle (5,1-25,0) High (>25,0)
Mg/K		Low (≤ 2,5) Middle (2,6-15,0) High (>15,0)
(Ca+Mg)/K		Low (≤ 10,0) Middle (10,1-40,0) High (>40,0)

Extractant: KCL 1N (Acidez, Ca,Mg) Olsen Modified: (K,P,Cu,Zn,Mn,Fe)

RESULTS AND DISCUSSION

Considering all the samples and in accordance with the criteria used, the vast majority have a low pH (89%) and high acidity interchangeable (69%) and acidity saturation percentages (62%) , calculated that 45% of the sampled area would need more than 2 mt /ha of calcium carbonate to correct soil acidity problems and only 20% would not require liming.

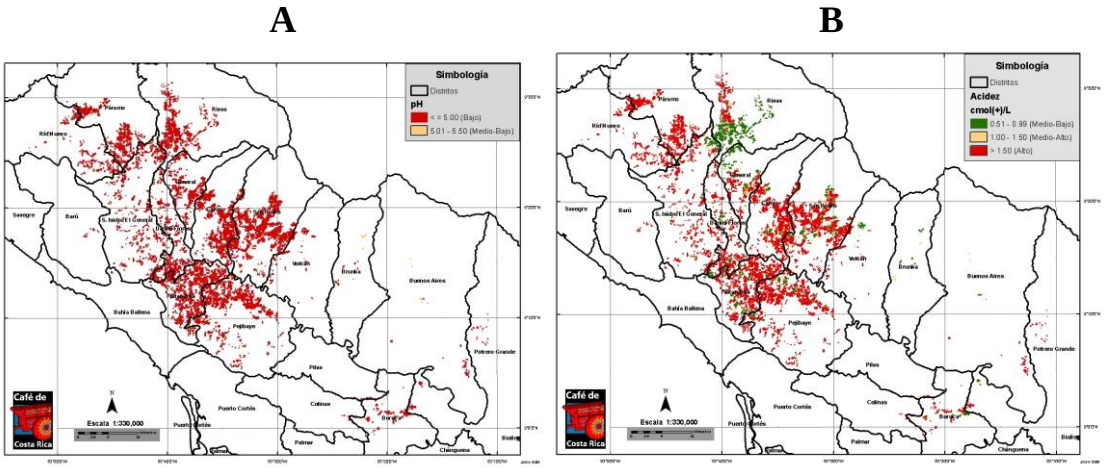

Figure 1. Fertility maps of pH (A) and acidity interchangeable (B).

Table 2 Percentage of samples low in pH, high in IA and high in % saturation of IA

Districts	n	% Samples		
		pH Low	IA (H+Al)	%IAS High
		< 5,0	> 1,50 cmol(+)/L	> 40 %
Boruca	18	72	61	44
Río Nuevo	30	90	83	40
El General	68	93	60	54
General	80	85	56	66
Daniel Flores	84	87	55	49
Páramo	104	97	89	74
Rivas	105	92	29	26
Pejibaye	167	90	80	66
Platanares	198	89	76	59
Cajón	204	93	71	81
San Pedro	276	84	72	69
Total	1364	89	68,6	62

When considering individual bases and taking the region as a whole, the elements calcium, magnesium and potassium values were lower by 73, 72 and 38% of cases, respectively.

In districts with more than 10 samples tested, Cajón, General, Rivas and Volcano to present all of them no less than 80% of samples with low contents of calcium and magnesium, where the district should add San Pedro.

In relation to potassium, Cajón and General clearly highlights as districts with major problems, achieving both low levels of the element in just over 80% of samples, followed by San Pedro and Rivas with 62 and 47% respectively.

Phosphorus was low in 70% of samples and all districts had at least 50% of their samples in this category reaching 87% in the case of Platanares. At the other extreme, the highest percentages of high samples were found in Rivas and San Isidro de El General, both with 19%.

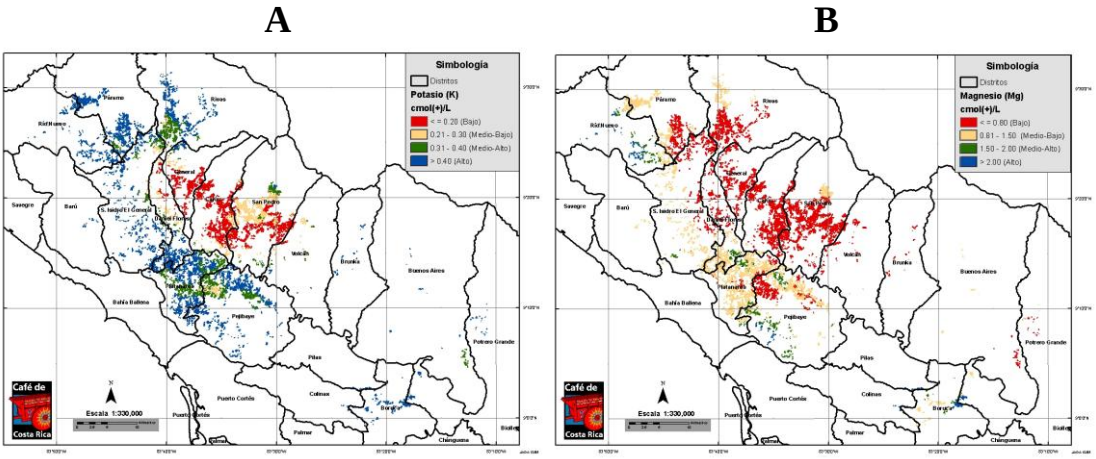

Figure 2. Fertility maps of K (A) and Mg (B).

Table 2 Percentage of samples low in K, Ca, Mg and P.

Districts	n	% Low Samples			
		Potassium	Calcium	Magnesium	Phosphorus
		< 0,20	< 3,00 cmol(+)/L	< 0,80 cmol(+)/L	< 0,20 mg/Kg
Boruca	18	0	39	33	72
Río Nuevo	30	0	27	30	50
El General	68	16	71	74	63
General	80	83	88	98	56
Daniel Flores	84	16	58	49	73
Páramo	104	9	75	68	67
Rivas	105	13	84	80	65
Pejibaye	167	18	67	57	80
Platanares	198	14	59	44	87
Cajón	204	82	92	99	62
San Pedro	276	62	75	87	66
Total	1364	38	73	72	70

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Liming Assessment. Correction of Soil Acidity in an Andisol

V. M. CHAVES-ARIAS, L. C. TORRES-MURILLO, C. E. DELGADO-CHAVARRÍA

Instituto del Café de Costa Rica

SUMMARY

With the purpose of evaluating the effect of different calcareous amendments on the acidity correction of an Andisol, a trial was conducted in ICAFE's experimental station (CICAFE), in San Pedro de Barva, province of Heredia, at 1100 m.a.s.l., in a coffee plot in full sunlight, with total pruning in February 2007. The treatments were applications of Calcium Carbonate, Dolomite, Hi-Cal-Mag, Triple Cal®, Surco Mejorador®, Nutrical and Tigsa Mag. The Calcium Carbonate and Dolomite were applied at a dose of 1.50 t/ha and the other five products at 0.75 t/ha. There was an eighth treatment: a control without liming. A randomized complete block design with five replicates was used in each one of the two sampling periods. The products were applied on the surface, spread over the alley, on September 12, 2008. After 236 and 426 days from the application of treatments (DAA), soil samples were taken at six depths (0-8, 8-16, 16-24, 24-32, 32-40 and 40-48 cm). Those samples were used to analyze the pH in water, exchangeable acidity, Ca and Mg extracted with 1N KCl; and K, P, Cu, Zn, Mn and Fe extracted with Modified Olsen. When examining each of the sampling depths individually, in terms of the parameters more related to soil acidity, such as pH, exchangeable acidity and acidity saturation percentage, significant differences were only found in the surface horizon (0-8 cm), and for the three variables the Calcium Carbonate was the one showing the highest acidity correction, differing statistically from the control treatment. When examining the sub-surface horizons (8-48 cm) together, the Dolomite and the Calcium Carbonate reported the highest acidity correction at 426 DAA, and they were the only ones differing statistically from the control treatment in terms of pH, exchangeable acidity and acidity saturation percentage.

INTRODUCTION

In recent years have appeared in our country a significant amount of commercial amendments, which are characterized as mixtures in different proportions and degrees of grinding, may contain two or more of the following products: carbonates, oxides and hydroxides of calcium and magnesium, and gypsum. The price of these amendments is much higher than that of calcium carbonate, but usually at lower doses are recommended to possess a higher PRNT and theoretically greater power of penetration into the soil profile.

The objective of this trial was to evaluate some alternative amendments to the calcium carbonate present in the Costa Rican market, in terms of its effectiveness in a correct acidity of the Andisoil soil.

MATERIALS AND METHODS

The trial was conducted in ICAFE's experimental station (CICAFE), in San Pedro de Barva, province of Heredia, at 1100 m.a.s.l., in a coffee plot in full sunlight, with total pruning in February 2007. The treatments were applications of Calcium Carbonate, Dolomite, Hi-Cal-Mag, Triple Cal®, Surco Mejorador®, Nutrical and Tigsa Mag. The Calcium Carbonate and Dolomite were applied at a dose of 1.50 t/ha and the other five products at 0.75 t/ha. There was an eighth treatment: a control without liming. A randomized complete block design with five replicates was used in each one of the two sampling periods. The products were applied on the surface, spread over the alley, on September 12, 2008. After 236 and 426 days from the application of treatments (DAA), soil samples were taken at six depths (0-8, 8-16, 16-24, 24-32, 32-40 and 40-48 cm). Those samples were used to analyze the pH in water, exchangeable acidity, Ca and Mg extracted with 1N KCl; and K, P, Cu, Zn, Mn and Fe extracted with Modified Olsen.

Table 1. Analysis of soil texture (average of two samples).

Profundity (cm)	%		
	Arena	***Limo***	***Arcilla***
0-8	41,1	36,0	23,0
8-16	37,9	34,8	27,3
16-24	35,4	34,8	29,8
24-32	34,8	36,6	28,6
32-40	38,6	36,0	25,5
40-48	31,1	33,5	35,5

Table 2. Characterization of amendments used.

Amendments	**Composition**	**Presentation**	**% Ca**	**% Mg**	**EG**	**EQ**	**LTRN**
Carboazul	Calcium Carbonate	Powder	35	-	83,19	88,2	73,4
Cal Dolomita	Calcium Carbonate and Magnesium	Powder	23	9	87,23	94,8	82,7
Hi Cal Mag	Hydroxide Calcium and Magnesium	Powder	32	16	70,92	145,4	103,1
Surco Mejorador	Carbonates, Hydroxides, Oxides and Calcium and Magnesium Sulphates	Powder	27	9	75,51	104,2	78,7
Nutrical	Carbonates and Hidróxides of Calcium and Magnesium , Calcium Sulphate	Powder	24	7	64,98	90,5	58,8
Triple Cal	Oxidos de Calcium and Magnesium , Calcium Sulphate	Powder	24	8	92,28	84,2	86,9
Tigsa Mag	Carbonates y Oxides of Calcium and Magnesium	Granulated					

RESULTS AND DISCUSSION

When examining each of the sampling depths individually, in terms of the parameters more related to soil acidity, such as pH, exchangeable acidity and acidity saturation percentage, significant differences were only found in the surface horizon (0-8 cm), and for the three variables the Calcium Carbonate was the one showing the highest acidity correction, differing statistically from the control treatment. When examining the sub-surface horizons (8-48 cm) together, the Dolomite and the Calcium Carbonate reported the highest acidity correction at 426 DAA, and they were the only ones differing statistically from the control treatment in terms of pH, exchangeable acidity and acidity saturation percentage.

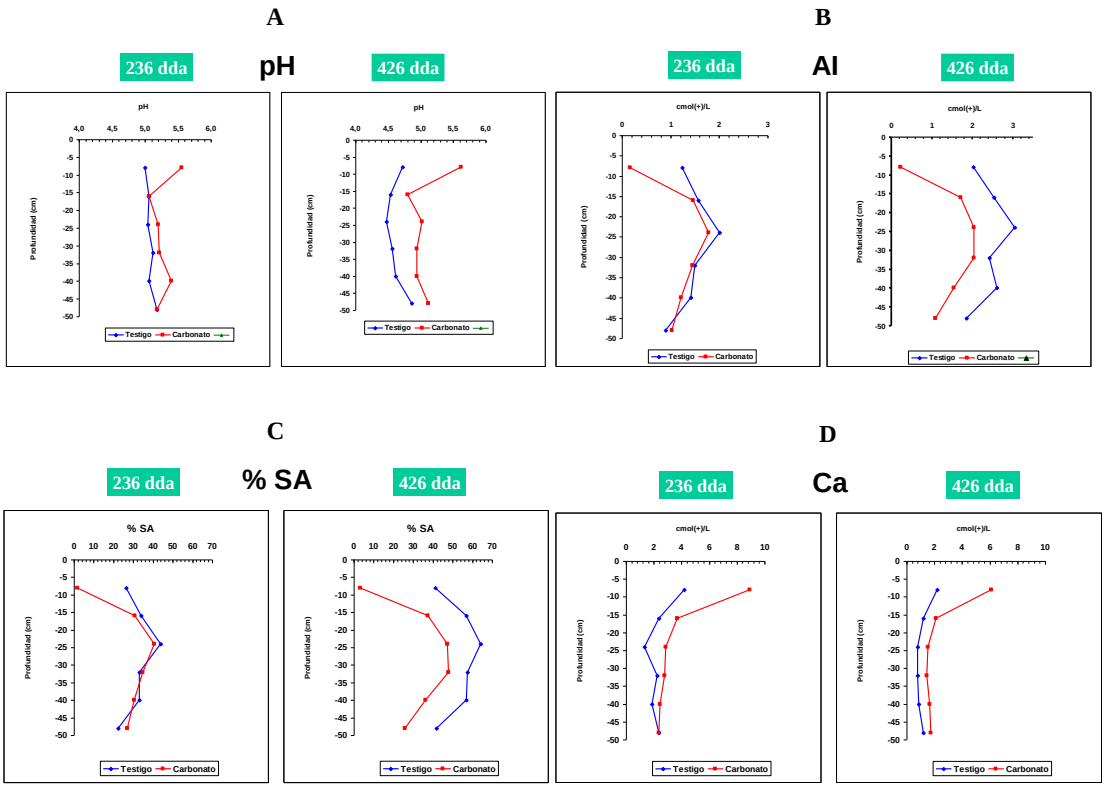

Figure 1. pH (A) exchangeable acidity (B), acidity saturation percentage (C) and calcium in the soil (D) in calcium carbonate and control treatments, 236 and 426 days after applying the treatments.

Table 3. PH, exchangeable acidity (AI), Ca, Mg and acidity saturation percentage (% SA) at different soil depths, to the 236 and 426 days after application of treatments.

236 días después de aplicación					depth.	*Treatment*	depth.	426 días después de aplicación				
pH	cmol(+)/L			%SA				pH	cmol(+)/L			%SA
	AI	Ca	Mg						AI	Ca	Mg	
5,00ns	1,24ns	4,20ns	0,79b	26,5ns	0-8cm	*Control*	0-8cm	4,72b	2,03ab	2,18b	0,75b	41,1a
5,56	0,16	8,91	1,22ab	1,7		*CarbonatodeCalcio*		5,62a	0,22c	6,12a	0,98ab	3,1b
5,16	0,69	5,50	1,38a	10,4		*Dolomita*		5,34ab	0,95abc	4,14ab	1,60a	17,3ab
5,66	0,59	6,27	1,54a	11,5		*HiCalMag*		5,20ab	0,94abc	3,60ab	1,29ab	17,2ab
4,98	0,81	4,99	1,01ab	12,2		*Nutrical*		4,72b	2,34a	2,39b	0,69b	44,1a
4,98	0,90	5,60	1,21ab	14,7		*Surcomejorador*		4,82b	1,81ab	2,34b	0,65b	37,2a
5,22	0,48	5,86	1,41a	6,8		*TigsaMag*		5,34ab	0,82bc	2,86ab	1,72a	16,2ab
5,06	0,59	5,16	1,24ab	8,3		*TripleCal*		5,18ab	1,31abc	2,65b	1,02ab	26,0ab
5,06ns	1,57ns	2,36ns	0,48b	34,0ns	8-16cm	*Control*	8-16cm	4,54ns	2,53ns	1,22ns	0,39ns	57,0ns
5,06	1,46	3,68	0,64ab	30,7		*CarbonatodeCalcio*		4,80	1,71	2,13	0,41	37,3
4,72	2,26	1,53	0,60b	46,6		*Dolomita*		4,94	1,99	1,33	0,52	45,8
4,96	1,67	2,43	0,99a	33,5		*HiCalMag*		4,82	2,20	1,47	0,59	46,8
4,64	2,47	1,49	0,46b	49,7		*Nutrical*		4,56	2,70	1,05	0,53	58,4
.5,00	2,03	1,38	0,56b	44,9		*Surcomejorador*		4,62	2,71	1,13	0,32	60,0
4,76	2,24	1,37	0,45b	50,1		*TigsaMag*		4,76	2,24	1,29	0,58	51,6
4,68	2,27	1,32	0,41b	50,3		*TripleCal*		4,74	2,38	1,06	0,41	55,1
5,04ns	2,01ns	1,31ns	0,32ns	43,9ns	16-24cm	*Control*	16-24cm	4,48ns	3,04ns	0,80ns	0,30ns	64,2ns
5,20	1,78	2,87	0,49	40,7		*CarbonatodeCalcio*		5,02	2,04	1,52	0,41	47,4
5,02	1,98	1,34	0,43	45,0		*Dolomita*		4,68	2,30	1,16	0,51	50,5
5,10	2,01	1,78	0,57	45,0		*HiCalMag*		4,72	2,43	0,98	0,45	55,8
4,98	2,67	1,04	0,35	56,0		*Nutrical*		4,46	2,57	1,08	0,34	57,1
5,02	2,03	1,17	0,39	48,5		*Surcomejorador*		4,62	2,91	0,85	0,23	66,2
4,84	2,33	1,23	0,31	53,5		*TigsaMag*		4,70	2,77	0,71	0,34	67,2
4,84	2,59	0,68	0,25	61,5		*TripleCal*		4,76	2,51	0,72	0,26	65,2
5,12ns	1,50ns	2,26ns	0,48ns	33,0ns	24-32cm	*Control*	24-32cm	4,56	2,43ns	0,77ns	0,33ns	57,6ns
5,22	1,45	2,79	0,53	34,8		*CarbonatodeCalcio*		4,94	2,04	1,46	0,39	47,8
4,88	1,79	1,62	0,53	41,6		*Dolomita*		4,98	1,28	1,90	0,85	29,1
5,14	1,66	1,79	0,53	38,7		*HiCalMag*		4,86	1,95	1,25	0,52	45,8
5,04	2,10	1,09	0,39	46,9		*Nutrical*		4,72	1,69	1,68	0,63	39,2
5,12	1,08	2,74	0,64	22,9		*Surcomejorador*		4,64	2,68	0,90	0,27	61,5

4,94	2,06	1,33	0,26	49,2		*TigsaMag*		4,86	2,66	0,74	0,33	63,1
4,94	2,15	1,03	0,28	50,9		*TripleCal*		4,66	2,52	0,63	0,22	66,9
5,06ns	1,42ns	1,87ns	0,45ns	33,1ns	32-40cm	*Control*	32-40cm	4,62	2,61ns	0,87ns	0,30ns	57,0ns
5,40	1,22	2,44	0,52	30,4		*CarbonatodeCalcio*		4,94	1,54	1,64	0,52	36,3
5,04	1,56	1,56	0,49	37,7		*Dolomita*		5,24	1,12	2,25	0,93	23,1
5,12	0,93	2,67	0,63	24,1		*HiCalMag*		5,00	1,36	1,48	0,53	35,7
5,18	1,27	1,96	0,62	30,1		*Nutrical*		4,90	1,22	2,19	0,89	28,7
5,60	1,28	1,76	0,48	30,1		*Surcomejorador*		4,74	2,01	1,22	0,49	46,2
5,16	1,60	1,69	0,45	35,2		*TigsaMag*		4,72	2,07	1,27	0,55	45,8
5,12	1,16	1,69	0,42	30,8		*TripleCal*		4,66	2,31	0,72	0,23	62,9
5,18ns	0,90ns	2,36ns	0,56ns	22,5ns	40-48cm	*Control*	40-48cm	4,86	1,86ns	1,22ns	0,56ns	41,8ns
5,18	1,03	2,35	0,48	26,9		*CarbonatodeCalcio*		5,12	1,09	1,75	0,73	25,9
5,08	0,95	2,04	0,60	24,0		*Dolomita*		5,52	0,71	2,19	0,94	16,0
5,02	0,66	2,57	0,63	16,9		*HiCalMag*		5,04	1,34	1,41	0,57	35,2
5,26	0,59	2,26	0,79	13,1		*Nutrical*		5,28	0,72	2,36	1,04	17,0
5,38	1,08	1,81	0,46	29,9		*Surcomejorador*		5,04	1,29	1,80	0,68	28,6
5,08	1,17	1,97	0,45	27,1		*TigsaMag*		5,18	1,16	1,78	0,85	25,2
4,98	0,65	2,23	0,57	17,1		*TripleCal*		4,74	1,89	0,93	0,28	53,0
5,09abc	1,48bc	2,03bc	0,46bc	33,3bc	8-48cm	*Control*	8-48cm	4,61d	2,49a	0,98cd	0,38cd	55,5ab
5,21ab	1,39c	2,83a	0,53b	32,7c		*CarbonatodeCalcio*		4,96ab	1,68c	1,70ab	0,49c	38,9de
4,95cd	1,71abc	1,62cd	0,53b	39,0abc		*Dolomita*		5,07a	1,48c	1,77a	0,75a	32,9e
5,07bc	1,39c	2,25b	0,67a	31,6c		*HiCalMag*		4,89bc	1,86bc	1,32bc	0,53bc	43,9cd
5,02cd	1,82ab	1,57cd	0,52bc	39,2abc		*Nutrical*		4,78bcd	1,78c	1,67ab	0,69ab	40,1de
5,22a	1,50abc	1,77bcd	0,51bc	35,1abc		*Surcomejorador*		4,73cd	2,32a	1,18cd	0,40cd	52,5abc
4,96cd	1,88a	1,52cd	0,38c	43,0a		*TigsaMag*		4,84bc	2,18ab	1,16cd	0,53bc	50,6bc
4,91d	1,76abc	1,39d	0,39c	42,1ab		*TripleCal*		4,71cd	2,32a	0,81d	0,28d	60,6a

Verification of Different Fertilizer Formulation for Sustainable Production of Coffee Seedling Growth in Ethiopia

T. KEBEDE, A. NESERE

Jimma Agricultural Research Center, P.O.Box 192, Jimma, Ethiopia

SUMMARY

Fertilizer use in Ethiopia has focused mainly on the use and application of nitrogen and phosphorous fertilizers in the form of di-ammonium phosphate (DAP) and Urea for almost all cultivated crops for both market and food security purposes for the last several years. The objective of this verification trial was therefore, to evaluate the effectiveness usage of the different fertilizer formulations for its roles in reduction of chemical fertilizers for sustainable use. Different Compo products have been recognized as inorganic fertilizer sources consisting of many macro and micro-nutrients in a single formula. These new Compo products *viz*. Bascot Plus®12M, Bascot LR®, Solugran 17-9-20 and Fetrilon Combi ® have been tasted under nursery at Jimma and Mettu Coffee Research Center using coffee as a test crop, in 2010. The results of the investigation revealed that growth of coffee seedlings evaluated by non-destructive (plant height, stem diameter, and number of primary branches and leaves), and destructive (leaf, stem and root fresh and dry weight, tap and lateral root length and lateral root number) growth parameter was significantly ($P \leq 0.01$or $P \leq 0.01$) affected by different fertilizer treatments at Jimma and Mettu. At Jimma the highest response of the aforementioned growth parameters were recorded from coffee seedlings treated by DAP and followed by Basacote® Plus and Basacote® LR. However, there was no significant growth variation among seedlings that were treated with Solugran 17-9-20. Seedlings grown using the Fetrilon Combi®, recommended media and control showed the lowest values of seedling growth at both locations. In general, the result obtained for this study showed that the current fertilizer recommendations for coffee using DAP gave better seedling growth at both locations compared to the new fertilizers. Among the introduced four new fertilizers Basacote® Plus and Basacote® LR has a promising results and need further investigation.

INTRODUCTION

In Ethiopia, coffee is a cash source to the majority subsistent farmers and foreign exchange earning to the country. Despite its decisive role, the national average is very low estimated to be 0.66 ton ha-1 green coffee (CSA, 2006). In western Ethiopia the area of devoted for coffee production is estimated to be 0.33 million ha (CSA, 2008). Studies executed on nutrient depletion rate in Central and Eastern African countries portrayed drastic decline of major nutrients that posed Ethiopia at an annual estimated net loss of 41 kg N, 6 kg P and 26 kg K per ha (Stoorvogel et, al. 1993). The common fertilizers used so far for both annual and perennial crops in Ethiopia are DAP and urea.

In general, low crop yields soil degradation and sub-optimal use of both N and P fertilizers because of high and rising costs, low availability of inorganic fertilizers and lack of knowledge of fertilizer management are among the factors accounting for low crop yields in most cases (AHI, 1997). Such unbalanced applications of nutrients might aggravate the depletion of other important nutrient elements such as K, Mg, Ca, S and micro-nutrients.

Consequently, lack of synergy among nutrient could occur and lead to severely inefficient nutrient use and low crop yields.

Hence, to keep up better synergy among macro and micro-nutrients, looking for mineral fertilizer sources that compose several micro-nutrients in a single formulation would eventually be a crucial task. In this regard, Compo products have been recognized as mineral fertilizer sources owing to constituents of many macro and micro-nutrients in a single formula. Thus, Compo products such as Solugran 17-9-20, Bascot Plus 12M, Basccote LR and Fetrilon Combi2 have been availed by Qoricha import/Export Pvt Ltd Company. These products have been agreed upon to be tested under field conditions at Jimma Agricultural Research Center, using coffee as a test crop in main cropping season of 2010. The objective of this verification trial was therefore, to evaluate the effectiveness of the different fertilizer formulations for their roles in reduction of chemical fertilizers as a sustainable solution.

MATERIALS AND METHODS

Based on the agreement made between EIAR and Qoricha Import/Export Pvt. Ltd. Company, new fertilizer formulations, have been tasted under nursery conditions at Jimma and Gera Research Center using coffee as a test crop. The study sites represents the dominant coffee based farming system in the south west Ethiopia. The geographical description of both sites is presented in Table 1.

Table 1. Geographical description of the study sites.

Center	Latitude	Longitude	Altitude (m.a.s.l)	Temperature (^{0}C)		Rainfall (mm)	Agro-ecological zone
				Minimum	*Maximum*		
Jimma	7^o 3'N	36^o 0'E	1753	13.5	25.9	1724.6	Tepid to cool humid mid high land altitude
Gera	7^0 7'N	36^0 0'E	1940	11.1	24.5	1837.2	Tepid to cool sub humid low to high altitude

The nursery experiment consisting of different fertilizer types, *viz.* Bascot Plus ® 12M, Bascot LR®, Solugran 17-9-20, DAP and Fetrilon Combi® was laid down in randomized complete block design with three replications at both sites'. Mode and rate of applications of each fertilizer are shown in Table 2.

Table 2. Mode and rate of applications of fertilizer treatment.

Fertilizer	Mode of application	Rate of application (g/pot)
Solugran 17-9-20,	Soil applied	4
Bascot Plus® 12M	Soil applied	4
Bascot Plus®	Soil applied	4
DAP	Soil applied	4
Fetrilon Combi®	Foliar	14 g diluted in 2 litter of water

Coffee seedlings were raised in polythene bag (pot) filled with top soil as growth media. The top soil was collected from open fields at the respective research centers. It was air-dried and crushed and passed through 2 mm size sieve to remove clods, plant roots and other foreign materials and filled in 12 cm wide and 22 cm long black polythene bags. Each experimental unit or plot consisted of 30 seedlings (pots) and arranged on nursery beds at 60 cm spacing and 3 m between replications. The fertilizer treatments were applied to each plot in to two splits with equal applications when the seedlings attained two and four pairs of true leaves. Besides, the above treatments, coffee seedlings raised in a recommended media (top soil and compost in 3:1 ratio) and control (seedlings raised in top soil alone or not treated with mineral fertilizer through out experimental period) were included in the trial as standard control for comparison.

Red ripe cherries were selectively harvested from coffee berry disease resistant selection 74110 at Jimma and 74165 at Gera and prepared for sowing using the recommended practices (Anteneh *et al*., 2008). To reduce the risk of germination failure and to obtain healthy seedlings the seeds were sorted for uniform size and sown at a rate of two per pot. All the nursery management practices were applied as recommended (according to the recommendations) (Tesfaye Shimber *et al*., 2006).

Growth of coffee seedlings was evaluated by non-destructive and destructive shoot and root parameters using on five randomly selected seedlings from the central rows of each plot when the seedlings attained normal transplanting stage (seven pair of true leaves).The attributes of shoot growth parameters such as plant height (cm), stem diameter (cm), at the base/collar of the stem number of nodes and leaves and internodes length were recorded for each individual seedling.

Each plant sampled for non-destructive parameters was taken to the laboratory with its polythene bag. The polythene bags containing the seedlings were immersed in a bucket of water and the roots were carefully separated from the soil. The seedlings were washed with clean water and the water on the surface of plant parts was dried with water adsorbent close. Shoots and roots were separated by cutting at collar point with scissors. Shoot was separated into leaves and stems. Fresh weight of each part was weighed using sensitive balance. Taproot length (cm) was measured using a ruler. The roots were traced on a clean transparent glass, by placing on a square paper. The length of individual lateral roots was counted by using squares paper covered by individual lateral root. Finally, all plant parts were oven dried at 100 ^{0}C for 24 hrs to constant weight as recommended by Adjei-Twum and Solomon (1982) and dry weight each part was measured separately using sensitive balance. The data was subjected to analysis of variance using SAS statistical soft ware Duncan's Multiple Range Test (DMRT) at 0.05 probability level was used to compare the difference between means.

RESULTS AND DISCUSSION

Both shoot and root growth parameters (plant height, stem diameter, and number of primary branches and leaves), leaf, stem and root fresh and dry weight, tap and lateral root length and lateral root number were significantly ($P \leq 0.05$) affected by fertilizer treatments at Jimma and Gera (Table 3, 4, 5 and 6).

Table 3. Effects of different fertilizers on growth of coffee seedlings at Jimma (2011 cropping season).

Treatment	Plant height (cm)	Girth (cm)	Number of main stem nodes	Mean internodes length (cm)	Number of primary branch	Number of leaves	Tap root length (cm)	Lateral root length (cm)	Lateral root No
Basacote®LR	30.78bc	4.60a	9.20a	3.30bcd	2.60ab	18.80bc	20.83a	15.33ab	101.00a
Basacote® Plus	34.40b	4.66a	9.28a	3.77b	3.00ab	20.27ab	21.43a	15.70ab	107.23a
Solugran 17-9-20	28.73bc	4.43a	9.13a	3.37bc	2.07bc	16.93bcd	20.97a	15.37a	105.07a
Fetrilon Combi®	27.87bcd	4.33	8.67b	3.20cde	0.80cd	15.40cd	19.33a	14.53ab	93.03a
Recommended media	25.10cd	4.40a	8.73b	2.87de	0.00d	14.67d	17.80a	13.26bc	61.03b
DAP	41.13a	4.70a	9.27a	4.27a	4.00a	23.13a	20.87a	16.80a	110.37a
Control	21.80d	3.70ab	8.47b	2.80e	0.27d	14.00d	12.50b	11.50c	50.70b

Means within a column followed by the same letter(s) are not significantly different at the $p \leq 0.05$ probability.

At Jimma, the highest values of all growth parameters were recorded for coffee seedlings treated with DAP followed by Basacote® Plus and Basacote® LR, However, Fetrilon Combi®, recommended media and control resulted in the lowest values of seedling growth parameters (Table 3).

Table 4. Effects of different fertilizers on growth of coffee seedlings at Gera (2011 cropping season).

Treatment	Plant height (cm)	Girth (cm)	Number of main stem nodes	Mean internodes length (cm)	Number of primary branch	Number of leaves	Tap root length (cm)	Lateral root length (cm)	Lateral root No
Basacote®LR	45.50a	5.50a	11.13a	3.77b	4.67	27.70ab	25.60ab	135.63ab	16.60ab
Basacote® Plus	44.90a	5.66a	11.07a	4.03ab	4.73	29.87a	25.67ab	121.13b	16.13b
Solugran 17-9-20	43.03a	5.10a	10.93a	3.83b	3.80	25.53abc	24.00b	120.23b	15.63b
Fetrilon Combi®	34.37b	3.09b	8.20b	4.87c	2.47	18.80bc	17.53c	99.50c	11.37c
Recommended media	46.67a	5.88a	11.23a	4.33ab	5.00	29.93a	25.60ab	120.13b	16.37ab
DAP	47.67a	5.96a	11.47a	4.50a	5.81	33.93a	26.76a	140.53a	17.50a
Control	28.47b	2.46b	7.53b	1.43c	1.67	15.30c	16.33c	93.80c	9.33d

Means within a column followed by the same letter(s) are not significantly different at the $p \leq 0.05$ probability.

Although, the difference between treatments was not significant for most of the root and shoot growth parameters, DAP, recommended media, Basacote® Plus and Basacote®LR resulted in a better performance, while Fetrilon had the lowest values at Gera (Table 4).

Table 5. Effects of different fertilizers on destructive growth parameters of coffee seedlings at Jimma in 2011 cropping season.

Treatment	Leaf		Root		Stem		Total Dry Matter (gm)	Root: Shoot
	Fresh weight (gm)	*Dry weight (gm)*	*Dry weight (gm)*	*Fresh weight (gm)*	*Dry weight (gm)*	*Fresh weight (gm)*		
Basacote®LR	9.60b	3.80a	0.867ab	6.500ab	1.765ab	2.34ab	4.97ab	2.83ab
Basacote® Plus	10.19b	3.92a	0.893ab	6.564ab	1.788ab	2.51a	5.19a	3.01a
Solugran 17-9-20	9.126b	3.75a	0.772bc	5.785ab	1.623abc	2.20ab	4.60ab	2.68ab
Fetrilon Combi®	6.00c	2.46b	0.696bc	4.229bc	1.058bcd	1.50bc	3.25bc	2.16b
Recommended media	5.32c	2.32b	0.591cd	3.532bc	0.907cd	1.38bc	2.88c	2.03bc
DAP	13.74a	4.18a	1.041a	8.785a	2.266a	2.71a	6.02a	3.17a
Control	4.51c	1.75b	0.470d	2.125c	0.625d	1.16c	2.26c	1.91c

Means within a column followed by the same letter(s) are not significantly different at the p≤ 0.05 probability.

Fresh weight of leaves, roots, stem and root dry weight were highly significantly (p≤ 0.01) affected by fertilizer treatments. The effect of treatments was also significant p≤ 0.05 for leaf and stem dry weight, total dry matter yield and root: shoot ratio (Table 5). In general, DAP followed by Basacote® Plus and Basacote®LR resulted in significantly higher leaf, stem and root dry weights, Total Dry Matter (TDM) yield and root: shoot ratio (R: S) ratio at Jimma (Table 5).

Table 6. Effects of different fertilizers on destructive growth parameters of coffee seedlings at Gera in 2011 cropping season

Treatment	Leaf		Root		Stem		Total Dry Matter (gm)	Root: Shoot
	Fresh weight (gm)	*Dry weight (gm)*	*Fresh weight (gm)*	*Dry weight (gm)*	*Fresh weight (gm)*	*Dry weight (gm)*		
Basacote®LR	16.30a	3.40a	4.70a	1.70a	8.23abc	2.30bcd	7.80a	4.14a
Basacote® Plus	15.97ab	3.80a	4.83a	1.67a	8.67ab	3.03abc	7.93a	4.35a
Solugran 17-9-20	14.90abc	3.23a	4.60ab	1.60a	7.33abc	2.53bcd	5.73b	3.86a
Fetrilon Combi®	8.10c	1.60b	2.40bc	0.77b	4.13bc	1.20d	5.04c	2.24c
Recommended media	18.40a	3.07a	4.17ab	1.67a	9.43a	1.60cd	6.87a	4.11a
DAP	19.07a	3.60a	5.67a	1.87a	11.76a	4.60a	8.47a	4.01ab
Control	8.20bc	2.00b	1.43c	0.50b	3.43c	1.33cd	3.83d	2.38bc

Means within a column followed by the same letter(s) are not significantly different at the p≤ 0.05 probability.

At Gera, there was a highly significant difference between the treatments for leaf dry weight, where the lowest value was recorded for Fertilon Combi®. Control and Fertilon Combi® also resulted in significantly lower root and stem dry weight as well as Total dry matter yield and root to shoot ratio (Table 6).

CONCLUSIONS AND RECOMMENDATIONS

The reduction in extension growth and dry matter yield of coffee seedlings grown in recommended media at Jimma might be attributed to the low organic matter content of the media. But this must be confirmed by soil chemical analysis. In general, the result of the present investigation showed that the current fertilizer recommendations for coffee in the country (DAP) gave better seedling growth at both locations compared to the new fertilizers. Total dry matter yield and its partitioning among plant parts (leaves, stems and shoots) was significantly affected by the treatments. In general, DAP, Basacote® Plus and Basacote®LR resulted in significantly higher dry weight of leaf, stem and root, Total Dry Matter (TDM) yield and root: shoot ratio (R: S) ratio of coffee seedlings at Jimma. The same thing was observed at Gera.

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Evaluation of Selections of Cultivar Bourbon Amarelo in Sebastião Da Grama-Sp.

E. S. MANTOVANI, L. C. FAZUOLI, M. T. BRAGHINI

Centro de Café "Alcides Carvalho", Instituto Agronômico de Campinas (IAC/APTA)

SUMMARY

The purpose of this study was to characterize the agronomic performance of Bourbon Amarelo progenies selected in the Breeding Program of the Agronomic Institute of Campinas (IAC). For this purpose, an experiment was carried out in a randomized block design with three replications and 10 plants per plot, in São Sebastião da Grama, São Paulo State. For three years, the yield, efficiency and technological characteristics of the beans were evaluated. Although the mean yields did not differ significantly among treatments, the yield of the selections Bourbon Amarelo IAC J 02-01, Bourbon Amarelo IAC J 09-16, Bourbon Amarelo IAC J 11-11, and Bourbon Amarelo IAC J 30-20 was considerable (35.7, 35.1, 25.7, and 22.1 bags of processed coffee per hectare and year, respectively).

INTRODUCTION

The cultivar Bourbon Vermelho was introduced in Brazil in 1859. In 1932, the Agronomic Institute of Campinas (IAC) initiated breeding work on plantations of Bourbon Vermelho and released several selections as of 1938. Until the early 50s, the Arabica coffee (*Coffea arabica* L.) cultivar Bourbon Vermelho was the most widely planted by farmers in Brazil. In 1957, several Bourbon Amarelo selections were released . Two hypotheses could explain the origin of Bourbon Amarelo: either a mutation of Bourbon Vermelho or a natural hybridization between this cultivar and Amarelo de Botucatu . It is noteworthy that, in recent years coffee consumers have become increasingly interested in a product with a better beverage quality. Since the yield of the selections of Bourbon Vermelho and Bourbon Amarelo is lower than that of other Arabica varieties, they have been recommended for growers who wish to produce coffee with high cup quality. The purpose of this study was to evaluate progenies of the cultivar Bourbon Amarelo developed by the Agronomic Institute of Campinas (IAC), to select higher-yielding coffee trees.

MATERIAL AND METHODS

The experiment was initiated in March 2006, in São Sebastião da Grama, São Paulo State, on the Fazenda Recreio. The experiment was arranged in a randomized block design with three replications, on plots with 10 plants in 3.0 x 0.8 m spacing. Thirty-one progenies of Bourbon Amarelo were evaluated and one selection of Icatu Vermelho and two of Mundo Novo Amarelo, as controls (agronomic data of this plant material see Table 1).

The following technological characteristics were assessed: yield, vigor, fruit maturation, yield, and bean properties. The yield of the cultivars in three growing seasons (from 2009 to 2011), was determined by weighing the coffee cherries harvested from each plot. Additionally, coffee was sampled from each plot, dried, weighed and processed in order to transform the whole bean weight into bags per hectare, according to the number of plants/ha, as given by the spacing. Of these samples, the bean type (flat beans, peaberry and elephant

beans) was also evaluated, as well as 100-bean weight, mean bean size and percentage of bean size above 16.

The VEI vigor (visual evaluation index for vigor) was evaluated before harvest, assigning scores on a 1-10 scale, where 1 corresponds to plants with reduced vigor and pronounced deficiency symptoms and 10 to plants with excellent vigor, dense canopy and intensive growth of the productive branches.

Maturation was evaluated before harvest, where 1 indicates early ripening, 2- average to early 3- average, 4 - average to late and 5 - late.

The data were subjected to analysis of variance by the F test. The treatment means were compared by the Scott-Knott test at 5%. Statistical analyses were performed using GENES, a software tool for genetics and statistics.

RESULTS AND DISCUSSION

The mean yield and agronomic characteristics of the three harvests are shown in Table 1. There was no significant difference between the yields of the progenies. But the yields of the progenies Bourbon Amarelo IAC J 02-01, Bourbon Amarelo IAC J 09-16 and Bourbon Amarelo IAC J 11-11, and Bourbon Amarelo IAC J 30-20 were the highest (35.7, 35, 1, 25.7, and 22.1 bags of processed coffee per hectare / year), respectively.

There were significant differences in the VEI- vigor of the coffee trees and the scores ranged between 6.1 and 7.6.

Fruit maturation ranged from 2.0 to 3.5, indicating that the maturation of some Bourbon selections is early and mean of others.

Table 1. Agronomic traits evaluated in three growing seasons (2009, 2010 and 2011) in the experiment with Bourbon Amarelo progenies, in São Sebastião da Grama - SP.

Progenies of Bourbon Amarelo	Mean yield (bags/ha/year)	[1]VEI - vigor	[2]Maturation
IAC J 02 01	35.7	6.1 b	2.9 a
IAC J 09 16	35.1	6.5 b	2.7 b
IAC J 11 11	25.7	7.0 a	2.7 b
IAC J 30 20	22.1	6.9 a	2.6 b
IAC J 13 08	21.9	6.8 b	3.2 a
IAC J 23 19	21.5	6.9 a	2.4 b
IAC J 09 8	21.1	7.2 a	2.7 b
IAC J 19 18 10	20.9	6.8 b	2.3 b
IAC J 07 20	20.9	6.7 b	2.8 b
IAC J 20 14 14	19.0	7.1 a	2.9 a
IAC J 03 01	19.0	7.2 a	3.0 a
IAC J 21 07	18.5	6.9 a	2.4 b
IAC J 08 02	18.3	6.2 b	3.1 a
IAC J 14 20	17.9	6.4 b	2.7 b
IAC J 17 10	17.8	7.5 a	2.7 b
IAC J 19 01	17.7	7.3 a	2.7 b
IAC J 26 6	17.5	6.9 a	2.9 a
IAC J 19 18 10	17.4	6.7 b	3.0 a
IAC J 22 06	16.4	6.9 a	2.8 b
IAC J 15 16 10	15.1	6.6 b	2.0 b
IAC J 28 08	14.7	6.7 b	2.7 b
Mundo Novo Amarelo IAC 4266	14.6	7.5 a	3.5 a
IAC J 20 17	14.5	6.4 b	2.5 b
IAC J 06 09	14.4	6.6 b	2.8 b
IAC J 18 02	14.4	7.1 a	2.8 b
Mundo Novo Amarelo	14.3	7.0 a	2.8 b
IAC J 26 6	13.8	6.9 a	3.3 a
IAC J 27 04	13.6	6.8 b	3.3 a
IAC J 26 08	13.4	6.9 a	2.3 b
Icatu Vermelho	12.4	7.0 a	2.8 b
IAC J 15 02	11.8	7.2 a	2.8 b
IAC J 04 05	11.3	6.8 b	3.1 a
IAC J 24 6	9.7	7.6 a	3.0 a
IAC J 10 03	7.6	6.5 b	3.1 a
F	1.43^{ns}	2.37^{A}	2.84^{A}
CV(%)	48.99	5.76	11.41

[A]Significant at 1% probability by the F test; ns = non significant. Means were compared by the Scott Knott test at 5%; [1]VEIvigor = Visual Evaluation Index for vigor ; score: 1 = plants with reduced vigor and pronounced deficiency symptoms; 10 = for plants with excellent vigor, dense canopy and intensive growth of the productive branches; [2]Maturation: 1 = early, 2 = average to early, 3 = average, 4 = average to late, 5 = late.

Table 2 presents data of bean properties. There was no significant difference between the bean types flat and peaberry and 100-bean weight. The percentages for flat beans ranged from 67.9 to 85.3% and for peaberry from 12.0 to 17.9%. The 100-bean weight ranged from 14.4 to 16.9 g and the mean bean size was high (17.7 - 19.0). The reason for the large bean size of these Bourbon Amarelo selections is mainly related to the altitude of the experimental location (1300m asl). The Bourbon Amarelo progenies did not differ significantly in bean size above 16 (87.1 - 98.5%).

Table 2 - Characteristics of beans evaluated in 2009, 2010 and 2011, in an experiment with Bourbon Amarelo progenies, in São Sebastião da Grama - SP.

Progenies of Bourbon Amarelo	Flat beans	Peaberry	Elephant beans	100-bean weight (g)	Mean bean size	Bean size over 16
	%					%
IAC J 02 01	80.7	14.6	4.7 c	14.9	18.5 b	95.8
IAC J 09 16	83.7	13.8	2.5 c	14.4	18.3 b	94.2
IAC J 11 11	80.0	16.2	3.8 c	15.4	18.8 a	96.7
IAC J 30 20	80.8	13.9	5.3 c	15.5	18.5 b	94.5
IAC J 13 08	81.4	14.5	4.1 c	16.9	17.7 a	97.2
IAC J 23 19	82.8	13.7	3.5 c	15.2	18.4 b	95.3
IAC J 09 8	80.8	14.9	4.4 c	15.9	18.9 a	97.6
IAC J 19 18 10	84.1	13.3	2.6 c	14.7	18.4 b	94.6
IAC J 07 20	81.7	15.1	3.2 c	15	18.3 b	94.5
IAC J 20 14 14	85.3	12.0	2.7 c	14.7	18.2 b	93.1
IAC J 03 01	80.3	13.2	6.5 b	14.6	18.6 a	96.6
IAC J 21 07	79.5	16.0	4.5 c	15.5	18.6 a	95.5
IAC J 08 02	83.0	12.9	4.2 c	14.9	18.5 b	95.7
IAC J 14 20	77.1	15.4	7.5 b	15.8	18.6 a	95.9
IAC J 17 10	80.1	15.9	4.0 c	15.4	18.8 a	97.3
IAC J 19 01	67.9	15.5	5.4 c	15.2	18.6 a	96.1
IAC J 26 6	83.3	13.5	3.2 c	14.8	18.4 b	95.0
IAC J 19 18 10	82.8	14.3	3.0 c	15.5	18.9 a	97.6
IAC J 22 06	83.9	12.8	3.3 c	15.3	18.6 a	96.7
IAC J 15 16 10	83.5	13.5	3.1 c	14.9	18.2 b	87.1
IAC J 28 08	78.7	17.0	4.4 c	15.3	18.6 a	96.1
Mundo Novo Amarelo IAC 4266	75.1	13.9	10.9 a	16.3	19.0 a	97.6
IAC J 20 17	82.1	15.1	2.7 c	15.6	18.8 a	97.7
IAC J 06 09	79.8	14.6	5.6 c	15.3	18.6 a	95.7
IAC J 18 02	81.7	14.5	3.4 c	14.7	18.6 a	97.0
Mundo Novo Amarelo	77.7	14.7	7.5 b	15.2	18.6 a	95.6
IAC J 26 6	77.9	17.9	4.2 c	15.1	18.9 a	97.7
IAC J 27 04	82.2	13.1	4.5 c	14.7	18.3 b	93.7
IAC J 26 08	82.8	14.6	2.7 c	15.6	18.6 a	95.8
Icatu Vermelho	80.6	15.2	4.2 c	15.4	18.6 a	97.4
IAC J 15 02	81.4	14.7	3.9 c	15.2	17.7 a	96.4
IAC J 04 05	74.7	14.7	10.6 a	15.5	18.8 a	97.2
IAC J 24 6	78.1	18.1	3.8 c	16.2	19.0 a	98.5
IAC J 10 03	76.4	14.9	8.6 b	15.4	18.5 b	93.6
F	1.60^{ns}	1.41^{ns}	2.97^{1}	1.30^{ns}	2.25^{1}	1.38^{ns}
CV(%)	5.81	13.55	46.06	5.21	1.31	3.16

[1] *Significant at 1% probability by the F test, ns = not significant. Means were compared by the Scott Knott test at 5%.*

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Cultivar IAC Obatã 4739, another Contribution of the Instituto Agronômico de Campinas for Brazilian Coffee Production

L. C. FAZUOLI[1], M. T. BRAGHINI[1], M. B. SILVAROLLA[1], W. GONÇALVES[1], J. C. MISTRO[1], O. GUERREIRO-FILHO[1], P. B. GALLO[2]

[1]Centro de Café 'Alcides Carvalho', Instituto Agronômico de Campinas (IAC/APTA), SP, BR
[2]APTA, Polo Nordeste Paulista, Mococa, SP, BR

SUMMARY

The aim of this study was to obtain a cultivar of arabica coffee with short stature, resistance to coffee leaf rust (rust) and yellow fruits. After several decades of studies at the Instituto Agronômico de Campinas and elsewhere we obtained the IAC Obatã 4739 cultivar. It originated from a natural cross of cv. Catuaí Amarelo with cv. Obatã IAC 1669-20, which occurred in an experiment established in Garça, SP in 1978. Seeds from the original plant 16B of 'Obatã IAC 1669-20', were selected and planted in various locations in 1984 and afterwards. We identified in subsequent segregating generations, plants with yellow fruits with the same other characteristics as the cultivar Obatã IAC 1669-20. The progeny selected in F6 with high productivity, dwarf stature, yellow fruits and rust resistance has been designated by 'IAC Obatã 4739'. The average productivity in the three years of consecutive harvests, in an experiment with irrigation in Gália - SP, was 67 bags of green coffee per ha/year, while the cv. Catuaí Amarelo IAC 62, used as a control, was 48 bags per ha/year. 'IAC Obatã 4739' is indicated mostly for plantations with irrigation and fertilizer-irrigation systems, because it is demanding in nutrients and water. Being resistant to rust, its use allows for less spending on fungicides, presents considerable savings for the producer giving more profit and financial stability, reduction of environmental pollution (soil and groundwater) while preserving the environment, reducing the risk to the health of applicators of the product and growers. It is therefore, in line with the growing demand for cultivars that use fewer chemicals. The cup quality is similar to 'Catuaí Amarelo IAC 62'. The IAC Obatã 4739 cultivar is also important for the smallholders and organic coffee growers.

INTRODUCTION

Rust is the main disease affecting coffee plantations of Brazil and in the world. The aim of this study was to obtain a cultivar of arabica coffee with high productivity, dwarf stature, yellow fruits and rust resistance.

MATERIALS AND METHODS

The materials used in the development of this work were based on the mother plant of 'Obatã IAC 1669-20' (C16B). The genealogy of obtaining the Obatã mother plant with red berries is indicated in Table 1.

Table 1. Genealogy of obtaining the mother plant Obatã IAC 1669-20 (C16B).

Year	Locality	Generation	Germplasm codes
1967	CIFC-PORTUGAL	F1	CIFC H3614 (Villa Sarchí CIFC 971/10) x Híbrido de Timor CIFC 832/2
1971	CAMPINAS-SP	F2	IAC 1669
1972/81	CAMPINAS-SP	F2	IAC 1669
1977	CAMPINAS-SP	F1RC1	IAC 1669-20 X Catuaí Amarelo IAC 62
1978/83	GARÇA-SP	F1RC1	IAC 1669-20 C16B (Obatã IAC 1669-20)

'IAC Obatã 4739' stemmed from a probable natural crossing of the original plant 16B of the mother plant of cultivar Obatã IAC 1669-20 with 'Catuaí Amarelo IAC 62', occurred in 1983 in an experiment established at the Cooperative of Coffee Growers of the Garça Region, SP (Garcafé) in the experimental area called Dr. Alcides Carvalho. The Catuaí Amarelo IAC 62 cultivar was present in the experiment and should have provided pollen for the cross used for further selection of 'IAC Obatã 4739'. Fruits of the original Obatã IAC 1669-20 16 B plants, with red berries, in this experiment were harvested in 1984. The method of selection was line selection. The experiments and or selection fields were established in various coffee regions in the states of São Paulo and Minas Gerais with irrigation and control of coffee rust, except in Campinas and Mococa - SP.

The selection traits observed over six generations of selection were: kilograms of green coffee per tree, vigor (visually evaluated using a 10-point scale: IAV vigor), productivity (also evaluated visually through a 10-point evaluation scale: IAV production), earliness of ripening, fruit size, outturn (percentage of green coffee in relation to the weight of dry coffee berries), percentage of flat beans, of peaberries, of elephant beans, 100 green coffee bean weight, bean size (sieve average) and rust resistance. The rust resistance was assessed by giving scores from 1 to 5, with 1 and 2 = resistant, 3 = moderately resistant, 4 and 5 = moderately susceptible and susceptible.

RESULTS AND DISCUSSION

Origin of "IAC Obatã 4739"

The trees obtained in 1986 (F1 RC2) of the original plant, were planted in various locations. Selections of the best plants were made in subsequent generations. In this process in the F2RC2 generation plants were identified with yellow fruits, with the same agronomic characteristics as the Obatã IAC 1669-20 cultivar. Selections of trees with yellow fruits were made in the State of São Paulo at the Experimental Center of IAC, Campinas, in the farmer Mococa Experimental Station (now Northeast Polo APTA) and several others localities. The selected trees in generation F6 RC2 that had high productivity, yellow fruits and moderate resistance to yellow rust were designated as IAC Obatã 4739. The genealogy, years, places and generations of selection are shown in Table 2.

Table 2. Genealogy of obtaining the IAC Obatã 4739 cultivar.

Year	Local	Generation	Germplasm
1983	GARÇA-SP	F1RC2	Obatã IAC 1669-20 C16B x Catuaí Amarelo IAC 62
1984	GARÇA-SP	F1RC2	Obatã IAC 1669-20 C16B x Catuaí Amarelo IAC 62
1986/91	GARÇA-SP	F2RC2	IAC 4092
1986/92	MOCOCA-SP	F1RC2	IAC 4092
1986/91	ALFENAS-MG	F1RC2	IAC 4092
1986/91	RIBEIRÃO CORRENTE-SP	F1RC2	IAC 4092
1989/96	MOCOCA-SP	F2RC2	IAC 4092
1992/96	GARÇA-SP	F2RC2	IAC 4092
1993/97	ALFENAS-MG	F2RC2	IAC 4092
1993/97	RIBEIRÃO CORRENTE-SP	F2RC2	IAC 4092
1998/01	RIBEIRÃO CORRENTE-SP	F3RC2	IAC 4092
1997/01	MOCOCA-SP	F3RC2	IAC 4739
2000/10	MOCOCA-SP	F4RC2	IAC 4739
2000/05	RIBEIRÃO CORRENTE-SP	F4RC2	IAC 4739
2001/08	CAMPINAS-SP	F4RC2	IAC 4739
2003/09	CAMPINAS-SP	F5RC2	IAC 4932
2004/09	CAMPINAS-SP	F5RC2	IAC 4932
2004/08	RIBEIRÃO CORRENTE-SP	F5RC2	IAC Obatã 4739
2006/11	CAMPINAS-SP	F6RC2	IAC Obatã 4739
2006/11	GÁLIA-SP	F6RC2	IAC Obatã 4739
2006/11	GARÇA-SP	F6RC2	IAC Obatã 4739
2008/11	RIBEIRÃO CORRENTE-SP	F6RC2	IAC Obatã 4739
2008/11	CAMPINAS-SP	F6RC2	IAC Obatã 4739

Evaluation of cv. IAC Obatã 4739 in final selection trials

During the evaluation of trees in selection, occurrence of the leaf miner, berry borer and *Cercospora* leaf spot were observed. The rust was observed in trees under selection and in the susceptible control cultivars. The rust resistance in 'IAC Obatã 4739' was moderate (type 3 on the assessment scale). In some years there have been dry spells and this cultivar was sensitive to lack of water. The evaluation sites of cv. IAC Obatã 4739 are representative of the areas recommended for commercial cultivation of *Coffea arabica*, mainly in the states of São Paulo and Minas Gerais. There were differences in the behavior of the cultivar under irrigated conditions or not in relation to 'Catuaí Amarelo IAC 62'. The data obtained in productivity per ha/year are shown in Table 3.

Table 3. Productivity of cv. IAC Obatã 4739 in bags of green coffee per hectare per year in experiments with and without irrigation in various locations of the State of São Paulo.

Irrigated coffee			
Local	**Harvest Years**	**Average productivity of 'IAC Obatã 4739'**	**Average productivity of Catuaí Amarelo IAC 62'**
Gália,	2	53.3	48.0
Gália	3	66.9	48.1
Non-irrigated coffee			
Local	**Harvest Years**	**Average productivity of 'IAC Obatã 4739'**	**Average productivity of control cultivars**
Garça	2	33.7	33.6 Catuaí Amarelo IAC 62
Mococa	8	33.8	32.3 Catuaí Vermelho IAC 24
Mococa	2	49.7	35.2 Catuaí Amarelo IAC 62
Garça	6	37.5	36.4 Catuaí Amarelo IAC 62
Campinas	3	60.0	38.8 Catuaí Amarelo IAC 62
Ribeirão Corrente	45.0		40.0 Catuaí Amarelo IAC 62

Table 3 shows that in irrigated areas, the cv. IAC Obatã 4739 produced 66.9 kilograms of coffee per ha/year on average of three crops and the control cv. Catuaí Amarelo IAC 62 produced 48.1 bags / ha / year.

In rainfed areas (no irrigation) the average yield Obatã 4739 ranged from 33.7 to 60.0 bags of green coffee per ha/year between the various locations studied, while the control cultivars ranged from 32.3 to 40.0 bags of coffee.

Major morphological, biological and / or physiological make possible the identification of 'IAC Obatã 4739'

The main feature of IAC Obatã 4739 is the yellow color of the fruit, which differs from the cultivar that originated it (Obatã IAC 1669-20). The other characteristics are therefore similar to cultivar Obatã IAC 1669-20. It is moderately resistant to rust, and later maturing in some regions than cv. Catuaí Amarelo IAC 62. It has a dwarf stature, short internodes, broad leaves, green color in leaves, and large yellow fruit dimensions and with height and canopy diameter being similar to the Catuaí Amarelo IAC 62 cultivar. The percentage of normal grains (flat) is greater more than 85% and the average sieve size about 17. The outturn is around 50%.

Range of adaptation

The IAC Obatã 4739 cultivar is well adapted in regions of arabica coffee plantation, provided there is no water deficit. It is preferable to use the cv. IAC Obatã 4739 in irrigated areas or ferti -irrigated. 'IAC Obatã 4739' also provides less health risk to farmers due to decreased use of chemicals to control coffee leaf rust. Thus, it represents considerable savings for producers and significant reduction of risks related to environmental pollution and the health of farmers.

Cup quality

The cup quality is very good similar to cv. Catuaí Amarelo IAC 62.

Seed availability

Currently, genetic seeds are being produced by IAC.

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Determining Nutrient Composition of NPK Fertilizer Applied on Coffee Based on the Nutrient Removed in Yield and Seasonal Phenology

A. SOETANTO, M. SURIP

Indonesian Coffee and Cocoa Research Institute, Jember, East Java, Indonesia

SUMMARY

The purpose of fertilization on coffee plants is to replace the nutrients removed in harvested yield and to fulfill nutrient needed by plants those are follow the seasonal phenology as well as plant development phases. Nutrients those are removed by arabica coffee cherries that equivalent to one tonne of green beans were about 64 kg N, 12 kg P_2O_5, and 68 kg K_2O, while in robusta coffee were about 36 kg N, 6 kg P_2O_5 and 50 kg K_2O. Seasonal phenology of coffee that are consisted of flower primordia formation phase, the blossoming, the development and filling of cherry as well as seed is influenced by the latitude and the distribution of rainfall in the planting area. Hence, the nutrients needed by coffee crops also follow the phases of the seasonal phenology. On the phase of flower primordia formation and blossoming required more P element than K, while the phase of cherry as well as seed development and filling are more K needed rather than P. The need of N element is relatively same in all the seasonal phenological phases. In coffee producing areas in Java (Indonesia, 5-8^{o}S of latitude), the phase of flower primordia formation and blossoming occurs in the second half of the year (July-December), that are overlap apartly with period of the rainy season (November-April), while the development of cherry as well as seed and the filling phases occurs in the first half of the year (January- June), that are overlap partly with period of the dry season (May-October). Fertilizer application is done twice a year to meet the optimum availability of water as a solvent for fertilizer, those are in the second half of the year (the beginning of the rainy season, November-December) and in the first half of the year (the end of rainy season, March-April). Based on the nutrient requirements in each phase of the seasonal phenology, the fertilizer needed by coffee plant in the second half of the year (November-December) consists of a half dose of N + full dose of P, whereas in the first half of the year (March-April) consists of a half dose of N + full dose of K. If it is assumed that the average productivity of coffee plants as much as 1 tonne per hectare of green coffee, the minimum dose and composition of nutrients that should be applied in the form of fertilizer for arabica coffee in the second half of the year is 32 kg N/ha and 12 kg P_2O_5/ha, while in the first half of the year is 32 kg N/ha and 68 kg K_2O/ha. With the same assumptions, the minimum dose and composition of nutrients that should applied in the form of fertilizer for robusta coffee in the second half of the year is 18 kg N/ha and 6 kg P_2O_5/ha, whereas in the first half of the year is 18 kg N/ha and 50 kg K_2O/ha.

INTRODUCTION

Nutrients are recycled within the environment. A 'closed' environment such as a rainforest, recycles its own nutrients and is more or less self-sufficient. However, where plants are grown in a commercial situation, it is necessary to replenish the nutrients that are removed from the system. Without additional nutrients in some form of fertiliser, coffee yields will remain very low as nutrients are removed with the coffee beans.

The aim of fertilization is to maintain good foliage, to promote growth, to promote flowering, to promote root development, to replace lost nutrients, to repair and strengthen damaged tissues, and to enhance fruit growth and development (Campbell, 2006). Fertilization is one of the factor that affect yield of coffee. The amount of fertilizer given to coffee can be calculated from the need of nutrients and the availability of nutrients in soil. The need of nutrients is depend on the age of coffee, the amount of nutrients removed in yield, and the phenology of coffee plant, respectively.

The age of coffee is a factor that affect nutrients needed. The immature coffee trees need less nutrients than mature one, because the last one besides use the nutrients for their growth also need it to produce coffee bean.

Nutrients accumulated in the fruits will be removed when cherries are harvested. This loss needs to be compensated by the addition of fertilisers, organic manures, leaf fall or prunings and leaves from shade trees. Recycling of pulp to the soil after composting can help to reduce the additional (chemical) fertiliser needed, but nutrients contained in beans must be fully returned to soil as fertilizer.

Seasonal phenology of coffee that are consisted of flower primordia formation phase, the blossoming, the development and filling of cherry as well as seed is influenced by the latitude and the distribution of rainfall in the planting area. During flower primordia formation and blossoming, coffee needs rather high amount of phosphorus; but in the phase of development and filling of cherry as well as seed, potassium need is higher than other nutrients.

This paper discussing the amount and kind of nutrients those should be given to coffee crops to replace nutrients removed in coffee cherries as well as beans. Time of fertilizer application and the relations to the kind of nutrients also discussed.

Nutrients removed by coffee

There are many research results regarding nutrients removed by coffee trees. Catani and de Moraes *cit.* Willson (1985) estimated that major nutrients removed in 1 tonne of arabica green beans were 34.0 kg N, 5.2 kg P_2O_5 and 47.8 kg K_2O. They also reported that an arabica tree removed from the soil during its fifth year 118 g N, 16 g P_2O_5, 120 g K_2O, 76.5 g CaO and 23.4 g MgO. According to Ripperton, Goto and Palau *cit.* Willson (1985), the nutrients removed in bean, pulp and parchment equivalent to 1 tonne of arabica green beans are: in bean, 45.5 kg N, 7.67 kg P_2O_5 and 37.9 kg K_2O; in parchment, 2.27 kg N, 0.3 kg P_2O_5 and 1.87 kg K_2O; in pulp 15.33 kg N, 3.67 kg P_2O_5 and 27.4 kg K_2O. Roelofsen and Coolhaas *cit.* Willson (1985) reported that total losses of nutrients from robusta plantations equivalent to 1 tonne green beans were: 35 kg N, 6 kg P_2O_5, 50 kg K_2O, 4 kg CaO, 4 kg MgO, 0.3 kg Fe_2O_3 and 0.02 kg Mn_3O_4. Forestier *cit.* Willson (1985) also reported that without returning pulp and parchment to the plantation, one tonne of robusta green bean removes 30 kg N, 3.75 kg P_2O_5 and 36.5 kg K_2O. A results in Brazil on Mundo Novo reported in Table 1.

Table 1. The quantities of macro- and micronutrients in the aerial part of a 10 year-old Mundo Novo coffee tree in Brazil.

Nutrient	Trunk	Branches	Leaves	Fruit	Total
Macronutrients (g)					
Nitrogen	61.2	59.5	98.6	21.0	240.3
Phosphorus	2.0	5.2	8.3	2.1	17.6
Potassium	33.0	46.1	100.8	26.7	206.6
Calcium	39.1	38.2	58.4	2.7	138.4
Magnesium	6.8	4.8	17.8	1.7	31.1
Sulphur	4.4	5.6	13.6	1.4	25.0
Micronutrients (mg)					
Boron	108	135	312	30	585
Chlorine	1732	2958	27067	4131	35888
Copper	64	70	80	26	240
Iron	899	946	1920	65	3830
Manganese	108	161	507	27	803
Molybdenum	0.4	0.3	0.9	0.1	1.7
Zinc	24	50	70	13	157
Weight of parts (g)	9718	5856	3834	1211	20619

Source: Anon. cit. Wrigley, 1988

Coffee phenology in Indonesia

Seasonal phenology of coffee that are consisted of flower primordia formation phase, the blossoming, the development and filling of cherry as well as seed is influenced by the latitude and the distribution of rainfall in the planting area. Coffee areas in Indonesia spreads from western to eastern part of this country, and in northern as well as southern hemisphere. The consequence of that spread, there is a difference on the phenological period between northern and southern hemisphere.

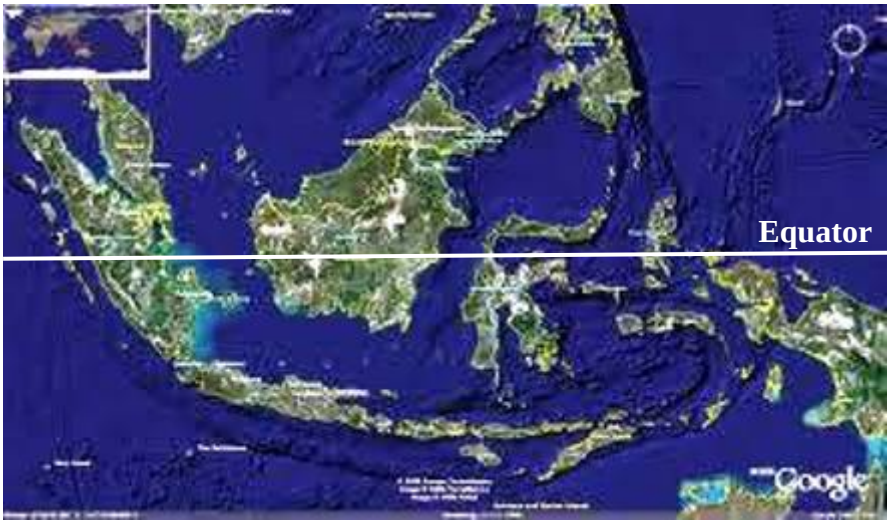

Figure 1. Map of Indonesia.

In southern hemisphere, the phase of flower primordia formation and blossoming occurs in the second half of the year (July-December), that are overlap apartly with period of the rainy season (November-April), while the development of cherry as well as seed and the filling phases occurs in the first half of the year (January- June), that are overlap partly with period of the dry season (May-October). This was opposite on coffee grown in northern hemisphere.

The majority of coffee areas in Indonesia located on southern hemisphere, i.e. Bengkulu, South Sumatera, Lampung, West Java, Central Java, East Java, Bali, West Nusa Tenggara, East Nusa Tenggara, South Sulawesi, West Sulawesi and Papua. Only two provinces that produce coffee in northern hemisphere of Indonesia, i.e. Aceh and North Sumatera.

Nutrients need for flower and seed development

Robusta coffee generally requires less nutrients than arabica (Harding, 1990). Nitrogen is needed for plant growth, synthesis of protein-enzymes-hormones, and involved in photosynthesis. Nitrogen is the most important plant nutrient and plants need it throughout the year. Nitrogen helps in the development of new shoots and berries. Nitrogen also helps in the production of large number of flowers and retention of leaves for a longer time. Adequately nitrogen supplied plants produce dense beans of higher quality (CCRI, 2008).

Nitrogen is essential for the development of stems, leaves and fruit (Willson, 1985). Applications of nitrogen to coffee which is not deficient in nitrogen will increase vegetative growth and yield. In arabica, nitrogen increased the number of of nodes per branch without affecting the internodal distance (Montoya *et al. cit.* Willson, 1985). In robusta, the increased yield was a consequence of an increase the number of fruiting nodeswhich was related to the application of nitrogen (Snoeck & de Reffye *cit.* Willson, 1985). Nitrogen also increased the number of flowers per node and the successful setting rate (Snoeck *cit.* Willson, 1985).

Phosphorus is essential for the synthesis of energy compounds, root development, flowering and ripening. Phosphorus is needed for the healthy and strong development of roots and shoots (CCRI, 2008).

Potassium holds a key position in the nutrition of plants and development of fruit. Potassium usually inadequate when coffee trees bearing a heavy cherries (Wrigley, 1988). K is particularly necessary during the period of fruit expansion and ripening. Inadequate potassium may increase the proportion of "floaters", or empty cherries (Harding, 1990). Potassium is another element which is essential for the fruit setting, bean filling, maturation and hardening of the beans. It improves the vigour and the pest and disease tolerance of the plants (CCRI, 2008).

In PNG, well managed rehabilitated coffee gardens and commercial plantations, producing more than 1 000 kg/ha green bean should be given a low-P/low-K compound in October and April/May, and straight N and K in December/January and February/March. Foliar sprays containing macro- and micronutrients should be given in July, August and September (Harding, 1990).

CONCLUSIONS

- Seasonal phenology of coffee that are consisted of flower primordia formation phase, the blossoming, the development and filling of cherry as well as seed is influenced by the latitude and the distribution of rainfall in the planting area.
- The nutrients needed by coffee crops follow the phases of the seasonal phenology.
- On the phase of flower primordia formation and blossoming required more P element than K, while the phase of cherry as well as seed development and filling are more K needed rather than P.
- The need of N element is relatively same in all the seasonal phenological phases.

- In coffee producing areas in Java (Indonesia, 5-8^{o}S of latitude), the phase of flower primordia formation and blossoming occurs in the second half of the year (July-December), that are overlap apartly with period of the rainy season (November-April), while the development of cherry as well as seed and the filling phases occurs in the first half of the year (January- June), that are overlap partly with period of the dry season (May-October).
- Fertilizer application is done twice a year to meet the optimum availability of water as a solvent for fertilizer, those are in the second half of the year (the beginning of the rainy season, November-December) and in the first half of the year (the end of rainy season, March-April).
- Based on the nutrient requirements in each phase of the seasonal phenology, the fertilizer needed by coffee plant in the second half of the year (November-December) consists of a half dose of N + full dose of P, whereas in the first half of the year (March-April) consists of a half dose of N + full dose of K.
- If it is assumed that the average productivity of coffee plants as much as 1 tonne per hectare of green coffee,

    - the minimum dose and composition of nutrients that should be applied in the form of fertilizer for arabica coffee in the second half of the year is 32 kg N/ha and 12 kg P_2O_5/ha, while in the first half of the year is 32 kg N/ha and 68 kg K_2O/ha.
    - the minimum dose and composition of nutrients that should applied in the form of fertilizer for robusta coffee in the second half of the year is 18 kg N/ha and 6 kg P_2O_5/ha, whereas in the first half of the year is 18 kg N/ha and 50 kg K_2O/ha.

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Spatial distribution of coffee from Minas Gerais State and their relation with quality

H. M. R. ALVES[1], J. N. BARBOSA[2], F. M. BORÉM[2], M. M. L. VOLPATO[3], M. A. CIRILLO[2], T. G. C. VIEIRA[3]

[1]Brazilian Agricultural Research Corporation, MG, BR
[2]Federal University of Lavras –MG, BR
[3]Agricultural Research Company of Minas Gerais, MG, BR

SUMMARY

The aim of this work was to assess the geographic distribution of coffee quality in Minas Gerais state, Brazil, and to study its interactions with chemical and environmental factors. Correlations between environmental factors, chemical compounds and sensory quality of participants of the Minas Coffee Quality Contest were made through Principal Component Analysis and Biplot Graphics. The results showed discriminations of high and low scores as a result of environmental variables, demonstrating a strong influence of temperature, rainfall, altitude and latitude on the quality of the coffees studied. In addition to the environmental characteristics, the chemical compounds trigonelline, caffeine, and especially the acid-5-cafeiolquinic were also relevant in discriminating the scores obtained through sensory analysis. This work is an initial indication of the factors that determine the quality of coffees produced in Minas Gerais.

INTRODUCTION

Brazilian coffee is mostly produced in the states of São Paulo, Espírito Santo, Bahia, Paraná, Rondônia and Minas Gerais, and coffee from each state has its own characteristics based on the environment and technological aspects of production. Minas Gerais is located in the Southeast region of Brazil, between the parallels 14º 13' 57'' and 22º 55' 47'' latitude South and between the meridians 39º 51' 24'' and 51º 02' 56'' longitude West, completely within the intertropical zone. With a territorial area of 582,586 km^2, Minas Gerais makes up 6.9% of the total area of Brazil and stands out as the country's largest coffee producer with a stake of 50.99% of the coffee produced in Brazil.

Its large territorial size and environmental variety makes it possible for the state of Minas Gerais to produce quality coffees with a great diversity of flavor and aroma. These differences are related to the particular characteristics of each municipality, mainly the climate variations, altitude and production systems.

Minas Gerais coffee lands are divided into the following four main macro-environments: Sul de Minas (South/Southwest region of the state), Matas de Minas (Zona da Mata and Rio Doce regions), Cerrados de Minas (Triangulo Mineiro and Alto Paranaiba regions) and Chapadas de Minas (Vale do Jequitinhonha and Mucuri regions).

Both domestic and international coffee markets have a growing demand for specialty coffees. Consumers seek exceptional taste and aroma as well as balanced characteristics of sweetness, acidity and body. In addition to the sensory qualities of the coffee, there is also a great interest

in products with marketable characteristics of production environment and geographic location.

The flavor and aroma of coffee are affected by the presence of various volatile and nonvolatile chemical constituents, such as proteins, amino acids, fatty acids and phenolic compounds, and also by the action of enzymes on some of these components. In addition to the chemical composition of the coffee, post-harvest processing also influences the final quality and characteristics of the product. Environmental factors, such as altitude and rainfall, have been highlighted as contributing to the quality of the coffee beverage, but further studies are needed to investigate additional environmental characteristics that affect coffee quality.

The aim of this work was to study the geographic distribution of the coffee in the Minas Coffee Quality Contest in 2007; also of interest was the relationship between the sensory quality and the chemical compounds trigonelline, caffeine and 5-caffeoylquinic acid (5-CQA) and the environmental characteristics of the municipalities of the 60 samples that were finalists in this contest.

MATERIALS AND METHODS

This work was carried out with data from the IV Minas Coffee Quality Contest (IV Concurso de Qualidade dos Cafés de Minas) held in 2007. Only coffee samples from the species *Coffea arabica* L., type 2 or better were accepted for the contest. The coffee beverage was required to be soft or superior, sieve 16 or above, with a maximum leakage of 5% and a maximum water content of 11.5%. The contest received coffee from everywhere in the state, and the samples were classified geographically according to the original municipality (Figure 1).

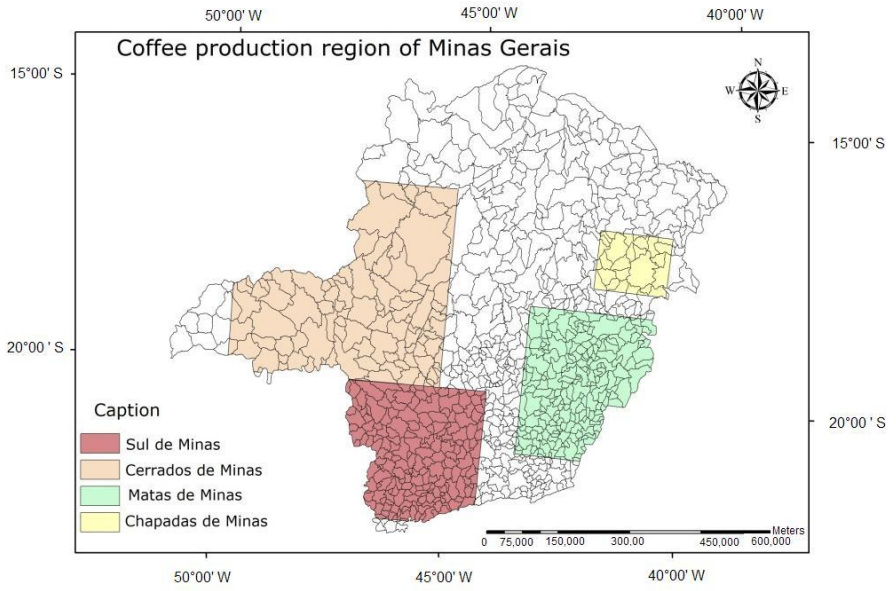

Figure 1. Map of the coffee regions in the State of Minas Gerais, adapted by Informe agropecuário.

The coffee samples underwent several stages of evaluation and classification. Samples were separated into two categories: natural coffee and cherry parchment coffee. The physical aspects of the samples were evaluated in the first stage, and the sensory attributes were evaluated from the second stage on. Each evaluation was conducted by a judging panel composed of at least ten classifiers and tasters. The sensory analysis was carried out according

to the Brazil Specialty Coffee Association – methodology, which evaluates the beverages based on taste, aroma, body, acidity, sweetness and fragrance and gives marks from 0 to 100. The contest data were provided by EMATER in the form of an electronic spreadsheet containing the following information about the samples: the municipality, category and evaluation score. In the 2007 edition, the total number of coffee samples registered in the contest was 1161.

Geographical distribution and environmental characteristics of the samples in the contest

The Geographic Information System open source TerraView, was used for the environmental characterization and analysis of the spatial distribution of the samples studied. The 1161 samples were evaluated spatially based on the geographic location (latitude and longitude) of the town where they originated. Using the GIS, the data were integrated with the state of Minas Gerais digital geographic base, made available by the GeoMinas. The temperature, rainfall and humidity index data were generated by the ZEE in Geotiff format.

Chemical Analysis: Trigonelline, Caffeine and 5-CQA

Among the samples approved in the fourth stage of the contest, 60 finalists' samples were randomly selected for chemical analysis of the following compounds: trigonelline, caffeine and 5-CQA.

The choice of these 60 finalists' coffee samples was based on a sampling plan that included 30 samples from each processing category (natural and cherry parchment). In each group of 30 samples, 15 coffee samples with scores over 80 points and 15 with scores below 75 points were selected. The samples were geo-referenced with a GPS using the geographic coordinates of each of the participants' farms.

The non-volatile compounds caffeine, trigonelline and 5-CQA were measured by high-performance liquid chromatography (HPLC) according the method of *Acta Scientiarum Agronomy*. For the extraction, 0.5 g samples of ground raw coffee diluted in boiling distilled water were used. The extract was then filtered with a Qualy® filter paper. A second filtration with a 0.45 µm Millipore membrane was performed before the HPLC readingsThe mobile phase consisted of a water: acetic acid ratio of 20:80:1, with 1 mL min-1 flux. For the identification and quantitative analysis, a standard curve was prepared using standards of caffeine, trigonelline and 5-CQA.

Principal Components Analysis (PCA) of the Chemical, Environmental and Sensory Quality Variables

The data were grouped in an electronic spreadsheet according to their town, category, latitude, longitude, altitude, temperature, rainfall, humidity index, trigonelline, chlorogenic acids, caffeine and sensory analysis scores.

Multivariate techniques, such as principal components analysis, were used and the results were displayed as biplot graphics.

The purpose of these techniques was to study the chemical and environmental variables that are important in contributing to the scores obtained in competition. The main objective of this multivariate analysis was to reduce the dimensionality of the original set of variables, with the

least possible loss of information and to allow the grouping of similar characteristics through graphic dispersions in a bi- or tri-dimensional space.

Geostatistic Analysis

The spatial dependence (based on the variables of altitude, latitude and longitude) of the scores obtained in the first stage of the contest was studied with the geostatistics program, R, using the Package GeoR (Note 2).

RESULTS AND DISCUSSION

Relationship between Altitude, Latitude and Coffee Quality

The results show that the distance between the spatially correlated samples is approximately 800 km; beyond this, there is no additional spatial correlation between samples.

It should be noted that the adequacy of the exponential model adjustment to the experimental data was considered acceptable because the estimated value $\hat{\gamma}(\mathbf{h})$repeated the expected trend with regard to the distance, h. Following this model, the adjustment of the area was performed using the kriging method because estimates based on this model are more precise and, therefore, more reliable.

Figure 2 shows the surface obtained from the altitude, latitude and sensory quality data. The results prove that the beverage quality scores varied with the altitude, as a function of the latitude. In other words, the higher the altitude, the higher the score and the higher the latitude, the lower the need for high altitude to get a better score.

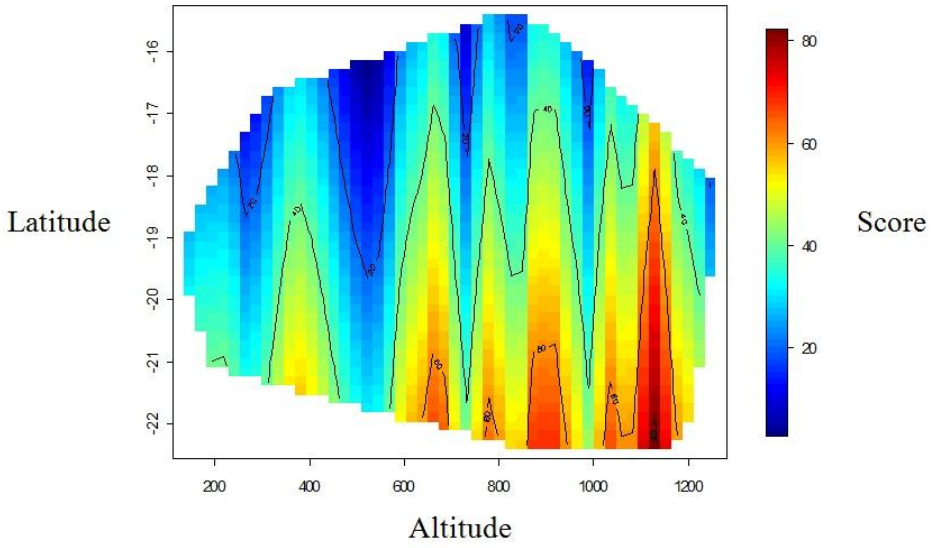

Figure 2. Coffee samples surface, showing the relationship of the beverage quality score, altitude and latitude.

This relationship was also noted by Avelino et al.who studied the effects of the exposure of the steeper slopes and different altitudes on the quality of the Costa Rica coffee terroirs. Other authors have also noted a relationship between geographical location and the influence of altitude on coffee characteristics in 20 regions of the world.

Discrimination between High and Low Scores by Principal Components Analysis

According to the principal components analysis, the determination of high and low scores is related to chemical, environmental and sensory quality variables.

The coefficient of greater numbers to the first component was given by the moisture index and of lower numbers was given by the trigonelline concentration, both for natural coffee and for cherry parchment coffee.

The equations of the first two main components, PC1 and PC2, were obtained using the method of *Applied Multivariate Statistical Analysis*, by creating a matrix of correlation of the coffee samples' chemical, environmental and sensory quality variables. Although values presented for the first and second component are not high (46.00 and 18.04%), respectively, these values were sufficient to discriminate between the different processing categories of coffee in relation to environmental factors and quality parameters. The environmental and chemical variables that showed better correlation with the scores are represented in the biplot graphics in Figure 3.

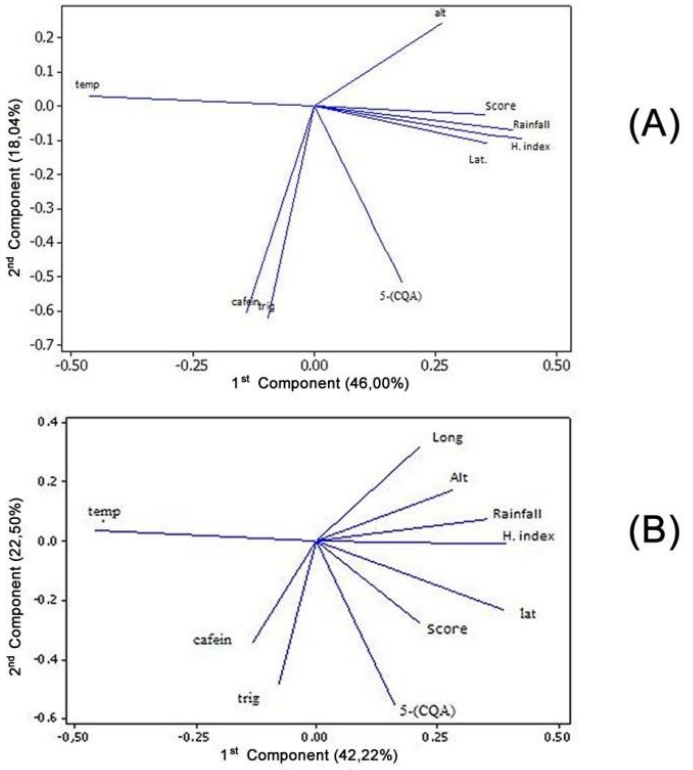

Figure 3. Biplot of the environmental, chemical and sensory quality variables for (A) natural coffee and (B) cherry parchment coffee. Score, rainfall, humidity index (H. index), temperature (temp), altitude (alt), latitude (lat), longitude (long), trigonelline (trig), caffeine (cafein) and 5-CQA.

The vectors indicate the variables that were determinant for the given score. To complement this, the scores graphic provided by each main component was able to discriminate the low scores (B) in the 1st and 4th quadrants and the high scores (A) in the 2nd and 3rd quadrants, as seen in Figure 4.

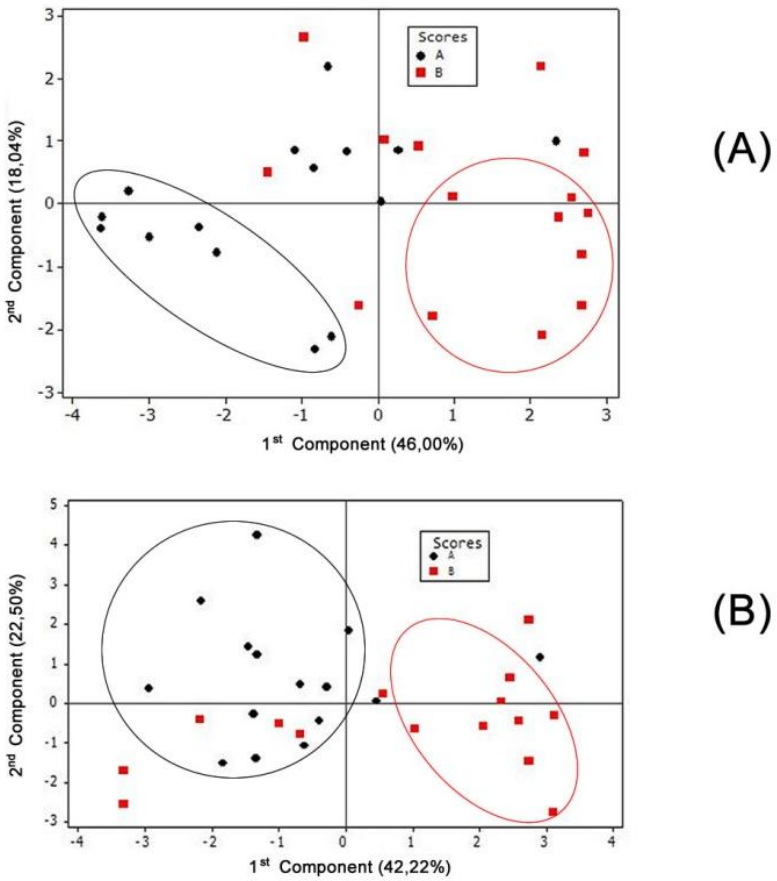

Figure 4. Scores of the two first main components for (A) natural coffee and (B) cherry parchment coffee. A = High scores; B = Low scores.

The low scores (Fig. 4) were mainly influenced by the following variables: humidity index, rainfall and 5-CQA, as indicated by the vectors in the biplot graphic in Fig. 3. While working with the correlation between the quality of coffee beverages and the presence of chemical compounds, Farah et al. observed that the presence of 5-CQA is associated with beverages of lower quality. According to the literature, a reduction in the quality of coffee correlates with an increase in phenolic substances.

Nature reported that chemical compounds, such as chlorogenic acids, exert a protective action. This action is explained by Cortez et al., who observed that in humid and hot areas, during maturation and harvest, the moisture in the air promotes the activity of microorganisms that detract from the quality of the drink. Fig. 3 (B) and Fig. 4 (B) show the results for cherry parchment coffee. As seen in the biplot graphic in Fig. 3 (B), the vectors that indicate the variables that best discriminate the high scores from the low scores were the chemical variables trigonelline and caffeine, and the environmental variable temperature. Temperature was the variable that most contributed to the discrimination between scores. According to *Acta Scientiarum Agronomy* trigonelline is an important precursor of the volatile compounds that contribute to the aroma and taste of roasted coffee. Working with the *cafés-terroir* in Honduras, Avelino et al. noted that the effect of temperature is conditioned by the latitude and altitude and that those attributes jointly favor coffee quality, producing the local characteristics of taste and aroma.

The graphics in Figure 3(B) and Figure 4(B) show a distribution similar to the graphics seen in Figure 3(A) and Figure 4(A) for natural coffee. The variables that contributed most to the

discrimination of high scores were temperature, trigonelline and caffeine and the variables that correlated with low scores were rainfall, humidity index and 5-CQA. However, for cherry parchment coffee, as seen in Figure 4(B), the component long (longitude) provided a small contribution to the discrimination of low scores when evaluating the whole set of nine variables. This contribution was not seen for natural coffee.

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Cultivars Obatã Iac 1669-20 and Iac Obatã Amarelo for Irrigated Cultivation

L. C. FAZUOLI[1], E. S. MANTOVANI[1], M. T. BRAGHINI[1], J. L. MISTRO[1], J. R. M. SERRA[2], P. LOSASSO[3]

[1]Centro de Café 'Alcides Carvalho', Instituto Agronômico de Campinas (IAC/APTA), SP, BR
[2]Agronomist, Fazenda Consuelo Gália, SP, BR
[3]Agronomist, Sítio Ouro Verde, Garça, SP, BR

SUMMARY

The present study aims to evaluate the arabica coffee cultivars, Obatã IAC 1669-20 and IAC Obatã Amarelo, are very productive, rust resistant and have been planted under fertigation and without irrigation in the cities of Gália and Garça, São Paulo State, Brazil, respectively. The variety with highest yield in the experiment was ferti-irrigated Obatã IAC 1669-20, with 76.9 bags/ha/year, not differing from IAC Obatã Amarelo (66.9). The susceptible cultivars Catuaí Amarelo IAC 62 and Catuaí Vermelho IAC 144 produced 48.1 and 50.5 bags/ha/year, respectively. Regarding the characteristics of the seeds, cultivars IAC Obatã 1669-20, IAC Obatã Amarelo had the highest percentages of flat beans and highest weight of 100 beans. And while for the values of the average bean size, the cultivars showed little variation (17.5 to 18.0). The cultivar Obatã IAC 1669-20 was also the most productive in the experiment without irrigation with 38.8 sac/ha/year, followed by the Catuaí Amarelo IAC 62 (32.0), IAC Obatã Amarelo (28.7) and Catuaí Vermelho IAC144 (21.5). Without irrigation, cultivar Obatã IAC 1669-20 was also vigorous and fruit maturity was the latest among the cultivars. Therefore, the cultivars Obatã IAC 1669-20 and IAC Obatã Amarelo are suitable for fertigation system which had the highest yield.

INTRODUCTION

The arabica coffee cultivars Obatã IAC 1669-20, with red fruit and IAC Obatã Amarelo, with yellow fruit, are high-yielding, rust resistant and were planted and evaluated under fertigation and without irrigation. The purpose of this study was to evaluate these cultivars under fertigation and rainfed cultivation in the counties of Gália and Garça, respectively.

Origin of Obatã IAC 1669-20

This cultivar was derived from the cross of cultivar Villa Sarchí with the Timor Hybrid (CIFC 832/2), which generated the F1 hybrid (H361/4), at the Coffee Rust Research Centre (CIFC), in Oeiras, Portugal. Of this hybrid, F2 coffee trees were planted in 1971 in Campinas, SP, with good yields. Progenies of selected coffee plants were evaluated in several experiments and selection was continued in this way for several generations. Cultivar Obatã IAC 1669-20 is the result of the, most likely natural, hybridization of a H 361/4 coffee tree with another of the cultivar Catuaí Vermelho. The cultivar Obatã IAC 1669-20 was officially released by IAC in 2000 and included in the National Register of Cultivars (RNC) in 1999. It is rust-resistant, late-maturing and in some regions even later than ‘Catuaí Vermelho’.

Origin of IAC Obatã Amarelo

IAC Obatã Amarelo is originated from a probably natural cross between cultivar Obatã IAC 1669-20 and 'Catuaí Amarelo', which occurred in an experiment established at the Cooperative of Coffee Growers in the region of Garça, SP (Garcafé), in the experimental area Dr. Alcides Carvalho. Beans of the original Obatã IAC 1669-20 tree, position 16 B, from this experiment were collected in 1984. In the following generations, plants with yellow fruit were observed, with the same characteristics as of cultivar Obatã IAC 1669-20. The progeny derived from cultivar Obatã with yellow fruit and rust resistance was designated IAC Obatã Amarelo.

MATERIAL AND METHODS

Two experiments with arabica coffee were installed, one without irrigation and the other under fertigation. The rainfed experiment was installed on March 8, 2006, in Garça, São Paulo State, on the Fazenda Ouro Verde, in a randomized block design with three replicates. Plots consisted of two rows with 17 plants each, of which 15 plants in either row were evaluated. At a spacing of 4.0 x 0.6 m, the cultivars Catuaí Amarelo IAC 62, Catuaí Vermelho IAC 144, Obatã IAC 1669-20, and IAC Obatã Amarelo were evaluated. The fertigation experiment was installed in Gália, São Paulo State, on March 7, 2006 on the Fazenda Consuelo, in a randomized block design with two replicates. The plots consisted of two rows with 27 plants each, of which two rows with 25 plants were evaluated and a third replication consisted of two rows with seven plants each, of which five plants in both rows were evaluated. The low-stature and rust-susceptible cultivars Catuaí Amarelo IAC 62 and Catuaí Vermelho IAC 144 and the rust-resistant were Obatã IAC 1669-20 and IAC Obatã Amarelo were assessed at a spacing of 3.30 x 0.6 m.

The following variables were evaluated: yield, plant vigor, fruit ripening, production of processed coffee, and bean characteristics. The yields of three years (2008, 2009 and 2010) were determined by weighing the green coffee harvested from each plot. Also, a sample of coffee beans was taken from each plot, dried, weighed (coffee bean weight) and processed to transform the green coffee weight into production of processed coffee (bags of processed coffee per hectare) considering the number of plants/ha according to the spacing. From these samples, the types of beans (flat, peaberry and elephant beans) and average size were also evaluated. The production was also visually assessed on a 1-10 grade scale, where 1 stands for low and 10 for high yield; this index was named visual evaluation index of yield (IAV yield).

The visual evaluation index of vigor (IAV vigor) was evaluated before harvest, on a 1-10 scale, where 1 corresponds to plants with low plant vigor and 10 for plants with excellent vigor, and intense vegetative growth of the productive branches.

Maturation was assessed before harvest, assigning grade 1 to early maturing, 2 to average to early, 3 to average, 4 to average to late and 5 to late.

The beans were evaluated for the characteristics: percentage of flat, peaberry and elephant beans, 100-bean weight and mean bean size.

RESULTS AND DISCUSSION

The results are presented separately for the fertigation and the rainfed experiment.

Fertigation experiment

The average yield and agronomic characteristics of the three growing seasons are shown inTable 1. The highest-yielding cultivar was Obatã IAC 1669-20, with 76.9 bags/ha/year, not significantly different from IAC Obatã Amarelo (66.9). The rust-susceptible cultivars Catuaí Amarelo IAC 62 and Catuaí Vermelho IAC 144 produced 48.1 and 50.5 bags/ha/year, respectively.

Cultivar Obatã IAC 1669-20 was the latest-maturing, but not significantly different from IAC Obatã Amarelo.

Table 1. Average yield of processed coffee of three growing seasons (in bags/ha/year), agronomic characteristics, visual assessment index (IAV) for vigor and fruit yield and maturation, assessed in 2008, 2009 and 2010 in the fertigation experiment in Gália - SP.

Cultivars	Productivity		Agronomic characteristics		
	Bags/ha/year	%	IAV		[3]Maturation
			[1]Vigor	[2]Yield	
Obatã IAC 1669-20	76.9	159.9	8.8	7.3	4.7
IAC Obatã Amarelo	66.9	139.1	8.8	7.6	4.4
Catuaí Amarelo IAC 62	48.1	100.0	8.5	6.3	3.9
Catuaí Vermelho IAC 144	50.5	105.0	9.1	6.7	3.7

[1]IAV vigor = Visual Evaluation Index for vigor grade: 1 = plants with reduced vigor and pronounced deficiency symptoms; 10 = for plants with excellent vigor, dense canopy and intensive growth of the productive branches; [2]IAV yield = Visual Evaluation Index for yield: grade 1 = no yield, 10 = high yield; [3]Maturation: 1 = early, 2 = average to early, 3 = average, 4 = average to late, 5 = late.

Regarding the bean characteristics (Table 2), the cultivars Obatã IAC 1669-20 (84.8), IAC Obatã Amarelo (83.0) had the highest percentages of flat beans and highest weight of 100 beans. In terms of average bean size, the cultivars varied but little (17.5 - 18.0).

Table 2. Average bean characteristics assessed in 2008, 2009 and 2010, in the fertigation experiment in Gália - SP.

Cultivars	Bean characteristics				
	Flat	Peaberry	Elephant bean	100 bean weight (g)	Average size
	%				
Obatã IAC 1669-20	84.8	11.1	4.1	15.0	17.6
IAC Obatã Amarelo	83.0	12.9	4.1	14.9	17.8
Catuaí Amarelo IAC 62	78.1	12.7	9.2	13.9	17.5
Catuaí Vermelho IAC 144	78.0	11.5	10.5	14.1	18.0

Experiment without irrigation

The yield data and agronomic characteristics obtained in three growing seasons are shown in Table 3. Cultivar Obatã IAC 1669-20 was the highest-yielding, with 38.8 bags/ha/year, followed by the Catuaí Amarelo IAC 62 (32.0), IAC Obatã Amarelo (28.7) and Catuaí IAC144 (21.5).

Cultivar Obatã IAC 1669-20 was more vigorous and fruit maturation was later than of the other cultivars.

Table 3. The average productivity of processed coffee (in bags/ha/year) and agronomic characteristics, assessed in 2008, 2009 and 2010, in the experiment without irrigation in Garça - SP.

Cultivars	Productivity		Agricultural characteristics		
	Bags/ha/year	%	IAV		[3]Maturation
			[1]Vigor	[2]Yield	
Obatã IAC 1669-20	38.8	180.5	7.6	6.3	4.2
IAC Obatã Amarelo	28.7	133.5	7.2	5.4	3.6
Catuaí Amarelo IAC 62	32.0	148.8	7.1	6.0	3.8
Catuaí Vermelho IAC 144	21.5	100.0	7.4	5.9	3.5

[1]IAV vigor = Visual Evaluation Index for vigor grade: 1 = plants with reduced vigor and pronounced deficiency symptoms; 10 = for plants with excellent vigor, dense canopy and intensive growth of the productive branches; [2]IAV yield = Visual Evaluation Index for yield: grade 1 = no yield, 10 = high yield; [3]Maturation: 1 = early, 2 = average to early, 3 = average, 4 = average to late, 5 = late.

Table 4 presents data of bean characteristics. The results indicate no significant variation between the cultivars.

Table 4. Characteristics of beans obtained in 2008, 2009 and 2010, in the rainfed experiment in Garça - SP.

Cultivars	Bean properties				
	Flat	Peaberry	Elephant bean	100 bean weight (g)	Average size
	%				
Obatã IAC 1669-20	81.5	17.0	1.5	13.8	17.5
IAC Obatã Amarelo	80.9	16.2	3.0	12.3	17.4
Catuaí Amarelo IAC 62	81.8	15.1	3.1	13.5	17.1
Catuaí Vermelho IAC 144	80.0	16.4	3.6	12.2	17.4

CONCLUSIONS

- The cultivars Obatã IAC 1669-20 and IAC Obatã Amarelo are suited for fertigation, under which yields were highest.
- The yield performance of cultivar Obatã IAC 1669-20 was 59.9% higher than that of Catuaí Amarelo IAC 62, in three growing seasons under fertigation.
- The yield performance of cultivar IAC Obatã Amarelo was 39.1% higher than that of Catuaí Amarelo IAC 62, in three growing seasons under fertigation.

- Without irrigation, the yield performance of cultivar Obatã IAC 1669-20 was higher than of the other cultivars.

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Iac 125 Rn, a New Cultivar of Coffea Arabica Resistant to Rust and to the Meloidogyne Exigua Nematode

L. C. FAZUOLI[1], M. T. BRAGHINI[1], M. B. SILVAROLLA[1], W. GONÇALVES[1], J. C. MISTRO[1], O. GUERREIRO-FILHO[1], P. B. GALLO[2], S. R. ALMEIDA[3]

[1]Centro de Café 'Alcides Carvalho', Instituto Agronômico de Campinas (IAC/APTA), SP, BR
[2]APTA, Polo Nordeste Paulista, Mococa, SP, BR
[3]Fundação PROCAFÉ, Varginha, MG

SUMMARY

The aim of this study was to develop a coffee cultivar with short stature with resistance to coffee leaf rust (*Hemileia vastatrix*) and root knot nematode (*Meloidogyne exigua)*. IAC received in 1971, the following F2 hybrid seeds from the cross CIFC H361/4 (cv. Villa Sarchí x Timor Hybrid, CIFC 832/2), which constitute the basis for obtaining the IAC 125 RN cultivar. The F2 hybrid H361/4 was assigned the IAC introduction number 1669. Throughout evaluation of the early experiments with this material in Campinas, it appeared that the F2 plant IAC 1669-13 and its progeny was superior than the others, due to its productivity, the size of seeds and resistance to rust and Meloidogyne exigua. Selections were made at various locations in segregating generations, and trees resulting from the F6 generation were assigned to as IAC 125 RN. In irrigated areas its average yield varied from 60 to 66 bags of coffee /ha/year and in non-irrigated areas yields varied from 33 to 59 bags /ha/year. This was substantially more than the Catuai control varieties used, especially so under irrigated conditions. The IAC 125 RN cultivar has large grains with average sieve size of 17.4 and 90.2%, 8.0% and 1.8% of flat bean types, peaberries and elephant beans, respectively. The young leaves are green colored. Its stature is dwarf and the berries are large, red, and show early maturation. Coffee seedlings were tested for resistance to the nematode *Meloidogyne exigua*. race 1. The gall index was 0.5 in this material, with small thickenings, however, without typical galls. In the control cv. Catuaí Vermelho IAC 144, the gall index was 4.5, with very typical galls. Plants were tested for race 2 of *M. exigua* and were also resistant. These results indicate that this cultivar is highly resistant to *M. exigua*. With regard to reaction to rust, it was found that, under field conditions, so far, the material remains resistant to races prevailing at the places where the cultivar was planted. It is recommended to be planted in soils with high fertility in highlands and mainly under irrigation or fertilizer-irrigation, since it is demanding in nutrients and water. Due of its multiple resistance to rust and nematode *M. exigua*, the IAC 125 RN cultivar may also be planted in areas infested with *M. exigua.* The cup quality is similar to Catuaí. It is another great option for all the Brazilian coffee growers, besides the well-known Catuai and Mundo Novo cultivars.

INTRODUCTION

Rust (*Hemileia vastatrix*) is the major disease of coffee. For Brazil to maintain its high coffee production it is important to have cultivars with high yield and resistant to major diseases and nematodes available to producers. The objective of this study was to develop a cultivar of arabica with rust resistance and resistance to *Meloidogyne exigua*.

MATERIALS AND METHODS

F2 seed was used descending from a F1 cross between Villa Sarchí and the Hybrid of Timor CIFC 832/2 (H361/4) that was obtained in 1967 by the Research Centre of Coffee Rusts (CIFC), Oeiras, Portugal. The Timor Hybrid CIFC 832/2 is a selection with rust resistance derived from a cross between *Coffea arabica* and *C. canephora*, with two probable backcrosses with *C. arabica*, that occurred in East Timor (former Portuguese colony). F1 hybrid seeds (F2) were sent to the Agricultural Institute in 1971, in Campinas, SP, BR, where they received the acronym IAC 1669 and constituted the basic genetic material for selection in Brazil according to line selection procedures.

The experiments and or selection fields were established in various coffee growing regions in the states of São Paulo and Minas Gerais. The selection traits observed over 6 generations of selection were: kg of green coffee per tree, vigor (visually evaluated using a 10-point scale: IAV vigor), productivity (also evaluated visually through a 10-point evaluation scale: IAV production), earliness of ripening, fruit size, outturn, percentage of flat beans, of peaberries, of elephant beans, 100 green coffee bean weight, bean size (sieve average) and rust resistance. Fungicide was applied in the experimental fields, except in Campinas and Mococa-SP.

The rust resistance was assessed by giving scores from 1 to 5, with 1 and 2 = resistant, 3 = moderately resistant, 4 and 5 = moderately susceptible and susceptible. The index for vigor (IAV vigor) was obtained by giving scores from 1 to 10, where 1 and 10 represent low and high vigor plants with few leaves (1) and many leaves (10) respectively. The index IAV production was obtained giving scores from 1 to 10, where 1 and 10 are unproductive and highly productive, respectively. The outturn is the percentage of green coffee in relation to the dry coffee berries.

Evaluations for resistance to *Meloidogyne exigua* (races 1 and 2) were made in experiments conducted with twenty repetitions in a greenhouse. The inoculations were done on 16 March 2005 and scoring was done 13 July 2005. The inoculum consisted of 5000 eggs and juveniles J2 per pot of 300 ml for the two races of *M. exigua*.

RESULTS AND DISCUSSION

Selection procedure of the IAC 125 RN cultivar

The F2 progeny consisted of 27 trees which production and other agronomic and technological characteristics were analyzed in the period from 1974 to 1981. The selection was done using the pedigree method. In 1985, the F2 plant IAC 1669-13 was selected for being more productive, vigorous and with dwarf stature. Other features of this tree were: new leaves were green, the berries were red and large, the green coffee had high sieve scores and the tree was highly resistance to rust.

Coffee plants that originated from IAC 1669-13 F2 (F3 generation) were planted in Varginha - MG, to study their adaptation and production, from where some trees were further selected. Seeds of the best F3 coffee tree (F4 generation) were included in an experiment in Patrocínio, MG, and the good productivity was confirmed in this experiment. From this generation onward, the name remained as IAC 1669-13. Subsequently a selection field (F5 generation) was established in Patrocínio, MG, conducted with irrigation. The result was excellent, with high yields and the material was considered promising also for other agronomic and technological characteristics. Since 2000, IAC 1669-13 was established in several selection

trials in Patrocínio and Patos de Minas, in Minas Gerais, and in Campinas and Mococa, in São Paulo.

The F6 generation, from selected F5 trees, was planted in 2006 in Campinas, SP and Mococa, SP. Selections were made in these two experiments, the trees being planted F6 generation in 2008 in Campinas from F5 generation. From the F6 generation, which is already fairly uniform, the name of IAC 125 RN was attributed to this cultivar.

Genealogy of the IAC 125 cultivar

The outline of the genealogy of IAC 125 RN is presented in Table 1.

Table 1. Genealogy of IAC 125 RN.

Year	Local	Generation	Germplasm
1967	Oeiras - Portugal	F1	CIFC H361/4 (Villa Sarchí CIFC 971/10x Híbrido de Timor CIFC 832/2
1971	Campinas - SP	F2	IAC 1669 (Sementes F2)
1972/81	Campinas - SP	F2	IAC 1669
1987/92	Varginha - MG	F3	IAC 1669-13
1994/99	Patrocínio - MG	F4	IAC 1669-13
2000/06	Patrocínio - MG	F5	IAC 1669-13
2005/2010	Campinas - SP	F6	IAC 1669-13
2006/10	Mococa - SP	F6	IAC 1669-13
2006/10	Campinas - SP	F6	IAC 125 RN
2008/10	Campinas - SP	F6	IAC 125 RN

Yield performance of IAC 125 RN

The IAC 125 RN cultivar was evaluated in field trials conducted in areas suitable for the cultivation of arabica coffee in the States of São Paulo and Minas Gerais. Important differences were observed in the development and production of plants of the cultivar due to the use or non-use of irrigation. IAC 125 RN shows substantially higher production under irrigated conditions in comparison to the control Catuaí cultivar IAC 144 (Table 2). In Patrocínio, MG, the productivity of the cultivar in irrigated field trials for the first five harvests was 91, 50, 89, 50 and 50 bags of green coffee per hectare per year and an average of 66 bags /ha/year, at spacings of 3.68 x 0.5 m.

Table 2 shows that average productivity in irrigated areas varies from 60 to 66 bags of coffee /ha/year in non-irrigated areas and from 33.1 to 59.4 bags /ha/year. In areas without irrigation the average productivity of the Catuaí control cultivar ranged from 31.1 to 36.4 bags of green coffee.

Table 2. Productivity of IAC 125 RN in bags of green coffee per hectare per year in irrigated and non-irrigated experiments in various coffee regions in the states of São Paulo and Minas Gerais.

Irrigated conditions			
Locality	**Harvest Years**	**Average productivity of IAC 125 RN cultivar**	**Average productivity IAC 144 cultivar of Catuaí Vermelho**
Patrocínio – MG	5	66.0	40.0
Patos de Minas – MG	3	60.0	40.0
Non-irrigated coffee			
Locality	**Harvest Years**	**Average productivity of IAC 125 RN cultivar**	**Average productivity of Catuaí control cultivar**
Mococa – SP	3	59.4	36.4 (Catuaí Vermelho IAC 144)
Campinas – SP	3	35.5	31.0 (Catuaí Vermelho IAC 144)
Patos de Minas – MG	3	40.0	35.0 (Catuaí Vermelho IAC 144)
Campinas – SP	6	33.1	34.7 (Catuaí Vermelho IAC 144)

Other selection traits

The cultivar presents large grains with average sieve size of 17.4 and 90.2%, 8% and 1.8% of the grain types being flat beans, peaberries and elephant beans, respectively. The young leaves are green. Its size is dwarf type (as Caturra) and the berries big and red, with early maturation. The dimensions of plant height and crown diameter are slightly smaller than those of red Catuaí IAC 144. It is demanding in nutrients. It has good cup quality, with its grains receiving broad market acceptance.

Resistance to root knot nematodes

Coffee cultivar IAC 125 RN seedlings were tested in 2005 for resistance to *M. exigua* races 1 and 2. A gall index of maximum 0.5 was observed for race 1 (Table 3), only with very small root hypertrophies, but without the typical galls caused by susceptible cultivars. No hypertrophy at all was observed in relation to race 2 (Table 4) In cultivar IAC 144 the gall index was equal to 4.5, with frequent presence of typical galls.

Table 3. Resistance IAC 125 RN Meloidogyne exigua race 1[1].

Cultivate	**IG[2]**	**Classification**	**NOSR[3]**	**NO/g raiz[4]**	**FR**	**Classification**
Catuaí Vermelho IAC 144	4.6	Susceptible	5550	2649.5	1.12	Susceptible
IAC 125 RN	0.5	Resistant	100	51.5	0.02	Resistant

[1]Race determined from tests with host range, being pathogenic to coffee and tomatoes, as well as by presenting phenotype esterase isozyme E1;IG[2] = IG Index for galls. Scale 0-5 proposed by Taylor & Sasser (1978). Plants with GI less than or equal to 2 are resistant; NOSR[3] = Number of eggs in the root system. Average of four plants with GI less than or equal to 2; NO [4] / g root = number of eggs per gram of root. Average of four plants with GI less or equal than 2.

Table 4. 125 RN cultivar resistance to Meloidogyne exigua race 2 [1].

Cultivate	IG[2]	Classification	NOSR[3]	NO/g raiz[4]	FR	Classification
Catuaí Vermelho IAC 144	4.1	Susceptible	1047	2585.3	2.10	Susceptible
IAC 125 RN	0.0	Resistant	25	16.4	0.01	Resistant

[1]Race determined from tests with host range, being pathogenic to coffee and tomatoes, as well as by presenting phenotype esterase isozyme E1; IG[2] = IG Index for galls. Scale 0-5 proposed by Taylor & Sasser (1978). Plants with GI less than or equal to 2 are resistant; NOSR[3] = Number of eggs in the root system. Average of four plants with GI less than or equal to 2; NO [4] / g root = number of eggs per gram of root. Average of four plants with GI less or equal than2.

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Seed Storage of Arabica and Robusta Coffee

M. T. BRAGHINI, L. C. FAZUOLI

Centro de Café "Alcides Carvalho"- Instituto Agronômico de Campinas (IAC)
P. O. Box 28, 13001-970, Campinas, SP, Brazil.

SUMMARY

The purpose of this study was to determine the parameters of Arabica and Robusta coffee seed storage, to preserve a good germination capacity for grafted seedling production. Beans of the cultivar Mundo Novo of *Coffea arabica* and Apoatã of *C. canephora* were used, at two initial moisture levels: high (35-37%) and medium (20-25%). They were placed in two types of wrapping: transparent polyethylene plastic bag (film thickness 0.16 mm) and in plastic mesh bags and stored at three different locations: in the laboratory, without control of temperature and relative humidity; in a cool container or with temperature control, and in a cold chamber, with temperature and relative air humidity control. The seed moisture content and germination rate were evaluated every 2 months for 12 months. The best treatment for seed storage of both cultivars was: cool container at 13±3°C, in a 0.16 mm polyethylene bag with an initial seed moisture content of around 35%.

INTRODUCTION

Under normal storage conditions (paper bag, room temperature and seed moisture of 15%), coffee seeds quickly lose their viability. The two economically most important coffee species, *Coffea arabica* and *C. canephora*, generally lose their germination capacity 3-6 months after harvest. For this reason, sowing normally occurs immediately after harvest or within six months at most. Due to this fact, coffee seed producers have difficulties in maintaining seed reserves. Consequently, sowing in nurseries must be performed shortly after harvest, which may not always be the most ideal moment. Furthermore, for seedling production with hypocotyledonary grafting technique, for planting in nematode-infested areas, it would be highly desirable to maintain a seed stock of Arabica and Robusta coffee for approximately one year, since grafted seedlings can be produced year-round. Thus, several studies have been conducted to establish technologies that can improve conditions for a longer maintenance of seed viability. However, these studies were not consistent and often conflicting in some aspects, for example with regard to the initial seed moisture content, wrapping and environmental storage conditions. Thus, a number of studies were conducted at the Agronomic Institute of Campinas (IAC) to determine the conditions for seed storage of Arabica and Robusta coffee that would preserve at least 70% of the germination capacity of Arabica and 60% of Robusta coffee for the longest possible period, as determined by the Norms and Standards for Coffee Seed Production, for the State of São Paulo.

MATERIALS AND METHODS

Seeds *C. arabica* cv. Mundo Novo IAC 388-6 and *C.canephora* cv. Apoatã IAC 2258 were obtained from coffee cherry harvest in 1999. Immediately after harvest, the cherries were mechanically depulped, mucilage removed in a natural 24-h fermentation and washed in tap water. The seeds were dried in the shade to two moisture levels: high (35-37%) and medium (20 - 25%) and wrapped in two packaging materials: plastic bag with transparent polyethylene

(film thickness 0.16 mm) and plastic mesh bag. Seeds were treated with Tecto 600 fungicide at a ratio of 1g/kg seeds. After the treatment, the seeds were stored at the three locations described below:

- Seed laboratory, without control of temperature and relative air humidity, with approximate temperature of 22-29 ° C and 70-95% relative air humidity throughout the experiment.
- Cool container controlled temperature of 13 □ 3 ° C and relative humidity 60-90%.
- Cold chamber with temperature and relative humidity of 9 ± 1 º C and 57 ± 1%, respectively.
- The statistical design was completely randomized with a 3x2x2 factorial design, with three environments, two types of wrapping material and two levels of initial seed moisture for each coffee species.

The germination capacity and seed moisture analyses were performed in compliance with the Norms for Seed Analysis, at the beginning of the experiment and then every two months during the storage period.

The moisture content was determined in two replications of 30g seed samples at 105±3ºC, for 24 hours. For the germination tests, seeds without the (manually removed) parchment were used at a constant temperature of 30 º C, with four replications of 50 seeds.

RESULTS AND DISCUSSION

The data of germination capacity and seed moisture of the cultivars Mundo Novo IAC 388-6 and Apoatã IAC 2258 obtained during storage are shown in Tables 1 and 2. An analysis of these two Tables indicates that the 0.16 mm polyethylene wrapping was efficient in preserving seed moisture throughout the experimental period in all environments except in the laboratory. Furthermore, the seeds in the plastic mesh bags lost moisture after two months of storage in all environments tested. The germination capacity of seeds maintained in a laboratory environment without control of temperature and relative humidity declined rapidly, as of the sixth month of storage. This drop, particularly marked for cv. Apoatã, was unrelated to the wrapping material and initial moisture level. The most effective conditions for the preservation of the germination capacity of *C. canephora* cv. Apoatã IAC 2258 were given by a high initial seed moisture content of 35% in polyethylene bags, in a refrigerator (13±3°C, 60 – 90% RH), with a germination rate of 76% after 10 months of storage. Interestingly, after 12 months, 51% of the seeds were still able to germinate, which is a higher value than recorded in the other treatments after 10 months of storage.

Generally, the conservation of seeds of *C. arabica* cv. Mundo Novo, in environments with controlled temperature (container and chamber) was very satisfactory. In the cold chamber, the seed germination rate was high until 10 months of storage, regardless of the wrapping and initial moisture. However after 12 months, the best conditions were given by wrapping seeds in plastic mesh bags, with high or medium initial moisture content. In the container, only the seeds stored in polythene bags with high initial moisture content preserved a germination rate of 87%. It should be remembered that viable seeds of both species are required to produce grafted seedlings, and especially to ensure minimum germination rates of 70% of Arabica and 60% of Robusta. In this experiment it was found that, after 10 months of storage, these germination rates are preserved under the conditions given by a cool container, at 13 ± 3°C, wrapping in polyethylene bags (thickness 0.16 mm) and a high initial seed moisture content (around 35%).

Table 1. Germination rate of *Coffea arabica* cv. Mundo Novo IAC 388-6 and moisture content (%), using seeds in two wrappings, stored in three environments.

Environment	Wrapping	Seed moisture (%)			Germination (%)			
		Beginning	6 months	12 months	Beginning	6 months	10 months	12 months
Room	Polyethylene	20	21	19	95	15	0	0
		37	29	30	95	76	7	0
	Mesh	20	13	12	95	16	0	0
		37	13	11	95	28	0	0
Cool container	Polyethylene	20	19	20	95	84	40	5
		37	35	33	95	94	87	87
	Mesh	20	13	16	95	92	74	55
		37	13	16	95	82	72	53
Cold chamber	Polyethylene	20	18	19	95	93	91	77
		37	34	33	95	95	92	58
	Mesh	20	10	10	95	94	83	83
		37	10	9	95	92	89	82

Table 2. Germination rate of seeds of *Coffea canephora* cv. Apoatã IAC 2258 and moisture content (%), using seeds in two wrappings stored in three environments

Room	Wrapping	Moisture (%)			Germination (%)			
		Beginning	6 months	12 months	Beginning	6 months	10 months	12 months
Room	Polyethylene	25	26	21	95	1	0	0
		35	34	30	95	9	0	0
	Mesh	25	11	11	95	1	0	0
		35	11	11	95	3	0	0
Cool container	Polyethylene	25	25	26	95	80	25	2
		35	32	33	95	91	76	51
	Mesh	25	12	17	95	81	28	4
		35	13	17	95	63	20	4
Cold chamber	Polyethylene	25	24	25	95	76	18	2
		35	32	34	95	76	23	21
	Mesh	25	11	10	95	59	47	47
		35	8	9	95	54	36	30

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Long-Term Evaluation of "Conilon" Coffee Yield from Plants Propagated by Cuttings and Seeds

F. L. PARTELLI[1], M. B. SILVA[1], I. GONTIJO[1], H. D. VIEIRA[2], J. C. RAMALHO[3], P. BATISTA-SANTOS[3], A. GOLYNSKI[4], M. C. ESPINDULA[5]

[1]Centro Universitário Norte do Espírito Santo - Universidade Federal do Espírito Santo, Rodovia BR 101 Norte, Km. 60, Bairro Litorâneo, 29932-540, São Mateus, Espírito Santo, Brazil
[2]Laboratório de Fitotecnia - Universidade Estadual do Norte Fluminense, Av. Alberto Lamego 2000, 28013-602 Campos dos Goytacazes, Rio de Janeiro, Brazil
[3]Centro de Ambiente, Agricultura e Desenvolvimento (BioTrop), Instituto de Investigação Científica Tropical (IICT), Qta Marquês, Av. República, 2784-505 Oeiras, Portugal
[4]Instituto Federal Goiano, *Campus* Morrinhos. Rodovia BR-153, Km 633, Zona Rural, Caixa Postal 92, 75650-000 Morrinhos, Goiás, Brazil
[5]Embrapa Rondônia, BR 364 - Km 5,5 - Zona Rural, Caixa Postal 127, 76815-800, Porto Velho, Rondônia, Brazil

SUMMARY

World coffee production in recent years has been around 7.5 to 8 million Ton, mostly in developing countries, supplying the total national demand, providing local employment and promoting rural development, with Brazil as the largest world producer and exporter. In 2011, the brazillian coffee production was 2.61 million Ton, on a cultivated area of 2.3 million ha, with a total of 6,400 millions coffee trees. The objective of this long-term experiment of 12 years of *C. canephora* cv. Conilon, in Vila Valério, Espírito Santo, Brazil, was to evaluate possible yield differences along that period between plants implanted from seed or cuttings.

The experiment was performed in randomized complete block design, with two treatments (seedlings originated from seeds and cuttings), and 12 replicates with five plants per plot, implanted by 2 x 1 m, in November 1999. The plant production was analyzed along a 12 year period, from 2001 (17 months) until 2012 (149 months).

The production from the plants obtained from cuttings was higher until the 10th harvest (although not significantly in the 3rd, 5th and 6th), except for the 7th and 9th, when plants propagated from seeds presented marginal (not significantly) higher yield, as observed also in the 11th and 12th harvest.

Although in the last 8 harvests only two were significantly higher in cutting plants, the accumulated production over a 12 year yields in these plants was higher in *ca*. 6214 kg ha^{-1}, when compared with plants implanted from seed over the same period. That reflects a strong yield advantage in the first years of production crop implanting using cuttings instead of seeds, although a convergence of yields seemed to occur after 10 years.

INTRODUCTION

The genus *Coffea* comprehends at least 103 species, with commercial relevance for *C. arabica* and *C. canephora*. World coffee production in recent years has been around 7.5 to 8 million Ton, mostly in developing countries. Brazil is the biggest world coffee producer and exporter, with this culture constituting an important source of incomes, employment and local development in the producing or processing regions. In 2011, the Brazilian coffee production was 2.61 million Ton, on a cultivated area of 2.3 million ha, with a total of *ca*. 6,400 millions trees.

When propagated through seeds, *C. canephora* cv. Conilon plants shows large variations concerning the productivity, plant architecture, disease resistance, fruit maturation, size and shape of seeds, fruits and leaves, as the *C. canephora* is a diploid (2n = 22 chromosomes), allogamous species, self-sterile with a gametophytic incompatibility. On the other hand, vegetative propagation maintain the genetic characteristics of a selected plant, granting a certain crop homogeneity concerning grain maturation, yield potential and other desirable agronomic characteristics, showing as well production precocity. In this way, the use of cuttings has become quite advantageous, using orthotropic branches. Nevertheless, the yield potential, resistance to environmental constraints and durability of such plants are still a matter of debate. It is known that the root system shows differential characteristics according to the species, genotype, plant age, year season, climate, crop density, biotic stresses, soil texture and structure, etc.. However, it was reported that until the 6th year Conilon plants did not show differences concerning the root system (superficial area and length) until a depth of 60 cm, either propagated from cuttings or seeds.

The objective of this work was to evaluate yield differences between *C. canephora* cv. Conilon plants implanted both from seed or cuttings in a long-term experiment of 12 years, in Vila Valério, Espírito Santo, Brazil.

MATERIALS AND METHODS

The experiment was carried out in the North of Espírito Santo State Brazil (Vila Valério -18º 57` South; 40º 17` West). In this region, the tropical climate is predominant (humid and hot summer, dry winter), with annual precipitation average of 1200 mm and annual temperature average of 23 ^{o}C. The predominant soil is a distrofic Red-Yellow Latosol (typic Hapludox).

The experiment was performed in randomized complete block design, with two treatments (seedlings originated from seeds and branches), and 12 replicates with five plants per plot, implanted by 2 x 1 m, in November 1999. The plant production was analyzed along a 12 year period, from 2001 (17 months) until 2012 (149 months). From 2002 onwards the conventional fertilization system was substituted by agricultural organic procedures. After 2006, chemical and organic fertilization were implemented, without pest/disease control products application.

A one-way ANOVA ($P < 0.05$) was applied to evaluate differences between the yield obtained from plants originated from seeds and cuttings. A further comparison was carried out using an F test (95% confidence level). This work had the collaboration of Valnei Marcos Partelli, Ufes, Capes, Fapes, CNPq and Heringer.

RESULTS AND DISCUSSION

The productivity of plants derived from cuttings was significantly higher in the 1st, 2nd, 4th, 8th and 10th) (Table 1), although it showed a tendency to higher yields also in the 3rd and 6th

years. On the other hand, the plants derived from seeds showed only marginal higher yields in the 7th, 9th, 11th and 12th. The annual average production for the experimental period (12 years) was 3,223 and 2,706 kg ha^{-1} $year^{-1}$, whereas the 12 year period yields were 38,681 and 32,467 kg ha^{-1}, respectively for the plants originated from cuttings and seeds. Therefore, on the experimental period an accumulated difference of 6214 kg ha^{-1} was obtained in favour of the plants originated from cuttings, clearly pointing a strong production advantage for these *C. canephora* cv. Conilon plants. Note that such differential was higher than 2 average annual yields from plants originated from seeds, and could be (at least partially) explained by the fact that the plants originated from cuttings are physiologically adults already at the implantation, but not from differences in root development. In fact, it was shown a similar development of the root system in the two types of plants, therefore the higher productivity of cutting plants might be related to their ability to emit a higher number of branches and nodes, responsible for the higher initial productions.

Furthermore, the better productivity in plants originated from cuttings might have arise from a good and uniform genetic material, possible due to this cloning process, while the plants originated from seeds would have presented genetic variability not always maximizing the yield capacity.

It was concluded that *C. canephora* cv. Conilon implantation through cuttings offer higher production advantages as compared to plants originated from seed, at least considering the first 10 years after implantation, whereas for longer periods a convergence of yields seemed to occur.

Table 1. Production (kg ha^{-1}) of *C. canephora* cv. Conilon, implanted from cuttings or seeds, from 2001 (17 months) until 2012 (149 months) after implant, in Vila Valério, Espírito Santo State Brazil.

Implantation	**Production (kg ha^{-1}) along time of implantation (year)**					
	1 (2001)	**2 (2002)**	**3 (2003)**	**4 (2004)**	**5 (2005)**	**6 (2006)**
Cutting	421 a	5795 a	1629 a	3135 a	1562 a	4325 a
Seed	73 b	3220 b	1269 a	2355 b	1549 a	3745 a
CV	50.2	16.9	35.3	26.2	34.9	27.4
Implantation	**Production (kg ha^{-1}) along time of implantation (year)**					
	7 (2007)	**8 (2008)**	**9 (2009)**	**10 (2010)**	**11 (2011)**	**12 (2012)**
Cutting	2330 a	4349 a	2861 a	4410 a	3812 a	4051 a
Seed	2513 a	3446 b	2977 a	3099 b	4051 a	4170 a
CV	22.2	26.1	45.6	17,66	33,23	26,26

In each harvest, means followed by different letters represent significant differences (F test, 95% confidence level).

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Coffee Rehabilitation in Ghana: Results and Implications of a Baseline Socio-economic Survey

V. M. ANCHIRINAH, F. K. OPPONG, F. BAAH, M. ASAMOAH, F. OWUSU-ANSAH, G. J. A. KWAPONG

Cocoa Research Institute of Ghana. P.O. Box 8, Tafo-Akim

SUMMARY

Coffees used to rival cocoa as far as production and incomes are concerned in Ghana. Low revenue resulting from falling world market prices resulted in the Ghana Cocoa Board divesting its interest in coffee and privatization of plantations following structural adjustment in 1992. However, positive outlook in world demand over the last decade has resulted in renewed interest with the launch of a 4-year coffee rehabilitation project involving coppicing of old farms and plantations, provision of inputs like fertilizer and improved materials among others being initiated. This study was carried out with the view of identifying current production and marketing practices and constraints and to use the results as a benchmark for future project impact. A total number of 291 farmers made up of 70% project beneficiaries and 30% non-project farmers sampled from all the 6 coffee growing regions in Ghana were interviewed using pretested survey questionnaires. The study revealed that though the project has resulted in renewed interest and enthusiasm of coffee farmers marketing still remains the single most important constraint(identified by 74% of respondents) that could adversely affect project impact.

INTRODUCTION

Coffee used to be one of the most important export crops in Ghana in the late sixties and early seventies with higher producer prices than even cocoa. However, following the period of free fall of global primary commodity prices, coffee prices fell sharply to the lowest level for a century in the period from 2000 to 2004(Brata, 2007: Anim-Kwapong and Osei-Bonsu 2000). This fall in world market prices resulting mostly from falling demand severely affected exporting countries like Ghana where domestic demand is virtually non-existent. This collapse in world market prices resulted in the Ghana COCOBOD divesting itself of both production and marketing activities. Recent global trends indicating increasing demand and prices particular over the past decade have encouraged the government of Ghana through the Board to again intervene directly by embarking on a three year coffee rehabilitation project aimed at increasing and sustaining coffee farmers' income especially small holders. The initial 3- year project phase involves helping farmers to coppice old moribund coffee farms, providing improved planting materials for new plantations and also providing technical and financial assistance for farm maintenance. In order to measure project impact upon completion, a socio-economic baseline survey was carried out in 2011/2012.

Objectives of the Survey

- To study farmers' current production practices, identify production and marketing constraints and farmers perceptions about the project generally.
- To use the information as a benchmark for studying future project impact on farmers' incomes and general standard of living.

Study Method and Areas Covered

The study was carried out in eleven districts in five regions of Ghana namely Ashanti, Brong Ahafo, Western, Eastern, and Volta regions using formal and informal survey methods. Apart from the Volta and Eastern and non-project farmers where simple random sampling was done because of higher numbers of project farmers, the team interviewed all project farmers in the other regions. In all a sample of 291 respondents made up of 203 and 88 project and non-project farmers respectively were interviewed. The analysis was mainly descriptive.

RESULTS AND DISCUSSION

Farmer characteristics

The majority (90.4%) of the respondents involved in this study were males. Other general characteristics of farmers are presented in table 1.

Table1. General demographics of respondents.

Demographics		Percentage
Gender	Male	90.4
	Female	9.6
Age	< 30 years	4.8
	31-60 years	57.1
	61> years	38.1
Marital Status	Married	91.8
	Single	2.4
	Widow/Widower	4.1
	Divorced	1.7
Level of Education	No formal education	28.9
	Primary school Middle/JHS	59.5
	Secondary/Vocational/Tertiary	11.4
	Non-Formal	0.3
Migration Status	Native	60.8
	Settler	39.2

Project Farm characteristics

Seventy percent of 325 farms have been completely coppiced and a few (13%) of the project beneficiaries have formed farmer groups. Currently the total land area under coffee production is 3944.73acres which forms 32% of total land use by the farmers interviewed. Average age and size of a coffee farm is 26years and 1.1ha respectively. Farmers also obtained free inputs such as fertilizer, pesticides, seedlings and some financial assistance for their farm maintenance.

Coffee production practices

Most of the project beneficiaries (66.9%) weed their farm three times in a year whiles the non project farmers weed twice. Ghana's coffee like the rest of West Africa is predominantly robusta (Adu-Ampomah *et al.,* 1993). From table 2, it can be said that the farmers involved in this project are applying the recommend practices on their farms.

Table 2. Coffee Production Practices

Activity		Project Farm (%)	Non Project Farm (%)
Wedding	Once	1.2	17.5
	Twice	16.2	42.3
	Trice	66.9	27.8
	More than three times	15.8	12.4
Variety planted	Robusta	78.1	77.1
	Arabica	5.4	4.6
	Robusta and Arabica	16.5	18.3
Did you provide shade?	Yes	52	19.6
	No	49	80.4
Land tenure	Owner	61.9	55.4
	Family	13.1	16.1
	Rented	9.6	7.1
	Sharecropper	13.5	17
	Allocated for free	1.9	4.5
Did you apply Fertilizer?	Yes	84.2	1.4
	No	15.8	98.6
Shade management methods	Natural vegetation	53.8	55.7
	Thinning of existing forest trees	26.2	26.2

Awareness on some coffee recommended practices

The study showed that between 2010 and 2011, most (96%) of the respondents have become aware of some agronomic recommendations for growing coffee which is shown in figure 1.

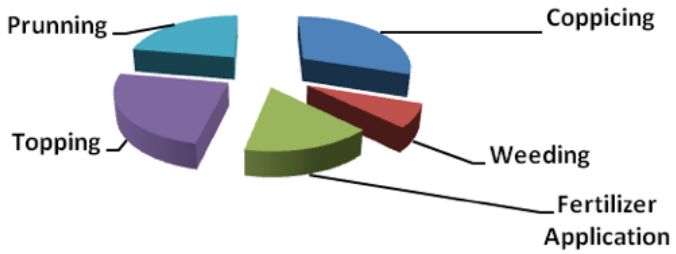

Figure 1. Assement of farmers awareness on coffee practicieses.

Marketing of coffee and general constraints of farmers

Even though there were no standardized methods of weighing coffee across the regions, majority (64.8%) of farmers sold to district local buying agents whiles farmers in towns along the borders sold to neighboring countries for lack of buying centers a better prices Farmers recommended a producer price of at least one cedi thirty-eight pesewas per kilogram (GH₵1.38) or $0.77. Figure 2 shows the quantities of coffee sold and price trends from 2009-201 whiles fig 3 shows the general constraints of farmers.

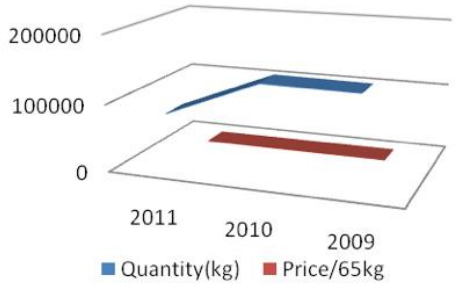

Figure 2. Price trend and quantites sold from 2009-2011.

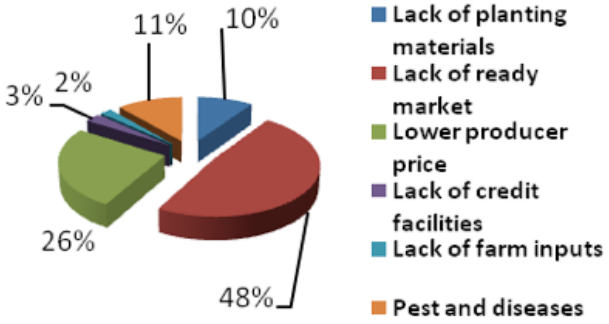

Figure 3. general constraints of farmers.

Table 3. Perceived impact of the rehabilitation project.

Perceptions	Strongly Agree	Agree	Disagree	Strongly Disagree	No Opinion
Project will increase coffee yield	38.8	59.1	0.7	0.7	0.7
Increase coffee price	30.9	64.9	2.8	0.3	2.1
Increase income of farmers	19.2	77.3	0.9	0.7	2.7
Government addresses problems of coffee farmers	29.9	58.8	2.8	1.0	1.4
Project has improved farmers knowledge on coffee farming	30.2	67.7	1.1	-	1.0
Project has increased enthusiasm of farmers	32.6	63.6	2.4	0.3	1.4
Being a coffee farmer is a hell	15.0	17.0	17.0	50.7	0.3

CONCLUSIONS

Marketing still remains the single most important constraint hampering coffee production in Ghana (identified by 74% of farmers). This is consistent with the findings of a similar study by Anchirinah et al (2009). This will need to be addressed if the project is to make the desired impact.

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Global Warming Impact of a Cup of Soluble Coffee

A. STOCKWELL, F. GUILMINEAU, J. MELROSE

Mondelēz International, Global Coffee Technology Centre
Ruscote Avenue, Banbury - OX16 2QU, UK

SUMMARY

Mondelēz International (as Kraft Foods) has been working on sustainability for many years. Sustainability is becoming a growing area of focus within our society and is of interest to our coffee consumers. Mondelēz International has worked both visibly by using Rainforest Alliance coffee beans and refill packaging and 'behind the scenes' improving factory and transport efficiencies. The poster will review our work on life cycle analysis of global warming potential (GWP) of soluble coffee products.

INTRODUCTION

Public awareness of, and concern for, the effects of climate change and other environmental impacts has increased dramatically in recent years. As an environmentally and socially responsible company, Mondelēz International is seeking to take the lead in understanding the environmental impacts associated with its products and operations. As part of its commitment to minimise the environmental impact of its operations and better understand the sustainability of its products, Mondelēz International (as Kraft Foods) has carried out a life cycle assessment study to investigate the global warming potential (GWP) of freeze dried soluble coffee with a range of coffee packaging options and how this has changed over time.

MATERIALS AND METHODS

The functional unit is the provision of a cup of freeze dried soluble coffee as drunk by a UK consumer. It is made from 1.4g soluble coffee with 200ml hot water, 50ml milk and 9g sugar. Packaging options of a glass jar, tin and refill bag all holding 100g of coffee were investigated. The system boundary is shown in

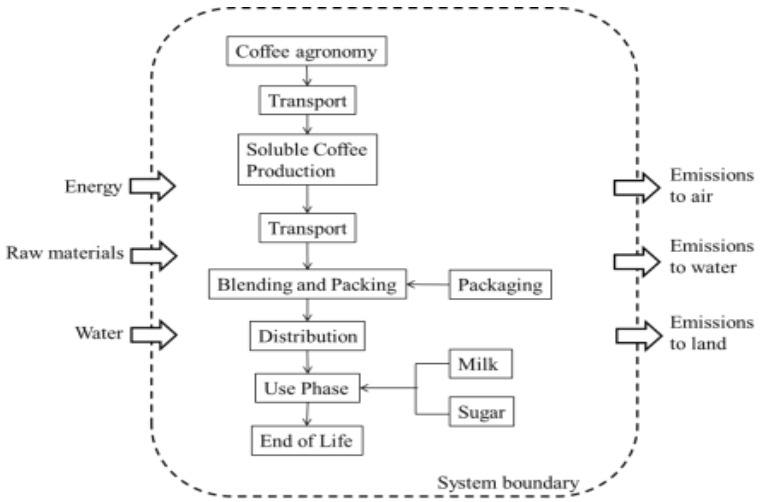

Figure 1.

Data was collected from a variety of sources. Coffee agronomy model was written by PE International based on literature about Brazilian coffee agronomy (Coltro et al 2006). Processing data came directly from Mondelēz International plants taking average 2011 production. Background data came from GaBi 5 datasets.

Packaging data used Mondelēz international specifications and GaBi 5 datasets. The 3 pack types were:

Glass jar
Glass container with PP lid

Tin
Tin plate steel container with PE lid

Refill
PET/Al/PE laminate bag

There are three scenarios for UK packaging waste at end of life – recycling, incineration with energy recovery and landfill. These were allocated based on average UK data (DEFRA 2009).

The milk dataset is based on production of raw milk at the farm, excluding pasteurisation and other post farm processing steps, based on data for Germany dairy farms in 2008 (PE International 2010). Therefore the impact of milk is likely an under estimate and to be used as indication only. Sugar dataset is based on production of refined sugar from sugar beet for average EU conditions (PE International, 2006). It was estimated that a consumer would boil on average 50% more water than needed ie 300ml boiled to prepare a 200ml serving.

The model followed ISO 14044:2006 for LCA and for specifics on GWP PAS 2050:2011. This paper is based on two ISO standard reports, which have been externally verified (Stockwell 2012; PE International 2011).

RESULTS AND DISCUSSION

Errore. L'origine riferimento non è stata trovata. shows that the biggest impact in this cup of soluble coffee is the milk accounting for approximately 50% of the GWP. Coffee is next accounting for approximately 25% followed by hot water (15%) and sugar (10%). Only 25% of the GWP of the beverage is within Mondelēz International control.

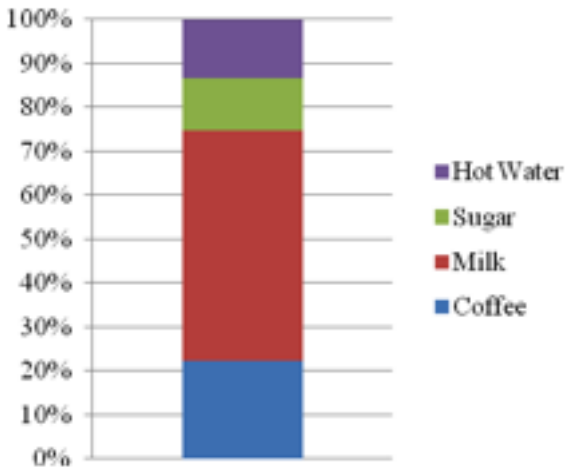

Figure 1. Relative GWP of a cup of soluble coffee prepared in the UK.

Focussing on the coffee itself in **Errore. L'origine riferimento non è stata trovata.**, shows that the majority of the GWP of soluble coffee is due to growing the coffee beans. Mondelēz International has worked with the Rainforest Alliance since 2007 to produce certified coffee products. Rainforest Alliance support farmers to improve environmental impact through education and discourage the use of certain chemicals. Sadly, data on how this improves GWP is not available. However there is data (COSA, 2012) which shows that Rainforest Alliance certified farms have on average a 15% increase in yield. If it is assumed that inputs remain the same, then this leads to the GWP of green coffee agronomy decreasing by 13%.

Soluble coffee production has the next biggest impact. Mondelēz International has an on-going target for all plants to reduce their energy and water consumption by 15% every 5 years which will be met through a combination of improving efficiencies and capital investment.

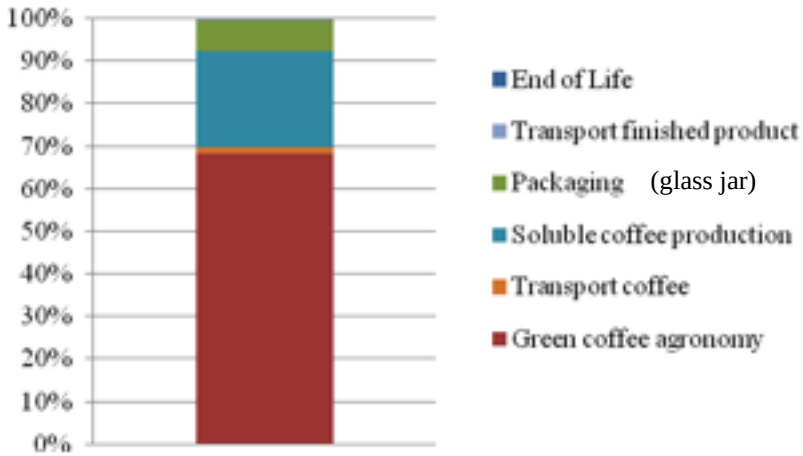

Figure 2. GWP of a 100g jar of soluble coffee.

Although packaging is very visible to the consumer and therefore a focus of this work, its impact is less than 3% of the cup of coffee and less than 10% of the footprint excluding consumer use.

For soluble coffee, a refill pack has been promoted as using 97% less packaging by weight than a glass jar. As shown in

, the refill pack also has 80% less carbon footprint. A tin used for whole bean instant products also uses less material than glass jar, but the refill pack is still the lightest and has the lowest GWP impact.

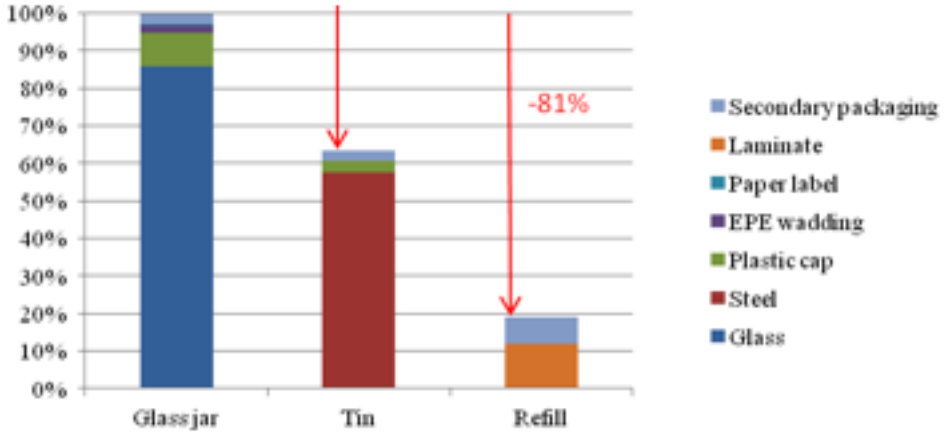

Figure 3. GWP of packaging options.

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Potential Productivity of Genotypes of Robusta Coffee in the São Paulo State, Brazil

J. C. MISTRO[1], L. C. FAZUOLI[1], M. T. BRAGHINI[1], E. S. MANTOVANI[1], G. S. GIOMO[1], R. VENCOVSKY[2], M. D. V. RESENDE[3]

[1]Centro de Café - Instituto Agronômico (IAC/APTA), Campinas, SP, Brasil
[2]Escola Superiorde Agricultura "Luiz de Queiroz" (ESALQ) - Universidade de São Paulo (USP) - Departamento de Genética, Piracicaba, SP, Brasil
[3]EMBRAPA Florestas, Colombo, PR, Brasil

SUMMARY

The aim of this work was to select genotypes of *Coffea canephora* (robusta) with high production potential to constitute future cultivars for the State of São Paulo (Brazil).Two experiments were carried out in Campinas (SP, Brazil) with random block designs, one consisting of seedling progenies and the other of clones, observing four harvests. In the clonal experiment the genotypes that performed best were 17, 24, 19, 13, 9, 6, 1 and 12. In the experiment with seedling progenies the following progenies performed best 844, 592, 439, 848, 721, 846, 49, 55 and 51. Genetic gains were estimated to be more than 30% in the clonal experiment and was 54% for the seedling progenies. The high yields obtained show that the continuity of the selection cycles will result in promising future cultivars of robusta coffee in the São Paulo State.

INTRODUCTION

The increase in the production of robusta coffee (*Coffea canephora* Pierre ex A. Froehner) in the world is notorious. Its participation in the world coffee production passed from 18% in 1965 to 35% in 2010. Some factors have been driving this growth, including its high yield potential, the promising Asian and European consumer markets and lower price fluctuation in the international coffee market compared to arabica coffee. A robusta cultivar can be developed sexually (seeds) and asexually (clones). The Espírito Santo State is the largest Brazilian producer (around 8-9 million bags/year). Cultivars developed by INCAPER are clonal. The robusta coffee is not cultivated in São Paulo State. Recently, interest from the part of coffee planters in the São Paulo State and by the coffee industry has been observed tostartrobusta cultivation in São Paulo, as there are advantages for the coffee grower and for the coffee industry.

The Instituto Agronômico de Campinas (IAC) is now intensifying the research for developingcultivarswith high yield potentialadapted to the climatic conditions of the São Paulo State.The IAC launched in 1987 the rootstock IAC 2258 (Apoatã) recommended for areas where arabica coffee is grown and which are infested with nematodes. Currently, the focus of the breeding program of robusta coffee in IAC is the development cultivars adapted to the State of São Paulo.

The objective of this work was to select genotypes with high productive potential, propagated asexually and sexually, and have the possibility of future cultivars.

MATERIALS AND METHODS

Two experiments were carried out in Campinas (SP, Brazil), in 2005, in a random block design. The treatments that comprise each of the experiments were selected from a Mococa population (SP, Brazil).The first experiment was composed of 28 clones, three replications and four plants per plot. The second experiment consisted of 30 progenies, four replications and eight plants per plot. The spacing in both experiments was 4.0 x 1.5 meters.There were four harvests (2008-2011).

In perennial crops, such as coffee, where imbalance occurs and data are taken for several years from the same experimental unit, the recommended procedure for the analysis is the methodology REML/BLUP (mixed model), where the estimation of the components of variance is done by restricted maximum likelihood (REML) and the prediction of genetic values by best linear unbiased prediction (BLUP). We used the SELEGEN computer program and adopted a linear mixed model, in which the matrix form is as follows:

$$Y = Xb + Zg + Wp + e$$

where Y = vector of phenotypic observations of the trait, X = incidence matrix for fixed effects (blocks and overall averages), b = vector of fixed effects, Z = incidence matrix for genotypic effects considered random; g = vector for genotypic effects, W = incidence matrix for the effects of the plots, p = vector for plot effects, e = vector of error effects generated randomly.

RESULTS AND DISCUSSION

Clonal experiment

The analysis of deviance, by the likelihood ratio test, revealed significant differences between clones at 1% probability, indicating the existence of genetic variability, a primary condition for successful selection. The interaction clones x harvests was also significant, reflecting the change in the order of production of the treatments over the 4 years.The average production for each harvesting year were: 0.98 kg/plant (1^{st}harvests), 4.07 kg/plant (2^{nd} harvest), 1.41 kg/plant (3^{rd}harvests) and 8.60 kg/plant (4^{th} harvest). The occurrence of dry periods in 2010 (3^{rd}harvests) drastically reduced production in that year, affecting also the average production for four harvests (3.76 kg/plant).

Tables 1 and 2 are presenting the ratings for yield data of the top ten best clones of robusta according to the harvests. There are two possibilities for selection: one based on an average of four harvests and another based only on the fourth harvest.Considering the top eight clones, only the number 8 was not among those selected in both situations.17 and 21 were the best clones, while 17 presented the first and second highest genotypic values, 6.30 and 15.88 kg/plant, in the joint analysis and in the fourth harvest alone, respectively. Clone 21 showed the best performance in the 2011 harvest, and was second in the joint4-year analysis. Selecting the best eight clones based on the average of four harvests, it is expected that in the next selection cycle, the average production will be close to 5.00 kg/plant with a genetic gain of above 30%. Considering only the fourth harvest, the average production will be near to 13.00 kg/plant and genetic gain of 50%. One should take care of that in a single harvest, the genotypic values may be inflated. It was also noted that the predicted values are not equal to the true genetic values of the clones.

Table 1. Ranking of best 10clones of *C. canephora* based on genotypic values (u + g) relating to the joint analysis of four harvest evaluated in clonal experiment.

Ranking	Clone	g	u + g	Gs	Gs	X_i	LIIC	LSIC	f
	nº	kg /plant			%	kg /plant			
1º	17	2.54	6.30	2.54	67.55	6.30	4.50	8.10	7.94
2º	21	2.28	6.04	2.41	64.10	6.17	4.25	7.84	7.52
3º	1	0.97	4.73	1.93	51.33	5.69	2.93	6.53	5.36
4º	19	0.95	4.71	1.69	44.95	5.45	2.92	6.51	5.33
5º	8	0.95	4.71	1.54	40.96	5.30	2.91	6.51	5.33
6º	13	0.83	4.59	1.42	37.77	5.18	2.79	6.39	5.12
7º	9	0.82	4.58	1.33	35.37	5.10	2.78	6.38	5.11
8º	24	0.78	4.54	1.26	33.51	5.03	2.74	6.34	5.04
9º	11	0.70	4.46	1.20	31.91	4.96	2.66	6.26	4.91
10º	6	0.61	4.37	1.14	30.32	4.90	2.57	6.17	4.76

g = genotypic effects, u + g = genotypic value; Gs = gain from selection; Xi = mean improved; LIIC = Lower limit of the confidence interval; LSIC = Upper limit of confidence interval; f = average phenotypic value.

Table 2. Ranking of best 10 clones of *C. canephora* based on genotypic values (u + g) regarding the use of fourth harvest in 2011, evaluated in clonal experiment.

Ranking	Clone	g	u + g	Gs	Gs	X_i	LIIC	LSIC	f
	nº	kg /plant			%	kg /plant			
1º	21	7,30	15,88	7,30	84,98	15,88	12,84	18,93	16,77
2º	17	6,65	15,24	6,98	81,26	15,56	12,19	18,29	16,05
3º	24	5,42	14,01	6,46	75,20	15,04	10,96	17,06	14,67
4º	19	5,13	13,71	6,12	71,25	14,71	10,66	16,76	14,34
5º	13	3,18	11,77	5,54	64,49	14,12	8,72	14,82	12,15
6º	9	3,12	11,71	5,13	59,72	13,72	8,66	14,76	12,09
7º	6	3,11	11,69	4,84	56,34	13,43	8,65	14,74	12,07
8º	1	2,55	11,14	4,56	53,08	13,14	8,09	14,18	11,45
9º	12	2,51	11,10	4,33	50,41	12,92	8,05	14,14	11,40
10º	26	1,31	9,89	4,03	46,92	12,61	6,84	12,94	10,05

g = genotypic effects, u + g = genotypic value; Gs = gain from selection; Xi = mean improved; LIIC = Lower limit of the confidence interval; LSIC = Upper Limit of Confidence Interval; f = average phenotypic value.

Seedling experiment

The analysis of deviance showed that only for the fourth harvest, in 2011, there weresignificant differences between progenies, and that there was no significant interaction between progenies x harvests.

The average production of each harvest were 0.56 kg/plant (1st harvest), 4.39 kg/plant (2nd harvest), 1.29 kg/plant (3rd harvest) and 7.35 kg/plant (4th harvest), and 3.40 kg/plant production average for the four harvests combined. The adverse climatic conditions in 2010 was observed in this experiment, as in the clonal experiment. Table 3 presents the ranking according to the additive effect, the best 15 of robusta coffee plants. The 15 best plants belong to only three different progenies: 4, 9 and 30, with a predominance of individuals from progeny 9 (60%). The most superior plants were 844, 592, 439, 848, 721, 846, 49, 55 and

51.The genetic gains with individual plant selection would raise the new average production after one cycle of selection of 7.35 kg/plant to 11.38 kg/plant (55% gain). Although this significant gain, it is advisable to select a larger number of plants to minimize the effects of inbreeding that may occur in the next selection cycle, if the number of selected progenies is small.If the selection intensity is lower, e.g. the best 50 plants, we would have 10 different progenies, but with a lower genetic gain (43%). If the interest of the breeder is to select new clones, the plant 721 has shown promise, together with 844, 49, 55, among others. In this case it is interesting to select progeny plants in order to minimize incompatibility problems.

Table 3. Classification of the 15 best plants of *C. canephora* based on additive effects (a) of the analysis performed on the fourth harvest, in 2011, in the seedlingexperiment.

Ranking	Plant	Progênie	f	a	u + a	Gs	Gs	X_i	g
	nº		kg /plant				%	kg /plant	
1º	844	9	25.00	4.61	11.96	4.61	62.66	11.96	5.97
2º	592	9	23.00	4.47	11.82	4.54	61.74	11.89	5.75
3º	439	9	24.00	4.38	11.73	4.49	61.02	11.84	5.60
4º	848	9	23.00	4.32	11.67	4.44	60.46	11.80	5.49
5º	721	30	30.00	4.27	11.62	4.41	59.98	11.76	6.34
6º	846	9	22.00	4.17	11.53	4.37	59.45	11.72	5.26
7º	49	4	28.00	4.16	11.51	4.34	59.04	11.69	5.71
8º	55	4	28.00	4.16	11.51	4.32	58.73	11.67	5.71
9º	51	4	27.00	4.02	11.37	4.28	58.28	11.64	5.48
10º	644	30	28.00	3.90	11.26	4.25	57.76	11.60	5.73
11º	436	9	20.00	3.81	11.16	4.21	57.22	11.56	4.64
12º	841	9	19.00	3.74	11.10	4.17	56.70	11.52	4.54
13º	782	4	21.00	3.66	11.01	4.13	56.16	11.48	4.88
14º	226	9	17.00	3.44	10.80	4.08	55.50	11.43	4.04
15º	437	9	17.00	3.38	10.73	4.03	54.86	11.38	3.92

f = phenotypic value, a = additive effects, u + a = additive genetic value; Gs = gain from selection; Xi = mean improved set of selected plants; g = genotypic effects.

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Multivariate Associations among Bean Yield and Agro-Morphological Traits in Robusta Coffee

E. ANIM-KWAPONG

Cocoa Research Institute of Ghana, P.O. BOX 8, New Tafo-Akim, Ghana.

SUMMARY

Inter-relationships among bean yield and agro-morphological traits are important in developing a strategy for indirect selection for bean yield in *Coffea canephora* (Robusta coffee). Multivariate associations among bean yield and several morphological, phenological and bean/fruit traits were determined to identify traits that may be included in a selection index for population and hybrid development. Ten females and four males from the base population at the Cocoa Research Institute of Ghana were crossed in a North Carolina Design II crossing programme to yield 14 half-sib groups and 40 full-sib progeny families. The 40 full-sib families were planted in a randomised complete block design with four replications. Factor analysis was used to explain dimensions in the data in relation to bean yield in terms of additive genetic effects. The additive genetic values of the 14 genotypes and 13 traits, which were genotypically correlated with bean yield, were projected on the same multivariate plane. Factor analysis showed that, the relationships among the traits, genotypes, and bean yield could be explained by two factors: general vigour and fertility; and branching habit and fertility. High breeding value for bean yield of five parental genotypes, by their positions on the multivariate plane, could be attributable to their progenies investing assimilates into the production of strong main stems and primary branches, long and erect primary branches, high fruit set, many fruits at the nodes and fewer proportions of empty locules; or many primary branches with shorter internodes lengths of the main stem and fewer proportions of empty locules. These factors could form a basis of a pre-selection index for bean yield in Robusta coffee.

INTRODUCTION

Most traits of economic importance are either linked and, therefore, transmitted together, or are governed by genes with pleiotropic effects (Falconer and Mackay, 1996; Kearsey and Pooni, 1996). As improvement in one trait causes simultaneous changes in other traits, hence, correlated response among traits has become a basis for indirect selection, to provide faster or cheaper genetic gains.

In coffee correlation studies were used to find yield prediction traits by correlating observed traits, mostly defining tree shape, growth and vigour with cumulative yield of four or more years, in both Arabica (Cannell, 1971; Walyaro and Van der Vossen, 1979; De Reffye, 1979; Snoeck and De Reffye, 1980; Cilas et al., 1998) and Robusta coffee (Leroy et al., 1994, 1997; Marandu et al., 2004; Cilas et al., 2006; Anim-Kwapong and Adomako, 2010). Most of these studies, however, concentrate on phenotypic and more or less genotypic correlations of morphological traits, with little mention of phenological or bean traits. Correlations based on morphological, phenological and bean traits should provide a better understanding of the relationships among these traits and their exploitation to improve yield. As Coffea canephora is predominantly out-crossing, further splitting of the genotypic correlations into its additive and dominance components would enable the additive component provide better insight into

the inter-relationships among the traits and their transmission for an efficient yield prediction strategy. Multivariate analysis of these relationships should provide insight into the underlying factors that affect the variation and association among the various traits and bean yield.

The objectives of this study were to: a) determine additive genetic associations among coffee bean yield and agro-morphological, phenological and bean traits using factor analysis; b) to determine the main factors underlying these associations to identify traits that may be included in a selection index for bean yield.

MATERIALS AND METHODS

Plant materials and experimental design

Fourteen genotypes from the base population at CRIG were used for the study. Ten genotypes used as female parents and four as male parents were crossed in a North Carolina Design II crossing programme to yield 40 full-sib progeny families and 14 half-sib family groups. The 40 families were planted at the experimental field of the Cocoa Research Institute of Ghana in May 2003. Planting was done in a randomised complete block design with four replicates and eight plants per progeny family per plot at spacing of 2m x 3m. Although many traits were observed, only those with significant genotypic correlations with bean yield (Anim-Kwapong, unpublished data) were included in this study. These traits and their abbreviations are shown in Table 1.

Statistical analysis

Factor analysis was performed after data standardisation, using the mean trait score of the 14 parental genotypes across their half-sib progeny for the 13 observed traits. Minitab statistical software (MINITAB, 1997), was employed for data analysis. Factor analysis was done to elucidate additive relationships among the variables in terms of the main underlying factors. After varimax rotation, the relationships between the factors and the traits or genotypes were assessed. Plotting of the values of the genotypes and the traits for the first and second factor loadings was done to give graphical representation of the relationships among the genotypes, the traits and combination of the genotypes and the traits.

RESULTS

Factor analysis showed that four factors accounted for 86.7 percent of the total variation among genotypes (Table 2), and 76.3 percent of the total variation among the traits (Table 3). The first, a factor of increase in fertility (reduced EMPTL and increased FS and F/N), bean yield and vigour (depicted by increased Girth, Span, DPB, and LPB), accouted for 31.4 percent of the total variation in the parents and 25.9 percent of the variation in the traits. The genotypes E138, 197 and E139, with high breeding values for bean yield, fertility and vigour correlated high and positively with this factor; whereas B170, B96 and B191, with low breeding values for bean yield, fertility and vigour correlated high and negatively with the factor. The second factor, a factor of reduction in ILST and EMPTL and increase in bean yield and NPB also accounted for 26.5 and 20.0 percent of the variation among the genotypes and the traits, respectively. Genotypes A129 and C134 with high breeding values for bean yield, NPB, and low breeding values for ILST and EMPTL correlated positively with the factor, whereas, D47 and E186 with low breeding values for bean yield and NPB and high breeding values for ILST and EMPTL correlated negatively with the factor.

Table 1. Summary of observed traits, their abbreviations and description.

Trait	Abbreviation	Description
Vegetative traits		
Stem diameter (girth)(mm)	Girth	Measured as diameter of main stem 10 cm from the ground
Crown diameter (span)(cm)	Span	Width of tree canopy measured at the widest portion of the tree
Total number of primary branches	NPB	Total number of primary branches counted per tree
Diameter of primary branches (mm)	DPB	Average of six primary branches measured at 10 cm from point of attachment to main stem
Length of primary branches (cm)	LPB	Average of six primary branches at the middle of the stem, measured from point of attachment to main stem to apex of branch
Inter-nodes length of primary branch (cm)	ILPB	Average of six primary branches at the middle of the stem per tree, calculated as length divided by the number of nodes
Inter-nodes length of orthotropic branch, or main stem (cm)	ILST	Calculated per tree as height of main stem, taken from the first primary branch counted from the ground, divided by NPB
Reproductive traits		
Number of fruits per node	F/N	Number of fruits counted per node at six months from initial flowering expressed as an average for total number of fruiting nodes on the same three primary branches at the middle of the stem, on which the flowers were counted
Fruit-set (%)	FS	Total fruits counted per three primary branches at the middle of the stem at six months after flowering divided by total flowers counted on the same branches
Bean yield (kg/ha/yr)	BYD	Weight of wet berries per tree x outturn per tree x number of trees per hectare averaged over the production cycle
Berry and bean quality traits		
Bean weight	BWT	Average weight of random samples of three 100-bean lots counted
Empty locules	EMPTL	Percentage of berries with an empty locule
Outturn	OT	Average weight of dry beans from 300 berries divided by weight of 300 wet berries

Table 2. Eigenvalues and Factor loadings for four factors for 13 important traits that determine bean yield.

Traits	Factors				Communality
	1. Increase in BYD, fertility, vigour	*2. Reduction in ILST, EMPTL & increase in BYD, NPB*	*3. Reduction in BWT and Outturn*	*4. Increase in ILPB, F/N*	
BYD	0.600	0.693	-0.125	0.261	0.923
Span	0.884	-0.121	-0.032	0.158	0.823
NPB	-0.071	0.945	0.073	0.271	0.976
Outturn	0.208	0.271	-0.901	0.180	0.961
EMPTL	-0.346	-0.838	0.227	0.166	0.901
F/N	0.605	-0.240	-0.365	0.548	0.858
FS	0.821	0.128	0.012	-0.007	0.691
Girth	0.615	0.431	0.002	0.362	0.696
DPB	0.834	0.243	-0.259	-0.115	0.835
BWT	-0.031	-0.114	-0.963	0.082	0.949
LPB	0.796	0.205	-0.021	0.218	0.723
ILST	-0.066	-0.951	-0.023	0.267	0.980
ILPB	0.138	0.009	-0.168	0.954	0.957
Variance	4.082	3.440	2.043	1.706	11.272
% variance	0.314	0.265	0.157	0.131	0.867

Table 3. Eigenvalues and Factor loadings for four factors for breeding value of 14 genotypes as determined by bean yield.

Genotypes	Factors				Communality
	1. Increase in BYD, fertility, vigour	*2. Reduction in ILST, EMPTL & increase in BYD, NPB*	*3. Reduction in BWT and Outturn*	*4. Increase in ILPB, F/N*	
A149	0.390	-0.058	0.659	-0.174	0.621
A197	0.724	-0.425	-0.098	0.316	0.815
A101	-0.151	0.036	-0.798	-0.184	0.694
A129	0.089	0.651	0.261	0.474	0.725
B170	-0.809	-0.358	0.141	0.005	0.803
B191	-0.662	0.070	0.061	0.596	0.802
B96	-0.779	-0.062	-0.199	0.131	0.667
E138	0.817	-0.067	0.192	-0.081	0.716
E139	0.595	0.118	-0.204	-0.657	0.842
E152	-0.015	-0.056	0.108	-0.886	0.800
C134	0.150	0.871	-0.239	-0.071	0.843
C193	-0.354	0.364	0.784	-0.020	0.872
D47	0.211	-0.636	-0.494	0.172	0.723
E186	0.009	-0.859	-0.109	-0.086	0.757
Variance	3.621	2.800	2.231	2.029	10.680
%variance	0.259	0.200	0.159	0.145	0.763

The third factor, a factor of reduction in bean weight and outturn explained 15.7 percent of the variation among the genotypes and 15.9 percent of the variation among the traits. Genotypes 149 and C193 had high breeding values for bean weight and outturn and correlated positively with the factor, whereas, A101 and D47 which had low breeding values for bean weight and outturn correlated negatively with the factor. The fourth factor, explaining the least amount of variation and accounting for 13.1 and 14.5 percent of the original variation among the genotypes and the traits, respectively, is a factor of increase in ILPB and F/N. The genotypes

B191 and A129, with high breeding value for ILPB and F/N, correlated positively with the factor, whereas, E152 and E139 with low breeding value for ILPB and F/N correlated negatively. Very high communalities were observed for the traits and the genotypes, indicating, all 13 traits and 14 genotypes contributed substantially to the variation in the data, with bean yield explained by the first two factors and bean weight by the third.

The positions of the traits and the genotypes projected on the multivariate plane for the two main factors also showed clearly, on the vertical axis on one hand, the positive association of breeding value for bean yield with NPB and genotypes A129 and C134, and negative association of breeding value for bean yield with EMPTL and ILST and genotypes E186 and D47. The horizontal axis depicts, on the other hand, the positive association of the breeding value for bean yield with Girth, DPB, LPB, Span, FS and F/N and parental genotypes E139, E138 and 197, and the high negative association of breeding value for bean yield with the genotypes B170, B96 and B191.

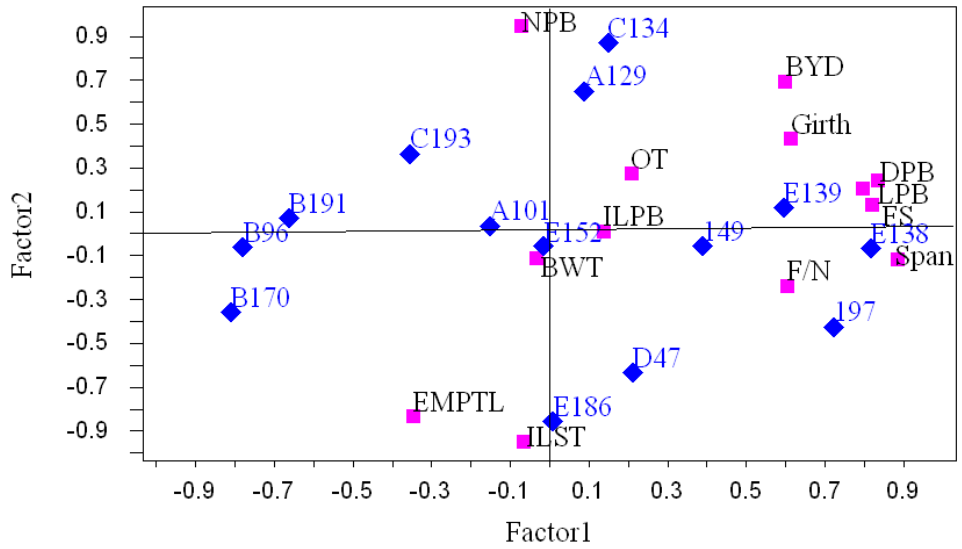

Figure 1. Plot of 14 parental genotypes and 13 traits each against values for the first two factors explaining bean yield.

DISCUSSION

From the positions on the multivariate plane of the two principal axis, the high breeding value for bean yield obtained by genotypes E138, E139, 197, A129 and C134 was apparently achieved by their progenies directing assimilates into the production of large stem diameter, many long, strong and erect primary branches, high percent fruit set and many fruits per node; or into the production of many primary branches with short internodes length of stem and high ovule fertility. Factor analysis also showed clearly, by the first two factors explaining bean yield, that the poor breeding value for bean yield of parental genotypes B96, B170, B191, D47 and E186, was attributable, mainly to, low biomass production or stem reserves, and poor ovule or pollen fertility of their progenies. Empty locules, however, determine fertility as they were found to result from endosperm failure (Krug, 1937; Mendes et al., 1954), possibly due to chromosomal disturbances.

Factor analysis also showed a positive association of F/N with ILPB as a factor, which indicated that, F/N compensated for the longer ILPB, as more assimilates were available for the development of the fruits. Grouping bean weight and outturn as a factor, with direct association with bean yield shows that, indirect selection for bean weight could be done

efficiently using outturn, and that, any index for simultaneous selection for bean weight and yield should include outturn for high genetic gains.

The significant positive associations observed for bean yield with Girth, Span, DPB, LPB, and NPB, at the vegetative phase agree with previous findings that, Robusta coffee bean yield (Bouharmont et al., 1986; Leroy et al., 1994, 1997; Marandu et al., 2004; Cilas et al., 2006; Anim-Kwapong and Adomako, 2010), as well as Arabica coffee yield (Walyaro and Van der Vossen, 1979; Cilas et al., 1998) were positively correlated with young plant vigour. Previous studies of phenotypic and genotypic correlations of reproductive traits with bean yield in both Robusta (Marandu et al., 2004; Anim-Kwapong and Adomako, 2010) and Arabica coffee (Walyaro and Van der Vossen, 1979) also showed that, fruit set percentage and to a lesser extent, fruits per node were positive and significantly associated with bean yield. Similar findings were made in the present study which showed further that these associations were due to additive genetic effects, hence, heritable.

CONCLUSIONS

Factor analysis showed that, the relationships among the traits and bean yield could be explained by two factors: general vigour and fertility; and branching habit and fertility. These factors could form a basis of a pre-selection index for bean yield in Robusta coffee.

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Productivity Coffea Arabica L. in Northwest Fluminense Region - Rio de Janeiro State

W. P. RODRIGUES[1], H. D. VIEIRA[1], D. H. S. G. BARBOSA[2], G. SOUSA-FILHO[1], S. J. FREITAS[1]

[1]Universidade Estadual do Norte Fluminense – Rio de Janeiro State – Brazil
[2]Centro Nacional de Pesquisa em Mandioca e Fruticultura – Bahia State – Brazil

SUMMARY

This work is to evaluate the productivity of 25 genotypes of arabica coffee, wishing in the future, recommend one or more cultivar (s) for the Northwest Fluminense region. We evaluated the productivity of 2009, 2010 and 2011. In the first harvest in 2009, the genotypes Catucaí amarelo 2 SL, Catiguá MG 02, Sabiá 398, IPR 103/Iapar, IPR 100/Iapar, Catucaí amarelo 24/137, Catucaí amarelo 20/15, IPR 104/Iapar and H 419-10-6-2-5-1, were higher in group averages. At the second harvest in 2010, the genotypes Catiguá MG 02, Acauã, Palma II, IPR 103/Iapar, IPR 100/Iapar, H 419-10-6-2-12-1, Iapar 59 and H 419-10-6-2-5-10-1 were higher in group averages. In the third harvest in 2011, the genotypes Catucaí amarelo 2 SL, Palma II, Sabiá 398, Catucaí amarelo 24/137, Oeiras, Catuaí Vermelho 144, Catucaí amarelo 20/15, H 419-10-6-2-5-10-1, IPR104/Iapar , Bourbon amarelo IAC and H 419-10-6-2-5-1 were higher in group averages. The average of the three crops, the genotypes Catucaí amarelo 2 SL, Catiguá MG 02, Palma II, Sabiá 398, IPR 103/Iapar, Catucaí amarelo 24/137, Catucaí amarelo 20/15, H 419-10-6-2-5-10-1 and H 419-10-6-2-5-1 were higher in group averages. Some genotypes. Some genotypes showed a more pronounced effect of biannuality such as Catucaí amarelo 2 SL , Sabiá 398, Catucaí amarelo 24/137, Oeiras, Catuaí Vermelho 144, Catucaí amarelo 20/15, IPR104/Iapar e Bourbon amarelo IAC and H 419-10-6-2-5-1. However, news genotypes such as Catucaí amarelo 2 SL, Catiguá MG 02, Palma II Sabiá 398, IPR 103/Iapar, IPR 100/Iapar, Catucaí amarelo 24/137, Catucaí amarelo 20/15, H 419-10-6-2-5-10-1 and H 419-10-6-2-5-1 have shown good agronomic performance in conditions in the Northwest Fluminense which may be recommended in the near future for this region.

INTRODUCTION

The Rio de Janeiro State, which has once been the largest coffee producer, is currently facing difficulties in promoting the expansion of this crop. In addition to old problems such as *Hemileia vastratrix* Berk et Br. and *Meloidogyne exigua* Goeldi, 1887, the maintenance of old and depleted fields and lack of improved cultivars adapted to the ecological conditions of Rio de Janeiro has hindered the recovery of coffee plantations in the state.

With the aim of increasing productivity, breeding programs have attempted to launch coffee cultivars adapted to each production region, different management and resistance to major pests and diseases. However, due to the great climatic diversity among production regions, it becomes necessary to study local adaptation with respect to these new cultivars to minimize future risks.

Thus, the objective of this study is to evaluate the production of 25 genotypes of arabica coffee in order to indicate, in the future, one or more cultivar (s) to the Northwest Fluminense region.

MATERIALS AND METHODS

The experiment was settled in 2007 in Fazenda Panorâmica 1, the city of Varre Sai – Rio de Janeiro State, in Oxisol, located at - 20 ° 55 '52" Latitude and - 41 ° 52' 07" Longitude, with an average altitude of 680 meters. Climate is typical tropical highland, showing average annual temperature of 19.0 °C and average rainfall of 1601 mm per year (Martorano *et al.*, 2003).

The seeds of 25 genotypes of C. arabica used in the experiment (Table 1) were provided by Empresa de Pesquisa Agropecuaria de Minas Gerais (EPAMIG).

For the formation of the seedlings polyethylene bags measuring 11 cm × 22 cm and the substrate formed were used as recommended by Thomaziello et al. (2000). Both fertilization (by soil analysis) and phytosanitary treatments were carried out according to Matiello *et al.* (2005).

The 25 genotypes were evaluated at a spacing of 2.5 × 0.8 m, and the design used was completely randomized experiment with five replications and eight plants in each repetition, totaling 1000 plants. We evaluated the harvests 2009, 2010 and 2011 when plants had 80% of ripe fruit (cherry). The volume collected was transformed into bags processed/ha (bag ha^{-1}), by use of the scale of 480 liters of coffee cherries harvested / processed 60 kg-bag. The variables were subjected to analysis of variance and means grouped by the Scott Knott test at 5% probability (Cruz, 2006).

RESULTS AND DISCUSSION

Analysing the table 1 we can to observe in the first harvest 2009 2009, two yellow genotypes Catucaí amarelo 2 SL, Catiguá MG 02, Sabiá 398, IPR 103/Iapar, IPR 100/Iapar, Catucaí amarelo 24/137, Catucaí amarelo 20/15, IPR 104/Iapar and H 419-10-6-2-5-1 were in the group of higher averages. . In the second harvest in 2010, genotypes Catiguá MG 02, Acauã, Palma II, IPR 103/Iapar, IPR 100/Iapar, H 419-10-6-2-12-1, Iapar 59 and H 419-10-6-2-5-10-1 were in the group of higher averages. In the third harvest in 2011, the genotypes Catucaí amarelo 2 SL, Palma II, Sabiá 398, Catucaí amarelo 24/137, Oeiras, Catuaí vermelho 144, Catucaí amarelo 20/15, H 419-10-6-2-5-10-1, IPR 104/Iapar, Bourbon amarelo IAC and H 419-10-6-2-5-1 were in the group of higher averages. The average product of three years, the genotypes Catucaí amarelo 2 SL, Catiguá MG 02, Palma II, Sabiá 398, IPR103/Iapar, Catucaí amarelo 24/137, Catucaí amarelo 20/15, H419-10-6-2-5 -10-1 and H 419-10-6-2-5-1 were in the group of higher averages. we emphasize that, exception of Catuaí vermelho 144 and Bourbon Amarelo IAC, the genotypes that showed good yield, considering the years and the mean, have some kind of resistence to *H. vastatrix*.

The genotypes Catucaí 785/15, IPR/Iapar, IPR99/Iapar, Araponga MG 01, H4193-3-3-716-4-1, Catiguá MG 01, Sacramento and Pau Brasil were lower in group averages in all years. Probably, these genotypes did not adapt well to the region or crop management, especially spacing and without irrigation.

Table 1. Average productivity (bags 60 Kg hectare^{-1}) of 2009, 2010 and 2011 and productivity average of three years of coffee in the Northwest Fluminense region – Rio de Janeiro State.

Genotypes	2009	2010	2011	Média
1-Catucaí vermelho 785/15	27.50 b	34.28 b	17.44 b	26.40 b
2-Catucaí amarelo 2 SL	57.63 a	32.22 b	53.12 a	47.66 a
3-IPR/Iapar	41.66 b	29.64 b	32.82 b	34.72 b
4-Catiguá MG 02	61.78 a	53.21 a	26.02 b	47.02 a
5-IPR 99/ Iapar	46.25 b	35.68 b	21.08 b	34.32 b
6-Acauã	43.75 b	45.83 a	27.58 b	39.06 b
7-Araponga MG 01	41.11 b	30.83 b	16.92 b	29.60 b
8-Palma II	41.80 b	49.82 a	36.70 a	42.80 a
9-Sabiá 398	65.28 a	32.50 b	52.08 a	49.96 a
10-IPR 103/Iapar	57.78 a	45.83 a	31.78 b	45.14 a
11-IPR 100/Iapar	52.22 a	40.28 a	20.98 b	37.84 b
12-H 4193-3-3-716-4-1	45.28 b	36.11 b	18.22 b	33.22 b
13-H 419-10-6-2-12-1	42.50 b	41.68 a	30.50 b	38.22 b
14-Catucaí amarelo 24/137	59.17 a	31.94 b	50.52 a	47.22 a
15-Iapar 59	45.89 b	41.79 a	23.68 b	37.14 b
16-Oeiras	46.11 b	28.33 b	41.68 a	38.70 b
17-Catuaí vermelho 144	44.03 b	25.62 b	46.08 a	38.60 b
18-Catucaí amarelo 20/15	61.67 a	35.28 b	63.02 a	53.32 a
19-Catiguá MG 01	38.47 b	28.61 b	22.64 b	29.92 b
20-H 419-10-6-2-5-10-1	47.78 b	41.87 a	37.52 a	42.38 a
21-IPR104/Iapar	54.82 a	25.00 b	41.42 a	40.42 b
22-Sacramento	45.28 b	29.17 b	27.86 b	34.08 b
23-Bourbon amarelo IAC	36.38 b	17.45 c	35.70 a	29.86 b
24-Pau Brasil	46.68 b	33.89 b	27.32 b	35.96 b
25-H 419-10-6-2-5-1	58.89 a	27.22 b	45.06 a	43.74 a

Scott Knott test 5% probability

The Catuaí vermelho 144. much planted in the region and. even that being in the group of higher productivity in 2011. was the group of low average productivity in 2009 and 2010.

Promising results indicating that new genotypes. some with resistant to *H. vastatrix* and *M. exigua* . will can be recommended for planting in the region. However. the evaluation of one or two crops and the management through pruning should be done to better understand the agronomic performance of genotypes that are genotypes are showing higher. since it can reduce productivity in the next crop or not respond to pruning because of some physiological or genetic factor. invalidating this planting. Examples are genotypes Catiguá MG 02. 103/Iapar IPR. IPR 100/Iapar Iapar and 59 which had good yields in 2009 and 2010 and greatly reduced productivity in the year 2011. Coincidentally. these genotypes descended from a cross involving hybrid of Timor. Plants derived from crosses involving the Timor Hybrid have good productivity in the first harvests. However. after the third or fourth crop plants begin to degenerate. due to low vigor. Good example is the 59 Iapar which in Mountainous Zone of eastern Minas Gerais has shown good response until the third harvest Matiello et al. (2009).

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Weed Control Under High Rainfall Regime In Kenya Coffee

D. A. ODENY

Coffee Research Foundation, P.O. Box 4-00232, Ruiru, Kenya

SUMMARY

Weeds pose a major challenge in coffee production in Kenya, especially in areas that experience high rainfall and high temperatures. The use of herbicides, while effective, is generally an expensive undertaking and therefore unaffordable to resource poor small holder farmers who produce about 75% of the country's coffee. This study was undertaken to explore various weed control strategies that would be affordable and sustainable. The study was carried out in Kisii and Koru substations of the Coffee Research Foundation in Nyanza province of Kenya. These areas experience rainfall throughout the year. The treatments included weed control methods such as use of herbicides, cultural and use of cover crops. Results showed that cover crops are as effective as the use herbicides and mechanical weed control methods. A cost benefit analysis of the various treatments is discussed.

INTRODUCTION

Coffee is Kenya's third most important agricultural export commodity after tea and horticulture. Besides contributing to foreign exchange earnings, it is also a source of livelihood for over half a million households. The natural environment provides opportunities for production of the top quality coffee in the world. However, the same environment also provides perfect conditions for the growth of weeds which pose a major challenge to coffee production among other constraints. Several methods have been established for the control of weeds in coffee (Anon, 2009). These include hand weeding, forking, slashing and use of chemicals (herbicides). The choice depends on the weed diversity and intensity, efficacy and the costs. New herbicides and more efficient application technology have been developed. Although these are important advances, weeds continue to provide considerable constraint to farmers especially in the high rainfall areas. Since weeds reduce coffee yields significantly and their management methods expensive, there is need to assess the most economical weed management regimes for the high rainfall areas in Kenya. Care should be taken that such methods are environmentally and health safety compliant.

MATERIALS AND METHODS

The trials were laid out at the Coffee Research Sub-Stations of Kisii and Koru. The annual rainfall data, for years 2008 to 2011 are shown in Figs 1, for Koru and Fig 2, for Kisii. In Koru, the mature coffee variety K7 spaced at 2.74m x 2.74m was used while mature variety Ruiru 11 coffee spaced at 2m x 2m was used. The trials were laid out in completely randomized block design (CRBD) with three replicates. The plot size consisted of 25 coffee trees. The herbicides were applied using a CP3 knapsack sprayer fitted with a low volume nozzle. Hand weeding was carried out using different implements such as slasher, forked hoes, and blade (known locally as panga). Mulching was carried out using dry Napier grass put to a depth of 15cm. The various treatments were applied at the 4th leaf stage and when weed cover reached 50% of the plot. The weed cover was recorded on a fortnightly. The data on weed cover (%) was based on the whole plot whereas coffee yields and quality were

recorded from the middle nine effective trees. Analysis of variance was used to determine the difference among treatments and means separated by least significant difference at 0.05 level of significant using Duncan's multiple range test. A Partial Budget and Marginal Rate of Return analysis was carried out to assess the most cost effective weed control method.

RESULTS AND DISCUSSION

The rainfall data for years 2009 to 2011 for Koru and Kisii are shown in Fig 1 and 2 respectively. The Kisii site received an average of 2160.4mm compared to Koru that received 1797mm. However, Koru had a higher average temperature of 28.13^0C compared to $27.3\ ^0$C. The weed pressure was higher Koru as shown by the higher numbers of application of all the weed control measures (Table 1a). In Koru, all treatments, except Paraquat applied at 1.5 l/ha, significantly reduced weeds compared to the unsprayed control (p=0.05) 42 days after spraying (Table 1a) . The least applied treatment was mulching. The herbicides in straight applications were applied more times compared to the other treatments. After 28 months, all the treatment except application of Paraquat at 1.5 and 3 l/ha, had significantly achieved better weed control than the untreated plots.

In Kisii, the least applied treatment was wild groundnut cover crop and most applied was herbicide, Paraquat at rate of 1.5 l/ha followed by Paraquat at 3 l/ha and slashing (Table b). At the end of 28 months, all treatments had significantly lower weed % cover compared to the control.

In terms of net benefits, the use of strawberry cover crop (Table 2a) in Koru and wild groundnuts cover crop in Kisii seemed to offer the best returns. These results show that there is considerable potential for use of cover crops to control weeds in these high rainfall areas. The clean coffee yields were relatively low and the untreated control had lowest yields at both sites, the differences were not statistically significant from most the treated plots (Tables 1a and b). The study will be up scaled to include other potential cover crops and to assess their long term effect on the soil.

Table 1a. Effect of different weed control methods on % weed cover and clean coffee yield – Koru substation.

Treatment	% Weed cover at 42 days after application	No. of applications	%Weed cover at 28 months	Clean coffee yield (Kg/ha)
Glyphosate 36% at 1.5 L/ha	62c	20	24bc	488 bcd
Glyphosate 36% at 3.0 L/ha	38d	21	10c	640 ab
Paraquat 20% at1.5 L/ha	88a	21	95a	445 bcd
Paraquat 20% at 3.0 L/ha	73b	21	84a	472 bcd
Forking	4f	20	40b	557 abcd
Slashing	33d	22	7c	560 abcd
Pangas	6f	18	6c	614 abc
Mulching	1f	14	18c	502 bcd
Forking + Glyphosate 36% at1.5 L/ha	63c	19	3c	638 ab
Slashing + Glyphosate 36% at 1.5 L/ha	88a	20	9c	418 cd
Strawberry cover crop	6f	20	10c	704 a
Wild groundnuts cover crop	17f	14	4c	539 abcd
Control	96a	-	96a	361 d

Values followed by the same letter down the column are not significantly different according to Duncan's Multiple Range Test (P=0.05).

Table 1b. The economic analysis of different weed control methods – Koru substation.

Treatment	Labour/material cost (KES)	Gross benefit (KES)	Net Benefit (KES)
Glyphosate 36% at 1.5 L/ha	47,220.00	269,727.00	222,507.00
Glyphosate 36% at 3.0 L/ha	83,850.00	353,741.00	269,891.00
Paraquat 20% at1.5 L/ha	44,566.00	245,960.00	201,394.00
Paraquat 20% at 3.0 L/ha	74,466.00	260,884.00	186,418.00
Forking	60,320.00	307,864.00	247,544.00
Slashing	35,728.00	309,523.00	273,795.00
Pangas	54,288.00	339,370.00	285,082.00
Mulching	81,200.00	277,465.00	196,265.00
Forking + Glyphosate 36% at1.5 L/ha	51,409.00	352,635.00	301,226.00
Slashing + Glyphosate 36% at 1.5 L/ha	39,850.00	231,037.00	191,187.00
Strawberry cover crop	60,320.00	389,115.00	328,795.00
Wild groundnuts cover crop	42,224.00	297,916.00	255,692.00
Control	-	199,531.00	199,531.00

Table 2a. Effect of different weed control methods on % weed cover and clean coffee yield – Kisii substation.

Treatment	% Weed cover at 42 days after application	No. of applications	%Weed cover at conclusion (28 months)	Clean coffee yield (Kg/ha)
Glyphosate 36% at 1.5 L/ha	17def	14	45cde	484abc
Glyphosate 36% at 3.0 L/ha	4f	14	37cde	336bc
Paraquat 20% at1.5 L/ha	63b	19	68b	436abc
Paraquat 20% at 3.0 L/ha	27cdef	17	53bcd	452abc
Forking	11ef	13	55bc	372abc
Slashing	52bc	17	70b	480abc
Pangas	27cdef	13	35def	586a
Mulching	42bcd	8	7g	524ab
Forking + Glyphosate 36% at1.5 L/ha	8f	14	30ef	425abc
Slashing + Glyphosate 36% at 1.5 L/ha	38bcd	12	48cde	391abc
Strawberry cover crop	37cde	12	53bcd	398abc
Wild groundnuts cover crop	28cdef	6	18fg	535ab
Control	95a	-	94a	263c

Values followed by the same letter down the column are not significantly different according to Duncan's Multiple Range Test (P=0.05).

Table 2b. The economic analysis of different weed control methods– Kisii substation.

Treatment	Labour/material cost (KES)	Gross benefit (KES)	Net Benefit (KES)
Glyphosate 36% at 1.5 L/ha	42,798.00	267,516.00	224,718.00
Glyphosate 36% at 3.0 L/ha	66,108.00	185,714.00	119,606.00
Paraquat 20% at1.5 L/ha	53,523.00	240,986.00	187,463.00
Paraquat 20% at 3.0 L/ha	72,114.00	249,829.00	177,715.00
Forking	75,400.00	205612.00	130,212.00
Slashing	51,272.00	265,305.00	214,033.00
Pangas	75,400.00	323,894.00	248,494.00
Mulching	46,400.00	289,625.00	243,225.00
Forking + Glyphosate 36% at1.5 L/ha	63,623.00	234,906.00	171,283.00
Slashing + Glyphosate 36% at 1.5 L/ha	36,438.00	216,114.00	179,676.00
Strawberry cover crop	69,600.00	219,982.00	150,382.00
Wild groundnuts cover crop	34,800.00	295,705.00	260,905.00
Control		145,365.00	145,365.00

ASSUMPTIONS

- Labour cost per man-day = KES 232.00
  - Tasks (trees/man-day) - Slashing – 200, Forking – 100, Panga weeding – 100, Chemical weeding – 450
  - Cost of chemicals (per litre) - Glyphosate KES 1110, Paraquat KES 950
- Average Price per 50Kg bag = US $ 329.24 (Exchange rate – 1 US$ = 85.00 KES)
- Plant density – Koru 1330trees/ha; Kisii 2500 trees/ha

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Integrating *Cedrela Odorata* into Robusta Coffee Production in Ghana – Impact on Soil Properties and Initial Yield

F. K. OPPONG, G. J. ANIM-KWAPONG, K. OFORI-FRIMPONG

Cocoa research institute of Ghana, p. O. Box 8, Tafo-Akim, Ghana

SUMMARY

The impact of integrating *Cedrela odorata* into the cultivation of Robusta coffee on some soil nutrients and initial yield was assessed in a field trial established in Ghana in June 2007. Robusta coffee clones, at a density of 1736 plants ha^{-1} were interplanted among *C. odorata* planted one year earlier, at densities of either 434 trees ha^{-1} (Treatment 1); 434 trees ha-1 to be thinned to 217 trees ha^{-1} in the 8^{th} year (Treatment 2); 217 trees ha^{-1} (Treatment 3); 434 trees ha^{-1} to be thinned to 108 trees ha^{-1} in the 8^{th} year (Treatment 4); or 192 trees ha^{-1} (Treatment 5). The standard treatment consisted of Robusta coffee interplanted among *Gliricidia sepium* planted initially at 192 trees ha^{-1} but thinned to 48 trees ha^{-1} in the 4^{th} year (Treatment 6). There were no significant treatment differences in soil pH and organic carbon three years after interplanting the coffee clones among the *C. odorata* and *G. sepium* trees. There was however a general decline in the nitrogen content of the soil in all the treatments during the initial two years after planting the coffee clones. There were no significant treatment differences in the available phosphorus and potassium content of the soil during the initial two years after planting but significantly higher ($P \leq 0.05$) available phosphorus and potassium were recorded after the third year in the soils where *C. Odorata* was planted at 192 trees ha^{-1} (Treatment 5) and in the control plot (Treatment 6). There were no treatment differences in soil moisture content during the dry season. The initial yield of coffee after the second and third year planting was significantly higher ($P \leq 0.05$) in Treatments 5 and 6 than in Treatments 1 and 2, giving early indications that *C. odorata* planted at a high density of 434 trees ha^{-1} could have adverse effects on the reproductive capacity of Robusta coffee plants.

INTRODUCTION

The coffee producer price crisis experienced globally between 1999 and 2004 brought to the fore, the need for developing strategies for diversification in coffee production in order to provide complementary income for coffee farmers (Greenhalgh *et al*., 2006). Integrating *Cedrela odorata*, a fast growing high quality commercial timber species (Lamb, 1968; FAO, 1986) into coffee cultivation was considered as a possible source of additional income for coffee farmers in the event of low producer prices on the international market (Oppong and Anim-Kwapong, 2011). An additional advantage envisaged in a coffee/*C. odorata* combination is the provision of overhead shade for the coffee by the latter since medium shade is recommended for young and mature coffee in Ghana, especially in the marginal areas (Amoah, *et al.*, 1999). The potential of *C. odorata* as overhead shade during the establishment phase of young Robusta coffee has been demonstrated in Ghana (Oppong and Anim-Kwapong, 2011). Earlier studies involving the use of *Gliricidia sepium*, a leguminous tree as shade in Robusta coffee resulted in the latter deriving some biological benefits from the association (Anim-Kwapong *et al*., 1999). However not much is known about the impact of coffee/*C. odorata* interplanting on soil nutrients even though the latter is known to shed a lot

of leaves onto the soil surface. This paper reports on the impact of the presence of *C. odorata* on soil properties and the initial yield of the Robusta coffee plants.

MATERIALS AND METHODS

The experiment was set up at the Afosu substation (06^{o}N; 0059^{o}W and 228m a.s.l.) of the Cocoa Research Institute of Ghana in the Eastern Region of Ghana. It is located in a moist semi-decidous forest zone with an average minimum temperature of 27^{o}C, average maximum temperature of 32 ^{o}C and an average annual rainfall of 1360 mm. The soils are classified as ferric lixisol (FAO/UNESCO, 1990) and the topsoil (0-15 cm) has a sandy clay loam texture. The treatments evaluated were:

- T1 - Coffee + *C. Odorata* (434 trees ha^{-1})
- T2 - Coffee + *C. Odorata* (434 trees ha^{-1}, to be thinned to 217 trees ha^{-1} in the 8th year)
- T3 - Coffee + *C. Odorata* (217 trees ha^{-1})
- T4 - Coffee + *C. Odorata* (434 trees ha^{-1}, to be thinned to 108 trees ha^{-1} in the 8th year)
- T5 - Coffee + *C. Odorata* (192 trees ha^{-1})
- T6 - Coffee + *G. sepium* (192 tree ha^{-1}, thinned to 48 trees ha^{-1} in the 4th year [Control]

Stumped seedlings of *C. odorata* and *G. sepium* cuttings were planted in July 2007 whereas the coffee clones were transplanted at a density of 1736 plants ha^{-1} in July 2008. The trial was designed as a randomized complete block with six treatments and four replications.

The initial (baseline) soil sampling was done in September 2007, two months after planting the *C. Odorata* stumps and nine months prior to the planting of the coffee stumps. Thereafter, samples of soil were taken from each treatment plot at a depth of 0-15 cm in June 2009, February 2011 and January 2012. The soil samples were prepared and analyzed for pH, Organic carbon, total nitrogen, available phosphorus, exchangeable potassium and soil moisture following the methods described by Anderson and Ingram (1993). The amount of litter fall from the *C. odorata* and *G. sepium* in the various treatment plots were estimated using a 0.25 m^{2} quadrat thrown at random three times in each plot and the litter collected and dried in an oven at 80^{o}C for 48 hours after which the weights were determined. Initial yield of hulled coffee from each plot were also recorded during the 2010/11 and 2011/12 cropping seasons. Analysis of Variance (ANOVA) was performed on all the data and the Least Significance Difference test used to separate significant means.

RESULTS AND DISCUSSION

There were no significant treatment differences in the soil pH at the different sampling times. There were generally slight increases in the soil pH values of the treatments above the baseline pH value of 5.64 in 2007, which falls within the acceptable soil pH range for Robusta coffee cultivation. There were also no significant treatment differences in % organic carbon and % nitrogen content of the soil at all the sampling times (Figure 1).

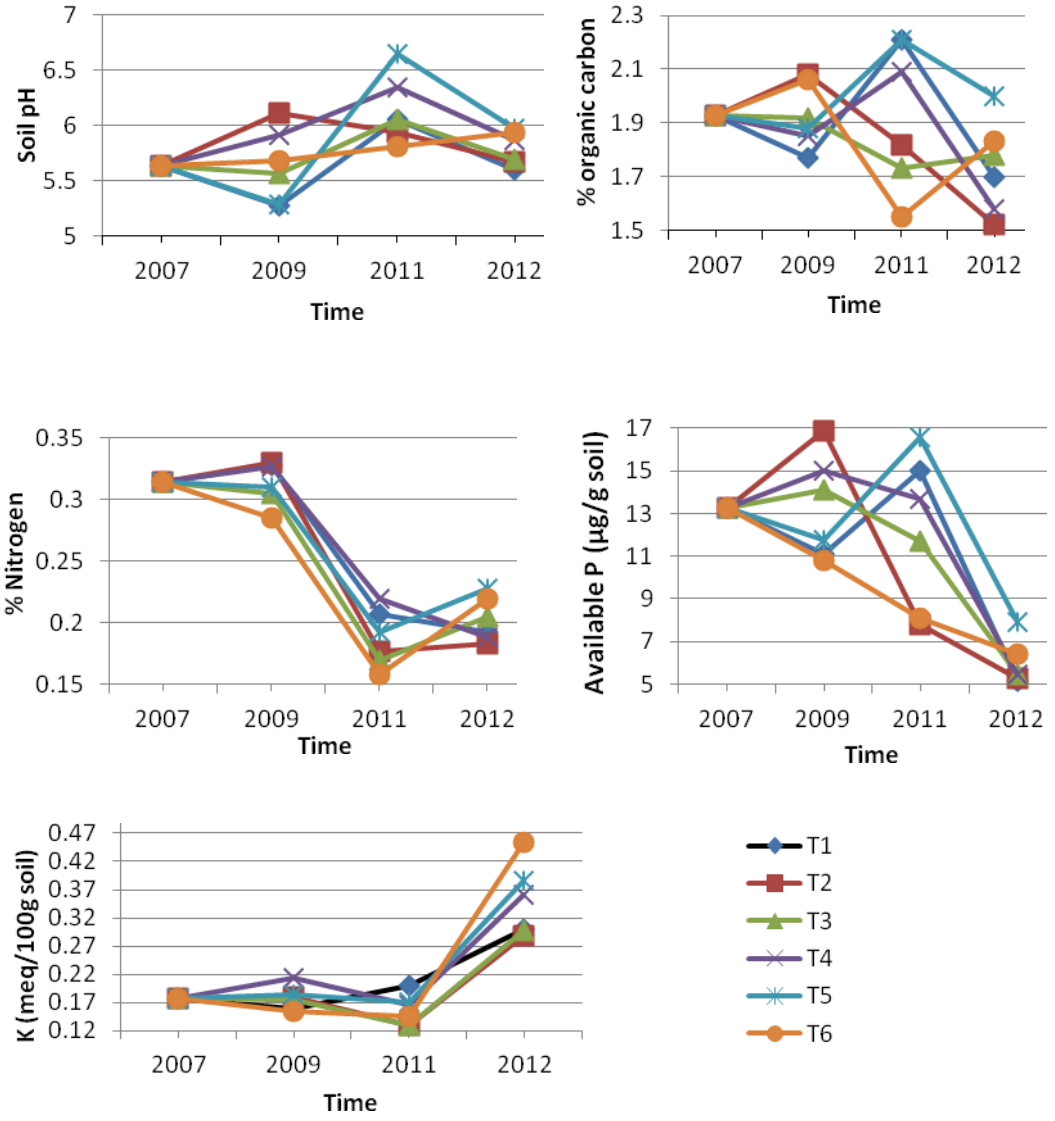

Figure 1. Effects of treatments on soil pH, % organic carbon, % nitrogen, available P and exchangeable K.

However, there was a general decline in the nitrogen content of the soils in the treatments from the second year after planting the coffee clones when compared to the initial value of nitrogen recorded in 2007. Reductions in soil nitrogen ranged between 29.9 to 49.7% in February 2011 and 27.4 to 41.7% in January 2012 across the treatments (Figure 1). The decline in N content of the soil may be attributed to the high requirement of nitrogen by the young coffee plants as well as the *C. odorata* and *G. sepium* for vegetative growth. The results obtained did not show that *G. sepium* which is a leguminous tree, played any significant role in adding nitrogen to the soil. Since there were no marked differences in the soil nitrogen content between Treatment 6 and the other treatments (Figure 1). The values recorded for available phosphorus and potassium in June 2009 and February 2011 did not show any significant differences. However, there were significant treatment differences in both the available P and K sampled in January 2012 (Figure 1). For the available P, significantly higher amounts were recorded in T5 than in the other treatments with the exception of T6. When compared to the initial value of available P, reductions ranged from 40.4 % (T5) to 61.3% (T1) across the treatments (Figure 1). This decline in available P could be ascribed to the fact that more P could have been used for reproductive growth by both the

coffee and the shade trees. This suggests the need for the application of P at this stage to enhance berry production by the coffee plants. Generally high levels of K were recorded in all the plots in January 2012 ranging between 61.2% in T2 and 154.5% in T6 as compared to the baseline value of K recorded in September 2007 (Figure 1). It is possible that mineralization of litter from *C. odorata* and *G. sepium* resulted in the release of more K into the soil hence the high K content in all the treatment plots.

Although leaf drop from *C. odorata* was higher [ranging between 25.6 to 32.9% across Treatments 1 to 5] than was recorded for the plot with *G. sepium* (T6) (Figure 2a), this did not have any clear impact on the organic carbon and nitrogen contents of the soil (Figure 1). No significant treatment differences were recorded in the moisture content of the soils during the third dry season after planting the coffee clones (Figure 2b). This implies that *C. odorata* is not inferior to *G. sepium* (the standard recommended shade tree for coffee in Ghana) with respect to conserving soil moisture during the dry season.

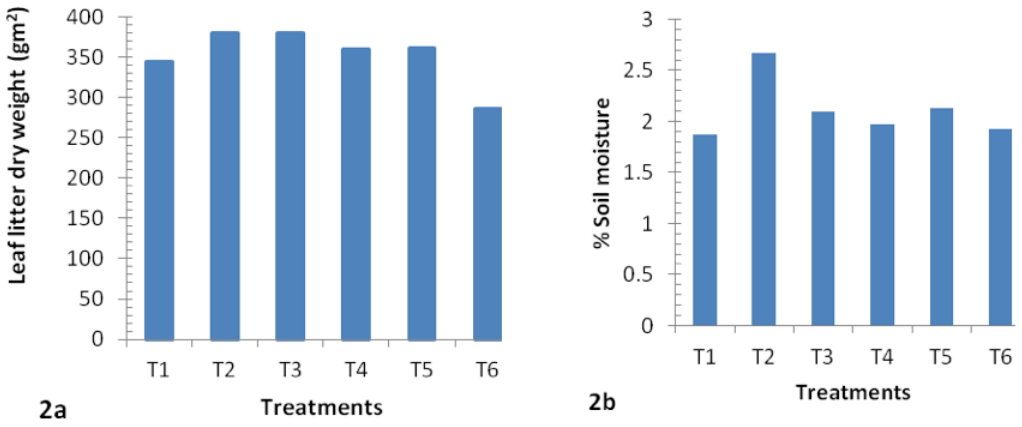

Figure 2. Effects of treatments on leaf litter from trees (a) and % soil moisture content (b) in the dry season in January 2012.

The initial yield of coffee in 2010/2011 season was significantly higher in Treatments 5 and 6 than in Treatments 1 and 2. The same trend was observed in the 2011/12 season but the differences were not significant. However, the cumulative yield of treatment 5 was significantly higher than that of treatments 1 and 2 (Figure 3), giving early indication that *C.odorata* planted at a high density of 434 trees ha^{-1} could have adverse effects on the reproductive capacity of Robusta coffee plants. This confirms earlier reports that the vegetative growth of young Robusta coffee could be negatively affected by high densities of *C. odorata* (Oppong and Anim-Kwapong, 2011).

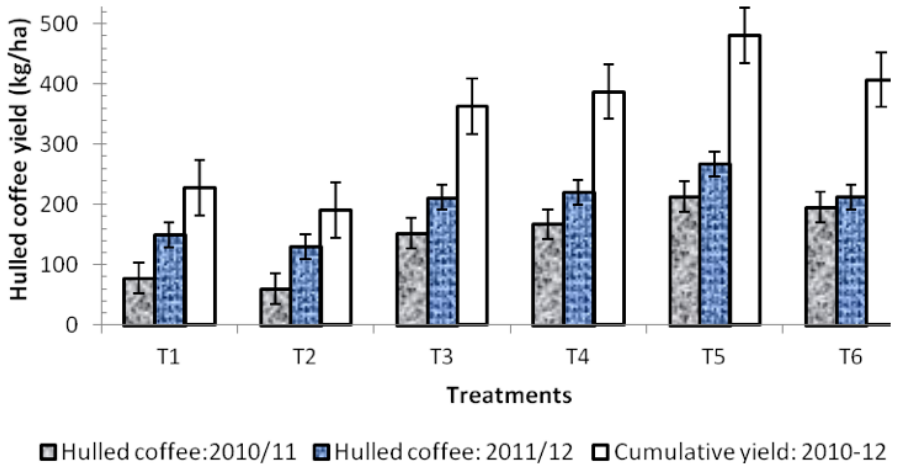

Figure 3. Effects of treatments on initial yield of hulled coffee (kg/ha).

CONCLUSIONS

Interplanting coffee with *C. odorata* is a feasible option in attempts to find complementary income for farmers in the event of a slump in market prices for coffee. There is however the need to critically monitor the nutrient status of the soil for the restoration of normal levels of N and P for enhanced growth and development of the coffee plant.

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Phytosociological Survey of Weeds in *Coffea Canephora* and *Hevea Brasiliensis* Intercropping

J. O. RODRIGUES[1], A. V. ARAUJO[1], P. RANGEL[1], J. A. ALTOÉ[2], F. L. PARTELLI[1]

[1]Centro Universitário Norte do Espírito Santo - Universidade Federal do Espírito Santo - Rodovia BR 101 Norte, Km. 60, Bairro Litorâneo, 29932-540, São Mateus, Espírito Santo, Brazil.
[2]Laboratório de Fitotecnia - Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego 2000, 28013-602 Campos dos Goytacazes, Rio de Janeiro, Brazil

SUMMARY

Weeds negatively interfere on coffee culture, causing to it direct and indirect economic losses. The study of weed population dynamics, based on phytosociological survey, is essential to the integrated management aiming to determine the time of application of control methods in coffee cultivation. This work aimed to carry out the phytosociological survey of the infesting community of plants in an area composed by intercropping between Conilon coffee and rubber tree according to the spacing. It was used a completely randomized design with six repetitions. It was determined six treatments corresponding to the distance from rubber trees to the sampling area, i.e., 1.5, 4.5, 7.5, 10.5, 13.5 and 16.5 m. The floristic survey of weeds present in the coffee and rubber trees intercropping area has evidenced the occurrence of 13 species being observed the highest number of weed species in the treatment four, totalizing 10 species. The highest importance value index in the total area was obtained for *Commelina benghalensis*, following by *Digitaria insularis* and *Coffea canephora*. Thus, the results suggest that these are the most problematic species able to reduce coffee tree yield in the studied area. There was difference in the similarity index. Near to the rubber trees there was predominance of dicotyledonous plants.

INTRODUCTION

Coffee is a perennial culture grown in row and can be productive for 30 years. However, it is extremely sensitive to weed occurrence, mainly, during rain period, being necessary have weeds under control during this period, since the production decreases in 80%.

The degree of interference is often calculated in relation to plant production and can be defined as the percent reduction of the economic production in a certain culture caused by the interference of the weed community. Among the factors that determine the competition level, the coexistence period, or competition period, and the weed density can be pointed out. The competition period refers to the time in which weeds compete with cultivated plants by growth factors. Weeds compete directly by the same requested resources with coffee tree, by this reason it is important to study their occurrence in coffee tree areas, by this way it is possible to conduct an efficient management, allowing the producer to interfere on competition balance and thus cultivated plants are favored on the competition for resources. Thus, this work aimed to carry out a phytosociological survey of the infesting plant community in a Conilon coffee and rubber tree intercropping area, according to the spacing of coffee row in relation to rubber trees.

MATERIALS AND METHODS

This work was carried out in Jaguaré, a prominent region in coffee production located in Espírito Santo state. The studied area was a Conilon coffee (*Coffea canephora*) farm, which plants were grown on in the year 2006, and maintained in intercropping system with an arboreous species rubber tree, grown in the year 2007. The rubber trees were grown in double-row (33.0 m x 3.0 m), with 2.3 m between plants, while coffee trees spacing was 3.0 m x 1.0 m. Both cultures were grown in the E x W direction. The collections were made on 2011. The experimental design was completely randomized with six repetitions. For sample collection six treatments were determined, each one corresponding to the distances from the arboreous species to the coffee rows, i.e., 1.5, 4.5, 7.5, 10.5, 13.5 and 16.5 m, respectively, treatments 1, 2, 3, 4, 5 and 6. The repetitions were spaced by 10 m from the row spacing of the culture, being a sampling area of 30 m^2. The square frames used have an inner area of 0.25 m^2 and were thrown 1.5 m from the row towards the row spacing.

The species enclosed by each square frame were cut near to the soil surface, enclosed in paper bags, taken to the laboratory for counting and identification using specialized literature and by means of comparison with herborized material. After identification, the plants were submitted to drying in stove at 70°C, for 72h, for dry mass determination.

It was evaluated absolute density (Da), relative density (Dr), absolute frequency (Fa), relative frequency (Fr), absolute dominance (DoA), relative dominance (DoR) and importance value index (IVI), using the following estimators: Absolute Density; Relative Density; Absolute Frequency; Relative Frequency, Absolute Dominancy; Relative Dominancy; Importance Value Index. This work had the collaboration of Ufes and Basf.

RESULTS AND DISCUSSION

The floristical survey of weeds occurring in coffee and rubber trees intercropping has showed the presence of 13 species. According to Figure 1A, it is observed the presence of six weed species, among them *Digitaria insularis* has showed the highest importance value index (IVI) with a value of 170 for the distance of 1.5 m from rubber trees. At the distance of 4.5 m from rubber trees (Figure 1B) the highest IVI was obtained for *Coffea canephora*, originated by fallen fruits in the row spacing that act as weeds competing with Conilon coffee matrices.

In the figure 2A can be observed the presence of six weed species, among them *Commelina benghalensis* is pointed out presenting IVI of 180. The treatment 10.5m of distance from rubber trees showed the highest number of weeds (Figure 2B) totalizing 10 species. In the figures 3A and 3B which correspond to the distances 13.5 and 16.5 m, respectively, it is observed the presence of five species of weeds, with *Commelina benghalensis* occurring in both treatments and presenting the highest IVI.

The weed which has presented the highest importance value index on the total area was *Commelina benghalensis*, followed by *Digitaria insularis* and *Coffea canephora*. The results suggest that these species are the most relevant in reducing coffee yield on the studied area. Near the rubber trees there was predominance of dicotiledoneous.

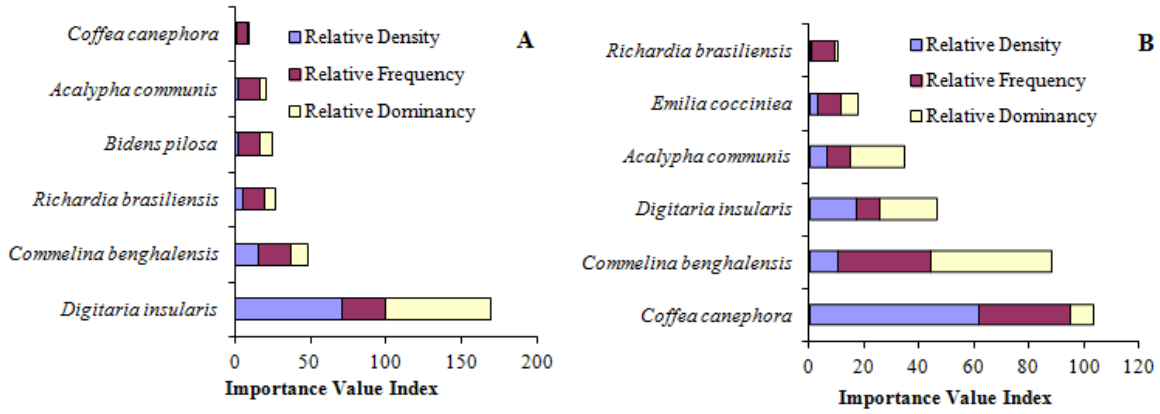

Figure 1. Representative histograms of estimation of the algorithms expressing the Importance Value Index (IVI) for the distance of 1.5 m (A) and 4,5 m (B) from the arboreous species in relation to coffee plants.

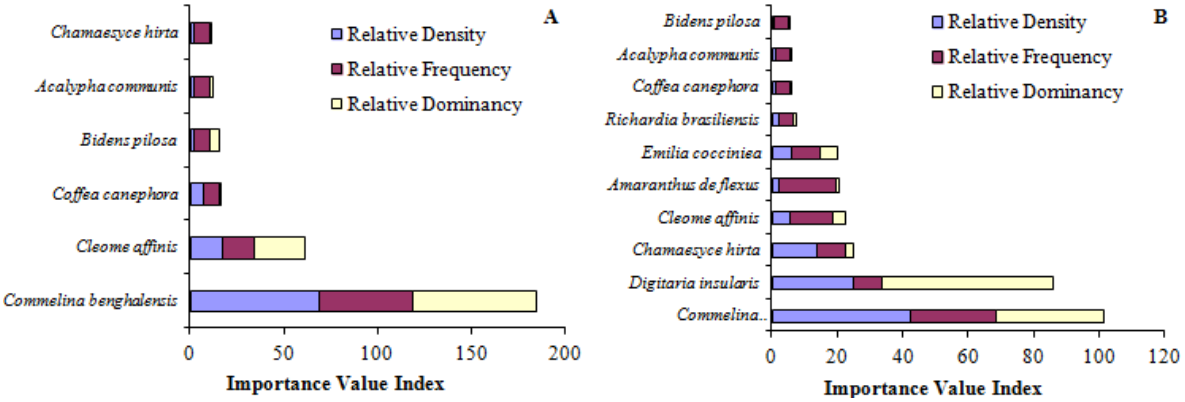

Figure 2. Representative histograms of estimation of the algorithms expressing the Importance Value Index (IVI) for the distance of 7.5 m (A) and 10,5 m (B) from the arboreous species in relation to coffee plants.

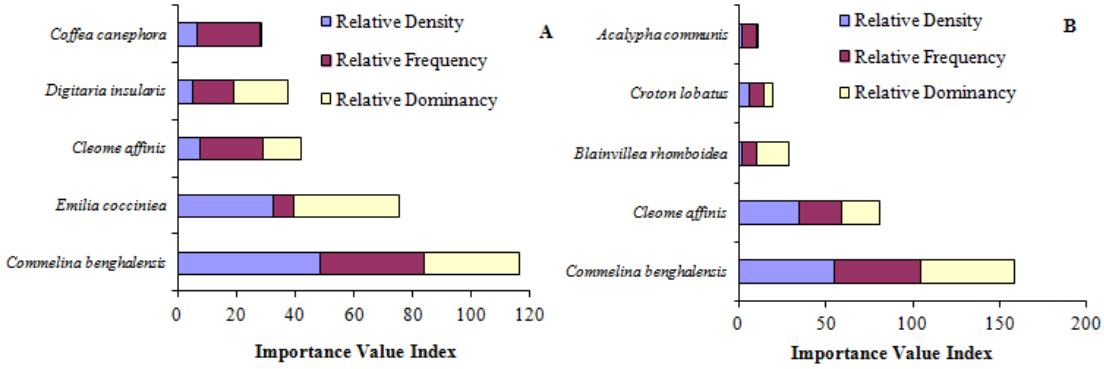

Figure 3. Representative histograms of estimation of the algorithms expressing the Importance Value Index (IVI) for the distance of 13.5 m (A) and 16,5 m (B) from the arboreous species in relation to coffee plants.

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Epidemiology of Coffee Leaf Rust: Influence of Shade on Microclimate and Ecophysiology of Coffee

R. N. MAYOLI, E. K. GICHURU

Coffee Research Foundation, P.O. BOX 4 00232 RUIRU

SUMMARY

The influence of shade trees on coffee productivity depends on many interacting factors such as soil and climatic conditions, coffee and shade tree species, fertilization regime, shade management, and pest and disease management. Evaluation of different shade levels provide the basis for identifying the optimum shade conditions which minimize the entire pest complex and maximize the effects of beneficial microflora and fauna acting against it. A field trial to study the epidemiology of the Coffee Leaf Rust (CLR) under different shade regimes was carried out in a medium altitude coffee growing zone in Kenya. Sites were selected with trees casting shades over susceptible coffee trees. A trend in photosynthetically active radiation (PAR), stomatal conductance, leaf temperatures and photosynthetic rates was observed. Coffee trees under moderate shade and in full sunlight had higher leaf temperature, stomatal conductance values and net photosynthetic rates compared to coffee grown directly under shade. The role of temperature, leaf wetness and reduced irradiance in relation to disease incidence are discussed.

INTRODUCTION

Arabica coffee, *Coffea arabica* L. originates from Ethiopian tropical forests (latitude of 6-9^{O} North, altitude of 1600-2800m) with average annual temperatures of 20^{O}C, well distributed annual rainfall (1500-2500mm) and a dry season of 2-3 months (Van Kanten and Vaast, 2006). Arabica coffee was traditionally grown under shade in complex agroforestry systems with up to three storeys of vegetation (Vaast et al., 2006). The influence of shade trees on coffee productivity depends on many interacting factors such as soil and climatic conditions, coffee and tree species, fertilization regime, shade management, and pest and disease management. The presence of shade trees is known to improve soil organic matter content (Beer et al., 1998) and agroforestry systems can bring about favourable changes in the microclimatic conditions by influencing radiation flux, air temperature and wind speed all of which have a significant impact on modifying the rate and duration of photosynthesis, transpiration and stomatal conductance (Monteith et al., 1991).

Limiting factors of coffee production include major diseases, such as the Coffee Leaf Rust (CLR or Orange rust) and the Coffee Berry Disease (CBD) caused by the fungi *Hemileia vastatrix* Berkeley and Broome and *Colletotrichum kahawae* Bridge and Waller, respectively (Silva et al., 2006). Coffee rust epidemics are based on simple infection cycle but develop polycyclic epidemics in a season and these processes involve a large number of environmental variables and as in any system involving a perennial crop, the physiology of the coffee crop. Crop management and particularly shade is therefore expected to have large effects on CLR epidemic.

MATERIALS AND METHODS

A field trial was conducted at the Coffee Research Station, Ruiru, Kenya, a medium altitude area situated 1.05^{O}S and 36.45^{O}E at an elevation of 1608m above the sea level. The soils at the site are classified as humic nitosols (Jaetzold et al., 2007). The study was carried out between February and November 2011. The site was selected with a tree casting shades over susceptible French mission coffee trees. Twenty two trees were selected along the path of the shadow and six control trees in full sun. Data was recorded on two selected branches for physiological parameters. Simultaneous records of photosynthetically active radiation (PAR) at the leaf surface, leaf temperature, stomatal conductance and leaf transpiration, and net photosynthetic rate were taken using a LCPro+ photosynthesis system (ADC BioScientific Ltd, UK). Disease incidence was assessed fortnightly by counting the total number of leaves, number of infected leaves and number of pustules.

RESULTS AND DISCUSSION

Some cropping practices may affect the development of the coffee leaf rust through their influence on the microclimate and the host, which in turn act on the life cycle of the fungus (Avelino et al, 2004). In this trial, shading buffered leaf temperature (Figure 1) with increasing irradiance (Figure 2). Stomatal responses to ambient humidity are very strong in both shade and sun adapted cultivars (Fanjul et al., 1985). Since stomatal aperture is greater under shade or on cloudy/rainy days (Fanjul et al., 1985), this may be the reason why high stomatal conductances (Q leaf) (Figure 3) were recorded on coffee in full sun during period of low irradiance. Shade may have resulted in lower net photosynthetic rate (Figure 4) due to insufficient light interception. Fahl et al. (1994) also observed higher net photosynthetic rate in sun-grown than in shade-grown Arabica coffee plants. Coffee leaves exhibit typical shade acclimation features theoretically allowing them to maintain net photosynthetic rates in low light. Limitation of photosynthesis by low light availability has been proposed as one of the main reasons for lower yields of coffee grown in agroforestry systems in optimal coffee production areas. Coffee under shade had higher chlorophyll levels than coffee grown under full sun (data not shown). It was observed that CLR initially increased on the coffee trees located away from the shade tree up to levels of 40% and above (Figure 2). In shaded coffee plantations, at low rainfall intensity and duration, many water droplets do not reach the coffee trees and spore liberation and dispersal are reduced (Avelino et al, 2004). This may have attributed to a low disease pressure near the shade tree. It is more likely that shade reduces the susceptibility of the plant to rust because yields are reduced; production of a heavy crop depletes the tree of nutrients and makes it more susceptible to the disease. Effects of shade would be expected to differ with agro-ecological zones and seasonal weather. These aspects will be investigated further by having more trials in different areas.

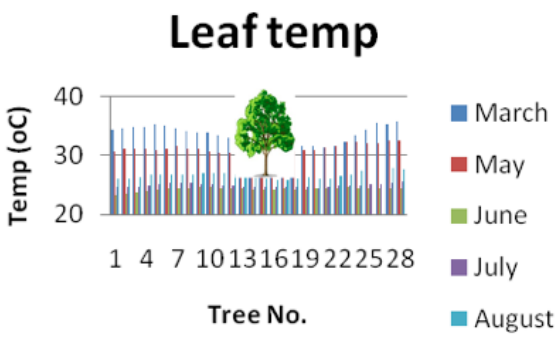

Figure 1.
Effect of shade on leaf temperature.

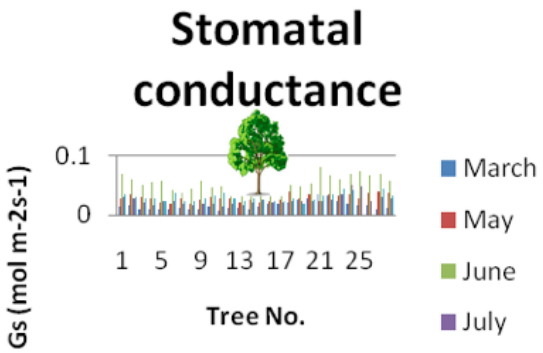

Figure 3.
Effect of shade on stomatal conductance.

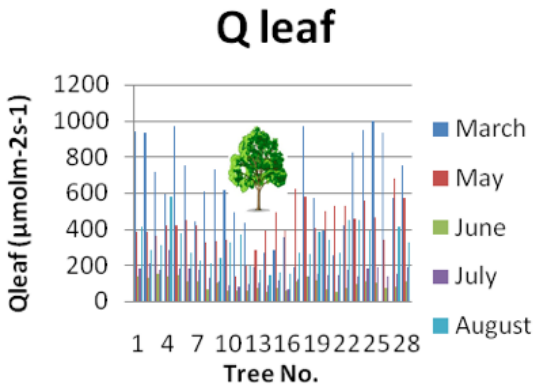

Figure 2.
Effect of shade on Qleaf.

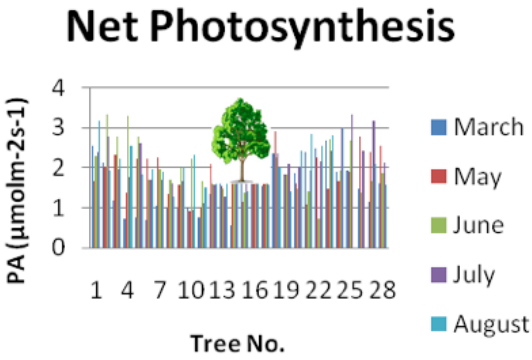

Figure 4.
Effect of shade on Net photosynthesis.

Note: The diagram of a tree indicates the position of the shade tree.

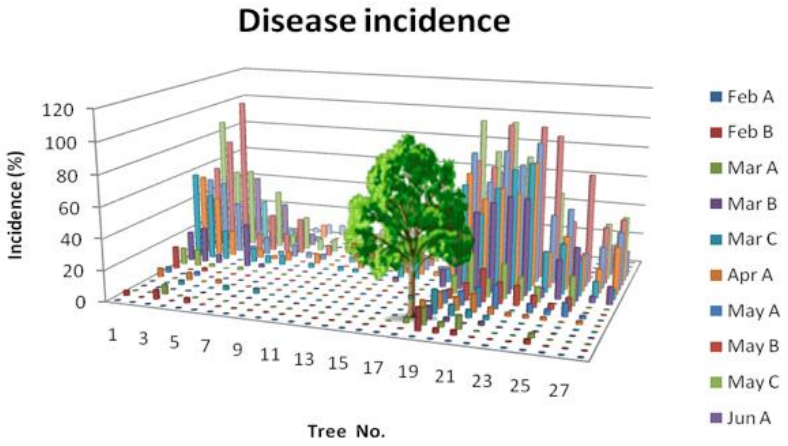

Figure 5. Effect of shade on CLR incidence.

ACKNOWLEDGEMENTS

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Coffee Rust Progress Curves in Clones of Conilon Coffee (*Coffea Canefora*) in the North Region of the State of Espírito Santo, Brazil

M. B. SILVA[1], F. L. PARTELLI[1], L. ZAMBOLIM[2], C. W. OLIVEIRA[1], L. CANAL[1], T. T. HERZOG[1]

[1]Centro Universitário Norte do Espírito Santo - Universidade Federal do Espírito Santo, Rodovia BR 101 Norte, Km. 60, Bairro Litorâneo, 29932-540, São Mateus, Espírito Santo, Brazil
[2]Universidade Federal de Viçosa, Laboratório de Proteção de Plantas, Av. P. H. Rolfs s/n, CEP 36570-000 Viçosa, Minas Gerais, Brazil

SUMMARY

Little is known about the epidemiology of rust on *C. canephora* in the north of the state of Espírito Santo, Brazil. Understanding the behavior of rust is one of the prerequisites to building a rational and sustainable disease management program. As a result of climatic fluctuations, changes in cropping systems, increased crop yields and other factors, behavior of rust has varied greatly over the years. This led to the indiscriminate use of fungicides which made it hard to control rust in the field. The objective of this study was to understand the behavior of coffee rust in four clones in the study area.

Coffee rust was evaluated in a coffee farm that uses a non-chemical approach to disease control. Rust was monitored in the clone 02 (susceptible to rust), 143 (considered as resistant), and clones G35 and "Verdinho". Data were obtained from three plots of 25 plants for each clone. Rust progress was evaluated on a monthly basis with six leaves collected from ten plants randomly chosen in each plot. The number of leaves with rust was recorded and the percentage of diseased leaves determined.

Based on rust incidence data it has been possible to obtain the curve of disease progress for each clone. The disease incidence increased from a value close to zero in January/February to around 80% in September/August. These data showed that coffee rust continues to grow even after the harvest. Genetic resistance to rust observed in clone 143 reduced by 62% the incidence of rust, showing the importance of the use of genetically resistant clones in the management program in the field.

INTRODUCTION

Brazil is the second largest producer of *C. canephora* accounting for 23% of the world production. The referred species is known in Espírito Santo as conilon coffee because it is the most commonly grown coffee variety of the *C. canephora* in the state. The high production of this coffee in Espírito Santo (72% of the Brazilian production) is largely due to the Incaper (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural) improvement program that began in 1985.

Coffee production, both arabic and conilon, is limited by several factors, particularly diseases. From planting to harvesting, the culture of conilon coffee can be affected infection by

phytopatogens, so that the use of pesticides is required. Among the diseases that infect this culture, not only in Brazil, but all over the world, rust caused by *Hemileia vastatrix* Berk. et Br. is a major concern because it causes leaves to fall from infected plants, and, thus, branches to dry. The progressive drought of the branches reduces the life of the crop, and, consequently, production is not economically viable.

The varieties of conilon coffee consist of a set of clones that have several common characteristics, such as homogeneity of production, but that differ in the level of rust resistance.

Little is known about the epidemiology of rust on *C. canephora* in the north of the state of Espírito Santo, Brazil. Understanding the behavior of rust is one of the prerequisites to building a rational and sustainable disease management program. As a result of climatic fluctuations, changes in cropping systems, increased crop yields and other factors, behavior of rust has varied greatly over the years. This led to the indiscriminate use of fungicides which made it hard to control rust in the field. The objective of this study was to understand the behavior of coffee rust in two clones in the study area.

MATERIALS AND METHODS

Coffee rust was evaluated in a coffee farm that uses a non-chemical approach to disease control. Rust was monitored in the clone 02 (susceptible to rust), 143 (considered as resistant), and clones G35 and "Verdinho". Data were obtained from three plots of 25 plants for each clone. Rust progress was evaluated on a monthly basis with six leaves collected from ten plants randomly chosen in each plot. The number of leaves with rust was recorded and the percentage of diseased leaves determined.

Rust was monitored for one year from October/2010 to August/2011 and the disease progress curve was built. The initial disease (Yo) and the maximum (Ymax) disease value and area under disease progress curve were obtained for each clone. Data was linearized and the rate of disease increase (r) was estimated. AUDPC was calculated and statistically compared between the clones using a statistical package.

RESULTS AND DISCUSSION

The disease progress curve for each clone can be seen in Figure 1. There was a decreased incidence of disease after October to January/February and increased incidence until August/September (Table 1). Harvest occurred in June. Unlike the disease progress curve observed in *C. arabica*, rust in *C. canephora* continued to increase after harvest because in the north region of the state of Espírito Santo the winter temperature is not very low and a greater number of diseased leaves remain in the plant after harvest.

The characteristics of each disease progress curve are shown in Table 1. The initial disease (Yo), the maximum disease severity value (Ymax), the rate of disease increase (r) and the Area Under Disease Progress Curve (AUDPC) can be seen. The highest values of Yo, Ymax and AUDPC were observed in clone 02 (susceptible). On the other hand, clone V-143 obtained the lowest values of Yo, Ymax and AUDPC. The results observed in the other clones were similar to those of clone V-02, indicating their susceptibility to rust. Only for the result obtained with the rate of disease progress the highest value was observed in clone 143, due to the significant increase in disease observed from July to September. This data showed that the rate of disease increase was not a good parameter to establish the differences between resistant and susceptible clones in this experiment.

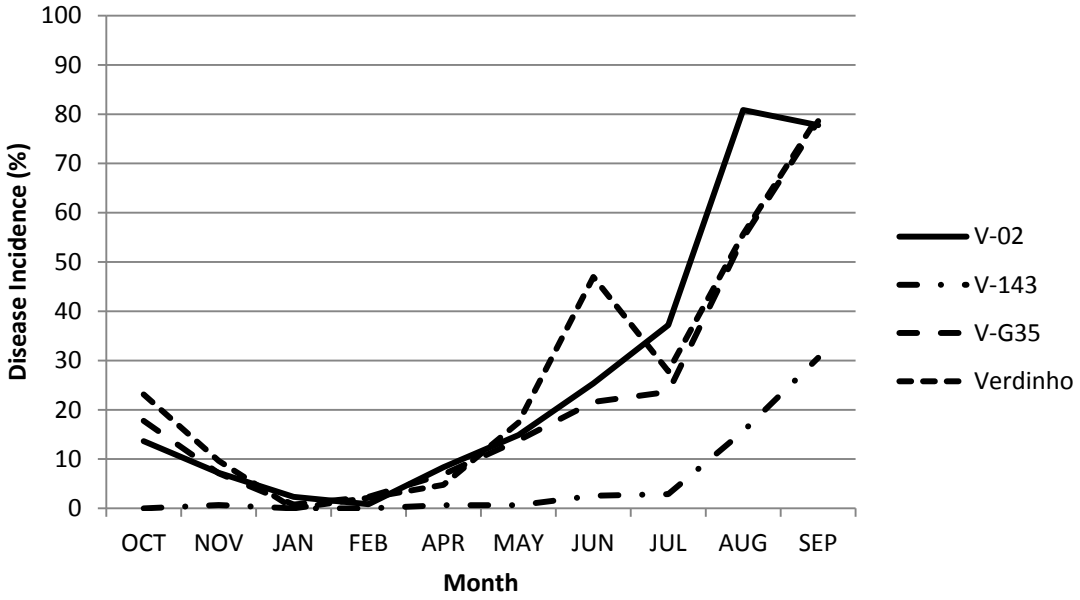

Figure 1. Disease progress of coffee rust for four different clones.

Table 1. Different components obtained from disease progress curve obtained in four clones of *C. canephora* (Clones 02, G35, 143 and "verdinho").

Clone	Variable			
	Yo (%)	***Ymax (%)***	***r***	***AUDPC***
V-02	Feb/0.84	Aug/85.85	0.206	6960 A
V-143	Jan/0.00	Sep/30.57	0.360	1169 B
V-G35	Jan/0.76	Sep/78.62	0.203	5634 A
"verdinho"	Jan/0.00	Sep/79.43	0.211	6719 A

In each AUDPC means followed by different letters represent significant differences (F test, 95% confidence level)

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Effectiveness of Cyantraniliprole on Control Coffee Berry Borer (*Hypothenemus Hampei*) in Indonesia

S. WIRYADIPUTRA, S. MAWARDI

Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java, Indonesia

SUMMARY

Cyantraniliprole is a novel pesticide and second generation ryanodine receptor insecticide. This insecticide is safer than others due to lower toxicity against mammalian and the others. Their effectiveness in controlling main coffee pests, especially against coffee berry borer (CBB, *Hypothenemus hampei*) is not known yet in Indonesia. An experiment has been set up to know the effectiveness of the pesticide against CBB on Arabica coffee in East Java Province, Indonesia, from November 2011 to March 2012. Eight treatments including untreated plot as a control and five levels of cyantraniliprole dosage, as well as single dosage of carbaryl and lamda sihalothrin have been applied in field condition on each plot composed 20 coffee trees of four years old. Each treatment has been replicated four times. Applications of insecticide have been conducted two times with interval of one month and started at the condition of coffee seed starting hardening. The results revealed that observation of CBB infestation on coffee cherry in the field, cyantraniliprole at the dose of 1750 ml and 2000 ml formulation (10% cyantraniliprole) were very effective in controlling both CBB infestation and population until two months after second application. The effectiveness was superior to carbaryl and lamda sihalothrin insecticides. Observation on green coffee and parchment coffee also showed lower infestation than untreated treatment as well as carbaryl and lamda sihalothrin treatments. Green coffee production on cyantraniliprole was also higher than the others treatments.

INTRODUCTION

Coffee berry borer (CBB), Hypothenemus hampei (Coleoptera: Curculionidae) is the most important insect pest on coffee in the world as well as in Indonesia. The pest causes yield losses significantly high and low coffee bean quality if the infestation was uncontrolled. Management of CBB infestation in Indonesian coffee is directed to the integrated pest management (IPM) program with emphasis on biological and cultural measures with minimal or safer pesticide application. Selection of pesticide used was mainly based on properties safer to environment and human health and it solved the resistance of target pest to other pesticides.

Application of insecticides for controlling CBB has been conducted for long time ago, using organochlorine pesticides such as lindane and endosufan in the several coffee producing countries in Asia Pacific, Africa and Central as well as South America (Brun et al., 1989; Damon, 2000; Perez et al., 2000). In Indonesia, although majority of coffee farmers do not apply endosulfan for controlling CBB in their garden, research results showed that endosulfan was very effective in controlling CBB (Wiryadiputra, 1996). Disadvantages of intensive application of endosulfan have appeared causing resistance of target pest in coffee plantation, especially CBB. Brun et al. (1989) reported that application of endosulfan in New Caledonia during 10 years with biannual frequency had been causing resistance to the insecticide in high levels of being up to 1000 fold.

In this paper, we reported an experiment of application of a novel pesticide belong to ryanodine-receptor targeting insecticides, which has characterized higher LD-50 value against mammalian than other insecticides generation before.

MATERIALS AND METHODS

Experiment has been conducted at Kalibendo Arabica coffee plantation in Banyuwangi Regency, East Java Province, Indonesia with elevation of about 650 m above sea level and has climate type of B according to Schmidt and Fergusson (1951) classification. Variety of "Composite" four years old Arabica coffee was used as plant material in the field. Eight treatments consist of five level dosages of cyantraniliprole, two compared insecticides and one untreated plot. Five dosages of cyantraniliprole were 1000, 1250, 1500, 1750 and 2000 ml cyantraniliprole 10% per hectare, and for the compared insecticides used were carbaryl 85% and lamda sihalotrin 25g/L with a dosage of 1000g and 1000 ml per hectare, respectively. Carbaryl 85 % was recommended for CBB control according to green book published by Department of Agriculture, Republic of Indonesia (Anonim, 2011), although in the field it was rarely used by the farmers.

Every plot consisting of 20 coffee trees was sprayed two times with interval of one month using insecticide treatments except the untreated plot. The first spraying was initiated when the CBB starting to bore on coffee berries. Observations were done on the parameters of CBB infestation, CBB population, green coffee production, and effect of insecticides on other pests (green scale and mealy bug) on five sample trees chosen in every plot. Infestation of CBB was observed on coffee berries in the field every two weeks and on harvested red coffee cherries as well as parchment and green coffee. Population of CBB was observed two times, at one day before first application and one month after second application of insecticide. Coffee production was observed during three harvesting times which covered around 60 % of total berries harvested. The data obtained then to be analyzed using SAS software and Duncan's Multiple Range Test (DMRT) for comparison of the treatments.

RESULTS AND DISCUSSION

Cyantraniliprole insecticide was very effective in controlling CBB infestation in the field as showed on figure 1A. Dosages of 1750 and 2000 ml cyantraniliprole 10% per hectare were effective in suppressing CBB infestation until the end of observation; however at lower dosages, effectiveness only occurred during 1.5 months after second application of insecticide. The same inclination also occurred on the compared insecticide, carbaryl and lamda sihalothrin. Effect on CBB population, cyantraniliprole 10 % also performed in better results compared to carbaryl and lamda sihalothrin (Figure 1B). Cyantraniliprole 10% also suppressed CBB infestation on green coffee harvested from the plot treated (Figure 2A), and the dosage of 2000 ml per ha was the best in suppressing CBB infestation. Application of cyantraniliprole has also increased the coffee bean production compared to untreated plot. The highest increase of production occurred on the treatment of cyantraniliprole at the dosage of 1000 ml/ha. Increasing of production of green coffee in this case was not only due to application of insecticides but also other factors; however, the treatments were affecting dominantly than the others.

Cyantraniliprole is a novel insecticide belong to the class of anthranilic diamides acting selectively on ryanodine receptors of a broad spectrum of lepidopteran (including caterpillars and potato beetle). When coupled with calcium channels, they induce muscles contraction in a target insect, what quickly results in paralysis and finally- insect death (Legocky *et al*., 2008). A novel class of anthranilic diamide insecticides has an excellent properties of high

toxicagainst insect pests and very low toxic to mammalian. It is also safe for natural enemies such as parasitoids and predators (Lahm, *et al*., 2007; Lahm, *et al*., 2009).

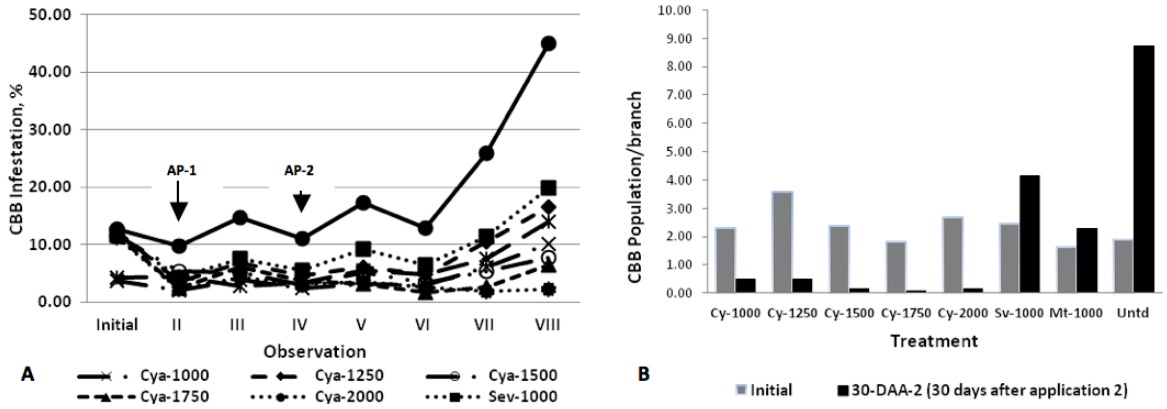

Figure 1. Effect of cyantraniliprole 10% and compared insecticides on CBB infestation (A) and CBB population (B) on Arabica coffee in East Java, Indonesia.

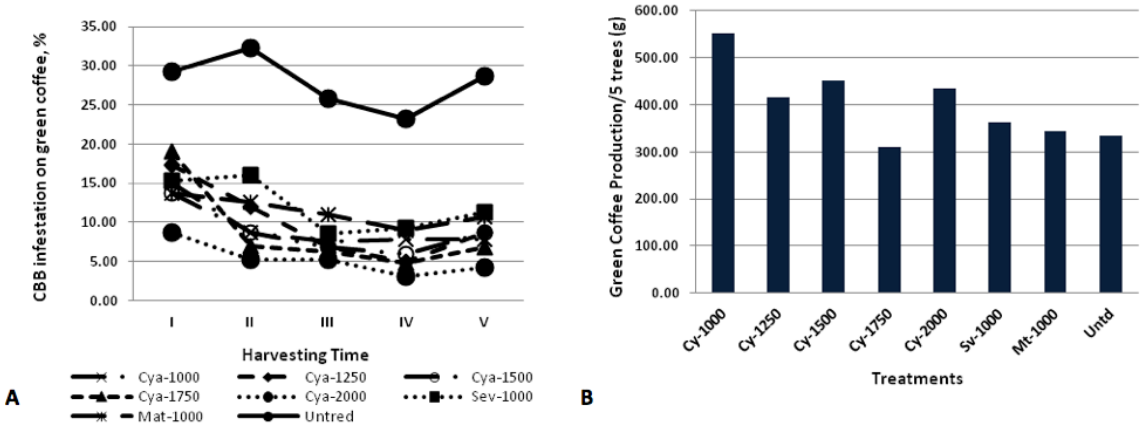

Figure 2. Effect of cyantraniliprole 10% and compared insecticides on CBB infestation in green coffee (A) and on green coffee production (B).

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Brazilian Coffee Free-Air Carbon Dioxide Enrichment (FACE) Facility: Predicting the Impact of Climate Change

R. GHINI[1], A. TORRE-NETO[2], A. F. M. DENTZIEN[1], W. BETTIOL[1], F. R. A. PATRÍCIO[3], O. GUERREIRO-FILHO[4], R. A. THOMAZIELLO[4], M. T. BRAGHINI[4], L. C. FAZUOLI[4]

[1]Embrapa Environment - CP 69 - 13820.000 Jaguariúna - SP - Brazil
[2]Embrapa Agricultural Instrumentation - São Carlos - SP - Brazil
[3]Instituto Biológico - Campinas - SP - Brazil
[4]IAC Café - Campinas - SP - Brazil

SUMMARY

The atmospheric CO_2 concentration has been increasing significantly in the last decades, despite the international efforts for the reduction of emissions. The Climapest FACE facility was established at Embrapa Environment (Jaguariúna, São Paulo State, Brazil), on August 25th, 2011, in order to generate field response data in broad-acre coffee to elevated CO_2 air concentration and water supply. Diseases, pests and weeds, as well as plant physiology of two coffee cultivars (Catuaí Vermelho IAC 144 and Obatã IAC 1669-20), multitrophic interactions and soil attributes have been monitored in twelve 10-m-diameter octagonal rings (plots) located within a 7-ha coffee field. Six rings, representing the control treatment, were left under untreated conditions (current atmosphere), whereas other six rings have been treated with pure CO_2 to achieve the concentration of 200 µmol mol^{-1} above ambient concentration, supplied by a bulk CO_2 container with the capacity of 20 t. The system instrumentation is based on wireless sensor network technology. Each octagon segment has individual gas valves to compensate the wind direction and a flow control device to compensate wind speed changes. The objective of this paper is to describe the first coffee FACE facility in the world.

INTRODUCTION

The average atmospheric concentration of carbon dioxide (CO2) reached 397 µmol mol-1 in 2012, exceeding the concentrations of the last 800,000 years (180 to 300 µmol mol-1).Projections indicate that CO2 concentration could reach 730 to 1020 µmol mol-1 by 2100 under the A2 scenario.

Despite the evidence of beneficial effects of CO2 on plants, it is not well known whether these effects will still take place in the presence of pathogens, pests and weeds or other limiting factors, particularly in tropical countries. Studies conducted under controlled conditions might not reflect plant responses in the field, where there are variations and interactions among temperature, precipitation, and other factors. The search for more realistic conditions has led to the use of Free Air Carbon-dioxide Enrichment (FACE) experiments.

FACE experiments are characterized by large-scale and long-term exposure of plants to elevated CO2 concentrations under field conditions without enclosure, allowing interdisciplinary evaluations. This paper discribes the first FACE facility stablished in order to generate field response data in broad-acre coffee to elevated CO2 air concentration and water supply.

MATERIALS AND METHODS

The Climapest FACE facility (Fig. 1) is located at Embrapa Environment, in Jaguariúna, São Paulo State, Brazil (latitude 22°41'S, longitude 47°W, altitude of 570 m a.s.l.) and became operational on August 25th, 2011.

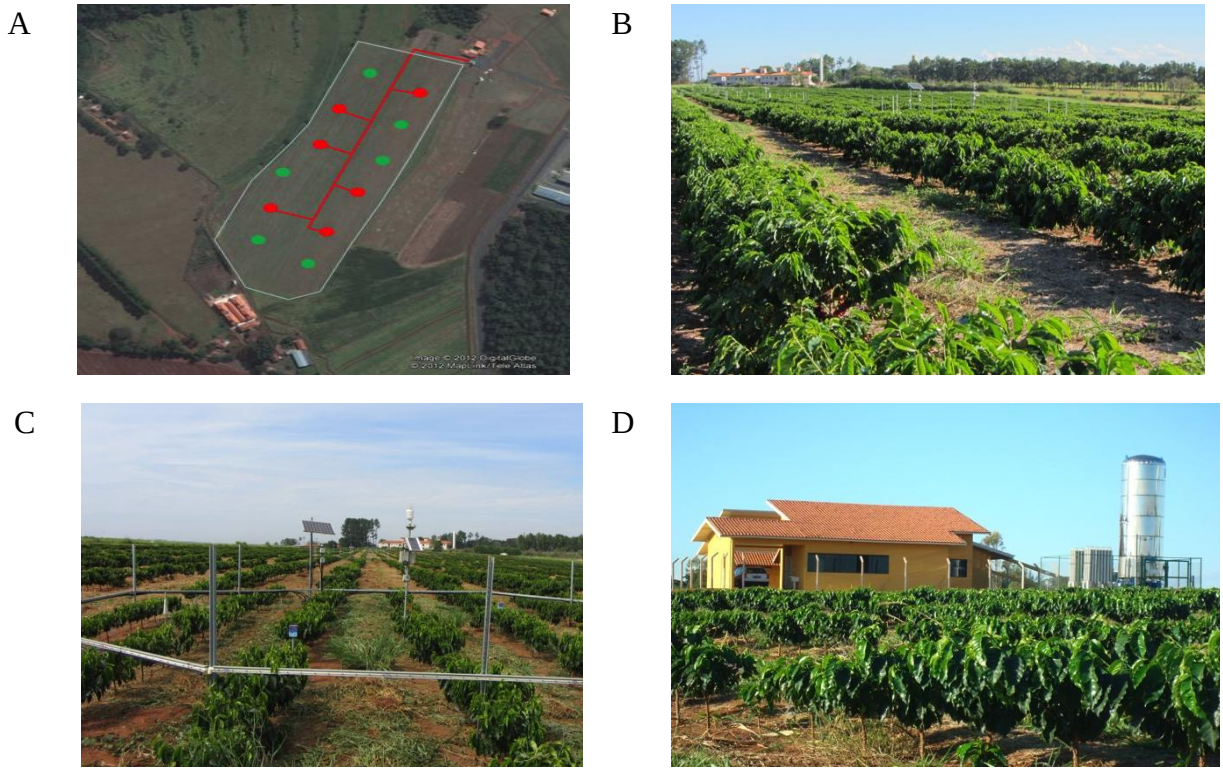

Figure 1. Climapest FACE facility: (A) Google aerial view of the total area (red rings are plots with elevated [CO_2] and green rings are plots with ambient [CO_2], (B) rings in the coffee field, (C) a octogonal ring and (D) bulk CO_2 container and laboratory.

The experiment was designed to study the effects of: CO_2 concentrations (current ~395 µmol mol^{-1} and 200 µmol mol^{-1} above current concentration) and water supply (with and without irrigation) on two coffee cultivars (Catuaí Vermelho IAC 144 and Obatã IAC 1669-20). Twelve 10-m-diameter octagonal rings (plots) were established within a 7-ha-coffee field; six of which with elevated [CO_2] and six with ambient [CO_2], separated at least 70 m from each other to minimize cross-plot contamination. Plots were built with eight chlorinated polyvinyl chloride (PVC) tubes (internal diameter of 18 mm).

The system instrumentation is based on wireless sensor network technology. Environmental sensors (infra-red gas analyzers – IRGA - to measure the CO_2 concentration, anemometers, sensors of air and soil temperature and humidity, solar radiation and precipitation) have been adapted to ZigBee modules. The wireless sensor network based instrumentation facilitated the system installation and maintenance, and increased its portability. Each octagon segment has individual gas valves to compensate the wind direction and a flow control device to compensate wind speed changes. The system has been adjusted to allow injection only for wind speeds within the range of 0.5 to 4.0 m s^{-1} and the CO_2 injection is run only during daylight hours.

The FACE facility is part of the project entitled "Impacts of climate change on plant diseases, pests and weeds - Climapest" (http://www.macroprograma1.cnptia.embrapa.br/climapest), which has been supported by Embrapa (Brazilian Agricultural Research Corporation).

RESULTS AND DISCUSSION

Since August 25th, 2011, plants of the two coffee cultivars have been treated with CO_2. Average CO_2 concentrations in the elevated [CO_2] were significatively higher than in ambient plots. Data from April 2012 are shown in Figure 2.

Understanding how coffee plants, pathogens, pests, weeds and related organisms respond to future increase in CO_2 concentration and their interaction with water supply will allow the development of adaptation strategies. The results can help minimize the negative impacts of climate change or provide new opportunities from the positive impacts.

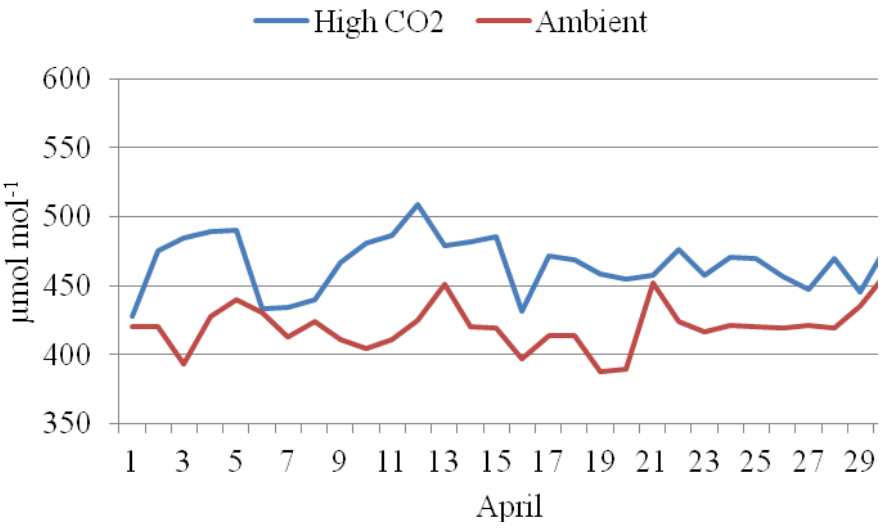

Figure 2. Average CO_2 concentrations in the elevated [CO_2] plots (High CO2) and in ambient plots in April 2012.

ACKNOWLEDGMENTS

The authors are thankful to CNPq for research grants.

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Field Study of the Attractant and Repellent Potential of Volatile Organic Compounds for the Coffee Berry Borer

B. P. DUFOUR[1], L. ETIENNE[2], F. RIBEYRE[1], J. AVELINO[1,3,4]

[1]CIRAD, Montpellier, France
[2]SupAgro, Montpellier, France
[3]CATIE, Turrialba, Costa Rica
[4]IICA-PROMECAFE, Guatemala

SUMMARY

Chemical analysis of effluvia emitted by coffee berries reveals the existence of numerous volatile organic compounds that are attractive to the coffee berry borer (CBB) *Hypothenemus hampei* Ferrari, and which are poorly understood. Only ethanol and methanol have a clearly demonstrated attractant potential when mixed; they are widely used as bait to trap this pest. Methylcyclohexane, ethylbenzene, nonane, octen-3-ol, phenylethanol, trans-2-hexenal and benzaldehyde induce an electrophysiological reaction and/or a positive olfactometric response in the CBB. Camphene and α-pinene show a repellent effect for the CBB when widely released and cis-3-hexenol and 1-hexanol disrupt attraction of the pine shoot beetle. These compounds were tested under field conditions, in order to confirm their attractant or repellent potential. The seven potentially attractive compounds were tested separately with BROCAP® traps in the field, in association or not with a blend of ethanol and methanol. The compounds tested alone did not attract significantly more than water; associated with a blend of ethanol and methanol, they did not attract significantly more than ethanol-methanol alone.

The four compounds selected for their repellent properties were tested in combination with a blend of ethanol and methanol, under the same field experiment conditions. We demonstrated that the repellent action of cis-3-hexenol and 1-hexanol was significant.

The results of field trials with the assumed attractive volatile organic compounds disagreed with those obtained under laboratory conditions, according to the literature. In addition, compounds whose repellent properties are confirmed offer the possibility of integrating them in pull-push experiments.

INTRODUCTION

Olfactory perception in Scolytidae of volatile organic compounds emitted by host plants can trigger the process of colonization (Byers, 1989). For the coffee berry borer (CBB), Hypothenemus hampei Ferrari, coffee berries are attractive. Thus, the perceived odor emissions direct females towards berries. They pierce berries and mine tunnels in the endosperm, in which their offspring develops. Due to the degree of damage caused by this species, it is considered the main pest of coffee.

Volatile organic compounds (VOCs) emitted by berries have been studied by several authors (Mathieu et al., 1996, 1998, Ortiz et al., 2004; Mendesil et al. 2009). Their number varies depending on the coffee variety and berry ripening stage (Mathieu et al. 1998). Among these compounds, methanol used alone has high attractant potential, which is enhanced when mixed with ethanol (Mendosa Mora, 1991, Dufour et al., 2008). These two alcohols are widely used

as bait in CBB trapping. Other compounds such as methylcyclohexane, ethylbenzene, nonane, octen-3-ol, phenylethanol, trans-2-hexenal and benzaldehyde, induce an electrophysiological reaction and/or a positive olfactometric response in CBB (Mendesil, 2009; Gomez de Lima et al., 2004). On the other hand, camphene and α-pinene show a repellent effect for the CBB when widely released (Dufour, unpublished) and cis-3-hexenol and 1-hexanol disrupt attraction of the pine shoot beetle which is of the same subfamily as CBB (Polland et al., 2004).

The purpose of this study was to test these compounds in the field using the BROCAP® trap to confirm their attractant or repellent potential.

MATERIALS AND METHODS

The experiments were conducted at the Aquiares and Maquina Vieja sites, in the Turrialba region, Costa Rica. The wet tropical climate is characterized by a uniform distribution of rainfall throughout the year and frequent cloud situations. Annual precipitation averages about 2698 mm, with temperatures of 21.8°C and 88.1% relative humidity.

The Aquiares coffee plantation is located at 1000 m above sea level. It consists exclusively of Arabica coffee var. Caturra, planted at a density of 6300 plants/ha, under a light shade composed mostly of *Erythrina* sp. At Maquina Vieja, the coffee is 650 m above sea level and has similar agronomic characteristics, except that shading is temporarily absent.

Volatile organic compounds tested

In the first CBB attraction trial (Aquiares site), seven synthetic volatile organic compounds (Sigma-Aldrich) were tested alone against a "water" absolute control. In a neighboring plot, the same compounds were associated with the diffusion of "ethanol-methanol in a 50:50 blend" and tested against an "ethanol-methanol" relative control (Table 1). The diffusion rate of the first four compounds was adjusted according to their volatility and concentration measurements provided by Mendesil et al. (2009). In the absence of baseline data, the rate of diffusion of the other three compounds studied by Gomez de Lima (2004) was arbitrarily set (Table 1). Four-milliliter glass dispensers were used for all the test compounds including water and the ethanol-methanol blend.

In the second trial (Maquina Vieja site), four synthetic compounds (Sigma-Aldrich) were tested for their repellent properties (Table 1) in association with an "ethanol-methanol (50:50) blend".

Table 1. Compound and diffusion data.

Property	Order	Compound	Purity (%)	Diameter of the diffusion hole (mm)	Diffusion rate (g/day)
Attraction	1	methylcyclohexane	99	1	0.05
	2	ethylbenzene	> 99	1.5	0.02
	3	nonane	99	1.5	0.01
	4	1-octen-3-ol	98	2	0.001
	5	2-phenylethanol	99	1	0.001
	6	Trans-2-hexen-1-al	95	1	0.004
	7	benzaldehyde	99	1	0.003
	8	ethanol-methanol (50:50)	> 99	1.5	0.20
Repulsion	1	α-pinene	99	3	0.02
	2	camphene	80	3	0.02
	3	cis-3-hexenol	98	1	0.01
	4	hexanol	98	1	0.01

Experimental conditions

The experimental designs are presented in Table 2. The BROCAP® trap was used for CBB capture. The number of captured females was assessed by direct counting.

Table 2. Terms of experimental designs.

Compound	Number of treatments (control included)	Number of replicates	Distance between two traps (m)	Duration of trapping (days)	Treatment distribution
Only attractant	8	12	20	34	Total randomization
Attractant + EM	8	10			
Repellent + EM	5	10	30	7	

RESULTS

Attractiveness of selected volatile organic compounds

For the diffusion rates studied, none of the CBB capture averages was significantly different from that of the "water" absolute control. Therefore, no compound used alone was attractive (Table 3). Also, none of the CBB capture averages was significantly different from that of the "ethanol-methanol" relative control. No compound increased the attractiveness of the ethanol-methanol blend.

Table 3. Kruskal Wallis test for attractiveness of compounds alone or associated with the ethanol-methanol blend.

Compounds	Number of traps	Number of CBB/trap	Standard deviation	Differences
Nonane	12	0.00	0.00	A
Benzaldehyde	12	0.25	0.45	A B
Trans-2-hexen-1-al	12	0.42	0.90	A B C
Methylcyclohexane	12	0.42	0.67	A B C
1-octen-3-ol	12	0.50	0.90	A B C D
Ethylbenzene	12	1.42	3.42	A B C D E
2-phenylethanol	12	0.75	0.75	A B C D E
Water (control)	12	0.83	0.72	A B C D E
1-octen-3-ol + EM[1]	10	32.20	16.88	B C D E F
Trans-2-hexen-1-al + EM	10	47.10	48.56	C D E F
Nonane + EM	10	46.40	33.11	D E F
Methylcyclohexane + EM	10	79.30	78.40	E F
Benzaldehyde + EM	10	98.90	111.57	E F
2-phenylethanol + EM	10	102.00	79.20	F
Ethylbenzene + EM	10	102.60	75.24	F
EM (control)	10	172.10	125.70	F

[1]*EM = ethanol-methanol blend (50:50); DF = 15, P < 0.0001.*

Repellent effect of selected volatile organic compounds

The repellent effect of cis-3-hexenol and hexanol was significant compared to the control (Table 4). However, α-pinene and camphene did not significantly decrease the attractiveness of the ethanol-methanol blend of the control. Hexanol was significantly more repellent than camphene.

Table 4. Kruskal Wallis test for the repellent effect of compounds associated with the ethanol-methanol blend.

Compounds	Number of traps	Number of CBB/trap	Standard deviation	Differences
Hexanol + EM	9	1.84	1.47	A
Cis-3-hexenol + EM	10	4.37	7.70	A B
α-pinene + EM	10	27.1	43.9	A B C
Camphene + EM	10	20.1	17.6	B C
EM (control)	10	27.9	15.5	C

EM = ethanol-methanol blend (50:50); DF = 4, P < 0.001.

CONCLUSIONS

The olfactory responses of *H. hampei* to the release of certain volatile organic compounds present in the aroma of coffee berries proved to be different in the laboratory and in the field. The compounds selected and tested individually did not generate an attractant effect in the field.

- The most likely hypothesis is that the CBB would seem to perceive and recognize these compounds among many others released by plants into the environment, but only react to the complex of odors from coffee berries. However, in a confined location, the perception of a single compound could cause a reaction that would resemble insect attraction.
- Also, the low level of migratory flights may have influenced the accuracy of the capture results.

Cis-3-hexenol and hexanol, which are known to disrupt attacks by coniferous bark beetles, are repellents for *H. hampei*. Hexanol, which is the more common of the two, may be advantageously used in push-pull experiments.

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The Sanitation Harvesting Included in a Coffee Berry Borer Management Plan should Eliminate almost all Residual Fruits from Branches to Be Efficient

F. RIBEYRE[1], B. P. DUFOUR[1], F. FRANCO-FRANCO[2]

[1]Fabienne.ribeyre@cirad.fr Cirad, Montpellier, France
[2]Universidad de El Salvador, San Salvador, El Salvador

SUMMARY

In a coffee plantation with one annual harvest and a marked dry period, how sanitation harvesting should be done to reduce damages caused by the coffee berry borer (CBB) on the harvest?

For two consecutive years, in El Salvador, we measured quantities of attacked and healthy residual fruits in March, as well as attacked and healthy new fruits before the harvest in September, in control plots and in plots with sanitation harvesting. In year 1, the sanitation harvesting was done by a team of farm workers without supervision, in year 2, sanitation harvesting was made by a team supervised by a leader previously trained.

None sanitation harvesting had reduced significantly residual berries on ground. Unsupervised sanitation harvesting led to a 65% reduction of residual berries on branches. Supervised sanitation harvesting led to a 93% reduction of residual berries on branches.

As a result, unsupervised sanitation harvesting doesn't lead to a significant reduction of infested berries on new harvest. On the contrary, a supervised sanitation harvesting leads to a significant reduction of infested berries on new harvest (70 % infested berries less than control plots).

Residual berry from branches seems to influence new harvest attacks and can be eliminated by supervised sanitary harvest. So, the elimination of residual berries, from branches made through a rigorous sanitation harvesting, is a useful contribution to the control of the CBB.

Figure 1. coffee berry borer on a dry bean (photo: Laurence Ollivier).

INTRODUCTION

Effective disease and pest management is more and more based on overall crop management practices to maintain disease and pest pressure at a low level using integrated pest management. Integrated pest management includes cultural, biological (trap, parasites or predators) and chemical control of pests and diseases in combination with early warning systems. For Coffee Berry Borer (CBB) management, the recommended control measures vary according to the status of CBB at a particular site. CBB find an interseason safe haven in the fruits that remain on the coffee trees or fall to the ground. Sanitation harvesting, which consists of collecting all the fruits after harvest, has been recommended since the 1940s.

So we wonder if this practice, extremely difficult and time-consuming, is efficient in a coffee plantation with one annual harvest and a marked dry period.

MATERIALS AND METHODS

Trials were conducted in a coffee plantation with one annual harvest and a marked dry period, in El Salvador. The effect of supervised and unsupervised sanitation harvesting was assessed in two consecutive years, on March for the residual berries, then on September for the new berries.

In 12 control plots and 12 plots with sanitation harvesting, we measured the quantities of attacked and healthy residual fruits, on the ground and on the trees, in March. In each plot 16 coffee trees were sampled. In year 1, sanitation harvesting was carried out by a team of farm workers without supervision; in year 2, sanitation harvesting was carried out by a team supervised by a previously trained leader.

We measured the quantities of attacked and healthy new berries on 16 trees per plot in September before harvesting in 6 plots with sanitation harvesting and 6 control plots.

Results were analyzed using two way analysis of variance with plot and treatment (sanitation or control) as factors. Analyses were performed using XLSTAT.

RESULTS AND DISCUSSION

In the first year, there were as much residual berries in plots with unsupervised sanitation harvesting than in the controls (figure 2). On the ground, there are no significant differences in number of residual berries with or without sanitation harvesting. This result reflected the ineffectiveness of unsupervised sanitation harvesting at ground level. On the other hand, when the unsupervised sanitation applied to the berries on branches, the reduction of those berries was significant. Unsupervised sanitation harvesting leads to a 65% reduction in residual berries on branches.

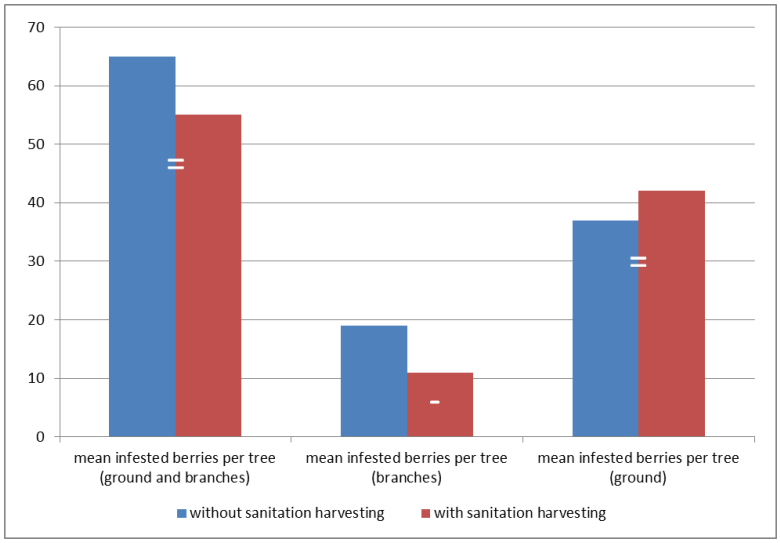

Figure 2. Effect of unsupervised sanitation harvesting on number of berries (= no significant difference; - or + significant difference).

In the second year, the supervised sanitation harvest didn't reduce significantly the number of infested berries on the ground but reduced the total number of berries (figure 3). When the supervised sanitation harvest concerned infested berries on branches, the reduction in the number of berries became significant. Supervised sanitation harvesting leads to a 93% reduction in residual berries on branches.

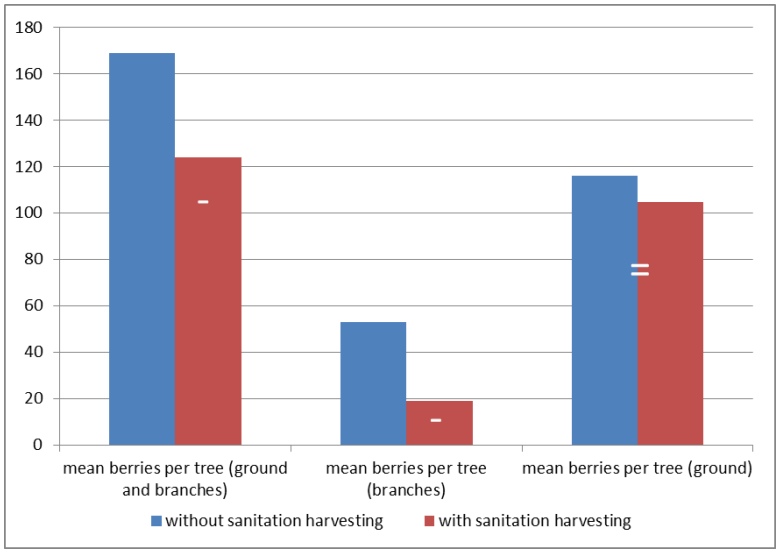

Figure 3. Effect of supervised sanitation harvesting on number of berries (= no significant difference; - or + significant difference).

In September the difference of infested new berries between control plot and unsupervised harvested plots was not significant (figure 4). It turned out that unsupervised sanitation harvesting did not lead to a significant reduction in infested berries in the next crop. On the other hand, supervised sanitation harvesting led to a significant reduction in infested berries in the next crop (70% fewer infested berries than the control plots). It seems that eliminating almost all residual fruits from branches is efficient However, the effect on next crop may depend on attack rate.

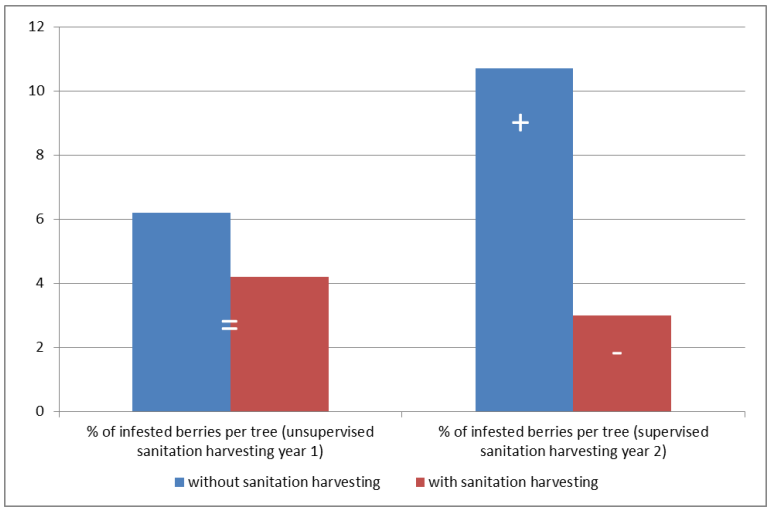

Figure 4. Effect of unsupervised and supervised sanitation harvesting on percentage of infested berries (= no significant difference; - or + significant difference).

None sanitation harvesting had reduced significantly residual berries on ground. We can suppose in the conditions of the plantation it is too difficult to be efficient. However, almost total elimination of residual berries from branches seems to reduce attacks on new crops. This can be achieved with supervised sanitation harvesting.

Only strict sanitation harvesting makes a useful contribution to CBB control. Further investigations should be done to investigate if sanitation harvesting of residuals berries on the ground may be suppressed in a coffee plantation with one annual harvest and a marked dry period.

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Characterization of the New Arabica Coffee IAC Ourama Cultivar

L. C. FAZUOLI[1], M. T. BRAGHINI[1], M. B. SILVAROLLA[1], W. GONÇALVES[1], J. C. MISTRO[1], O. GUERREIRO-FILHO[1], P. B. GALLO[2]

[1]Centro de Café "Alcides Carvalho", Instituto Agronômico de Campinas (IAC/APTA), SP, BR
[2]APTA, Polo Nordeste Paulista, Mococa, SP, BR

SUMMARY

The cultivar IAC Ourama was developed from the backcross between selected F3 trees of 'Catuaí Amarelo' IAC H2077-2-12-70 and S4 trees of 'Mundo Novo IAC 515-2'. The backcross (H5010) was made at the IAC, Campinas, SP, Brazil, in 1961. The aim was to create a dwarf Catuaí cultivar with more vigor and more yield, in combination with other favorable agronomic characteristics. The contribution of the Bourbon coffee variety in the genome of 'IAC Ourama' is around 62.5% and 37.5% is Typica. Line selection was carried out for high yielding plants with yellow berries for 6 generations (F6BC1), which has been named 'IAC Ourama'. This cultivar is productive or more productive than 'Catuai Amarelo IAC 62'. In areas without irrigation its average yield is 30 to 44 bags of coffee /ha/year and in irrigated areas 40 to 56 bags. For cv. Catuai Amarelo IAC 62 these yields varied from 23-32 and 30-46, respectively. The size of plant is short, the internodes are small and the secondary branching is abundant. The root system is well developed, giving appropriate physiological balance with the vigorous upper part of the plants. The young leaves are green, the adult leaves bright dark green and the berries are yellow. The average period, from fertilization to full ripeness of the berries of the IAC Ourama cultivar is approximately 225 days (7.5 months). The average sieve value is between 16 and 17 and the percentage of seeds of the flat type is of the order of 95%. The cup quality is similar to 'Catuaí Amarelo IAC 62'. It is adapted to be cultivated by large coffee growers and by smallholders. Its dwarfness allows it to be planted at up to 5,000 plants per hectare. It is adapted to manual or mechanical harvesting and reacts well to any type of pruning. It can be used successfully under irrigated cropping or fertilized-irrigated cropping systems. The IAC Ourama cultivar is susceptible to coffee leaf rust.

INTRODUCTION

Arabica coffee has considerable weight in the Brazilian economy. The use of dwarf cultivars has been responsible for the high production of arabica in the country, which in 2012 was around 38 million bags of coffee. The aim of this study was to make available information to growers on a new arabica coffee cultivar with high productivity, dwarfness, vigor, yellow fruits and with excellent cup quality.

MATERIALS AND METHODS

The cross leading to the Ourama cultivar was conducted in 1961, at the Instituto Agronômico de Campinas (IAC), Campinas, SP. The coffee breeding lines used were 'Catuaí Amarelo' IAC H 20077-12-70 (F3) and 'Mundo Novo IAC 515-20' (S4). This represents a backcross generation, because Catuaí is derived from a cross between 'Mundo Novo IAC 374-19' and

'Caturra Amarelo IAC 476'. The breeding method used was line selection. The breeding trials were established in various coffee growing regions of São Paulo and Minas Gerais, with control of rust and ferti-irrigation, except in Campinas and Mococa-SP.

The traits observed over six generations of selection were: kilograms of green coffee per tree, a visual vigor score (IAV vigor), a productivity score (IAV production), earliness of ripening, fruit size, outturn, percentage of flat beans, of peaberries, of elephant beans, 100 green coffee bean weight and bean size (sieve average).

IAV vigor was assessed by giving scores from 1 to 10, where 1 and 10 represent low and high vigor plants with few leaves (1) and many leaves (10) respectively. The index IAV production was obtained giving scores from 1 to 10, where 1 and 10 are unproductive and highly productive, respectively. The outturn is the percentage of green coffee in relation to the dry coffee berries.

RESULTS AND DISCUSSION

Genealogy of cv. IAC Ourama

The genealogy, years, places and generations of selection of the IAC Ourama cultivar are shown in Table 1.

Table 1. Genealogy of IAC Ourama cultivar.

Year	Locality	Generation	Germplasm codes	
1961	CAMPINAS-SP	F1	H 5010	
	(cv. Catuaí Amarelo IAC H2077-2-12-70 x cv. Mundo Novo IAC 515-20)			
1962	CAMPINAS-SP	F1	H 5010-5	
1964/70	CAMPINAS-SP	F1	H 5010-5	
1967	CAMPINAS-SP	F2	H 5010-5	
1969/85	CAMPINAS-SP	F2	H 5010-5	
1985/92	CAMPINAS-SP	F3	H 5010-5	
1987/94	CAMPINAS-SP	F3	H 5010-5	
1987/96	ALFENAS-MG	F3	H 5010-5	
1995/99	PATROCÍNIO-MG	F3	H 5010-5	
1997/03	CAMPINAS-SP	F4	H 5010-5	
2004/09	CAMPINAS-SP	F5	IAC 4397	
2005/10	CAMPINAS-SP	F5	IAC 4397	
2006/10	PATROCÍNIO-MG	F5	IAC 4397	
2006/10	CAMPINAS-SP	F6	IAC 4397	
2006/10	MOCOCA-SP	F6	IAC 4397	
2006/11	GÁLIA-SP	F6		IAC Ourama
2006/11	GARÇA-SP	F6		IAC Ourama
2008/11	CAMPINAS-SP	F6	IAC Ourama	

In segregating generations, trees were mainly selected for high yield, vigor and with yellow fruits. After selection in six generations, the name "IAC Ourama" was given to the selection (Table 1).

Final selection trials

The final selection of cv. IAC Ourama was done in representative areas recommended for commercial cultivation of *Coffea arabica*, mainly in the states of São Paulo and Minas Gerais (Table 2). The data on yield are therefore indicative of the potential average yield per hectare per year. Data on yield in irrigated and non-irrigated trials are presented in Table 2. These data show that in areas without irrigation the average yield of 'IAC Ourama' is 30.1 to 43.6 bags of coffee / ha / year in irrigated areas and 40.0 to 55, 8 bags of coffee / ha / year. The yield of cv. Catuaí Amarelo IAC 62 was lower, varying from 22.6 to 33.7 bags under non-irrigated conditions and from 30.0 to 45.6 in areas with irrigation. Therefore, the cv. IAC Ourama can be considered to have excellent yields under irrigated and non-irrigated conditions.

Table 2. Average yield of 'IAC Ouro Amarelo' and of 'Catuaí Amarelo IAC 62' (control) in bags of green coffee per hectare per year in irrigated and non-irrigated trials in various coffee regions in the states of São Paulo and Minas Gerais.

Irrigated coffee			
Local	**Harvesting Years**	**Average yield 'IAC Ourama'**	**Average yield 'Catuaí Amarelo IAC 62' (control)**
Gália -SP	2	55.8	45.6
Patrocínio - MG	2	40.0	30.0

Coffee without irrigation			
Local	**Harvesting Years**	**Average yield 'IAC Ourama'**	**Average yield 'Catuaí Amarelo IAC 62' (control)**
Campinas - SP	2	41.2	30.3
Patrocínio - MG	2	36.6	22.6
Garça - SP	2	30.1	33.7
Mococa -SP	2	43.6	31.5

Major morphological, biological and/or physiological traits to identify "IAC Ourama"

The IAC Ourama cultivar presents dwarf plants, with short internodes and abundant secondary branching. In an experiment in irrigated Gália-SP, plant height reached 170 cm and 150 cm canopy diameter within three years, while 'Catuaí Amarelo IAC 62', used as a control, reached the values of 170 and 160 cm, respectively. New leaves are green in color and the adult, dark green shiny. The root system is well developed. Usually the two major blooms occur in September and October and the ripening of fruits, which are yellow, in May and June. The average time of fertilization until complete maturation of the berries in Campinas is about 225 days. The average value of the sieve is about 16.5 and the percentage normal flat beans is approximately 95%.

Reaction to major pests and diseases

"IAC Ourama" is susceptible to rust (*Hemileia vastatrix*), to cercosporiose (*Cercospora coffeicola*), to phoma (*Phoma tarda*), to coffee berry borer (*Hypothenemus hampei*) and coffee leaf miner (*Leucoptera coffeella*).

Adaptation to coffee growing regions

"IAC Ourama" is recommended to be planted in all regions where arabica coffee is grown, especially in the states of São Paulo and Minas Gerais.

Beverage quality

The cup quality is excellent, as good as 'Catuaí Amarelo IAC 62'. This can be explained because of the cultivar Bourbon Vermelho participated with around 62.5% in the genealogy of "IAC Ourama".

Seed availability

Currently, genetic seeds are being produced by IAC.

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Relationship Between Coffee and Environmental Conservation in the Serra da Mantiqueira, Minas Gerais, Brazil

L. ZANELLA[1], R. A. T. BORÉM[1], C. G. SOUZA[1], F. M. BORÉM[1], H. M. R. ALVES[2]

[1]Universidade Federal de Lavras- UFLA, CxP 3037, 37200-000, Lavras, MG, Brasil
[2]Empresa Brasileira de Pesquisa Agropecuária- EMPRABA, CxP 40315, 70770-901, Brasília, DF, Brasil

SUMMARY

This work was addressed to analyze the relationship between coffee and environmental preservation in the Serra da Mantiqueira, Minas Gerais, Brazil, using geotechnologies. The physiographic structure of the study area was characterized aiming at analyzing and crossing biotic and abiotic variables that act on the local landscape configuration and to access how coffee plantation are distribute in this landscape. The results showed the study area is rich in natural resources, with high drainage density, high variation in height and geomorphological features quite different. The human impacts changed the local landscape structure over time, and limited the ecosystems ability to perform their ecological functions. Coffee plantation contributed to modify the landscape structure and affect natural resources in the study area. However, pasture was the human activity of greater negative environmental impact, due to the inadequate management of some areas. The change of land-uses for agroforestry systems, based on sustainable development, can represent an alternative to the compatibility of agricultural production and conservation of local natural resources.

INTRODUCTION

The Brazilian Atlantic Forest is considered one of the richest ecosystems on the planet. This forest has been converted into man-dominated areas, largely for agricultural production. The consequences of this conversion are the habitat loss and fragmentation, which endanger biodiversity maintenance.

In order to seek the proper use of natural resources, balancing the conservation of ecological systems and the economic interests of modern society, environmental planning is an important tool to guide decision makers. To this end, information on the physical characteristics and the land use dynamics of are important information to be used in the characterization of a landscape.

The southern region of Minas Gerais is considered as the largest coffee producing region of Brazil. The microregion of the Serra da Mantiqueira is a major coffee producing regions of the state and the country, and is characterized by mountainous terrain, and internationally known for the production of specialty coffees.

The city of Carmo de Minas has physical characteristics that represent the microregion of the Serra da Mantiqueira and therefore was used as a pilot area for this work.

In this context, this paper analysed the relationship between coffee plantations and environmental conservation in the Mountain range, Minas Gerais, Brazil.

MATERIALS AND METHODS

Study site

The study area comprises the municipality of Carmo de Minas, located at 22 ° 07'21 "S and 45 º 07'45" W, in the microregion of the Serra da Mantiqueira, South state of Minas Gerais. This municipality has 32,332 ha, minimum altitude and maximum altitude of 856 and 1645 meters, respectively. The mean annual temperature is 19.1 º C and average annual precipitation is 1,568 mm. The climate is Cwb, subtropical high, according to the Köppen system. The municipality is within the Atlantic Forest biome according to the Brazilian classification.

Image processing and data analysis

Hydrography and roads were extracted from IBGE planialtimétic charts. We obtained the total drainage density (DDT = Σh / A) and total roads density (DET = Σe / A), based on the hydrography and roads quantification, using the total length of watercourses/roads and the total area.

We conducted a field campaign for recognition of the study area, and the survey of secondary information. We used biophysical maps and IBGE 1:50,000 scale planialtimetric charts (SF23VDVI4 - Conceição do Rio Verde; SF23YBIII1 - Cristina; SF23YBIII2 – São Lourenço). This information was adjusted and inserted in a geographic database in the softwares SPRING 5.1.5 and ArcGIS 9.3.1 ®.

We used maps from NASA SRTM (90m resolution, quadrants: sf-23-vd and SF-23-yb on 1:250,000 scale). The SRTM data were interpolated to 30 m. Later we generate the Digital Elevation Model - DEM to obtain the maps of elevation and slope. The altitude was divided into nine classes in units of 100 meters between 856 and 1645 m. The first and last class contained values below 900 m above 1600 m (<900m, 900-1000m, 1000-1100m, 1100-1200m 1200-1300m, 1300-1400m, 1400-1500m, 1500-1600m,> 1600m). The relief classes were divided in five classes: plane (0-3% slope), softly wavy (3-8%), moderately wavy (8-13%), wavy (13-20%), strongly wavy (20-45%); mountain (45-100%), steep (> 100%).

We use a HCR SPOT5 image, with 2.5 m spatial resolution from 2008 to conduct a visual interpretation using SPRING 5.1.5. We mapped eight land use classes: (i) natural vegetation: old growth secondary forest remnants, (ii) coffee: coffee plantations, (iii) annual agriculture: corn and pasture crops, (iv) pasture: livestock areas, (v) secondary forest: early growth secondary forest patches on abandoned cropland and pasture, (vi) watercourse: ponds and rivers, (vii) silviculture: eucalyptus plantations; and (viii) other uses: urban areas and agricultural buildings. Field survey was carried out in order to guarantee high map accuracy with Kappa index of 0.92.

All these biophysical information were used to a final analysis to explore the relationships between coffee plantations and environmental conservation in the study area.

RESULTS AND DISCUSSION

The municipality of Carmo de Minas belongs to the Rio Verde basin, which belongs to the of the Rio Grande basin. Carmo de Minas is rich in springs and streams possibly due to its mountainous terrain. Hydrography was estimated at 740.66 km, including 10 m average width water courses and a part of the Rio Verde, which has an average width greater than 20 m.

Drainage density was estimated to 22.97 m/ha, and was considered high according to the DENAEE classification. So, we can infer that the volume of water drained is compatible to the extent of water courses, reducing vulnerability to flooding. Besides this, a high drainage density is closely related to the presence of riparian corridors, which act as structural connectors in the landscapes. The riparian corridors in agricultural matrices may facilitate biological fluxes and prevent the extinction of species in fragmented habitats. According to the Brazilian Environmental legislation (Brazil, 1965) riparian corridors are known as Permanent Protected Areas - PPAs. The PPAs vary in length according to the average width of the water bodies and should be preserved.

We also accounted the roads, which totalized 397.21 km. The density of roads was estimated at 12.28 m / ha, which is considered high. A high density of roads can be considered positive in terms of greater accessibility to rural human communities. But, it is considered negative in terms of biodiversity conservation, because it increases the probability of being run over wildlife, promotes access to natural areas (facilitating the plants and animals collection) and the entrance of impurities and pollutants, and erosion processes along the roads.

The study area had a high altitudinal range (789 m). The highest percentage of the study area (80%), found in altitudes lower than 1,100 m. Class 900 to 1000 m occupies almost 50% of the municipal territory. The altitude along with hydrography determines the existence of most of the remaining natural vegetation in the area of study. The difficulties to implement agricultural activities at high altitudes represent the most important factor for the maintenance of forest areas.

We also calculate the percentage area of natural vegetation in each altitude range and compared with the area of Carmo de Minas in each altitude range. There was an increase in the area at higher altitudes when comparing the values of natural vegetation to the values of the municipal area.

the amount of natural vegetation has doubled in almost all ranges of altitude above 1,100 m, compared to the total area of the municipality. The vegetation amount at altitudes below 1,100 m was kept because these altitudinal ranges occupy larger areas.

This information highlights that some vegetation types found at lower altitudes were lost, leaving only those adapted to higher altitudes. This was due to the development model the region where the study area is located. In this model, agricultural activities are widely associated with the slope, and altitude. There are large areas with softly wavy slope, wavy and moderately wavy (80%) that allow the development of agricultural activities, such as pastures and annual crops. However, areas with steep slopes more related to coffee require proper management to prevent erosion, since this activity is well developed and relevant to the region's economy.

The terrain is very irregular, varying from plane to mountainous. Most of the area (62.13%) can be used for agricultural activities, using practices for controlling erosion. The other areas (37.87%) show strong or severe susceptibility to erosion. These areas are not suitable for farming practices, because erosion control may be costly or even uneconomic. We observed in the field, that most coffee crops were associated with high slope, but they were well structured from a soil conservation perspective.

We evaluated the compatibility of land use, especially coffee plantations, with the conservation of Atlantic Forest in the study area and verified the natural vegetation remnants

are dispersed in a matrix of agricultural activities, consisting mainly of pasture. Pasture occupied about 45% of the total area. We observed that most pastures were poorly managed and highly compacted, with bare ground and no protection against the erosive action of rain and winds. Natural vegetation is extremely fragmented. There remain few remaining and most of them are very small.

Coffee plantations occupied about 15% of the landscape. In comparison with the areas occupied by pasture, the negative environmental impact of coffee production, especially in soil conservation, can be considered moderate to low. Furthermore, according to the cultivation of organic coffee positively affects the biomass, population density and species diversity of soil organisms (earthworms, in this case), contributing to soil conservation.

We found a geographical proximity between the natural vegetation and coffee plantations, due to the relationship of these two classes with altitude.

There is strong evidence that altitude and latitude directly influence the quality of coffee beverage. The study by legitimized fluctuations in scores of cafes, which varied with height as a function of latitude. The results showed that the higher the altitude, the greater the sensory quality of the coffee beverage.

Pasture is the human activity of greater negative environmental impact, due to inadequate management. Coffee plantations are well structured, but could be optimized from the point of view of conservation, changing from traditionally productive character to agroforestry systems, based on sustainable development. This change is an alternative to the compatibility of agricultural production and biodiversity conservation and local water resources.

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Integrated Communication and Information Flow in the Integration between University-Industry-Government (Triple Helix)

C. M. G. AGUIAR[1], S. P. PEREIRA[2], J. Y. SUGANO[1], N. CARVALHO[3]

[1]Federal University of Lavras – UFLA
[2]Agronomic Institute of Campinas – IAC
[3]Federal University of Juiz de Fora – UFJF

SUMMARY

This is a period of growing demands for innovations that can bring solutions for bottlenecks, as well as competitive advantages for different productive chain links. This work proposes a new view on the role of communication and coordination models of the actors in an interorganizational innovation network. Used as an object of analysis the Polo Excellence Coffee (PEC), as representative of the relationship between university, business and government (UBG), highlighted by the Triple Helix Theory. For the efficiency of a sectorial innovation system, in this study confirms the importance of an articulating central agent, to support communication and coordination the space for conversation between to all stakeholders. As a result, it is suggested the creation of an information architecture that promotes parity between the information needs of the enterprises and the knowledge generated by research centers and universities, and the contribution of the government incentive to their interrelations. Thus, the theoretical contribution of labor, which is supported by the empirical results of the study, is located in defense of the joint function of the Triple Helix increases in capability if it is continuously incorporated into the vision of integrated communications and hybrid organizations, because these concepts just keep contributions to thinking at systematically and the opening of channels between UBG, aiming at the success of the coffee activity.

INTRODUCTION

Being more present and even more necessary in the Knowledge Society, innovation occurs, mostly, as a result of the interaction University-Business-Government (UBG), which is an action that coordinates the core of the Triple Helix Theory. This relationship has motivated several means of investigation under different facet and angles. However, it can be noticed that there are many topics yet to be unveiled so that this triple relation can show an optimized performance. It was aimed to understand the role that communication plays in the process of articulation among UBG, specifically, by a study of case in Polo de Excelência do Café, which shall be hereby named PEC. Specifically, the complexity of the context of innovation on the coffee sector will be the main issue, being emphasized in relation to the triple helix in places for idea sharing, such as a Ba[2], described by Nonaka and Toyoma (2008) or conversation spaces, suggested by Martinho (2003). In this collaboration environment, communication is used to bond the actors, being a fundamental element for the existence of an organic interaction net. Thus, this work aimed to evaluate the interviewees' perception as for the means of communication used for information sharing among different actors.

METHODOLOGY

In order to comply with the proposed objectives, the qualitative and explorative research was adopted. As for the method, the case study was chosen, which, according to Lazzarini (1997), is particularly useful for this kind of approach. The case study implies in a higher level of details of the relationships between individuals and organizations, as well as the exchange with the environment where they are inserted. The data collection was made by means of in-depth interviews, in order to clarify and make good use of the interviewee's opinion on the topic. Qualitative Content analysis (BARDIN, 2004) was used to analyze the interviews. Seven research projects aimed at innovations and developed with PEC's aid were selected for the case study. The choice of coordinators was made according to the representation of the analyzed groups (UBG), in different stages on the innovative cycle, in different ways of cooperation and viewpoints about the role of communication in this process. The interviews were made from November to December 2010. After the data collection, the answers were analyzed comparatively, being attached to the observations and documental collections. The analysis of the given projects and of the business models used has also been used to base the results described as follows.

RESULTS AND DISCUSSION

By means of this case study, it could be identified, within the PEC scope and the context of coffee sector, the tendency to a wider and more cooperative innovation process. However, this relation is full of challenges that involve the essence of interinstitutional teamwork and, mainly, interaction between distinct segments, as in the relationship between academy and market. The actions of the researchers involved in collaborative project in PEC reinforce Chesbrough's idea (2006) that the open model broadens the innovation potential of a given business model for creation and collection of values.

It can be observed that for PEC there are synergies with authors' quotes, such as Kline and Rosenberg (1986), Furtado (2003), Berkhout et al. (2006) that defend the Innovation Cyclic Model (MCI), in which science is not in the beginning of a chain where Market is one of the extremes. According to these quotes, it is necessary ability to handle the proper tools for communicative actions, demanded by the innovation context.

As Etzkowitz and Leydesdorff (2000) highlighted on the Triple Helix Theory, the interviewees also visualized the PEC's action with emphasis on the sum of actions in and between institutional spheres, namely the interaction link among the three helixes, as suggested by Mello (2004), when he highlights the importance of an articulator agent in the middle of the relationship. In these new spaces, knowledge is changed into economic development, resulting from the gathering of different areas, organizations and innovative perspectives.

As for PEC specifically, it occurs what Leydesdorff (2003) names positive entropy, or disorder, i.e., a tendency to relationship disorder or balance. According to this author, the mutual information among these three institutional dimensions can provide a performance indicator, having as its aim, the means of communications between the actors. That is due to the fact that, although PEC is being stated as a connective interface between the segments and the actors, the perception, in general, is still the absence of open and systematic channels between UBG. This unbalance can be noticed within the agroindustrial coffee system, which influences the way how agents from different segments relate.

The majority considers that the communication between university and government is easier, since there is a traditional approach between public and research learning institutions and funding organizations and reference secretariats. However, they argue that this perception on communication should not be generalized.

In the interviewed entrepreneurs' perception, the communication between university and business is still full of barriers. The analysis of this perception points out a dynamic that can be seen in Figure 1, which, according to Leydesdorff (2003), could settle a condition of positive entropy, i.e., through the unbalanced relationships, the system would tend to chaos.

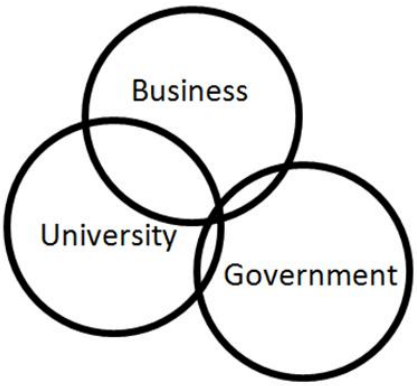

Figure 1. Dynamic Communication between triple helix in view of the interviewed

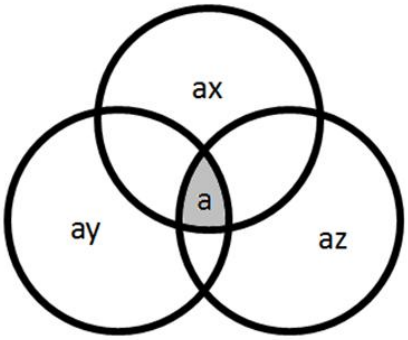

Figure 2. Setup with three dynamic

In Figure 1, it is possible to observe the communication channels more systematically between Academy and Government, mostly, through formal channels of accountability about projects financed with public funds. It can yet be observed that the alignment to Hofer's (2006) results, that universities show a passive attitude regarding technological diffusion. This representation differs from the configuration suggested by Leydesdorff (2003), Figure 2, where three helixes are put upon, creating a common space in the superimposed area, indicated as "a" in the Figure. This representation is known as Venn's Diagram, which points out the superimposition among subsystems. However, in normal conditions, this superimposition may be null or even negative, as described in Figure 3. In such case, the integration of different communication interfaces could result in a hypercycle, symbolically represented. That means that communication could represent the link between sectors.

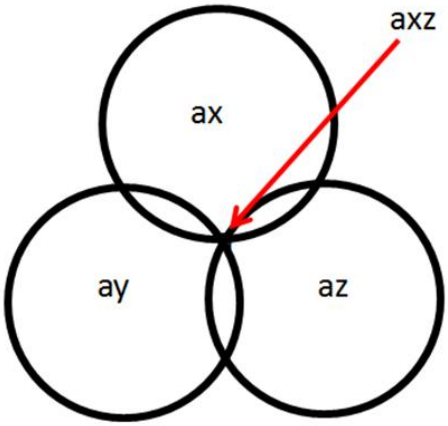

Figure 3. Setup with three dynamic overlays with bilateral

Figure 4. Design of interfaces associated with the presence of PEC

The analysis of this case also allows us to infer that the construction of conversation areas among these different spheres promotes a virtuous circle of information that strengthens the innovation net, intended by PEC. It represents a search for a new configuration, in which the organizational design, represented by three spheres, becomes as it is displayed on Figure 4. Therefore, achieving the main objective of this study, it is identified that the information flows represent an important interface in the relationship among university, business and government. In the current innovation system pattern of the coffee sector, the need for a neutral element that is responsible for this articulation is observed. It would be as if we imagined a fourth sphere resulting from the intersection of the others. This affirmation is based upon the fact that the innovation process, especially through complex articulations necessary for its development, shows an optimized performance when there is a central articulator, which represents neither the university, nor the business, but exactly the interaction between these areas.

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Water Excess in Coffee Seedlings (*Coffea Arabica* L.): Effects in the Growth

J. D. ALVES, H. R. O. SILVEIRA, K. R. D. DE SOUZA, M. O. SANTOS, A. C. ANDRADE, R. G. M. ALVES

Universidade Federal de Lavras, Lavras, MG, Brasil

SUMMARY

The objective of this study was to evaluate the influence of 19 weeks of soil-waterlogging on the growth and biomass accumulation in plants of Mundo Novo and Catuai Vermelho cultivars. Plants, with eight pairs of leaves, were submitted to three different conditions of water availability in the substrate: field capacit (FC), continuous waterlogging of the substrate (CW), intermittent waterlogging of the substrate (IW). The combined analysis revealed that, even the seedlings of Mundo Novo had shown to be more tolerant to the excess of water in the soil than those of Catuaí, when analyzing the non-destructive growth variables of aerial part, both varieties, after 19 weeks of stress, could not increase the dry matter in the same proportion of those that were in CC. This sensibility was due to the death of radicels with two months of stress. Anyway, coffee seedlings had a good capacity of tolerating the soil waterlogging, since both varieties recovered after a period with the soil in field capacity.

INTRODUCTION

In the water relations of coffee plant, there are no works that specifically discuss the effect of excess of water in the development of the culture. Most of them study the use of irrigation in order to solve problems related to water deficit. It is observed in these works a quadratic effect between production and applied water depth, especially in high harvest years. In low years, the irrigation negatively influenced the production.

Even though the authors do not discuss the causes of decreasing the yield of coffee plant, other articles explain the reason why most of cultures do not tolerate excess of water in the soil. In general, the waterlogging causes anoxia or hypoxia of the soil where there is no deviation of the metabolism for fermentative pathway, producing a minimum amount of energy and toxic products resulting from anaerobic respiration.

In general, nurserymen irrigate the beds up to three times a day without worrying about the amount of water applied. As there is a high concentration of roots in a small substrate volume, many of them choose to add too much water due to the quantitative principle – the more the better. In this context, this work aimed to evaluate the effects of excess of water in the soil about the growth of coffee seedlings of Mundo Novo IAC and Catuaí Vermelho cultivars.

MATERIALS AND METHODS

Seedlings of Mundo Novo IAC 379-19 and Catuaí Vermelho IAC 44 cultivars, were cultivated in perforated polyethylene bags, black color, dimensions of 15 x 25 cm and volume of 4,4 L. Once reaching eight pairs of leaves, they were submitted to three conditions of water in the substrate: seedlings with moisture of the substrate close to the field capacity (FC); continuous waterlogging of the substrate (CW), in which the seedlings were stored in buckets

with a permanent water depth covering two thirds of the height of the polyethylene bag and intermittent waterlogging of the substrate (IW) where the the seedlings remained alternately, three days under continuous waterlogging and four days under field capacity throughout the entire experimetal period.

The radicular system, the height of the plants, the diameter of the stem, leaf area, total number of leaves, number of plagiotropic shoots and dry matter of root, stem and leaf were weekly analyzed. The experimental design was in randomized blocks, three water conditions and two cultivars. The experimental plot was of eight plants.

RESULTS AND DISCUSSION

The coffee seedlings had their growth affected by substrate-waterlogging. In general, all the characteristics had losses proportional to the intensity of stress, that is, seedlings under CW were more affected than those under IW. Of the five growth characteristics of aerial part studied in Mundo Novo cultivar under IW, four of them (height of the plants – Figure 1, number of plagiotropic shoots – Figure 2, number of leaves – Figure 3 and diameter of the stem – Figure 4) had a positive linear growth standard. In Catuai this number was of three (height of the plants – Figure 1, number of plagiotropic shoots – Figure 2 and diameter of the stem – Figure 4). When the seedlings of Mundo Novo and Catuaí were submitted to CW, the only characteristic with linear standard was the diameter of the stem – Figure 4. For Catuaí, the only feature that showed a negative linear standard was the number of leaves – Figure 3 that decreased with the time of waterlogging.

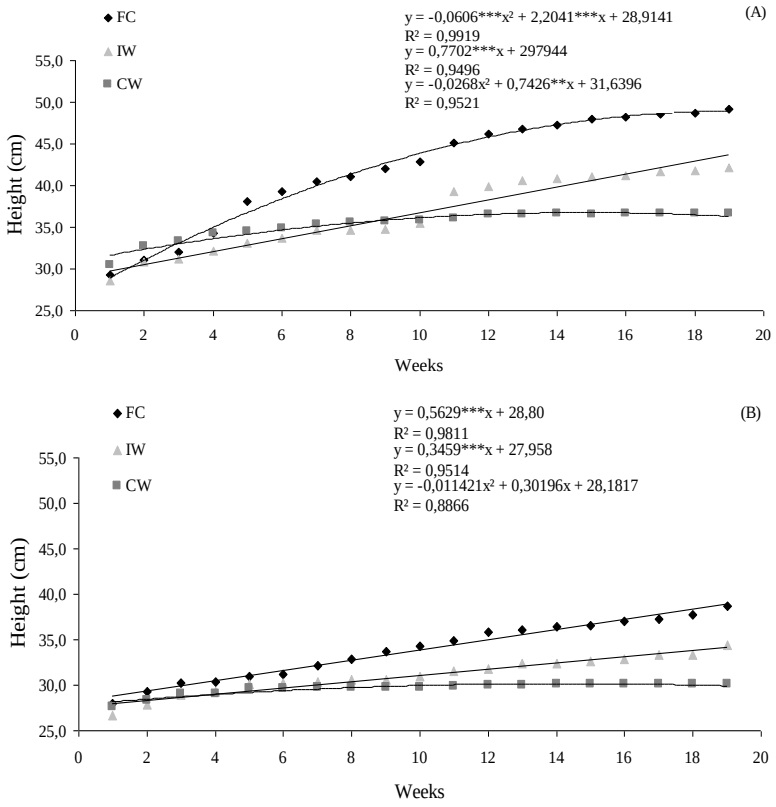

Figure 1. Height of coffee seedlings Mundo Novo (A) and Catuaí (B) submitted for 19 weeks to three conditions of water availability in the substrate: field capacity (FC), intermittent waterlogging (IW) and continuous waterlogging (CW).

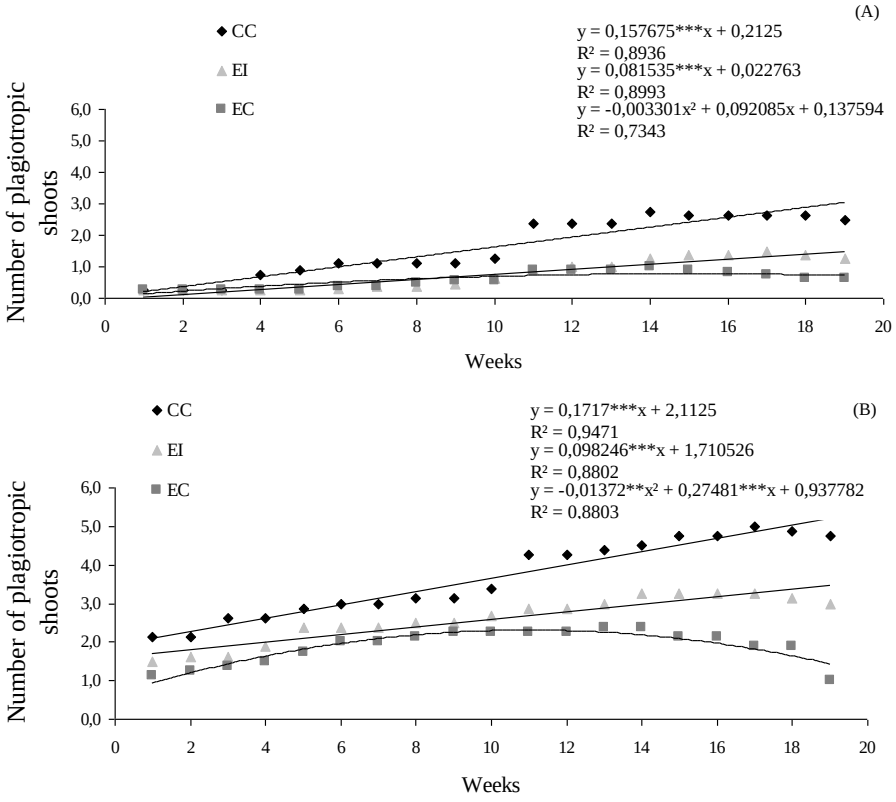

Figure 2. Number of plagiotropic shoots of coffee seedlings Mundo Novo (A) and Catuaí (B) submitted for 19 weeks to three water availability in the substrate. Legend in Figure 1.

The results show that although in lower rates than those of control, the seedlings could grow fairly well during a period of 19 weeks under IW. This alternation between periods of hypoxia and normoxia relieves the pressure of stress of O_2, making the anaerobic respiration return, reestablishing the energy level necessary for the plants growth. With the pressure of stress (CW), the cultivars had, depending on the characteristic, growth inhibition, aggravated by premature leaf fall. Possibly, the lowest leaf area undermined the photosynthesis and the recomposition of energy, even at the level of seedlings under IW.

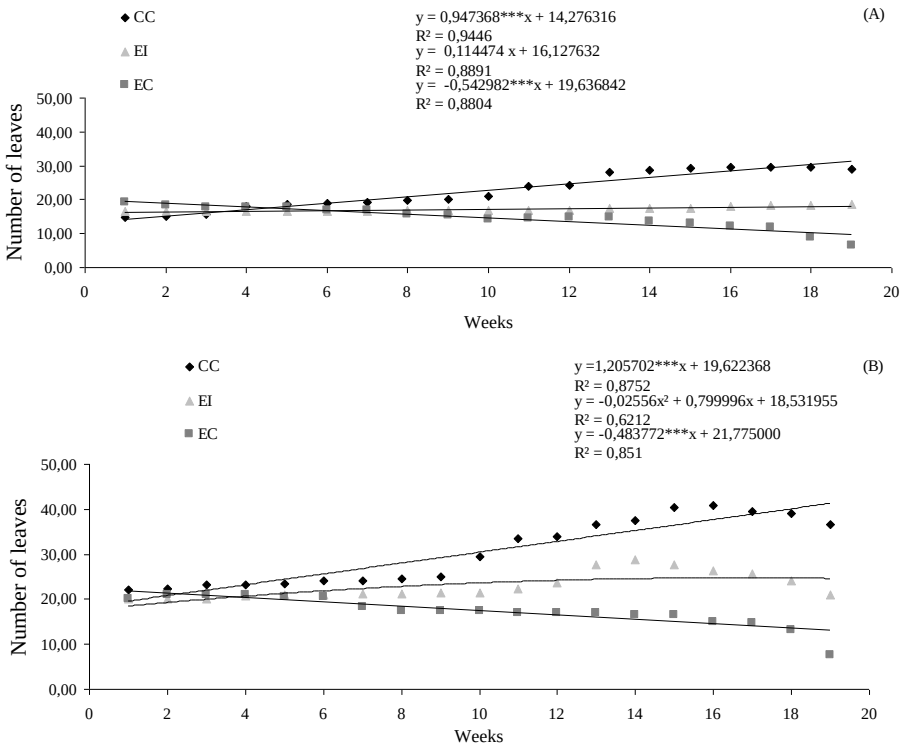

Figure 3. Number of leaves of coffee seedlings Mundo Novo (A) and Catuaí (B) submitted for 19 weeks to three water availability in the substrate. Legend in Figure 1

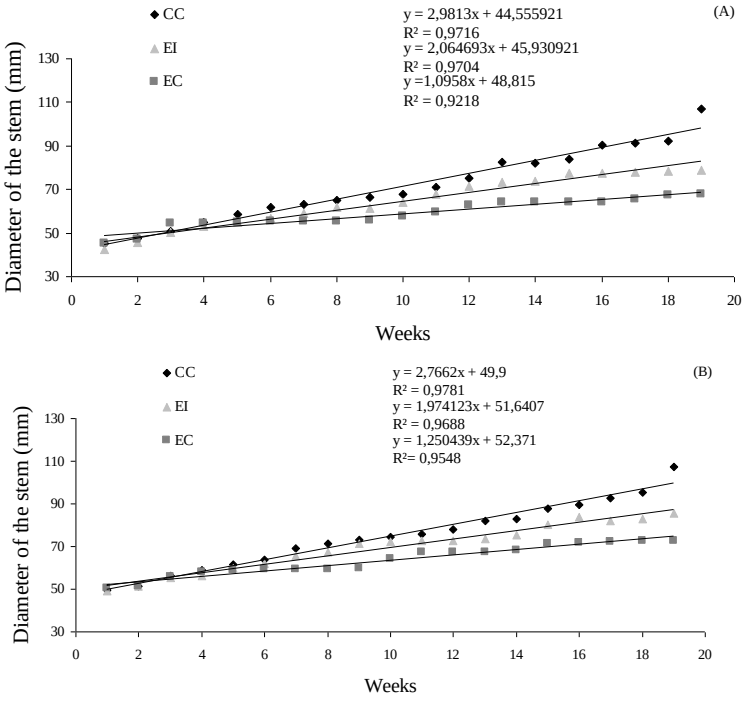

Figure 4. Diameter of the stem of coffee seedlings Mundo Novo (A) and Catuaí (B) submitted for 19 weeks to three water availability in the substrate. Legend in Figure 1

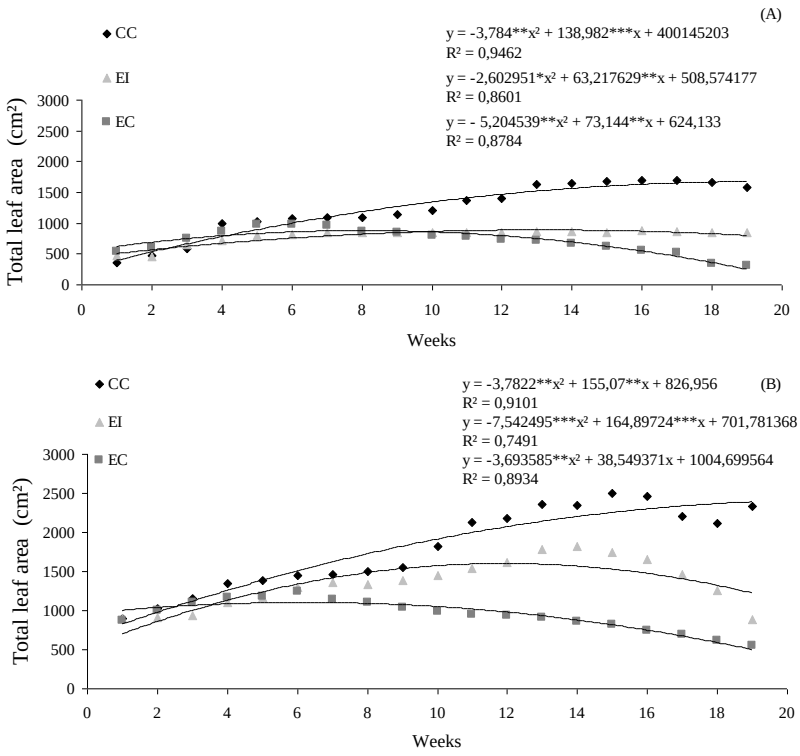

Figure 5. Total leaf area of coffee seedlings Mundo Novo (A) and Catuaí (B) submitted for 19 weeks to three water availability in the substrate. Legend in Figure 1

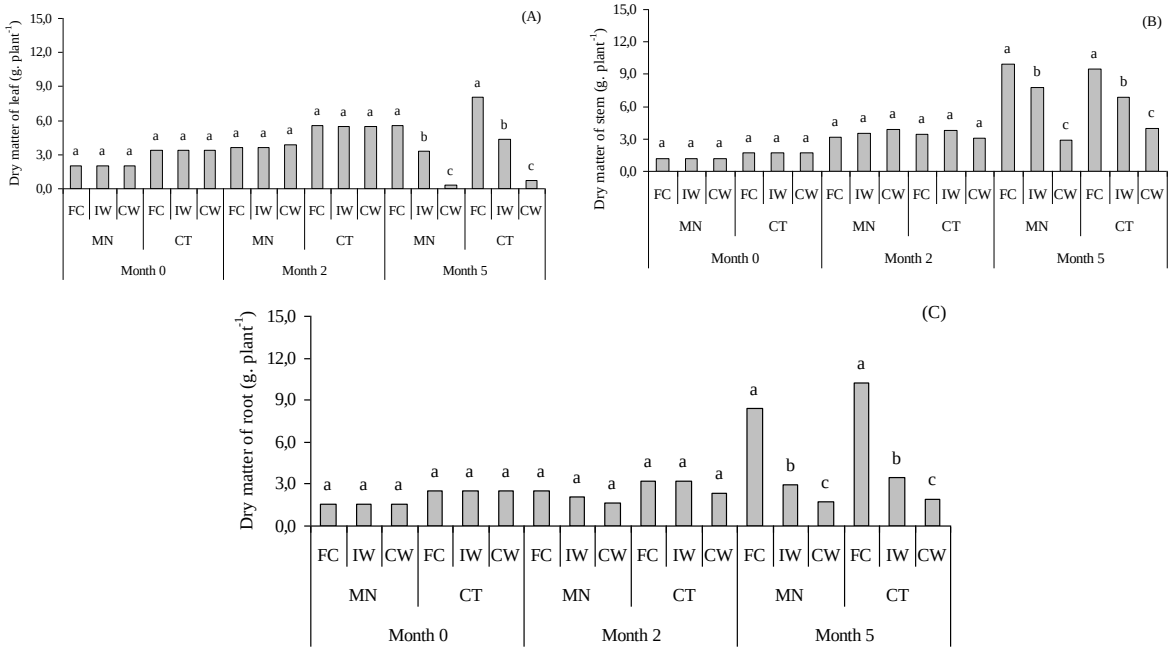

Figure 6. Leaf dry matter of leaf (A), stem (B) and root (C) of two coffee cultivars Mundo Novo (MN) and Catuaí (CT) submitted to three conditions of water availability in the substrate. Legend in Figure1. Letters compare the means between the water systems in each period for each cultivar (Scott-Knott (p≤ 0,05))

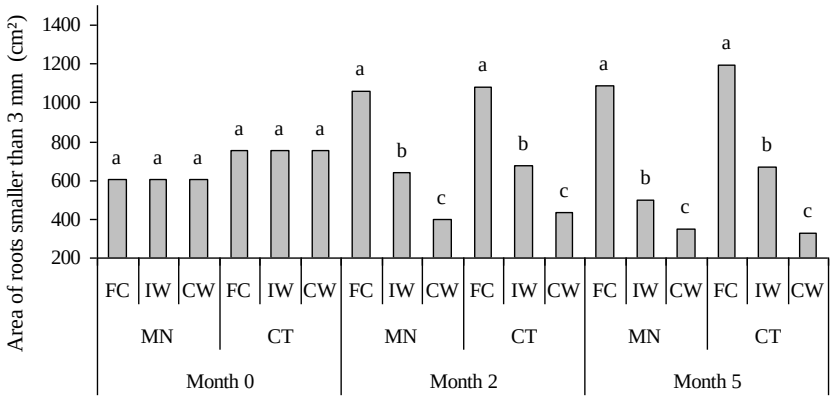

Figure 7. Area of roots smaller than 3 mm of two coffee cultivars Mundo Novo (MN) and Catuaí (CT) submitted to three conditions of water availability in the substrate. Legend in Figure 1. Letters compare the means between the water systems in each period for each cultivar (Scott-Knott (p≤ 0,05))

Then based on the points of maximum presented for these characteristics, seedlings of Mundo Novo and Catuaí tolerate IW for 12 and 11 weeks, respectively – Figure 5. Under CW, the tolerance period falls to seven weeks in the seedlings of Mundo Novo and to five weeks for those of Catuaí. However, the analysis of dry matter showed that up to the end of the second month of stress, the seedlings had high tolerance to waterlogging, once there were no significant differences with the control – Figure 6. Nevertheless, when the variation of dry matter of control seedlings are compared with those that were under stress after five months, it is realized the roots followed by the leaves were more affected. This sensibility of these organs to waterlogging was a result of decreasing the area of absorbent roots that occurred three months before, that is, with two months of waterlogging – Figure 7.

As related, even the seedlings of Mundo Novo had been more tolerant to the excess of water in the soil than those of Catuaí, when the non-destructive growth variables of the aerial part are analyzed, both varieties, at the end of 19 weeks of stress, can not increase the dry matter in the same proportion of those that were in FC. This sensitivity is due to the death of radicels with two months of stress. Anyway, the coffee seedlings showed a good capacity of tolerating the soil-waterlogging, since both varieties recovered after a period with the soil in field capacity.

ACKNOWLEDGEMENTS

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Control of Coffee Berry Borer (*Hypothenemus hampei*) and increase of coffee yields using Surround WP (kaolin)

S. STEIMAN[1], E. BURBANO-GRECO[2]

[1]Coffea Consulting, Honolulu, HI, USA
[2]University of Hawai'i, Honolulu, HI, USA

INTRODUCTION

Surround WP, a finely ground, white, kaolin clay product, is an effective mechanism for controlling a variety of insect pests on crops (Glenn and Puterka, 2005). This on-farm trial tests its effectiveness against the Coffee Berry Borer (CBB) (*Hypothenemus hampei*) also known as "la broca". On one farm, Surround WP is also compared to the use of Mycotrol, a solution containing the common CBB control fungus, *Beauveria bassiana*.

Surround WP has also been shown to increase coffee yields in the second year of production following two years of spray treatments (Steiman et al., 2011). This experiment also attempts to repeat that yield-increasing effect.

This poster reports on the first year of data from a 2-year experiment.

MATERIALS AND METHODS

- 4 farms in Kona, Hawai'i; 6-tree experimental units; 3 replications or blocks per farm.
- Farm elevations range from 260 m – 500 m above sea level.
- Rainy season occurs during fruit maturation (April to September), coinciding with the spray cycle.
- Treatments:
  - Control.
  - Surround WP (5% water solution with .3% NuFilm sticker).
  - Mycotrol (*Beauveria bassiana* solution; only on one farm; .06% water solution with .03% spreader).
  - Surround WP + Mycotrol (only on one farm).
- Spray trees every 2 weeks, beginning 6 weeks after flowering until the end of harvest.

Measurements

- CBB control: Percentage of cherries with CBB holes on 4 randomly selected branches, counted about every month.
- Yield: Harvest and weigh all ripe coffee about once per month and add values at end of the season.

RESULTS AND DISCUSSION

- With diligent application of Surround WP (farms 2 and 4), CBB infestation was reduced 59-79% (Table 1).
- Non-diligent spraying (farms 1 and 3) show no CBB control.
- Surround WP in combination with Mycotrol demonstrates the greatest level of CBB control.
- In the first year of application, with diligent spraying, Surround WP increased yields or trended towards increased yields (Table 2).

Table 1. Percent of cherries infested by CBB.

Farm	Treatment	Infested berries (%)[1]
1	Control	21.4 A
	Kaolin	31.2 A
2	Control	33.8 A
	Kaolin	13.7 B
3	Control	18.9 A
	Kaolin	15.4 A
4	Control	4.7 A
	Mycotrol	3.0 AB
	Kaolin	1.0 B
	Kaolin + Mycotrol	0.6 B

[1] *Different letters within a farm are significantly different at $p < 0.05$.*

Table 2. Total yield for season 1.

Farm	Treatment	Average yield (kg)[1]
1	Control	5.4A
	Kaolin	4.3A
2	Control	30.7A
	Kaolin	46.1A
3	Data not available, trees were accidentally harvested.	
4	Kaolin	22.2A
	Kaolin + Mycotrol	18.6AB
	Control	12.4AB
	Mycotrol	9.2B

[1] *Different letters within a farm are significantly different at $p < 0.05$.*

- Surround WP may be an effective CBB control tool.
- Surround WP seems to increase coffee yields after application.
- Sufficient coverage is necessary to ensure effectiveness.
- Cost of materials and labor must be factored into any decision to use Surround WP.

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Consumption and Bioprotection of Coffee Components in the Presence of Mycotoxins on Wistar Rats

J. S. ROCHA[1], P. DE F. P. GOULART[2], C. J. PIMENTA[3], S. M. CHALFOUN[4], R. M. EVANGELISTA[3], P. S. ABREU[3], T. A. REIS[3]

[1]University of São Paulo, Ribeirão Preto, BR
[2]University Center of Lavras, Lavras, BR
[3]Federal University of Lavras, Lavras, BR
[4]EPAMIG,Lavras

SUMMARY

Confronted with evidence found in literature, this study sought to confront, by "in vivo" testing, the bioprotective action of the most important chemical compounds in coffee (i.e. chlorogenic acids) and two known mycotoxins (ochratoxin A and aflatoxin B1). The experiment was composed of four biological tests. Young Wistar male rats were used in this study, being kept in a controlled environment with 12 hours photoperiod and ambient temperature of about 25 oC during the whole experiment. The bioprotective action of coffee torwards the mycotoxins, especially by chlorogenic acids, was clearly note, since in the groups that received the addition of chlorogenic acids, a significant improvement in weight gain was noted. It was not found whatsoever, any differences on biochemical and histopathological exams.

INTRODUCTION

Nothing less than the second most beverage consumed in the world, staying behind only water, coffee is currently pointed out as part of those called functional foods. Its attractive flavor and aroma explain all its acceptance, its grain has a huge variety of minerals, amino acids, lipids, polysaccharides and vitamins, especially niacin. Several authors argue that coffee must be in the list of functional foods due to its effects of bioprotection. Bioprotector foods have components whose regular consumption brings benefits to health, such as: regulating the metabolism, performing antioxidant function and preventing or mitigating symptoms of some illnesses .Chlorogenic acids (CGA), the biggest family of phenolic compounds that represents 6–12% of coffee constituents in mass, are known to be responsible for coffee pigmentation and astringency. The foods can be contaminated by various different microorganisms.

Fungi can proliferate in foods very easily, and when this occurs, it is common to find toxin produced by the most different species, mycotoxins.

Aflatoxins and ochratoxins are toxic secondary metabolites, produced by some lineages of Aspergillus flavus; Aspergillus parasiticus, and Aspergillus ochraceus besides other species. Under favorable conditions of temperature and moisture, these fungi can grow in certain foods, resulting in the production of aflatoxins . Through many years, coffee was seen as a beverage that could cause damage to the humans, and due to all these available evidences nowadays, it is necessary a full dissemination, a demystification, which can show that coffee is surely considered a functional beverage, in relation to the human health.

MATERIALS AND METHODS

For a better result, the biological assays were realized separately, with each combination of chlorogenic acid and mycotoxins used. Each one of them was conducted using 32 young male Wistar rats with initial weight of approximately 130g. The experiment was composed of four biological tests. Young Wistar male rats were used in this study, being kept in a controlled environment with 12 photoperiod and room temperature of about 25C during the whole experiment. All mice were maintained in individual metabolic cages with access to controlled food (15g/day) and "ad libtum" water. During each test, which lasted for about four weeks, each animal received 50ng of toxin/day on 3g of powdered milk that was shaped into a sphere by adding 1.5 ml of distilled water. Animals from the negative toxin groups received the same treatment without adding the mycotoxins. Recorded data (weight gain in grs) were compared by Scott Knott and Student t tests at 5% of probability.The feed with different amounts of bioprotector used was called: control, that one with zero concentration of bioprotector; 1%, that one with concentration relating to low consumption of coffee; 2%, that one with concentration regarding average consumption of coffee; and 3%, for that one with concentration concerning the high consumption of coffee.At the end of the assay, all rats were sacrificed according to the ethical principles and practices of use of experimentation animals. After the collection, the blood of each animal was immediately directed for realizing biochemical exams in an accredited laboratory. Glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT).

RESULTS AND DISCUSSION

Some authors report that the consumption of AFB1 by Wistar rats interferes in the growth and development of the animals. At the end of 10 weeks, animals that consumed AFB1 showed lower weight in relation to the other animals that did not. Results that corroborate the ones found in (Table 1).

Table 1. Average weight gain (g) of the animals submitted to different concentrations of chlorogenic acids in absence or presence of AFB1.

Levels of[1] Chlorogenic acids	**Aflatoxin B1[2]**					
	Presence			***Absence***		
0	205,375 (± 5,083)	a	B	184,875 (± 5,083)	b	A
1	197,625 (± 5,083)	a	B	178,500 (± 5,083)	b	A
2	197,750 (± 5,083)	a	A	222,875 (± 5,083)	c	B
3	209,500 (± 5,083)	a	B	156,875 (± 5,083)	a	A
CV = 5,24						

Averages followed by the same lower case in the column and capital letters in the line do not differ among them by Scott Knott test (1) and T of Student (2) at level of 5% of probability.

It was observed that the animals belonging to the negative group for the consumption of AFB1 did not show weight gain, thus the consumption of chlorogenic acids by itself did not cause alteration in weight gain. At the time the animals that belong to the positive groups for consumption of AFB1, a standard with significant differences was showed in the weight gain. The animals that consumed the average quantity of chlorogenic acids demonstrated weight gain higher than the others, maybe being this concentration the ideal one for protecting it against the toxic of this toxin.

There was no statistics difference in weight gain of the animals that consumed OTA or not. (Table 2).

Table 2. Average weight gain (g) of the animals submitted to different concentrations of chlorogenic acids in absence or presence of OTA.

Levels of[1] Chlorogenic acids	Ochratoxin A[2]					
	Absence			***Presence***		
0	120,875 (± 5,808)	a	A	130,000 (± 5,808)	a	A
1	136,625 (± 5,808)	a	A	126,250 (± 5,808)	a	A
2	124,000 (± 5,808)	a	A	126,750 (± 5,808)	a	A
3	131,125 (± 5,808)	a	A	124,000 (± 5,808)	a	A
CV = 9,11						

Averages followed by the same lower case in the column and capital letters in the line do not differ among them by Scott Knott test (1) and T of Student (2) at level of 5% of probability.

Glutamic-oxaloacetic transaminase

The serum levels of GOT aid in the detection and differential diagnosis for acute liver disease and also monitor the progress of the patient and the prognostic of heart and liver diseases. Table 3 represents serum levels of GOT of animals submitted to different treatments.

There was no difference among the treatments, showing safety in the consumption of chlorogenic acids in the doses studied. In relation to the effect of mycotoxins, a short time of exposure and / or low dose of intake are viable hypotheses due to the results.

Table 3 Average levels of GOT in the blood (U/L) of animals submitted to different treatments

Different levels of chlorogenic acids in absence or presence of AFB1: Assay I						
Levels of[1] Chlorogenic acids	AflatoxinB1[2]					
	Absence			***Presence***		
0	298,00 (± 4,7912)	a	A	305,00 (± 4,7912)	a	A
1	292,00 (± 4,7912)	a	A	309,50 (± 4,7912)	a	B
2	294,75 (± 4,7912)	a	A	301,00 (± 4,7912)	a	A
3	307,50 (± 4,7912)	a	A	298,50 (± 4,7912)	a	A
CV = 3,19						

Different levels of chlorogenic acids in absence or presence of OTA: Assay II						
Levels of[1] Chlorogenic acids	Ocratoxin A[2]					
	Absence			***Presence***		
0	281,50 (± 7,2410)	a	A	304,75 (± 7,2410)	a	B
1	293,00 (± 7,2410)	a	A	301,50 (± 7,2410)	a	A
2	300,50 (± 7,2410)	a	A	301,00 (± 7,2410)	a	A
3	283,50 (± 7,2410)	a	A	303,25 (± 7,2410)	a	A
CV = 4,89						

Averages followed by the same lower case in the column and capital letters in the line do not differ among them by Scott Knott test (1) and T of Student (2) at level of 5% of probability.

Glutamic-pyruvic transaminase

GPT, the second of the two enzymes that catalyze a transfer reaction of amine group reversible in the cycle of Krebs, firstly appears in cytoplasm hepatocellular with lower amounts in the kidneys, heart and skeletal muscles, and it is an indicator of complication acute hepatocellular. Its measurement has the aim of detecting and evaluating the treatment of acute

liver disease, especially hepatitis or cirrhosis without icterus, distinguishing between myocardial tissue complication and hepatic, besides evaluating the hepatoxicity of some substances. Table 4 represents the serum levels of GPT of animals submitted to different treatments.

Table 4 Average levels of GPT in the blood (U/L) of animals submitted to different treatments

Different levels of chlorogenic acids in absence or presence of AFB1: Assay I						
Levels of Chlorogenic acids	**Aflatoxin B1[2]**					
	Absence			***Presence***		
0	71,000 (± 1,6638)	a	A	68,250 (± 1,6638)	a	A
1	71,500 (± 1,6638)	a	A	72,500 (± 1,6638)	a	A
2	70,250 (± 1,6638)	a	A	69,750 (± 1,6638)	a	A
3	69,750 (± 1,6638)	a	A	69,750 (± 1,6638)	a	A
CV = 4,73						

Different levels of chlorogenic acids in absence or presence of OTA: Assay II						
Levels of Chlorogenic acids	**Ocratoxin A[2]**					
	Absence			***Presence***		
0	68,500 (± 1,8435)	a	A	69,500 (± 1,8435)	a	A
1	68,000 (± 1,8435)	a	A	69,000 (± 1,8435)	a	A
2	68,250 (± 1,8435)	a	A	69,000 (± 1,8435)	a	A
3	68,750 (± 1,8435)	a	A	70,750 (± 1,8435)	a	A
CV = 5,37						

Averages followed by the same lower case in the column and capital letters in the line do not differ among them by Scott Knott test (1) and T of Student (2) at level of 5% of probability.

CONCLUSIONS

The consumption of chlorogenic acids promoted gain weight of the animals. The absence of the difference found in the biochemical exams, all of them within the normal patterns, suggest time of exposure and /or insufficient dosage of toxin during the experiment.

ACKNOWLEDGEMENTS

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Productivity and Root System of Coffee Cultivated under Different Population Arrangements, with and without Drip Irrigation

J. M. C. SILVEIRA[1], E. SAKAI[2], E. A. A. BARBOSA[3]

[1]Sao Paulo State Agribusiness Technology Agency, Department of Development Decentralization, Technological Development Cluster of Northeast of Sao Paulo State, Mococa, SP, BR
[2]Agronomic Institute of Campinas, Campinas, SP, BR
[3]State University of Campinas, Campinas, SP, BR

SUMMARY

This work was addressed to study the yield and development of root system of coffee submitted to application water by drip irrigation in different population arrangements during two years. The irrigated coffee in the two cycles showed higher production of processed coffee, with emphasis on the coffee grown in the smaller spacing. The treatments irrigated presented a higher concentration of roots when compared to non-irrigated, it was noted that on the treatments with smaller spacing between the rows, the roots concentrated more in the 0-0.5 m layer.

INTRODUCTION

The use of drip irrigation is becoming a very common technique in the coffee yield once it provides a more controlled production environment, avoiding production losses due to water deficit in soil. Coffee production increase through the use of irrigation has been proven by several authors along the years. Another advantage of irrigation, mainly with the use of trickle irrigation as drip irrigation is the nutrient application during the cycle according to the nutrient absorption rate. Generally, the use of fertirrigation improves the efficiency in the use of nutrients because they are applied gradually, according to the nutrient absorption rate.

The coffee trees are sensitive to microclimate changes and small alterations in the production system influence their development. In this way, the application of water by irrigation in the coffee production needs population arrangements studies in order to verify which system provides higher coffee grain yield.

In order to obtain good yields and a higher rationalization of the water and nutrient use, it is necessary to know the distribution of the root system for a more efficient application of irrigation depth. The lack of information regarding the root distribution of irrigated coffee trees brings about the use of information from rain fed system when designing a project. Thus, this research aimed at evaluating the coffee production during two cycles and the root system of *Coffea arabica* L. (cv. Yellow Catuai) grown under different population arrangements, with and without drip irrigation.

MATERIALS AND METHODS

The experiment was carried out in the Agribusiness Technological Development Cluster of Northeast of São Paulo State, located at latitude 21°28'S, longitude 47°00'W and altitude 663 m. The climate according to the Köppen classification is Cwa. Seedlings of *Coffea arabica* L. cultivar Yellow Catuaí were transplanted between March 6th and 7th, 2006, and the 2009/2010 and 2010/2011 crop cycles were assessed. The experimental design was a 6 x 2 factorial scheme in randomized blocks, with four replications. The six densities of plants were E1 (1.60 x 0.50); E2 (1.60 x 0.75); E3 (1.60 x 1.00); E4 (3.20 x 0.50); E5 (3.20 x 0.75) and E6 (3.20 x 1.00), corresponding to 12,500; 8,333; 6,250; 6,250; 4,127 and 3,125 plants ha^{-1}, respectively, which were divided according to the availability of water (irrigated – I – or non-irrigated – NI – groups).

Fertilization was performed according to the Bulletin 200 of the Campinas Agronomic Institute based on the result of the soil chemical analysis. Fertirrigation was carried out once a week, except during water restriction period (July-August). In the non-irrigated treatments, three applications were manually performed, along rainy months (October, November and January).

The amount of water applied depended on the irrigation interval, the climatic demand (reference evapotranspiration) undertaken by the Penman-Monteith method according to meteorological data which were daily collected from the Automatic Weather Station located approximately 500 m from the experimental area. Irrigation was suspended for 60 days during July and August for the imposition of water deficit, in order to promote uniformity of flowering. The irrigation system was surface drip irrigation, with emitter flow rate of 2.3 l h^{-1} and emitter spacing of 0.50 m.

The harvest of irrigated coffee was performed in April and that of non-irrigated one in June. The early harvest in the irrigated group was due to the early fruit maturation. The crop was harvested in a sieve thus preventing fruit falling on the ground. The processing of freshly harvested coffee was done using the conventional terrace drying method. After 45 days of drying, the husk and parchment were removed.

After harvest of the second cycle, with the aid of an auger roots, samples of roots were collected in layers of 0.1 m up 1.0 m of depth, at points at 0, 0.4 and 0.8 m distance from orthotropic branch in the treatments E1, E2 and E3 and 0, 0.4, 0.8, 1.2 and 1.6 m in the treatments E4, E5 and E6. In laboratory the samples were immersed in water and diluted successively, aiming at promoting suspension of organic material, including roots. This material was collected in a sieve of 0.5 mm mesh and placed on a sheet of paper to dry at ambient temperature. Then the fine roots were separated, cleaned and dried at 60°C for 48 hours. Data were submitted to analysis of variance and averages compared by Tukey test at 5% of probability.

RESULTS AND DISCUSSION

In both cycles there was a significant effect from the population arrangement and from the yield of processed coffee irrigation as well as a significant effect from the interaction between the two variation sources in the 2010/2011 cycle (Table 1). Analyzing the population arrangement effect on the yield of processed coffee, it is noted that in the 2009/2010 cycle the E1 treatment presented a higher coffee yield (4,631 Kg ha^{-1}), different from the E4, E5, E6 treatments. In the 2010/2011 cycle the E2 and E3 treatments presented the higher yield of

processed coffee, 1,051 and 1,515 Kg ha^{-1}, respectively, different from the coffee tree grown in the E4, E5 and E6 population arrangements.

The higher yield of processed coffee in the high density treatments, in the first cycles or when there is no influence on the canopy overlay due to pruning, happens because of a high density population being an alternative growth when it is expected good yields in first cycles.

Table 1. Analysis of variance for yield of processed coffee cv. Catuaí, cultivated in different population arrangements, with or without irrigation, in Mococa – SP, Brazil.

		Yield of processed coffee (kg ha^{-1})	
		2009/2010	***2010/2011***
Population arrangements (m)	E1 - 1.60 x 0.50	4631 a	929 ab
	E2 - 1.60 x 0.75	4201 ab	1051 ab
	E3 - 1.60 x 1.00	3590 ab	1515 a
	E4 - 3.20 x 0.50	3463 b	712 ab
	E5 - 3.20 x 0.75	3287 bc	693 ab
	E6 - 3.20 x 1.00	2268 c	610 b
F test for PA		$10,3^{2}$	$2,61^{1}$
Irrigation	With	4162 a	1702 a
	Without	2985 b	134 b
F test for I		$32,3^{2}$	$85,7^{1}$
F test for I x PA		$1,03^{ns}$	$2,85^{1}$
C.V.%		20,1	63,8

[1]Significant at 5% of probability; [2]Significant at 1% of probability; ns – non significant. S.A.D = Significant average deviation; C.V. = Coefficient of variation.

The irrigated coffee tree in the two cycles showed a higher yield of processed coffee producing 4,162 and 1,702 kg ha^{-1} in the 2009/2010 and 2010/2011 cycles respectively when compared to the non-irrigated growth which obtained yields of 2,985 and 134 kg ha^{-1} in the 2009/2010 and 2010/2011 cycles respectively. Comparing the total yield of processed coffee in the two cycles it is noted that the irrigated coffee tree presented an increase of 53% when compared to the non-irrigated one.

Through the analysis of the interaction effect between irrigation and population arrangement, it is noted that in the 2010/2011 cycle the coffee tree grown in the irrigated E2 and E3 spacing presented a higher yield of processed coffee differing from all the treatments with the exception of the irrigated E1 which showed a yield similar to the coffee tree grown in the irrigated 3.2 m spacing.

Table 2. Analysis of the effects of irrigated coffee tree in different population arrangements, in the 2010/2011 cycle in Mococa-SP.

Population arrangements (m)	**Yield of processed coffee (kg ha^{-1})**		
	With	***Without***	
F test	**5,35[1]**	**0,98[ns]**	**F test**
E1 - 1,60 x 0,50	1695 ab A	163 a B	13[1]
E2- 1,60 x 0,75	1926 a A	176 a B	17[1]
E3 -1,60 x 1,00	2931 a A	99 a B	46[1]
E4 - 3,20 x 0,50	1160 b A	264 a B	4,6[1]
E5 - 3,20 x 0,75	1386 b A	0 a B	11[1]
E6 - 3,20 x 1,00	1116 b A	104 a B	5,9[1]

[1]*Significant at 5% of probability; ns – non significant. Small letters represent the average values in the vertical and capital letters represent the average values in the horizontal.*

The irrigated coffee tree presented a higher root concentration when compared to the non-irrigated, with the exception of the E2 (1.6x0.75) treatment and it is noted that there was no effect from the irrigation in the root system depth development. In general, the irrigated treatments presented a depth similar to the non-irrigated in each population arrangement.

In the 1.6 m rows spacing, 90% of the roots concentrated in the 0 to 0.5 m layer. In the 3.2 m rows spacing, except for the E6 treatment, 90% of the roots concentrated in the 0 to 0.6 m layer while in the E4 and E5 treatments more than 80% of the roots were concentrated in 0 to 0.5 m layer.

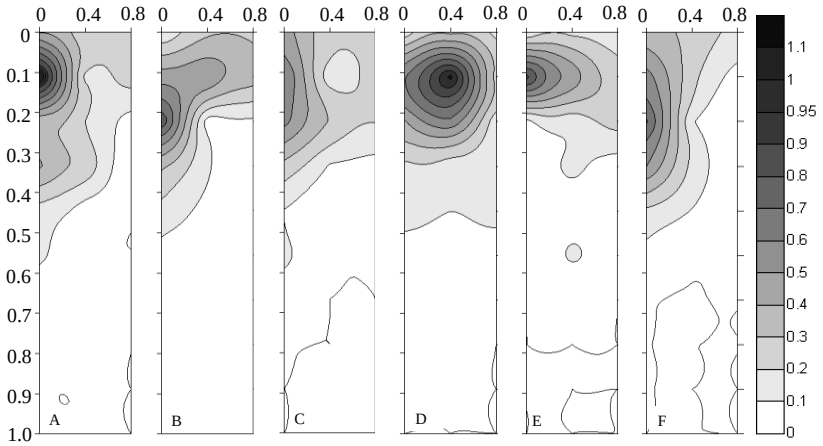

Figure 1. Distribution of the coffee tree root system (in grams of dried matter per cubic decimeter of soil) grown in irrigated 1.6 x 0.5 m (A) and non-irrigated (B); irrigated 1.6 x 0.75 m (C) and non-irrigated (D); irrigated 1.6 x 1.0 m (E) and non-irrigated (F) population arrangements.

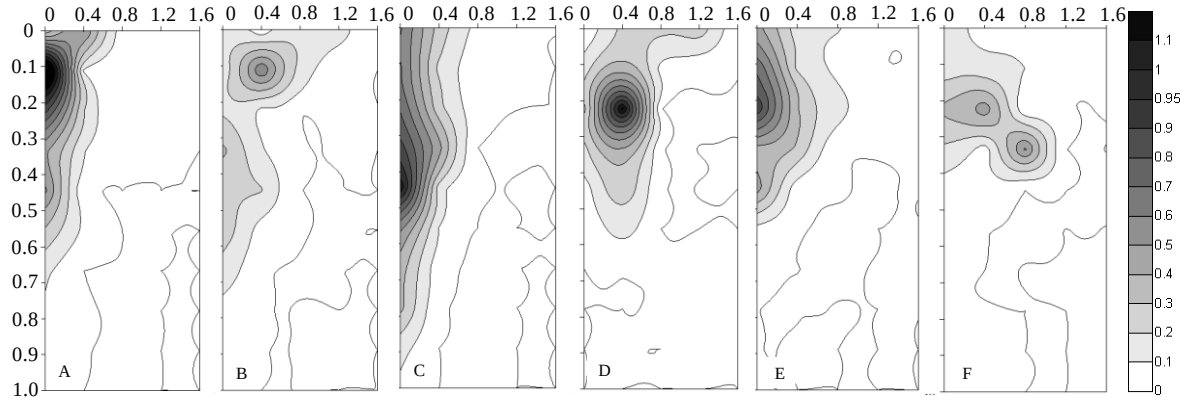

Figure 2. Distribution of the coffee tree root system ((in grams of dried matter per cubic decimeter of soil) grown in irrigated 3.2 x 0.5 m (A) and non-irrigated (B); irrigated 3.2 x 0.75 m (C) and non-irrigated (D); irrigated 3.2 x 1.0 m (E) and non-irrigated (F) population arrangements.

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Flotation Population of Coffee-Leaf-Miner *Leucoptera Coffeella* (Guérin-Mèneville, 1842) (Lepidoptera: Lyonetiidae) in the Southern Region Minas Gerais State - Brazil

R. A. SILVA[1], J. L. MACHADO[2], J. C. SOUZA[1], E. A. ALCÂNTARA[1], T. A. F. CARVALHO[2]

[1]Minas Gerais Agricultural Research Institution, Brazil, FAPEMIG's scholarship holders
[2]Federal University of Lavras, Minas Gerais, Brazil

SUMMARY

The objective of this study was to evaluate the population dynamics of the coffee-leaf-miner - CLM for 10 consecutive years, to verify management actions to reduce losses provided by this pest. The monitoring was conducted at the EPAMIG's Experimental Station in São Sebastião do Paraíso. The leaf samples were collected monthly from 2002 until 2012. Pest infestation data were correlated with rainfall in the region. The CLM percentage of infestation was higher in the upper third of the plant. The highest infestation levels occurred during the dry season. Therefore, the implementation of monitoring of the CLM in coffee plantations is important because the time to make control can change each year.

INTRODUCTION

Brazil is the largest world producer and exporter of coffee. The coffee production prediction for the 2012 indicates that the country will harvest 50.48 million bags in an area of 2,339,600 ha. Among the factors that affect coffee production pests as Coffee-leaf-miner – CLM is been considered a major coffee pest in Brazil, due to its widespread occurrence in coffee plantations and the economic losses caused by this insect in relation to coffee production.

The damages caused by this insect vary depending on several factors like as time of year and growing region. Adverse weather conditions and long drought periods associated with high temperatures as well as the ecological imbalance caused by inappropriate chemicals use are the main causes of large infestations observed. The temperature influences on the CLM infestation is large and has a positive correlation, but rainfall and relative humidity show a negative correlation.

Therefore, monitoring the crops is an important tool because it allows tracking the onset and progression of the pest infestation through regular sampling enabling the application of control measures when necessary, reducing costs and environmental damage. For this it is very important to know the factors determining the intensity of pest attack to make the management planning strategies and tactics.

MATERIALS AND METHODS

The experiment was conducted at the EPAMIG Experimental Station in São Sebastião do Paraíso, in the southern of Minas Gerais state, Brazil. To monitor the CLM implementation was demarcated a plot with 1000 plants, with coffee cultivar Catiguá MG1 in the spacing of

3.0 x 0.70 m. This area has received no insecticide treatment during the evaluation period, to avoid interference in the pest dynamics population. Other cultural practices were usually held in the crop at appropriate times. Within this area 10 plants were randomly and representative chosen as samples. Were collected from each plant five leaves in the third pair of leaves by branch, from the tip to plant apex of in each plant quadrant (north, south, east and west) in the upper, middle and lower third plant separately, totalizing 20 leaves / third and 60 leaves / plant. Data were recorded on a spreadsheet for later tabulation. The samples were collected each two week.

The sum of quadrants upper, middle and bottom of the 10 plants were made separately to evaluate possible differences in the infested plant parts. Was also computed the average of the results in the two assessments within each month.

Meteorological data were collected in the same study period at the EPAMIG's Meteorological Station.

With the infestation percentage and meteorological data were created graphs in order to compare the behavior of the insect with the climatic data in the region over the past 10 years.

RESULTS AND DISCUSSION

The CLM attack can occur throughout the year in coffee producing regions and not only in the dry season. However, note that the borer damage is higher in dry periods with intermediate or high temperatures.

Climate changes, as the occurrence of longer periods of drought and presence of high temperatures, even in winter, are directly related to population fluctuation of the Bug-Miner. Looking at Figure 1 was possible to notice that the CLM infestation showed a similar behavior in the years studied, with few exceptions. The years 2003, 2008, 2010 and 2011 had higher levels of infestation above the control level.

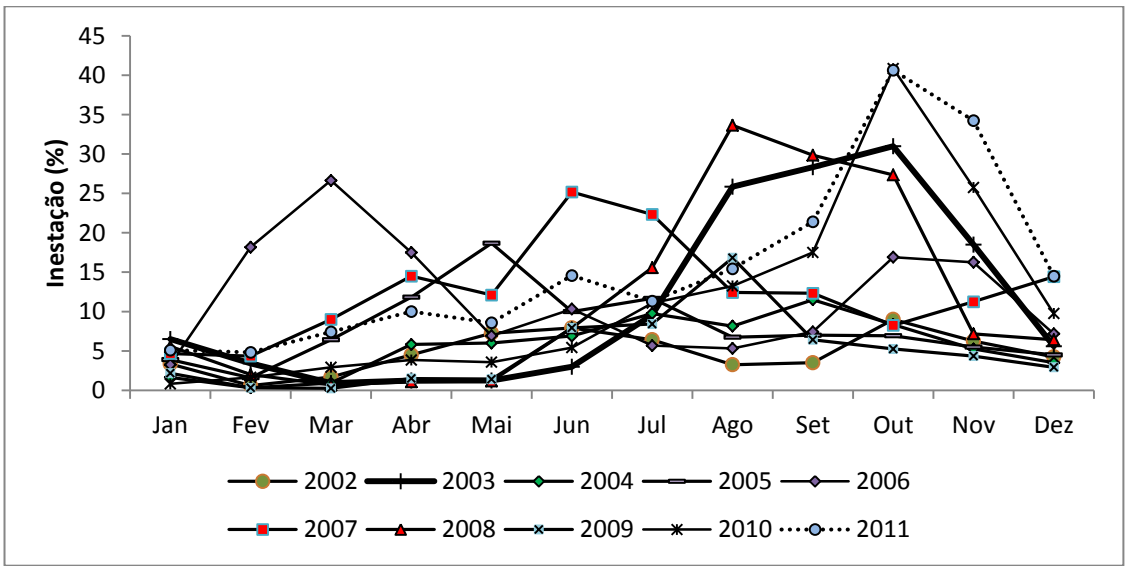

Figure 1. Percentage infestation of CLM from 2002 to 2011, in the southern region of the state of Minas Gerais.

In the region under study the onset of rains usually occurs from October and goes until March. In this period, is generally do not occur the leaf-miner attack since precipitation has direct influence on the infestation.

As can be observed in Figure 2, the drought characteristic of the region generally is April / May to September / October. Within this period is observed a peak of infestation that usually occurs in August. Given the climatic conditions of each year the maximum level of infestation can vary as can be seen in this work. In 2006 there was a higher infestation level of leaf-miner in March, noting that this month there was a low rate of rainfall (66.0 mm) compared to other years.

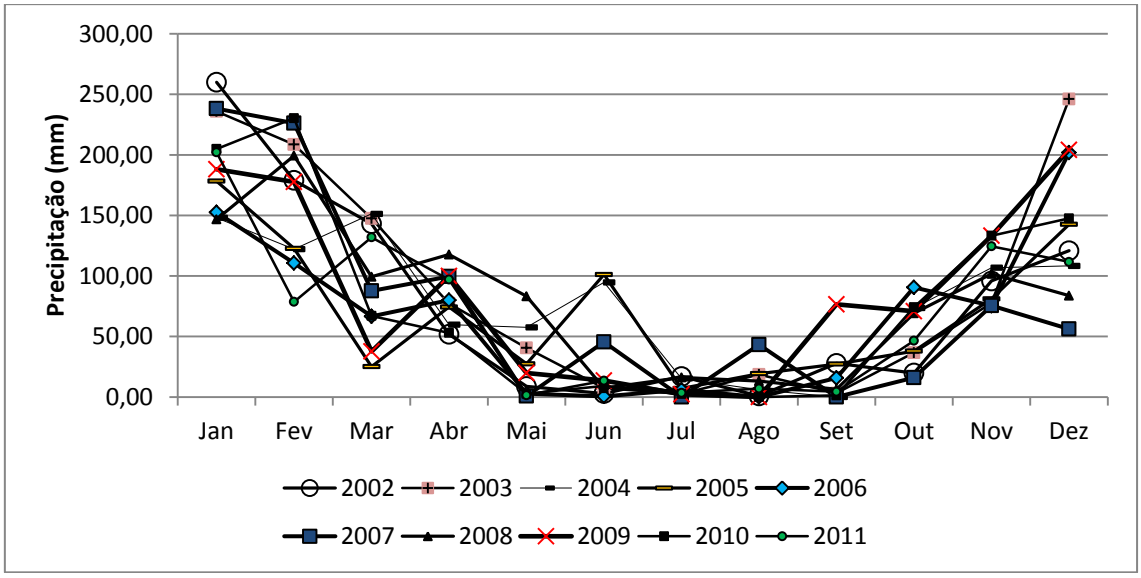

Figure 2. Rainfall (mm) recorded at the Meteorological Station of the Experimental Farm Epamig in southern Minas Gerais, in the period from 2002 to 2011.

The temperature also has an influence on the leaf-miner infestation. In figure 3 it is observed that in the period of lower temperature (17 to 22ºC) coincide with the greatest infestation of leaf-miner observed in figure 1. The incidence of high temperatures and long periods without rain increases the occurrence of injury and damage. Climatic factors can directly affect the physiology (eg, rate of development and water regulation), or behavior (locomotion, orientation and dispersion) of the insect. Similar results were found in previous work.

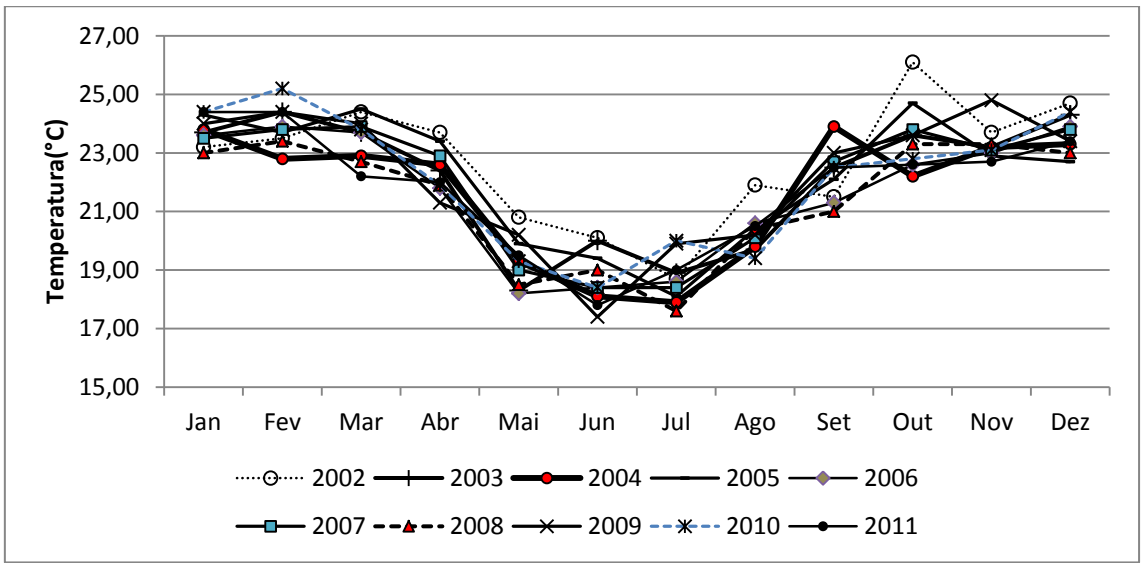

Figure 3. Average Temperature (°C) recorded at the Meteorological Station of the Experimental Farm Epamig in southern Minas Gerais, in the period from 2002 to 2011.

CONCLUSIONS

The results presented here emphasize the importance of monitoring the evolution of pest attack in the properties because the local environmental characteristics can affect the pest occurrence so that there are differences in the infestation throughout the year and between years.

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Flotation Population of Coffee Berry Borer Hypothenemus Hampei (Ferrari, 1867) (Coleoptera - Scolytidae) in Southern State of Minas Gerais – Brazil

R. A. SILVA[1], J. L. MACHADO[2], J. C. SOUZA[1], E. A. ALCÂNTARA[1], T. A. F. CARVALHO[2]

[1]Minas Gerais Agricultural Research Instituition, Brazil, FAPEMIG's scholarship holders
[2]Federal University of Lavras, Minas Gerais, Brazil

SUMMARY

The coffee berry borer attacks the coffee fruits with different intensities depending on weather conditions and the stage of fruit development. So, it is necessary to monitor the insect population in crops to be taken preventive control measures. In this sense, the objective of this study was to evaluate the infestation of the coffee berry borer in southern Minas Gerais state by monitoring over the past seven years. The incidence of coffee berry borer proved to be coincident years of evaluation in relation to the time of incidence, in other words the highest incidence was observed in insect months before harvest. The monitoring also has shown that preventive actions like picking performed well, each remaining fruit on the plant and the ground survey e removed during the offseason culture.

INTRODUCTION

The coffee culture is one of the most important agricultural activities in agriculture in Brazil highlighting at state of Minas Gerais, which is the largest producing state and accounts for half of all domestic production. Among the factors contributing to affect the coffee production stand out pests every year causing major losses burdening the exploitation of this activity. Among the main pest the coffee berry borer coffee *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera - Scolytidae) is considered one of the most important due to direct and indirect damages they cause. Being a pest that feeds exclusively of coffee fruits and reproduce, this insect attacks the coffee fruits at any ripeness stage, from green to dry them punching through the crown until the seeds where they form galleries and oviposit. When larvae feed on the seeds emerge destroying them partially or completely. In the southern region of Minas Gerais with wetter winter in coffee plantations with different spacing and uneven crop, the survival of the drill has been favored and usually chemical control required.

In this context, the aim of this study was to evaluate the fluctuation of the coffee berry borer in the southern region of Minas Gerais in the last seven eight.

MATERIALS AND METHODS

The experiment was conducted at EPAMIG Experimental Station, São Sebastião do Paraíso in southern Minas Gerais State, Brazil. To conduct the monitoring, was used a field with 1500 graves properly marked. The cultivar used was MG1474 Acaiá deployed in 2002 at a spacing of 3.20 x 0.70 m. Within this area 50 were selected at random in a representative way. This

area received no insecticide control during the monitoring period, and all other cultural practices routinely performed normally.

Were collected monthly 40 fruits per plant, being sampled 20 fruits on each side of the plant, varying harvest from the middle third to the lower third of 2000 totaled fruits. After was performed separation and counting of damaged fruits, subsequently making the determination of the percentage of damaged fruits. This procedure was performed monthly and started three months after the first bloom, beginning in December and ending sampling at harvest. The first sampling was done at the top of the plants, where fruits found were from the first flowering, the remaining samples followed as before.

The fluctuation was conducted from 2004 to 2011 and climate data were obtained from the meteorological station located at the EPAMIG Experimental Station in São Sebastião do Paraíso. With the data infestation percentage in seven years, were mounted graphs in order to compare the behavior of the insect with the climatic data of the southern state of Minas Gerais.

RESULTS AND DISCUSSION

In this study, there was some variation in the intensity of the attack of the coffee berry borer (Figure 1), but it was possible to see that there is a repeatable behavior in this insect throughout the year. Its incidence occurs in the same period and, thus, the producer can make use of monitoring and, if necessary, chemical control. The infestation starts to increase from November and varies from year to year, with greater or lesser intensity, reaching a peak at harvest time because the coffee fruits at later stages of development become increasingly favorable for the development of the coffee berry borer.

In the months from May to July are generally observed high levels of infestation. This period corresponds to the period in which the majority of coffee beans have reached the stage of ripening cherry, demonstrating that the increased infestation is related to the availability and properties of the fruit as a food source and location for oviposition.

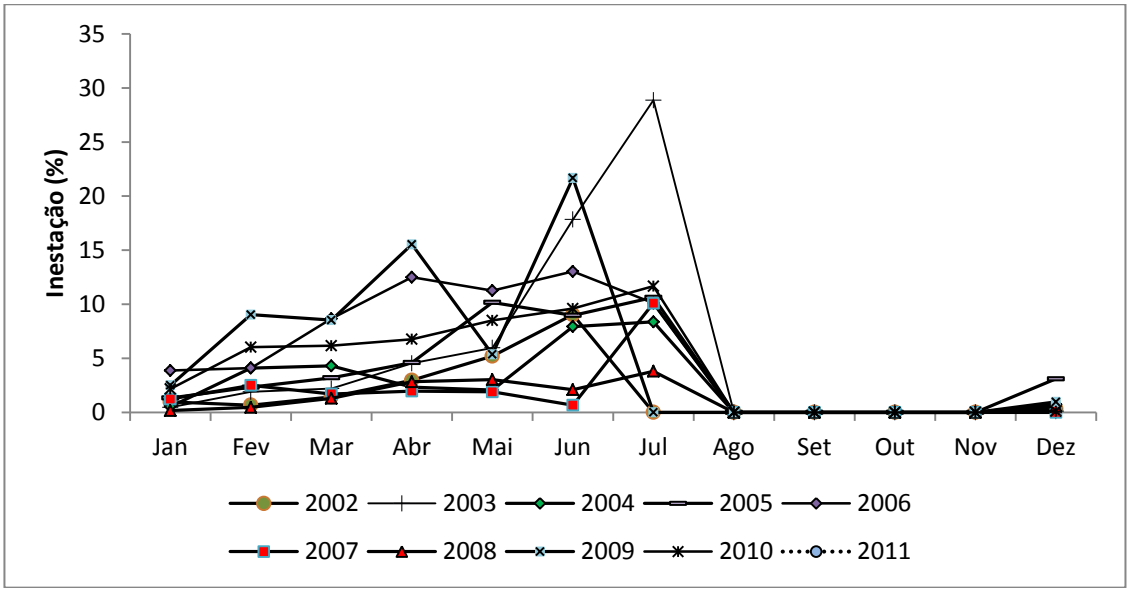

Figure 1. Percentage of borer infestation from 2004 to 2011, in the southern state of Minas Gerais.

In 2003 there was a high infestation levels in the month of July, it may be because the high levels of precipitation in the previous year (Figure 2) because the moisture is a limiting factor for this insect to survive and reproduce in a season for the next harvest.

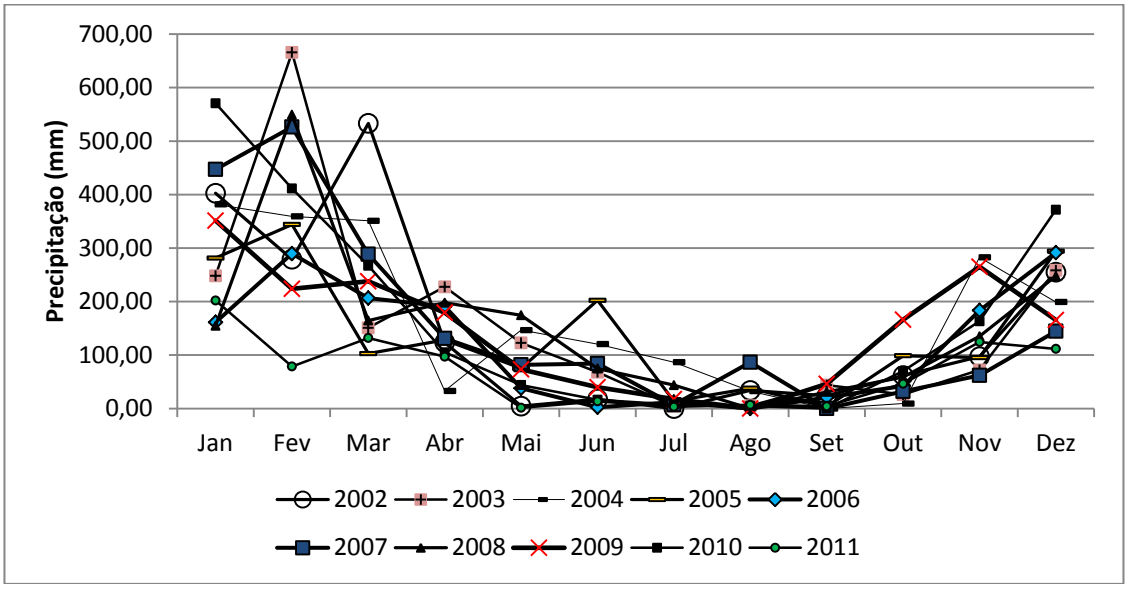

Figure 2. Rainfall (mm) recorded at the Meteorological Station of the Experimental Farm Epamig in southern Minas Gerais, in the period from 2004 to 2011.

The temperature influences directly the length cycle of the drill. High temperatures cause a reduction of the insect life cycle and thereby increase the generation number and larger losses during coffee harvestings. In the work in question is verified that there was a discrepancy in the temperature in the years studied (Figure 3).

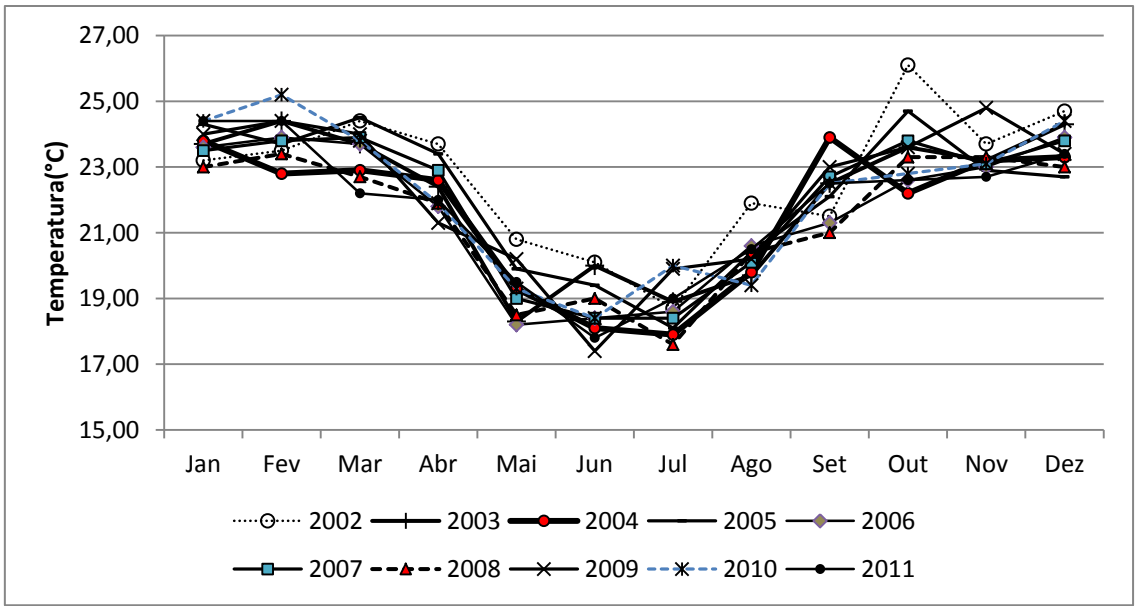

Figure 3. Average Temperature (° C) recorded at the Meteorological Station of the Experimental Farm Epamig in southern Minas Gerais, in the period from 2004 to 2011.

Several management practices aimed at the prevention of the incidence of this pest can be adopted and one of the most important is the good harvest in the previous season then made the transfer with the picking of the fruit that remained in the plant and the ground. Other

practices can be considered as the completion of the harvest as soon as possible by starting early maturing plants, disposal of abandoned farms present on the property and that can serve as the focus of plague, use of larger spacing, weed control.

CONCLUSIONS

In this context, monitoring the coffee berry borer is necessary to know the behavior of the insect over the years thus enabling the application of control measures at the right time and in an appropriate manner since many times the level of infestation is not reaches the level of control that is 3 to 5%.

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Environmental and Socioeconomic Impacts of Coffee Cultivars Resistant to Diseases and Pests in the Development of Brazilian Coffee Regions

F. M. M. BLISKA, P. H. N. TURCO, C. L. R. VEGRO, T. FRONZAGLIA, L. C. FAZUOLI

Centro de Café "Alcides Carvalho", Instituto Agronômico, Av. Barão de Itapura, 1421, Campinas, Brazil, Postal Box 28, 13012-970

SUMMARY

Immunity or tolerance to pests and diseases can provide less use of chemicals and therefore significant savings for producers, and significant reduction of risks related to environmental pollution and health of farmers and consumers. Consequently, the innovative characteristics of cultivars with resistance to diseases and pests to meet growing global demand for agricultural production that minimizes waste, unnecessary costs and result in significant and positive impacts on health of workers, farmers and consumers, contributing to sustainable rural development. This study examines the social-economic and environmental impacts of the adoption of cultivars resistant to pests and diseases in developing sustainable coffee regions based on the assessment of resistant cultivars developed by Instituto Agronômico (IAC): Tupi IAC 1669-33, IAC 125 RN, Obatã IAC 1669-20 and Apoatã IAC 2258 (rootstock). These cultivars have high yield potential and good agronomic and technological characteristics, and stand out for use in conventional or organic crops, family or business farms, in areas with soil and climatic conditions favorable to the spread of specific diseases and pests. Moreover, the cultivar IAC 125 RN presents multiple resistance, which provides additional advantages compared to other resistant cultivars.

INTRODUCTION

In Brazil, coffee is one of the most important crops on the generation of employment and trade. Among the major innovations for the production of coffee beans, stand out cultivars adapted to different soil conditions and climate, with good agronomic and technological characteristics, in addition to good quality of beverage. However, the impact of these cultivars has not been adequately measured, that is, until the moment is not properly valued, in what extent they can actually assist in the transformation of Brazilian coffee production. Among the newer cultivars, those with resistance to pests and diseases, and especially with multiple resistance, have aroused great sectorial interest. The immunity or tolerance to diseases and pests provides less use of chemical products, so considerable savings for producers, as well as significant reduction of environmental risks and on human health. The innovative characteristics of these cultivars are consistent with the growing global demand for products cultivated in order to minimize residues, wastages, and unnecessary costs that result in significant and positive impacts on the health of workers, farmers and consumers, contributing to rural sustainable development. The objective of this study is to analyze the social, environmental and economic impacts of adoption of cultivars resistant to pests and diseases in the sustainable development of Brazilian coffee regions.

MATERIALS AND METHODS

To assess the impacts of technologies - coffee cultivars resistant to diseases and pests – we used the ex post analysis of four cultivars: Tupi IAC 1669-33, IAC 125 RN, Obatã IAC 1669-20 and Apoatã IAC 2258 (rootstock), considered recent when compared to cultivars that are widely used in Brazil – Mundo Novo and Catuaí. The socioeconomic factors were assessed via Social System of Impact Assessment of Agricultural Technology Innovations (Ambitec-Social) and environmental factors, through the System of Environmental Impact Assessment of Agricultural Technology Innovations (Ambitec-Agro) or System Ambitec.

The system involves: 1) General data collection, about the technology (scope and influence, geographical area and population of adopters); 2) Application of questionnaires in individual interviews with adopters, and inclusion of data on impact indicators (range, efficiency, conservation and rehabilitation) in spreadsheets System components (platform MS-Excel ®), obtaining quantitative results of the impacts, and partial and aggregates impacts indices; 3) Analysis and interpretation of these indices and indication of alternative management practices and technologies, which will minimize negative impacts and maximize the positive effects, contributing to sustainable local development. The coefficient of impact of technology results from the aggregation of several impact indicators and their measurement criteria. The results of the impact assessment are expressed graphically in the worksheet and range between -15 and +15. We defined an intentional sample of respondents from the universe of adopters of technologies, based on the scope and boundaries identified for each cultivar analyzed, with the following profiles: family and business producers, seedling producers, consultants and extension agents. Additionally we interviewed the researcher who developed the technologies (Table 1).

Table 1. Geographical limits of adopters of Tupi IAC 1669-33, IAC 125 RN, Obatã IAC 1669-20 and Apoatã IAC 2258, and number of users interviewed (U). Cropping intensity (CI): 0 (uncultivated / experimental cultivation), 1 (rarely cultivated), 2 (cultivated) and 3 (very cultivated).

Regiões Cafeeiras		Tupi		IAC 125		Obatã		Apoatã	
		CI	U	CI	U	CI	U	CI	U
Minas Gerais	South	1	1	0	0	1	1	0	0
	Cerrado	1	2	2	11	1	1	0	0
	Zona da Mata	0	0	0	0	0	0	0	0
	Jequitinhonha	0	0	0	0	0	0	0	0
São Paulo	Mogiana	1	4	0	0	2	4	0	0
	Alta Paulista	0	0	0	0	0	-	3	5
	Garça-Marília	1	1	0	0	2	2	3	3
	Piraju-Avaré	1	1	0	0	1	2	0	0
Paraná	North	1	1	0	0	1	1	0	0
	Northwest	0	0	0	0	0	0	1	1
Bahia	Planalto	1	1	0	0	1	1	0	0
	West	0	0	0	0	0	0	0	0
Total users			11		11		12		9

Source: Survey conducted for this study.

RESULTS AND DISCUSSION

The impacts indices of the cultivars analyzed are presented in Table 2. Tupi and Obatã not result in significant environmental and socioeconomic impacts for regional development of Brazilian coffee regions. IAC 125 RN shows promising results and could become strategic for the expansion or maintenance of coffee areas in the Cerrado. Apoatã (rootstock) results in larger regional impacts. The questionnaire responses were homogeneous among respondents with the same profile. Summary on the performance of these cultivars in the coffee regions.

IAC 125 RN

Registered in 2012 but intensively planted in Cerrado, mainly from 2007. Then the plantations are very new to us evaluate your effective potential. We identified great interest in this cultivar due to its good physical, agronomic and beverage characteristics, but most producers do not know its resistance to *Meloidogyne exigua*. The major requirement in water and nutrients is related to its high productivity. When irrigated is more productive, but it was not too stressed with drought. Some producers observe additional resistance to Phoma. Currently there are few areas with nematodes in the Cerrado, but the level of infestation grows every year. It is expected that in the medium term this cultivar will be strategic to the survival of the coffee in this region, with consequent expansion of its cultivated area.

Apoatã IAC 2258

Used as rootstock. There is unanimity as to its importance for the survival of farmers in large area of São Paulo State, mainly in Alta Paulista. The growing infestation of their soils by nematodes contributes considerably to the decline of coffee production, resulting in negative impacts on regional socioeconomic indicators. The planting of grafted seedlings led to the renovation of most coffee plantations in the region and less use of chemicals. The Apoatã rekindled farmers' expectations about the possibility to reach sustainable regional development. Positive impacts of this cultivar were also identified in the region of Garça-Marília and the Northwest of Paraná State.

Tupi IAC 1669-33

The questionnaire responses were heterogeneous, particularly with respect to the characteristics of the beverage and its productivity. Some producers observe additional resistance to Phoma and Ascochyta.

Obatã IAC 1669-20

Extremely productive, consequently demanding on nutrients and water. This has been champion of beverage quality.

Coefficients of impacts perceived by producers are comparable to those obtained in other similar evaluations. As an example, two wheat cultivars released after 1986 showed social impact indices respectively equal to 0.93 and 1.39, and indices of environmental impact of 1.91 and 0.01, respectively. We found that the rates obtained in this study contrasted different respondent profiles and technologies. However, the technological content of these coffee cultivars may be subject to the forecast of other potential impacts.

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Table 2. Impact indices of cultivars Tupi, IAC 125 RN, Obatã e Apoatã, in Brazilian coffee regions (creator and users of technologies): Environmental (Env), Economics (Eco), Social (Soc), Total (Tot) and Creator (Cre).

State	Arábica coffee region	Impacts indices of cultivars																			
		IAC 125					Apoatã					Tupi					Obatã				
		Env	*Eco*	*Soc*	*Tot*	*Cre*	*Env*	*Eco*	*Soc*	*Tot*	*Cre*	*Env*	*Eco*	*Soc*	*Tot*	*Cre*	*Env*	*Eco*	*Soc*	*Tot*	*Cre*
Minas Gerais	Sul	-	-	-	-	-	-	-	-	-	-	0,13	0,92	0,92	0,13	0,92	-0,13	0,92	0,92	0,52	0,75
	Cerrado	1,74	5,25	5,13	3,98	1,19	-	-	-	-	-	0,76	1,48	2,03	0,76	1,48	0,13	0,89	0,83	0,57	0,75
	Zona da Mata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Jequitinhonha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
São Paulo	Mogiana	-	-	-	-	-	-	-	-	-	-	0,65	2,84	2,41	0,65	2,84	0,23	2,17	2,46	1,24	0,75
	Alta Paulista	-	-	-	-	-	3,02	7,90	5,03	3,02	7,90	-	-	-	-	-	-	-	-	-	-
	Garça-Marília	-	-	-	-	-	2,15	7,66	4,95	2,15	7,66	0,63	2,79	2,84	0,63	2,79	0,34	5,13	2,86	2,35	0,75
	Piraju-Avaré	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,59	3,12	1,93	1,59	0,75
Paraná	Norte	-	-	-	-	-	1,16	7,62	4,57	3,96	1,65	0,33	1,58	1,25	0,33	1,58	0,21	0,92	0,92	0,62	0,75
	Noroeste	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bahia	Planalto	-	-	-	-	-	-	-	-	-	-	0,88	1,43	2,03	0,88	1,43	0,13	0,89	0,83	0,60	0,75
	Oeste	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Source: Results obtained in this study (Methodology Ambitec-Agro).

Succession Process in Family Farms: Case Studies in Brazilian Coffee Farms

W. M. OLIVEIRA[1], L. F. ALMEIDA[2]

[1]Economist and Coffee Producer
[2]Professor of the Business Course at School of Higher Education in Marketing & Communication Management, Sao Paulo, Brazil

SUMMARY

This paper examines the succession process in family businesses, particularly in coffee producing farms located in Minas Gerais state, the leading producer of Arabica coffee in Brazil. Based on the three-circle theory of family businesses (DAVIS et al, 1987), the paper analyzes two cases of farmers who are dealing with succession issues at the moment. The main conclusions are that the continuity of the family business depends on the prevalence of good communication skills and that preparing a successor among family members is important to coffee estate owners.

INTRODUCTION

Coffee production is a traditional economic activity in Brazil. It occupies an area of 2,3 million hectares, being the most important source of income of 1.900 cities (AGROANALYSIS, 2009). The country has produced an average of 45 million 60 kg bags of coffee in the last years (CONAB, 2012), and its production is spread over seven different states: Minas Gerais, Espírito Santo, São Paulo, Bahia, Paraná, Rondônia and Rio de Janeiro. The main producer, Minas Gerais, is responsible for 70% of the arabica national production and half of the total coffee production.

The urbanization and industrialization processes which took place after the 1950's provided Brazil with a diversified economic basis. Agriculture is still very important, but nowadays its characteristics are very different from those of the past; innovation, use of technology, economies of scale and competitiveness are concepts assimilated by Brazilian producers (VIEIRA FILHO, 2009).

Since the beginning of the 1990's institutional and structural transformations in the country have had relevant impacts on the coffee sector. Deregulation of the national market with the extinction of IBC – the National Bureau of Coffee – represented an incentive to private initiative and meant valorization of quality attributes of coffee. Stabilization of currency, opening to foreign investments and the increase of population's income, among others, impelled producers to be more professional in their coffee plantations. The rising of labor costs, price of land and a changing and strict legislation in environmental and social fields are also part of the reality of the Brazilian coffee producer nowadays. Besides technical matters, understanding financial and commercialization aspects is important in order to be efficient.

In this context of a dynamic activity, that demands knowledge and dedication of the producer and is characterized by mid and long-term investments, this research intends to examine the preparation of the succession process of family farms, especially those involved in coffee production.

OBJECTIVES

The objective of this research is to look into the way the preparation of the succession process is carried out in families who own rural estates. The specific objectives are: (i) to analyze the main aspects involving succession in family owned companies; (ii) to identify possible particularities of succession in coffee farms.

MATERIALS AND METHODS

Based on the theoretical model of analysis of family owned companies (DAVIS et al, 1987), this research, which is of exploratory nature, investigates two case studies of rural producers undergoing a family succession process. In addition, thirty people involved in the coffee business (producers, managers of cooperatives, agronomists and specialized technicians) answered a questionnaire regarding the topic of succession.

According to Lourenzo (2007) and Bornholdt (2005), family businesses are present worldwide and represent a significant part of the economy in every country:

Country	Participation (%)
Spain	80
England	75
Italy	95
Portugal	70
Sweden	90
Switzerland	85-90
Brazil (estimation)	85

Figure 1. Percentage of family business per country. Source: Adapted from Lourenzo M. Jr. (2007, p.2) and Bornholdt (2005, p. 35).

According to the Brazilian Institute of Corporate Governance (IBGC, 2007), the three-circle model of family business is widely used to explain the dynamic of such companies. This model considers that the development of a family business leads to the existence of three subsystems: ownership, family and business.

There are seven possible positions an individual may occupy in this interdependent subsystems: one can be an owner and family member at the same time, but not participate in the management of the company. Another can be a manager but not participate in the family or the ownership. It is possible to be a family member, owner and manager at the same time. Each position corresponds to a particular interest and expectation, and that can be a source of possible conflicts affecting the entire system.

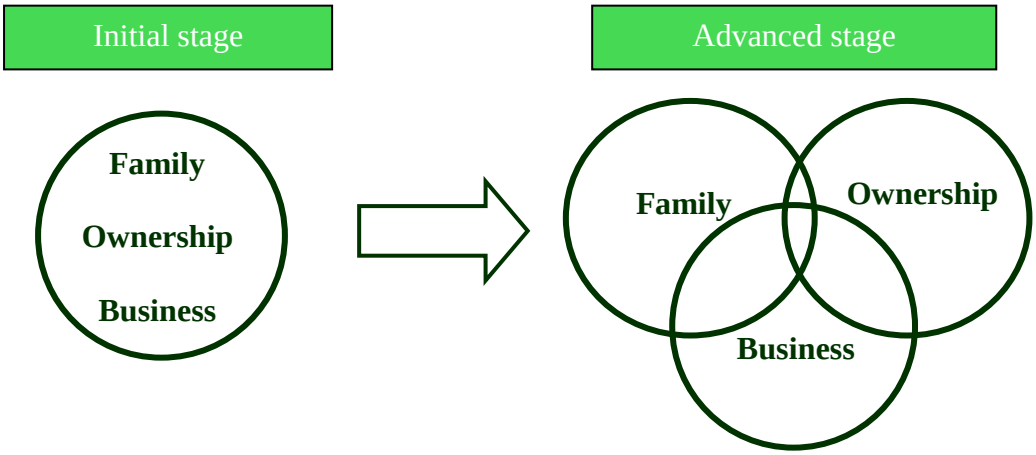

Figure 2. Three-circle model of family business. Source: Davis et al. Generation to generation: Life Cycles of the Family Business. Harvard Business School Press. 1a.ed., 1987.

Potential conflicts are thus an intrinsic element of family businesses. Commitment and good communication skills are essential to prevent and overcome those disagreements. For Ward (2004), the continuity of the family business requires consistent planning, which comprises simultaneous planning of the business strategy, estate and personal financial planning of the founders/owners, a leadership and ownership succession plan and a family continuity plan.

RESULTS AND DISCUSSION

The results suggest that the continuity of the farm as an entrepreneurship, though desirable, is not part of the main concerns of the rural producer. The dynamics of the coffee production nowadays involves intensive use of technology, appreciation of quality attributes, environmental issues, among other aspects which absorb his time and dedication. Furthermore, communication among family members over the topic of succession is highly complex.

In spite of that, the subjects interviewed admit that preparing a successor that belongs to the family is crucial to the future of a coffee farm. According to them, one reason is that volatility of coffee prices and the occurrence of climate events (droughts, frosts, heavy rains) make the coffee farm quite an unpredictable business. Another is that investment decisions take a long time to mature. Therefore, the farm cannot rely on professional executives that have little emotional connection to it and could leave at anytime. Besides, hiring such professionals would require economies of scale that most coffee farms do not have.

The two estate owners interviewed have had a son working with them for at least five years. According to them, differences in style and points of view are common and sometimes make working together difficult. However, the presence of the sons in the management of the farms has contributed to their confidence in investing and expanding each one´s business.

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Drought Tolerant Coffee Varieties: Development Programme in Tanzania

D. MTENGA[1], D. L. KILAMBO[1], R. NGOMUO[1], E. MNDOLWA[2], E. O. NKYA[1], J. M. TERI[1]

[1]Tanzania Coffee Research Institute (TaCRI), P.O. Box 3004 Moshi, Tanzania
[2]Sokoine University of Agriculture, P. O. Box 3005 Morogoro

SUMMARY

Drought due to the global warming is becoming a serious constraint on *Coffea arabica* production worldwide with serious threats on producers' earnings. In most developing countries like Tanzania small-scale producers are the main coffee producers; they lack irrigation facilities and therefore they are the most vulnerable group. Development of drought tolerant coffee arabica varieties could be a good strategy to assist them especially those who are in coffee production marginal areas. Genetic improvement may sound the most appropriate approach but due to the complexity of the trait, integrated approaches may be more relevant. In this work an attempt on horticultural approach as short term solution was tried out. Several genotypes of both *Coffea arabica* and *Coffea canephora* root stock known to be drought tolerant from literature were grafted with scions from improved disease resistant *Coffea arabica* hybrid variety. The grafted seedlings together with lines from the breeding programme were evaluated in drought prone coffee growing marginal areas to establish their levels of tolerance. Preliminary results are reported in this work.

INTRODUCTION

Coffee is one of Tanzania's primary agricultural export crop generating export earnings averaging USD 100 million per annum over the last thirty years. More than 90 percent of Tanzanian coffee comes from smallholder farmers. The industry provides direct income to more than 400 000 farmer families and also benefits indirectly the livelihoods of over 2.5 million Tanzanians (TCB and TaCRI. 2010). One of the noticeable challenges to Tanzanian coffee industry is the changing climatic conditions resulting into unreliable and unpredictable rainfall in the areas traditionally cultivating coffee. Mean annual temperatures have increased and total precipitation has declined in all eight East African countries over the past 40 years. This change to a hotter and drier environment has coincided with a decline in both the number of optimal and tolerable arabica coffee growing locations across the region (Ridley, 2011). Arabica coffee is native to Ethiopian tropical forests at altitudes of 1600-2800 m. In this region, air temperature shows little seasonal fluctuation, with an annual average of about 20ºC. Rainfall is well distributed ranging from1600 - 2000 mm with dry season lasting three to four months. Coffee cultivation in producing countries has spread towards marginal lands where rainfall and water shortage and unfavorable temperatures is a limit coffee yield (DaMatta and Ramalho, 2006). Drought refers only to a period in which rainfall fails to keep up with potential evapotranspiration (DaMatta, 2003). Drought inhibits growth, yield and reduces quality of the produce. Although coffee requires a defined period of drought, extended water stress damages the plant. Crop yields in rainfed systems may decrease 40–80% during drier El Nin˜o years (DaMatta et al., 2003). Slow imposition of drought allows the development of a range of time-dependent morphological and physiological acclimation responses. Root characteristics and growth play a crucial role in maintaining the water supply

to the plant, and drought adapted plants are often characterized by deep and vigorous root systems. Development of coffee cultivars that could withstand severe drought spells with acceptable yields is the first requirement for a successful breeding programme for drought tolerance (DaMatta and Ramalho, 2006).

MATERIALS AND METHODS

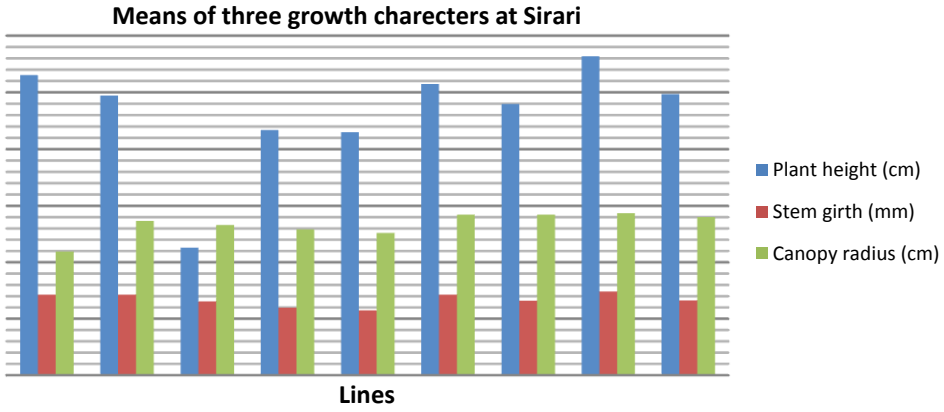
Nine treatments comprising of three lines of improved diseases resistant coffee arabica hybrids derived from the crosses involving the variety SL28 (Tanganyika drought tolerant), one improved officially released variety and five lines obtained by grafting a scion of improved arabica hybrid on the root stocks of K7, Salvatrix, Robusta, KP423, SL28 and Catuai were used in this study. These materials were obtained from the Tanzania coffee research institute (TaCRI) breeding programme. The seedlings of the hybrids were raised from the clones from the selected mother plants. The rootstocks were raised from seeds of drought tolerant selections while scion was taken from improved released hybrids. A randomized complete block design with three replications with eight central trees per treatment was adopted. Trials were established at Sirari, Rombo and Mbozi representing the main coffee arabica growing zones in Tanzania.

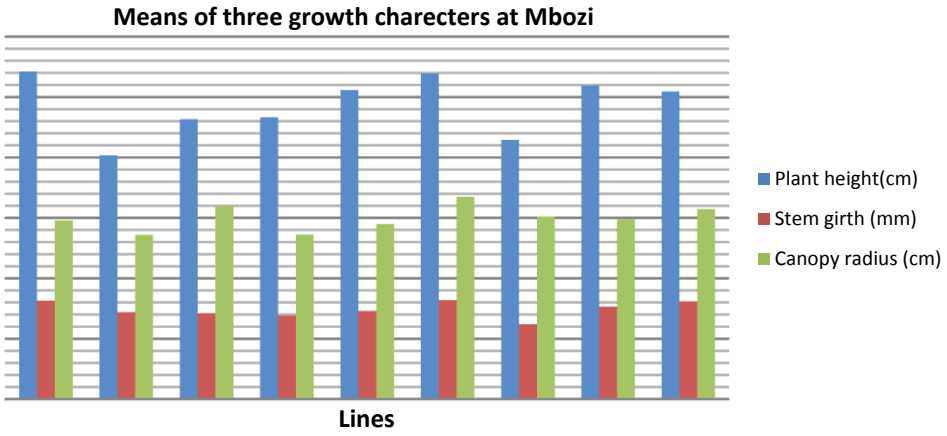
Data was recorded on stem girth, measured using vernier caliper at five centimeter from the ground, plant height was measured using a two meter ruler from the ground to the growing tip and canopy diameter was obtained by measuring the length of the alternate primaries at the upper third of the randomly selected plants for each treatment.

Line	Type of material
1	SL28 hybrid
2	Compact SL28 hybrid
3	K7 rootstock + N39-3 scion
4	Robusta rootstock + KP423-1 scion
5	Salvatrix rootstock + KP423-1 scion
6	Catuai rootstock + Kp423-1 scion
7	Tall hybrid N39-3
8	SL28 rootstock + N39-3
9	KP423 rootstock + N39-3 scion

List of Lines used in the evaluation.

RESULTS AND DISCUSSION





Preliminary observation on growth behavior under limited moisture in field condition showed that lines 1, 6, 8 and 9 had good vigour compared with the rest in both sites from different coffee arabica growing zones in Tanzania. The control lines 8 and 9 had good performance similar to lines 1 and 6. Stem girth was consistent for almost all line in the two sites. Canopy radius was generally better at Mbozi site than at Sirari for all lines.

CONCLUSIONS

Since this is the first data just after establishment, more data on yield and how the lines can withstand the drought under good crop is needed to guide selection of the candidate variety.

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Progress with Somatic Embryogenesis of Improved Hybrids Coffee Varieties in Tanzania

R. NGOMUO, D. L. MTENGA, D. L. KILAMBO, J. M. TERI

Tanzania Coffee Research Institute P.O. Box 3004 Moshi, Tanzania

SUMMARY

Experiments to evaluate the response of different Tanzania *Coffea arabica* hybrids and *Coffea canephora* clone to multiplication by somatic embryogenesis (SE) at Tanzania Coffee Research Institute (TaCRI) Lyamungo from September 2009 to 2011. Fourteen genotypes were *C. arabica* hybrids and one was *C. canephora* clone. The initiation of callus followed standard procedures adopted from Nestlé Research & Development Centre, France. The research materials were fully expanded fresh leaves sampled from the trees of the selected genotypes from the field as well as the clonal garden. The response to disinfection reflected the sources from which the initial material came from; with the ones from the clonal garden giving better results. High variations in calli formation were observed among the genotypes. Nine *C. arabica* hybrids and the *C. canephora* clone produced aqueous callus within the first month of culture. The other five *C. arabica* hybrids produced aqueous calli in the second month. Six months after culture initiation, embryogenic calli were abundant (70-80%) in five *C. arabica* hybrids and the *C. canephora* clone. The embryogenic calli were multiplied per genotypes and converted to embryos in RITA system. The somatic embryos were developed, successfully harvested, and hardened with their response to acclimatization recorded. Potted fully developed embryos were raised in the nursery for field planting.

INTRODUCTION

Due to its considerable multiplication potential, somatic embryogenesis constitutes the greatest possibility for large-scale clonal propagation of elite plants (Ammirato and Styer 1985). The use of liquid media enables the embryogenic tissue proliferation and mass production of somatic embryos in bioreactors or Erlenmeyer flasks of about 20 species, including coffee (Van Boxtel and Berthouly 1996). Tanzania has released 14 *C. arabica* hybrids and five *C.canephora* varieties and more are in pipe line for release with good yield, cup quality and having resistance to the major diseases of coffee leaf rust (CLR) and coffee berry disease (CBD) for *C.arabica* and coffee wilt disease (CWD) for *C.canephora*. The major challenge is rapid multiplication and distribution of these varieties to farmers to enable them replaces the old disease susceptible varieties. The demand for replacement of old trees with improved clones is in the area of 200 million trees. The current potential for replacement through grafting and cutting is an annual production of 14 million seedlings. Among in vitro culture techniques, somatic embryogenesis has been used for micro propagation and genetic modification of higher plants. (Ogita et al. 2002). In coffee, this process can be achieved via direct somatic embryogenesis (DSE) from proembryogenic cells of the tissue in the absence of embryogenic callus or by indirect somatic embryogenesis (ISE) via callus formation (Jiménez 2001, Molina et al. 2002). Somatic embryogenesis is expected to contribute significantly to this effort.

MATERIALS AND METHODS

Leaf samples were collected from field and clonal garden. The samples were brought in the tissue culture Laboratory, washed with tap water then rinsed with distilled water before dipping for 30 seconds in a 70% ethanol solution. They were then surface sterilized for 30 minutes under agitation in a solution of 3.5% Sodium hypochlorite ($NaCl_2O_2$) and finally rinsed three times in sterile water. The leaves were cut in small explants (1cm²) avoiding mid veins, margins, apical and basal portions. A glass beads sterilizer maintained at 250°C and absolute ethanol and flame were used frequently to sterilize the tools during the manipulation. Five explants were plated per Magenta bottle (diameter 6cm), containing 20 ml of T1 media (Murashige and Skoog, 1962) media for *C. arabica*. For *C.canephora* clones, they were transferred to 23A8 (Yasuda *et al*., 1985) media. The upper epidermis was in contact with the medium. The explants were incubated in darkness at 25±1°C for 1-2 months after which they were sub cultured on fresh media of the same protocol. Then after two sub cultures, the media was changed to T2 media of the same protocol and cultures were placed in the same conditions. During sub culture the aqueous callus and contaminated Petri dishes were discarded. Evaluation was done at 10 and 7 months for *C.arabica* and *C.canephora* respectively. Somatic embryos were placed in a temporary immersion liquid media to produce torpedo shaped embryos following Van Boxtel protocol. Maturation of torpedo to cotyledonary stage embryos was conducted using RITA system. After 2 months the cotyledonary embryos were harvested rinsed with NPK solution (1g/L) for 5 minutes. Finally green somatic embryos which are able to photosynthesize transplanted to the greenhouse for acclimatization to get full germinated plantlets.

RESULTS AND DISCUSSION

From table 1, both samples from clonal garden and field showed high level of contamination but were worse for the samples coming from field with range of 56% to 90 % with an average contamination of 73.87%. On the other hands samples from clonal garden slightly showed low level of contamination which ranged from 38% to 70 % with an average contamination of 46.93% which was low compared to field samples by 26.84%. However from the two studies at pre-selection stage isolation of clean explants showed that the percentage of callogenesis was closely the same regarding where the materials came from. Material from clonal garden has a range of 0% to 45.16% with an average of 21.08% while the materials direct from field ranged from 0% to 50% with an average of 24.43%. Observation of individual genotype from table 1 genotype N39-1 and 27-V-15-7 failed to produce callus till time of evaluation for both materials from field and clonal garden, also genotype N39-3 for the materials from clonal garden but showed low level of callogenesis (6.67%) for materials from field in contrast with genotype N39-3 which showed 0% callogenesis for materials from field and 3.85% for materials from clonal garden.

Table 1. Callus formation and rate of contamination of explants from clonal garden and Field 1 month after Culture.

Genotype	Clonal Garden						Field					
	A	***B***	***b***	***C***	***D***	***E***	***A***	***B***	***b***	***C***	***D***	***E***
KP423-2	50.0	28.0	22.0	8.0	28.6	44.0	50.0	10.0	40.0	2.0	20.0	80.0
N39-5	50.0	30.0	20.0	6.0	20.0	40.0	50.0	8.0	42.0	1.0	12.5	84.0
CVT2-3	50.0	32.0	18.0	4.0	12.5	36.0	50.0	5.0	45.0	1.0	20.0	90.0
N39-2	50.0	20.0	30.0	0.0	0.0	60.0	50.0	12.0	38.0	0.0	0.0	76.0
CVT2-11	50.0	25.0	25.0	5.0	20.0	50.0	50.0	20.0	30.0	5.0	25.0	60.0
N39-1	50.0	27.0	23.0	11.0	40.7	46.0	50.0	17.0	33.0	7.0	41.2	66.0
N39-7	50.0	30.0	20.0	10.0	33.3	40.0	50.0	22.0	28.0	10.0	45.5	56.0
KP423-3	50.0	23.0	27.0	7.0	30.4	54.0	50.0	21.0	29.0	9.0	42.9	58.0
N39-3	50.0	26.0	24.0	1.0	3.8	48.0	50.0	10.0	40.0	0.0	0.0	80.0
27-V-6-4	50.0	38.0	12.0	6.0	15.8	24.0	50.0	5.0	45.0	1.0	20.0	90.0
CVT1-2	50.0	31.0	19.0	8.0	25.8	38.0	50.0	10.0	40.0	4.0	40.0	80.0
27-V-15-7	50.0	22.0	28.0	0.0	0.0	56.0	50.0	12.0	38.0	0.0	0.0	76.0
CVT1-1	50.0	31.0	19.0	14.0	45.2	38.0	50.0	21.0	29.0	9.0	42.9	58.0
N39-6	50.0	20.0	30.0	0.0	0.0	60.0	50.0	15.0	35.0	1.0	6.7	70.0
ROBUSTA	50.0	15.0	35.0	6.0	40.0	70.0	50.0	8.0	42.0	4.0	50.0	84.0
MEAN	50.0	26.5	23.5	5.7	21.1	46.9	50.0	13.1	36.9	3.6	24.4	73.9

A=Number of initial explants, B=Number of clean explants, b=Number of contaminated explants, C=Number of explants with callus, D=% explants with callus E=% of contaminated explants.

Most of the explants developed reasonably amount of callus and few were lagging behind (Table.1). On other hands the rate of contamination gave an average of 0% for the materials coming from clonal garden while for the materials from field showed an average of 2.56% and this may be explained by the fact of slow growing contaminants. Both samples gave a range of 10-90% callus development giving the evidence that no difference of callus formation with regards to the source of material but rather the difference is explained by the rate of contamination. The highest rate of callogenesis was observed by CVT1-2 (90%), N39-1 (88.89%) and 27-V-6-4 (86.64%) while the lowest by genotype N39-1 and N39-3 which gives 10%, and N39-6 (11.54%).

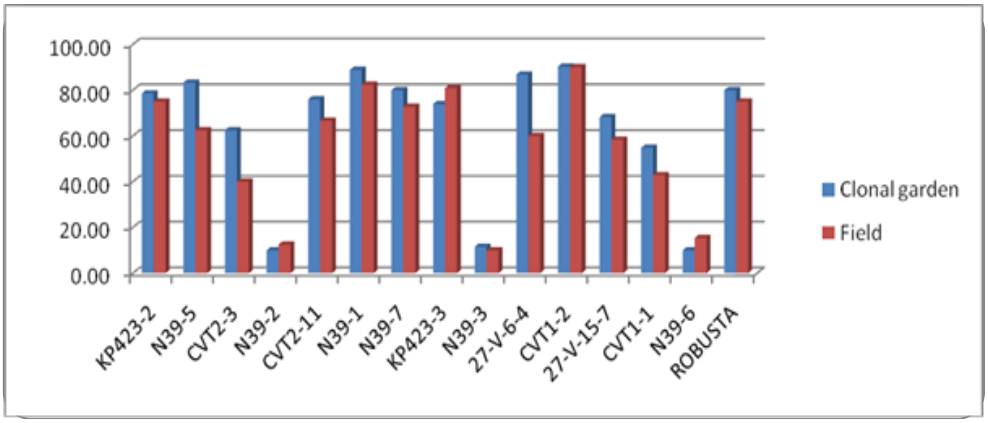

Figure 1. Callogenesis of different genotypes as observe 2 month after culture.

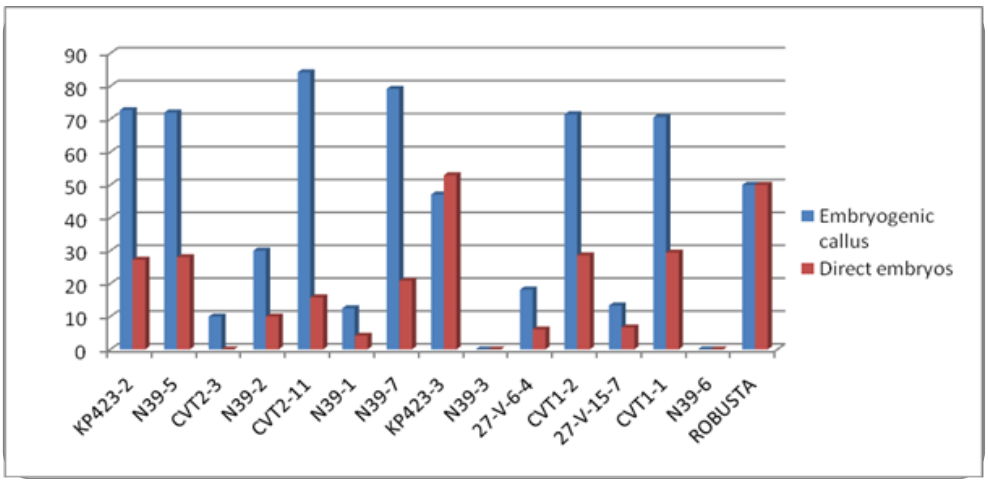

Figure 2. Embryogenic callus (HFSE) and direct embryos (LFSE) formation as assessed 10 month after culture.

The Figure 2 shows the percentages of embryogenic callus (HFSE) as well as direct embryos (LFSE) formed per each genotype as assessed at the end of 10 month of culture. Seven genotypes gave good prospect by producing embryogenic callus with up to 83% for genotype CVT2-11, 78% for N39-7 and 50% for Robusta genotype. Furthermore direct embryos were found to be 21.1%, 20.8% and 21.1% for CVT2-11, N39-7 and 27-V-6-4 genotypes respectively. Poorly embryogenic callus development was observed to be 0% for genotype N39-2, N39-3 and N39-6.

CONCLUSIONS

- The tested TaCRI genotypes responded well to S.E.
- It is clear that the enormous embryogenic potential of coffee is far from being fully exploited and that several improvements could be expected to reduce the cost production.

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Evaluation of Tanzanian Robusta Coffee Varieties on Cup Taste and Bean Sizes

N. M. NG'HOMA[1], J. M. TERI[2], P. KUSOLWA[3], D. P. MAMIRO[3], D. L. KILAMBO[2]

[1]Tanzania Coffee Research Institute (TaCRI) Maruku: P. O. Box 127 Bukoba, Tanzania
[2]Tanzania Coffee Research Institute (TaCRI) Lyamungu:
P.O.Box 3004, Moshi, Tanzania
[3]Sokoine University of Agriculture, Morogoro, Tanzania
P.O.Box 3005, Sokoine University of Agriculture, Morogoro, Tanzania,
department of Crop Science and Production

SUMMARY

Robusta coffee accounts for 30-44% of the total Tanzania coffee exports and about 68.13% farm-families in Kagera depend on this crop for their income security and livelihoods. One of the great achievements of the Tanzania Coffee Research Institute (TaCRI) was the release this year of four improved Robusta varieties that combine good beverage quality with resistance to the devastating coffee wilt disease (CWD). The Tanzania robusta coffee is known for its beverage quality that we strive to maintain and/or improve in our efforts to develop high yielding varieties with resistance to diseases. We report here results of our work to evaluate the beverage quality and bean sizes of the newly released varieties. The experiment was in a Randomized Complete Block Design (RCBD) with three replicates. The treatments were the four new varieties, Bukoba1, Maruku1, Maruku2 and Muleba1 with MS1, a CWD susceptible commercial variety as a check. Ripe cherries were harvested, sun dried, hulled, winnowed, sorted to remove broken and black beans. Clean beans were graded using screen sieves numbers 18, 16, 14 and 12. Samples from the treatments were subjected to beverage tasting. Results showed significant ($P \leq 0.001$) variations on the bean sizes among the treatments. With sieve number 18, Muleba1 had 91% of its beans retained. The results also showed significant ($P \leq 0.001$) different in the bean sizes of Robusta varieties using screen sieves numbers 16 and 14. Beverage assessment of these varieties varied with Maruku1, Maruku2, Bukoba1 and Muleba1 cups described as of natural Robusta flavour. Beverage assessment of MS1 was described as of bitter Robusta type. Varieties with high proportions retained in sieve 18 were described as of natural Robusta flavour and one of the varieties was described as having Arabica taste.

INTRODUCTION

Robusta coffee is one of the two types of coffee (Arabica and robusta) grown in Tanzania. Robusta coffee is grown in Kagera region by more than 250,000 farm families and accounts for 30– 44% of the national coffee export. Production of robusta coffee has several constraints (NEI 1994), and Kilambo *et.al.* 2010). These included small seeds with poor quality cultivars, low productivity due ageing of coffee trees, poor coffee husbandry practices and invasion of coffee wilt disease. The later being a serious problem. In 2011 the Tanzania Coffee Research Institute (TaCRI) released four Robusta varieties based on yielding ability and resistant to CWD. These are: Maruku 1, Maruku2, Muleba 1 and Bukoba 1. The varieties were evaluated at on farm on CWD hot spot areas to verify their performance. This study therefore, was conducted to evaluate the bean sizes and cup quality of the newly released robusta varieties that are resistant to coffee wilt disease for commercial production.

MATERIALS AND METHODS

An experiment was superimposed in the coffee field established at Maruku in 1998 using Randomized Complete Block Design (RCBD) with three replicates. Four varieties which were released for commercial multiplication in 2011 based on their resistant to coffee wilt disease were selected for evaluation for bean sizes and beverage quality. These varieties were Bukoba1, Maruku1, Maruku 2 and Muleba 1. Maruku selection 1 (MS1) susceptible to CWD was included as a check.

Ripe cherries were harvested and one kilogramme per each treatment was taken as sample for bean sizes and cup quality analysis. The samples were sun dried for 21 days. Dried samples were hulled using hand huller. Hulled samples were winnowed to remove coffee husks and other chaffs. Green beans were sorted to remove black and broken beans. Sorted green beans were graded using screens with dimensions 7.10 mm (screen over 18), 6.30 mm (superior, screen 16), 5.60mm (screen 14) and 4.75 mm (screen 12). Samples of 0.25 kg of green beans from each treatment were sent to cuppers in Moshi for cup quality analysis (beverage assessment). Data for different bean sizes were computed to percentages and then analyzed using GENSTAT statistical package.

RESULTS AND DISCUSSION

Bean sizes

Figure 1 summarizes the bean sizes of the five Robusta coffee varieties grown in Bukoba district in Kagera region. The bean sizes of these varieties varied significantly ($P \leq 0.001$). The variety Muleba 1 had the largest beans of which more than 91% were retained in screen of 7.10 mm (over 18) followed by Maruku, control variety (MS1), Bukoba 1 and Maruku 2. In general, the beans of tested varieties were retained on over 18 and superior 16 screens that qualifying them to be grade 1 coffee. These findings conform to those reported by Kilambo *et. al* (2010).

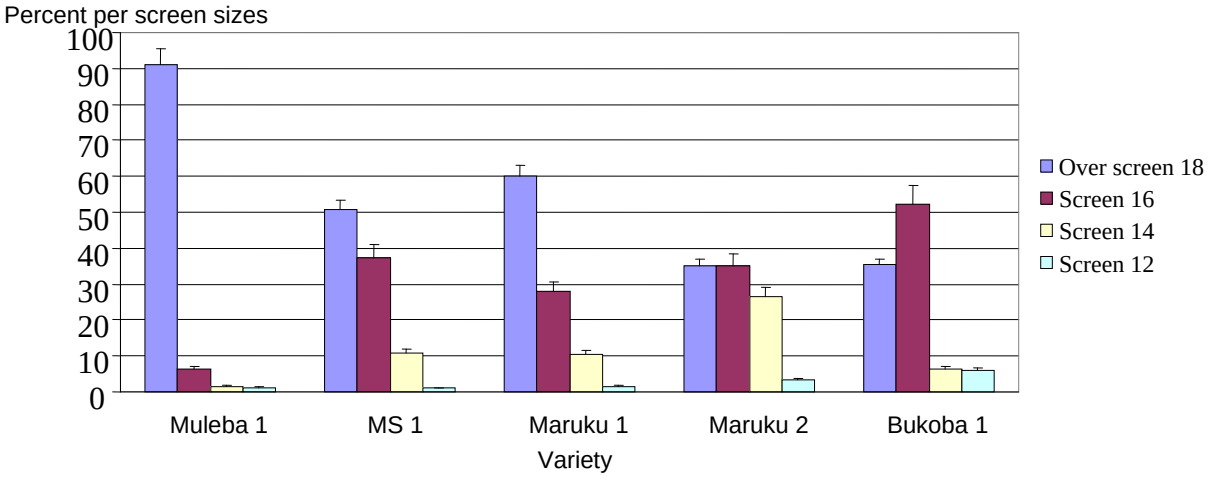

Figure 1. Bean sizes of the five Robusta varieties grown in Bukoba district Kagera region, Tanzania.

Beverage quality

The results of beverage quality of the five evaluated robusta materials are presented in table 1.

Table 1. Summary of beverage assessment of the five evaluated Tanzanian Robusta varieties.

S/no.	Variety	Description	Remarks
1	Maruku1	Typical natural robusta coffee	neutral cup
2	Bukoba1	Natural robusta coffee	clean/ smooth cup
3	Maruku2	Smooth cup, balanced cup, nice aroma like arabica	clean cup
4	Muleba1	Clean cup, typical natural robusta coffee	clean cup
5	Maruku section 1	Bitterness, unusually robusta acid	average cup

The beverage assessment revealed the diversity of Tanzania robusta varieties. These findings are similar to those reported by Moschetto *et. al.*, (1996) and Ky *et. al*., (2001) who described the presence of diversity in the cup taste of *C. canephora* in terms of aroma, acidity, body and bitterness ranging from average to excellent cup taste. The findings from this study showed that most of the Tanzanian robusta coffee from Kagera region have clean cup. These finding conform to those reports reported by EFCA during the exhibition competitions held in Uganda in 2004 and Ethiopia 2012 whereby the robusta coffee from Kagera region in Tanzania ranked number one among the best robusta coffee in the world.

CONCLUSIONS

The study shows that the newly released Robusta varieties: Maruku 1, Maruku 2 and Muleba 1 combine high yields and resistance to coffee wilt disease with large bean sizes and good beverage quality. The variety, Maruku 2 was the most outstanding in beverage quality that was described as smooth and balanced cup with aroma like Arabica.

ACKNOWLEDGEMENTS

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The Nitrogen Mineralization Potential of Two Coffee Soil Systems of Northern Tanzania when Treated with Different Organic Materials

G. P. MARO[1], J. P. MREMA[2], B. M. MSANYA[2], J. M. TERI[1]

[1]Tanzania Coffee Research Institute (TaCRI), P.O.Box 3004, Moshi, Tanzania
[2]Soil Science Department, Sokoine University of Agriculture, P.O.Box 3008, Morogoro, Tanzania

SUMMARY

An incubation experiment was conducted at TaCRI Lyamungu Screenhouse to establish the nitrogen release potential of two contrasting soil systems of Northern Tanzania, when treated with different types of organic substrates. Soils were Eutric Nitisols of volcanic origin obtained from Lyamungu, Hai district, and Humi-Umbric Acrisols of gneiss origin from Yoghoi Prisons Farm, Lushoto district. The tested organics were cattle manure, coffee leaves, pulp and husks, Albizzia leaves and four green manure plants – *Mucuna pruriens*, *Lupinus pilosus*, *Canavalia ensiformis* and *Crotalaria ochroleuca*. They were mixed with the soils at 5% ratio, moistened to FC and incubated in 10 litre plastic containers arranged in RCBD (10 treatments and 3 replications) in the screenhouse at room temperature. Duplicate soil samples were taken at day 0, 3, 8, 15, 26, 45, 74, 112 and 180 and analyzed for NH_4^+-N and NO_3-N. Release trends were exposed to descriptive statistics while the Nmin values resulting from the treatments were exposed to 2-way ANOVA under COSTAT statistical package, with means separated by Tukay's HSD at 0.05 level of significance.

The Nmin release trends showed that NH_4^+-N reaches peak release earlier than NO_3-N. Nmin release varied significantly ($P<0.001$) among the organics and between the two soil types. The Yoghoi Acrisols were slightly more efficient in Nmin release than the Lyamungu Nitisols. The green manure plants (*Crotalaria*, *Mucuna*, *Canavalia* and *Lupine*) were tentatively picked as best bets for inclusion in the coffee ISFM programme, and future research will focus on the most appropriate method of application in a coffee field.

INTRODUCTION

Coffee is one of the major export crops in Tanzania. Average coffee production is variably pegged between 45,000 and 52,000 metric tons, while smallholder coffee productivity per tree ranges between 250 and 300g of parchment (Hella *et al*., 2005) which is very low. Soil fertility degradation in coffee farms and escalating costs of industrial fertilizers constitute one of the major problems facing coffee productivity in Tanzania (TCB, 2009; Maro *et al.* 2010).

Tanzania Coffee Research Institute (TaCRI) aims at promoting integrated soil fertility management (ISFM), which includes use of organic materials in the coffee ecosystems, for improved and sustainable productivity. Efforts in coffee ISFM include research on coffee by-products done in India (Korikanthimath and Hosmani, 1998) and Zimbabwe (Chemura *et al*. 2008) among others. Such studies have not been intensively done in Tanzania. An experiment was therefore done to investigate the nitrogen release potential of two contrasting coffee soils of Northern Tanzania when treated with different types of organic materials available in a

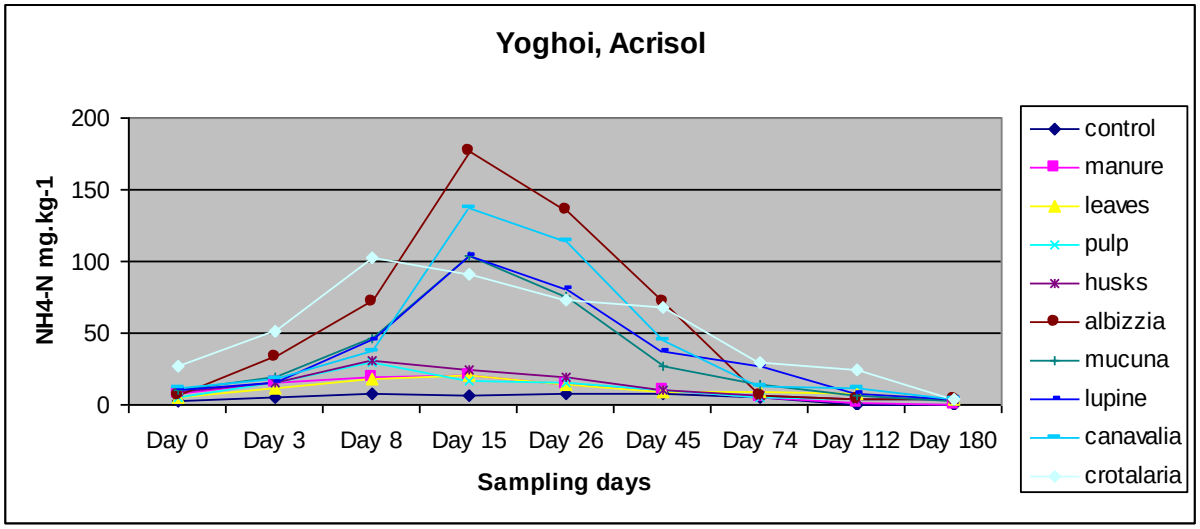
coffee farming system and identify appropriate organic materials to include in the coffee ISFM programme.

MATERIALS AND METHODS

The incubation experiment was conducted at TaCRI Lyamungu Screenhouse. Soils were collected from Lyamungu, Hai district (Field 46), representing Eutric Nitisols of volcanic origin, and Yoghoi Prisons Farm, Lushoto district, representing Humi-Umbric Acrisols of gneiss origin. The tested organics were cattle manure, coffee leaves, pulp and husks, Albizzia leaves and four green manure plants – Velvet bean (*Mucuna pruriens*), Lupine (*Lupinus pilosus*), Jackbean (*Canavalia ensiformis*) and Sunhemp (*Crotalaria ochroleuca*). They were mixed at 5% organics to soil ratio, moistened to field capacity (FC) and incubated in 10 litre plastic containers arranged in RCBD (10 treatments and 3 replications) in the screenhouse at room temperature. Moisture level was maintained around FC by misting twice a week with a hand sprayer. Duplicate soil samples were taken at day 0, 3, 8, 15, 26, 45, 74, 112 and 180. 20g of moist soils were placed in 500 ml Erlenmeyer flasks and 200 ml of 2M KCl solution were added to the flasks, shaken for 40 minutes and filtered through Whatman filter paper no 42. NH_4^+-N and NO_3-N from soil extracts were measured by steam distillation procedure using MgO and Devarda's alloy. Results were exposed to descriptive statistics where means were plotted against the dates sampled. Cumulative total Nmin was calculated and values for Day 0 were subtracted from the totals to get the Nmin released only during the time of the experiment. The values for the untreated control were also subtracted to remain with the Nmin resulting from the treatments. These were exposed to 2-way ANOVA under COSTAT statistical package, with means separated by Tukay's HSD at 0.05 level of significance.

RESULTS AND DISCUSSION

Figures 1 and 2 show the trends in release of NH_4-N and NO_3-N for the two soil types. Peak NH_4^+-N release was attained between Day 8 and Day 45 for both Yoghoi and Lyamungu, accounting for 62-89% and 58-90% respectively of the total NH_4^+-N released. With NO_3-N, the two soil types differed in the time of peak release. Lyamungu attained peak release between Day 15 and Day 74, which accounted for 41-92%, while Yoghoi attained peak realease between Day 26 and Day 102, accounting for 34-87%. Similar trends were observed by Vimlesh and Giri (2009) in their study on domestic sludge.



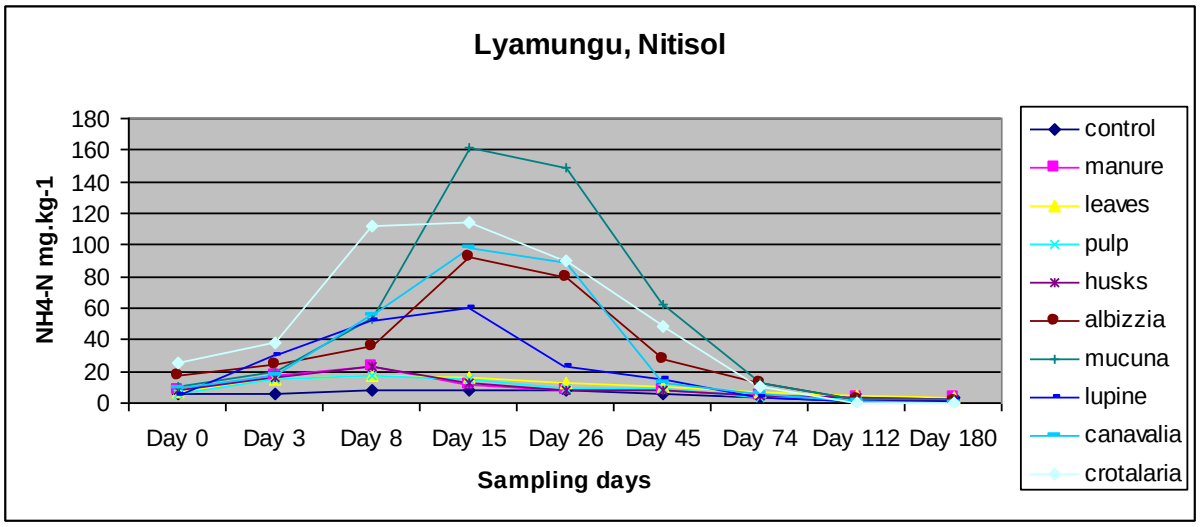
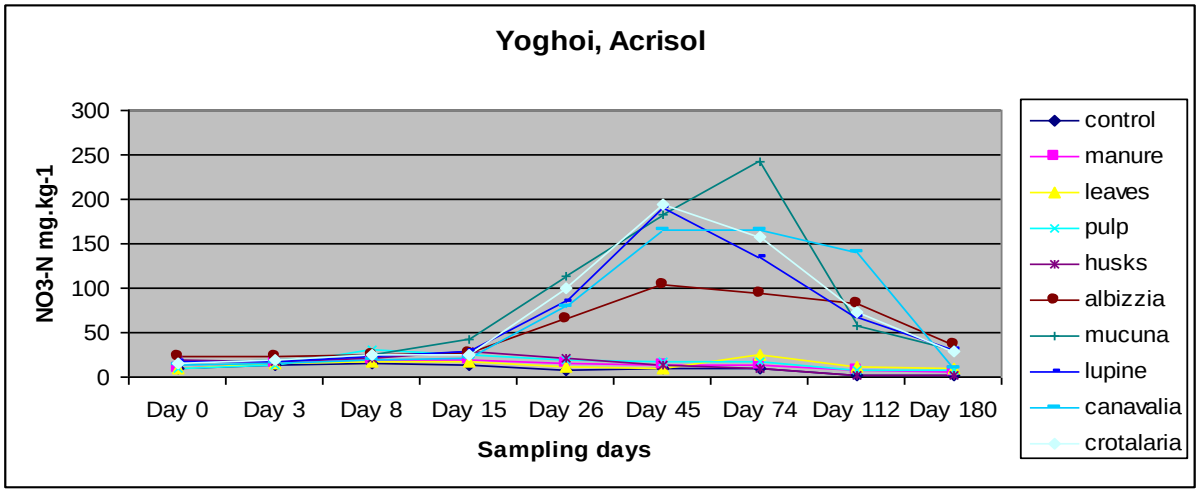


Figure 1. The ammonium N release trends for the two coffee soils.



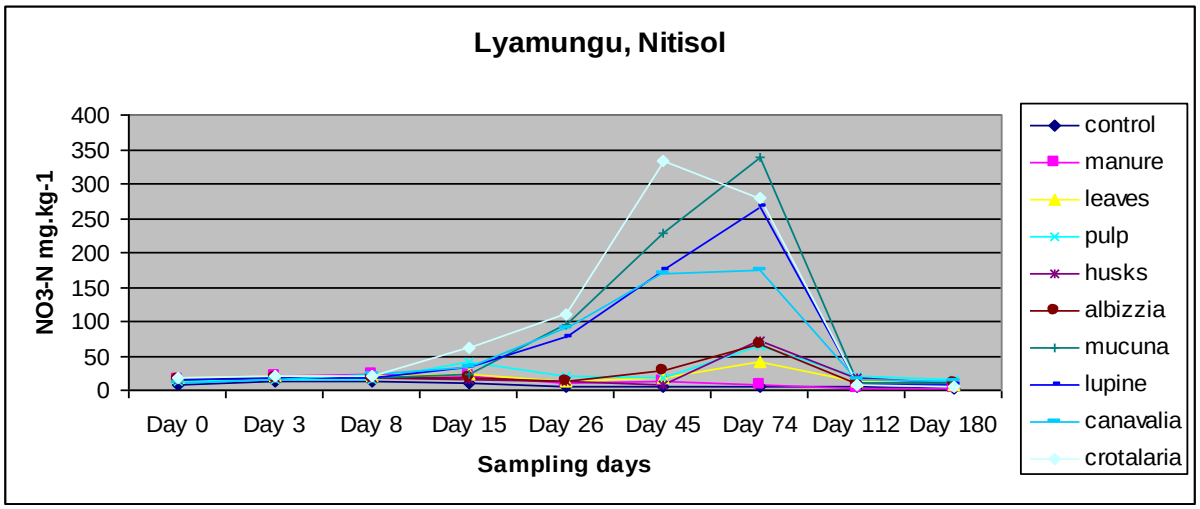


Figure 2. The nitrate N release trends for the two coffee soils.

The observed stagger in peak release time between NH_4^+-N and NO_3-N is in line with the nitrogen cycle explained by Brady and Weil (2002) and Pidwirny (2006). Nitrogen mineralization from organic materials starts with NH_4^+-N formation and a further

transformation is needed through NO_2-N to NO_3-N, hence the delay in NO_3-N accumulation.

Table 1. Analysis of Variance for total Nmin release.

Source	df	Type II SS	MS	F	P	Sign
Replicates	2	70.37029939	35.18515	0.0769784	.9261	ns
Main Effects Organics	8	5930957.369	741369.67	1621.9752	.0000	***
Soil types	1	31460.63787	31460.638	68.829867	.0000	***
Interaction Organics x soils	8	472963.4233	59120.428	129.3442	.0000	***
Error	34	15540.66183	457.07829			
Total	53	6450992.462				
Model	19	6435451.8	338707.99	741.02839	.0000	***

The Nmin release from the tested organics showed highly significant variations ($P<0.001$) among the organics and between the two soil types (Table 1). As expected, replicates were not significant ($P >0.05$) as they belonged to the same combinations. These results are in line with those of Kwabiah *et al* (2001) in their work on decomposing leaves. Slight replicate variation was noted, which could be attributed to possible variations in moisture conditions (Moyano and Chenu, 2011). The model was also highly significant with R^2 of 0.9976, RMSE of 21.3794 and CV of 4.6%. From Tukey's HSD, the four green manure plants emerged top of the list, in the order *Crotalaria* > *Mucuna* > *Canavalia* > *Lupine*. *Albizzia* leaves came next in the list, performing better with the Acrisols than the Nitisols. There was a clear distinction between these and the last four (Pulp> Husks> Leaves > Farmyard Manure), whereby *Albizzia*, the last in the upper list, was about 5 times coffee pulp, the first in the lower list. The Acrisols of Yoghoi gave average Nmin of 488.56 $mg.kg^{-1}$, which was higher than the average Nmin of 440.29 $mg.kg^{-1}$ from the Nitisols of Lyamungu.

CONCLUSIONS

The nitrogen release potential of two contrasting coffee soils of Northern Tanzania when treated with nine types of organic materials available in a coffee farming system was studied in this work. It was noted that the Yoghoi Acrisols are slightly more efficient in Nmin release than the Lyamungu Nitisols. The release trends showed that NH_4^+-N reaches peak release earlier than NO_3-N. Four green manure plants (*Crotalaria*, *Mucuna*, *Canavalia* and *Lupine*) were picked as best bets for inclusion in the coffee ISFM programme. The challenge remains the appropriate application techniques in coffee farms, which will be pursued in later phases of this work.

ACKNOWLEDGEMENTS

This work is part of a PhD research programme of Sokoine University of Agriculture, and the authors are indebted to the TaCRI management and the staff of Soil Science Department (SUA) for their guidance. Special thanks to the European Commission (EC) and Tanzanian coffee stakeholders for financing this activity, and to TaCRI staff E. Mosi, E. Mdoe and J. Kiwia who helped in the screenhouse and laboratory work.

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Survey for Natural Enemies of Coffee Berry Borer, *Hypothenemus Hampei* in Kilimanjaro Region, Tanzania

F. L. MAGINA[1], A. P. MAERERE[2], G. P. MARO[1], J. M. TERI[1]

[1]Tanzania Coffee Research Institute (TaCRI), P.O Box 3004, Moshi, Tanzania
[2]Sokoine University of Agriculture (SUA), P.O Box 3005, Morogoro, Tanzania

SUMMARY

A survey was carried out in coffee fields' at Tanzania Coffee Research Institute (TaCRI), Lyamungu to determine potential of natural enemies of coffee berry borer (CBB) from April 2010 to September 2011. The objective of this study was to identify natural enemies of coffee berry borer (CBB) as one of the TaCRI strategies to manage the pest. Total of 1590 full expanded green and ripe berries were collected in the field and incubated in boxes (30cm x 30cm x 10cm) at TaCRI, Lyamungu. These parasitoids were preserved in 40% Formaldehyde solution in plastic vials and sent for identification at the International Centre of Insect Physiology and Ecology (*icipe*) Nairobi, Kenya. Two bethylid wasps (*Cephalonomia stephanoderis* and *Prorops nasuta*) were identified as potential parasitoids of CBB for Arabica and Robusta coffee in the study area. Additionally, 110 invasive fruit flies emerged from the same coffee berries and were preserved in 75% and taken to Sokoine University of Agriculture (SUA), for identification. These were identified as *Ceratitis capitata, Ceratitis rosa* and *Trirhithrum coffeae.* It is the first time that invasive fruit-flies were identified in coffee in Tanzania.

INTRODUCTION

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytidae) is one of the most important and widespread insect pests affecting coffee production worldwide (Jaramillo, *et al;* 2006). In Tanzania, it is a major insect pest of Robusta and low altitude Arabica coffee (Le Pelley, 1968). A recent study by Magina *et al.*, (2010) reported infestation of farms by the pest at medium altitudes (1200-1600 m. a. s. l.) in Kilimanjaro region. Both adult and larval stages cause damage by feeding inside immature and mature berries, resulting in staggering crop loss of up to 60% or more, as well as reduction in quality of the remaining crop (Le Pelley, 1968). Present control strategies of the pest mostly rely on application of broad spectrum synthetic insecticide like Endosulfan and Chlorpyrifos (Jaramillo *et al.,* 2005; Magina, *et al.;* 2010). However, these insecticides are expensive and hazardous to the environment, the farmers who use them and the community living adjacent to the treated coffee plantations (Jaramillo *et al.*, 2005). Additionally, they seem to be surface-active, and thereby unable to control the pest that has entered the berry. This may have possibly resulted to problems of pest resistance to insecticides.

Currently the pest is managed by use of cultural methods such as: prune regularly to reduce heavy shade which is preferred by the pest, pick ripe berries regularly, at least two weeks to reduce more breeding of the pest, removal of leftover of berries and off season crop immediately prior to flowering, destruction of infested berries by burning, so as to prevent larvae to develop in fallen fruits, mulching to encourage natural enemies, which are usually sufficient for control and also causing berries and the pest to rot, hot water treatments to the

infested crop before drying, and proper drying of coffee, picking by use of mats on the ground and use of traps (Magina, *et al.,* 2010; Kucel *et al*., 2009; Jaramillo et al., 2005; Baker, *et al.,* 2002). The cultural methods are on their own inadequate and cumbersome for small holder farmers to apply. Due to the insects' nature of infestation, biological control is the most promising management option against this pest.

Previous explorations of their natural enemies in Kenya revealed the presence of various parasitoids, including *Heterospilus coffeicola*, *Cephalonomia stephanoderis* and *Prorops nasuta* (Jaramillo, 2005). It is known that the parasitoids reduce the population of *H. hampei* and therefore no effect on green maturing berries and ripe cherries. However no information is available in regard to presence of these natural enemies in coffee in Tanzania which hinders its research in biological control strategies. Due to the lack of information on natural enemies the study on natural enemies infesting the pest was initiated in collaboration with IPM Collaborative Research Supportive Program, (IPM/CRSP) in East Africa (financed by USAID) and Tanzania Coffee Research Institute (TaCRI) as part of an attempt to develop efficient, economically feasible, and environmentally sustainable control strategies.

MATERIALS AND METHODS

The study was carried out in the insectary at TaCRI, Lyamungu, Tanzania. Coffee berries were collected from the coffee fields on the station (1268 m.a.s.l). The two farms were chosen based on management practices (sprayed and unsprayed) and different varieties of coffee (Arabica and Robusta) to see if there is any difference in parasitoid infestations. A total of 15,190 fully expanded green, ripe and overripe berries infested with CBB in the field were collected on Arabica and Robusta coffee fields and brought to the insectary and incubated in boxes (30 cm x 30 cm x 10 cm) at TaCRI, Lyamungu from September 2010 to January 2012 (Table 1). Forty two bethylid wasps were emerged and were preserved in 40% Formaldehyde solution and sent for identification at *icipe*, Kenya. 110 fruit flies were emerged from the same berries and were preserved in 75 % Ethanol and send for identification at SUA, Morogoro.

RESULTS AND DISCUSSION

Table 1 presents data on parasitoids and invasive fruit flies collected from coffee berries of Arabica and Robusta coffee under different management practices. Fourty two parasitoids emerged between 21 to 25 days after incubation and were normally observed in September to November when berries are overripe and dry (Table 1). Out of 42, two bethylid wasps were identified as *Cephalonomia stephanoderis, Prorops nasuta* as potential parasitoids of CBB for Arabica and Robusta coffee in the study area. From the literature *C. stephanoderis* and *P. nasuta* are ectoparasitoids of adult *H. hampei* and also prey on *H. hampei* eggs. *C. stephanoderis* also attacks and feeds on the adult female *H. hampei*, whereas *P. nasuta* use their bodies (abdomens) to block the entrance to infested coffee berries (Jaramillo *et al.,* 2005).

Additionally three invasive fruit flies emerged from the same berries between 5 - 7 days after incubation and were identified as *Ceratitis capitata*, *Ceratitis rosa* and *Trirhithrum coffeae*. The pests are of economic importance by causing fruit damage therefore causing rapid deterioration resulting from the feeding and fruits are quickly rendered unmarketable and inedible (Duke, 2005). The pests are endemic in the tropics, subtropical and temperate regions. In Tanzania this is the first time for those invasive fruit flies to be identified in coffee.

Table 1. Number of CBB Parasitoids and fruit fly emerged from coffee berries collected from the field on the station.

Period	Field	Coffee type	No. of berries	No. of Parasitoids	No. of fruit flies
Sept. 2010	VC	Robusta	571	0	2
Oct. 2010	VC	Robusta	658	3	5
Nov. 2010	VC	Robusta	394	5	10
Dec. 2010	VC	Robusta	264	0	0
Jan. 2011	VC	Robusta	401	0	0
Feb. 2011	VC	Robusta	283	0	0
Apr. 2011	18Z	Arabica	439	0	0
Jun. 2011	VC	Robusta	990	0	0
Jul. 2011	18Z	Arabica	1499	0	0
Aug. 2011	18Z	Arabica	2601	0	0
Sep. 2011	18Z	Arabica	720	0	21
Oct. 2011	VC	Robusta	134	9	0
Oct. 2011	VC	Arabica	359	6	22
Nov. 2011	VC	Robusta	3481	15	49
Nov. 2011	VC	Arabica	364	4	1
Dec. 2011	VC	Robusta	1641	0	0
Feb. 2012	VC	Robusta	391	0	0
			15190	42	110

CONCLUSIONS

We identified that potential parasitoids for CBB are available in coffee farms in Kilimanjaro region and CBB becoming potential in the medium altitude suggesting for exploring biological control measures through mass multiplication of these natural enemies and later release in the field for management of the pest is in progress. In addition the invasive fruit flies which were identified for the first time in coffee in the country is recommended to be evaluated for its economic importance as they could be a problem for coffee in the future.

ACKNOWLEDGEMENTS

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Coffee Farming Systems, Productivity Constraints, Quality and Profitability to Smallholder Farmers in Two Contracting Zones in Tanzania

L. KIWELU, J. MAGESA, J. M. TERI

Tanzania Coffee Research Institute (TaCRI), P.O. Box. 3004, Moshi, Tanzania

SUMMARY

Assessment of coffee farming systems, productivity constraints, quality and profitability to smallholder farmers was conducted in Southern and Northern Zones of Tanzania. Both primary data from 120 households and secondary data's were collected. Statistical Package for Social Science (SPSS) was used to analyse the quantitative data. It was found that the average area under coffee in Southern Zone was (1.0 ha) compared to the Northern Zone (0.8 ha) with mono-cropping system dominating in the Southern Zone as opposed to agroforestry system practiced in the Northern Zone. In both farming systems it was found that, high input costs; prevalence of pests and diseases; aged coffee trees, aged coffee farmers; lack of price differential on quality; volatile coffee prices; lack of market information and prolonged drought are the major constraints to coffee productivity, quality and profitability. Meanwhile in both areas, coffee was found to be the major source of income to the households. About 33% of households' income comes from coffee, 27% from livestock; other crops 23% and off-farm sources contribute 17%. Therefore the sustainability of Tanzanian coffee industry is through farmers adopting high yielding and disease resistant coffee varieties with good beverage quality developed and released by TaCRI.

INTRODUCTION

The economy of Tanzania depends heavily on agriculture which employs around three-quarters of the Tanzanian labour force (URT, 2011). Coffee sub-sector employs about 450,000 smallholder families. 90% of coffee is produced by smallholder farmers and the 10% is produced by 110 estates. About 2.5 million other people depend on coffee directly or indirectly. The average coffee production in Tanzania is 50,000 tons (figure 1) and the prices of this commodity continue to increase since 2001/02 to date due to increase demand. The coffee sub-sector contributes about 22% (figure 2) to the country foreign exchange earnings (BOT, 2012).

Coffee production and exports have stagnated since the mid-1990s. During that time the livelihood of coffee producers were affected as the world and domestic coffee prices declined further followed by policy reform in Tanzania which affected producers in access of inputs Mwakalobo (1998). Other curial factors noted by (Bafes, 2003; Sarris and Savastano, 2006; Amani 2005) includes low prices compared to production cost, low use of farm inputs such as fertilizer, old age of coffee trees, pests and diseases, low level of capital especially for small scale farmers and policy reform. However, coffee sub-sector is still important as the source of income to coffee grower (Sarris and Savastano, 2006).

MATERIALS AND METHODS

Structured questionnaire was used to collect primary data such as socio-economic characteristics which were age of the household head, sex, household size and education), data on coffee productivity, constraints in coffee farming and household source of income from 120 coffee farmers. The study employed the farming system approach which is the common method used to study farming systems as it was used by FAO, (1995). The sustainable livelihood approach which is used to provides the main factors that affect people's livelihoods and typical relationships between them as formally used by (Scoones, 1998 and D'Souza, 2001). In assessing the profitability, the study adopted the gross margin computation which is the difference between gross income "revenue" and operating "variable" costs. According to CTAHR (1998) gross margin is a good measure for comparing the economic and productive efficiency of similar sized farms. Olagunju *et al.* (2007) used gross margin to study economic viability of Cat fish production and CTAHR (1998) used gross margin analysis to determine economics of coffee production. Statistical Package for Social Science (SPSS) was used to analyse the quantitative data. The gross margin was computed as:

$$GM = TR - TVC$$

where:

GM = Gross Margin ,TR = Total revenue, TVC = Total variable costs.

RESULTS AND DISCUSSION

Household characteristics

During survey it was founded that about 62% of respondents had age between 18 and 60 years old whereas 48% have age above 60 years old. Age has significant influence on farm productivity and technology adoption. Results also showed that the average household size was 6 people. Meanwhile (75.8%) of respondent's attained primary education, (15%) secondary education, (2.5%) college education and (5%) had no formal education.

The farm size and farming system

The study founded that, the average area under coffee in Southern Zone (Mbinga) was (1.0 ha) compared to the Northern Zone (0.8 ha) due to land availability. The type of farming system in Mbinga was mono-cropping (96.7%) as opposed to agro-forestry system practiced in the Northern Zone (97.8%). Farmer in coffee growing zones also drives their livelihood from other crops such as bananas, maize and beans. The agro-forestry system in North zone includes trees for shading and livestock folders which also contribute to household income.

Factors affecting coffee productivity and quality

The study found out that coffee leaf rust (97%) and coffee berry disease (65%) were reported as the major drawbacks for coffee quality and productivity while Antestia (89.7%) reported in Mbinga and white stem bore (79%) and (89.7%) are pests reported in Arumeru and Hai districts respectively. Other drawbacks were, high input costs (34%); aged coffee trees (22%) and prolonged drought (18%). Also lack of price differential on quality to farmer who strives to increase quality (16%); volatile coffee prices and lack of market information (10%) affect the improvement of coffee quality in the study area.

Household Income Composition

Result in figure 1 indicates that, coffee contributes the largest share (33%) in the household incomes followed by livestock, other crops and off-farm activities. This implies that coffee is an important pillar in the livelihood of coffee farmers as formerly noted by (Sarris and Savastano, 2006). Large share of coffee in the household income composition is due to increased coffee prices. TaCRI (2005) reported that, coffee contributes 11.5% to the household income in 2004 which was relative small compared to livestock (40.7%), banana (26.4%) and other crop (21.4%). The low contribution of coffee in the household income was due to low coffee prices emerging during coffee crisis (Amani 2005).

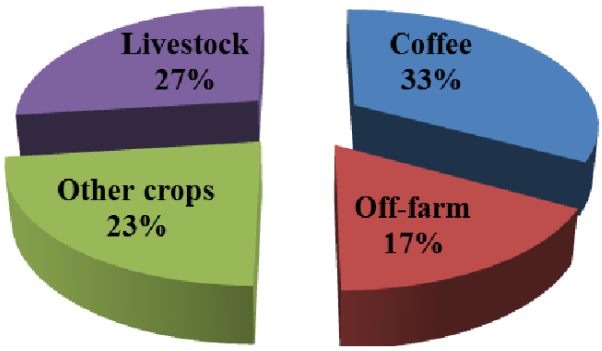

Figure 1. The Contribution of coffee to household income.

Trend of coffee production in the study area

Results in figure 2 indicate the trend of coffee production in the study area which implies that, coffee production is high at Mbinga and stagnant at Hai, Arumeru and Moshi due to land availability. There are several factors affecting coffee production in the study area such as: high input costs; prevalence of pests and diseases; aged coffee trees, aged coffee farmers; lack of price differential on quality; volatile coffee prices; lack of market information and prolonged drought.

There is opportunity of increasing coffee productivity and quality in the study areas through planting TaCRI high-yield and disease-resistant varieties. The study noted that all respondents are aware about the improved coffee varieties from TaCRI and about 57.4% of interviewed respondents have planted improved varieties from TaCRI. Meanwhile 55.5% of respondents challenge the low supply of these varieties in the study areas as compared with demand.

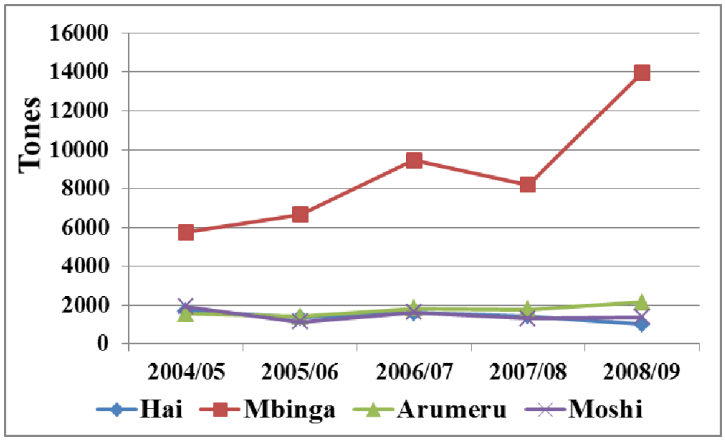

Figure 2. Trends of coffee production in the study area.

CONCLUSIONS

It was clearly noted that coffee is important source of income to the household. More emphasis is needed to mobilize farmers to plant improved varieties from TaCRI and adopting good agricultural practices as formerly recommended by TaCRI (2005). Hence this will contribute to lower cost of production and boost household income from coffee.

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Assessment of Compatibility by Grafting Arabica Improved Coffee Varieties on Robusta Rootstock

A. H. MSHIHIRI, N. M. NG'HOMA, G. P. MARO

Tanzania Coffee Research Institute (TaCRI) Maruku, P.O Box 127 Bukoba, Tanzania

SUMMARY

Most of the coffee growing areas in Kagera Region, Tanzania experience 3 to 4 months of prolonged dry spell. This affects Arabica coffee production in the region as Arabica coffee requires higher amount of soil moisture than Robusta. On the other hand Robusta cultivars have an added advantage of greater root system for water absorption than Arabica cultivars. Top - working Arabica scions on Robusta rootstocks can overcome the problem. An experiment was therefore conducted to assess the compatibility of Arabica coffee as a scions onto Robusta rootstocks as a measure to support Arabica coffee production in this region. The Arabica variety used was KP423-1 as a scion grafted onto six Maruku Robusta cultivars (Selections MS1, MS2, MS3, MS4, MS5, and MS6) as rootstocks using six months old seedlings of both rootstock and scion. Grafted seedlings were maintained in humid boxes to encourage union of the scion and the rootstock. Percentage take -off was observed after 15 weeks to determine which among the grafted MS Robusta varieties has high compatibility with Arabica. Results show that, compatibility was confirmed 16 weeks after grafting. Rootstock MS 6 showed high compatibility with KP423-1 whereby the percentage take - off was more than 80 percent. The grafted seedlings are expected to be experimented in Arabica growing areas experiencing prolonged drought conditions. It is expecting the findings to mitigate drought in Arabica coffee areas in Kagera.

INTRODUCTION

In Tanzania methods of propagation that have been used for accelerated multiplication of coffee planting materials include use of clonal cuttings, seeds to raise seedlings and grafting. The methods assist to produce true to type planting materials of either *Coffea arabica* or *Coffea canephora*. Out of these methods, use of clonal propagation has been practised to produce true to type experimental materials since 1960s (Fernie, 1962), and it is currently used commercially in accelerated multiplication of improved Arabica hybrids. Extensive research work has been done recently to adopt grafting by converting old Arabica coffee trees to new hybrids in Tanzania (Magina *et. al.*, 2004). Application of grafting have been very successful and well adapted in most of the coffee growing areas in Tanzania (TaCRI, 2009; 2010, 2011). With the recent problem of climate change, grafting has also been experimenting to overcome the problem of surviving coffee plants to a sustainable production in water stressed areas. It is known that some of the coffee cutivars such as K7, *Coffea racemosa*, *Coffea canephora*, and SL28 have deeper roots therefore able to survive under limited amount of water (Millot, 1969). It is for this reason that investigation involving grafting scions of Arabica coffee onto rootstocks of *C. Canephora* was initiated at Maruku with the purpose of having enough supply of Arabica planting materials in water stressed areas. The experiment involved grafting a scion of Arabica hybrid KP423-1 onto six Maruku cultivars as rootstocks to test the level of compatibility eventually to be evaluated in water stressed areas. The levels of compatibility and preliminary observation on field performance are discussed in this report.

MATERIALS AND METHODS

An experiment of grafting arabica coffee onto Robusta rootstock was conducted at Maruku substation in 2010/11. One hundred and eight rooted cuttings nine month old of Maruku selections cultivars namely: Maruku selection 1 (MS 1), Maruku selection 2 (MS2), Maruku selection 3, (MS3) Maruku selection 4 (MS4), Maruku selection 5 (MS5) and Maruku selection 6 (MS6) were used on this study. Thirty six scions of arabica coffee hybrids KP 423-1 were grafted to each Robusta cultivar. The scions of improved arabica coffee KP423-1 were collected from mother trees at Maruku substation. Using a sharp knife, a vertical slit was cut at the centre of the rootstocks of Robusta cultivars seedlings. The scions harvested from the mother trees at Maruku were cut at the basal end to make v- shaped edges. The scions were firmly inserted into the Robusta rootstocks matching the vascular cambia of both rootstocks and scions. Using polythene strip, the rootstocks and scions were tied firmly and arranged in the Randomized Complete Design (RCD) replicated three times in the rooting boxes and then covered with transparent polythene sheeting (750 G). Grafted coffee materials were regularly irrigated and misted for eight month. After eight month survived grafted coffee were planted to the field. Data collected included percentage take off of grafted coffee in both rooting boxes and main field. Data for percentage take off were analyzed using GENSTAT statistical package, and presented in a bar chart.

RESULT AND DISCUSSION

Figure 1 below presents the summary of percentage take off of Arabica scions grafted onto Robusta cultivars in the nursery. There were significant differences (P > 0.05%) on the take off arabica scions among the six tested rootstocks. The survival of arabica scions grafted to Maruku selections 1 and 3 were 50% compared to 100% for those grafted onto Maruku selection 6 indicating that MS6 is more compatible to arabica KP 423-1. Survived scions from MS 1, 3 and 6 were planted in the field for performance assessment. In the field, significant difference (P < 0.05%) noted on the performance of grafted coffee (Figure, 2). The results showed that coffee scions grafted onto MS6 performed better in the field. Eleven months after planting the grafted coffee in the field survival ranged from 33% for arabica scions grafted to MS1 and MS3 to 100% those grafted onto MS6, this could be probably due to differences in the scion- rootstock compatibility.

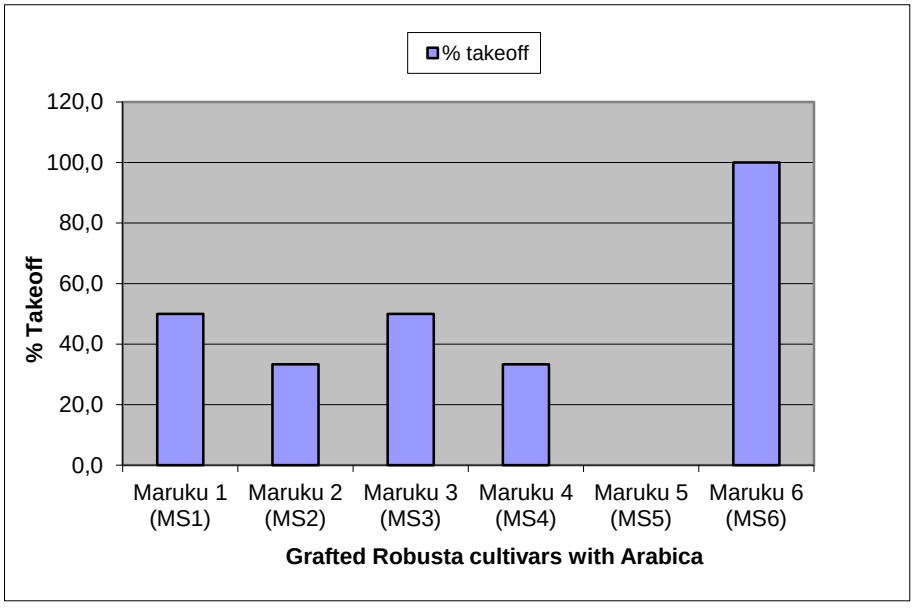

Figure 1. % Take off Arabica scion grafted onto Robusta rootstocks in the nursery.

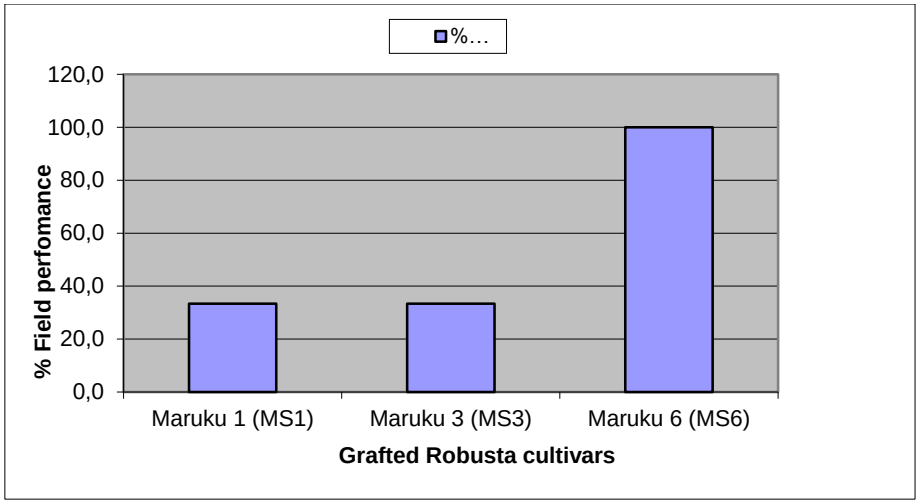

Figure 2. % Field perfomance of Grafted Robusta with Arabica.

The advantages of using grafting method have been previously reported for Arabica coffee trees. For instance, in nematode (*Meloidogyne incognita*) infested areas, the use of resistant rootstocks has increased the development and yield of grafted plant, compared to non - grafted controls (Marcelo *et. al.*, 2002). Grafted has not only been of advantage to coffee, but also in grapevine whereby, grafted plant show higher yield than non - grafted (Edwards, 1988). This was attributed to better root and canopy development of grafted plants. Higher compatibility and preliminary survival rate in the field shown by KP423-1 onto MS6 predicts excellent performance of this graft in water stressed areas, and therefore can be recommended for a massive grafting.

CONCLUSIONS

The results obtained from this study showed that among the six Robusta cultivars grafted with arabica variety; Maruku selections MS1, MS3 and MS6 could be used as reliable Robusta rootstocks for grafting with arabica varieties, to cutter for water stresses Arabica areas.

ACKNOWLEDGEMENTS

We are grateful to European Commission (EC, Tanzania) and Tanzania coffee growers for their financial support for this study.

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Potential of Yellow Bourbon Variety to Improve the Green Bean Physical Quality of Specialty Coffees in Brazil

G. S. GIOMO, R. SAATH, J. C. MISTRO, A. DE C. IOBBI, L. C. FAZUOLI

Instituto Agronômico (IAC), P.O. Box 28, 13012-970, Campinas, São Paulo, Brazil

SUMMARY

The coffee bean size constitutes an important physical attribute for coffee grading aiming to obtain homogeneous lots during processing and for coffee prices determination, but it is not yet totally elucidated the extend of green bean size effects on beverage quality. Considering the importance of the Yellow Bourbon variety for specialty coffee production in Brazil the Instituto Agronômico de Campinas (IAC) has intensified researches into Bourbon Germplasm Collection in order to identify new promising genotypes to supply the needs of the specialty coffee producers. Information regarding green bean physical quality of Yellow Bourbon variety is very scarce in the literature, thus this research was carried out to evaluate the green bean physical characteristics of thirty four arabica coffee genotypes comprising of 31 Yellow Bourbon progenies, one Red Icatu cultivar and two Yellow Mundo Novo advanced selections. The experiment was performed in 2010/2011 crop year in the Northwest Sao Paulo State, Brazil and the coffee samples were obtained by semi washed processing method. It was observed that the percentages of flat and peaberry beans were affected by genotype. The significant differences on green bean physical quality among the genotypes indicate the possibility of to select new Yellow Bourbon cultivar to improve the specialty coffee production in Brazil. Considering that all coffees were from the same environmental and processing procedures, it is supposed that the green bean physical characteristics differences could have occurred due genetic effects. However, considering the natural biennial cycle of arabica coffee would be necessary to evaluate these genotypes during at least more two crop years. This will contribute to understand the real genotype effects on the green bean physical characteristics, avoiding any mistakes or inappropriate conclusions concerning the Yellow Bourbon coffee quality.

INTRODUCTION

It is generally claimed that Bourbon coffee enjoys a high intrinsic quality profile to the point of being considered as an international benchmark that any new variety should aim to reach in order to be considered as suited for the specialty coffee market. The green bean size, shape and density are so important characteristics for coffee processing and grading aiming to get homogeneous lot and constitute parameters for coffee price determination because in the specialty coffee market are request coffee that present high cup quality and good physical quality.

Several studies have indicated that Bourbon variety could offer frequently better cup quality than the other commercial arabica coffee variety in the same environmental conditions, confirming the real genetic potential of Bourbon to produce high coffee quality. The first Brazilian Bourbon variety, named Red Bourbon IAC662, was released by Instituto Agronômico de Campinas (IAC) in 1939 and during the 1940's it were selected several Yellow Bourbon cultivars (IAC J2, IAC J9, IAC J10, IAC J19, IAC J20, IAC J22 and IAC J24), which showed yield potential 40% superior than the first Red Bourbon selection,

constituting nowadays the major Bourbon coffees in Brazil. Considering the scarcity of information regarding Yellow Bourbon coffee bean physical quality, to attend the recent needs of specialty coffee producers in Brazil, the IAC restarted its Bourbon Breeding Program and at this time several Bourbon progenies are being studied in field trials to identify the promising ones to improve the specialty coffee production in Brazil.

This research was carried out to evaluate the green bean physical characteristics of *Coffea arabica* genotypes aiming to get reliable information about physical quality by genotype comparisons. Considering the natural biennial cycle of arabica coffee and the probable effects of genotype × environment interactions would be necessary to evaluate these genotypes during several years, being so important that all genotypes have been cultivated in the same site. This will contribute to better understanding of the real genotype effect of on bean physical characteristics, avoiding any mistakes or inappropriate conclusions concerning the Bourbon coffee quality.

MATERIALS AND METHODS

Study site and test varieties

The experiment was carried out at Recreio Farm Estate Coffee in the Northwest São Paulo State, Brazil. A total of thirty four *Coffea arabica* genotypes were used in this study and many of them are Yellow Bourbon progenies that present high genetic potential for use in the coffee breeding programs aiming to improve the cup quality, including Brazilian cultivars 'Icatu Vermelho' and 'Mundo Novo Amarelo' as control for quality and yield comparisons. Each of the genotype was represented by ten plants per plot disposed in the field in a Randomized Complete Block Design with three replications.

Harvest, processing and physical evaluations

Fruits samples were collected during the peak harvesting period of June-July in 2010/2011 crop year. Ripe health cherries were harvested by hand from each of the experimental plot and processed using semi washed processing procedures (pulped natural coffee). The full ripe fruits were pulped and the parchment coffees were sun dried over elevated screens until the green beans reached moisture content of 11.5% (wb). After hulling the clean beans were classified by size using five screens with circular perforations of 19, 18, 17, 16 and 15/64 inches and three screens with oblong perforations of 12, 11 and 10 x ¾ inches according current recommendations.

RESULTS AND DISCUSSION

Based on the values of green bean grading (Table 1), it was observed that there was significant effect of the genotypes on coffee bean size. For larger bean, retained over screen 19, it was observed the highest value for genotype 34 (18.3%) and the lowest values for genotypes 4, 12 and 30 (2.4%, 3.5% and 3.7%, respectively). The genotypes 1, 9, 10, 14, 16, 20 and 33 were similar to treatment 34 showing intermediate values that did not differ from the better or worst treatments.

Table 1. Effects of *Coffea arabica* genotype on bean size. Percentage of flat beans retained over round screens 19, 18, 17, 16 and 15/64 inch) and peaberry beans retained over oblong screens 10, 11 and 12 x ¾ inch in 2010/2011crop year. São Sebastião da Grama, São Paulo, Brazil.

Genotype [1]	Screen 19	Screen 18	Screen 17	Screen 16	Screen 15	Screen 10/11/12
	%					
01. BA 19.18.10-1043A	10.69 a-d	31.75 a-c	29.34 a-c	8.91 b-d	0.67 b	15.99 ab
02. BA 20.14.14	4.27 b-d	18.81 b-d	36.45 a-c	22.57 ab	2.76 b	10.84 ab
03. BA 09.08	8.83 b-d	28.07 a-c	33.15 a-c	11.54 b-d	1.76 b	11.03 ab
04. BA 15.16.05	2.40 d	12.18 d	33.78 a-c	28.10 a	6.45 a	10.89 ab
05. BA 19.18.10-1043B	4.54 b-d	24.46 a-d	40.85 ab	13.46 b-d	1.57 b	12.04 ab
06. BA 26.06-108	5.60 b-d	26.21 a-d	35.77 a-c	14.14 b-d	2.22 b	13.48 ab
07. BA 26.06-705	8.03 b-d	29.24 a-c	34.35 a-c	9.17 b-d	0.98 b	15.38 ab
08. BA 24.06-813	6.79 b-d	28.29 a-c	33.81 a-c	9.45 b-d	1.02 b	15.50 ab
09. IV 4782.16.82.03 [2]	9.32 a-d	28.86 a-c	29.09 a-c	8.01 cd	0.97 b	19.33 a
10. BA 28.08	10.69 a-d	31.85 a-c	29.94 a-c	9.25 b-d	0.90 b	14.37 ab
11. BA 27.04	5.46 b-d	27.03 a-c	40.42 ab	11.86 b-d	1.56 b	10.33 ab
12. BA 26.08	3.49 cd	18.44 cd	38.28 ab	20.68 a-c	3.06 b	12.37 ab
13. BA 23.19	4.31 b-d	25.90 a-d	38.01 a-c	15.24 a-d	1.78 b	11.31 ab
14. BA 30.20	9.65 a-d	30.46 a-c	30.17 a-c	9.27 b-d	1.95 b	14.66 ab
15. BA 21.07	9.05 b-d	32.83 ab	34.74 a-c	8.89 b-d	1.35 b	10.31 ab
16. BA 22.06	12.05 a-c	34.16 a	30.71 a-c	7.45 cd	0.91 b	13.34 ab
17. BA 19.01	6.96 b-d	26.79 a-c	35.50 a-c	12.59 b-d	1.87 b	13.47 ab
18. BA 17.10	8.32 b-d	31.77 a-c	32.94 a-c	8.76 cd	1.03 b	14.79 ab
19. BA 18.02	6.11 b-d	25.04 a-d	37.86 a-c	12.28 b-d	1.10 b	15.07 ab
20. BA 15.02	10.72 a-d	27.86 a-c	29.33 a-c	10.91 b-d	1.79 b	15.47 ab
21. BA 14.20	4.41 b-d	18.87 b-d	38.31 ab	18.98 a-d	3.11 b	11.51 ab
22. BA 10.03	5.51 b-d	24.52 a-d	35.36 a-c	14.04 b-d	1.40 b	15.91 ab
23. BA 13.08	6.86 b-d	32.00 a-c	33.85 a-c	9.48 b-d	0.85 b	14.73 ab
24. BA 09.16	6.65 b-d	26.84 a-c	33.93 a-c	12.65 b-d	2.16 b	13.25 ab
25. BA 20.17	6.72 b-d	29.02 a-c	35.74 a-c	10.09 b-d	1.08 b	13.91 ab
26. BA 11.11	6.25 b-d	25.51 a-d	39.04 ab	13.14 b-d	1.28 b	11.87 ab
27. BA 07.20	4.90 b-d	25.29 a-d	36.52 a-c	13.06 b-d	1.35 b	16.77 ab
28. BA 06.09	4.46 b-d	26.04 a-d	39.50 ab	13.45 b-d	2.05 b	11.95 ab
29. BA 08.02	4.75 b-d	22.73 a-d	33.03 a-c	17.65 a-d	3.02 b	14.39 ab
30. BA 02.01	3.71 cd	17.80 cd	37.06 a-c	20.16 a-d	2.70 b	14.99 ab
31. BA 03.01	4.00 b-d	20.31 a-d	42.49 a	17.05 a-d	2.59 b	8.75 b
32. BA 04.10	12.99 ab	30.09 a-c	26.40 bc	10.54 b-d	1.53 b	11.31 ab
33. MNA 4266-MD [2]	12.50 a-c	34.12 a	27.04 bc	8.37 cd	1.49 b	13.33 ab
34. MNA 4266 [2]	18.30 a	34.13 a	23.14 c	6.66 d	1.03 b	13.86 ab
Means	7.33	26.68	34.29	12.88	1.80	13.43
$F_{genotype}$	4.37[3]	4.25[3]	2.77[3]	3.93[3]	4.46[3]	1.57[4]
CV (%)	38.56	16.55	13.46	32.80	48.93	22.87

[4] and [3] = significant differences by F test at level of 5% and 1% of probability, respectively. Values followed by the same letters in the columns did not present significant difference by Tukey test at level of 5% of probability. [1] BA=Yellow Bourbon genotypes under selection at Agronomic Institute (IAC) Coffee Breeding Program. [2] IV=Icatu Vermelho and MNA=Mundo Novo Amarelo are Brazilian cultivars used as control for quality comparisons.

For 18 bean grade, the highest values were observed in genotypes 16, 33 and 34 differing significantly from the genotype 4, 30 and 12 that had been showed the lowest values for 19 bean grade. For 17 bean grade it was observed the highest values in genotypes 31, 5, 11, 12, 21, 26 and 28, with significant difference from treatment 34. According these results it was concluded that genotype 4 (BA 15.16.05) produces highest values of small beans. In contrast, the genotypes 5, 11 and 31 present high potential to produce large beans. The control variety Icatu Vermelho (genotype 9) showed the highest value for peaberry beans, differing from the genotype 31 (BA 03.01). The remaining genotypes showed intermediate values that did not differ from the 9 or 31 genotypes. These results indicate that there is variability among Yellow Bourbon genotypes concerning green bean physical quality. However, considering that it is not yet totally elucidated the extend of green bean size effects on beverage quality it is not recommended to select new Yellow Bourbon variety for quality based on only green bean physical characteristics. Considering the natural biennial cycle of arabica coffee would be necessary to evaluate these genotypes during at least more two crop years. This will contribute to understand the real genotype effect on the green bean physical characteristics, avoiding any mistakes or inappropriate conclusions concerning the Yellow Bourbon coffee quality.

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Determining Effects of Time, Temperature, and Humidity on Mortality of Coffee Berry Borer (*Hypothenemus hampei)*

L. D. GAUTZ, A. J. BOWLES

Department of Molecular Bioscience & Bioengineering - University of Hawaii at Mānoa
1955 East West Rd. Honolulu, HI, USA

SUMMARY

With the identification of *H. hampei* in Kona Districts of Hawai‘i, movement of coffee in any form short of roasted was restricted by the Hawai'i Department of Agriculture. Disinfestation of green bean by dry hot air was explored. Using sequential two level fractional factorial experiments a response surface of *H. hampei* mortality on time and temperature of recirculated air was determined. A treatment of 50°C for 25 minutes is recommended for .985 disinfestation.

BACKGROUND

Problem

The beetle *Hypothenemus hampei* was collected for the first time in the South Kona District of the State of Hawai‘i in August 2010. At the time of this writing, November 2012, *H. hampei* has not spread outside of the Kona and Ka‘u coffee growing areas. Within the North and South Kona districts that comprise the Kona Coffee region, damage has been quite severe. The Ka‘u Coffee region has not had substantial damage which has been attributed to aggressive field sanitation (HCA, 2012).

Legal

A quarantine on movement of coffee in any form out of infested areas was imposed by the Hawai‘i Department of Agriculture in 2010 and renewed in 2011. Under the quarantine, green bean coffee is not to be transported out of Kona without being treated to assure no living *H. hampei* are in the shipment. Currently the only treatment is methyl bromide fumigation as is required for unroasted coffee coming from outside of the State of Hawai‘i.

Technical

Beans

Sivets and Desrosier (1979) stated beans can be exposed to temperatures of 50°C for up to 120 minutes and 60°C for 60 minutes without affecting cup quality. The length of time falls quickly to 1 minute or less at 70°C. Processors in Kona generally do not operate dryers in excess of 50°C air.

Coffee green beans are traded internationally at 12% wb moisture content. Storage and transfer at this moisture is desirable. Any treatment should maintain the original moisture content.

H. Hampei

Early reports were that *H. hampei* did not survive drying on sun decks or forced hot air dryers (Trujillo, 1991). Current observations do not substantiate this. Trujillo (1991) reported no survival of adult *H. hampei* held at 65°C and saturation air moisture content for 15 minutes with some survivors at lower times. Jaramillo (2010) reported low survival and life progression at temperatures in excess of 30°C and saturation air moisture content.

Because *H. hampei* thrive only in moist coffee beans, imposing a low vapor pressure environment should assist in mortality of the beetle.

OBJECTIVE

Find a mathematical model for *H. hampei* mortality in green bean coffee using the treatment variables time, temperature, and relative humidity.

MATERIALS AND METHODS

Green bean coffee was obtained from a Kona processor for the experiments. A sample was placed in a 300mm square box with 50% opening screen on the bottom to a depth of 50mm.

The sample box was placed in an insulated box with a heater and fan arranged with baffles forcing the heated air through the sample and returning the exhaust from the sample back to the fan intake.

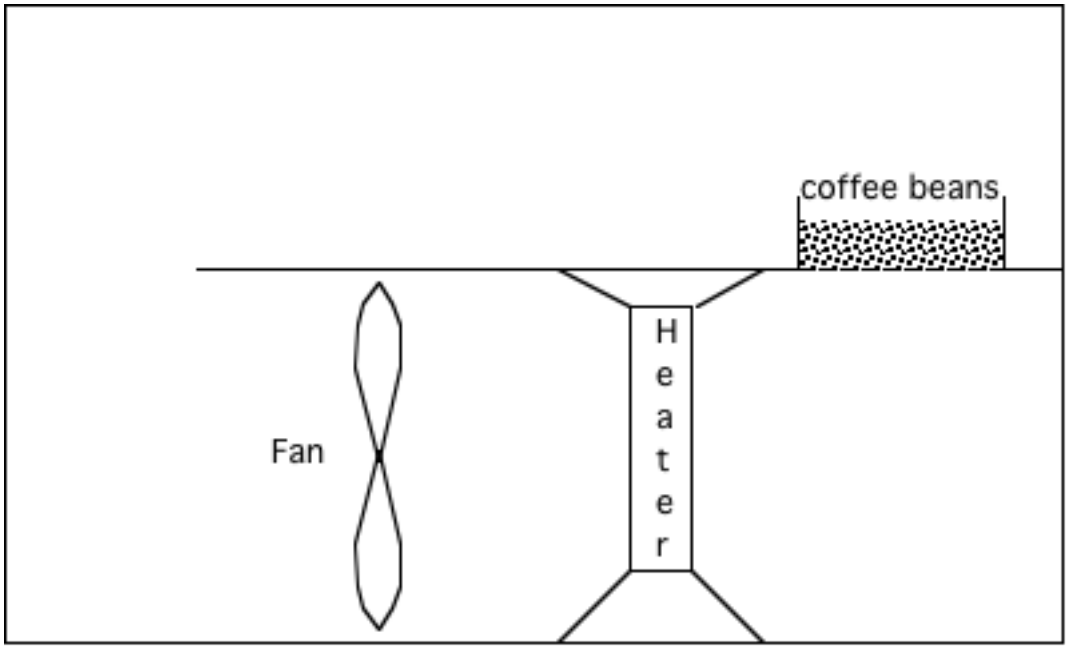

Figure 1. Heat treatment test box schematic showing fan, heater and coffee sample box arrangement.

A hole 1 mm in diameter was drilled from the end of beans into the center. A type T thermocouple was inserted into two beans. One was placed at the bottom of the sample and the other on the top. A bare type T thermocouple was placed in the plenum below the sample with another bare type T thermocouple in the plenum above the sample.

The temperature of the air was controlled with an on-off controller with a set point and 1°C offset. All temperatures were monitored every second and the average recorded every minute with an electronic data logger.

H. hampei adults were harvested from fresh infested cherry coffee by cutting or agitating the coffee. One live adult each was introduced into 1mm drilled holes of 20 green beans with a fine artist paint brush. The adults crawled into the hole which was initially closed with paper tape and in later trials with plastic tape (Beetles would bore through paper tape during trial). The artificially infested beans were placed in a screen bag with 600 micron holes so that all beetles could be found following treatment.

A two level factorial experiment with the three variables air temperature, time, and air humidity to estimate the gradient of mortality followed by a search along the gradient established a region for more thorough exploration. A central composite design in time and air temperature in this region provided the data for determining a mathematical model of the mortality.

Analogous to the WSU thermal block described by Tang and Ikediala (2000), a 50mm diameter capsule made from two pieces of 12mm aluminum plate with 1mm space for *H. hampe*i life stages was used to establish which life stage was least vulnerable to the heat treatment. Temperature of the capsule was monitored with a type T thermocouple embedded in the far side of the capsule with the heat applied with a halogen light to the near side. Trials using 20 *H. hampei* at each stage of life, egg to adult, at time and temperature that had been determined to yield survival of at least two adults, established most vulnerable stage of development.

The model was determined by applying the cumulative Gaussian distribution to the mortality results. An algorithm was implemented in MatLab® to determine the coefficients and models for the 50% mortality time and standard time deviation on temperature for the cumulative mortality. The models and coefficients that gave the sum of least squared residuals when compared with actual mortality were chosen. Search for coefficients of the model was based on Marquadt-Levenberg method with the modification of estimating the gradient using two level fractional factorials rather than steps along the axes. Line searches along the gradient of the model coefficients used a fibonacci search.

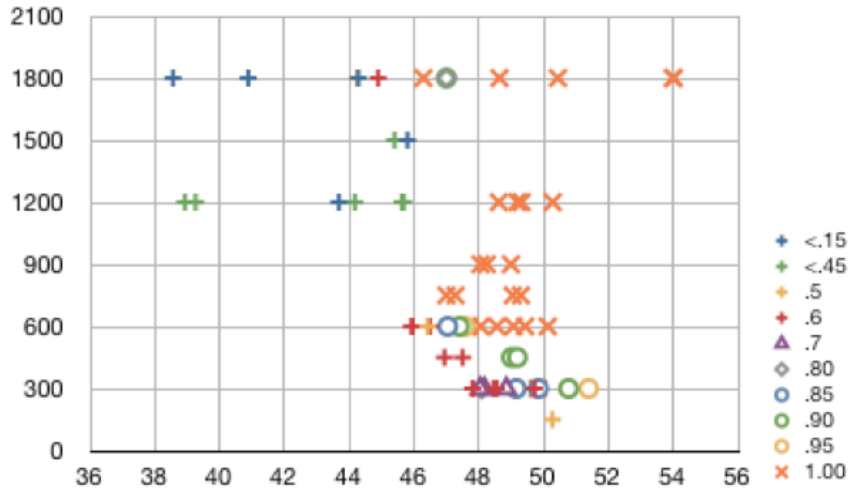

Figure 2. Mortality as ratio from trials on the experimental space with x-axis in °C and y-axis in s.

RESULTS AND DISCUSSION

The search and central composite with appropriate replication required 64 trials. Mortality with respect to actual time and temperature are displayed in Figure 2. Note when treatment was over 600s (10 minutes) and 48°C there were no survivors.

A line drawn through the approximate 50% mortality contour implies a hyperbolic relationship for time as a function of temperature for the line. Similarly, The second parameter, standard deviation, of the cumulative normal distribution used to model mortality is hyperbolic in nature. This second parameter is directly related to the gradient of mortality at the 50% line.

Time was transformed using its inverse (has units of frequency) with a scaling factor of 1000. A plot of mortality with inverse time and temperature in Figure 3 implies a linear relationship for both the cumulative distribution mean and the standard deviation.

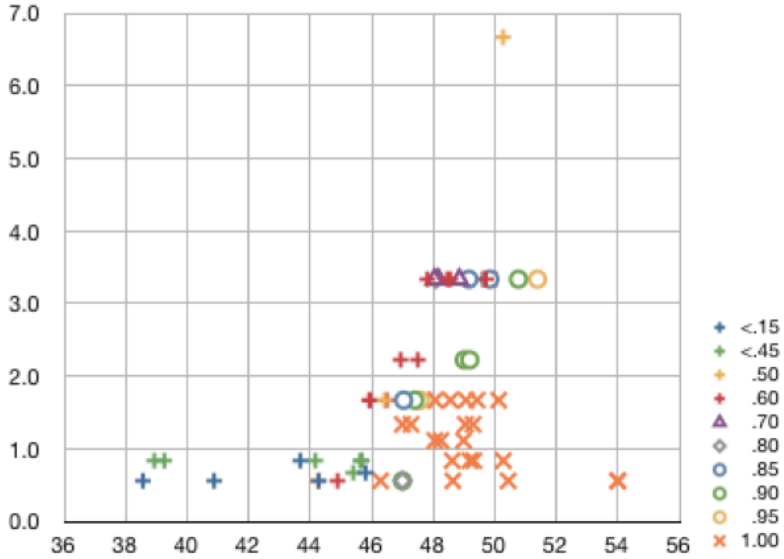

Figure 3. Mortality ratio from trials on the experimental space with x-axis in °C and y-axis in 1000/s.

The model for the mean (50% mortality) was found to be:

$$fm(p50) = 0.980*Temp - 43.272 \quad (1)$$

with the standard deviation being:

$$sd = 0.229*Temp - 9.127 \quad (2)$$

Mortality is found by supplying these two parameters to a normal distribution function along with scaled inverse time, mHz, at the temperature, °C, used above and subtracting from 1. The cumulative normal distribution with the above parameters returns the probability of survival.

In Excel®, the equation is:

$$\text{mortality} = 1 - \text{Norm.Dist}(1000/t,\text{fm},\text{sd},\text{cumulative}) \quad (3)$$

where t is time at temperature in seconds.

A plot of actual data and predicted mortality is shown in Figure 4.

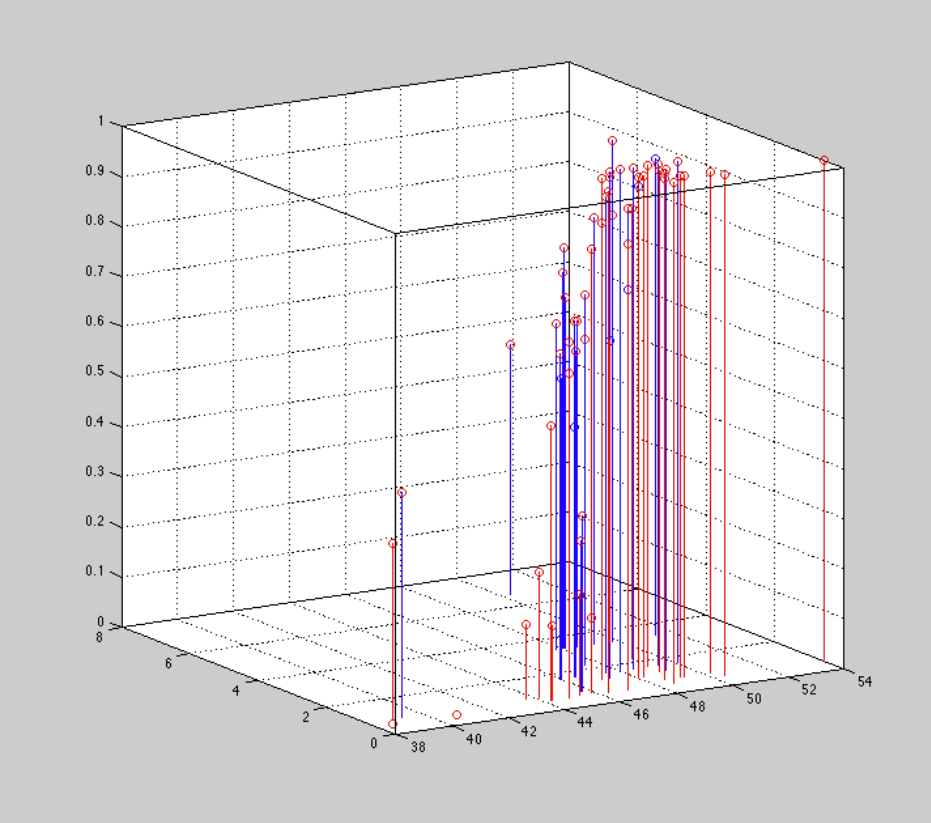

Figure 4. Measured mortality as ratio is shown in red, predicted mortality as blue, against the vertical axis. Temperature in °C is the horizontal axis going to the right. Scaled inverse of time in mHz is the horizontal axis going left.

At 50°C the mean (50% mortality) is 5.278 mHz with a standard deviation of 2.323 with 25 min resulting in a mortality of .985.

Using the aluminum capsule 20 pre-adult *H. hampei* died at 47°C for two minutes. Over 50% of adult *H. hampei* survived this treatment. If no adults survive a heat treatment then no pre-adult life forms will survive.

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Rain Effect on Coffee Berry Borer Mortality Present in Berries Fallen to the Ground at the Bramón Experimental Station in Táchira, Venezuela

A. N. TORRES, B. LOZADA, Y. ACEVEDO, M. E. ZAMBRANO, L. BAUTISTA, W. CAMACHO, S. ESPAÑA

INIA-TACHIRA. Carretera Rubio – Delicias, Bramón, Táchira, Venezuela

SUMMARY

The coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae, Scolitynae) is the main coffee insect pest worldwide. This insect spends the dry season, both in the remaining coffee berries left in coffee plants after harvesting and in berries fallen to the ground. At the starting of the rain season, the female adults initiate the flight activity and the infestation of new berries begins. When rain events occur during the dry season, the coffee berry borer (cbb) adults may activate its flight and die because of the lack of suitable coffee berries to be infested. In the other hand, the rain favors all those living organisms present in the soil that may affect the cbb, either eggs, larvae, pupa, or adults. Determining the mortality effect of rainfall on cbb present into the fallen berries is the main objective of this study. Every two days, starting on March 1st, both in 2010 and 2011, female adults were captured in alcohol traps, and berries picked from the soil were dissected to count cbb individuals and their health status was registered. The precipitation onsite was registered every day. Results show that the female adults captured in alcohol traps increased significantly after any rain event, especially those with a considerable amount of rain. The dissected berries or grains indicated the presence of entomopathogenic fungi in cbb adults, as well as parasitized cbb by *Cephalonomia stephanoderis* in several samples. This results prove that rain events occurred during the dry season is capable of reducing the cbb adult female population that wait for the new harvesting season, either by stimulating the flight activity before the berries are ripped enough to be infested or by increasing the natural mortality factors due to the biological activity in the humid soil. These results may help coffee growers to foresight the population of cbb just by knowing the precipitation charts or rainfall occurred in their area during the dry season and, entomologists may refine their strategies for controlling this pest based on IPM programs.

INTRODUCTION

The coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae, Scolitynae) constitute one of the most damaging insects of coffee plantations capable of devastating the whole harvest. This insect, originating in Africa, only reproduce in coffee beans (Le pelley 1968). At the end of the harvest, the coffee berry borer (CBB) can be found in dry berries, either in plants or in the soil. Females bore berries and lay their eggs in, there. The emerging larvae feed in one of the two grains that comprise the bean leaving the coffee berry exposed to the invasion of pathogenic fungi. In most of the coffee growing area of Táchira, the rainfall pattern is unimodal, from May to October. During this period of time the CBB reproduces very actively coinciding with coffee berries in all the developmental stages. In the dry season, from November to April, the CBB is found inside dry beans, either in plants or in the soil. There, the borer remains in a resting stage waiting for the onset of moisture to activate its

flight and initiate reproducing. (Baker *et al*. 1994). Once the females emerge they orient themselves to coffee fruits for reproduction preferring those in a ripening stage (Igboekwe 1984; Cure *et al*. 1998).

In this region the coffee harvesting finishes from December to January, then, plants start blooming between January and March induced by water deficit causing stress. Three months later coffee berries become susceptible to cbb infestation, from May on, approximately. In recent years climate changes have occurred that has altered the normal rainfall pattern. The extemporaneous rainfall may induce flight activity on cbb females too early. That is to say, before the coffee berries are ripe enough to be infested by the insect. When this occur, the cbb female may die before reproducing because of the lack of suitable coffee berries with 20% dry matter, at least (Montoya 1994, Penados 1979) . Under these circumstances, the cbb females may be captured in alcohol traps. Methanol and ethanol mixed in a 3:1 proportion becomes a good attractant of *H. hampei* females provided there are not coffee berries ready to be infested (Batista *et al*.1988). In the coffee growing area where this study was conducted, the cbb female is attracted to these traps during the months of January to July, however, captures are more abundant between February and April (INIA 2007). Moreover, when the soil moisture increases due to rainfall all the soil-borne organisms become active, which may affect cbb present in fallen coffee berries. This study pretends to determine the influence of extemporaneous rainfall on cbb populations, which is waiting to infest the new coffee harvest season.

MATERIALS AND METHODS

Capture of coffee berry borer adult females

Adult females of the coffee berry borer were captured every 2-4 days using alcohol traps. Captures were made in several coffee plots located at the Bramón Experimental Station (INIA-TACHIRA). In 2010, captures were made in three coffee plots from March until May, and in 2011, captures were made in five plots from February until May. Five traps were installed at random per plot, which were baited every 15 days.

Dissection of coffee berries

Ten coffee berries per plant were collected in 10 plants selected at random. The percentage of infested berries was determined before the dissection procedure for determining the number of individuals of each stage and their health status.

Weather data

Records of rainfall, temperature, relative humidity, and sunshine provided by INIA TACHIRA Agrometeorological Station were used. This station is located at the Bramón Experimental Station in the same place where the coffee berry borer was captured.

RESULTS AND DISCUSSION

The relationship between cbb flight activity and rainfall was very variable from March through May in 2010, and from February through May in 2011. However, in March of 2010 and February of 2011 a positive correlation between cbb captures and rainfall was observed (2010: $F=31,96$; $df=1$; $P<0,0005$, 2011: $F=8,98$; $df=1$; $P=0,017$). This results coincide with those months where the cbb capture was greater in alcohol traps. Reports from INIA (2007) indicate captures of 90.000 and 110.000 adults in 25 traps, during March and April,

respectively. Females of cbb activate their flight when the coffee grain moisture increases as a consequence of intense and frequent rainfall. Results from this study indicate that in March of 2010 the adult females were captured more abundantly after intense rainfall (figure 1), Nonetheless, this relationship does not exist in the following months of April and May. In the same way, in 2011, the greater captures of cbb females occurred in February after intense rainfalls, and the lower captures occurred when rainfall events diminished or ceased, noticeably (figure 2). During the months of March, April, and May of 2011 there was not observed a positive correlation between flight activity and rainfall, even in March when a great amount of cbb females were captured. This behavior indicates that cbb is stimulated to fly during the first 4 weeks with rainfall of some magnitude, and once, all the adult females receive the stimulus to fly, then they undertake their flight obeying to multiple factors, besides the relative humidity. One way to clarify the other factors that influence in the cbb flight activity is by recording hourly captures during sunny, rainy, cool, and wet days. In the process of massive reproduction of cbb parasitoids, naturally infested coffee berries in the field are used. These berries are placed in just one layer in sieves, then, they are treated with fungicide and acaricide and immediately dried by using fans during one or two weeks, and finally, they are stored in a well aereated place protected from the rain. Forty five days later, the infested coffee berries are sprayed with abundant water to simulate rain in order to stimulate the cbb to fly. Only the adult female can fly, the adult male does not have functional wings, just fertilizes the female and dies. Occasionally, the temperature must be increased, as well as, the light intensity to promote the female flight in the emergency cage.

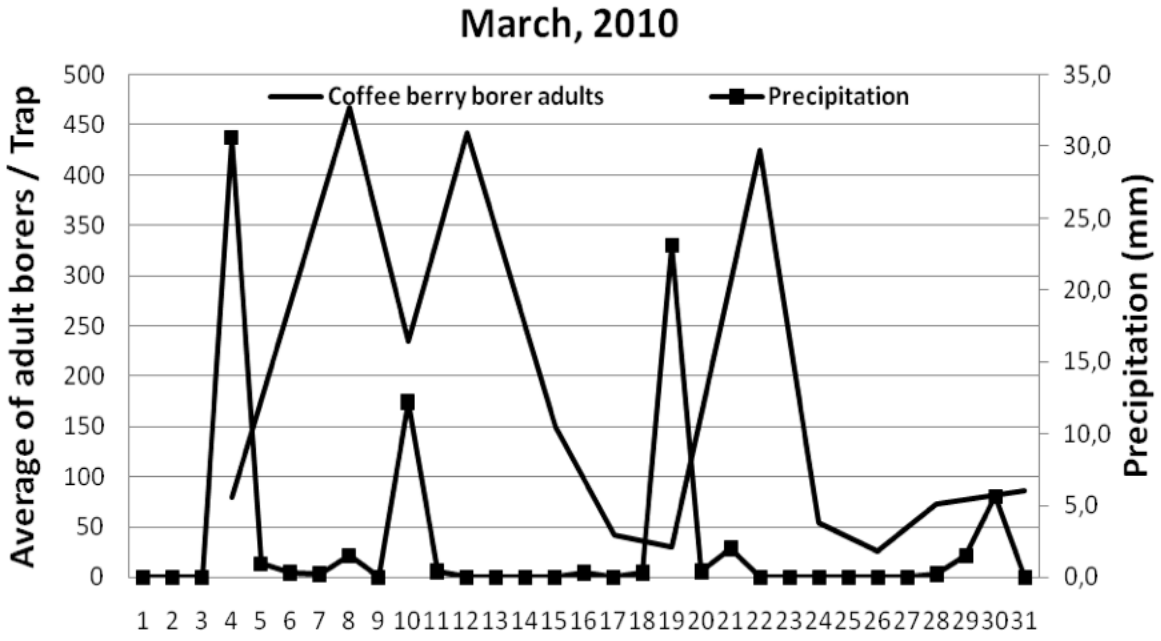

Figure 1. Hypothenemus *hampei* adults captured in alcohol traps and its relation to rainfall in March 2010, Bramón, Táchira, Venezuela.

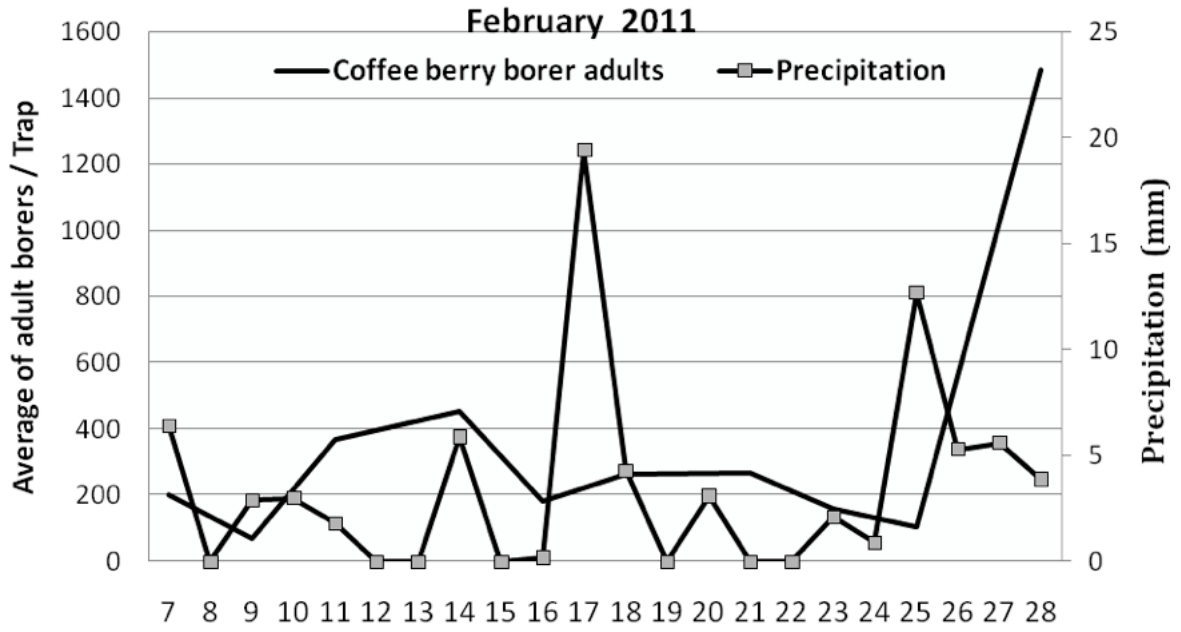

Figure 2. Hypothenemus *hampei* adults captured in alcohol traps and its relation to rainfall in February 2011, Bramón, Táchira, Venezuela.

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Identification of Areas for Permanent Preservation in Coffee Producing Regions of South Minas Gerais, Brazil

R. A. T. BORÉM[1], L. F. M. SILVA[1], H. M. R. ALVES[2], T. G. C. VIEIRA[3], M. M. L. VOLPATO[1,2], F. M. BORÉM[1]

[1]Universidade Federal de Lavras, CxP 3037, 37200-000, Lavras, MG, Brasil
[2]Empresa Brasileira de Pesquisa Agropecuária, CxP 40315, 70770-901, Brasília, DF, Brasil
[3]Empresa de Pesquisa Agropecuária de Minas Gerais, CxP 176, 37200-000, Lavras, MG, Brasil

SUMMARY

Planning for the sustainable use of natural resources requires knowledge and organization of up-to-date information about the environment. Due to the need for rural properties to comply with environmental legislation, the aim of this work was to developed a methodology to identify Permanent Preservation Areas (PPA) of hill tops and river banks occupied by coffee production. A digital elevation model of the municipality of Lambari, Minas Gerais, Brazil was created in a geographic Information System (GIS), using data from contour and system drainage of topographic charts from IBGE. The classes of PPA studied were hill tops and river banks. A map of copy from the INPE was used to identify areas occupied by coffee production, which can be viewed on the CAFESAT website. This mapping was conducted using Lansat images from 2005 and 2006. The municipality of Lambary shows an area of 21,380 hectares (ha), out of which 2,724.66 are PPA of river banks and 4,461.26 are PPA of hill tops. The PPA represent 33.61% of the total area of the municipality, being 12.74% of riverbanks and 20.87% of hill tops. In this region, coffee occupies an area of 3,936.77 ha, corresponding to 18.42% of the total area. Coffee is present in 381.36 ha of the PPA of margins of river banks and 1,116.92 ha of hill tops. That is, it occupies 14 and 25.04% of the respective PPA. This study enabled the development of methodologies to quantify the PPA of hill tops and riverbanks, which can be used for planning sustainable mountain coffee farms and compliance with environmental legislation, especially as regards to Permanent Preservation Areas.

INTRODUCTION

The planning of the agricultural activities is an action of extreme importance for the preservation of the natural resources in our country. The Brazilian legislation, in essence, is composed of command and control instruments. However, for being a territory of continental proportions, the monitoring of activities linked to the rural environment do not always present the necessary efficiency required to comply with current legislation.

The discussions concerning the high forest fragmentation and deforestation indices, and the debates about preservation and conservation of the natural vegetation, above all those situated along the water courses, springs and in areas of altered topography, have been prominent, being one of the pillars for technicians, researchers and environmentalists that extol their importance for protection of the hydric resources.

To understand the real situation of the plant covering and the degradation state of the landscape in a specific area, it is of fundamental importance to accomplish the land use and covering analysis. To know its dynamics favors the proper administration of the space through the adoption of measures that will guarantee the preservation and maintenance of the environment. The current environmental and socioeconomic impacts of these uses cause concern on a local, regional and global scale. Therefore, soil use should relate to the purpose attributed to its covering and vocation, be it an agricultural production area or as an environmental preservation area. And in this sense, geotechnologies offer a series of tools that greatly assist the adequacy of land use on rural properties, classifying the productive areas and the Permanent Preservation Areas.

The Permanent Preservation Areas (PPAs) are defined by the CONAMA Resolution 303/2002 as areas with forests or other forms of vegetation destined to biodiversity protection and soil and water conservation, and that by their topographical position within the property, should be preserved.

Even being protected by the legislation, it is observed that these areas have been anthropically altered. Countless factors were responsible for the suppression of a large part of the areas destined for permanent preservation. The expansion of the agricultural borders due to economic pressure was one of the great motivating factors for the change in the land use.

Recomposing PPAs constitute an important action in view of the benefits that they provide to the environment. According to in countries of continental dimensions, the representation and characterization of PPAs on maps becomes indispensable, because it aids in the territorial planning, inspection and field actions.

In line with that, the present study aimed to develop a methodology to identify hilltops and stream margins occupied by coffee plantation PPAs and assess their compliance with the Brazilian Forest Code in those areas by analyzing the land use and cover using geotechnology.

MATERIALS AND METHODS

The studied area encompasses the municipal district of Lambari located in the South of the state of Minas Gerais, Rio Verde Basin, São Lourenço Microregion. Lambari is between the geographical coordinates 45° 21' 00" W and 21° 58' 33" S.

Land use mapping was used to verify the anthropic activities that generate PPA occupation conflict. A field survey was conducted for reconnaissance of the area.

For this work, contour curves and drainage networks from the Brazilian Institute of Geography and Statistics were used (IBGE) corresponding to the municipal district of Lambari, besides coffee mapping data gathered by the National Institute of Space Research (INPE) available on the CAFESAT Website and the resolution number 303/02 of CONAMA that defines the parameters for PPA delimitation. All of the water bodies (rivers, channels, lakes, ponds, reservoirs and flooded areas) were vectorized. The topographical chart SF-23-V-D-VI-3, in digital format (scale 1:50.000), was also used. Analyses and processing were generated in ArcGis 9.3.

From the contour curves and drainage networks, a Digital Elevation Model (DEM) of the municipal district was generated. This procedure was conducted through the *topo to raster* interpolator, generating a DEM with spactial resolution of 20 meters.

With the data from DEM the hilltop methodology was used for delimitation of the Permanent Preservation Areas, in other words, in agreement with resolution No. 303/02 of CONAMA, when there are occurrence of two or more hills or mountains in which the summits are separated by distances under five hundred meters, the PPA will be equal to the group of hills or mountains, that are delimited from the contour curve that represents two thirds of the height of the hill in relation to its base.

For the PPA map of water courses, a 30 m wide buffer was applied to each margin of the river, for water courses with margins of up to10 meters, and a buffer of 50 m of width to each margin, for water courses with margins from 10 to 50 meters, in agreement with the CONAMA Resolution 303/2002.

The data obtained individually in the mapping of the PPA class of were contained in a single map, generating a Permanent Preservation Area map. The quantitative analysis of the preservation areas was conducted through the direct comparison of the total value found per APP class.

The results of the permanent preservation area delimitation were crossed with the coffee mapping, obtaining the areas which are in conflict with the existing environmental legislation.

RESULTS AND DISCUSSION

The municipal district of Lambari presents an altimetric variation of approximately 707 meters. The total area of the municipal district is 21,380 hectares (ha), of these 2,724.66 ha are water course PPAs and 4,461.26 ha are hilltop PPAs.

PPAs represent 33.61% of the total area of the municipal district, 12.74% of these being water course margins and 20.87% hilltops. In the municipal district, coffee occupies an area of 3,937.77 ha, that corresponds to 18.42% of the total area. Coffee is present on 381.36 ha of water course margin PPAs and 1,116.92 ha of hilltops, in other words, 14 and 25.04% of respective PPAs. The Figure 1 presents the PPA and coffee spatialization in the Lambari municipal district.

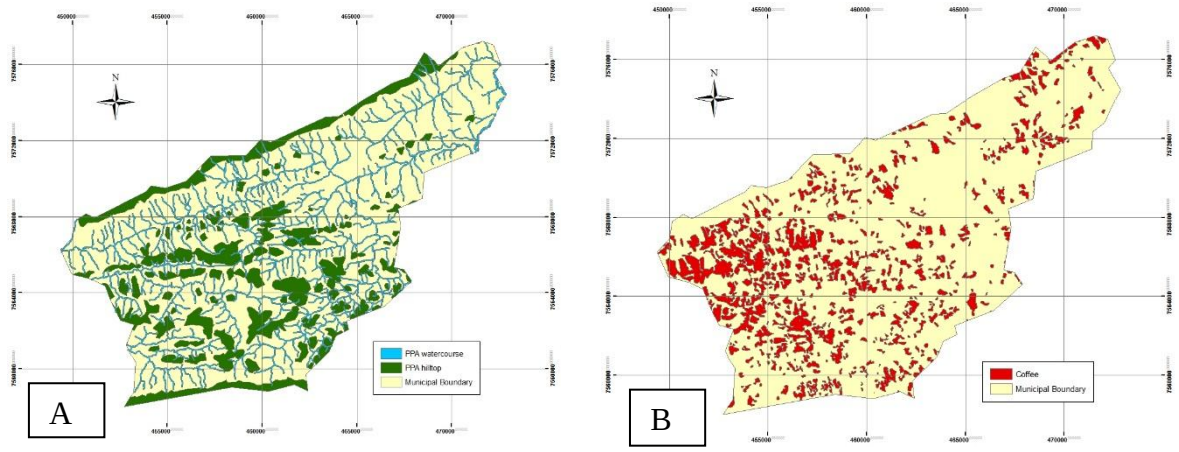

Figure 1. Map of hilltop and stream Permanent Preservation Areas (A) and coffee plantation location map in the municipality of Lambari, Minas Gerais, Brazil (B).

In spite of the ecological importance of PPAs, conflict exists between those areas and coffee growing in the municipal district of Lambari. also verified that cultivated areas occupied a large part of PPAs.

Figure 2 presents the intersection of the of PPA and coffee maps of the municipal district, demonstrating the areas where coffee production infringes the legislation. It can be seen that the coffee growing practiced in the area is one of the main factors responsible for the transformation of the natural landscape. It is important to emphasize that that activity is considered one of the largest economic resources of the area. However, farming activities are considered a great threat to the maintenance of the biodiversity in the tropics.

The PPAs basically prevent the erosion of sloped land and the silting the rivers, they ensure the hydric resources, propitiate gene pool flow, thus providing environmental services of utmost importance. The current use of PPAs generates serious environmental problems such as the erosion and compacting of the soil, besides damaging the springs and beds of the water courses.

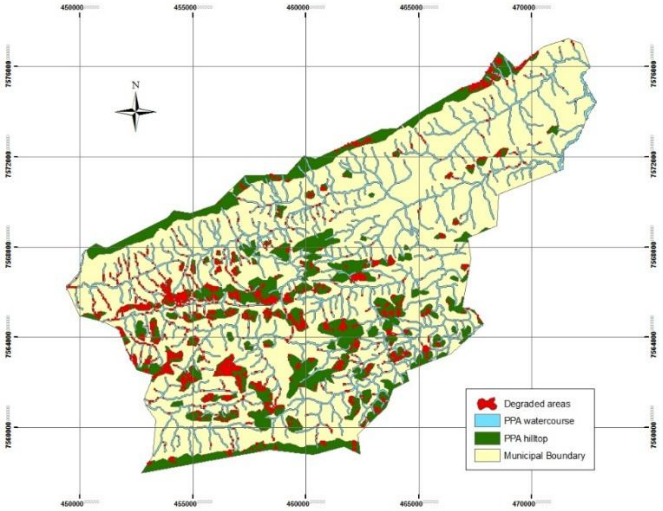

Figure 2. Map of the intersection between the permanent preservation areas and coffee production in the municipality of Lambari, Minas Gerais, Brazil.

The irregular occupation compromises hydric resources, due to the incompatibility of those agricultural activities resource conservation and protection. The conflicting areas should be converted to natural vegetation. However, it is worth noting that a large part of the coffee producing farms precede this law, therefore, the main focus of the supervisory agencies should be the monitoring, so that the increase of the deforestation due to coffee production in this municipal district does not occur. Promoting the control and monitoring of natural resource use, mainly in PPAs, is indispensable, thus allowing the execution of the environmental legislation and the preservation of biodiversity.

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Mapping of Areas for Permanent Preservation in Coffee Producing Regions of South Minas Gerais, Brazil and Identification of Land Use Conflicts

L. DE O. SILVA[1], R. A. T. BORÉM[1], G. N. LEITE[1], L. F. M. SILVA[1], M. M. L. VOLPATO[2], H. M. R. ALVES[3], T. G. C. VIEIRA[2], F. M. BORÉM[1]

[1]Universidade Federal de Lavras, CxP 3037, 37200-000, Lavras, MG, Brasil
[2]Empresa de Pesquisa Agropecuária de Minas Gerais, CxP 176, 37200-000, Lavras, MG, Brasil
[3]Empresa Brasileira de Pesquisa Agropecuária, CxP 40315, 70770-901, Brasília, DF, Brasil

SUMMARY

Climate changes and land use discussions has subsidized studies for soil and environmental characterization of regions. Geotechnologies have proved be a good tool for this kind of approach. The coffee consume in the world is in continuous expansion and increasing the consumer market requires for a high quality coffee. According to Brazilian forest code (Federal Law N^{o}. 12.651/2012 and CONAMA N^{o} 303/02), these areas are for environmental preservation. Thus, to keep a quality product, it's necessary also a sustainable techniques of production. In south of Minas Gerais, Brazil, was carried out a mapping of Permanent Preservation Areas (PPA) and coffee plantations in municipalities of Lambari, Heliodora and Jesuânia aiming to identify the land use and forest fragments. The mapping was done by ArcGis 9.2 software using as base RapidEye images. Analysis of results showed that Coffee is the second largest agricultural activity present in PPA, with few areas that have been preserved, showing low levels of compliance with resolution CONAMA N^{o}. 303/02 the large size of Brazil and its lack of information for making decisions on the rural environment and the geotechnologies present themselves as an important tool, fast and accurate. The data discussed are pre-existing models which allows evaluating the land use serving as bases for environmental planning.

INTRODUCTION

Currently environmental issues such as the soil conservation and microbasin preservation have been much discussed. However, in Brazil there are still few studies involving monitoring of PPA's, which contributes to the occurrence of problems such as the occupation of inappropriate areas with erosion risks and of the soil exposure, pollution of hydric resources and invasion of permanent preservation areas. The integrated management of the natural resources using geoprocessing techniques, has had significant progress in view of the problems resulting from the occupation of the basins, the increase of water use and the impact on the environment.

The consumption of coffee in the world is in continous expansion and the consumer market increasingly demands a coffee drink of high quality. Brazil is the largest world producer of coffee, responsible for 30% of the international market. The state of Minas Gerais stands out in the Brazilian scenario as the largest national producer of coffee, involving more than 104 thousand rural establishments and directly influencing 75% of the municipal district

economies, the south of Minas Gerais being the largest producer of the state. The the majority of coffee producers of the south of Minas Gerais are small producers, producing in altitude areas, such as hilltops and hillsides, where there are the better quality coffees. Those areas, according to the Environmental Legislation (Federal law 12.651/12 and Resolution CONAMA nº303/2002), are intended for environmental preservation.

The Permanent Preservation Areas (PPAs) are defined by the Resolution CONAMA 303/2002 areas with forests or other forms of vegetation destined to biodiversity protection and soil and water conservation, and that by their topographical position within the property, should be preserved.

The demarcation of the preservation areas on hilltops, mountains, along ridgelines and river courses is a complicated process to undertake using only conventional methods. Studies that seek to aid the environmental characterization of a specific region using geotecnologies have been conducted with good results, due to the data generation speed and its low cost.

In this regard, mapping of soil use and covering of the municipal districts of Heliodora, Jesuânia and Lambari situated in the South of the state of Minas Gerais, Brazil, was conducted with the objective of mapping and identifying the coffee growing use conflict in the Permanent Preservation Areas, defined by the effective environmental legislation, generating data to evaluate the coffee growing suitability in the area and to support a plan for sustainable environmental management.

MATERIALS AND METHODS

The studied area comprises the municipal districts of Heliodora (22º04'01''S and 45º32'31''O), Jesuânia (22º04'01''S and 45º32'31''O) and Lambari (22º04'01''S and 45º32'31''O), located in the South of the state of Minas Gerais, Rio Verde Basin.

A field survey was conducted for reconnaissance of the area. The land use mapping was used to verify the antropic activities that generate the PPA occupation conflicts.

To generate the survey maps of the land use and occupation, SRTM satellite images were used, with a resolution of 90 m, in the TIFF format and with a articulation compatible to the scale of 1:250.000, acquired through the website Brazil in Relief (http://www.relevobr.cnpm.embrapa.br/), of the Brazilian Agricultural Research Company (EMBRAPA).

All of the water bodies (rivers, channels, lakes, ponds, reservoirs and flooded areas) were vectorized. With the objective of carrying out the correction of the hydrographic network the planialtimetric topographical charts SF-23-V-D and SF-23-Y-B were used. The digital processing of the images and vectorization of the thematic maps were conducted in ArcMap 9.3.

The Permanent Preservation Area (PPAs) maps were generated for hillsides, hilltops, water courses and springs; the parameters for delimitation of these areas were given by the Law 12.651/2012 and Resolution CONAMA 303/2002. The data obtained individually in the mapping of the PPA classes were contained in a single map, generating a map of Permanent Preservation Areas. The quantitative analysis of the preservation areas was conducted through the direct comparison of the total value found per PPA class. The analyses and processing were generated in ArcGis 9.3.

For the mapping of the coffee growing areas, images from the RapidEye satellite were used, following the methodology used by the CAFESAT Project.

RESULTS AND DISCUSSION

The region presents a total area of 521.00 km², of this total 281.25 km² are made up of PPAs, that corresponds to 53.96% of the studied area.

According to the Brazilian Forest Code (6) PPAs have the environmental function of preserving the hydric resources, thus guaranteeing the conservation of elements of nature and human well-being, PPAs thus being areas that should be preserved.

Figure 1 presents the spatialization of PPAs (PPA classes studied) in the municipal districts of Heliodora, Jesuânia and Lambari.

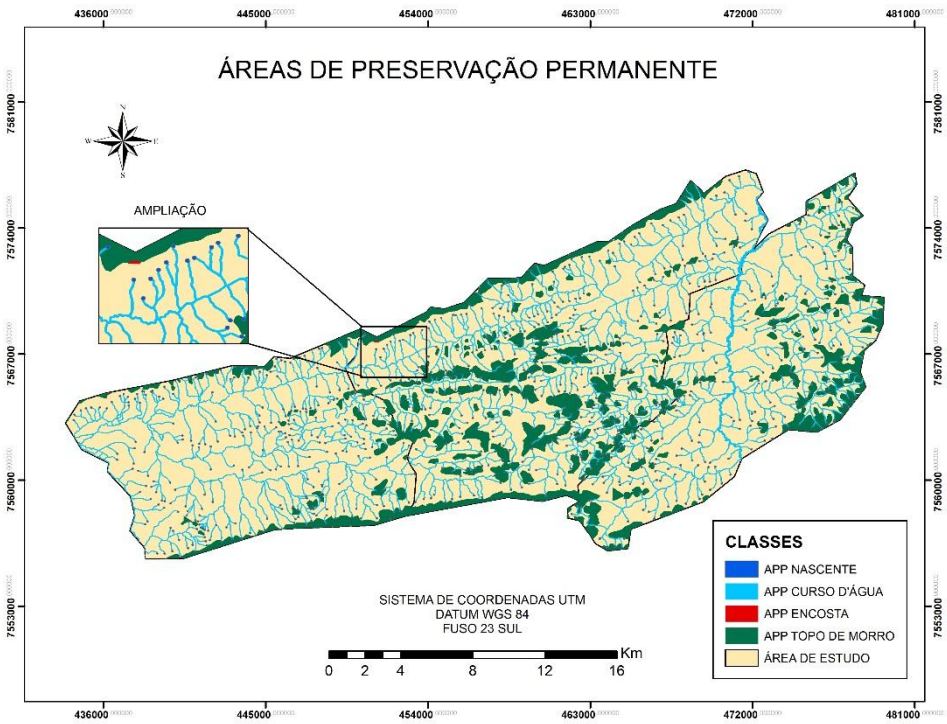

Figure 1. Map of Permanent Preservation Areas of municipalities Heliodora, Jesuânia and Lambari, Minas Gerais, Brasil.

The PPA class hilltop presents the largest area among the PPAs, corresponding to 48.75% of the total microbasin, while the PPA class that has the lowest representation in area is the hillside (over 45º), representing 0.13% of the total of the PPA areas. Those two PPAs classes present as very deteriorated, and this is a serious environmental problem, because the steepness, or degree of inclination of the land, has great influence on the soil erosion process, and in these areas the presence of the native vegetation would have a primordial role of soil protection and erosive process minimization.

PPAs of springs and water courses present high representativeness, not only for the extension of the area, but also for their fundamental role in the preservation of the environment and for dealing with a hydric resource, primordial in the maintenance of life. The PPA areas that

involve the preservation of springs correspond to 2.79% and those of water courses represent 48.32%.

Figure 2 presents the coffee growing spatialization in the areas of the studied municipal districts.

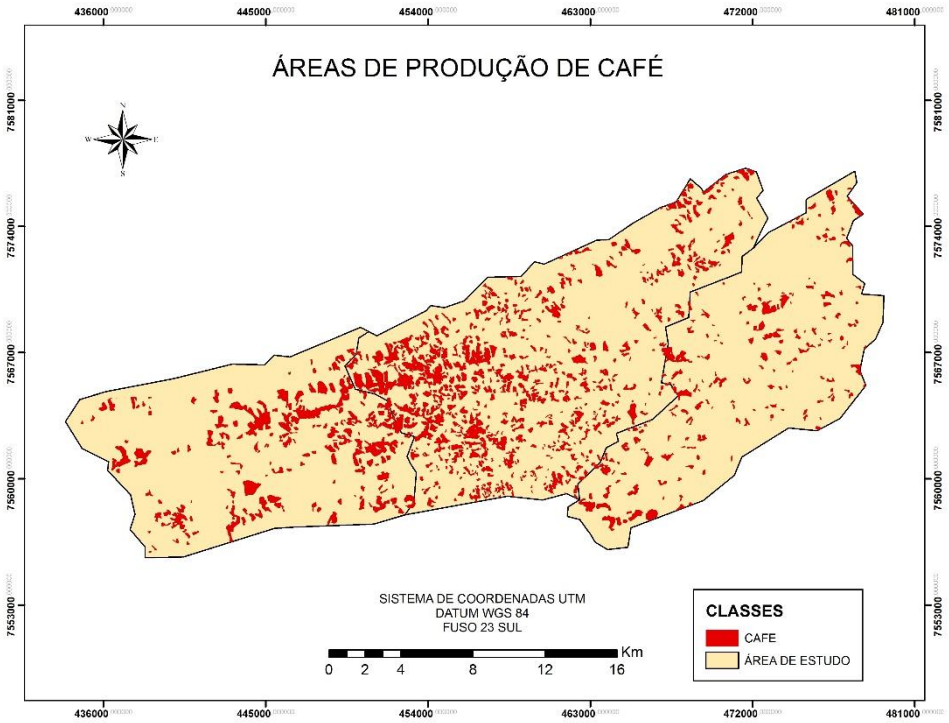

Figure 2. Map of coffee crop location in municipalities Heliodora, Jesuânia and Lambari, Minas Gerais, Brazil.

A large part of the agricultural cultivation occupies Permanent Preservation Areas, and in spite of the ecological importance of PPAs, those areas conflict with the coffee growing in the studied area, since coffee growing was the dominant use class, occupying 14.5% of the total PPAs. Various works show that cultivated areas occupy a good part of the PPAs.

These results are a relevant contribution for decision making regarding the correct administration and planning of the land occupation and use in the region, allowing the indication of priority areas for conservation and restoration, because PPAs possess important ecological functions.

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Iac Ouro Verde, a New Cultivar of *Coffea Arabica*

L. C. FAZUOLI[1], O. GUERREIRO-FILHO[1], H. P. MEDINA-FILHO[1], W. GONÇALVES[1], M. B. SILVAROLLA[1], M. T. BRAGHINI[1], J. C. MISTRO[1], P. B. GALLO[2]

[1]Centro de Café - Instituto Agronômico de Campinas (IAC/APTA), SP, BR
[2]APTA. Pólo Nordeste Paulista, Mococa, SP, BR

SUMMARY

The IAC Ouro Verde variety was developed from the backcross between cultivar Catuaí Amarelo IAC H2077-2-12-70 and Mundo Novo IAC 515-20, which was carried out at the IAC, Campinas, SP, Brazil in 1961 (H5010). The aim was to obtain a type of dwarf 'Catuaí' cultivar, but with more vigor and more productivity, while maintaining other favorable agronomic characteristics. In the selection process, from the F2 to the F6, plants were selected for high yield and red fruits. The product was named IAC Ouro Verde (IAC Green Gold), because the importance of coffee in the Brazilian agro-business. In areas irrigated or fertilized-irrigated its average productivity ranges from 40,0 to 53.4 bags of coffee /ha/year and in areas without irrigation from 30.8 to 44.0 bags. These yields are slightly superior than the yields of Catuaí Vermelho (Red Catuaí), that produced 30-48 and 23-32 bags per ha and per year, respectively. The size of the plants are short, the internodes small and secondary branching is abundant. The young leaves are green and/or bronze and the adult bright dark green. The average period, from fertilization to full ripeness of the berries is approximately 225 days, which is slightly shorter than for Catuaí Vermelho. The average sieve value is approximately 16.5 and the percentage of seeds of the normal flat type is of the order of 95%. The cup quality is excellent, as good as Catuaí. It is recommended for cultivation in coffee estates as well as by smallholders. Its short plant size allows planting density of up to 5,000 plants per hectare. It is also adapted to manual or mechanical harvesting and reacts well to any type of pruning. It can be used successfully under irrigated or under fertilized-irrigated conditions.

INTRODUCTION

Brazil is the largest producer of arabica coffee. The estimated production for 2012, made by CONAB, is 38.13 million bags of coffee. One of the factors responsible for this high production is the use of arabica coffee cultivars with high productivity. There is a continuous need for the growers to have new varieties of arabica coffee available to maintain this level of production. The objective of the research performed is to make available to growers information on a new cultivar of arabica coffee with high productivity, dwarfness, great vigor, red berries, and excellent cup quality.

MATERIALS AND METHODS

The cross leading to the IAC Ouro Verde (IAC Green Gold) cultivar was conducted in 1961, at the Instituto Agronômico de Campinas (IAC), Campinas, SP. The coffee breeding lines used were Yellow Catuaí IAC H 20077-12-70 (F3) and Mundo Novo IAC 515-20 (S4). This represents a backcross generation, because Catuai is derived from a cross between Mundo Novo IAC374-19 and Caturra Amarelo IAC476. The breeding method used was line

selection. The breeding trials were established in various coffee growing regions of São Paulo and Minas Gerais with control of rust and ferti-irrigation except in Campinas and Mococa-SP. The traits observed over 6 generations of selection were: kg of green coffee per tree, a visual vigor score (IAV vigor), a productivity score (IAV production), earliness of ripening, fruit size, outturn percentage of flat beans, of peaberries, of elephant beans, 100 green coffee bean weight and bean size (sieve average).

Vigor (IAV vigor) was assessed by giving scores from 1 to 10, where 1 and 10 represent low and highly vigorous plants with few leaves (1) and many leaves (10) respectively. The index IAV production was obtained giving scores from 1 to 10, where 1 and 10 are unproductive and highly productive, respectively. The outturn is the percentage of green coffee in relation to the dry coffee berries.

RESULTS AND DISCUSSION

Geneology of IAC Ouro Verde

The genealogy, years, places and generations of selection of IAC Ouro Verde are shown in Table 1. In successive segregating generations, trees were mainly selected for high yield, vigor and with red fruits. After 6 generations, the name IAC Ouro Verde was given to the selection (Table 1). The name, meaning 'IAC Green Gold' is associated to the Brazilian agro-business.

Table 1. Genealogy of the IAC Ouro Verde cultivar.

Year	Locality	Generation	Germplasm codes
1961	CAMPINAS-SP	F1	H 5010
			(cv. Catuaí Amarelo IAC H2077-2-12-70 x cv. Mundo Novo IAC 515-20)
1962	CAMPINAS-SP	F1	H 5010-5 (Seeds F1)
1964/70	CAMPINAS-SP	F1	H 5010-5
1967	CAMPINAS-SP	F2	H 5010-5 (Seeds F2)
1969/85	CAMPINAS-SP	F2	H 5010-5
1985/92	CAMPINAS-SP	F3	H 5010-5
1987/94	CAMPINAS-SP	F3	H 5010-5
1989/96	ALFENAS-MG	F3	H 5010-5
1995/99	PATROCÍNIO-MG	F3	H 5010-5
1997/03	CAMPINAS-SP	F4	H 5010-5
1998/03	CAMPINAS-SP	F4	H 5010-5
2001/06	CAMPINAS-SP	F5	H 5010-5
2002/08	CAMPINAS-SP	F5	H 5010-5
2004/09	CAMPINAS-SP	F5	H 5010-5
2005/10	CAMPINAS-SP	F6	H 5010-5
2006/10	MOCOCA-SP	F6	H 5010-5
2006/11	PATROCÍNIO-MG	F6	H 5010-5
2006/11	GÁLIA-SP	F6	IAC Ouro Verde
2006/11	GARÇA-SP	F6	IAC Ouro Verde
2006/11	CAMPINAS-SP	F6	IAC Ouro Verde

Final selection

The final selection of IAC Ouro Verde was done in representative areas recommended for commercial cultivation of *Coffea arabica*, mainly in the States of São Paulo and Minas Gerais (Table 2). The data on yield are therefore indicative of the potential average yield per hectare per year. Data on yield in irrigated and non-irrigated trials are presented in Table 2.

Table 2. Average yield of IAC Ouro Verde and of control Catuaí cultivars in bags of green coffee per hectare per year in irrigated and non-irrigated trials in various coffee regions in the States of São Paulo and Minas Gerais.

Irrigated Coffee			
		Average productivity of cultivar IAC Ouro Verde	**Average productivity of control cultivar**
Local	**Harvest years IAC**	**Ouro Verde**	**Catuaí Vermelho IAC 144**
Gália, SP	2	53.4	48.0
Patrocínio, MG	2	40.0	30.0
Non-irrigated Coffee			
		Average productivity of cultivar IAC Ouro Verde	**Average productivity of control cultivar**
Local	**Harvest years IAC**	**Ouro Verde**	**Catuaí Vermelho IAC 144**
Garça, SP	2	31.0	30.0 Catuaí Vermelho IAC 144
Campinas, SP	2	40.0	29.1 Catuaí Vermelho IAC 144
Campinas, SP	6	42.5	35.4 Catuaí Vermelho IAC 81
Patrocínio, MG	3	30.8	22.6 Catuaí Vermelho IAC 81
Mococa, SP	2	44.0	31.5 Catuaí Amarelo IAC 62

Analyzing the Table2 shows that the average productivity in non-irrigated areas is 30.8 to 44.0 bags of coffee /ha/year in irrigated areas and from 40.0 to 53.4 bags of coffee / ha/year. The coffee's IAC Ouro Verde, generally presented a higher productivity than the control cultivars.

Main morphological, biological and/or physiological conditions that are characteristic for identification of cultivar IAC Ouro Verde

The IAC Ouro Verde is a dwarf cultivar, but due to its great vigor, the trees have become a bit larger in size than the control cultivar Red Catuaí IAC 81. In an experiment at Heron SP, plant height at eight years reached 192 cm and 202 cm canopy diameter while the cultivar IAC 81, used as a control, the values were 185 and 184 cm, respectively. The internodes are short, secondary branching is intense resulting in a high leaf area index. The new leaves are green or bronze, while adult leaves are bright dark green. The root system is well developed. Usually, the two major blooms occur in September and October, and the ripening of fruits in May-June. The average period from fertilization to full maturation of the fruit, in Campinas, SP, is approximately 225 days, which is slightly shorter than for the Catuaí control cultivars. The berries are red. The average sieve value of the green beans is about 16.5 and the percentage normal flat beans is approximately 95%.

Behavior or reaction to major pests and diseases

As Catuaí, IAC Ouro Verde is susceptible to rust (*Hemileia vastatrix*), to the leaf spot (*Cercospora coffeicola*), to phoma (*Phoma tarda*), to the berry borer (*Hypothenemus hampei*) and to coffee leaf miner (*Leucoptera coffeella*).

Regional adaptation

The variety IAC Ouron Verde is expected to be adapted to all regions where arabica coffee grows well, and especially so in the States of São Paulo and Minas Gerais, where this variety was selected.

Beverage quality

The cup quality is excellent, similar to Catuai.

Seed availability

Currently, basic genetic seeds are being produced by IAC.

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- Instituto Nacional de Ciência e Tecnologia do Café – INCT.

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Effectiveness of Trapping and Entomopathogenic Fungus as Management Alternatives for the Coffee Berry Borer in Hawaii

E. BURBANO-GRECO, M. G. WRIGHT

**Department of Plant and Environmental ProtectionSciences,
University of Hawaii at Manoa, Honolulu, HI. USA.**

SUMMARY

In August 2010, the CBB was reported in Kona, Hawaii. Reports from processing mills estimated losses to be over 85%. Owing to the fact that the CBB has only recently invaded Hawaii, there is a dearth of information on this pest under local conditions in Hawaii. Management techniques are used without knowledge of their effectiveness. Kona coffee is grown at different elevations and fruit production is perennial, which facilitates the establishment and reproduction of the CBB year-round. An overview of several studies including trapping studies, effectiveness of B. bassiana and seasonal fluctuation are discussed here.

INTRODUCTION

Hawaii is the only state in the United States that grows coffee commercially. The largest area of production is in the Kona district on the Island of Hawaii (Table 1). The Coffee Berry Borer (CBB), Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae: Scolytinae) is the most important pest of coffee. Feeding damage on the coffee seed reduces both the crop yield and quality. CBB was reported in South Kona in August 2010 and the infestation has extended from North Kona to the district of Kau, on the south east side of the island. Climatic conditions, production practices, labor costs and the presence of feral coffee and abandoned coffee farms have made Hawaii an attractive environment for the CBB. Mill processors have reported CBB infestations from 20 to 85%. Since the CBB has only recently invaded Hawaii, management techniques are limited and the development of a strategic plan to reduce populations is an urgent matter. Beauveria bassiana (formulated as Botanigard® or Mycotrol O) was licensed for use in Hawaii (February 2011). The objectives of this study were 1) compare the effectiveness of different trap designs, 2) test the effectiveness of trap location, 3) determine the CBB seasonal fluctuation and 4) determine the effectiveness of B. bassiana.

Table 1. Number of Farms, Acreage, Yield, Marketing, Price, and Value, State of Hawaii, 2011/12.

Farms	In Crop	Harvested	Yield	Marketing[1]	Farm price[1]	Value of sales	Green Production[1]
830	8,000 ac	6,300 ac	1,300Lb/ac	8.3 M	$ 4.15/Lb	$34,4 M	6.6 M

[1]*Expressed in parchment equivalent pounds (HDoA, 2012).*

MATERIALS AND METHODS

Farm description

The study was conducted in two commercial Kona coffee plantations located at 472 m and 568 m (variety Guatemalan, also known as Kona typica) in the district of Kona. The coffee berry borer infestation ranged between 5% and 25% respectively. The first flowering was observed on April 2012 and the main harvest started on October 12, 2012.

Evaluation of trap design

The traps tested were the Broca® trap (Agroindustrias Unidas de México), the green and red Japanese beetle trap (TRÉCÉ Company, Salinas, CA) and a homemade trap. Homemade traps consisted of 2.0 L red painted gallon (recycled from milk container) with four windows (8 x 6 cm) at 12 cm above the bottom. Thirty two traps were randomly placed in a zigzag pattern with 20 m spacing between trap locations. Traps were baited with methanol: ethanol (3:1) and suspended from the coffee trees at a height of 1.5 m. Collection cups and bottom of the homemade traps had diluted glycol with water (1:1) to kill and preserve the insects. Traps were monitored from April 1 to July 27 2012 and checked every two to three weeks. An additional 10 traps were used to monitor seasonal fluctuation in CBB numbers.

Trap position

Thirty Broca® traps were randomly spaced and hug in the coffee trees at approximately 0.5, 1.5 and 2.0 m. Ten traps for each height were placed on April 17, 2012 and beetles were collected monthly until April 2013 (see above for further detail).

Beauveria bassiana

The commercial strain of B. bassiana, Botanigard® (with Widespread Max) was applied at: 16, 24 and 32 oz per acre (30 gallons of water), Widespread Max (9.5 ml per acre) alone and the untreated control. CBB infestation was sampled every 10 days for three months after treatment. Forty infested berries were collected and dissected per treatment. Number of dead CBB females was recorded.

Statistical analysis

A randomized complete block design was used for all the experiments with three replicates per treatment. ANOVA was performed followed by the Tukey test ($P < 0.05$) to compare means (SAS 2002).

RESULTS AND DISCUSSION

Evaluation of trap type

The mean number of CBB captured in the traps ranged from 115 to 13,425 beetles. None of the traps tested were consistently better than the others (Table 2). The green Japanese beetle trap captured the highest number of beetles from April 1th to May 12th however; there were no significant differences among the traps. The homemade trap captured the highest number of beetles from May 12th to July 7th. The coffee berry borer did not show a strong preference for green or red color or the design of the traps. Farmers could use any of the traps effectively,

however, price and durability are important to consider. Commercial traps are more expensive and last longer that the homemade traps.

Table 2. Mean number (± SE) of coffee berry borer captured in different traps. Farm located at 472 m. Kona district, Island of Hawaii. (May 1 to July 7, 2012).

	April-1-17	April-17-May-12	May-12-27	May-27-Jun-21	Jun-21-Jul-7
Green Japanese Beetle trap	15,795 ± 4863.5a	9,248 ± 2340.8a	623 ± 182.1a	138 ± 64.4a	271 ± 84.9b
Red Japanese Beetle trap	13,425 ± 5250.9a	3,938 ± 945.9a	1,070 ± 200.0a	295 ± 30.0a	311 ± 62.3b
Broca trap	10,495 ± 1852.5a	3,689 ± 868.5a	498 ± 71.9a	111 ± 16.95a	115 ± 2.8b
Homemade trap	5,351 ± 1606.7a	8,313 ± 1746.3a	1,101 ± 224.0a	485 ± 176.36a	683 ± 52.9a

Different letters in the same column indicate statistical differences according to Tukey test with $P < 0.05$, $n = 10$.

Height of traps

From April 17 to May 14th (flowering season), the traps placed at heights of 0.5 and 1.5 m captured significantly more CBB than traps located at 2.0 m ($F= 6.82$; $P < 0.0035$) (Table 3). During the flowering and fruit development season, CBB prefers the fruits that are located at the lower and middle part of the plant. This may be due to the presence of coffee berries present on the ground and left on the trees from the previous harvest or the pruning (February 14, 2012). However, during the harvest season the number of CBB caught at the three heights is similar. Apparently, the attractants released by the berries can interfere with the attractant released by the traps.

Table 3. Mean number (± SE) of coffee berry borer captured at 0.5, 1.0 and 2.0 m above the ground. Farm located at 472 m. Kona district, Island of Hawaii. (April 17 to August 30, 2012).

	Apr-17-May-1s	May-1-May-14	May-14-Jun-11	Jun-11-Jul-11	Jul-11-Aug-30
0.5 m	3367 ± 695.92a	2577 ± 361.07a	1179 ± 160.05a	1021 ± 97.05a	763 ± 152a
1.5 m	2473 ± 469.81a	1694 ± 368.61a	1032 ± 145.70a	677 ± 61.29b	542 ± 82.11a
2.0 m	1416 ± 255.97b	873 ± 109.97b	761 ± 77.65a	737 ± 81.40b	524 ± 46.66a

Different letters in the same column indicate statistical differences according to Tukey test with $P < 0.05$, $n = 10$.

CBB seasonal fluctuation

CBB populations were present all year round with a quickly developing peak in numbers March-May. (Fig. 1). After May, the number of females caught in traps decreased drastically and remained low until the next spring. The highest number of CBB was caught during the flowering and fruit development season.

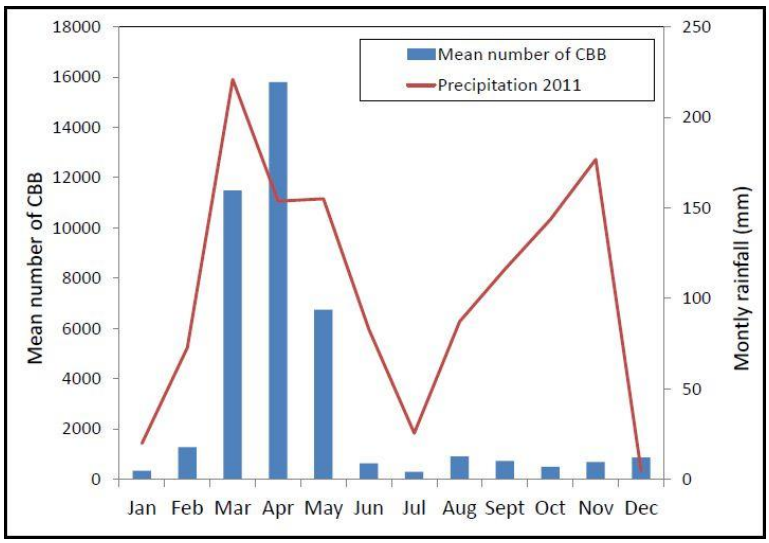

Figure 1. Seasonal beetle captures of coffee berry borer collected in Broca traps. Farm located at 568 m. 2011.

Beauveria bassiana

Before the application of Botanigard® the average percentage of mortality of CBB ranged from 3.92% to 13.69%. With one fungus application, there were no significant differences among the treatments. However, the highest mean percentage of CBB mortality was obtained with the higher doses (31 ml or 62 ml of Botanigard + 9.5 ml of Widespread Max) at 27.67% approximately after one month of the application (F= 6.32; P > 0.0135). (Fig. 2). After the second application (July 11th), the highest mortality was obtained with the high dose at 59.62% approximately 5 days after treatment.

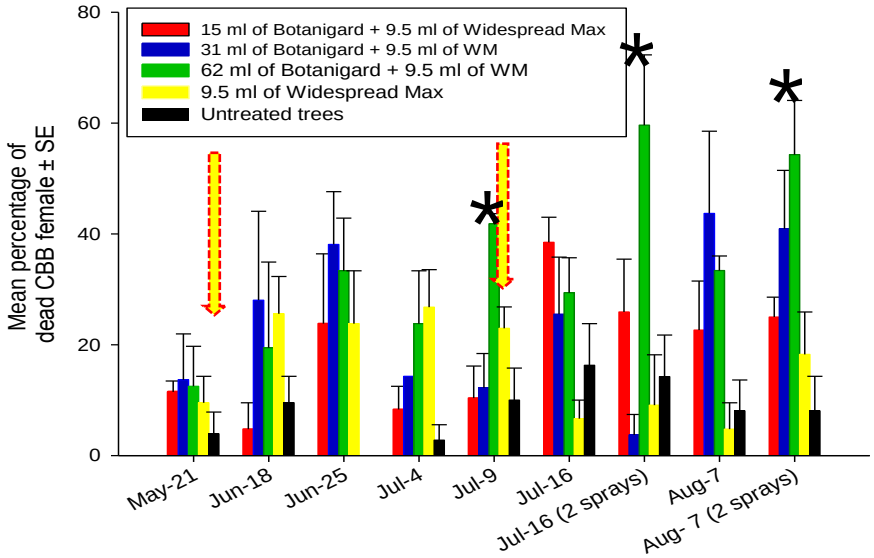

Figure 2. Effectiveness of three doses of B. bassiana (Botanigard®) on the CBB. Botanigard 1st spray: June, 12, 2012, 2nd spray: July 11, 2012. Comparisons significant at the 0.05 level are indicated by *. Farm located at 568 m. 2012.

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Water Stress in Genotypes of *Coffea Arabica*[1]

L. GALDINO[1], J. A. S. ALMEIDA[1], E. SAKAI[2], D. C. MARTINS[1], F. A. LODOVICO[1], M. B. SILVAROLLA[1]

[1]Centro de análise e Pesquisa Tecnológica do Agronegócio do Café "Alcides Carvalho", Instituto Agronômico de Campinas, CP 28, 13.001-970. Campinas, SP, Brasil
[2]Centro de Pesquisa e Desenvolvimento de Ecofisiologia e Biofísica, Instituto Agronômico de Campinas, CP 28, 13.001-970, Campinas, SP, Brasil

SUMMARY

It was evaluated the effect of water restriction on vegetative growth of plants of *Coffea arabica.* The plants were obtained from seeds germinated and kept individually in pots like soft plastic, with 1.3 kg of gravel deposited at the bottom and 3 kg of soil mix. The soil mix consisted of sieved soil, sand and coconut fiber (3:1:1). The treatments applied were water restriction and continues hydration. Each treatment consisted of four plants, maintained in greenhouse conditions and evaluated every seven days. The treatments were evaluated to plant height, number of pair of leaves and weight of pots. Besides, it was determined the leaf water potential at the last available. The results obtained showed that the Semperflorens and Catuaí were more tolerant than BA10 and Bourbon Vermelho to water restriction.

INTRODUCTION

Coffee is the most important "commodity" of world trade in agricultural products, representing an important source of income for many countries of Latin America, Africa and Ásia. But, the production of coffee can suffer reduction in relation to climate changes on the planet, such as, for example, drought resulting from the scarcity of precipitation, due to global warming. The coffee is affected by drought, which can hinder growth and, consequently, the production. Almeida et al. (2007) submitted *C. arabica* plants to water restriction for 40 days to water restriction, in condition of pot. These plants were classified according to their response to water restriction as Resistant (Semperflorens); intermediate (BA10, Catuai 81, Obatã) and sensitive (BV).

Galdino et al. (2011) also submitted plants of *C. arabica* water restriction, over 40 days, in condition of pot, and found that the genotypes Semperflorens and Catuaí were more tolerant to lack of water that the BV, Obatã e BA10. The objective of this study was to verify the effect of other treatment fluid restriction on the same plants used for the study of the "Caracterização de estresse hídrico em plantas de *Coffea arabica* resistentes e susceptíveis à restrição hídrica".

MATERIALS AND METHODS

Plants used were from the germination of seeds of *Coffea arabica* of genotypes Catuaí, BA10, Semperflorens and Bourbon Vermelho (BV) maintained, individually, in plastic pots, whose substrate consisted of 1.3 Kg of gravel, deposited in the fund and 3 Kg of mixture of soil. The mixture of soil consisted of sifted soil, sand and coconut fiber (3:1:1). Each pot was irrigated

[1] Supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

with the same volume of water. The plants of the four genotypes were subjected to treatments of water restriction and hydration continues for 21 days, and each one had four repetitions.

The treatments were evaluated each week as plant height, number of leaves, degree of leaf wilting and weight of the pots. To the degree of leaf wilting were conferred on notes, being: 1. turgid leaf; 2. leaf rolling; 3. leaf wilt. Moreover, in the last evaluation was determined the leaf water potential, by means of pump to Scholander, at the half day, with four replicates per treatment.

RESULTS AND DISCUSSION

The plants used in this study had already been subjected to water restriction for 40 days, in previous experiments "Caracterização de estresse hídrico em plantas de *Coffea arabica* resistentes e susceptíveis à restrição hídrica". After the water restriction, the plants were hydrated and nourished again.

In the present study, these same plants were subjected again to another cycle of water restriction for 21 days.

Initially, it was observed that the pots of the plants under water restriction presented weight reduction over the conduct of the experiment, since that this was more intense in the treatments of Catuaí and BA10 genotypes in relation to the others. These results indicate that the substrate of the plants was subject to gradual loss of moisture along the conduction of the experiment. In addition to this, note also that the vessels of the treatment under hydration showed some loss of moisture (Figure 1).

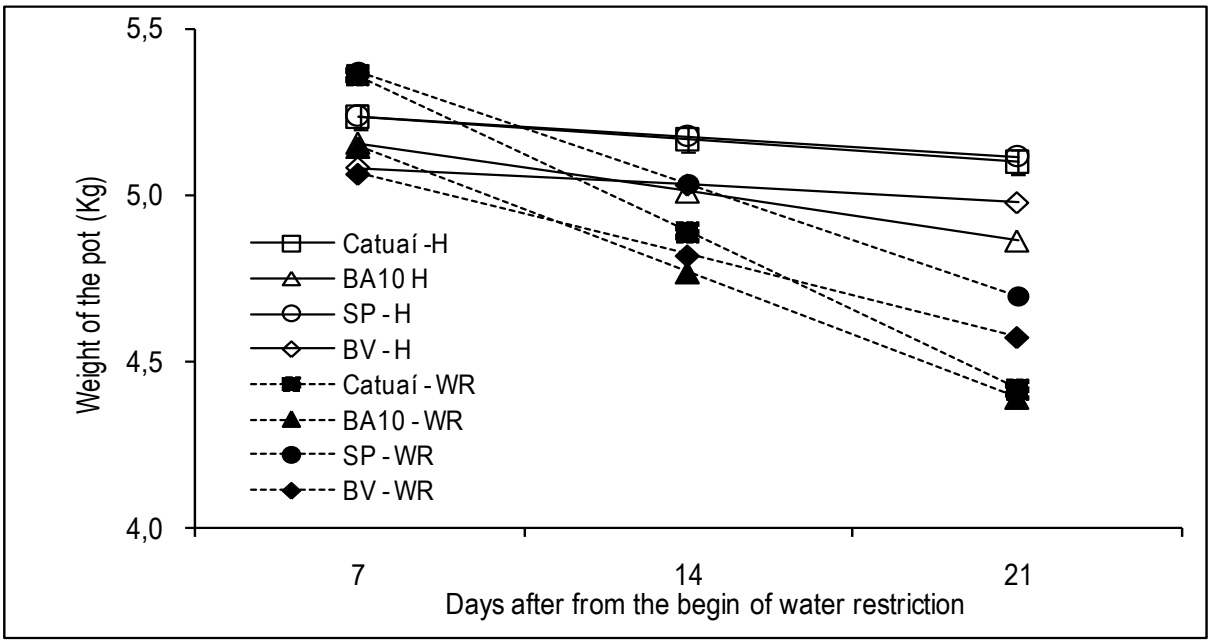

Figure 1. Determination of the weight of pots of the plants of C. arabica under water restriction (WR) and continuous hydration (H).

In Figure 2, note that in the beginning of the experiment, the control plants of all genotypes showed greater height than those under water restriction, whose difference is due to the effect of the lack of water applied the same throughout the 40 days, in the previous experiments. Another aspect to this difference, and that even after being re-hydrated and nourished these plants do not have reached height as those that have been hydrated continuously. Although

this result has been obtained in condition of pot, the same seems to indicate that the lack of water for a long period of time cause metabolic changes that may harm the future growth and development of a young plant. The water deficiency causes changes in the behavior plant whose irreversibility will depend on the genotype, the duration, severity and stage of plant development.

In Figure 2 it is also observed that the plants under water restriction still showed some growth in height which was greater for the genotypes BA10 e BV (Figures 2B e 2D) and less expressive for Catuaí and the Semperflorens (Figures 2A e 2C). In addition, also there is the formation of new leaves on the plants of genotypes Catuaí, BA10 and Borboun Vermelho under water restriction (Figuras 3A, 3B e 3D) while to the Semperflorens this response occurred in a less intense (Figura 3C). A plant under condition of restriction of humidity tends to be reduction of growth throughout the duration of treatment. Possibly, in this study, the observation of the occurrence of growth under water restriction can be attributed, possibly, the residual moisture in the mixture of soil, which was not lost by evaporation in the period of driving the experiment which may have been sufficient to keep the metabolic activity on the production of plant mass.

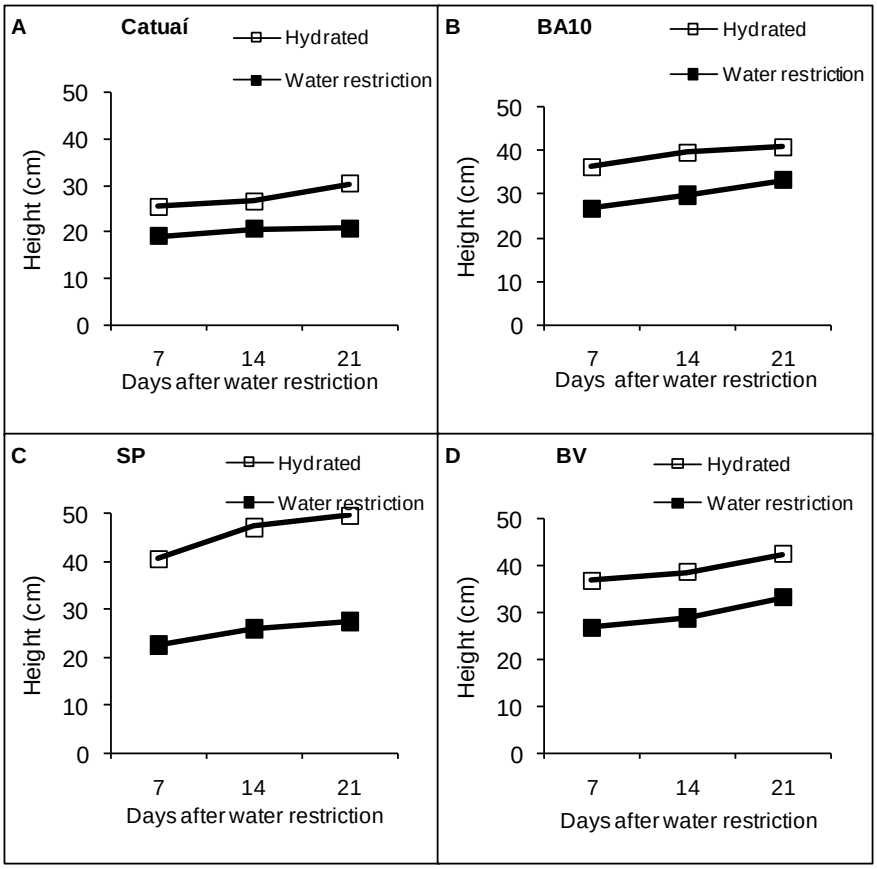

Figure 2. Effect of water restriction on height of plants of *C. arabica* kept in condition of the vessel in greenhouse.

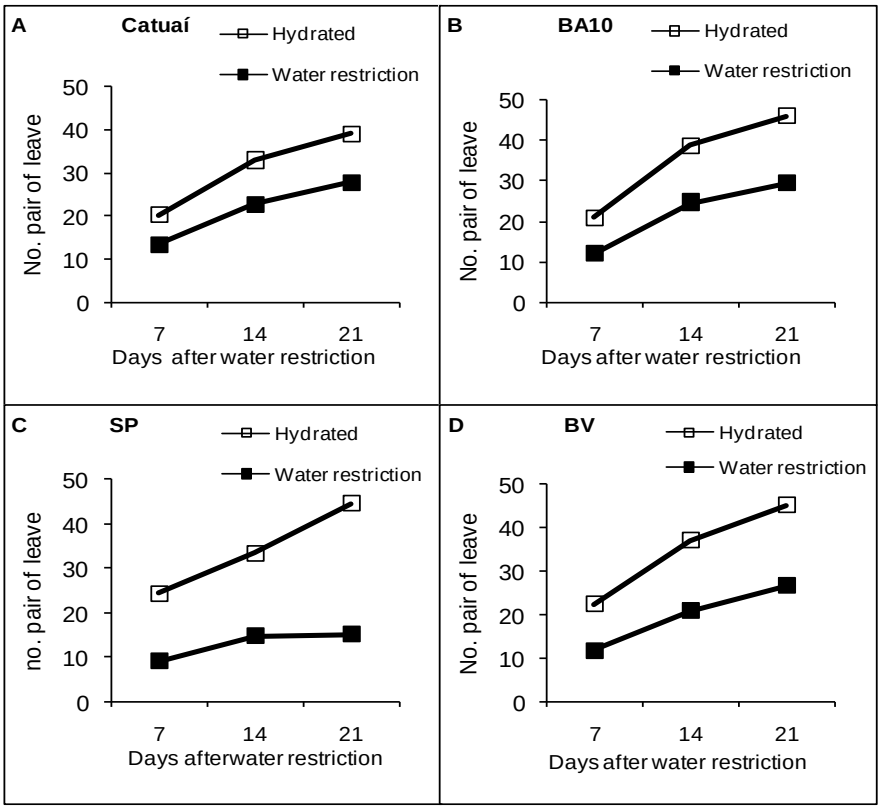

Figure 3. Effect of water restriction on number of leave of plants of *C. arabica* kept in condition of the vessel in greenhouse.

It was verified that the plants of the BA10 and BV under water restriction had leaf wilt, whose notes were respectively 1.5 and 1.75 while the genotypes Semperflorens and Catuaí showed no symptom (Table 1). The results indicated that the Semperflorens and Catuaí were tolerant to the treatment of water restriction.

Table 1. Degree of leaf wilting of plants of *C. arabica* subjected to water restriction in greenhouse.

	Hydrated	Water Restriction
Catuaí	1,00	1,00
BA10	1,00	1,50
SP	1,00	1,00
BV	1,00	1,75

Notes: 1. turgid leaf; 2. leaves rolled; 3. leaf wilt.

In Figure 4 are the results of the water potential of leaf plants after 21 days the application of the treatments. In plants under hydration the water potential was on average -2.0 Mpa. In addition, the water potential was lower for the Semperflorens and Catuaí, respectively, of -2,60 and -2,95 MPa and more negative to the Bourbon Red, -3,38 MPa.

The results highlight the Semperflorens genotypes and Catuaí under water restriction had no occurrence of leaf wilting along the most driving of the experiment while the BV was quite affected.

The results highlight the Semperflorens genotypes and Catuaí under water restriction had no occurrence of leaf wilting along the most driving of the experiment while the BV was quite affected. In addition, the water potential was lower for the Semperflorens and Catuaí, respectively, of -2,60 and -2,95 MPa and more negative to the Bourbon Red, -3,38 MPa.

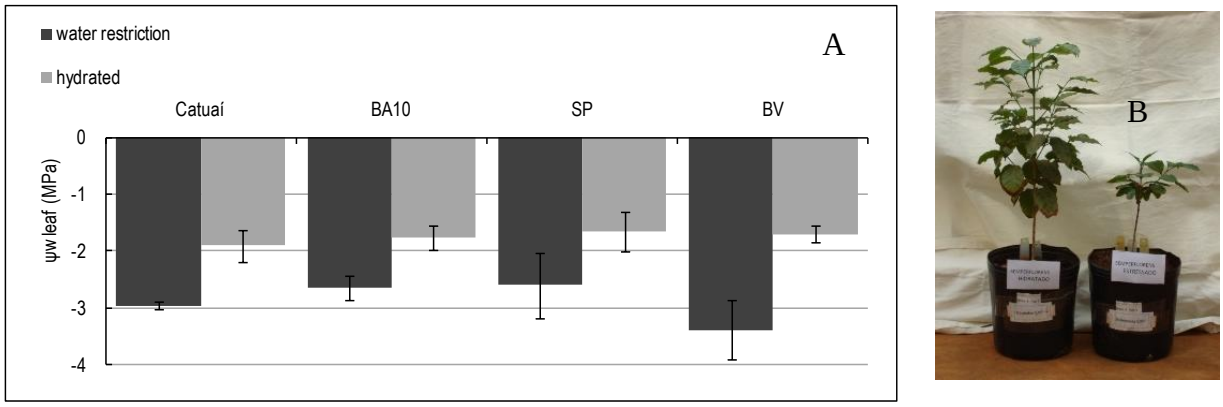

Figure 4. A. Determination of leaf water potential of plants of *C. arabica* subjected to water restriction in greenhouse. B. Plants of Semperflorens.

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Reduced Spraying Liquid Volumes for *Leucoptera Coffeella* (Lepidoptera: Lyonetiidae) Control in Coffee Plants

S. T. DECARO-JUNIOR, M. C. FERREIRA, O. LASMAR

FCAV/UNESP - Department of Fitossanidade
Access road Prof. Paulo Donato Castellane, s/n 14884-900 Jaboticabal, SP - Brazil

SUMMARY

In Brazil, coffee plantations suffer a decrease in production due to infestation of *Leucoptera coffeella*, bringing the necessity of pesticides applications to control this insect, burdening production costs. This control is characterized by the use of high volumes that generate waste and pollution of the environment. With the emergence of new equipments and spray nozzles capable of producing smaller and more uniform droplets, it is possible to succeed in low volumes application, contributing to reduce expenses of the phytosanitary treatment. In area containing coffee plants in the municipality of Altinópolis - São Paulo State, previous coffee leaves collection was realized to check the level of *L. coffeella* infestation wherein it was performed spray tests with 20, 30, 40 and 200 $L.ha^{-1}$, in comparison with conventional spray at 400 $L.ha^{-1}$ and an untreated area as control, to reduce the insect population that was presented above the level of control. The experiment design was a randomized block with four replications, performed between October 17th and November 7th, 2011. After the application, from 7 to 7 days, three samples of leaves were made in the area for each treatment. The leaves were taken to the laboratory of the Núcleo de Ensino e Desenvolvimento em Tecnologia de Aplicação (NEDTA), UNESP, Jaboticabal - São Paulo State, and were analyzed with the aid of a stereomicroscope. The variables analyzed were the number of live caterpillars and dead caterpillars. It was performed an analysis of variance and comparison of the means by Tukey test at 5% significance level for all treatments. The variables were different with respect to the different treatments, so that the treatment 200 $L.ha^{-1}$ contained the smallest number of live caterpillars and greater number of dead caterpillars for the three assessments, followed by treatment 400 $L.ha^{-1}$. It is concluded that, even reducing the conventional spray volume by half, while maintaining the amount of active ingredient of phytosanitary products per unit area, it is possible to obtain a better result in the control of *L. coffeella* infesting coffee plants.

INTRODUCTION

The Brazilian coffee production for 2011/12 harvest must hit 43.15 million bags, a very good expectancy for cycles of low productivity since yield of the 1999/2000. This forecast appears in the third survey this year, according to the Companhia Nacional de Abastecimento (CONAB), remaining Brazil as the greatest coffee producer.

It is highlighted in coffee plantations the coffee leaf miner, *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae), considered the most important pest on the culture. This insect, in its immature phase, builds mines in the epidermis of the leaf, due to the destruction of the palisade tissue, used by caterpillars as food.

It is common that in a phytosanitary application aiming to control L. coffeella by spraying, some areas of the treated plants are not sufficiently covered by spraying liquid. Thus, the

insect can select these areas to move, feed themselves and grow, with minimal or no contact with the pesticides which they lose their toxicity over time.

In order to suppress the occurrence of these failures, are traditionally used applications at high spraying volumes, whose liquids are applied beyond the point of runoff as a result of the difficulties to cover adequately the parts of plants, due to plant diversity and density of leaves, branches and fruits.

Some adjuvants when added to a spray at high volume, because of its amphoteric nature, reduce the surface tension of the liquid, increasing the runoff. In applications at medium and low volume its use can promote a better distribution of the applied droplets.

Making use of smaller spraying volumes it increases considerably the autonomy and operational capabilities of atomizers, also reducing the risks of environmental contamination because it minimizes the runoff, if adequately used, evaporation and drift. With this gain in operational capability, it is possible to work larger areas spraying with less time under good weather conditions. This reduction, however, brings the need for application technology improvements.

The aim of this study was to evaluate the control of L. coffeella in coffee plantation with applications at reduced spraying liquid volumes.

MATERIALS AND METHODS

In an area containing coffee plants in the municipality of Altinópolis - San Paolo State, Brazil, previous coffee leaves were collected in order to check the level of *L. coffeella* infestation which was above the level of control, hence requiring phytosanitary treatment for the whole coffee plantation sampled.

Aiming to decrease the infestation, the experiment performed spray tests with reduced spraying liquids volumes at 20, 30, 40 and 200 $L.ha^{-1}$, in comparison with conventional spray volume at 400 $L.ha^{-1}$ (Figure 1), currently taken by the property. An untreated area was adopted as control wherein no phytosanitary product was sprayed. For the ultra low volumes of 20, 30 and 40 $L.ha^{-1}$ an atomizer equipped with pneumatic nozzles was adopted, whereas for 200 and 400 $L.ha^{-1}$ it was used an atomizer fitted out with hydraulic nozzles. For all the treatments, the spraying liquid based on an admixture of LUFENURON+PROFENOFOS as active ingredient at a dosage of 800 $mL.ha^{-1}$, mineral oil as adjuvant at a dosage of 5 $L.ha^{-1}$ and water to complete the spray volumes.

The experiment had 6 treatments in randomized block design with four replications, performed between October 17th and November 7th, 2011. After the application, from 7 to 7 days, three samples of leaves were made in the area for each treatment. The leaves were conduced to the laboratory of the Núcleo de Ensino e Desenvolvimento em Tecnologia de Aplicação (NEDTA), UNESP, Jaboticabal - San Paolo State, Brazil, and were analyzed with the aid of a stereomicroscope.

The values were transformed by $(x+1)^{0,5}$ expression and the variables analyzed were the number of live caterpillars and dead caterpillars of each treatment. It was performed an analysis of variance and comparison of the means by Tukey test at 5% significance level for all treatments.

RESULTS AND DISCUSSION

The experiment has shown much difference between the treatments, highlighting the dosage at 200 L.ha^{-1} of spraying liquid, based on live and dead caterpillars and according to the three collections made after the spray in the area (Tables 1). The treatments using ultra low volumes sprayed cannot be adopted on coffee plantation because they showed lower efficacy on control, in configuration adopted.

Table 1. Means of *L. coffeella* live and dead caterpillars in coffee leaves for each treatment and collection done before and from 7 to 7 days after the spray. Altinópolis – SP State, Brazil, 2011.

Treatment	Previous	oct/24	oct/31	nov/07	General
No spray	4.09 b[1]	3.57 d	3.24 f	1.80 e	2.97 f
20 L.ha^{-1}	4.90 e	3.97 f	2.60 c	1.58 d	2.89 d
30 L.ha^{-1}	4.36 d	2.91 c	2.87 d	1.22 b	2.46 c
40 L.ha^{-1}	3.74 a	3.67 e	3.20 e	1.50 c	2.94 e
200 L.ha^{-1}	4.90 e	2.18 a	2.12 b	1.12 a	1.87 a
400 L.ha^{-1}	4.24 c	2.55 b	2.06 a	1.12 a	2.00 b
CV $_{live}$ (%)	36.66	47.25	32.52	29.41	33.6
No spray	2.34 a[1]	1.32 e	2.55 e	2.24 d	2.10 e
20 L.ha^{-1}	1.22 e	1.32 e	2.78 d	2.18 e	2.18 d
30 L.ha^{-1}	1.41 d	1.73 d	2.50 f	2.00 f	2.10 e
40 L.ha^{-1}	1.58 c	2.29 c	3.16 c	2.36 c	2.63 c
200 L.ha^{-1}	2.06 b	4.72 a	3.87 a	3.43 a	4.04 a
400 L.ha^{-1}	1.58 c	3.74 b	3.35 b	3.24 b	3.45 b
CV $_{dead}$ (%)	37.23	75.82	36.10	34.87	42.71

[1]*Means transformed followed by the same letter on the column have no difference according to Tukey test at 5% of propability*

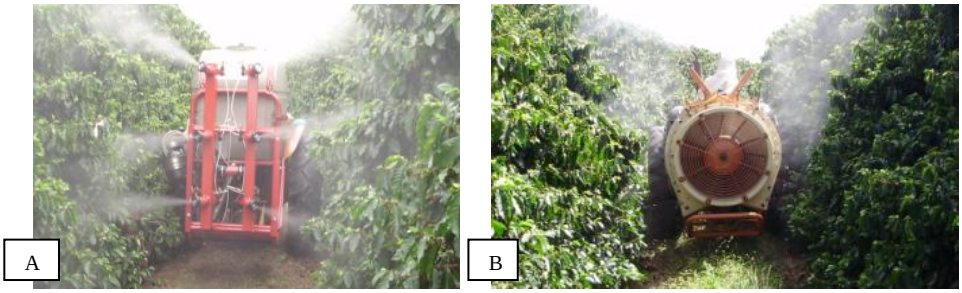

Figure 1. A. ULV sprayer used for the 20, 30 and 40 L.ha^{-1} volumes. B. Conventional sprayer used for the 200 and 400 L.ha^{-1} volumes. Altinópolis – San Paolo, Brazil, 2011.

The vehicle used (oil or water) on spraying liquids has no relation with the efficacy of phytosanitary products, so that in an ultra low volume application, using the indicated products of high concentration, it is possible to obtain similar or even better results in comparison to high volumes. Therefore, the quantity of active ingredient per unit area should be responsible for the success on the control.

With the production of small and uniform droplets during a spray, the coverage of the target may be better and the volume of spraying liquids may also be reduced; however there is the necessity of application technology improvements.

The admixture of oil could have contributed to a better coverage on the leaves once it is known that its use protects the droplets from the drift and promotes a decrease on the surface tension of the spraying liquid, making possible for the treatment to have the same efficacy on *L. coffeella* control using a volume reduced by half. Further studies about combination of oil in spray liquid and other configuration of equipment to spray on ultra low volume have been carried out.

Concluding, the conventional spray volume at 400 $L.ha^{-1}$ may be reduced by half, at 200 $L.ha^{-1}$, with a better control for *L. coffeella*, proving that it is possible to use reduced volumes per area through new researches on application technology.

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Kinetics of Surface Tension and Contact Angle of Droplets from Spraying Liquids with Adjuvants on Coffee Plant Leaves

O. LASMAR, M. C. FERREIRA

FCAV/UNESP - Department Phytosanitary
Access road Prof. Paulo Donato Castellane, s/n 14884-900 Jaboticabal, SP - Brazil

SUMMARY

We evaluated the kinetics of surface tension and contact angle of droplets formed from aqueous spraying liquids with different chemical groups of commercial adjuvants: LI 700 (fosfatidilcoline and propionic acid), Agral (ethoxylated alkyl phenols), TA 35 (sodium lauryl ether sulphate), Veget Oil (ester fatty acids), Agridex (aliphatic hydrocarbons) and MSO (methyl ester of vegetable origin) and water on artificial (glass slide) and natural (coffee plant leaves) surfaces. The experiment was carried out at the Laboratory of the Center for Research and Technology Development Application – NEDTA, Dept. Phytosanitary - UNESP, Campus Jaboticabal-SP, Brazil, in February 2012. Fragments of leaves of coffee plant with one cm² were cut, fixed in glass slide and taken to an automatic tensiometer (model OCA-20 Dataphysics Germany), for five minutes, to obtain image analysis and using software were obtained the kinetics of surface tension and the contact angle formed between the spraying liquids and the surfaces analyzed. The data were subjected to analysis of variance by F test and the averages were compared by Tukey test at 5% probability, besides a graphical analysis. All spraying liquids with the adjuvants evaluated spread on the surface of the coffee plant leaves, therefore, the contact angles were lower at 90º. The adjuvants - LI 700, Agral, TA 35, Veget Oil, Agridexand MSO reduced the surface tension of water decreasing the contact angle between the spray and the surfaces, this caused a better wetting of the coffee plant leaves than the water, which effectively contributes to a better coverage and spray efficiency.

INTRODUCTION

The first estimate of production of coffee (arabica and conilon) for the 2012 season indicates that Brazil should harvest an average of 50.61 million of bags of 60 kilograms of the processed product, representing an increase of 16.4% when compared with that obtained at the previous season which was 43.48 million bags. This growth is mainly due to the high biannuality year. Confirming the result, this will be the largest crop ever produced in Brazil, surpassing the volume of 48.48 million bags harvested in 2002/03.

However, due to the large extent of continuous areas occupied by the culture, there were developed a series of problems that require phytosanitary treatments, predominating the chemical method, typically by the use of sprayings.

In a spray, the spraying liquids formed by the commercial product added to water, undergoes fragmentation and usually causes the formed particles (droplets) become susceptible to drift and evaporation.

In Brazil, there is still much space for studies related to application technology, and the development of technologies can contribute to the current system faced serious problems due to large-scale applications with high volumes of syrup and under adverse weather conditions

of high temperatures and low relative humidity of air. Furthermore, developments in this sector can lead us to an agriculture more competitive with less risks to the environment.

In spraying at the field, it is common for some areas in the plants do not receive adequate spray coverage. With this, the target organisms, like insects, can select areas to walk and feed themselves, having little or none contact with the pesticides, which lose their biological activity over time. Therefore, to achieve better coverage, the producers have made use of some tools such as adjuvants.

Adjuvants are substances or compounds without phytosanitary property, which are added in an agricultural preparation (except water) to increase the efficiency or modify certain physico-chemical properties of the solution to facilitate the application or minimize potential problems.

Among the effects of adjuvants, stands out the reduction of surface tension of the droplet, causing flattening, which increases the surface contact with the biological target and improving coverage. The surface tension refers to forces that exist at the interface of immiscible liquid, preventing them from mixing.

When a drop of water is on a surface, the contact angle depends on the characteristics of that surface. If it is water repellent, the contact will be less and the droplet will be more spherical. If the surface is more hydrophilic, the drop spreads, and may even form a uniform film.

The contact angle influences the distribution of water or a solution in a surface, thereby determining the wetness of the same. When this angle is 0° this is an extreme case of maximum chemical affinity between the surface and the liquid and, therefore there will be full spreading of liquid on the surface. When it is equal or close to 180° is the other extreme case, where the liquid has no interaction with the surface. When the contact angle is less than 90° can be considered that the surface is wetted by the liquid.

The use of different chemical groups of adjuvants in the spray can favor the breaking of the surface tension of water and increase the affinity between the spray liquid and the leaf surface of plants.

In this way, this study aimed to evaluate the kinetics of the surface tension and contact angle of droplets formed by different groups of pesticides containing the chemical additives in coffe leaves.

MATERIALS AND METHODS

Evaluations of the kinetics of surface tension and contact angle of droplets on artificial surfaces (glass) and natural (coffee leaves) were carried out at the Laboratory of the Center for Research and Technology Development Application – NEDTA, Dept. Phytosanitary - UNESP, Campus Jaboticabal-SP, Brazil, in February 2012.

The leaves used in the evaluations were collected from plants presents in own campus. They were then cut into 1 cm^2 and to make them without roughness and not compromise the evaluation, the leaves were trapped in a glass slide using double-sided tape.

For the evaluations, the test solutions were prepared with common water of supply network, comprising the following adjuvants: LI 700 (fosfatidilcoline and propionic acid), Agral

(ethoxylated alkyl phenols), TA 35 (sodium lauryl ether sulphate), Veget Oil (ester fatty acids), Agridex (aliphatic hydrocarbons) and MSO (methyl ester of vegetable origin), at dosages of 500, 30, 50, 500, 200 and 200 mL c.p. 100 L^{-1}, respectively. Ultra-pure water and tap water were used as control.

Measurements were made every second in a total time of five minutes using an automatic tensiometer, Model OCA-20, of Dataphysics Germany where the surface tension is determined by the pendant drop method. The image of the drop is captured by camera and the equipment analyzes the shape of the pendant drop at the extremity of a needle attached to a syringe of the liquid emission to be analyzed by asymmetry of axes (ADSA axisymmetric drop shape analysis). A specific software that uses an ideal position as a reference line in the image field is used to identify the key point to start the images recording. The surface tension is determined by scanning and analysis of the drop profile, using the Young-Laplace equation for the adjust.

To prevent evaporation of the droplets, we used a glass plaque containing water, positioned below the pendant drop throughout the period of data collection.

To evaluate the kinetics of the contact angle of droplets, we used the same equipment (OCA-20), which also receives these values by image analysis. The measurements also occurred every second a total time of five minutes after deposition of each drop in two surfaces (artificial and natural).

The data were subjected to analysis of variance by F test and the averages were compared by Tukey test at 5% probability, besides a graphical analysis.

RESULTS AND DISCUSSION

The values of surface tension (IFT mN m^{-1}) were reduced in all treatments, except for (T 7) ultra pure water and (T 8) ordinary water (Figure 1a, Table 1).

Among adjuvants, (T 5) Agridex presented the highest final value of surface tension, followed by (T 2) Agral, (T 3) TA 35, (T 4) Veget Oil, (T 6) MSO and (T 1) LI 700 (Figure 1a, Table 1).

The results observed in this study are similar to those observed by Iost that evaluated the surface tension of pesticides with adjuvant LI 700 and verified that with 100% and 200% of the dose recommended by the manufacturer, obtained respectively 32.90 and 32.98 mN m-1 of surface tension and Xu et al., who observed that the vegetable oil concentrate and the modified seed oil (MSO) tested at various concentrations, showed mean values of surface tension of 36.38 and 34.00 mN m^{-1}, respectively.

The behavior of the droplets in relation to the kinetics of contact angle measured in both surfaces (glass sheet and coffee) showed that there was a significant reduction in contact angle of droplets in all treatments (Figures 1b and 1c, Table 1). Furthermore, none of treatments showed water contact angle exceeding 90°, indicating that the two surfaces are characterized as hydrophilic.

In determining the contact angle of droplets in natural surface (coffee leaf), observed that the final values were higher in the treatments (T 8) tap water and (T 7) ultra-pure water. The treatments (T 6) MSO, (T 5) Agridex, (T 4) Veget Oil and (T 3) TA 35, showed intermediate

final values and finally, treatments (T 2) Agral and (T 1) LI 700 were those who had the lowest final values of the contact angle of droplets after five minutes of assessment (Table 1).

There is a strong relationship between surface tension and contact angle of droplets. The higher the surface tension, lower is the surface wettability and thus greater will be the value of contact angle obtained.

This relationship was observed in this study, since the treatments (T 7) ultra pure water and (T 8) common water had the highest values of surface tension and contact angle of droplets, providing an lower wettability of the surfaces in relation to other treatments, constituted by adjuvant LI 700, Agral, TA 35, Veget Oil, Agridex and MSO (Table 1).

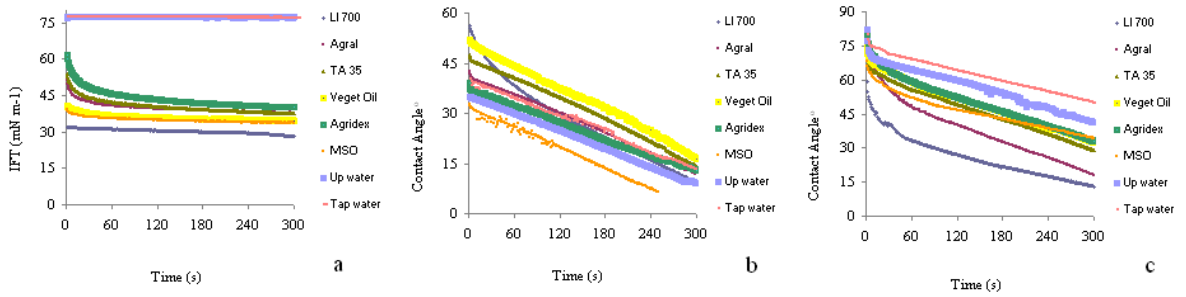

Figure 1. Kinetics of surface tension (a) and the contact angle of droplets on artificial surfaces (glass) (b) and natural (coffee leaf) (c), from the spraying liquids with adjuvants LI 700, Agral, TA 35, Veget Oil, Agridex, MSO, ultra-pure water and commom water. Jaboticabal, SP, 2012.

Table 1. Initial, middle and final values (iv, mv and fv) of surface tension measurements (IFT mN m^{-1}) and contact angles (CA°) of droplets to artificial (glass) and natural (leaf coffee) surfaces, in function of treatments. Jaboticabal, SP, 2012.

Trat.	IFT (mN m^{-1})			Contact Angle° (glass)			Contact Angle° (coffe leaf)		
	iv	mv	fv	iv	mv	fv	iv	mv	fv
1.	31,78 e	30,25 e	28,02 e	56,24 a	27,34 ab	9,17 ab	59,27 b	36,17 d	13,07 d
2.	51,30 c	39,70 c	37,76 bc	42,38 abc	26,79 abc	12,32 ab	79,14 a	48,57 cd	18,00 cd
3.	53,92 c	40,48 c	37,44 c	47,53 abc	31,09 ab	13,98 ab	75,29 a	52,12 bc	28,95 bc
4.	40,73 d	36,02 d	34,76 d	52,03 ab	34,29 a	16,46 a	74,45 ab	53,44 abc	32,44 b
5.	61,50 b	43,61 b	40,02 b	39,17 bc	24,77 abc	13,00 ab	80,34 a	56,69 abc	33,05 b
6.	38,99 d	34,76 d	33,71 d	32,65 c	19,04 c	6,53 b	68,01 ab	51,19 bc	34,37 b
7.	77,11 a	77,30 a	77,14 a	35,04 c	21,98 bc	9,15 ab	82,12 a	61,65 ab	41,19 ab
8.	77,15 a	77,24 a	76,90 a	40,34 abc	26,61 abc	12,23 ab	80,08 a	64,95 a	49,83 a
Mean	54,06	47,42	45,72	43,17	26,06	11,61	74,83	53,09	31,36
CV	1,88	1,66	1,86	13,76	14,35	22,89	7,49	8,44	14,07

Means followed by same letter do not differ by Tukey test (p <0.05), CV (%): coefficient of variation

Therefore, the results obtained in this study showed that the adjuvants - LI 700, Agral, TA 35, Veget Oil, Agridex and MSO reduced the surface tension of water decreasing the contact angle between the spray and the surfaces, this caused a better wetting of the coffee plant leaves than the water, which effectively contributes to a better coverage and spray efficiency.

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Coverage of Spraying Liquids in Coffee Plants Sprayed with Original and Adapted Equipment for Tall Plants

M. C. FERREIRA, G. J. LEITE, O. LASMAR

FCAV/UNESP - Department Phytosanitary
Access road Prof. Paulo Donato Castellane, s/n 14884-900 Jaboticabal, SP - Brazil

SUMMARY

It were evaluated the coverage of spray droplets of coffee plants by using a spray with and without a branch auxiliar for tall plants. The experiment was conducted in October 2011 in Patrocinio, Minas Gerais state, Brazil, and the experimental plots consisted of 30 plants of Catuaí IAC-99 variety, with about 12 years old, and average height of 3.50 m. The experiment was conducted following a randomized block design with seven treatments, four replications and two spray volumes (450 and 750 L ha-1). To check the distribution provided by droplets sprayed, water sensitive papers were used in one plant per plot, placed in four quadrants, and in two heights, representing the regions of the plant. After spraying in each parcel of four replicates, the papers were removed and placed in Petri dishes to keep them well away from exposure to moisture. Later, these papers were scanned and evaluated in specific program by determining the percentage of coverage. The data were statistically analyzed by F test and the averages compared by Tukey test at 5% probability. The auxiliary branch installed in the rear of the equipment is best, when compared to use of equipment without this feature, or installed in the front of the equipment and the application volume of 450 L ha-1 results in larger coverages of spray on the coffee plants, compared to 750 L ha-1, considered sufficient according to the parameters evaluated.

INTRODUCTION

The first estimate of production of coffee (arabica and conilon) for the 2012 season indicates that Brazil should harvest an average of 50.61 million of bags of 60 kilograms of the processed product, representing an increase of 16.4% when compared with that obtained at the previous season which was 43.48 million bags. This growth is mainly due to the high biannuality year.

Although the numbers are favorable, the crop faces constant difficulties, mainly due to the occurrence of phytosanitary problems, such as insects, mites and pathogens.

In the coffee production process, the application technology of plant protection products becomes increasingly important, especially due to the fact that the chemical control occurs mostly through net. This type of application has been suffering for decades a pressure from users and society in order to maintain the efficiency of biological treatment, to improve the operational performance of equipment, reduce the volumes and costs of applications and to minimize environmental contamination.

The pest management in tree crops such as coffee, citrus fruit and many other species should be based on some aspects of application technology, in particular, a proper range of the plant canopy, the choice of equipment and the application volume used. As the higher plants are also increases the difficulties in evaluating the efficiency of application.

In sprayers for tree crops, any design that maintains a minimal effective distance between each nozzle and their respective target improves deposit uniformity and reduces drift. Furthermore, the use of pipelines (e.g. towers and vertical spears) are growing in popularity as operators pursue better work rates, more accurate deposition, smaller buffer zone requirements and minimal wastage.

For the evaluation of agricultural sprayers, there are several options of methodologies. Coverage measurements and droplet deposition can be performed using target as the water sensitive paper, artificial and natural targets such as the leaves of the plants themselves.

The objective of this study was to evaluate the coverage of spray droplets on coffee plants using a spray with and without adaptation of an auxiliary branch for tall plants.

MATERIALS AND METHODS

Experiment was carried out on October 2011 in Patrocinio, Minas Gerais State, Brazil, and the experimental plots consisted of 30 plants of Catuaí IAC-99 variety, with about 12 years old, and average of 3.50 m height.

The machine space for scroll was 1.5 to 1.8 m between rows, moving nearly touches the culture (Figure 1).

Figure 1. Positioning of auxiliary branch in Speed Jet: A) behind auxiliary branch, B) without auxiliary branch, C) with suitable auxiliary branch ahead.

Was carried out the experiment following a randomized block design with seven treatments, four replications and two spray volumes, 450 and 750 L ha-1. (Table 1).

Table 1. Treatments for evaluation of the coverage in the culture of coffee (*Coffea arabica*). Patrocínio-MG, 2011.

Treatments[A]	Nozzles	Pressure (kg cm^{-2})	Speed (km h $^{-1}$)
		Application rate = 750 L ha^{-1}	
1 - Speed Jet without branch	TX 8001 - 22 nozzles	7,0	5,29
2 - Speed Jet branch ahead	TX 8001 - 22 + 3 nozzles	6,0	5,29
3 - Speed Jet branch behind	TX 8001 - 22 + 3 nozzles	6,0	5,29
		Application rate = 450 L ha^{-1}	
4 - Speed Jet without branch	TX 80067 - 22 nozzles	6,0	5,29
5 - Speed Jet branch ahead	TX 80067 - 22 + 6 nozzles	5,5	5,29
6 - Speed Jet branch behind	TX 80067 - 22 + 6 nozzles	5,5	5,29

[A]*The Hoefix adjuvant (sodium lauryl ether sulfate – Bayer S/A) 0,2% v.v. was added to the treatments*

The sprayer was equipped with 22 nozzles opened in the side branches, being three open and one closed successively from bottom to top, around the extension. In the auxiliary branch, when used, has been installed the model of spray tip TX 8001. Both for the volume of 750 L ha^{-1} as to the volume of 450 L ha^{-1} were used 3 copies open.

To check the distribution provided by droplets sprayed, water sensitive papers were used in one plant per plot, placed in four quadrants, and in two heights, representing the regions of the plant. After spraying in each parcel of four replicates, the papers were removed and placed in Petri dishes to keep them well away from exposure to moisture. Later, these papers were scanned and evaluated in specific program by determining the percentage of coverage.

The data were statistically analyzed by F test and the averages compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

The percentage of coverage in the superior third of the coffee plants showed significant differences only in the overall average of the four quadrants (Table 2).

Table 2. Coverage rates (%) on leaves of each quadrant of the superior and middle parts of the four quadrants in the culture of coffee. Patrocínio-MG, 2011.

Treatments[1]	Coverage (%) on coffee leaves				Means quadrants
	Sup. I	Sup. II	Sup. III	Sup. IV	
	Application rate = 450 L ha^{-1}				
1 - Speed Jet without branch	24,44 a	17,94 a	20,76 a	19,93 a	20,98 b
2 - Speed Jet branch ahead	24,03 a	19,20 a	15,07 a	19,58 a	19,80 b
3 - Speed Jet branch behind	26,81 a	24,51 a	29,11 a	28,90 a	28,14 ab
	Application rate = 750 L ha^{-1}				
4 - Speed Jet without branch	30,37 a	19,31 a	21,98 a	29,63 a	25,66 ab
5 - Speed Jet branch ahead	32,23 a	18,63 a	27,56 a	19,51 a	25,01 ab
6 - Speed Jet branch behind	32,67 a	29,46 a	23,02 a	33,46 a	30,45 a
F	$0,87^{ns}$	$1,26^{ns}$	$2,07^{ns}$	$2,67^{ns}$	$4,30^{A}$
CV	29,70	37,92	30,61	30,80	15,48
DMS	19,42	18,73	16,14	17,75	8,89

[1]*Transformed data in arc.sen* $(x + 0,50)^{1/2}$

To coverage the inferior third of the plants are not followed the same trends occurring in the superior (Table 3). The largest coverages are observed when it was used the highest application rate, alternating to the quadrants, indicating that in this case, the volume is more important that the position of the auxiliary branch. That was observed higher spray coverage with the use a sprayer calibrated with the assistance of air to a volume of 686 L ha^{-1}, both the top and the bottom parts of the coffee plants.

It is important to note, the main difficulty is to cover the region from the top of the tall plants of coffee. Higher coverage of the lower part of plants do not always translate into better control of pest problems may be in excess of deposit arising from applications. It is imperative, in this case, get a good coverage uniformity throughout the plant, not favoring one region or another, unless the problem is located in some regions, something not observed for the general field occurrences.

Table 3. Coverage rates (%) on leaves of each quadrant of the inferior and middle parts of the four quadrants in the culture of coffee. Patrocínio-MG, 2011.

Treatments[1]	Coverage (%) on coffee leaves				Means quadrants
	Inf. I	Inf. II	Inf. III	Inf. IV	
	Application rate = 450 L ha^{-1}				
1 - Speed Jet without branch	37,57 ab	19,55 b	35,82 a	17,47 c	27,95 a
2 - Speed Jet branch ahead	36,87 ab	22,41 ab	29,62 a	19,74 abc	27,70 a
3 - Speed Jet branch behind	29,50 ab	25,51 ab	47,61 a	20,95 abc	31,06 a
	Application rate = 750 L ha^{-1}				
4 - Speed Jet without branch	20,87 b	33,02 a	38,52 a	26,38 ab	30,10 a
5 - Speed Jet branch ahead	36,48 ab	23,50 ab	41,06 a	27,20 a	32,39 a
6 - Speed Jet branch behind	55,82 a	18,31 b	32,31 a	19,10 bc	30,80 a
F	2,67^{ns}	4,16^{A}	2,00^{ns}	6,06^{A}	0,21^{ns}
CV	39,16	21,77	24,37	14,75	25,33
DMS	32,55	11,86	20,99	7,37	17,53

[1]*Transformers data in arc.sen (x + 0,5)$^{1/2}$*

On the top part of the canopy of the plants, the largest coverages are checked for the configuration of the sprayer with the auxiliary branch installed behind the air baffle (Figure 1).

The lowest values of coverage are observed when the auxiliary branchs are installed in front of the equipment used for both volumes. Considering the general average of the quadrants at the bottom of the canopy of coffee plants, there is no significant difference between treatments regarding coverage (Table 3).

So, the auxiliary branch installed in the rear of the equipment is best, when compared to use of equipment without this feature, or installed in the front of the equipment and the application volume of 450 L ha^{-1} results in larger coverages of spray on the coffee plants, compared to 750 L ha^{-1}, considered sufficient according to the parameters evaluated.

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Rate of Recovery of Tracers Used in the Measurement of Deposition of Spraying Liquids in Coffee Leaves

O. LASMAR, M. C. FERREIRA, J. R. LORENÇON

FCAV/UNESP - Department Phytosanitary
Access road Prof. Paulo Donato Castellane, s/n 14884-900 Jaboticabal, SP - Brazil

SUMMARY

In order to study the feasibility of using different micronutrients as tracers in studies of deposition of spraying liquids in coffee leaves, recovery tests were performed from stock solutions of three micronutrients. The experiments were carried out at the Laboratory of the Center for Research and Technology Development Application - NEDTA, Dept. Phytosanitary - UNESP, Campus Jaboticabal-SP, Brazil, in April 2012. The evaluated micronutrients were: manganese sulfate (31% Mn^{2+}), copper oxychloride (50% Cu^{2+}) and copper hydroxide (35% Cu^{2+}). Were added by microsyringe to adaxial surface of leaves 0.006, 0.012, 0.025, 0.05 and 0.1 mL of each suspension formed from each micronutrient in four replicates. After drying, the leaves were placed in plastic bags duly identified and then received 100 mL of a solution of 0.2 N HCl, which acted for 60 minutes to dissolve the salts used. Immediately, these extracts were filtered for quantification of ions through atomic absorption spectrophotometry, resulting in the amount of label recovered expressed in $\mu g\ mL^{-1}$. The data were subjected to analysis of variance by F test and means were compared by regression test at 5% probability. The estimated results showed a recovery rate above 99% for the five micronutrients evaluated, validating the method used in coffee leaves.

INTRODUCTION

The technology of application of pesticides is one of the most interdisciplinary sector in agriculture, since it relates to the control of insects, mites, weeds and pathogens, which considers aspects of biology, chemistry, engineering, ecology, sociology and economy. This sector can be expressed also as the use of all scientific knowledge to provide the correct placement of the product biologically active in the target, in sufficient quantity, cost-effectively and with minimal contamination of other areas.

To evaluate the quality of spray, various parameters must be considered, in particular, the coverage and deposition of spraying liquids on the target.

Some studies were conducted to search a better methodology to evaluation the deposition of spraying liquids, by tracer elements as cooper and manganese. However, there is a lack of studies related to the recovery of these tracers in spraying liquids, especially when extracted from leaves.

Thus, the objective was to study the feasibility of using different micronutrients as tracers in studies of deposition of pesticides on coffee leaves.

MATERIALS AND METHODS

The experiments were carried out at the Laboratory of the Nucleus of Research and Development Technology of Application - NEDTA, Dept. Phytosanitary - UNESP, Campus Jaboticabal-SP, Brazil, in April 2012.

The evaluated micronutrients were manganese sulfate (31% Mn^{2+}), copper oxychloride (50% Cu^{2+}) and copper hydroxide (35% Cu^{2+}).

Were added by microsyringe to adaxial surface of leaves 0.006, 0.012, 0.025, 0.05 and 0.1 mL of each suspension formed from each micronutrient in four replicates. After drying, the leaves were placed in plastic bags duly identified and then received 100 mL of a solution of 0.2 N HCl, which acted for 60 minutes to dissolve the salts used. Immediately, these extracts were filtered for quantification of ions through atomic absorption spectrophotometry, being used a hollow multi element cathode lamp with wavelengths of 324.8 and 279.5 nm for Cu and Mn, respectively, resulting in the amount of label recovered expressed in $\mu g\ mL^{-1}$.

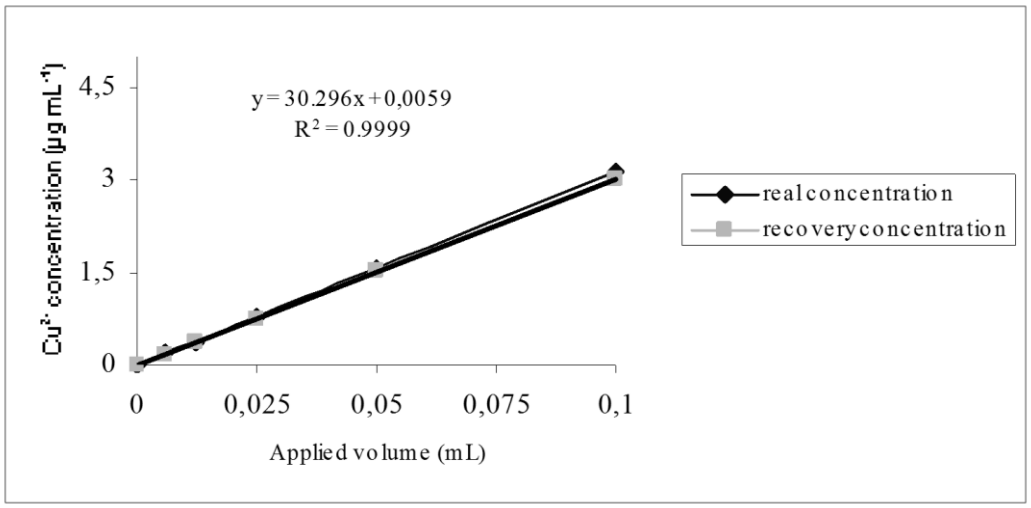
The data were subjected to analysis of variance by F test and means were compared by regression test at 5% probability.

RESULTS AND DISCUSSION

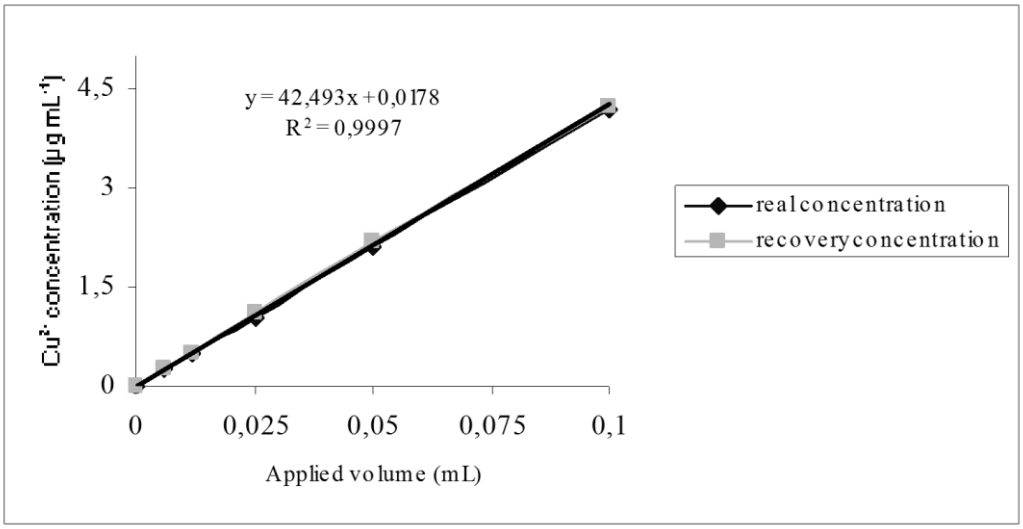
In the Figure 1 A, B and C are showed the results of the recovery rate of tracers copper oxychloride, copper hydroxide and manganese sulfate in $\mu g\ mL^{-1}$ on coffee plants leaves. These results demonstrate that both of the micronutrients evaluated can be used as tracers in deposition tests on plant coffee leaves, since their recovery rates were of over 99%.

Evaluated the feasibility of using a intimate absorbent as a sampler for studies of occupational exposure of applicators of pesticides through a recovery test. These authors concluded that it is possible to obtain a recovery rate of over than 99% when using copper oxychloride as a marker.

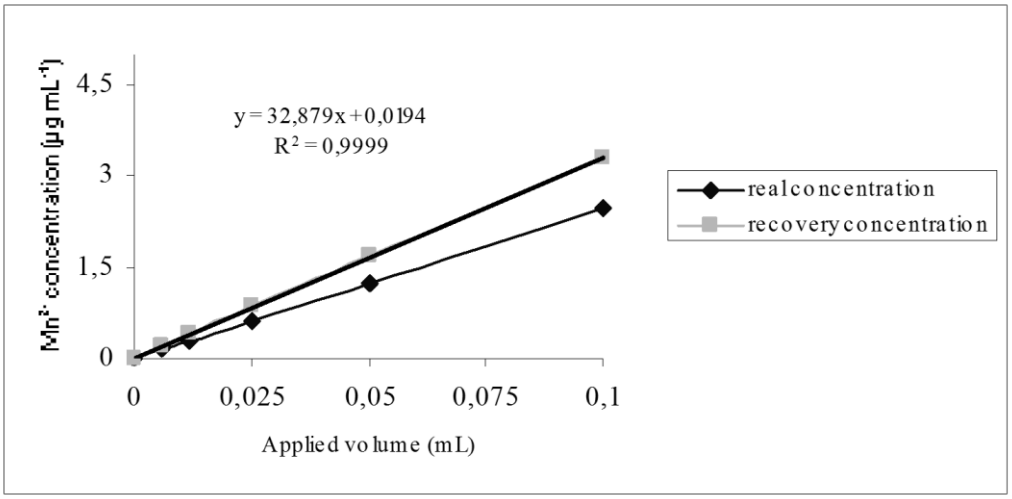
Evaluated the recovery of manganese in tests on dermal and respiratory exposure of workers during spraying in citrus, was adopted as collectors feminine pads and cellulose filters, respectively. The example of the present study and considering the proper proportions, the authors also obtained a high recovery rate of the tracer values over than 90%.



(A)



(B)



(C)

Figure 1. Rate of recovery of tracers: copper oxychloride (A), copper hydroxide (B) and manganese sulfate (C), used in the measurement of deposition of spraying liquids in coffee leaves. Jaboticabal-SP, 2012.

So, the estimated results showed a recovery rate above 99% for the three micronutrients evaluated, validating the method used in coffee leaves.

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Retainement of Copper Hydroxide Spraying Liquids on Coffee Plant Leaves with Adjuvants Addition

O. LASMAR, S. T. DECARO-JUNIOR, L. H. AZEVEDO, S. S. NEVES, M. C. FERREIRA

FCAV/UNESP - Department Phytosanitary
Access road Prof. Paulo Donato Castellane, s/n 14884-900 Jaboticabal, SP - Brazil

SUMMARY

We evaluated the retention of fungicide spraying liquids on coffee plant leaves (*Coffea arabica*) using different adjuvants. The experiment was carried out at the Laboratory of the Center for Research and Technology Development Application - NEDTA Dept. Phytosanitary - UNESP, Campus Jaboticabal - SP, Brazil, in November 2011. The treatments were based on four copper hydroxide mixtures (5 mL L^{-1}) with the adjuvants Tensor Plus (nonilfenol), GL1 (glicerina) and Vertex RS (tributilcitrat + polidimetilsiloxano), at 1.0, 10.0 and 0.2 mL L^{-1} dosages, respectively. Coffee plant leaves were collected and fixed on a pole attached to a precision scale. The sprayer used was fitted to CO_2, at constant pressure, with hydraulic valves and two TLX2 model nozzles for small droplets production. For data collection referred to each leaf weight saturated by spraying liquids, it was adopted the 1:1 ratio (weight: volume). After the spraying, each leaf had its area measured for retention calculation in μL cm^{-2}. The experimental design was completely randomized with four treatments and six replications. The results have shown that all spraying liquids tested acted similarly according to the amount of fungicide spraying liquid retained on coffee plant leaves.

INTRODUCTION

The coffee production for the biennium 2011/12 should be 43.15 million bags, the best result for cycles of low productivity since the period 1999/2000. The forecast appears in the third survey this year by the CONAB.

The coffee plantations usually alternate every year cycles of low and high productivity. For the harvest 2011/12, is expected to drop 10.3% over the previous cycle, with a reduction of 4.94 million bags. The Conab evaluates that the loss is due to drought verified between January and February this year in Minas Gerais, Bahia and Rondonia, States of Brazil. In addition, one of the biggest impediments to ensuring good productivity and product quality is still high infestation of pest problems, such as insects, mites and pathogens.

One of the strategies currently adopted to try to minimize these problems is the phytosanitary management which makes use of various tools such as the application technology of plant protection.In Brazil, there is still much space for studies related to application technology, and the development of technologies can contribute to the current system facing serious problems due to large-scale applications, with high volumes of spraying liquids and under adverse weather conditions of high temperatures and low relative humidity of air. Furthermore, developments in this sector could lead to an agriculture more competitive with less risks to the environment.

In coffee plant crop, studies related to quality of aplication and a lower spray volume used in phytosanitary treatments are still scarce and deserve attention owing to the large number of sprayings that are taken to minimize the effects of phytosanitary problems.

In this context, one of the tools used to obtain a better quality of spraying is the use of adjuvants, are advantages of using adjuvants: the rapid absorption of products with lower losses caused by rain after application, ease of coverage of hydro-repellents surfaces such as leaves, fruits or even insects with waxy surfaces.

In this way, the aim of this study was to evaluate the retention of pesticides based in copper hydroxide in coffee leaves (*Coffea arabica*) in function of different adjuvants.

MATERIALS AND METHODS

The experiment was carried out at the Laboratory of the Center for Research and Technology Development Application - NEDTA Dept. Phytosanitary - UNESP, Campus Jaboticabal - SP, Brazil, in November 2011.

To compare the retention of spraying liquids, were collected leaves with visually similar sizes of plants present in Campus UNESP of Jaboticabal.

The treatments were based on four copper hydroxide mixtures (5 mL L^{-1}) with the adjuvants Tensor Plus (nonilfenol), GL1 (glicerina) and Vertex RS (tributilcitrat + polidimetilsiloxano), at 1.0, 10.0 and 0.2 mL L^{-1} dosages, respectively, as recommended by the manufacturers.

Spraying with the spraying liquids was done with two nozzles, model TLX2 positioned laterally for the leaves, supported on a pedestal and placed inside a metal tray to collect excess. Was sprayed a sheet at a time, to beyond the point of runoff.

In applying the spraying liquids, each leaf was placed in a vertical plate placed on a digital scale with precision of 1 mg, proceeding to the same tare. Then, were performed applications on the leaves in order to allow the spray reached uniformly the adaxial and abaxial surfaces of the leaves. Immediately after stopping the runoff of excess spraying liquids was noted the mass of each leaf (Figure 1). Was adopted the ratio 1:1 (weight: volume) and then each leaf had its area measured by the equipment LI-3100C (Figure 2). To calculate the retention, the leaf area value was multiplied by two, considering the two sides of the leaf and ultimately, the results were expressed in μL cm^{-2}.

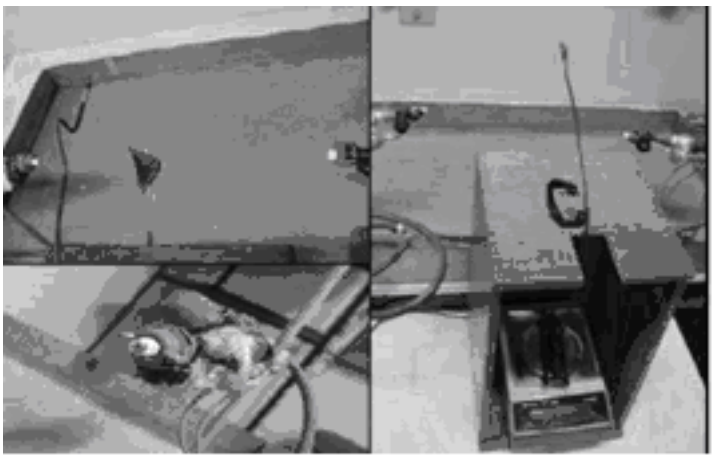

Figure 1. Equipment used for application of spraying liquids. Jaboticabal, 2011.

The experimental design was completely randomized with four treatments and six repetitions. The data were subjected to analysis of variance, the F test and means were compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

The retention results of spraying liquids based on copper hydroxide in coffee leaves not presented significant difference according to the mixture with different adjuvants (Figure 3).

The control treatment based on spraying liquids by copper hydroxide, at a dose of 5 mL p.c. L^{-1} without adjuvant, had an average of 3.4 cm^{-2}, while the other treatments, syrups represented by the base copper hydroxide in admixture with adjuvants Tensor Plus, GL1 e Vertex RS at doses de 1.0, 10.0 e 0.2 mL de p.c. L^{-1}, showed average of 2.7, 4.1 and 3.9 µL cm^{-2}, respectively (Figure 3).

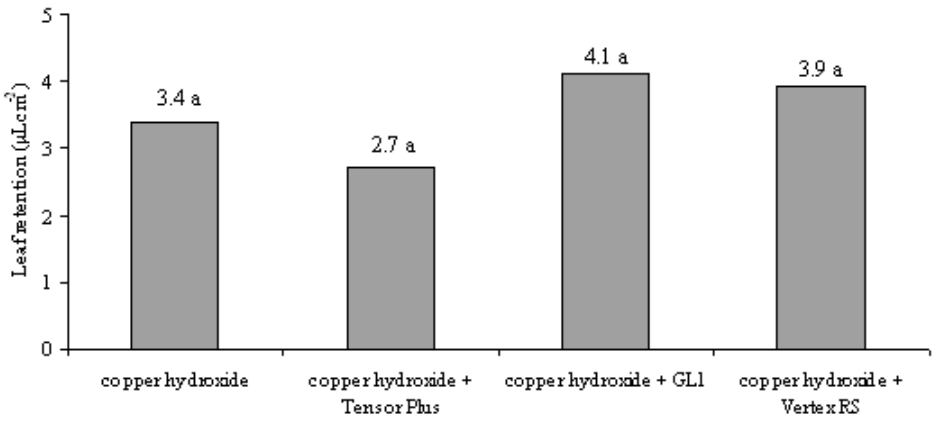

Figure 2. Retention of spraying liquids based on copper hydroxide with and without adjuvants in coffee leaves. Jaboticabal, SP, 2011. (CV = 44.09%).

Although there are numerical differences between the means of retention, these were not confirmed statistically. This may be due to the formulation of copper fungicide used, which alone already has good retention on coffee leaves.

Water is incompatible with the wax, making it dificult to grip and, therefore, does not occur coverage of the pesticide on the leaves. Thus, it is necessary to use together with water, products which among other things, reduce the surface tension of spraying liquids, improving the spreading of the leaf surface. Adjuvants execute this function and may also perform many other actions like stimulate the physiological activity of plants, improves the affinity of the drops with the waxy layer of the leaf surface, acidify and neutralize ions of the water used in the application, prevent evaporation of the droplets, and ensures the formation of these larger diameter, by increasing the density of the solution, can also avoid the drift.

Typically, adjuvants have one or two of these qualities, since it is rare to have adjuvants with these qualities all at once. The activity of pesticides is generally dependent on the constituents of the spray, which, although not comprise the active ingredient, improve its effectiveness. Thus, some adjuvants found in the market can favor the performance of plant protection products.

Therefore, the results obtained in this study show that there was no difference between withholding spraying liquids based on copper hydroxide, possibly due to good retention resulting from the very formulation of this fungicide.

ACKNOWLEDGMENTS

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Optimizing Timber Production and Carbon Storage of Cedrela Odorata and Switenia Macrophylla in Coffee Agroforestry

G. JIMENEZ[1], P. SILES[2], O. BUSTAMANTE[2], C. STAVER[3], B. RAPIDEL[4]

[1]Instituto Hondureño del Café (IHCAFE), Honduras
[2]Bioversity International, Turrialba, Costa Rica
[3]Bioversity International, Montpellier, France
[4]CIRAD, UMR SYSTEM, Montpellier, France

SUMMARY

Over 95% of Honduran coffee producers grow shaded coffee primarily with nitrogen-fixing shade trees. Some growers have begun to replace legume trees with timber trees to improve profitability. However, shade greater than 50% significantly reduces coffee yields. Knowledge of species-specific timber growth rate is needed to identify optimum densities to maximize tree growth at densities with sufficient light transmission for coffee production. The objective of this study was to identify the optimum density of *Cedrela odorata* (Co) and *Swietenia macrophylla* (Sm) in coffee agro-forestry based both on light levels to coffee and carbon accumulation in timber growth. Tree diameter and height growth were measured in 244 coffee farms with plantations ranging from 2 to 32 years in Comayagua and Santa Barbara, Honduras. In 46 fields, tree distribution and dasometic characteristics were mapped and light interception measured in a plot of 25×25 m. These data were used to parameterize a spatially explicit individual-based forest simulator model (SExI-FS) of light transmission and growth for multi-species agroforests. Simulations ranging from 3m×3m to 12m×12m showed that increasing the tree density reduced available understory light after 3 years of growth. For small farmers, wider space arrangements with 2-3 thinning appeared more suitable. With a limitation of more than 50% light transmission to coffee, the model predicts a potential volume of wood for *Sm* of 22-29 m^3ha^{-1} and for *Co* of 28-32 m^3ha^{-1} after 21 years. The potential carbon storage is 11 (*Sm*: 100 tree.ha^{-1}) and 22 (*Co*: 65 tree.ha^{-1}) Mg C ha^{-1}.

INTRODUCTION

Over 95% of Honduran coffee producers grow shaded coffee primarily in association with nitrogen-fixing shade trees. In recent years, some growers have tended to replace legume trees with timber trees to improve profitability, especially during periods of low coffee prices. Preferred timber trees for association with coffee are *Cordia alliodora*, *Terminalia amazonia*, *Cedrela odorata* and *Switenia macrophylla*. The Honduran Coffee Institute has also promoted the substitution of timber species for legume trees through a network of on-farm plots. However, shade cover higher than 50% significantly reduces coffee yields. Management of tree density, thinning and pruning in those systems must consider this shade limit to maintain a balance between coffee production and income from trees. Knowledge of species-specific timber growth rate in these systems is needed to identify optimum densities to maximize tree growth at densities with sufficient light transmission for coffee production. Coffee growers want guidelines on spacing, thinning and pruning for the different species to prepare plans for plantation design and management. The objective of this study was to identify the optimum density *Cedrela odorata* (Co) and *Swietenia macrophylla* (Sm) in coffee agro-forestry systems based both on critical light levels to coffee and carbon accumulation in timber growth in Comayagua and Santa Barbara, Honduras.

MATERIALS AND METHODS

The field work for this study was conducted in the coffee regions of Comayagua and Santa Barbara in central Honduras. The complete study consisted of three stages.

Tree growth analysis

Coffee fields were selected from the IHCAFE (Instituto Hondureño del Cafe) farms data base with plantations of *Co* and *Sm* greater than three years. Tree diameter at breast height (DBH), height and crown width were measured in 20 trees in 244 coffee fields. DBH growth models were parameterized based on three allometric relationships: (*i*) height as a function of DBH; (*ii*) crown depth as a fraction of DBH; and (*iii*) crown radius as a function of DBH.

Tree light interception model validation and tests

For a test of the light model, we measured additional 46 coffee plots ($625m^2$) where *Co, Sm* or both were present. In each plot all trees ≥5 cm DBH were mapped (X, Y coordinates) and DBH, total height, crown width, crown depth were measured. Canopy openness was measured in five points in the plot with a hemispherical densiometer. The *X, Y* coordinates, stem diameters, heights, canopy width and depth of the mapped trees were then entered into SExI-FS model (Spatially Explicit Individual-based Forest Simulator), along with the empirically estimated crown porosity (estimated from digital photographs), to generate overall canopy openness values predicted by the model for each location.

Tree management scenarios

The simulation of the scenarios was initiated from year one through year 20 when harvest was hypothesized. The initial stand structure was given by five planting densities, 3x3m, 6x6, 8x8m, 10x10 and 12x12m. For each year the DBH, H and canopy width were calculated for each tree and canopy openness was estimated at regular spacings to obtain plot average canopy openness. In order to maintain greater than 50% canopy openness, thinning was simulated when canopy openness was less than 50%.

RESULTS AND DISCUSSION

Tree growth analysis

For DBH five growth models were tested. The Chapman-Richards function best fit the data, but logistic models and Gompertz also provided reasonable fit. The model of Chapman-Richards demonstrated coefficients of determination (R^2) of above 80 % for both species (Figure 1). In general the exponential model seemed to overestimate the tree DBH at older plantation ages, while the Shumacher model tended to underestimate the DBH at all ages. Maximum crown radius was a linear function of stem diameter and was quite similar for both species (R^2=0.77 for *Co*, R^2=0.87 for *Sm*). The height–diameter relationships showed that *Co* presented greater height than *Sm* at the same DBH.

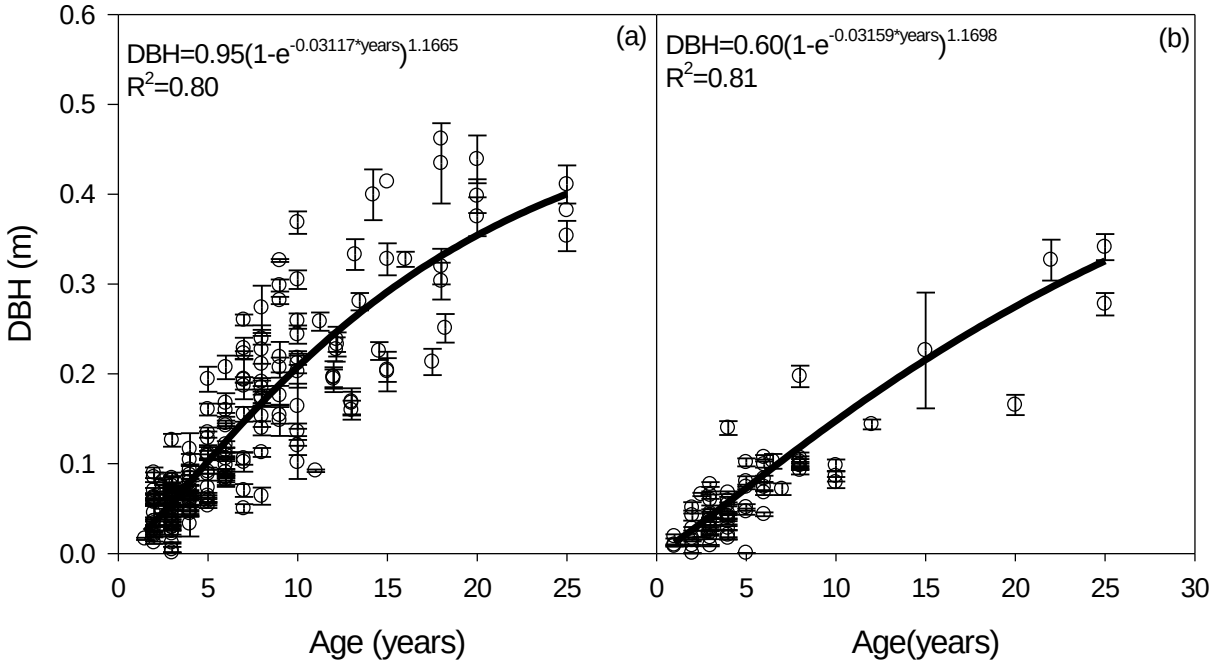

Figure 1. Relationship between DBH and plantation age for both species. *Co* (a) and *Sm* (b), each point represents an average of 20 trees for each coffee field.

Tree light interception model validation and tests

In 46 plots tree density ranged between 64 to 520 trees ha^{-1}; with stem basal areas from 1.7 to 16 m^2ha^{-1}. Canopy openness varied widely from 10% to 97% with most plot shade concentrated between 40 and 60%. Canopy openness presented low fit to simple variables of the tree stand, including tree density (R^2=0.11), stem basal area (R^2=0.37) and tree crown area (R^2=0.47). The canopy openness estimated by The SExI-FS model using 46 mapped plots estimated a good fit with canopy openness measured by hemispheric densiometer (R^2 = 0.76, p <0.047). The model underestimates canopy openness at low cover values (< 40 %) (Figure 2).

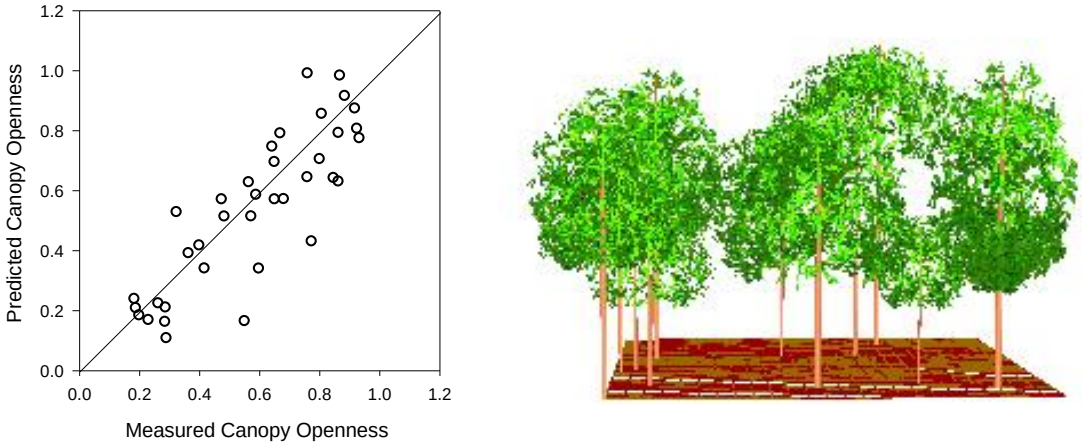

Figure 2. Relationships between observed and predicted canopy openness (a) and (b) visualization of a plot with high density of *Co*.

Tree management scenarios

Denser timber tree plantations with spacing regimes of 3x3m seemed to be less convenient in shaded coffee. These densities showed the highest timber volume with crown closure beginning as soon as the third year. At high initial densities growers must thin and prune more often with higher management costs. According to the simulated scenarios, intermediate densities are suitable in coffee AFS (spacing regimes of 6m or 8m) (Table 1.). Wider spacing regimes (10m and 12m) result in later crown closure and increased weed control for the first five to ten years. Intermediate tree densities required less thinning than denser tree plantation, but also require thinning (Table 1). Training for smallholders on visual criteria for thinning based on multi-objective tree management is needed.

Table 1. Timber potential volume (TPV), carbon capture (CC) and proposed thinning for different simulated management scenarios for *Co* and *Sm* in coffee plantations.

Management scenario	*Cedrela odorata*			*Switenia macrophylla*		
	TPV (m^3ha^{-1})	CC ($Mgha^{-1}$)	Proposed thinning	TPV (m^3ha^{-1})	CC ($Mgha^{-1}$)	Proposed thinning
6x6	32	22	at 7 and 12 years old	29	11	at 12 and 18 years old
8x8	31	22	at 9 and 18 years old	27	11	at 12 years old
10x10	30	22	at 12 years old	25	11	
12x12	28	21		23	9	

CONCLUSIONS

Predicting tree stand dynamic and light interception in agroforestry systems cannot be easily accomplished by field experiments. We used the spatially explicit model (SExI-FS) to simulate tree management in coffee systems. For small farmers, wider initial planting densities with 2-3 thinning appear more suitable. Under these scenarios and with the limitation of maintaining canopy openness higher than 50% light transmission for coffee, the model predicts a potential volume of wood for *Sm* of 22-29 m^3ha^{-1} and for *Co* of 28-32 m^3ha^{-1} after 21 years. The potential carbon storage is 11 (*Sm*: 100 $tree.ha^{-1}$) and 22 (*Co*: 65 $tree.ha^{-1}$) Mg C ha^{-1}. These results can contribute to the improvement of the productivity and ecological services of multi-strata coffee agroforestry. We conclude that models as SExI-FS are useful to explore tree management in complex systems such as multi-strata coffee agroforestry.

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Effects of Weed Control Methods in Coffee Interrows on Growth Of Coffee

E. N. DE ALCÂNTARA[1], R. A. SILVA[2], J. N. ALCÂNTARA[1]

[1]Southern of Minas Gerais state, Brazil
[2]EPAMIG Southern of Minas Gerais state, Brazil

SUMMARY

The culture of coffee is very sensitive to weed competition. The weed control in coffee rows and between rows is responsible for a high cost of production. Due to this, various weed control methods have been used as a way to minimize or eliminate weed competition on rows and coffee interrows, at compatible cost, management and yield sustainable. In order to verify the effects of several weed control methods on the coffee, was implemented in January 2006, at the EPAMIG Experimental Station in São Sebastião do Paraíso, MG, an experiment area planted with the cultivar Paradise MGH 419, using 2300 plants in the area Oxisol with 8% slope. A randomized complete block design was used with seven methods (treatments) of weed control at coffee interrows, namely: mower, harrow, rotary tiller, post-emergence herbicide (glyphosate at a rate of 720g/ ha), pre-emergence herbicide (using as pre-emergence herbicide oxyfluorfen to 3,0 liters of commercial product / ha), hand weeding and control without weeding, in three replications. The coffee rows were always kept clean by hand weeding and / or herbicide application. Several parameters about soil qualities and coffee yield in this experiment were evaluated, however, this work will be focused only on the effects on coffee growth at 2007, 2008, 2009 and 2010.The data show that treatment with pre-emergence herbicide at coffee interrows was always superior to the use of the mower, harrow, rotary tiller, post-emergence herbicide, hand hoe and the interrows with no weed control treatment, which was inferior to all other treatments, especially in the initial cropping years. It was evident that the development of coffee tends to be equal, despite the influence of weeding methods, from the fourth year of planting, eliminating in the fifth year already all the significant differences.

INTRODUCTION

The coffee is sensitive to competition from weeds reducing production in 55-77%, BLANCO, OLIVEIRA and Pupo, 1982. Given the rising cost of weed control, several alternative methods have been introduced (SILVEIRA and Kurachi, 1981 and Muzilli, 1987), but only a few studies focus on the impacts of different methods of coffee weed control in a long term on the soil quality (LAL, 1993) and its production.

AVATRAMANI suggested in 1974 an integrated control with reduced cultivation and training of "mulching" organic. The importance of these trends was grounded on the existence of soil organic matter highlighted by STEVENSON in 1986, which affects all of the other attributes of soil quality, (Fernandes et al. 1997).

Indeed, the improvement in the quality of soil, have also been observed by ALCÂNTARA and FERREIRA, 2000, however it wasn't observed direct relations between the improvement of soil conditions on the growth and production of coffee. Moreover, it has been found that the greatest effect on the coffee has been due to competition for water that occurs during the

dry season, (Njoroge 1994) and mainly due to the presence of perennial weeds that remain infesting the coffee in dry periods, as the Brachiaria (ALCÂNTARA et al 2003b and ALCÂNTARA and SILVA, 2011).

The effect of the weeding methods on the growth of coffee in formation was evaluated in a study conducted in a cerrado soil in Patrocinio, MG, from 1999 to 2003 and the results showed that weed competition on the early growth of the coffee in the first three years reduces drastically the stem diameter, canopy, height and also the vigor after five years of planting, it was found, however, that the statistical differences disappeared, ALCÂNTARA et al. 2003b.

The aim of this study is evaluate the effect of the methods of weeding on the growth of coffee, deployed in the southern Minas Gerais.

MATERIALS AND METHODS

A experiment was implemented in January 2006 at the EPAMIG Experimental Station São Sebastião do Paraíso in southern Minas Gerais, in an Oxisol area with 8% slope, using the cultivar Paraíso MGH 419 at 4 m inter rows and 0.70 between plants. A randomized block design, with seven methods (treatments) for weed control between the lines were used: mower, disk harrow, rotary tiller, post-emergence herbicides (glyphosate applied at 720g a.i./ ha), pre-emergence herbicide (oxyfluorfen) at 720 kg a.i./ ha, beyond hand weeding the rows with and hoeing without witness, in three replications.

The coffee rows always remained free of weeds by hand weeding and /or herbicides applications. To evaluate the diameter of stem, was used a digital caliper at 2 cm height of from the ground, the canopy diameter and plant height, was measured using a tape fixed in a ruler of 1.5 m, and vigor evaluated by visual notes using by two experienced evaluators using in 20 plants per plot in may of 2007 2008, 2009, 2010, 2011.

From 2010, it was observed that the measured parameters started not providing statistical differences, so it was decided to stop measuring, so that the 2011 results are not presented in this study.

Table 1. Number of annual operations to keep weeds at inters rows controlled in weed control methods experiment, in São Sebastião do Paraíso, MG, 2011.

Interrows treatments	Operations number/year
Mower	Five
Disk harrow	Three
Rotary tiller	Three
Post Emergence herbicide	Three
PRE Emergence herbicide	Two
Manual weeding	Five
No weed control	---------

RESULTS

The Tables 2, 3, 4, 5 shown that PRE emergence herbicide in coffee inter rows presented since 2007 best vigor, greater stem diameter, canopy and plant height that mower, disk harrow, rotary tiller, post E herbicide, manual weeding and no weed control from 2007 to 2009. The no control treatment show n that the coffee plant growth was inferior to all kind of weed tested control tested. In 2010 and 2011, however, although in absolute values, evaluated parameters presented did not statistical differences. It was evident therefore, that coffee growth tend be equal, besides the weed control method influences, noted at forty and fifth year from planting eliminating the statistical differences.

Table 2. Means of growth parameter at weed control methods experiment in EPAMIG. Experimental Station. S. Sebastião do Paraíso, MG 2007.

Inter rows treatment	Vigour	Stem diameter (mm)	Canopy diameter (cm)	Plant height (cm)
Mower	5,8 b	16,65 b	55,75 c	58,1 a
Disk harrow	5,8 b	18,25 b	59,25 b	59,8 a
Rotary tiller	6,1 b	20,30 a	62,47 b	61,7 a
Post Emergence herbicide	5,7 b	17,35 b	54,28 c	60,4 a
PRE Emergence herbicide	6,6 a	22,21 a	67,35 a	64,2 a
Manual weeding	6,3 a	19,48 a	57,94 b	61,4 a
No weed control	5,7 b	16,53 b	52,39 d	64,4 a
Variation coefficient (%)	5,5	7,5	2,86	4,5

Means followed by the same letters do not differ between them by Scott Knott at 5%.

Table 3. Means of growth parameters in weed control methods experiment in EPAMIG. Experimental Station. S. Sebastião do Paraíso, 2008.

Inter rows treatment	Vigor	Stem diameter (mm)	Canopy diameter (cm)	Plant height (cm)
Mower	5,61 c	32,02 b	69,3 a	95,39 a
Disk harrow	6,22 b	32,27 b	71,5 a	98,36 a
Rotary tiller	6,39 b	34,58 a	77,8 a	102,25 a
Post emergence herbicide	5,80 b	31,05 b	68,2 a	95,89 a
PRE emergence herbicide	7,17 a	34,05 a	86,2 a	104,76 a
Manual weeding	6,14 b	33,55 a	73,7 a	98,86 a
No weed control	5,75 c	29,25 c	65,8 a	97,14 a
Variation coefficient (%)	3,73	2,52	8,8	1,95

Means followed by the same letters do not differ between them by Scott Knott at 5%.

Table 4. Means of growth parameters in weed control methods experiment in EPAMIG. Experimental Station. S. Sebastião do Paraíso, 2009.

Inter rows treatment	Vigor	Stem diameter (mm)	Canopy diameter (cm)	Plant height (cm)
Mower	7,53 ab	42,23 ab	82,27 a	130,94 bc
Disk harrow	7,75 abc	42,66 ab	120,39 a	133,77 abc
Rotary tiller	8,00 ab	46,70 a	115,76 a	138,56 ab
Post emergence herbicide	7,34 c	43,19 ab	110,00 a	127,86 c
PRE emergence herbicide	8,15 a	45,10 ab	121,16 a	140,60 a
Manual weeding	7,69 abc	45,08 ab	120,28 a	136,19 bc
No weed control	7,38 c	41,98 b	111,94 a	132,94 abc
Variation coefficient (%)	1,9	1,59	12,97	3,16

Means followed by the same letters do not differ between them by Scott Knott at 5%.

Table 5. Means of growth parameter at weed control methods experiment in EPAMIG. Experimental Station. S. Sebastião do Paraíso, 2010.

Inter rows treatment	Vigor	Stem diameter (mm)	Canopy diameter (cm)	Plant height (cm)
Mower	7,61 a	45,00 a	137,11 a	154,92 a
Disk harrow	6,55 a	44,57 a	139,69 a	156,97 a
Rotary tiller	7,61 a	47,55 a	144,55 a	162,19 a
Post emergence herbicide	6,78 a	46,07 a	136,67 a	148,03 a
PRE emergence herbicide	7,22 a	48,30 a	144,72 a	161,50 a
Manual weeding	7,47 a	46,07 a	138,39 a	157,61 a
No weed control	7,19 a	44,85 a	135,39 a	157,31 a
Variation coefficient (%)	4,24	2,01	2,51	1,42

Means followed by the same letters do not differ between them by Scott Knott at 5%.

Pelos trabalhos já apresentados, ficou evidenciado que o controle das plantas daninhas, realizado previamente com o uso de herbicida de pré-emergência, evita a infestação e a concorrência do mato, 'a priori', com a lavoura em todo o ano, ALCÂNTARA & FERREIRA, 2000 e ALCÂNTARA et al 2003a.

The use of PRE emergence herbicide presented a fast growth of the plants in the field, as demonstrated by ALCANTARA et al 2003b, giving also greater yield.

This result is explained by the weed control timing, in other words, the weed control application that depends on whether conditions and anthropogenic factors that are affect good weed control as post emergence herbicide application and manual weeding in which intervals allow new weed competition occurrence.

In 2007 there were statistical differences in vigor, stem and canopy diameters, Table 2. In 2008 these differences were observed only in vigor and stem diameter, Table 3. In 2009 there were differences in vigor, stem diameter and plant height, Table 4. In 2010, Table 5 there was no statistical differences between all factors. In 2011 there were no differences in all analyzed

parameters, so the data are not presented. This result also were observed in Patrocínio, MG, in coffee cerrado soil, ALCÂNTARA, et al. 2006.

CONCLUSIONS

The influence of the weed control methods affected the initial coffee growth until the third year of the planting.

After the forty year the differences due to weed control methods diminish and disappear at fifth year.

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Increase in Incidence of Bacterial Halo Blight (*Pseudomonas syringae* pv. *garcae*) in Coffee Producing Areas in Brazil

I. M. G. ALMEIDA, K. W. MACIEL, L. O. S. BERIAM, L. M. R. RODRIGUES, S. A. L. DESTÉFANO, J. RODRIGUES-NETO, F. R. A. PATRÍCIO

Instituto Biológico, Caixa Postal 70, 13012-970 - Campinas, São Paulo, Brazil

SUMMARY

Since 2008, the importance of bacterial halo blight, caused by *Pseudomonas syringae* pv. *garcae*, has increased in several coffee producing areas in Brazil. In the last summer this disease was especially severe in the States of Minas Gerais and São Paulo. Because of the re-emergence of the disease, studies are being conducted at Instituto Biológico aiming to evaluate the main causal agent and help coffee growers in the chemical control of the disease. During the last four years, about 50 new strains of *P. syringae* pv. *garcae* were obtained from leafs, flowers, pin-head berries and branches with die-back, belonging the cultivars Mundo Novo, Bourbon, Catuaí, Catucaí, Obatã, Acaiá, Tupi, and Icatu, from 11 diferent counties of the State of Minas Gerais and 15 from the State of São Paulo. The cultures were deposited into IBSBF-Phytobacteria Culture Collection of Instituto Biológico (www.biologico.sp.gov.br/bacterias/php) and will be used for further studies. One experiment with chemical control of the disease was conducted with coffee seedlings inoculated with *P. syringae* pv. *garcae* and two experiments were carried out in coffee crops naturally infested, in the counties of Altinópolis and Caconde, State of São Paulo. In the seedlings experiment the most effective treatment was with kasugamicin (0.030 g a.i./ha), followed by copper hydroxide (1.25 kg a.i./ha). In the field experiments, the best results were obtained with 3 to 4 treatments with copper hydroxide (1.25 kg a.i./ha) + silicone adhesive (0.025%), copper oxychloride (2.0 kg a.i./ha) and kasugamicin. However, the chemicals reduced only partially the incidence of the disease and were more efficient in the plots less exposed to winds. Therefore, other control practices such as the use of wind-break-plants and healthy seedlings are suggested in combination with chemical treatments for the management of bacterial blight disease.

INTRODUCTION

In Brazil, several bacterial diseases have been described in coffee crops, such as "bacterial halo blight" caused by *Pseudomonas syringae* pv. *garcae* , "bacterial leaf-blight", caused by *Pseudomonas cichorii* , "bacterial brown spot", caused by *Burkholderia andropogonis* , "coffee leaf scorch"**,** caused by *Xylella fastidiosa* and, more recently, **"**bacterial leaf spot", which causal agent is *Pseudomonas syringae* pv. *tabaci* . *P. s.* pv. *garcae* was described in 1956 and was considered a secondary pathogen for many years, causing damages more frequently in nurseries.

However, since 2008 the incidence of bacterial halo blight has increased in nurseries and several coffee producing areas in Brazil, especially in the States of Minas Gerais and São Paulo. From December 2011 to January 2012 the disease was severe in many coffee

producing areas of these regions. The disease is characterized by lesions in leaves, flowers and pin-head berries, and die-back of twigs and branches (Figure 1).

Due to the recent re-emergence of the disease, some studies are being conducted at Instituto Biologico, São Paulo, Brazil, aiming to obtain different strains of *P. syringae* pv. *garcae* for further studies of genetic diversity and chemical control of the disease in nurseries and field conditions.

Figure 1. Symptoms of "bacterial halo blight" in leaves (A), branches (B) and pin-head berries (C).

MATERIALS AND METHODS

P. syringae pv. *garcae* strains were isolated from samples of coffee showing symptoms of lesions in leaves, flowers and pin-head berries and die back of twigs and branches. Small pieces of infected tissues were examined under light microscope for the presence of bacterial oozes. The pieces were macerated in sterile distilled water and the resulting suspension was streaked on Nutrient Agar medium. After incubation at 28ºC for 48-72h, the bacterial colonies were purified and the isolates were characterized by cultural, physiological and biochemical tests.

For evaluation of the chemical control of the disease, one experiment was carried out with coffee seedlings and two experiments were conducted in coffee crops in the counties of Caconde and Altinópolis, State of São Paulo (SP).

Nursery experiment

Seedlings of coffee cultivar Mundo Novo with five pairs of leaves were used in the experiments. A group of four seedlings representing a replication was arranged in small trays, which were randomly disposed on greenhouse benches. Chemicals were applied with a hand sprayer until run-off. The treatments and active ingredient per ha were: kasugamicin (0.040 kg), kasugamicin (0.030 kg) + copper hydroxide (1.25 kg), copper hydroxide (0.025 kg), calcium oxychloride (0.250 kg) and benzalkonium chloride (0.250 mL). Control plants were treated with distilled water. After 24h of the chemical treatments, the seedlings were inoculated with bacterial suspension, consisting of a mixture of two *P. syringae* pv. *garcae*, strains IBSBF 1664 and IBSBF 1665, containing approximately 9.10^7 $CFU.mL^{-1}$. The suspension was sprayed on the upper and underside surfaces of the leaves.

Incidence and severity of the disease were evaluated from 7 to 21 days after the inoculations in the three upper pairs of the leaves of the seedlings. Severity was assessed with a graded scale (1-5).

Field experiments

The first experiment was conducted in county of Altinópolis (SP). The chemicals were applied in November, December 2009, and February 2010. The treatments were: kasugamicin (0.030 kg) + copper hydroxide (1.25 kg) + silicone adhesive (0.025%), copper hydroxide (1.25 kg) + mineral oil (0.25%), copper hydroxide (1.25 kg) + silicone adhesive (0.025%), copper hydroxide (1.25 g) + benzalkonium chloride (0.100 L) + silicone adhesive (0.025%).

The second experiment was carried out in Caconde (SP). The chemicals were used in four applications in September, November, December 2009 and March 2010. The applied products with respective active ingredients per ha were: kasugamicin (0.030 kg), kasugamicin (0.030 kg) + copper hydroxide (1.25 kg), copper hydroxide (1.25 kg) + vegetal oil (0.25%), copper hydroxide (1.25 kg) + mineral oil (0.25%), copper hydroxide (1.25 kg) + silicone adhesive (0.025%), copper hydroxide (1.25 kg) + acibenzolar S-methyl (ASM) (0.025 kg), copper oxychloride (2.0 kg), ASM (0.025 kg) and ASM (0.0125 kg).

In both experiments the incidence of the disease was evaluated in leaves of ten branches from ten plants per plot. In the experiment carried out in Caconde the disease was evaluated in November 2009, January, March and April 2010. In the experiment conducted in Altinópolis the disease was evaluated in December 2009, January and April 2010.

RESULTS AND DISCUSSION

The isolations showed circular, slightly raised, cream coloured bacterial colonies which produced light fluorescence on King's B medium and incited hypersensitivity reaction in tobacco leaves. They were oxidase and arginine dihydrolase negative, produce levan and did not cause soft rot in potato slices and according to Lelliott *et al*. these bacterial isolates belong to the group Ia of the *Pseudomonas syringae* pathovars. Some other tests mainly the utilization of lactate and no utilization of trigoneline and L-tartrate confirmed the classification of the bacterium isolates as *Pseudomonas syringae* pv. *garcae*. Fifty new bacterial strains from the cultivars Mundo Novo, Bourbon, Catuaí, Catucaí, Obatã, Acaiá, Tupi, and Icatu, originated from 11 diferent counties of the State of Minas Gerais and 15 from State of São Paulo were deposited into IBSBF-Phytobacteria Culture Collection of Instituto Biológico (www.biologico.sp.gov.br/bacteria.php).

For the control of bacterial halo blight, the best treatment in the nursery experiment was with kasugamicin, followed by the treatment with copper hydroxide. Calcium oxychloride and benzalkonium chloride reduced the incidence of the disease, but were less efficient (Figure 2).

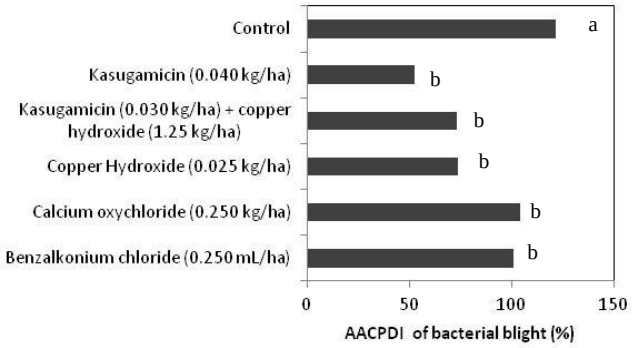

Figure 2. Area under the progress curve of the severity of bacterial halo blight, caused by *P. syringae* pv. *garcae,* assessed by a graded scale (1-5), after application of chemicals in coffee seedlings.

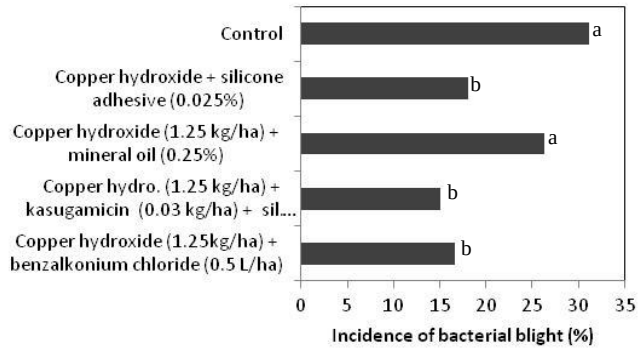

Figure 3. Incidence of bacterial halo blight in a field experiment carried out in Altinópolis, State of São Paulo, Brazil, from November 2009 to June 2010.

In the field experiment carried out in Altinópolis (SP), the treatments with copper hydroxide + silicone adhesive, copper hydroxide + kasugamicin + silicone adhesive and copper hydroxide + benzalkonium chloride + silicone adhesive reduced the incidence of the disease (Figure 3). In the experiment conducted in Caconde (SP), the treatments with kasugamicin + silicone adhesive, kasugamicin + copper hydroxide, copper hydroxide + vegetal oil, copper hydroxide + mineral oil, copper hydroxide + silicone adhesive, copper hydroxide + ASM reduced the incidence of the disease, but in this experiment chemical control was more effective in the plots less exposed to winds (Figure 4).

Nowadays it is unknown why the importance and damages caused by bacterial halo blight have increased in these last few years in several coffee producing areas in Brazil. Although other bacteria have been detected in coffee plants and seedlings in previous studies, the studies carried out herein showed that *P. syringae* pv. *garcae* is the most important pathogen associated with halo blight disease. The strains obtained will be subjected for further studies of genetic diversity of the pathogen.

Even though chemical control for bacterial plant disease usually shows less efficiency than for diseases caused by fungi, in the present study seedling treatments with kasugamicin and copper hydroxide reduced the incidence of the disease.

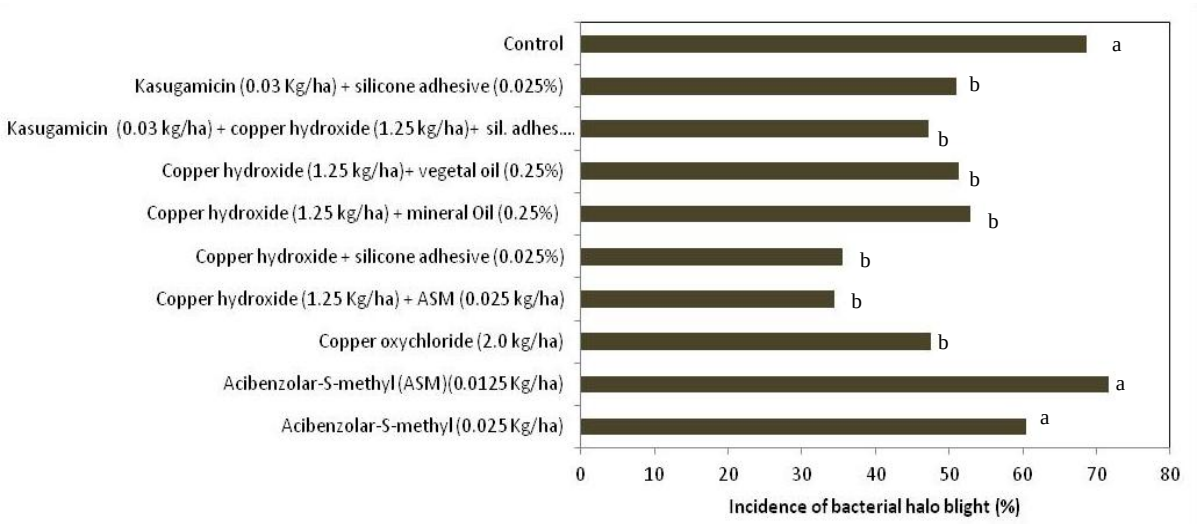

Figure 4. Incidence of bacterial blight in a experiment carried out in Caconde, State of São Paulo, Brazil, from September 2009 to April 2010.

In the field experiments the treatment with copper hydroxide + silicone adhesive showed less incidence of the disease than the treatments with copper hydroxide + mineral oil (in both experiments) or vegetal oil in one experiment. In this study, the chemical treatments with copper hydroxide, copper oxychloride or kasugamicin reduced the incidence of bacterial blight. Other management practices such as the planting of wind breaks and using healthy seedlings can also be extremely important for the control of this disease.

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Morphological and Molecular Characterization of *Meloidogyne* spp. in Coffee Plantations of Costa Rica

M. ROJAS

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica

SUMMARY

In order to make a morphological and molecular characterization of the *Meloidogyne* species found in coffee plantations of Costa Rica in 2007, 100 root samples were proportionally distributed in different regions of the country. Each sample consisted of 200g of fine roots from 10 plants randomly selected from plots of 0.5-1 ha, following a zig-zag line. Information collected included geographic location, height, conditions of the plantation and data on recent applications such as product and dose.

The samples were transferred in fresh media to the Nematology Laboratory of the University of Costa Rica. The laboratory personnel determined the population density of *Meloidogyne* and *Pratylenchus*, extracted the *Meloidogyne* females for characterization and made a morphological identification by studying their perineal design. *Meloidogyne* females extracted from the roots were used for molecular characterization in the Biotechnology Laboratory of the Agronomic Research Center, University of Costa Rica. This identification was made through the Polymerase Chain Reaction technique (PCR) and PCR-RFLP analysis of mitochondrial DNA.

The samples showed high densities of *Meloidogyne*: 90 percent in the Turrialba region, 56 percent in the Central Valley, 45 percent in Coto Brus, 40 percent in Perez Zeledon, 26 percent in West Valley, 13 percent in Los Santos and, in the northern area, no samples exceeded the 20000 *Meloidogyne* J_2/100g of root. In the case of the *Pratylenchus* genus, only one sample had a population density higher than 8000/100g of root. Field observations on possible aerial symptoms as chlorosis and death of branches were not clearly related to *Meloidogyne* high populations.

The morphological characterization of *Meloidogyne* identified the *exigua* species in 82 percent of the samples and *arabicida* in 4 percent. The molecular characterization identified *exigua* in 78 percent of the samples and did not identify the *arabicida* species.

INTRODUCTION

Plant parasitic nematodes have been considered an important obstacle to the production of coffee in Costa Rica. It can detect all coffee growing regions of the country and its distribution has been mainly through nursery plants that have been infested areas to regions unpopulated by the plague (ICAFE 1998b).

In Costa Rica, ICAFE (1998b) mentions the presence of *Meloidogyne exigua*, *M. arabicida* and *M*. spp. Hernandez (2007) also reported the presence of *M. incognita* and *M. javanica* in samples of Costa Rica. Villain et al. (2006) also reported *M. incognita*, in addition to a new report from *M. mayaguensis*.

As pathogenic *M. arabicida* was reported by Lopez and Salazar in 1989, severely attacking coffee plantations in the region of Juan Viñas, Cartago. Other authors such as Villain et al. (1999) and Bertrand et al. (2000) support its high pathogenicity. *M. exigua* is predominant in the coffee plantations in Costa Rica (Villain et al. 1999; Alpízar and Alvarado 1999; Figueroa 1984, Flores and López 1989 cited by Bertrand et al. 2000) and according to Bertrand et al. (1998) its attack can lead to a decrease of 15% of the crop.

Many experts insist that nematodes should be managed in an integrated way, starting with the identification of species present in each particular region, since it is basic for control purposes. Caution should be used to control a genus or species, for not to unleash attacks another genus or species that appear less important. The objective of the research was to characterize morphologically and molecularly *Meloidogyne* species found in coffee plantations in Costa Rica.

MATERIALS AND METHODS

It took a total of 100 samples of roots, nationwide between August and November 2007, covering the entire area national coffee. Each sample consisted of 200 g of fine roots, from 10 randomly selected plants in batches of 0.5-1 ha, making travel in a zig-zag. As part of the sampling, noted the location, height, state of the plantation and recent applications (products and doses).

Fresh samples were transported to the Laboratory of Nematology, University of Costa Rica. Here was determined population density of *Meloidogyne* and *Pratylenchus*. They also performed the extraction of females for characterization and identified the species by studying perineal patterns of females.

The other part of females extracted from the roots was used for molecular characterization in the Biotechnology Laboratory of Agricultural Research Center of the University of Costa Rica. This part was performed by the technique of Polymerase Chain Reaction (PCR), and PCR-RFLP analysis of mitochondrial DNA.

RESULTS AND DISCUSSION

The regions with the highest population density of *Meloidogyne* were Turrialba, Valle Central, Coto Brus and Pérez Zeledón and generally *Pratylenchus* density was low (Table 1). Morphological characterization of *Meloidogyne* identified the presence of *arabicida* in Cachí, Naranjo, Tarrazú and Pérez Zeledón, while *exigua* was identified in 82 samples and 16 presented no galls (Table 2). Molecular characterization identified *exigua* in 78 samples, 16 no showed galls and 6 were not identified (Table 3).

The result of this study corroborates and expands the information that *Meloidogyne exigua* is the most common species in coffee plantations in Costa Rica. It also shows the presence of *M. arabicida* in places where it not had been reported before. This species was found in a home in Juan Viñas and then in other areas of the region of Turrialba (ICAFE, 1998a). Their presence in Naranjo had been reported by Alpízar and Alvarado (1999) and more recently by Lopez (2008). In infested areas the use of practices such as grafting is an alternative to continue production.

Field observations relate by 35% with air possible symptoms such as chlorosis or exhaustion, with *Meloidogyne* populations above 20000 J_2/100 g of root, but also related by 54% with

populations less than 20000 J_2/100 g root. Therefore, the symptoms observed in this case not attributable to "high" populations of Meloidogyne, but rather to other causes.

About the dissemination of nematodes from affected areas to other where yet is not the problem, it can be given by different means. However, it is noted that moving nursery plants is how these pathogens can be moved more easily. It is therefore important to study and monitor the condition of plant nursery before making transfers from one place to another.

Table 1. Percentage of samples with "high" *M. exigua* (> 20000 J_2/100 g root) and *Pratylenchus* density (> 8000/100 g of root) for each coffee region (Rojas 2008).

Coffee region	*M. exigua* (%)	*Pratylenchus* (%)
Valle Central	56	0
Valle Central Occidental	26	0
Turrialba	90	0
Pérez Zeledón	40	0
Los Santos	13	4
Coto Brus	45	0
Zona Norte	0	0

Table 2. Samples identified morphologically by coffee region.

Region	Total samples	*M. exigua*	*M. arabicida*	No galls
Valle Central	16	16	0	0
Valle Central Occidental	19	14	1	4
Turrialba	10	10	1	0
Pérez Zeledón	15	12	1	2
Los Santos	23	19	1	3
Coto Brus	11	9	0	2
Zona Norte	6	2	0	4

Table 3. Samples identified molecularly by coffee region.

Region	Total samples	*M. exigua*	*M. arabicida*	No galls
Valle Central	16	16	0	0
Valle Central Occidental	19	14	0	4
Turrialba	10	10	0	0
Pérez Zeledón	15	11	0	2
Los Santos	23	16	0	4
Coto Brus	11	8	0	2
Zona Norte	6	3	0	3

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Population Dynamics of Coffee Berry Borer *(Hypothenemus hampei)* in the Remaining Fruits on the Ground During Postharvest

M. ROJAS, H. ROJAS, R. MESÉN

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica

SUMMARY

The coffee berry borer populations growing in the remaining fruits left on the ground after harvest represent a serious infestation source for the next harvest. Two trials were conducted in the Perez Zeledon region and other two in the Turrialba region, both under a slightly marked dry period, during 2005-2007, with the objective of determining the behavior of the coffee berry borer populations in the fruits on the ground during the postharvest period.

Periodical evaluations were conducted on the number of fruits infected on the ground, using a 50 x 50 cm frame to make a calculation per site, and this resulted in a total of 15 sub-samples. Fruits infected were collected from the ground and taken to the laboratory; 30 fruits were randomly selected from them and dissected to determine the coffee berry borer growth stages and number. The pest attack in the following harvest and periodical capture of coffee berry borer insects in traps were registered.

The behavior of populations was similar in the four sites of the study, reaching the higher number of insect stages per fruit two or three months after the end of the harvest, until the rainy season started, which stimulated the flight of females and the activity of natural enemies, and the population gradually decreased during the postharvest period. The flight of females under these conditions lasted for five months after harvest and the captures decreased at the same time the number of adult insects in remaining fruits decreased and the percentage of attack in the new crops increased. This study demonstrates the importance of collecting the fruits from the ground to reduce the coffee berry borer population that will attack the next crop.

INTRODUCTION

Coffee regions of Pérez Zeledón and Turrialba have areas that are characterized by a prolonged period of rain. This condition, which promotes rapid decay of the fruits of the ground, can also cause the death of the CBB by an unfavorable condition for pest development and activation of native natural enemies at ground level.

The coffee berry borer prefers the old fruits, where the grain is compact and low humidity (Ticheler, 1961 y Haarer, 1963, cited by Borbón, 1991). This type of fruit is characteristic of the post-harvest period, which have remained uncollected ground. The coffee berry fruits can attack healthy ground and reproduce in them and dry periods after harvest, contributing to increased insect population in the fruits that fall during harvest (Bustillo *et al*. 1998).

Baker et al. (1992) cited by Bustillo et al. (1998) indicate that the emergence of the CBB in infested grains increases under conditions between 90-100% relative humidity and 20-25 °C.

Bustillo et al. (1998) add that when the rainy season comes the CBB begins its emergence from infested fruit to plant new fruit.

In the study areas CBB starts attacking the fruits from about 40 days after blooming. Around 120 days after blooming, the fruits reach 20% dry matter and insect reproduction begins. Weather conditions affect the behavior of the blooms, fruit development, ripening time and the behavior of the coffee berry borer. The aim of the study was to determine the behavior of *Hypothenemus hampei* population in the fruits of the ground in regions with prolonged rainy season during the postharvest period.

MATERIALS AND METHODS

Two trials were conducted in the region of Pérez Zeledón (Platanares, General Viejo) and two in the region of Turrialba (CATIE, Santa Rosa), both with slightly marked dry period during the years 2005 to 2007.

Periodic evaluations were made of the number of attacked fruits present in the ground using a frame of 50 x 50 cm, for a total of 15 sub-samples. We collected ground attacked fruits to take to the lab, randomly selecting 30 fruits to review and determine the different stages of development of the CBB and their quantities (Rojas *et al*. 2007, Rojas y Cordero 2008, Rojas 2008). Was recorded the CBB capture in traps regularly.

In Pérez Zeledón during the study period there was a total annual precipitation exceeding 3100 mm in Platanares and 4372 mm in General Viejo. The total annual rainfall of Turrialba exceeds 2600 mm. All study sites were managed with *Erythrina* shade and density planting of coffee close to 6000 plants/ha.

RESULTS AND DISCUSSION

The sites studied in Turrialba, Santa Rosa and General Viejo experiencing Caribbean influence characterized by rains that occur towards the end and beginning of each year, differing characteristics of drought in the rest of the country during that time (data not shown). In Platanares rains occurs more magnitude from March, stimulating the flight of the coffee berry borer that has remained in the residuals fruits after harvest.

In the three Caribbean-influenced areas the largest populations of CBB in the fruits of the ground after harvest occurred between January and February. The maximum amount of biological stages CBB ranged from 6 to 10 individuals per fruit. In Platanares maximum population reached nearly 40 individuals per fruit in March (Figure 1). The early season rains in these regions affecting the multiplication of the pest in the ground residual fruits, but stimulate blooms of coffee and the flight of the coffee berry borer in search of fruits.

Generally, the largest populations of insects on fruits ground after harvest coincided with peak flight of females, which was evidenced in the catch curve of females in traps (Figure 2). In areas with Caribbean influence peak capture was presented earlier (February-March), compared with Platanares which was presented in April.

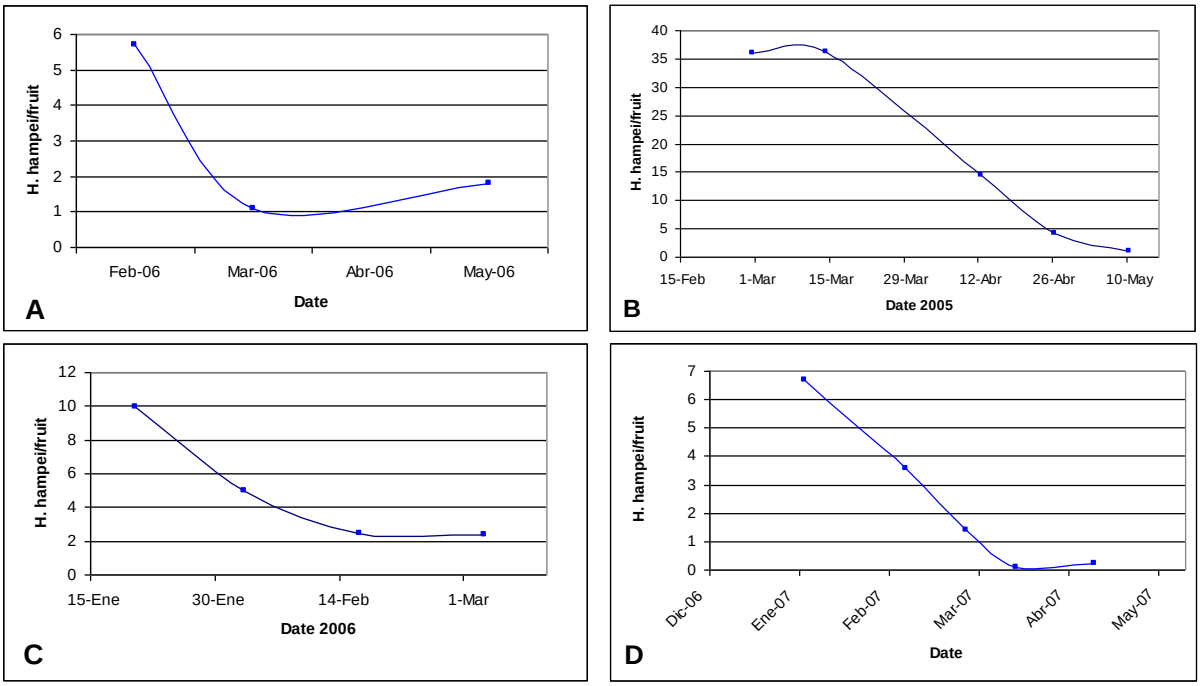

Figure 1. Insect population (*H. hampei*) per fruit attacked of ground during the postharvest period in CATIE (A), Platanares (B), General Viejo (C) and Santa Rosa (D).

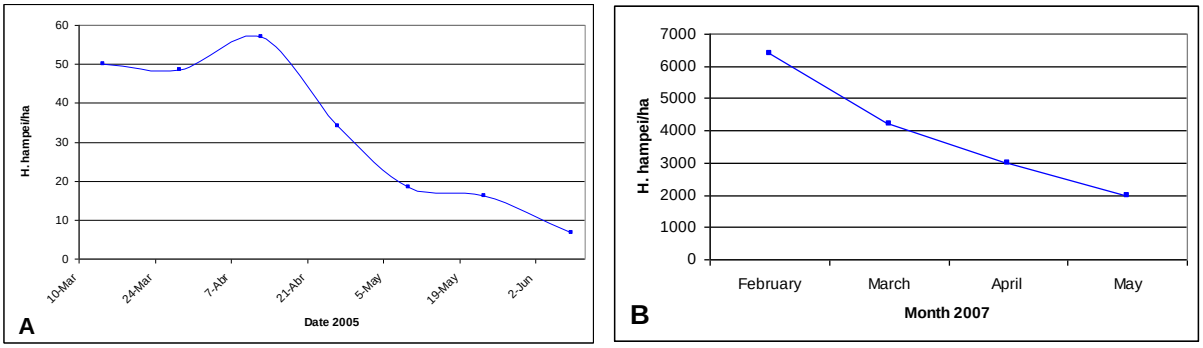

Figure 2. Catch curve of CBB in 20 multiple funnel traps per hectare during the postharvest period in Platanares (A) and Santa Rosa (B).

The CBB capture in traps was closely related to the behavior of rainfall mainly occurring after a dry period. From May onwards the catch was declining with decreasing coffee berry borer population, increasing precipitation and competition from the green fruits of the plant with more than 20% dry matter accumulated.

In coffee plantations the amount of fruit left on the ground without gathering after harvest, the attack level and CBB populations per fruit during postharvest period ensure pest surviving, reproducing and significantly attack the next harvest. The harvesting practices and collecting the fruits of the ground during and after harvest are the most effective way to reduce CBB populations and the attack to the next crop.

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Chemical Control of *Mycena Citricolor*

M. BARQUERO-MIRANDA, N. ARRIETA-ESPINOZA, A. ROBLES-CHAVES

Costa Rica Coffee Institute, Coffee Research Center (CICAFE)

SUMMARY

The search for efficient, economically viable alternatives to the chemical control of *Mycena citricolor*, pathogen causing the disease known as Ojo de Gallo or also called American leaf spot of coffee, is a constant in the studies on chemical control for this disease, in Costa Rica. For this reason, during 2009-2010, four different mixtures of penetrating fungicides were subjected to evaluation on the control of incidence and progress of this disease. The fungicides of this evaluation: Cyproconazol + Validamycin; Tebuconazol - Triadimenol + Validamycin; AEC656948 - Trifloxystrobin; AEC656948 - Tebuconazol; and Tebuconazol - Trifloxystrobin were applied in the field, in four different coffee regions, mixed with Cenebol, Flint, Alcaplant and Bordeaux mixture,. The best disease control was achieved with the Cyproconazol + Validamycin fungicide mixture and the Tebuconazol - Triadimenol + Validamycin mixture. Both mixtures achieved the lower incidence of disease in the four regions of the study, which was statistically different from the other treatments. Contact fungicides reached the highest development of the disease, even to total defoliation of plants. This study discusses the importance of chemical control and the choice of more appropriate products for an integrated disease management, focusing on the sustainable management of farms regularly affected by this disease in coffee trees.

INTRODUCTION

The disease caused by Ojo de Gallo is responsible for major problems in the coffee plant where it is present. For control, we recommend the integration of cultural and agronomic practices to reduce environments for the pathogen, along with the application of a chemical, which plays an important role in the handling of the pathogen (Barquero, 2007). However, no existing molecules eradicant effect, as known to the case of some products used for controlling the coffee leaf rust. This situation forces apply fungicide mixtures with preventive characteristics (limiting gems germination) and / or "curative" (limiting the formation of reproductive structures) in defined moments of disease development, thus achieving a protection the widest possible.

At the end of the 1990s, the Costa Rica Coffee Institute (ICAFE) determined that the use of systemic fungicides: Tebuconazol-Triadimenol or Cyproconazol, mixed with the Validamicina-A, is able to achieve adequate control of the disease. Subsequent work carried out during the period 2006 - 2011, confirms the effectiveness of this mixture of fungicides on control of the Ojo de gallo, although a high initial incidence. The success of this mixture of fungicides is to reduce significantly the reproduction of the pathogen for a period of approximately 40-45 days (ICAFE 2009; ICAFE 2011).

The problems and losses caused by the Ojo de Gallo (Barquero, 2011) and difficulty of control, require a constant search for handling, evaluating and implementing new forms for new chemical molecules that can be more efficient and less toxic to the environment, is a constant need in the Costa Rican coffee industry.

MATERIALS AND METHODS

The field test was conducted in four regions of Costa Rica, Table 1. The treatments evaluated are observed in Table 2. The experiment design was a randomized complete block of 5 treatments with 5 replications. The treatments were applied in the months of June, August and September, on commercial batches of Catuaí or Caturra varieties. The experimental plot consisted of 30 plants distributed in three rows of 10 plants each. We evaluated the total number of diseased and healthy leaves on a branch of the middle stratum of 10 plants. Evaluations were performed every 30 days during the months of june to november 2009-2010. For analysis of the data was analyzed the incidence variation obtained in the last evaluation.

Table 1. General characteristics of the experimental plots located in each coffee-growing region.

Regions	Precipitation (mm)	Temperature (ºC)			elevation above sea level (m)	distance sowing (m)
		Mean	Maxima	Minima		
Tarrazú	3300	17,5	23,6	14,2	1450	1,0 x 2,0
Poás	3500	18, 5	24,9	14,5	1450	1,0 x 2,0
Páramo, PZ	3000	19,0	23,5	14,5	1300	1,0 x 2,0
Sabalito	3400	20,2	25,8	16.7	1100	0,90 x 1,90

Table 2. Name and dose of the fungicides used.

Treatments	Doses / ha
Cyproconazol, 10SL + Validamycin, 10SL	0,5 L + 2 L
Tebuconazol-Triadimenol, 30EC + Validamycin, 10SL	0,7 L + 2 L
AEC656948-Trifloxystrobin	0,5 L
AEC656948-Tebuconazol	0,6 L
Tebuconazol-trifloxystrobin, 75 WG	0,5 kg
Oil mustard, 4,82EC [1]	0,4 L
Trifloxystrobin, 50 WG[1]	0,5 kg
Bordeaux mixture[1]	1,0 L
Alcaplant (colloidal suspension of calcium)[1]	2,0 kg

[1] *Contact fungicides used as control treatment. We used one per region.*

RESULTS

The environmental conditions that were presented during the year 2009 were not very favorable for the development of the disease. However, precipitation conditions presented during 2010 throughout the country, allowed to evaluate the biological efficiency of the proposed fungicides.

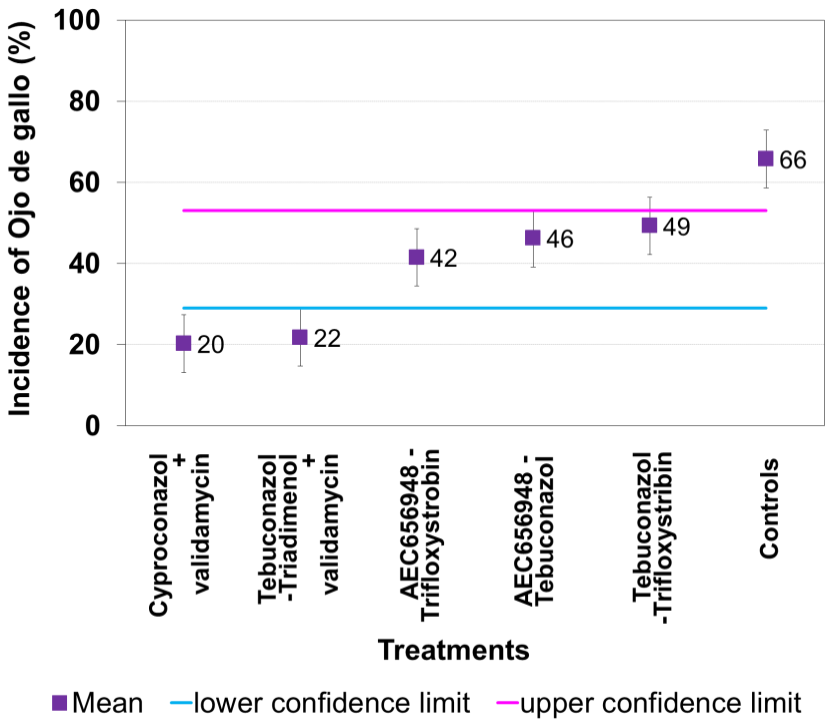

Figure 1. Average incidence of *M. citricolor*, evaluated with five treatments applied in four regions coffee plantations in Costa Rica, during the years 2009 and 2010.

Table 3. Influence of the design of planting of coffee, en la incidence of Ojo de gallo.

Treatments	Distance sowing (m)	
	1 x 2	0,9 x 1,9
Tebuconazol + Validamycin	9	53
Cyproconazol + Validamycin	10	56
Tebuconazol-Trifloxystrobin	27	85
AEC656948-Tebuconazol	34	84
AEC656948-Trifloxystrobin	36	88
Control (contact fungicides)	54	100
Means	29	78
Stand. deviation	19	19
Stand. error	5	8

DISCUSSION AND CONCLUSIONS

Results confirm good efficiency of the combination of fungicides Tebuconazole-Triadimenol or Cyproconazol in mixture with Validamicina-A. Better control of the disease is reflected not only in the final appearance of the coffee plants, but also in the protection of present and future harvest. Moreover demonstrates that, despite the good efficacy in disease control obtained with the mixture of the Triazole fungicides + Validamicina, it is evident that planting density plays an important role in the final result. A smaller distance between plants, will undoubtedly cause a greater permanence of water over the leaves and a higher relative humidity within the coffee plantation, especially in coffee-growing regions where the number of rainy days is greater between the months of august to november.

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Daily Growth Rate of *Ceratocystis Fimbriata* Isolates on Caturra and Catuai Stakes

M. BARQUERO-MIRANDA, G. MENESES-ROJAS

Costa Rica Coffee Institute, Coffee Research Center (CICAFE)

SUMMARY

Diseases in the stem of the coffee trees caused by the coffee canker known as "Llaga macana" are mentioned by the Costa Rican coffee growers since the early nineteenth century. Currently, this disease represents for coffee growers an increasing loss in the potential coffee yield, since the coffee trees productive life is reduced per unit area. With the purpose of learning more about the variability of pathogenicity in a sample of the population of this pathogen, we determined the growth rate of 10 isolates of *C. fimbriata* on coffee stakes of the Caturra and Catuaí varieties, and the efficacy of biological products: Butrol 31.5 EC, Aliette 80 WP and *Trichoderma* sp. There were no differences when comparing the average daily growth rate of the isolates studied on the stakes of the varieties evaluated and the source of the pathogen. It was indicated that the susceptibility of the coffee varieties to the *C. fimbriata* pathogen is the same of the sample analyzed. However, there were differences in the individual growth rate of each isolate, according to the genotype used as a host. Application of the treatment of *Trichoderma* sp. reduced the mortality of infected plants *C. fimbriata* by 87%.

INTRODUCTION

Diseases of the trunk and root in the trees mentioned in the coffee growing in Costa Rica since the early nineteenth century, caused by different pathogens and cause serious problems in both young plants and in adult plantations (Bianchini, 1955). In Indonesia (Java) at the beginning of the nineteenth century is mentioned on "Kanker" of coffee trees *Ceratostomella fimbriata* attributed to the fungus. In Costa Rica, Eddie Echandi (1955) first reported the presence of the fungus in *Ceratostomella* fimbriata (now classified as *Ceratocystis fimbriata*) symptoms caused by the "Llaga macana" of coffee, indicating that the disease appeared more frequently in older plantations older than 40 years, therefore considered that the disease was present many years before the trees in the country's central valley (Echandi, 1955).

MATERIALS AND METHODS

The study was conducted at the Plant Pathology Laboratory of the Coffee Research Center (CICAFE). In coffee plants of the variety Caturra and Catuai; orthotropic axes were selected with 1 cm of diameter. Each axis was cut into segments of 15 cm, the surface segments disinfected with 70% alcohol. Each segment was removed 50% of their cortex longitudinally. Six segments of each variety were inoculated with 10 µl of an isolate of *C. fimbriata*, at a concentration of 2.75 $x10^5$ spores / ml, then placed in a moist chamber at 23 °C and 12 h photoperiod. Mycelium growth was measured longitudinally every three days for 2 weeks. The assay was performed in duplicate. Was calculated daily growth rate of each strain by regression analysis.

The efficacy of biological products: Butrol 31.5 EC (1ml / L), Aliette 80 WP (4.5 g / L) and *Trichoderma* sp ($3x10^5$ spores / ml) of CICAFE Collection, was evaluated with the *C. fimbriata* isolate with the higher growth rate. Nine days after applying the product, 20 stems Catuaí plant to 8 months of age, were inoculated with $8.5x10^5$ spores / ml of *C.fimbriata*. Mortality was assessed after four months of treatment application.

Table 1. List of *Ceratocystis fimbriata* isolates.

Code	Province	City	District	Altitude
CF10	Heredia	Santa Bárbara	Purabá	1655
CF11	Heredia	Santa Bárbara	Purabá	1655
CF12	Alajuela	Poás	S.Redonda	1450
CF13	Alajuela	Poás	S.Redonda	1450
CF14	Alajuela	Poás	S.Redonda	1450
CF15	Alajuela	Poás	S.Redonda	1450
CF26	Alajuela	Central	Sabanilla	1360
CF30	Alajuela	Central	Carrizal	1400
CF53	San José	Pérez Zeledón	San Pedro	800
CF54	San José	Pérez Zeledón	San Pedro	800
CF67	Cartago	Unión	Tres Ríos	1450
CF81	Heredia	Barva	Getsemaní	1300

RESULTS

The intercept of the trend line with the axis, the value of x in the equation of the trend line which represents the daily growth rate (DGR), the linear correlation coefficient (R2) and the probability ($P<0.05$) for each isolate are presented in Table 2.

Isolates that grow on Caturra had higher growth rates than those obtained on Catuaí.
When the isolates grown on Caturra segments, the largest growth rate occurs daily with CF12 and CF15 isolates. When the isolates grown on Catuaí segments, the greater tendency of growth of *C. fimbriata* isolates occurs in CF53 and CF15. The values of the daily growth rate isolates CF12, CF15 Caturra were higher than the obtained on Catuaí.

The results of the application of *Trichoderma*, Aliette and Butrol on wounds inoculated with *C. fimbriata*, seen in Figure 1. Chemical fungicides: Butrol and Aliette, obtained similar results to the control treatment (no fungicide applications), 70 and 81% mortality respectively. While the application of *Trichoderma* showed a mortality of 10% of plants, 87% lower mortality compared to treatment Butrol, Aliette and control.

Table 2. Regression coefficients growth of *Ceratocystis fimbriata* isolates in coffee stakes var. Caturra and Catuaí.

Variety	Isolate	Intercept	DGR[1]	R^2	P
Caturra	CF11	-0,8521	0,5501	0,9795	0,0002
	CF12	**-1,3888**	**1,0493**	**0,9583**	**0,0007**
	CF13	-0,4859	0,7236	0,9885	0,0000
	CF15	-0,3139	0,9642	0,9716	0,0003
	CF26	0,0080	0,7763	0,9697	0,0003
	CF30	-0,1146	0,4884	0,9589	0,0006
	CF53	-0,1381	0,6590	0,9705	0,0003
	CF54	-1,0521	0,8932	0,9876	0,0001
	CF67	-0,0641	0,6272	0,9706	0,0003
	CF81	-0,4158	0,7360	0,9828	0,0001
Catuaí	CF11	0,2883	0,3442	0,8827	0,0054
	CF12	-0,4905	0,6817	0,9860	0,0001
	CF13	0,1146	0,6887	0,9280	0,0020
	CF15	0,9741	0,7064	0,9826	0,0001
	CF26	0,3427	0,6716	0,9615	0,0006
	CF30	0,6576	0,4017	0,9592	0,0006
	CF53	0,2195	0,7814	0,9786	0,0002
	CF54	0,6675	0,6882	0,9632	0,0005
	CF67	1,0374	0,5047	0,9297	0,0019
	CF81	2,1802	0,5067	0,7637	0,0228

[1]*DGR = Daily Grow Rate*

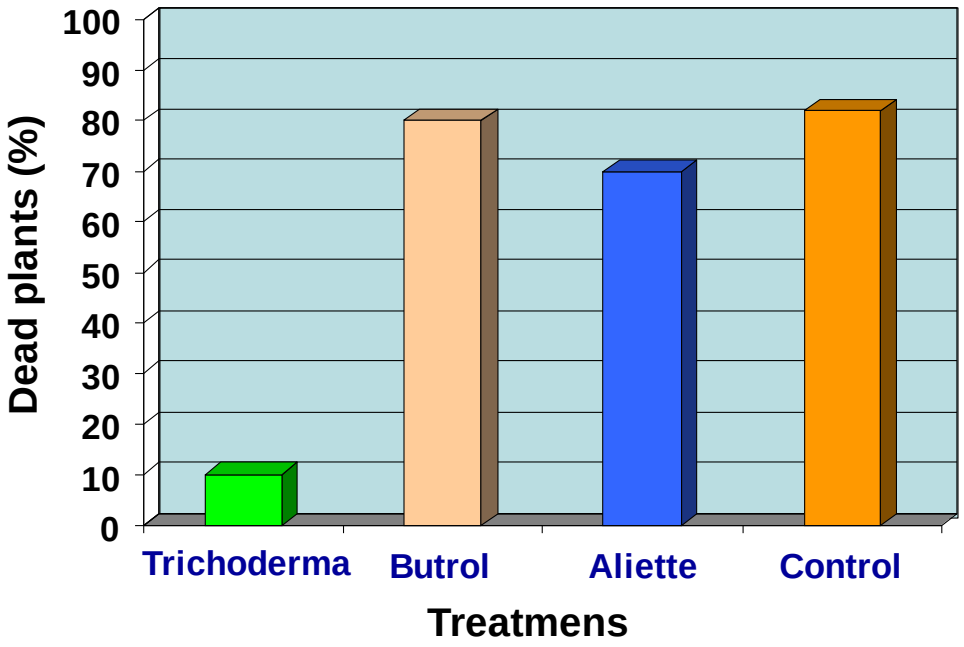

Figure 1. Biological efficacy of various products on the development of "Llaga macana" disease.

DISCUSSION AND CONCLUSIONS

Plants have various mechanisms of defense against pathogenic fungi, one of them is the production of growth inhibitory substances as fungi-toxic phenols, like chlorogenic acid which are produced in greater quantities at the time of infection . This acid is responsible for the genetic resistance of Cocoa and Coffee to *C. fimbriata*, explaining why the isolation that had higher growth trend in the stakes of the variety Caturra (CF12) was not the same in the variety Catuaí (CF53) (Solis and Herrera, 2005). The studies carried out by Ma et al (2007), showed the antifungal activity of the majority of chlorogenic acid derivatives and found that the mode of action of these acids is due to inhibition of 1,3-β-glucan synthase and 1,6-β-glucan synthase, essential enzymes for the biosynthesis of glucan cell wall component of fungi.

This research was possible to analyze the features in the aggressiveness of the isolates studied, determined that there difference in the population of isolates of *C. fimbriata* studied on the development and growth of the disease of "Llaga macana" in coffee plants varieties Caturra and Catuaí.

Moreover, preventive treatment with *Trichoderma* sp. was able to reduce the mortality of plants infected with the disease of “Llaga macana”. This result opens an opportunity for the management and control of *C. fimbriata* in soils of the coffee plantations in Costa Rica.

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Evaluation of Biological Products for *Mycena Citricolor* Control

M. BARQUERO-MIRANDA, A. ROBLES-CHAVES, A. N. ESPINOZA

Costa Rica Coffee Institute, Coffee Research Center (CICAFE)

SUMMARY

Traditionally the use of chemicals to control for the Ojo de Gallo, coffee disease caused by pathogen *M. citricolor*, has been supported by technicians and coffee growers due to the effectiveness of these products. Moreover, there is not much information on biological products and the reference on commercial products is very limited. Therefore, the purpose of this study was to evaluate the residual characteristics and action of several biological products on the formation and germination of the gemmae, in the field and under controlled laboratory conditions, during 2009-2010. Tree biological products were applied in the field: *Trichoderma lignorum, T.harzianum* and *Bacillus subtilis*; while in the Phytopathology Laboratory of the Center for Coffee Research (CICAFE) the following treatments were evaluated: *Trichoderma lignorum*, a leachate from compost supplemented with molasses, *Bacillus subtilis* and the bacterial microorganisms BacLC09A, BacLC09B and P16 codes; in addition to a treatment with no application of products. The results obtained from the applications in the field indicate that the *T. lignorum* and *B. subtilis* microorganisms are not efficient in reducing the disease progress. The incidence rates of "Ojo de Gallo" with these microorganisms were statistically equal to the treatment without application of products. The results obtained in the laboratory reported a variable response of the microorganisms on the germination of *Mycena citricolor*. The best efficiency in reducing the germination of the *M. citricolor* gemmae was observed with the *Bacillus subtilis*, while the P16 code microorganism caused the lower formation of gemmae on the lesions of "Ojo de Gallo". The other treatments did not differ from the treatment without application of products. The results obtained in this experiment demonstrate the efficiency of biological products or microorganisms to control the "Ojo de Gallo" disease, although their capacity is reduced in field. For this reason, it is necessary to make more research on formulation processes to increase the permanence of these microorganisms on the plants.

INTRODUCTION

Traditionally the use of chemicals to control of the Ojo de Gallo, coffee disease caused by pathogen *M. citricolor*, has been supported by technicians and coffee growers due to the effectiveness of these products. Moreover, there is not much information on biological products and the reference on commercial products is very limited. Therefore, the purpose of this study was to evaluate the residual characteristics and action of several biological products on the formation and germination of the gemmae, in the field and under controlled laboratory conditions.

MATHERIALS AND METHODS

Microorganisms and treatments

Bacteria P16, Bac LC09A, BacLC09B were isolated from coffee leaves by Salas (2010) and demonstrated its growth inhibiting effect in vitro *M.citricolor*. Also used an isolation of *Trichoderma lignorum* (Mycobac®), *Bacillus subtilis (*Serenade) and 1 compost leachate.

Laboratory evaluations

Non commercial bacteria were growth by liquid fermentation for 5 days at constant stirring. The inhibitory power of bacteria was determined *in vitro* in presence of the fungus (Figure 1).

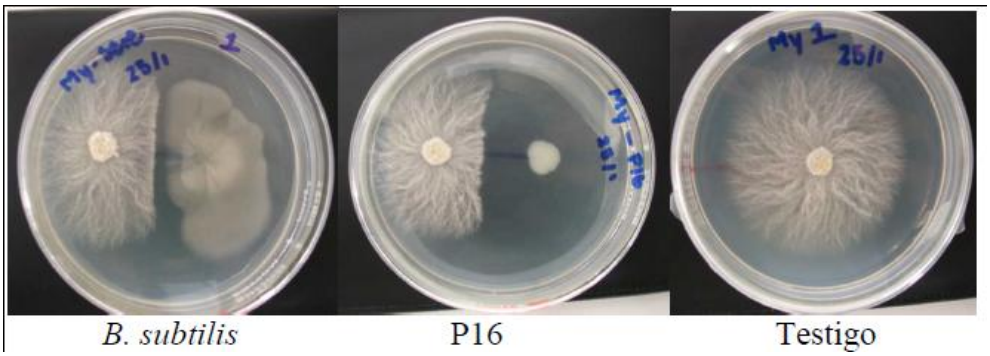

Figure 1. Inhibitory effect of bacterial broth on *Mycena citricolor* growth *in vitro* conditions.

$1x10^8$ CFU / mL solution was applied to 5 healthy leaves and allowed to dry for 2 h. The coffee leaves were maintained in a growth chamber with micro-sprinkler irrigation every 30 min, 21 ± 1 °C temperature, a brightness of 760 lx and a photoperiod of 12 hours. Evaluation included quantification of lesions formed in healthy leaves and quantity of gems in those lesions.

Field evaluations

The treatments: *Trichoderma lignorum* and *T. harzianum* ($2x10^8$ CFU/mL) and *Bacillus subtilis* ($1x10^8$ CFU/mL) were applied 2 to 3 times per year. The test was performed on Catuaí variety, planted at 2x1 meters, in a coffee plantation located at 2400 meters above sea level, with average annual conditions of 18 ° C, 90% RH and 3200 mm.

The experiment design was randomized complete block of 6 treatments with 4 replications; 30 plants distributed in three rows of 10 plants each. Was assessed the total number of healthy and diseased leaves in branches of the middle stratum of 10 plants, evaluation was every 15 days from may to october.

RESULTS

The results obtained in the laboratory, showed a differential responses between microorganism and treatments in the development and sporulation of *M. citricolor* (Table 1). The best efficiency reducing the germination of the *M. citricolor* gemmae was observed with the *Bacillus subtilis*, while the P16 code microorganism caused the least formation of gemmae on the lesions of "Ojo de gallo".

Table 1. Average sporulation and Incidencia of *Mycena citricolor* on leaves treated with different microorganism and compost leachate.

Treatments	Incidence (%)	Sporulation (%)
Bacillus subtilis	7	87
Trichoderma lignorum	60	30
P16	67	25
BacLC09B	67	40
BacLC09A	67	40
Compost	77	40
Control	100	100

The collected data show that the application of *Trichoderma lignorum*, *T. harzianum* and *Bacillus subtilis* in two and three applications in field had no effect in reducing the development *of Mycena citricolor* (Figure 2).

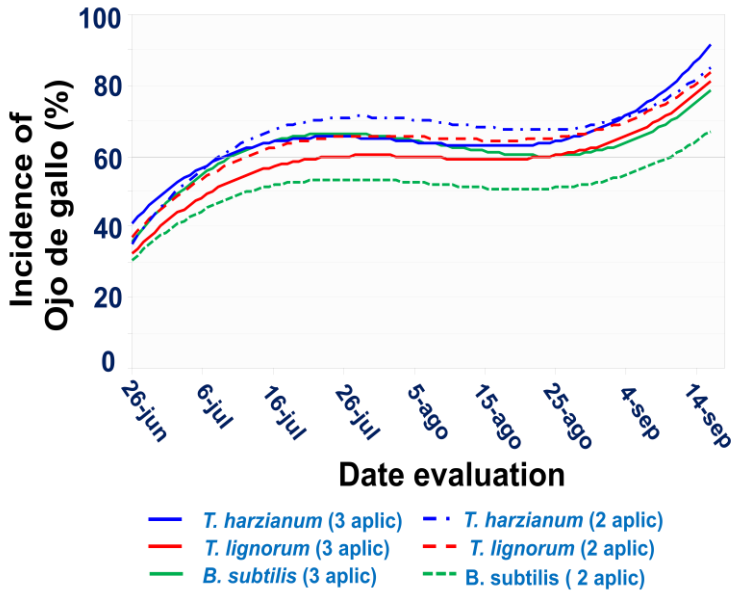

Figure 2. Incidence of *Mycena citricolor* in plants treated with biological products.

DISCUSSION AND CONCLUSIONS

It is known bacterias potential as biocontrol fungi and plant pathogens (Teng et al.; 2006; Wller and Masaba, 2006; Gebreel et al. 2008).

Antagonistic effect of bacterial P16 isolate may be due to releasing a diffusible antibiotic compound in the medium, as evidenced by a halo of inhibition which visually presents no bacterial or mycelial growth (Figure 1) (Gutterson, 1990).

The results obtained from the applications in the field indicate that the *Trichoderma lignorum* and *B. subtilis* microorganisms are not efficient in reducing the disease progress.

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Evaluation of Transmission of Crespera through Cicadellidae

A. N. ESPINOZA, A. ROBLES-CHAVES, M. BARQUERO-MIRANDA, R. CHACON

Costa Rica Coffee Institute, Coffee Research Center (CICAFE)

SUMMARY

In Costa Rica, the "Crespera del café" is a physiological disorder which symptoms are not always present in the entire plant. Sometimes, it may be possible to observe only a portion of the affected tissue in the orthotropic axes, plagiotropic branches and the leaf blades affected. Because of this behavior of the symptoms, a study was conducted in 2007-2011, in the Phytopathology Laboratory of the Center for Coffee Research (CICAFE), to evaluate the isolated inoculation of *Xylella fastidiosa* in the coffee plants with the disorder, by Cicadellidae and mechanics (internodes and leafstalk) in 3-month old plants from an indirect somatic embryogenesis propagation; with the purpose of reproducing the evolution and development of the Crespera's symptoms. It was determined that both the mechanical and vector inoculation methods are efficient to achieve the *X. fastidiosa* infection; however, after three months of inoculation, the more efficient infection was through vector and mechanical inoculation on leafstalks. The infection of 100% of the plants after 4 years was confirmed through DAS-ELISA. No symptoms related to "Crespera del café" were observed during this period. No significant differences were observed in growth between inoculated and uninoculated plants.

INTRODUCTION

The Crespera coffee plant is a physiological disorder which symptoms are not always present in the entire plant. Rodriguez and collaborators (2001) related *Xylella fastidiosa* bacterium with the symptoms of Crespera plant in Costa Rica. However, studies by Fournier (2007) and Chacón (2008) indicate that it is possible to detect *X. fastidiosa* in asymptomatic Crespera plants, although there are high concentrations of bacteria. Moreover, Chacón (2008) cites differences in the description of the symptoms of coffee Crespera made in countries like Colombia and Costa Rica, in turn indicating that these are different to those described for coffee plants infected with *X. fastidiosa* in Brazil. Because of the inconsistent behavior between Crespera symptoms and the presence of *X.fastidiosa* in the plants, a study was conducted with the purpose of completing Koch's postulates.

MATERIALS AND METHODS

With the purpose of select insects presence of the bacterium *X. fastidiosa*, was captured leafhoppers plants located in the presence of symptoms of coffee Crespera in an infected farm located in Naranjo of Alajuela in 2008.The trial was conducted in the laboratory and greenhouse of Plant Pathology by Research coffee Center (CICAFE), located in Barva of Heredia. It took young coffee plants propagated by somatic embryogenesis and acclimatized for two months, free of *Xylella fastidiosa*. The plants were exposed to leafhoppers captured in the field. We determined the presence of the bacterium *X. fastidiosa* in 15 insect dead bodies and survivors recovered in inoculated coffee plants. We selected twenty plants in greenhouse acclimated incidence free *Xylella fastidiosa*. Ten plants were inoculated with the bacterium *X. fastidiosa* by two mechanical forms: abaxial side of the leaf petioles of the second, third and

fourth internode in direction apical-radical with an incision petiole, as well as in the posterior region of the fourth, fifth and sixth internode in direction apical-radical with two incisions internode. In both methods was used 0,5ųl volume of inoculum at a concentration of $2{,}0x10^7$ CFU / ml cell *Xylella fastidiosa*. The serologic test was performed 3, 12 and 48 months after inoculation *X.fastidiosa*, in leaf petioles of the middle of the plants. While infect plants were evaluated each year to determine the presence of Crespera symptoms in the period 2007-2011.

RESULTS

The DAS-ELISA test applied to the bodies and survivors of grasshoppers, detected the presence of *X. fastidiosa* in 100% of the insects at the end of 45 days of exposure, Figure 1.

The results of serological tests indicate that *X.fastidiosa* infection was achieved, especially with infection method: insects and mechanic in leaf petioles. However, the proportion of positive *X.fastidiosa* plants was lower at the end of the study. Plants inoculated mechanically on petioles, were negative ELISA test after 4 years. While plants subjected to infection mechanical of internodes, kept positive 60% of plants, Table 1.

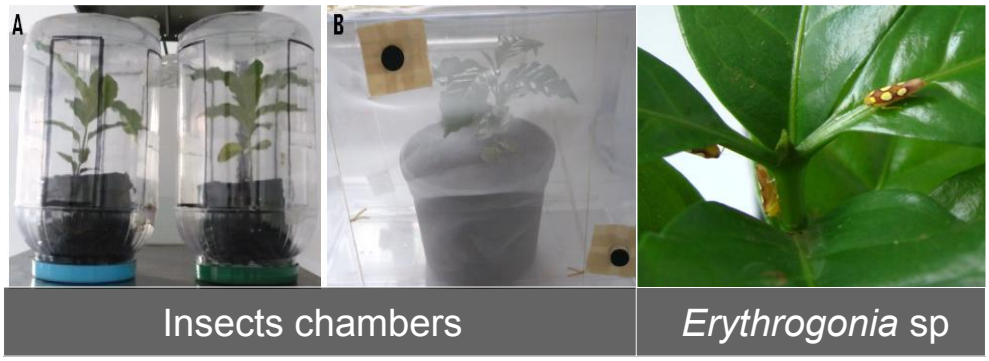

Figure 1. Appearance of the chambers used to perform the process of infection *X.fastidiosa* by insect vectors.

Table 1. Changes in the percentage of positive samples of *X.fastidiosa*, in coffee plants infected by use of vectors and two methods of mechanical inoculation.

Inoculation	**Infection *X. fastidiosa* (%), months after inoculation**		
	3	**12**	**48**
Mechanical			
Petioles	100	60	0
Internodes	60	60	60
Vector	100	100	60

Figure 2. Appearance of plants inoculated with *X. fastidiosa* after 4 year. No symptoms are observed of Crespera.

DISCUSSION AND CONCLUSIONS

Xylella fastidiosa growth is associated with the colonization and invasion of adjacent tissues inoculum and biofilm formation characteristic affecting transport across xylem division due to abnormal vascular tissue in the cortex and petiole and stem mesophyll reducing the number of chloroplasts and increasing accumulation of calcium oxalate crystals in the latter, at these levels of invasion is when you allow to see the symptoms of the diseases (Benetti et al, 1998). According to Alves and collaborators (2004), the colonization percentage varies among hosts. Research in plants peach, citrus and coffee, it was determined that the relationship expressed symptoms increase over time, they reported that the presence of the bacterium in little noticeable symptomatic plants is 26% in tissue colonization and reaches a severe symptoms when it exceeds 51,6% of colonization. However, this condition is not obtained in this test since it was found X.fastidiosa detectable concentrations in asymptomatic plants Crespera.

In the field, the most abundant leafhopper populations collected showed higher concentrations of *Xylella fastidiosa*. The leafhopper *Erytrogonia* sp was the most abundant, and shown to be an efficient vector for transmission of *X. fastidiosa*, because it detected the presence of bacteria 45 days after vector inoculation.

The wound inoculation method in petioles showed mechanical efficiency (96.7%) than in the internode inoculation method (66.7%) after three months of evaluation. However, after 48 months was determined greater presence of bacteria with intenudos method, compared to petiole inoculation method.

At the end of four years of years of study, it was possible to observe the emergence of symptoms related to Crespera coffee. There was also no significant differences in the growth of the inoculated plants (mechanical and vector form) with the untreated.

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Selection of Chemical for *Mycena Citricolor* Control

A. ROBLES-CHAVES, M. BARQUERO-MIRANDA, N. ARRIETA-ESPINOZA

Costa Rica Coffee Institute (ICAFE), Coffee Research Center (CICAFE).

SUMMARY

It is not always possible to test in the field effectiveness of fungicides to control *Mycena citricolor*, because of the changes in the climatic conditions that affect the development and progress of the disease. For this reason in this study the evaluation of effectiveness and residual activity of fungicides on the development of Ojo de gallo was made under controlled laboratory conditions. Were probed 43 different fungicides and mixtures of two or more fungicides. Evaluation included the residual effectiveness during a month on formation of lesions and gemmae of "Ojo de Gallo". The results allow to separate the tested fungicides in tree groups according to its effectiveness rate controlling Ojo de gallo sporulation and formation of new lesions. Better results were obtained with the products Silvacur combi and Atemi mixed with Cepex. Those and seven products more had an effectiveness rate over 62%.

INTRODUCTION

Mycena citricolor causes important damages in Costa Rican coffee plantations, producing decreases on plantation yield due to plant defoliation (Barquero 2007). For this reason evaluation of new chemical fungicides that help with the control of this disease is an important topic in the Coffee Research program of ICAFE.

During the last ten years ICAFE has evaluated different commercial fungicides in field conditions to identify which has the higher effectiveness against Ojo de Gallo. However the evaluations of fungicides eficiency in field imply an extensive evaluation, generally more than two years, to obtain a result. Neither helps to distinguish the real action of the different fungicides or chemical molecules on the formation of fruiting bodies of the pathogen or on the germination of these structures. For this reason ICAFE implemented an evaluation method in laboratory that provide as same as in field the optimal conditions of temperature, relative humidity and rain for the *Mycena citricolor* development. More than a hundred different fungicides have been evaluated using this method during the last five years (ICAFE, 2011).

MATERIALS AND METHODS

Evaluations were conducted in laboratories and experimental coffee plantation of ICAFE located in Barva, province of Heredia. The treatments consisted in chemical and organic fungicides (table 1) and mixtures of two or more products, also antibiotics and adjuvants (table 2).

The products were applied in field over three free-disease coffee plants variety catuaí, three years old and without products application before. Verdadero was applied in drench 15cm around plant base.

Table 1. Chemical products evaluated and dose.

Treatment	Product	Dose
1	Bellis 38WG	2,5ml/L
2	A166090D	1,5ml/L
3	AE C638206	1,6ml/L
4	AEC656948-TBZ	1,25ml/L
5	AEC656948-TFX	1,25ml/L
6	AgroSept	41,75ml/L
7	Amistar 50 WG	1,25ml/L
8	Atemi 10 SL	1,25ml/L
9	BCS 589	5ml/L
10	BYF 00587	1,5ml/L
11	Cepex 10 SL	5ml/L
12	Duett 25SC	1,2 gl/L
13	ECOFRUT	24ml/L

Treatment	Product	Dose
14	Flint 50WG	1,25g/L
15	Infinito 68.75SC	1,5ml/L
16	KBR 2738	1,25ml/L
17	Verdadero 600 WG	4g/L
18	Stratego 25 EC	2,5ml/L
19	Trehazolin	5ml/L
20	Monceren 25 WP	1,25g/L
21	Nativo 75WG	1,25g/L
22	Opera 18.3 SE	2,5ml/L
23	Score 25EC	1ml/L
24	Silvacur Combi 30 EC	1,75 ml/L
25	Esfera 26,7 EC	1,5ml/L

Table 2. Mixes of chemical products evaluated and dose.

Treatment	Product	Dose		Product	Dose
26	Silvacur Combi 30 EC	1,75 ml/L	+	Cenebol 4.82 EC	1,25 ml/L
27	Silvacur Combi 30 EC	1,75 ml/L	+	Agry-mycin 44.3 WP	6,25g/L
28	PureSprayGreen (PSG)	12,5ml/L	+	Biophytomax (BFT)	3,125ml/L
29	Folicur 25EC	1,25ml/L	+	Caporal 25DC	0,5ml/L
30	Atemi + Cepex	1,25ml/L	+	Cepex 10 SL	5ml/L
31	Cyprosol 10SL	1,25ml/L	+	Cepex 10 SL	5ml/L
32	Caporal 25DC	1,75 ml/L	+	Cepex 10 SL	5ml/L
33	Folicur 25EC	1,25ml/L	+	Cepex 10 SL	5ml/L
34	Orius 25EW	1,75 ml/L	+	Cepex 10 SL	5ml/L
35	Propicon 25EC	1,875ml/L	+	Cepex 10 SL	5ml/L
36	Silvacur Combi 30 EC	1,75 ml/L	+	Cepex 10 SL	5ml/L
37	Silvacur Combi 30 EC	1,75 ml/L	+	Cepex 10 SL	2,5ml/L
38	Silvacur Combi 30 EC	1,75 ml/L	+	Cepex 10 SL	3,75ml/L
39	Tebutriazell 30EC	1,75 ml/L	+	Cepex 10 SL	5ml/L
40	Silvacur Combi 30 EC	1,75 ml/L	+	Kasumin 2SL	5ml/L
41	Silvacur Combi 30 EC	1,75 ml/L	+	Validacin 5SL	5ml/L
42	Mixture St: Enlazador (5g/L) + Mangesium Nitrate (5g/L) + Silvacur Combi 30EC (0,5ml/L)+NP7(0,3ml/L)				
43	Control without applications				

Leaves were taken every seven days during four week after application. The leaves collect consisted in diseased and healthy leaves from medium stratus of plant, from the third node of the branch.

Healthy leaves were inoculated in laboratory with *Mycena citricolor* gems, four gems per leaf. Diseased leaves were selected by size and similarity of lesion. Healthy and diseased leaves were incubated during two weeks under 21±2°C temperature, 100% relative humidity and irrigation every hour to maintain permanent leaf wetness (Figure 1).

Evaluation consists on counting new lesions formed in healthy leaves and gems produced in diseased leaves during two weeks. The results were used to obtain the infection rate and sporulation rate of *Mycena citricolor* on leaves treated with different chemical products and potential control of the product reducing "Ojo de gallo" development.

Figure 1. Moist chamber used for development Ojo de gallo, in laboratory evaluation of effectiveness of chemical products.

RESULTS

Evaluation of effectiveness of the products on reducing the formation of "Ojo de gallo" lesion in treated leaves indicates difference between treatments in both factors evaluated, gemmae and lesions production and also the residual effect of those products. Table 3 summarized the average gemmae and lesions of Ojo de Gallo formed on leaves treated with the different products.

Better results were obtained with fungicides that reduce the gemmae formation during long time. According with those results compared to control treatment without applications was obtained the effectiveness rate for each treatment.

Table 3. Germination, sporulation and control of *Mycena citricolor* on leaves treatments with different fungicides.

Treatment and dose	Germinación (%)	Esporulación (gems/Lesion)	Efectiveness controlling "Ojo de Gallo" (%)
Sin aplicación	95	7,7	0,0
Duett	94	5,1	7,7
Mixture (St)	88	8,3	8,7
Infinito	92	7,7	9,9
Verdadero	94	5,2	19,2
Score	56	9,2	21,3
AEC656948-TBZ	39	2,7	25,2
ECOFRUT	98	2,8	25,5
AEC656948-TFX	13	1,5	26,4
AE C638206	52	5,1	27,1
AgroSept	92	2,8	28,4
PSG + BFT	68	3,9	31,4
BCS 589	56	3,0	39,0
Caporal + Cepex	84	2,0	39,3
Silvacur + Cenebol	69	2,9	39,4
Bellis	10	9,2	45,9
Cepex	75	1,7	46,8
BYF 00587	58	1,5	48,2
Orius + Cepex	50	2,2	49,1
Silvacur + Kasumin	60	2,2	49,9
Opera	63	1,9	51,4
Cyprosol + Cepex	70	1,4	52,0
Amistar	55	2,9	54,5
Flint	23	2,7	55,5
Silvacur + Agrymicin	53	2,0	55,7
Trehazolin	53	0,4	57,1
KBR 2738	54	2,5	57,3
Silvacur + Cepex (2,5ml/L)	72	0,6	57,4
Folicur + Caporal	45	2,1	58,6
Atemi	40	1,8	59,2
Esfera	35	1,9	60,4
Propicon + Cepex	64	0,7	60,7
Silvacur + Cepex (3,7ml/L)	44	1,8	61,9
Monceren	44	3,0	63,7
Silvacur + Validacin	48	1,2	64,6
Tebutriazell + Cepex	53	0,9	64,9
Nativo	86	0,8	65,4
Folicur + Cepex	50	1,0	65,5
Stratego	100	0,0	67,4
Silvacur	27	1,7	69,0
A166090D	30	1,7	69,8
Atemi + Cepex	32	0,6	73,7
Silvacur + Cepex (5ml/L)	23	0,3	80,1

Three groups are easily separated according to effectiveness rate value. Better results were obtained with products containing Tebuconazole and Cyproconazole as active ingredients mixed with Validacin. Also are include in these group: Stratego, Nativo 75WG and the sample product A166090D (not commercially available almost) where the percentage of efficiency is over 62%. Products included in the first group induce less gemmae production in old lesion and produce few new lesion (Figure 2). A second group are the products with an intermediate control efficiency between 62 and 32%. With intermediate potential to reduce gemmae formation and variable effect reducing new lesion formation. The last group do not achieve an efficiency of over 32%, with a low effect on production of gemmae and lesions.

DISCUSSION AND CONCLUSIONS

The results indicates a major control of Ojo de Gallo with Silvacur Combi 30EC (1,875ml/L) and Atemi 10SL (1,25ml/L) mixed with Cepex 10 SL (5ml/L), principally those products reduce the gemmae formation. The effect of those mixtures on *Mycena citricolor* sporulation in the optimal conditions used in this laboratory assay suggest a good control of Ojo de gallo development in field conditions. Field condition are less constant and favorable than laboratory condition, however evaluations in field during the years 2009-2011 (ICAFE, 2011) indicates an disease control around 80 % when those mixtures are used.The effectiveness of Silvacur Combi 30EC and Atemi 10SL is less when used alone than when using mixed with Cepex. Products included in the second group according to effectiveness rate, has lower control of gemmae formation than those located in group 1. According with Barquero (2007) the capability of *Mycena citricolor* of affect seriously all the coffee plantation depends principally of the sporulation rate that related with weather condition. In the Ojo de gallo control reducing gemmae formation is the most important aspect to obtain with a fungicide. Effect of products in group 2 over these parameter is not enough to be considerate for a good control on field conditions.

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Submerged Fermentation of *Beauveria Bassiana*

A. ROBLES-CHAVES, M. BARQUERO-MIRANDA, N. ARRIETA-ESPINOZA

Costa Rica Coffee Institute, Coffee Research Center (CICAFE)

SUMMARY

Beauveria basiana used as a biopesticide against the coffee berry borer (*Hypothenemus hampei*). Different culture conditions for Beauveria bassiana production by submerged fermentation were evaluated to determinate the optimal temperature, pH, and light for spore production on 1L flask scale. The results indicate that at 28 ° C temperature is achieved the highest concentration of spores. The pH and the light have no effect on spore production. After, an analysis was made considering the effect of the aeration rate and different substrate sources on the blastospore concentration. With 8-vvm airflow and a 4:1 carbon/nitrogen relationship, maximum concentration obtained was $9x10^8$ blastospores/ml using yeast extract as carbon source. Finally, proves were made for scale up system in 8 liters tank, separation of spores by centrifugation and preparation of an oil-based formula, an efficient control of the coffee berry borer was achieved, statistically similar to that obtained through the *B. bassiana* conidia produced in solid substrate.

INTRODUCTION

Since 2003, the Costa Rican Coffee Institute, through its Center for Coffee Research (CICAFE), started the production of *Beauveria bassiana* through solid substrate fermentation. This project aimed at having a biopesticide, and providing technical assistance and training to the national coffee growers for a biological control alternative that would create opportunities for the sustainable control of coffee berry borer in Costa Rica. Due to the increased demand for this biopesticide, the difficulties to obtain the solid substrate required and increase of space and work-time, which entails an cost increase in the entomopathogenic production through solid fermentation, studies conducted between 2008 and 2012 were made to examine the potential for production and scale up of the *B. bassiana* fungus, through the use of the liquid fermentation technique.

MATERIALS AND METHODS

Studies were made in CICAFE laboratories between 2008-2012 for evaluation of the submerged fermentation technique for massive production of *Beauveria bassiana*. Initial studies were made to determinate the optimal conditions of temperature, pH, and light for spore production. These evaluations were made in 1L flasks with an air flow entrance for agitation and aeration and an air outlet. The working volume was 800ml and determination of concentration of *Beauveria bassiana* spores was made in a Neubauer counting.

In the last two years studies included incubation time, aeration rate, substrate source. For aeration, were used aquarium air pumps which add an air flow between 2-9vvm. Incubation time was 24-96 hours. The substrates evaluated were: peptone, triptone, yeast extract (Hy-412 Sigma®), fish flour, corn flour, baking yeast, soy protein powder and flaxseed powder, all mixed with sucrose 30%. Quantification of spores concentration were made by Neubauer counting chamber.

Finally spores were separated for media culture by centrifugation and then formulated in oil derivated of petroleum plus tween 20 0,75%.

Bioassays of biological activity against coffee berry borer were conducted in greenhouses and in the field. Coffee plants in field were applied with the product, branches were collected 7,15 and 22 days after application and were infected with coffee berry borer in laboratory. Mortality of coffee berry borer in beans was evaluated in by dissection of coffee beans.

RESULTS

The optimal temperature for *Beauveria bassiana* was 28°C, with the higher spore production in the shortest time. pH and light seem to be not significative in spore production (Figure 1).

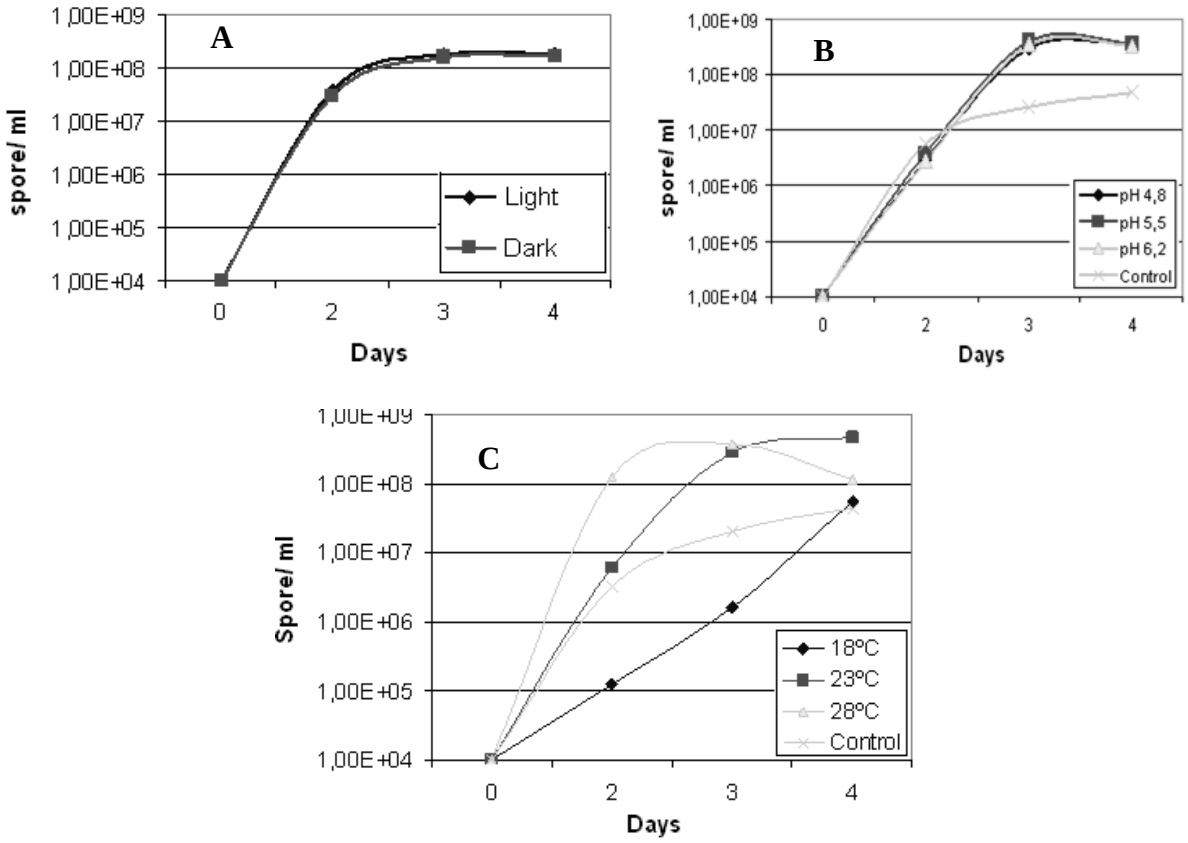

Figure 1. *Beauveria bassiana* spore production. A. Under light and dark condition. B. Different initial medium pH. C.Incubation temperature.

The spore production increased with the aeration rate (Table 1). The best spore concentration obtained was 8.00 x10^8 blastospore/ml with the higher airflow rate (>8vvm). The optimal incubation time was 48 hours, increases in incubation more incubation time (more than 48 hours) causes decrease in blastospore production, probably due to mycelia development.

Table 1. Producción of *B.bassiana* blastospores in four different condition of air flow rate-volume and four different incubation times.

vvm	Blastospore/ml[1]	Incubation time (hours)	Blastospore/ml[1]
>8	8.00 x10^8	24	7.00 x10^8
7	5.67 x10^8	48	8.89 x10^8
5	5.35 x10^8	72	4.68 x10^8
3	5.10 x10^8	96	5.79 x10^8

[1]*Mean*

Higher concentration of blastospores was obtained with the substrates sources: Soy powder protein, yeast extract and flaxseed powder (Table 2).

Table 2. *B.bassiana* blastospores produced in a submerged fermentation system with different substrate source (highest concentration obtained).

Substrate source	Blastospore/ml
Triptone	2,20E+08
Corn flour	2,60E+08
Baking yeast	4,50E+08
Fish flour	5,10E+08
Peptone	6,15E+08
Soy powder protein	9,00E+08
Yeast extract (Sigma ®)	9,20E+08
Flaxsed powder	9,80E+08

The mortality of the coffee berry borer by oil formulated B.bassiana blastospores was 38%, shortly higher than the use of Conidia of solid fermentation. Also was determinate a durable effect of oil formulate blastospores killing coffee berry borer after 15 days of application (Figure 2).

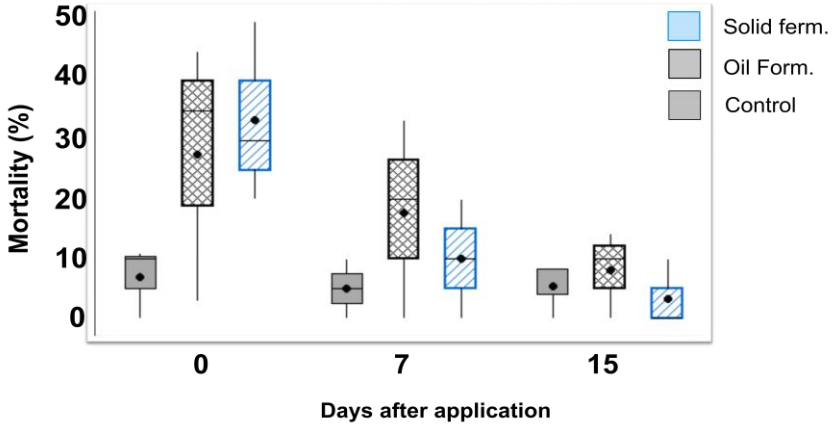

Figure 2. Bioactivity shown by *B.bassiana* blastospores formulated in oil and conidia of solid fermentation against coffee berry borer 15 days after application in field.

DISCUSSION AND CONCLUSIONS

Temperature has a determinant effect on the growth of *B.bassiana*, 28 ° C seems to be the right temperature for the strain studied B.bassiana which coincides with results of similar studies. (Shimadzu, 2004). The pH and light showed no effect on the growth of *B.bassiana*. We know the capacity of the medium pH regulation by entomopathogenic fungi to maintain a pH optimum for development (Hallsworth and Magan, 1996). The pH range evaluated at the beginning of the fermentation is suitable for microorganism growth, however it was observed a tendency of the organism to maintain the pH at 4.5 (Mata, 2008).

Increased biomass *B.bassiana* consumption depends directly on the nutrient medium. The substrates tested exhibit great variation in composition, so there is a wide variability in biomass production, this study suggest two substrate sources of low economic cost as potential candidates for use in production of *B.bassiana* by submerged fermentation, replacing yeast extract as currently used base substrate.

The oil formulation from the liquid fermentation blastospores promotes a greater permanence of the fungus on the leaf, remaining in effect until 15 days after the application, as indicated by several authors in the oil formulation of entomopathogenic fungi spores allows greater preservation of the viability of spores in the field. (Feng *et al,* 1994; Langewald *et al,* 1997).

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Susceptibility of Different Coffee Genotypes to *Ceratocystis Fimbriata*

A. ROBLES-CHAVES, N. ARRIETA-ESPINOZA, M. BARQUERO-MIRANDA

Costa Rica Coffee Institute, Coffee Research Center (CICAFE)

SUMMARY

The *Ceratocystis fimbriata* pathogen is responsible for the most serious problems of coffee canker known as *"Llaga macana"* in Costa Rica. Considering the economic losses caused by this disease, a study was conducted in 2011, in the Phytopathology Laboratory of the Center for Coffee Research (CICAFE), to evaluate the susceptibility of 42 different coffee genotypes. The purpose of the study was to determine the resistance of coffee to this disease. The infection was calculated by using an isolate of *Ceratocystis fimbriata* with highest pathogenicity, according to its daily growth rate. It was observed variability in the development of *Ceratocystis fimbriata* in the orthotropic axes of the coffee genotypes evaluated. Two genotypes of *C. arabica* showed similar immunity to that observed in two accessions of *C. liberica* var. *dewevrei* used as a pattern with high resistance or immunity. Other genotypes showed a response to infection similar or higher than Caturra and Catuaí varieties used as a comparison pattern. This paper discusses the potential of some coffee genotypes for a genetic improvement program to control this disease.

INTRODUCTION

The disease caused by the fungus *Ceratocystis fimbriata* is highly important in the Costa Rican coffee industry, because it caused losses to the producer by reducing the productive potential of the plantation, due to the gradual death of plants long before completing its productive cycle or of plantation renovation. In previous studies in this research center, was determinted differences in the susceptibility in some genotypes of *C. arabica* and in some forward lines of genetic improvement, to the infection of "Llaga macana" in controlled conditions (Barquero et al, 2011; ICAFE, 2009). Other research has reported the existence of resistance in species *C. liberica* and *C. arabica* (Echandi and Fernández, 1962).

Due to the wide distribution of the pathogen and the low efficiency of control products in the soil and the plant, the use of coffee materials with less susceptibility to the fungus *Ceratocystis fimbriata* could result in a cost reduction for combating this disease by eliminating or reducing the use of chemical or biological. The search of materials of coffee with tolerance or complete or partial resistance to the disease of "Llaga macana", becomes an improvement necessary for our country, in order to bring new easy of managing this stems disease for national coffee producers.

MATERIALS AND METHODS

The study was conducted in 2011 at the Plant Pathology Laboratory of the Coffee Research Center (CICAFE), located in Barva of Heredia. Orthotropic exes around 1 cm diameter were colleted from coffee plants in 7 genotypes of the species of coffee *C. arabica* (two commercial varieties Caturra and Catuaí), 15 genotypes of the species *C. liberica* x *C. arabica* (natural crosses), 17 genotypes from crosses between *C.arabica* x *C. arabica* (advanced

breeding lines) and 3 genotypes of the species *C. liberica*. For each genotype used, orthotropic exes were cut into segments 10 to 15 cm long. Each segment was inoculate with 10µl of a dilution $1x10^6$ spores / ml by *C. fimbriata* cultivated in liquid medium and 0.3% of yeast 1.3% sucrose for 5 days at 23 ° C under constant agitation. All segments were placed in humidity chamber at 23 ° C for 15 days. During this period was measured longitudinal growth of the lesion. We analyzed the daily growth rate by calculating growth regression curves, placing the results according to five categories: 1 - resistant, 2 - tolerant, 3 - moderately tolerant, 4 – moderately susceptible and 5- susceptible.

RESULTS

The results indicate the existence of differences in the response of coffee genotypes to *C. fimbriata* infection. The Figure 1 shows the observed frequency of genotypes in accordance with the classification categories used in this study.

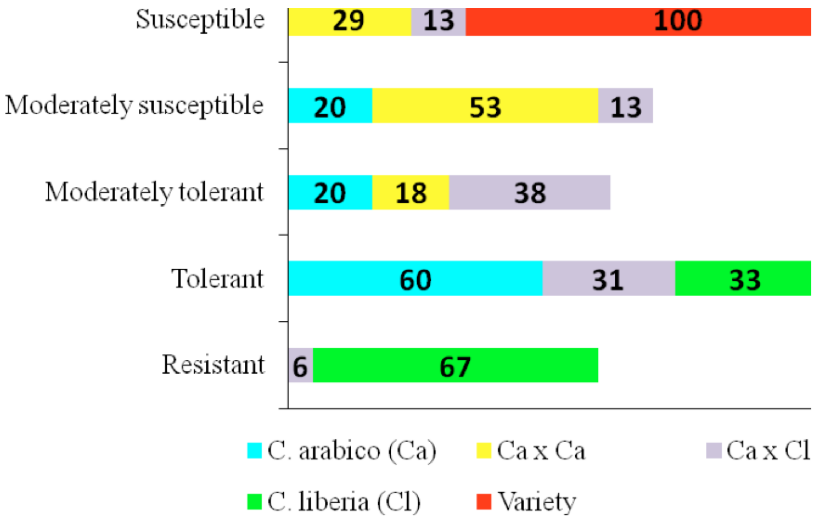

Figure 1. Distribution of the resistance, tolerance and susceptibility response to Llaga macana in different genotypes of coffee. Barva of Heredia, Costa Rica 2011.

Most individuals with complete resistance or immunity was obtained in the studied genotypes of the species *C. liberica*, was 67% of individuals immunes and 33% of tolerant. In a second group contains genotypes from crosses between species *C. arabica* x *C. liberica*, showing a 6% immune individuals, 31% tolerant, 38% moderately tolerant, 13 % moderately susceptible and 13% susceptible. A third group is composed of the selected genotypes *C. arabica*, which determined a 60% individual tolerance and response segregation distributed in 20 % moderately tolerant and 20 % moderately susceptible. Finally, in the group of individuals *C.arabica* x *C.arabica* the response of the genotypes to the Llaga macana was 18% moderately tolerant, 53% moderately susceptible and 29% susceptible. In the latter group, comprise commercial coffee varieties Caturra and Catuaí (100 % susceptible).

DISCUSSION AND CONCLUSIONS

The results obtained in this study demonstrate the existence of segregation in resistance or susceptibility of coffee materials from crosses between species *C. arabica* and *C. liberica* to *Ceratocystis. fimbriata*. The coffea *liberica* species; according to studies by Echandi and Fernandez (1962) show immunity properties to Llaga macana.

In this study, it was possible to find a complete resistance (immunity) and partial resistance to Llaga macana, that could be related to two points to consider in the future, a segregation of the genetic factors involved in resistance to Llaga macana in genotypes propagated by seed or the existence of virulence factors in the pathogen. Moreover, of particular interest are the genotypes of *C. arabica* showed high tolerability, very similar to the full or strong immunity in some genotypes *C. liberica*. Continuing with the study of these genotypes will explore possibilities for agronomic use in order to obtain materials with higher coffee defenses against pathogen *C. fimbriata*. This information may be used in the future as a tool for implementing a program to improve varieties of high quality production value and rate, with a view to obtaining coffee plants with resistance to Llaga macana of coffee plant.

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Isolation, Identification and Utilization of Phosphate Solubilizing Bacteria Isolated From Coffee Plant Rhizosphere

J. BAKO BAON[1], S. MAWARDI[1], S. WEDHASTRI[2], YUSIANTO[1], A. KURNIAWAN[2]

[1]Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia
[2]Agriculture Faculty, Gadjah Mada University, YogyakartaIndonesia

SUMMARY

Phosphate solubilizing bacteria isolated from rhizosphere of coffee plants may play important role in improving phosphate availability for the plants. However, one of the factor influencing the degree of phosphate solubilization by these bacteria is the ability of the microorganisms to utilize phosphate. The objective of this study was to determine the ability of phosphate solubilizing bacteria isolated from coffee plant rhizosphere and their effects on robusta coffee seedling growth. This research was carried out by taking soil samples from Andungsari (Bondowoso District) and Kaliwining (Jember District) coffee plantations, both located in East Java. Liquid medium of Pikovskaya was used for isolation of phosphate solubilizing bacteria from the soil samples. Results of this study showed that twelve phosphate solubilizing bacteria were obtained from this isolation, eight isolates from Andungsari and four isolates from Kaliwining. Selection of those bacteria isolates was based on the qualitative ability in phosphate solubilizing by measuring the clear zone surrounding the colonies and quantitatively by measuring the solubilized phosphate using spectrophotometer. The results showed that four isolates, in the order of PFpKW1, PFpC61, PFsC62a, and PFsB11, had the highest qualitative ability in solubilizing phosphate, while for the highest quantitative ability the order was PF_pKW1, PF_pC61, PF_sC62a, and PF_sB11. In a green house study, inoculation of these selected isolates onto Robusta coffee seedlings positively enhanced the coffee seedling growth. Phenotypic test indicated that the four isolates are similar to the genus of *Pseudomonas*.

INTRODUCTION

One of the main reasons is that many of Indonesian soils have lower availability of phosphorus which is due to the main process of fixation and immobilisation (Goenadi et al., 2000). The soils are often high in insoluble mineral and organic phosphates but deficient in available orthophosphate (Pi) (Anderson & Magdoff, 2005). Phosphate solubilizing bacteria isolated from rhizosphere of coffee plants may play important role in improving phosphate availability for the plants. However, one of the factors influencing the degree of phosphate solubilization by these bacteria is the ability of the microorganisms to utilize phosphate (Rodrıguez & Fraga, 1999). The objective of this study was to determine the ability of phosphate solubilizing bacteria isolated from coffee plant rhizosphere and their effects on robusta coffee seedling growth.

MATERIALS AND METHODS

This research was carried out by taking soil samples from Andungsari (Bondowoso District) and Kaliwining (Jember District) coffee plantations, both located in East Java. Type of soil in Andungsari plantation is dominated by Andisol; whereas type of soil in Kaliwining plantation

is dominated by Inceptisol. Liquid medium of Pikovskaya (1948) was used for isolation of phosphate solubilizing bacteria from the soil samples.

The presence of phosphate solubilizing bacteria was indicated by the formation of transparent ('halo') zone around its colonies in the media contained insoluble calcium phosphate as the phosphorus source. Those separated colonies were then purified on the same media before being kept in slant media in room temperature.

The best four bacterial isolates were selected from qualitative selection followed by testing for their quantitative ability in solubilizing phosphate by using Pikovskaya broth medium with $Ca_3(PO_4)_2$ as the phosphate source. The solubilized phosphate was measured on day 0, 2, 4, 6, 8, 10 by Yoshida et al. method (8). Morphological and biochemical characterization of the isolates were carried out (Seeley and VanDemark, 1972).

RESULTS AND DISCUSSION

Phosphate Solubilizing Bacteria Isolation

Based on their colony characteristics, this study obtained 12 different isolates of phosphate solubilizing bacteria from rhizosphere of coffee when plated out on agar plates supplemented with glucose as shown in Table 1. The twelve phosphate solubilizing bacteria obtained from this isolation eight isolates derived from Andungsari and four isolates from Kaliwining.

Selection of those bacteria isolates was based on the qualitative ability in phosphate solubilizing by measuring the clear zone surrounding the colonies and quantitatively by measuring the solubilized phosphate using spectrophotometer. The results showed that four isolates, in the order of PFpKW1, PFpC61, PFsC62a, and PFsB11, had the highest qualitative ability in solubilizing phosphate, while for the highest quantitative ability the order was PF_pKW1, PF_pC61, PF_sC62a, and PF_sB11. In a green house study, inoculation of these selected isolates onto Robusta coffee seedlings positively enhanced the coffee seedling growth. Phenotypic test indicated that the four isolates are similar to the genus of *Pseudomonas*.

Table 1. Isolates of phosphate solubilizing bacteria obtained from rhizosphere of coffee plants.

Locations of isolates source	Isolate code	Colony characteristics
Block B1	PFsB1.1	Irregular round, yellow
Block C6	PFsC6.1	Round, glossy, white
	PFsC6.2.a	Round, large, white, yellow precipitation
	PFsC6.2.b	Round, small, yellow
	PFpC6.1	Round, white, glossy, slimy
	PFpC6.2	Round, brown, small, thin
Block E6	PFsE6.1	Thin, small, glossy, slightly transparent
	PFsE6.2	Round, small, transparent, glossy
Block KW	PFsKW1	Round, large, slimy, milky brown
	PFpKW1	Round, small, thick, slimy, creamy brown
	PFpKW2	Round, small, white
	PFpKW1a	Round, small, thin, glossy

Phosphate Solubilizing Ability Selection

The phosphate solubilising index test result (Table 2) showed that there was a difference on the ability of phosphate solubilizing. Phosphate solubilizing index was formed because there was a solubilization of fine particles sediment from $Ca_3(PO_4)_2$ as a phosphate source. Four isolates were selected from this qualitative test, they were PFpKW1, PFpC61, PFsC62a, and PFsB11.

Table 2. Phosphate solubilizing indices of phosphate solubilising bacteria isolates from rhizosphere of coffee plants.

Isolate	Phosphate Solubilizing Index[1]
Burkholderia sp	1,19
PFpC61	1,30
PFsB11	1,04
PFsC62a	1,11
PFpC62	1,03
PFsC62b	1,04
PFsC61	1,00
PFs E62	1,01
PFsE61	1,00
PFpKW1	3,60
PFpKW1a	1,00
PFpKW2	1.26
PFsKW1a	1.25

Note: [1]Phosphate solubilizing index is the ratio of clear zone diameter over colony diameter.

The four selected isolates were then tested for their quantitative ability in solubilizing phosphate with *Burkholderia* sp. as bacteria that able to solubilize phosphate. The test result (Figure 1) showed that the highest phosphate solubilizing was showed on PFpKW1, followed by PFpC61, *Burkholderia* sp., PFsC62a, and PFsB11 culture respectively.

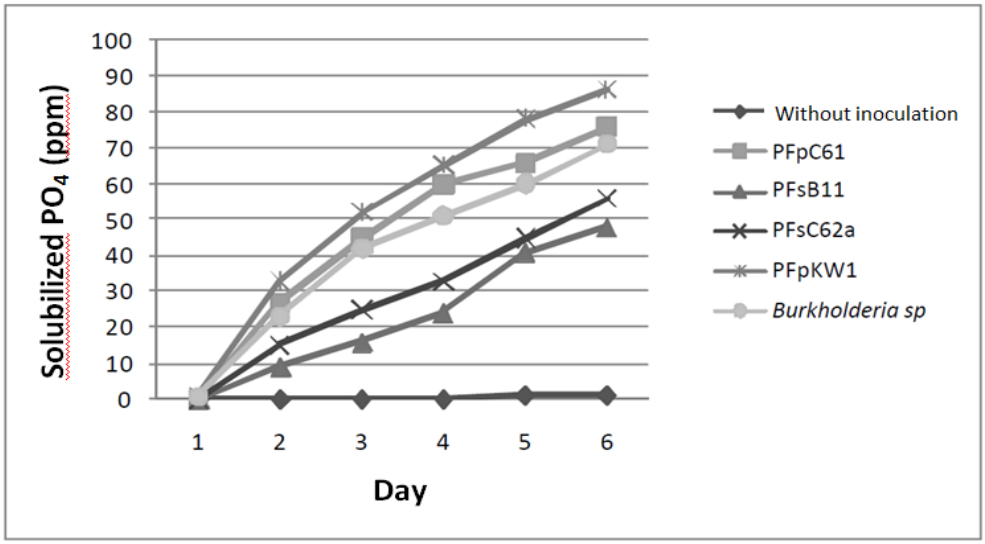

Figure 1. Phosphate solubilised from $CO_3(PO_4)_2$ by four selected isolates of phosphate solubilizing bacteria derived from coffee plant rhizosphere with no inoculation and *Burkholderia* sp. as control.

Bacterial Isolates Characterization

The characterization test result showed that there were similarities between the four tested isolates (Table 3). The PFpC61 had similarity with PFpKW1 isolate. It could be seen on their cell shape, gram, colony shape, elevation, and edges by morphological test. While PFsC62a isolate had similarity with PFsB11 isolate based on their cell shape, gram, colony shape, elevation, inner structure, and edges, and their growth characteristic on stab culture in morphological test. In addition there was a control, *Burkholderia* sp, had similarity seen on cell morphological characterization and biochemical characterization especially in catalase, oxidase, and indole acetic acid test. Based on descriptive chart by using Bergey's Manual of Determinative Bacteriology, seen on cell size and shape, gram, and biochemical characterization, there were expected that the selected isolates had similarity with genus *Pseudomonas* sp.

Table 3. Characteristics of selected isolates of phosphate solubilizing bacteria derived from rhizosphere of coffee plants.

Parameters	Isolates					
	PFpC61	**PFpKW1**	**PFsC62a**	**PFsB11**	**Burkholderia sp.**	**Pseudomonas sp.**
Cell size (mm)	0.6 - 0.8	0.7 - 0.9	0.7 - 1.4	0.5 - 0.8	0.6 - 0.7	0.5 - 1.5
Cell shape	Coccus	coccus	coccus	coccus	coccus	coccus, rods, coccobacillus
Gram stain	Negative	negative	negative	negative	negative	Negative
Colony shape	Circular	circular	irregular	irregular	circular	-
Edge	Entire	entire	undulate	undulate	undulate	-
Inner structure	transparent	smooth	smooth	smooth	smooth	-
Elevation	Convex	convex	convex	convex	convex	-
Agar stab	villous	echinulate	arborescent	arborescent	villous	-
Agar slant	filiform	filiform	Echinulate	arborescent	filiform	-
Catalase test	+	+	+	+	+	+
Starch hydrolysis	+	-	+	+	+	+/-
Indole acetic acid test	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Nitrate reduction	+	-	-	-	+	+/-

CONCLUSIONS

- Twelve phosphate solubilizing bacteria were obtained from this isolation, eight isolates from Andungsari and four isolates from Kaliwining.
- Four isolates, in the order of PFpKW1, PFpC61, PFsC62a, and PFsB11, had the highest qualitative ability in solubilizing phosphate.
- The highest quantitative ability the order was PF_pKW1, PF_pC61, PF_sC62a, and PF_sB11.

ACKNOWLEDGEMENTS

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Up-To-Date Knowledge on the "Potato Taste" of the Arabica Coffee Coming from African Great Lakes Region

D. GUEULE[1], G. FOURNY[1], E. AGERON[2], A. LEFLECHE[2], P. GRIMONT[2], C. CILAS[1]

[1]CIRAD, Montpellier, France
[2]The Pasteur Institute

SUMMARY

The potato taste of the coffee coming from the African Great Lakes region, although of low occurrence, represents a serious risk for the coffee sector of this region. Indeed, the coffee from this region is among the best in the world and it is important to avoid this off-flavor from the produced coffee.

This off-flavor is due to the presence of the molecule: isopropyl-2-methoxyl-3-pyrazine, produced following the introduction of a bacterium of the family of Enterobacteriaceae into the cherries. The taxonomy of this unknown bacterium is presented in several phylogenetic trees. On a RNA 16S phylogenetic tree, this bacterium is close to *Escherichia coli*; on a rpoB phylogenetic tree it is near *Enterobacter agglomerans* and *Erwinia psidii*. The introduction of this bacterium is facilitated by the stings of the insect *Antestiopsis orbitalis*. Indeed, it was shown that the protection of the coffee plantations against this insect decreased the occurrence of the "Potato Taste"; on the other hand it was never shown that this insect was a vector of the involved bacterium. This assumption of vector role will have to be tested. The insect causes other damages and its aggregate distribution makes difficult the estimation of the population levels.

More work has to be done to remove this threat from the coffee of the African Great Lakes region. Hand sorting of coffee beans is effective in reducing the incidence of the potato taste and there are now modern color sorting machines highly sensitive at different coffee export companies, which detect defective beans before further cupping tests. Nevertheless, the identification of the bacterium with the new tools of molecular biology is essential and will allow a better understanding of the ecology of this bacterium in the African Great Lakes region landscape.

INTRODUCTION

Potato taste, which is also known as "peasy" and translates as *pomme de terre* or *patate* in French (Becker et al., 1988) or *erbsig* in German, is an off-flavour that detracts from the quality of the Arabica coffee produced in the Great Lakes region of Africa. The potato taste of the coffee coming from the African Great Lakes region, although of low occurrence, represents a serious risk for the coffee sector of this region. It is important to avoid this off-flavor from the produced coffee. Currently, what is it known about this problem?

KNOWLEDGE

This off-flavor is due to the presence of the molecule: isopropyl-2-methoxyl-3-pyrazine, produced following the introduction of a bacterium of the family of Enterobacteriaceae into

the cherries. The taxonomy of this unknown bacterium is presented in several phylogenetic trees (Figures 1 & 2).

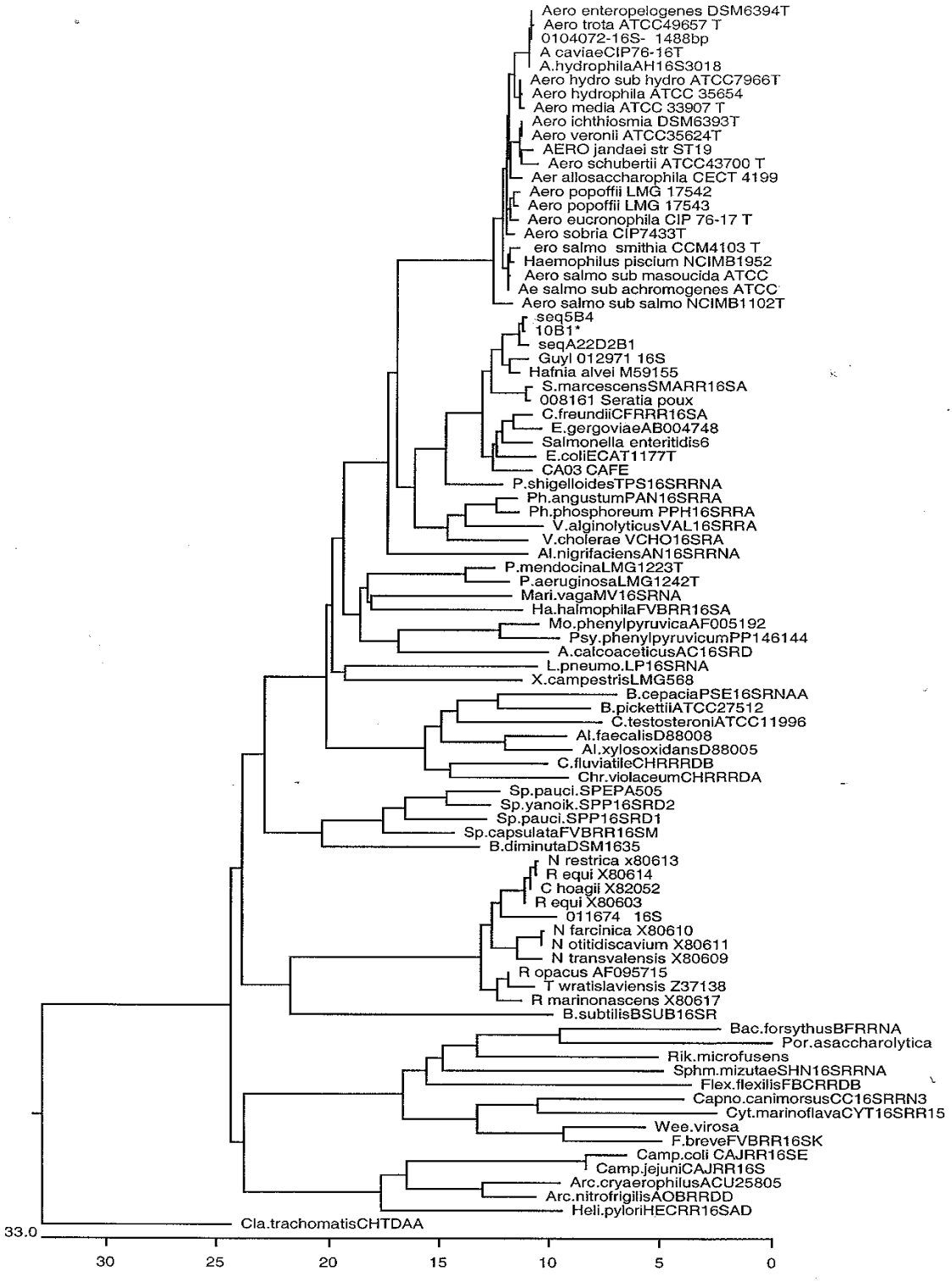

Figure 1. Classification of the PT bacterium in the RNA 16S phylogenetic tree.

On the RNA 16S phylogenetic tree, this bacterium is close to *Escherichia coli.*

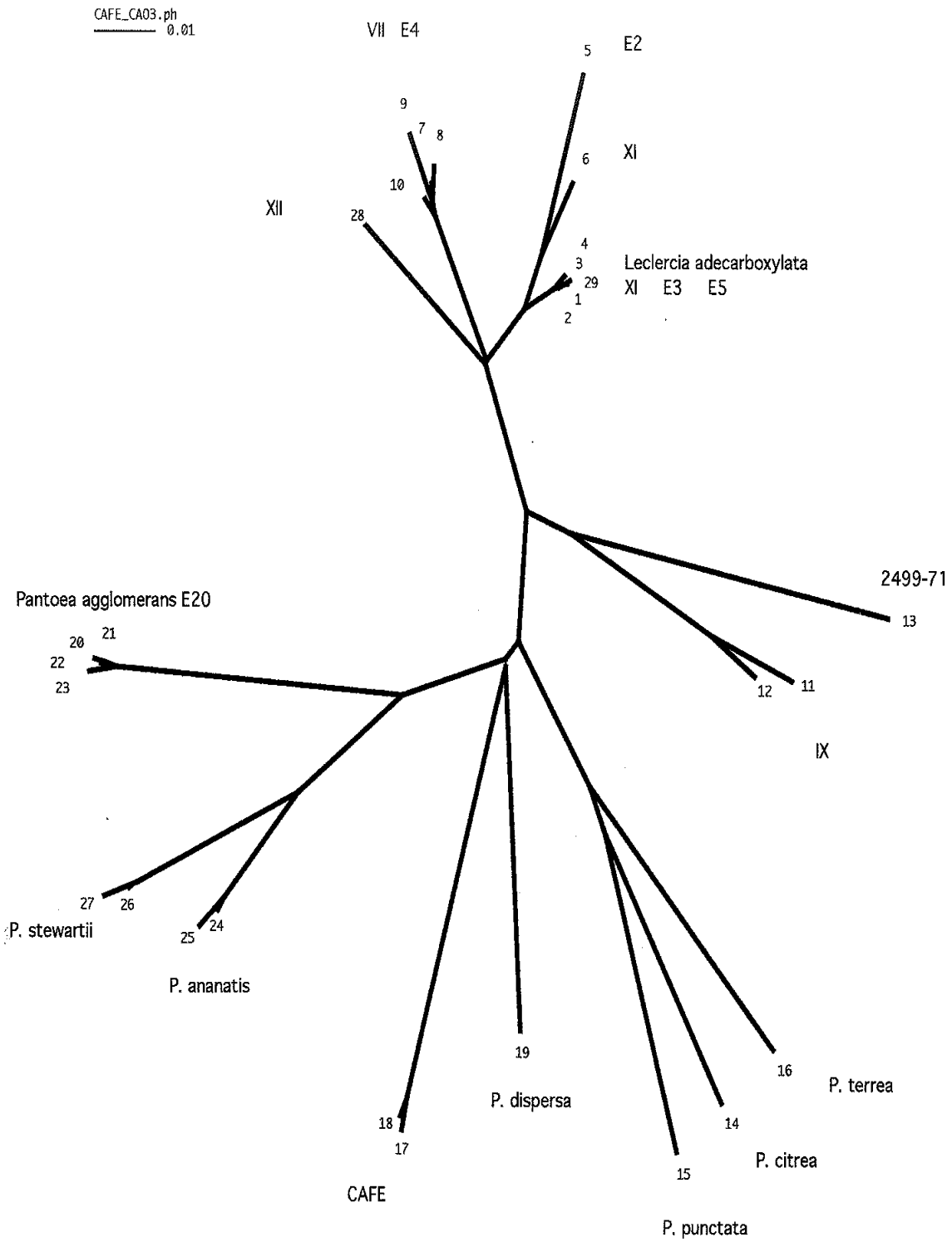

Figure 2. Classification of the PT bacterium in the *Pantoea* family.

The genus Pantoea is divided into seven different species named *Pantoea agglomerans, Pantoea ananas, Pantoea citrea, Pantoea dispersa, Pantoea punctata, Pantoea stewartii* and *P. terrea*. Some of these species have only recently been separated from other genera, e.g. the genus Erwinia.

The most prominent species of the genus Pantoea is *P. agglomerans*, formerly named *Enterobacter agglomerans*. The species involved in the potato test seems a new one.

The introduction of this bacterium is facilitated by the stings of the insect *Antestiopsis orbitalis*, a bug of the *Pentatomidae* family; it was shown that the protection of the coffee plantations against this insect decreased the occurrence of the "Potato Taste" (Bouyjou et al., 1999). The insect causes other damages and its aggregate distribution makes difficult the estimation of the population levels (Cilas et al. 1998).

PERSPECTIVES

More work has to be done to remove this threat from the coffee of the African Great Lakes region. Hand sorting of coffee beans is effective in reducing the incidence of the potato taste. There are now modern color sorting machines highly sensitive at different coffee export companies, which detect defective beans before further cupping tests.

In the context of a varied geographic and ecological landscape, mapping antestia bug attacks and potato taste occurrence would enable creation of a country-level risk map. To construct this map, a sampling of plots across all Burundian and Rwandan coffee-producing regions must be conducted.

Nevertheless, the identification of the bacterium with the new tools of molecular biology is essential and will allow a better understanding of the ecology of this bacterium in the African Great Lakes region landscape. It is also important to determine if the insect (A.orbitalis) is a vector of the involved bacterium, or only a "facilitator".

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The Income Content in the World Coffee Exports

O. M. DA SILVA, C. A. M. LEITE

Department of Economics and Rural Economics; Universidade Federal de Viçosa. CEP 36570-000. Viçosa, MG. Brasil

SUMMARY

Coffee is the most widely commercialized tropical product on the international market. The 2009/10 crop had an estimated value of $15.4 billion, with 93.4 million bags exported. But how would coffee products rank, in terms of income content, in relation to other commercialized products, and how have they evolved? To answer this question, the annual income content of 5,111 products exported by 167 countries from the period between 2000 and 2009, was calculated. The results showed that non-roasted, non-decaffeinated, whole grain coffee is still the most commercialized product, but with the lowest income content of all coffee products, occupying the twenty-fourth worst position in terms of income content in 2009. Products with the least amount of processing presented a loss in relative market share, with the addition of values to the production chain occurring outside those countries producing the raw materials.

INTRODUCTION

According to the International Coffee Organization – ICO (2011), coffee is the most widely commercialized tropical product on the international market. The 2009/10 crop had an estimated value of $15.4 billion, with 93.4 million bags exported. It is also estimated that in 2010 the number of people employed in the coffee sector was around 26 million, in 56 producing countries.

Coffee product exports make an important contribution to foreign exchange revenues for various countries. They also contribute to tax collection and Gross Domestic Product growth. It is known that primary products have had their share in the international market reduced and that technologically intensive products have grown more than others among manufactured products. It is also believed that the technologically intensive products bring greater benefits to the exporting countries, by requiring greater allocation of human and physical capital, which involves faster transfer and diffusion of technology. Therefore, there is a growing interest in analyzing the technological structure of exports.

According to Lall *et al*. (2006), the greater the average income of the exporting country, the more sophisticated the product. The argument behind this ratio is based on the fact that products exported by rich countries have characteristics that allow producers to pay high salaries and still be competitive in world markets.

An analysis of the sophistication indicators can bring interesting information about the functioning of the coffee market, in which there are different exported products and for which the number of exporting countries is much greater than the number of producing countries. Thus, the present study has as its main objective to calculate and analyze the income (sophistication) content of the different exported products of coffee on the international market between the years of 2000 and 2009. For this, an income content, or “sophistication”,

that depends only on product export information and the exporting countries' per capita incomes, is used.

MATERIALS AND METHODS

A country's export basket encompasses a great number of different products. In spite of there being no information for the Research and Development content on each of these products, the literature presents some methods that allow for a measurement of sophistication of each product (Hausmann et. al.). One of these methods calculates the Income Content Index of each product in the international market. For each i product, the sophistication index commonly called PRODY, can be calculated as:

$$PRODY_i = \sum_{c \epsilon C} \sigma_{ic} * Y_c$$

in which $\sigma_{ic} = VCR_{ic} / \sum_{d \epsilon C} VCR_{id}$, with $VCR = {}^{X_{ic}}/_{X_C} \Big/ {}^{X_i}/_{X}$, c = (1,2,3,...,M) and Y_c = per capita gross domestic product (GDP) of each country. M is the number of countries, and the weights (σ_{ci}) normalize the Balassa's index of revealed comparative advantage (RCA) of country C, in relation to all other countries that export product i.

In equation (1), $PRODY_i$ indicates the sophistication level of product i and it is measured as a weighted average of the per capita GDP of all countries that export product i. In the weighting variable (σ_{ci}), (${}^{X_{ic}}/_{X_C}$) corresponds to the share of product i in the total value of exports of country C, reflecting the importance of this product in the total exports of that country. The $VCR_{ic} / \sum_{d \epsilon C} VCR_{id}$ ratio makes the sum of weights equal to unit in such a way that the weights reflect the importance of product i in the exports of country C, in relation to all countries that export that product.

Products with high PRODY values are those for which the countries with high incomes have a preponderant role in relation to the other commercial partners. The implicit assumption is that high salaries are present where the comparative advantages are determined by factors such as technology, public infrastructure, research centers, etc., instead of the labor costs.

The data used in this work are annual and refer to the period from year 2000 through 2009. Trade data are from the United Nations Commodity Trade Statistics (COMTRADE) and income data (GDP) from the World Bank (World Development Indicators). For calculation of PRODY indices, export data encompassed 5,111 different products from 167 countries and corresponded to the Harmonized System's 6 digit level classification of goods (HS6). Under this classification, the coffee products considered were: 090111 – Non-roasted, non-decaffeinated, whole grain coffee; 090112 –Non-roasted, decaffeinated, whole grain coffee; 090121 – Roasted, decaffeinated coffee; 090122 –Roasted, non-decaffeinated coffee; 210111 – Soluble coffee, even if decaffeinated. It was not considered the classification products: 090190 – other coffee products; and 293930 – caffeine. The database utilized does not present any distinction between Robusta and Arabica coffee types.

RESULTS AND DISCUSSION

Most coffee exporting countries operate in the non-decaffeinated (whole grain and roasted) coffee market, but the greatest growth in the number of exporting countries occurred precisely

within the decaffeinated, roasted coffee market, which grew from 82 countries in the year 2000 to 102 in 2009. It is interesting to note that among the 102 decaffeinated, roasted coffee exporting countries in the year 2009, only 56 were producing countries (ICO, 2011). The number of non-roasted, non-decaffeinated, whole grain coffee exporting countries was 132 in the year 2000, reached 144 in 1996, and fell to133 in 2009. The non-roasted, decaffeinated coffee product has the smallest number of exporting countries, varying from 77 in the year 2000 to 84 in 2009. In this same year, the number of exporting countries of the roasted, non-decaffeinated and of the soluble coffees was 124 and 106, respectively.

PRODY values (income contents) calculated for the five coffee products considered are presented in Table 1. Non-roasted, non-decaffeinated, whole grain coffee had the smallest income content. This was expected, considering the low degree of product processing ("sophistication") in relation to the others products. An income content corresponding to $562.15 in the year 2000 increased to $787.76 in 2009, with a nominal growth of 40% throughout the period. The non-roasted, non-decaffeinated and the soluble coffees presented very small decreases in the PRODY values, whereas the others were not affected by the crisis. The decrease in the number of exporting countries could explain this fact. The product with the greatest income content was the decaffeinated, roasted coffee. It presented a nominal growth of 192% throughout the period, and enlarged the difference in relation to the other products. The income content growth of soluble coffee achieved 114% in the period.

Table 1. Income contents calculated for the coffee products. US dollars. 2000-2009.

Year	090111	090112	090121	090122	210111
2000	562.15	1776.77	11015.89	6122.44	3824.87
2001	492.20	2181.13	7094.19	7149.77	3296.61
2002	546.09	974.60	8556.23	11968.61	4079.48
2003	472.77	1617.26	11808.80	13858.73	5726.10
2004	578.87	1664.04	10379.35	13560.43	6771.56
2005	575.92	1930.53	10385.02	16332.53	7808.67
2006	679.09	1740.52	8744.43	14455.73	7148.61
2007	741.75	1948.10	10374.11	14536.44	7228.79
2008	870.66	2264.83	11385.64	15573.28	8243.45
2009	787.76	2660.50	11103.98	17903.42	8202.14

Source: Authors' calculation.

Table 2 presents the PRODY values of the coffee products calculated for the years 2000 and 2009, and those of the five products with the lowest and highest income contents.

In a universe of 5,111 different products, the group of five coffee products represented just 0.2% ($23.5 billion) of the total value of the international traded goods in 2009. According to the ICO, coffee continues to be the most widely traded product at the international level, after oil. In the group of coffee products, the non-roasted, non-decaffeinated, whole grain coffee still has the greatest share in the world market (0.14%).

Traditionally, coffee was exported as a green grain (The Global Coffee Trade, 2004), with the processing occurring in the importing countries. For lack of infrastructure and even processing technology, the producing exporting countries had a minimum share in the process of adding value to the production. Also, according to the order of classification (from the lowest to the highest) shown in Table 2, non-roasted, non-decaffeinated, whole grain coffee, which occupied the 29th position in the year 2000, fell to the 24th in 2009. The roasted, non-

decaffeinated coffee, which occupied the best position of the group with an income content of $11,015.80 in 2000, also lost its position in 2009, falling from place nº 2,751 to 1,816.

Table 2. Income contents, classification and shares in the world exports of selected products. 2000 and 2009.

Code	PRODY	Order Classif.	% of Exports	Code	PRODY	Order Classif.	% das Exports
2000				*2009*			
Products with smaller values[1]							
130120	234.8	1	000017	**901041**	333.4	1	000022
261590	317.0	2	000044	**071390**	360.4	2	000013
120792	327.6	3	000001	**410611**	387.6	3	000000
090700	350.2	4	000022	**530490**	391.1	4	000012
080131	369.6	5	000049	**261590**	452.9	5	000051
Coffee Products							
090111	562.1	29	001417	**090111**	787.7	24	001264
090112	1776.7	229	000062	**090112**	2260.5	252	000047
090121	11015.8	2751	000194	**090121**	11103.9	1816	000400
090122	6112.4	1434	000020	**090122**	17903.4	3309	000029
210111	3824.8	813	000248	**210111**	8202.1	1247	000301
Products with greater values							
560312	37107.7	5107	000159	**721069**	57204.2	5107	000167
721061	37668.4	5108	000102	**590210**	58547.6	5108	000104
741011	37856.1	5109	000143	**721633**	65787.6	5109	000149
721633	38788.1	5110	000272	**730110**	75498.3	5110	000257
730110	42458.9	5111	000076	**590290**	78029.6	5111	000098

Source: Authors calculation.

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[1] 130120 – Gum arabic; 261590 – Zirconium ores and their concentrates; 120792 – Other seeds and oleaginous fruits, even triturated; 090700 – Clove (fruits, flowers and peduncles; 080131 – Cashew nut, with peel; 560312 – False fabrics, even impregnated, coated, covered or stratified; 721061 – Flat laminated products, of iron or steel with no alloy, coated with aluminum-zinc alloys; 741011 – Sheets and stripes, thin, of refined copper **;** 721633 – Iron or steel profiles with no alloy , in H; 730110 – Iron or steel poles/boards, even perforated or made from assembled elements; profiles obtained through hard welding.

Determination of Soil Water Conditions Triggering Mass Wasting in the Colombian Coffee Region

L. F. SALAZAR[1], F. HOYOS[2], O. RAMIREZ[2], E. HINCAPIÉ[1]

[1]Centro Nacional de Investigaciones del Café (Cenicafé), Plan Alto Km 4 - vía antigua a Manizales, Chinchiná, Caldas, Colombia
[2]Universidad Nacional de Colombia, Medellín - Colombia

SUMMARY

For the soils of the Colombian coffee region there is poor knowledge about the contributing factors of the mass wasting in relation to the soil water content. In order to contribute to this knowledge, three tons of altered soil derived from granite located in the municipality of Ibagué – Colombia were selected until the 0.16 m of depth. Seven physical slopes model according to field bulk density and soil horizons disposition were building. The dimensions of the slope model were of 1.0 m of height, with base of 1.5 m² and slope of 32°. In each model, eight suction tensimeters (0 to –85 kPa) were located. Rainfall and infiltration simulated were applied. The results reveal a link of mass movements to hydrological processes occurring in the slope, related to soil permeability, to rainfall intensity and duration and water table changes. The major portion of soil slope instability cases was related to saturated condition of the slope bottom.

INTRODUCTION

Soil and water conservation are priorities for the Colombian coffee growers and essential for sustainability. Applied research on soil and water conservation has been conducted for decades in CENICAFÉ, a research institution backed by the Colombian Coffee Growers Federation, oriented to prevent and mitigate the soil erosion and mass movement through cultural practices and ecological restoration. Both soil erosion and mass movement affect the coffee growing sustainability in the Andean slopes. Landslide frequency has a close relationship with high precipitation associated with the Southern Oscillation periods known as La Niña, and it will be with increased rainfall associated with the climate change.

Mass movements are caused by several factors, including geological, geomorphologic and anthropogenic ones; the rainfall and soil water dynamic have strong potenctial to cause slope failure. In this sense, several researchers have used experimental physical models combining hydrological and geotechnical aspects and slope stability.

In tropical residual soils landslides are related to the reduction of matric suction induced by rainfall (Fredlund et al., 1978, Miyazaki, 1993). The soil water conditions as a mass wasting factor in the soils of the Colombian Coffee Region are yet poorly understood. The objective of this research was to determine the soil water conditions with potential to trigger mass wasting in the Colombian coffee region by mean of experimental physical models.

MATERIALS AND METHODS

Samples of altered soil derived from granite located in Ibagué, Colombia (04° 28’ 36’’ N, 75° 09’59’’W, 1220 m altitude) were taken from a coffee farm.

Seven physical slopes model according to field bulk density and soil horizons were prepared in a rectangular glass case. The dimensions of the slope model were: 1.0 m height, 1.5 m² base, and 32° slope. In each model, eight suction tensiometers (0 to –85 kPa) were located (Figure 1). The saturation level was determined by means of the water retention curves.

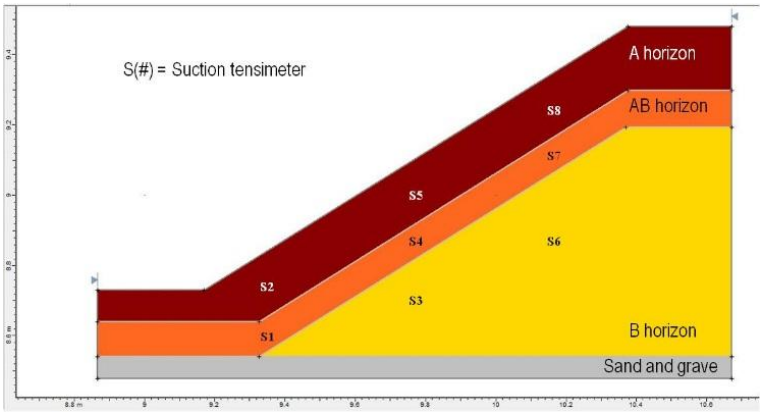

Figure 1. Experimental model and location of suction tensiometers.

Simulated rainfall was applied as follows:

- 680 mm during 34 h
- 685 mm during 180 h
- 150 mm during 14 h.

Seepage at the top of the slope was simulated as follows:

Rising water level

- 0.70 to 0.74 m in 8,2 h
- 0.70 to 0.75 m in 29.5 h followed by rising from 0.75 to 0.87 m in 0.16 h
- 0.70 to 0.92 m in 1.5 h
- A combination of infiltration and simulated rainfall were evaluated.

RESULTS AND DISCUSSION

The subsoil showed an effective cohesion of 10 kPa and an effective friction angle of 26°. The soil was characterized as low plasticity silt, with low to very low permeability. The saturation value was approximately 0.3, the soil properties may have negative implications for its stability and susceptibility to erosion because the pore spaces fill up with rainfall water and both the pore pressure and runoff increase (Table 1).

Table 1. Soil properties.

Soil depth	Sand	Clay	w_L	w_p	G_s	ρ_b	K	OM
(m)	(%)				(kg.m^{-3})		(cm.s^{-1})	(%)
0 –0.35	51.8	20.6	44.7	30.1	2.63	1.28	1.0 x10^{-4}	5.0
0.35 – 0.45	50.4	22.8	45.8	33.6	2.64	1.46	2.4 x 10 $^{-5}$	1.8
0.45 m - >1.60	58.0	19.9	45.9	27.6	2.66	1.54	3.4*10^{-7}	0.3

w_L: Liquid limit, w_p: Plasticity limit, G_s: Particle density; ρ_b: Bulk density, k: Permeability index, OM: Organic matter.

When 680 mm rainfall in 34 h was simulated, the slope bottom was the first section getting saturated, and both shallow landslides and severe laminar erosion were caused (Figure 2).

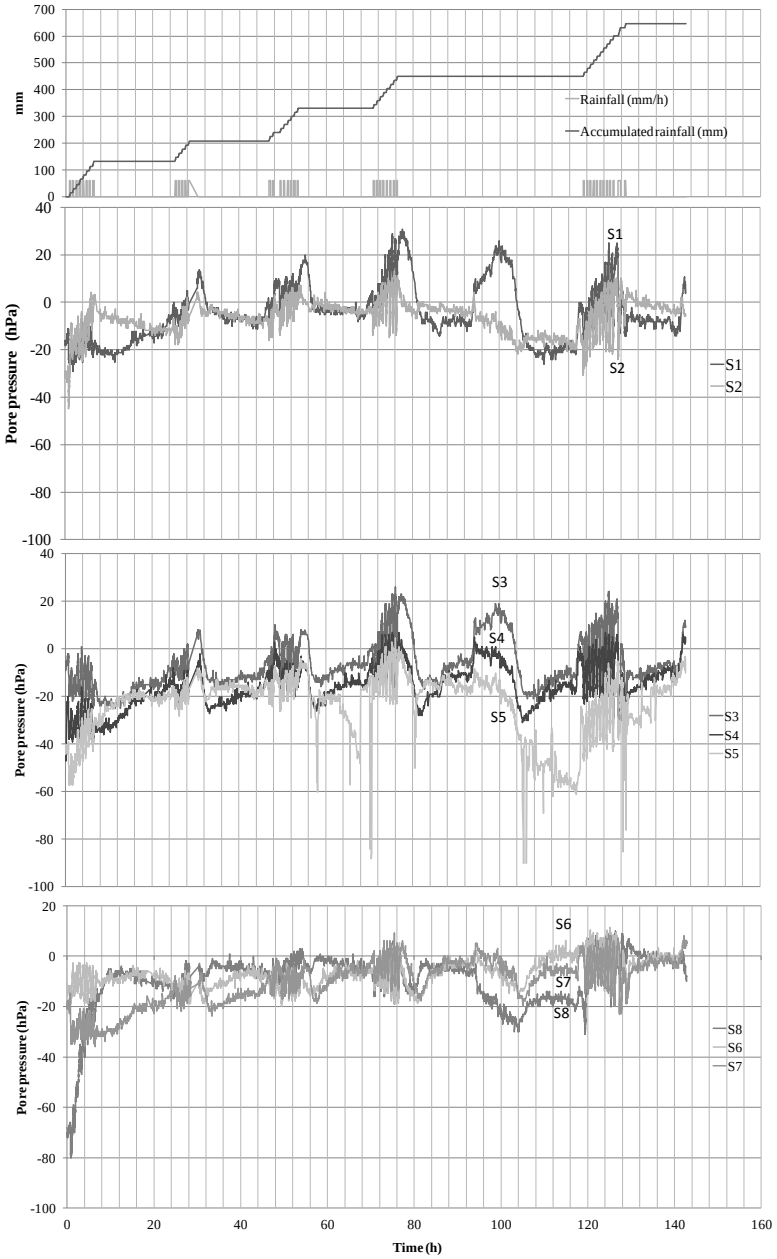

Figure 2. Rainfall effect on soil pore pressure on a model slope.

The 680 mm rainfall in 180 h caused the saturation of the slope bottom and the fastest subsoil saturation. As rainfall started suction was increased, followed by a drop below the initial value when it stopped.When rainfall stopped, the suction continued diminishing both at bottom and at the subsoil slope, whereas the tater one was increased again at the slope top section. When rainfall started under high soil suction conditions (-300 hPa) and in presence of soil cracks caused by drying and humidification, the rainfall caused both saturation of the bottom and of the superficial soil horizon. While simulating the sub-surface flow at the top of the slope, the soil failure was caused by suction loss of the slope bottom. The failure was controlled by the soil horizons permeability and by the changes in the soil water table.

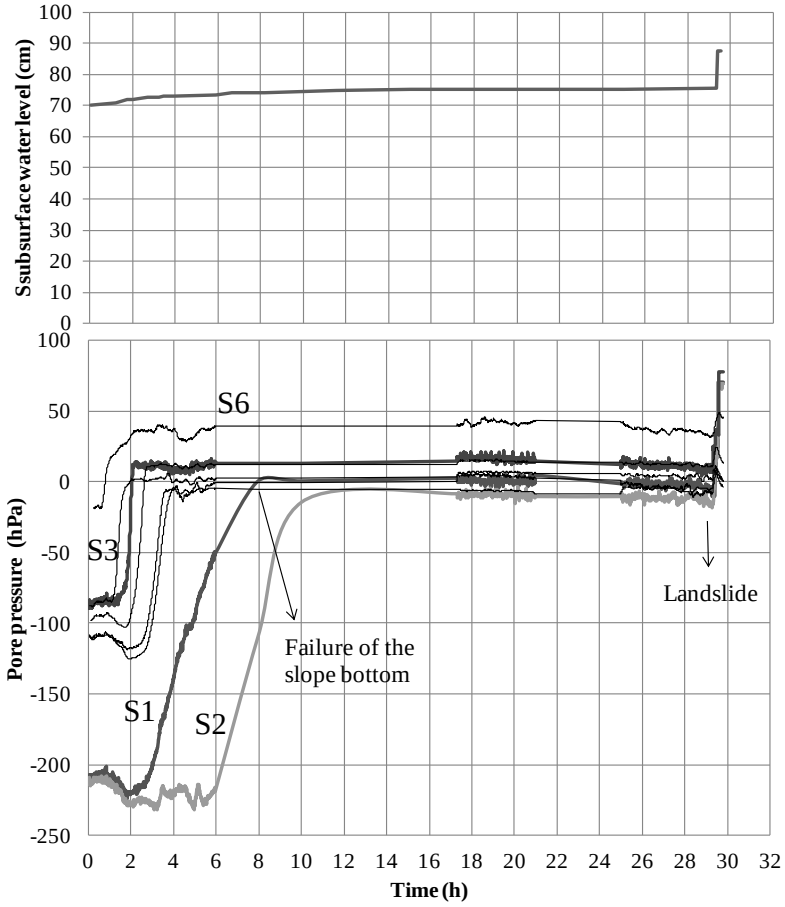

Figure 3. Effect of the subsurface water level rising on the soil pore pressure on a model slope.

Soil and water management practices on coffee crops should prevent soil saturation by means of drainage systems and planting of protection trees at the slope bottom. During long drought periods on coffee corps it is necessary to cover the soil with mulch in order to reduce formation of expansion cracks which could have slope stability implications.

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Herbaceous Legumes Intercropping in Weed Management in Newly Pruned Coffee Plantation

J. C. F. SANTOS[1], A. J. DA CUNHA[2], F. A. FERREIRA[3], R. H. S. SANTOS[3], N. S. SAKIYAMA[3], P. C. DE LIMA[4]

[1]EMBRAPA Café, Sede EMBRAPA Final, Av. W/3 Norte, 70.770-901, Brasília, DF
[2]UNICERP Univ.Cerrado Patrocínio, Av. Líria Lassi S/N, 38.740-000, Patrocínio, MG
[3]UFV Universidade Federal de Viçosa, Depart. de Fitotecnia, 36.570-000,Viçosa, MG
[4]EPAMIG Empresa de Pesq. Agro. de MG,UFV-Vila Gianetti, 36.571-000,Viçosa, MG

SUMMARY

Weed infestation in pruned coffee plantation is enhanced in the open and light-exposed interrows. The objective of this work was to evaluate soil covered with herbaceous legumes for reducing weeds in newly pruned coffee crop. The experiment was set up inViçosa, MG, after the second pruning of a Catuaí Amarelo coffee plantation spaced by 3 x 1 m. It was used a random block experimental design with four replicates, made up by eight treatments in 3 x 2 + 2 factorial scheme: three legumes (forage peanut (*Arachis pintoi*), siratro (*Macroptilium atropurpureum*) and lablabe (*Dolichos lablab*)) and two planting forms in the interrows of the coffee crop and three rows of legumes spaced by 1.0 and 0.50 m, respectively. The two additional treatments consisted of hand weeding with a hoe and the chemical control with glyphosate. It was found that lablabe at 90 and 120 DAP provided the greatest soil cover, the greatest predominance of vegetation on the weeds and the smallest weed infestation. Both lablabe and forage peanut presented the highest biomass yield in the two years. Density and biomass of the weeds were reduced by lablabe and siratro in the dry period and with no difference between them in the rainy period in the first year and by forage peanut, with higher effect in the rainy period in the second year, respectively. Cultivation of two or three rows of legumes did not differ among each other in soil cover, in the weeds and in the coffee crop. There were no differences in soil moisture, plant height and in the first coffee yield among legumes plants and among the additional treatments. Herbaceous legume intercropping reduces weeds of the newly pruned coffee plantation.

INTRODUCTION

The cultivation of plants of soil cover for weed control is constituted in an alternative component of the integrated management system, contributing to the reduction of the use of herbicides and of hand weeding. In the choice of the legume as plant of soil cover and of weed control, should be considered his establishment capacity, physiologic demands, management system, biomass production and allelopathic influence. This work aimed at to evaluate the effect of the soil cover with herbaceous legume in the weed control and in a crop with newly pruned coffee plants.

MATERIALS AND METHODS

The experiment consisted of eight treatments, implanted only time in the factorial outline of 3 x 2 + 2. The first factor was composed by the herbaceous legumes forage peanut (*Arachis pintoi*), siratro (*Macroptilium atropurpureum*) and lablabe (*Lablab purpureus*). The second factor was formed by two and three rows of legumes spaced by 1,00 m and 0,50 m

respectively. The additional treatments were the hand weeding (hoe) and the chemical control (glifosato). It was used a random block experimental design with four replicates in 32 plots. The treatments were applied in the two interrows of the plot of three lines of seven coffee plants with spacing of 3 x 1 m, being useful the five central plants. The soil cover, the predominance of the legumes and the infestation of the weeds were evaluate to the 90 and 120 DAP (Dias After Planting) for the method of intersections of strings in a picture, forming a net of same squares, told for [4], adapted for a plastic net of (2 x 6) m, formed by a drained group of 200 squares, disposed in the center of each interrow of the plot. The soil cover is the sum of the numbers of squares on the vegetation of the legume. The predominance of the legumes on the weeds is the sum of the numbers of squares of soil cover for the legume without the presence of the weeds. The infestation of the weeds is the sum of the numbers of squares on all the weeds. The biomass of the legumes was evaluated in may and december in the two years by the sampling of the study of the plants population, extracted of the sample of 0,5 m² of the plot, resulting from the picture of wood of 0,25 m² thrown in the two interrows. The density and biomass of the weeds were evaluated every two months, for two years, in the dry and rainy period for the same method of the study of the plants population, extracted of the sample of 0,5 m² of the plot, resulting from the picture of wood of 0,25 m² thrown in the two interrows.

RESULTS AND DISCUSSION

The forage peanut and the lablabe to the 90 and 120 DAP provided the greatest soil cover, however the lablabe to 120 DAP provided the greatest predominance of the vegetation without the presence of the weed, implicating the smallest weed infestation (Table 1). Although, the weed control for the legumes, can be conditioned to her biomass production and allelopathic potential. The forage peanut it was shown with similar acting in the work of PERIN et al., providing the greatest capacity of soil cover in the same establishment period whose planting conditions were identical as for the interrows spacing and the plants density.

Table 1. Soil cover and predominance of the legumes intercropping in weed management in newly pruned coffee plantation, March and April of 2008.

Treatments	Cover(%)		Predominance(%)		Infestation(%)	
	90 DAP	*120 DAP*	*90 DAP*	*120 DAP*	*90 DAP*	*120 DAP*
Legume						
Forage peanut	52,9 a	63,4 ab	28,9 a	33,0 b	40,0 a	42,1 a
Siratro	34,8 b	50,3 b	28,1 a	36,5 b	18,1 b	23,0 b
Lablabe	50,3 ab	74,3 a	45,3 a	70,6 a	13,2 b	9,0 c
DMS	17,9	21,4	17,4	18,5	8,1	13,1
Row						
Two	43,0 a	61,2 a	32,0 a	45,3 a	24,0 a	26,3 a
Three	48,9 a	64,1 a	36,1 a	48,2 a	23,6 a	23,1 a
DMS	12,1	14,4	11,7	12,5	5,4	8,8
C.V.(%)	36,74	32,78	46,27	36,89	28,86	44,40

Means followed by different letters inside of each factor in the column differ amongst themselves for the Tukey test 5%.

The biomass produced by the forage peanut, following for the lablabe was superior, shown in the two years, after the discount of the area 60% busy to be considered by the coffee plants in the calculation of the productivity of that biomass (Table 2). Among the perennial legumes, it was observed that the forage peanut, in those two periods, it maintained the production of

higher biomass than the one of the siratro, being divergent of the results obtained for ESPINDOLA et al. in the consortium of those legumes in the banana culture, in which there was not significant difference.

Table 2 – Biomass of the herbaceous legumes intercropping in weed management in newly pruned coffee plantation, March and April of 2008.

Treatments	Year 2008 Biomass (kg/ha)	Year 2009 Biomass (kg/ha)
Legume		
Forage Peanut	1851,90 a	1735,78 a
Siratro	1314,71 b	1152,52 b
Lablabe	1607,36 ab	1537,73 ab
DMS	483,22	406,36
Row		
Two	1670,57 a	1433,21 a
Three	1512,07 a	1517,48 a
DMS	323,58	272,12
C. V. (%)	23,37	21,20

Means followed by different letters inside of each factor in the column differ amongst themselves for the Tukey test 5%.

The forage peanut it allowed in the first year higher infestation density and biomass production of the weeds, happening higher influence of suppression of those species in the second year, mainly in the rainy period (Table 3). As that legume presented higher biomass production in the second year, the results combine with obtained them for [2] in the management of the consortium of this species with the coffee plantation, reducing the weed biomass.

Table 3. Density and biomass of the weeds in the dry and rainy period in two year intercropping of the newly pruned coffee plantation with legumes.

Treatments	First Year 2008/2009		Second Year 2009/2010	
	Dry Period	***Rainy Period***	***Dry Period***	***Rainy Period***
Contrasts[1]	**Density (plants/m^2)**			
Additional	2,79^{ns}	8,73	2,55^{ns}	4,81^{ns}
Legumes	3,33	6,72[1]	3,31	4,48
Hand Weeding	3,64^{ns}	8,33^{ns}	3,10^{ns}	4,77^{ns}
Chemical	1,95	9,13	2,00	4,86
Control				
Legume[2]				
Forage peanut	4,39 a	7,42 a	3,12 a	2,97 b
Siratro	3,08 b	7,34 a	3,43 a	4,68 ab
Lablabe	2,52 b	5,41 a	3,39 a	5,78 a
DMS	1,21	2,96	1,99	2,07
Row[2]				
Two	3,27 a	6,30 a	3,52 a	4,54 a
Three	3,57 a	7,24 a	3,10 a	4,71 a
DMS	1,03	1,99	1,34	1,39
C. V. (%)	43,64	34,48	56,96	37,98
Treatments	**First Year 2008/2009**		**Second Year 2009/2010**	
	Dry Period	***Rainy Period***	***Dry Period***	***Rainy Period***
Contrasts[1]	**Biomass (g/m^2)**			
Additional	3,49^{ns}	8,14	2,41^{ns}	4,49^{ns}
Legumes	4,89	5,77[1]	3,40	5,44
Hand Weeding	4,91^{ns}	8,12^{ns}	2,97	4,93^{ns}
Chemical	2,07	8,16	1,85[1]	4,06
Control				
Legume[2]				
Forage peanut	7,97a	6,97a	3,56a	3,61b
Siratro	4,08b	5,88a	3,61a	5,78a
Lablabe	2,62b	4,46a	3,03a	6,92a
DMS	2,66	2,55	1,37	2,09
Row[2]				
Two	5,30a	5,57a	3,68a	5,57a
Three	5,47a	6,14a	3,10a	5,64a
DMS	1,79	1,71	0,92	1,41
C. V. (%)	53,44	33,05	38,89	33,54

[1]Análise of contrast = significant; and ns = no significant, for the test F to 5% of probability.
[2]Means followed by same letter in the column they don't differ amongst themselves in the Tukey test 5%.

In works of the forage peanut legume management he has if registered effects in the reduction in the number and in the biomass of the weed of the coffee plantation.

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Duration of the Biological Cycle of the Coffee Berry Borer *(Hypothenemus hampei)* in Field Conditions

M. ROJAS, A. GAMBOA, M. BARQUERO-MIRANDA, M. MORA, O. BORBÓN

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica

SUMMARY

The duration of the biological cycle of the coffee berry borer and its reproduction depends on the temperature, which is influenced by altitude. The objective of this study was to determine the biological cycle of the coffee berry borer under field conditions in nine coffee areas of Costa Rica.

Research activities were conducted between 2002 and 2007 in Heredia (two locations), Perez Zeledón (two locations), Turrialba, Poás, Desamparados, Tres Ríos and León Cortés, in ranges from 700 to 1740 m.a.s.l. The experimental area was a plot of 0.5 ha where coffee plants were marked after the main bloom. Every 15 days, 100 infected fruits were randomly collected and 30 of them were dissected at random in the laboratory, indicating first the position of the insect in the fruit, and then calculating the number of adults, eggs, larvae and pupae in each fruit until the biological cycle was completed.

The completion time of the life cycle from egg to adult differed depending on the area and the prevailing weather conditions. In general, it was found that the cycle duration is shorter in the lower areas, while at higher altitudes the cycle needs more time to be completed. According to the results, the cycle is completed in a period of 40-45 days in areas between 700 and 1200 m.a.s.l., and approximately 90 days above 1300 m.a.s.l.

The low temperatures prevailing in the coffee plantations restrict the insect growth and reduce its potential as a pest. In the low areas, with higher temperatures, reproduction is fast and damage is more severe. During the development of infected fruits, the number of stages of the coffee berry borer gradually increased inside, which caused the presence of new adults in the lower areas before the harvest, and that results in re-infestation of the crop and more damage. In areas above 1300 m.a.s.l. it is possible to start the harvest before the pest's biological cycle is completed inside the fruits.

INTRODUCTION

Many researchers have studied the biological cycle of the CBB and agree that their duration depends on temperature. The low temperatures prevailing in coffee height plantations limit insect development, reducing its potential as a pest. In the lowlands, with higher temperatures, insect reproduction is rapid and the damage is more severe.

In Costa Rica the coffee berry borer was detected in 2000. As part of the research strategy of the pest in the national coffee environment, from the year 2002 were developed several jobs in order to determine the duration of the life cycle of the insect at different altitudes and its possible impact on coffee production.

MATERIALS AND METHODS

Investigations were conducted from 2002 to 2007 in Heredia, Perez Zeledón, Turrialba, Poás, Alajuela, Tres Ríos and León Cortés, in ranges from 700-1740 masl (Table 1). The experimental plot consisted of a lot of 0.5 ha of Caturra or Catuaí where coffee plants were labeled after the main flowering. Every 15 days, 100 infected fruits were randomly collected and 30 of them were dissected at random in the laboratory, indicating first the position of the insect in the fruit, and then calculating the number of adults, eggs, larvae and pupae in each fruit until the biological cycle was completed.

Table 1. Environmental characteristics of sites and biological cycle duration of *Hypothenemus hampei* in different coffee regions of Costa Rica.

Site	Year	Altitude (masl)	Temperature (°C)	Total rainfall per year (mm)	Biological cycle (days)
Ulloa, Heredia	2002	1150	20	2500	45
Ulloa, Heredia	2002	1000	20.5	2500	44
Poás	2003	1100	21	3000	44
Desamparados	2003	1300	19	2000	90
Dulce Nombre, Cartago	2003	1380	19	2300	91
San Isidro, P. Zeledón	2004	700	23	3000	40
Platanares, P. Zeledón	2005	746	23	3000	40
León Cortés	2006	1740	17.8	3000	93
Turrialba	2007	900	22	2600	43

RESULTS AND DISCUSSION

The results indicate that in localities located between 700 and 1150 masl, the cycle from egg to adult was completed between 40 and 45 days, while over 1200 masl cycle took about 90 days to complete (Table 1). The field performance has two levels of duration, which are clearly marked in Figure 1. The answer to the altitude (temperature) is not as straightforward as in laboratory studies (Borbón 1991), indicating that changes between day and night and other factors affecting the biology of the insect.

Constantino (sf) reported similar results in Colombia, indicating that low altitude localities (1200-1300 masl) with average temperatures above 21 °C shows a further development of the coffee berry borer, while development is lower in sites located on the 1600 masl, with temperatures below 20 °C. For field conditions of the Colombian coffee zone, Bustillo (2006) estimated that the time it takes to start another generation of CBB is 45 days at an average temperature of 22 ° C and about 60 days to a temperature of 19 ° C.

During the development of the fruits infested, the amount of the insect stages increases inside the grains, coming to be in low areas more adults before initiating collection, causing re-infestation of the crop and increased damage. In areas above 1200 m is achieved start collecting before the completion of the life cycle of the pest in infested fruits, facilitating control of the pest if done efficiently and timely harvesting (Rojas 2007).

The results of this research indicate that in regions with altitude below 1200 masl the risk of major attacks CBB is greater and therefore, should strengthen all control measures involved in the integrated pest.

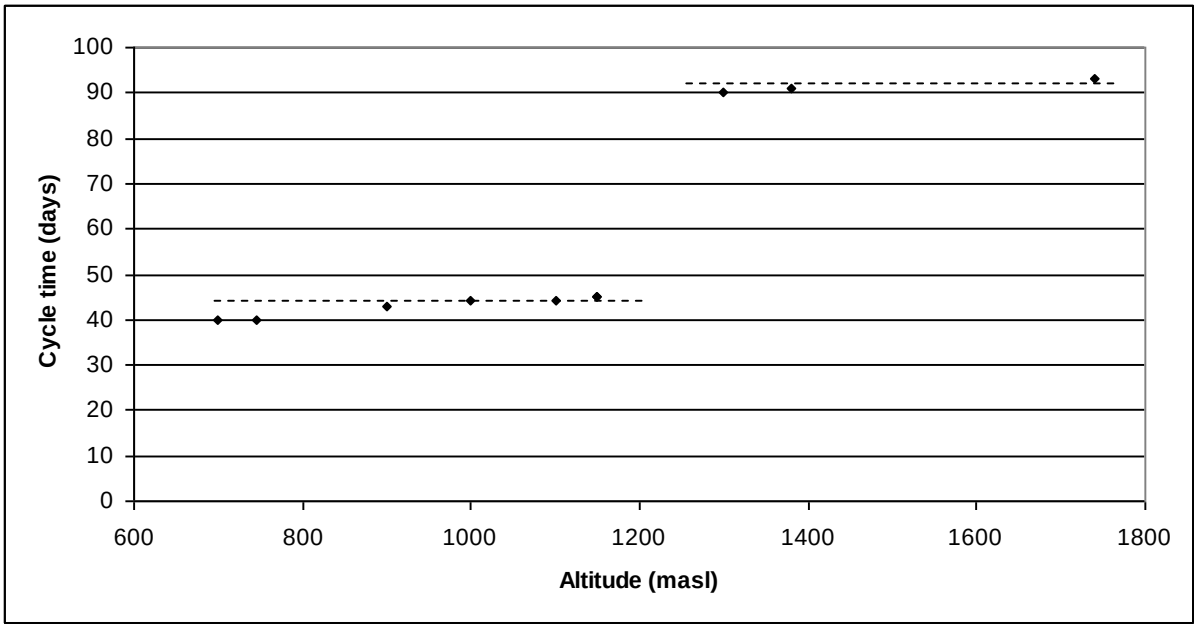

Figure 1. Levels of biological cycle duration of the coffee berry borer according to altitude.

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The Role of Irrigation in Coffee Production: A Review of Research Findings in Ethiopia

S. G. TESFAYE

Ethiopian Institute of Agricultural Research, Jimma Research Center, P.O.Box, 192, Jimma, Ethiopia

SUMMARY

Results of different irrigation experiments, which have been carried out on Arabica coffee in Ethiopia, have indicated that watering coffee seed beds at two days interval until seedling emergence, twice a week after emergence until two to four pairs of true leaves and then at a week interval during the dry season may promote vegetative growth and dry matter yield of coffee seedlings during the nursery period. Among the different irrigation regimes, partial root zone drying (PRD) and normal deficit irrigation (NDI) significantly decreased shoot growth and total dry matter yield of young coffee plants. On the other hand, full irrigation or well watering (WW) has resulted in considerably higher crop yield, compared to PRD and NDI. However, the difference between WW and PRD was not significant for crop yield, and yet PRD and NDI significantly improved the quality of coffee beans. In addition, PRD also resulted in significantly higher irrigation water use efficiency (IWUE). In a separate field experiment, supplemental full irrigation (SFI) during the dry season has also resulted in higher crop yield, as compared to supplemental deficit irrigation (SDI) and a rain-fed culture, though the difference between SFI and SDI was not significant. Besides yield advantage, overall quality of the crop was substantially improved by SDI compared to the SFI practice. Hence, it was concluded that PRD and SDI are effective deficit irrigation strategies that could save water, increase IWUE and improve crop quality without a significant reduction in crop.

INTRODUCTION

Reduced amount and erratic distribution of the seasonal precipitation brought about frequent drought incidences in Ethiopia. This situation makes crop production impossible or at least difficult without irrigation. Therefore, there is an urgent need to identify and adopt effective irrigation management strategies that can save water and increase water use efficiency. Like other crop plants, Arabica coffee is sensitive to water deficit stress and its growth and yield potential is greatly affected by seasonal droughts. It is well known that coffee requires an adequate supply of water, especially at early stages of growth when the seedlings develop new vegetative parts, at flowering and during rapid fruit growth period, and moisture deficit stress during these sensitive stages may cause considerable reductions in growth and subsequent yield of the crop. This condition necessitates supplemental irrigation. On the other hand, water is becoming increasingly scarce in most coffee growing areas of Ethiopia and, thus, management practices that increase irrigation water use efficiency (IWUE) without a significant adverse effect on plant growth and development (Davies *et al.*, 2000; Kirda *et al.*, 2004) would save water and decrease the likelihood of severe water deficits during sensitive phenological stages of the crop.

This paper is, therefore, intended to review research works, which have been carried out in the past, and document their results, regarding the impact of different frequencies, application

methods and amounts of irrigation on growth, yield, quality and IWUE of Arabica coffee in Ethiopia.

RESEARCH FINDINGS

Vegetative growth and dry matter yield

The influence of watering frequency on coffee seed germination, seedling emergence, vegetative growth, and dry matter yield and partitioning has been studied in standard nursery beds at different occasions using released cultivars at Jimma, Awada/Sidamo and Tepi. Results of these studies show that germination of coffee seeds and seedling vigor significantly improved when water was applied every day or at two day intervals. But, watering every three or four days further improved growth of coffee seedlings (Taye et al., 2001). In another experiment, seed germination and both growth and dry matter yield of coffee seedlings considerably increased, but root:shoot ratio decreased by watering the seedbeds at four days interval (Tesfaye, 1995). The increases in vegetative growth and dry matter yield of coffee seedlings with frequent watering could be attributed to increases in plant and soil water status and, thus, enhanced rate of physiological processes, mainly photosynthesis (Tesfaye, 1995; 2005).

Similar results have been obtained with coffee seedlings grown in a rain shelter with different irrigation regimes, involving well-watering (WW, control), partial root zone drying (PRD) and normal deficit irrigation (NDI). In the PRD treatment, the root system of each plant was divided in to two parts/compartments and half of the irrigation water used in the WW plot was applied to one side of the root system during each application, while the other side of the root was allowed to dry until the soil moisture content declined to less than 35% of the field capacity (FC). Then, the dry compartment was rewatered with half the volume of water applied to the WW treatment by allowing the wet compartment to dry for the same soil drying cycle. On the other hand, water was applied evenly to both sides of the root system in NDI plots during each application. Except the method of application, the amount of water applied to PRD and NDI treatments was the same during each irrigation application (Tesfaye, 2005; Tesfaye et al., 2008).

Results of The PRD experiment show that almost all the vegetative growth parameters were significantly affected by irrigation regimes. Plants in WW plots exhibited significantly higher shoot growth, expressed as plant height, girth at the base, number of nodes, inter node length, number of branches and total leaf area. The difference between treatments was also significant for root volume. (Table 1) (Tesfaye, 2005; Tesfaye et al., 2008). In agreement with the results of these findings, the reduction in shoot growth of plants under less frequent watering and with NDI and PRD practices has been reported for grapevines (dos Santos et al., 2003), tomatoes (Kirda et al., 2004; Zegbe et al., 2004) and hot pepper plants (Dorji et al., 2005). Besides chemical signal (the plant growth regulator, ABA, which is believed to have a central role in the long distance drought signaling process in many plants) (Davies et al., 2000), decreased plant water status is also believed to be responsible for reduced vegetative vigor and limited leaf growth in plants under water deficit conditions or in NDI and PRD practices (dos Santos et al., 2003; Wakrim et al., 2005).

Total dry matter (TDM) production was significantly higher for the WW treatment. However, there was no significant difference between the treatments for root: shoot ratio, although PRD followed by NDI resulted in considerably higher root: shoot ratio than did the WW treatment (Table 1) (Tesfaye, 2005; Tesfaye et al., 2008).

Table 1. Vegetative growth, dry matter yield (DMY) and IWUE of coffee seedlings as affected by irrigation regimes (WW = well-watering, PRD = partial root drying and NDI = normal deficit irrigation).

Growth parameters	Irrigation regimes		
	WW	***PRD***	***NDI***
Plant height (cm)	65.48 a	49.18 b	47.65 b
Girth (cm)	1.21 a	0.88 b	0.80 b
Number of branches	10.33 a	8.33 a	6.00 b
Total leaf area (cm^2)	2170.05 a	1318.82 b	1089.69 c
Root volume (cm^3)	61.67 a	43.33 ab	35.00 b
Total DMY (g)	52.13 a	28.54 b	26.00 b
Root:Shoot ratio	0.33 a	0.42 a	0.38 a
IWUE (10^{-3}) (g ml^{-1})	1.20 b	1.75 a	1.48 ab

Figures followed by same letter (s) within a row are not significantly different at P = 0.05. Source: Tesfaye, 2005.

Results of this study were also in agreement with what has been reported by Zegbe *et al.* (2004) for tomatoes, where vegetative dry mass was considerably lowered in PRD compared with full irrigation. Similarly, it has been observed that PRD and regulated deficit irrigation resulted in a significant reduction in shoot biomass of common bean plants (Wakrim *et al.*, 2005). It has also been observed that PRD has increased root: shoot ratio of hot pepper plants (Kang *et al.*, 2001). The decrease in TDM yield of coffee seedlings in NDI and PRD plots could be attributed to the reduced total leaf area, which might have decreased the photosynthetic capacity of plants (Tesfaye, 1995; 2005).

Crop Yield and Quality

The three irrigation practices have also been evaluated in the field during the dry spells using uniformly grown and well managed mature coffee stands of cultivar F-59 at Jimma. It was observed that clean coffee yield significantly increased in the WW treatment, but it was considerably affected by NDI (Table 2). However, the difference between NDI and PRD, as well as among WW and PRD treatments, was not statistically significant. On the other hand, the overall raw and liquor quality, as well as summation of the two, significantly increased by PRD and NDI treatments compared with coffee beans from WW plants (Table 2) (Tesfaye, 2005; Tesfaye et al., 2008). In agreement with these results, it has been reported that fruit quality of grapevines increased without a significant yield reduction for the PRD practice (dos Santos et al., 2003), which has also enhanced fruit quality of tomatoes by way of increasing water soluble dry matter in fruits compared to those harvested from fully irrigated treatments (Kirda et al., 2004). Davies et al. (2000) and Zegbe et al. (2004) have also observed advancement in fruit maturity and enhancement of quality (higher dry mass and total soluble solids concentrations, TSSC) in fruits from PRD treatment compared to those from fully irrigated tomato plants.

On the other hand, two supplemental irrigation treatments (supplemental full irrigation, SFI and supplemental deficit irrigation, SDI) were tested along with a rain fed (RF) control on cultivar F-59, 74110 and 75227 during the peak dry period in two sets of experiment. In both experiments, irrigation water was applied evenly to the root system of coffee trees in the conventional way when the soil moisture content at 30 cm depth declined to less than 35% of the field capacity (FC) (SFI replenishing the soil moisture to 100% FC, while SDI receiving half of the amount of water applied to SFI treatment during each application) (Tesfaye, 2005).

With regard to this, among the irrigation practices, SFI resulted in significantly higher coffee yield compared to that obtained from RF plots (Table 4). The difference between SFI and SDI or among SDI and RF treatments was not significant. On the other hand, in the second field experiment with SDI and RF treatments, SDI significantly increased coffee yields and resulted in 24.59% yield advantage over the RF treatment (Table 3) (Tesfaye, 2005; Tesfaye *et al.,* 2008).

Table 2. Coffee yield, quality and irrigation water use efficiency (IWUE) of cv. F-59 as influenced by different deficit irrigation methods (WW = well-watered control; PRD = partial root zone drying; NDI = normal deficit irrigation).

Irrigation Treatment	Yield (Clean Coffee, kg/ha)	IWUE (g/kg)	Quality (%)		
			Raw	***Liquor***	***Overall***
WW	1,431.78 a	8.98 c	25.44 b	38.12 b	63.56 b
PRD	1,229.50 ab	15.24 a	29.25 a	42.81 a	72.06 a
NDI	986.75 b	12.24 b	29.50 a	42.50 a	72.00 a

Figures followed by same letter or letters with in a column are not significantly different at P = 0.05. Source: Tesfaye, 2005.

In general, coffee yield was relatively higher in the first than in the second set of experiment (Table 3), because trees in the latter were younger than those in the former. In agreement with these findings, it has been reported that pod growth and yield of common bean (Wakrim et al., 2005) and total fresh mass of pepper fruits (Dorji et al., 2005) were considerably reduced by soil drying or deficit irrigation compared to well irrigated treatments. Significant reductions in fruit dry mass yield under deficit irrigation and in fruit fresh and dry masses as a result of partial soil drying have also been reported for tomatoes (Kirda et al., 2004). Hence, some previous reports on different crops support the results of these studies. On the other hand, RF plots had significantly lower coffee yields, probably because of reduced rate of physiological activities (mainly reduction in photosynthetic rate) associated with total dry matter production and its partitioning to fruits as a result of water deficit at critical berry development stages (Kirda et al., 2004; Tesfaye, 2005).

Liquor quality was unaffected by the treatments, but it was higher for SDI and RF than in SFI treatment. However, as compared to SFI, SDI and RF treatments significantly increased both raw appearance and overall quality of coffee beans (Table 3). Such a significant improvement in crop quality due to SDI has also been observed by different workers on various crops. For instance, it has been reported that deficit irrigation improved the quality of tomato fruits because of redder color and higher concentration of total soluble solids (TSSC) compared to those fruits from full irrigated control plants (Davies et al. 2000; Zegbe et al., 2004). Similarly, as compared to full irrigation, deficit irrigation has resulted in 21% higher TSSC and better color development in hot pepper fruits (Dorji et al., 2005).

Table 3. Effect of conventional irrigation (supplemental full irrigation (SFI), supplemental deficit irrigation (SDI) and rain fed (RF)) on yield and crop quality of three coffee cultivars (cv. F-59, 75227 and 74110).

Irrigation Treatment	Clean Coffee Yield (Kg ha^{-1}) (cv. F-59)	Quality (%) (cv. F-59)			Clean Coffee Yield (Kg ha^{-1})	
		Raw	*Liquor*	*Overall*	*cv. 75227*	*cv. 74110*
SFI	1,346.31 a	26.75 b	34.37 a	61.12 b		
SDI	1,120.77 ab	29.50 a	38.12 a	67.62 a	483.43 a	326.73 a
RF	881.55 b	29.25 a	36.25 a	65.50 a	346.74 b	241.72 b

Figures followed by same letters with in a column are not significantly different at P = 0.05. Source: Tesfaye, 2005.

Irrigation Water Use Efficiency

Irrigation-water-use efficiency (IWUE) was determined in both rain shelter and field experiments. It was calculated as total dry matter (TDM) yield or total crop (fresh cherry) yield divided by total volume of (gross) irrigation water applied during the study period (Kang et al., 2001). Accordingly, IWUE for TDM yield of coffee seedlings significantly increased with the PRD practice, but the difference between PRD and NDI or NDI and WW treatments was not significant (Table 1). In the field experiment, IWUE for crop yield was also significantly higher for the PRD treatment compared with NDI and WW plots. The difference between NDI and WW was also significant, where the former increased IWUE by 26.63% over the later treatment. Besides saving 50% of irrigation water, the PRD practice resulted in 41.08% and 19.68% more IWUE than the WW and NDI treatments, respectively (Table 2). Similar results have been reported for different crops. For example, the PRD practice increased IWUE by 56% (Kirda et al., 2004) and 70% (Zegbe et al., 2004) in glasshouse-grown tomatoes, and by about 50% in common bean plants (Wakrim et al., 2005). Such substantial increases in IWUE and a significant saving in irrigation water without significant yield reduction with the PRD treatment have also been reported for tomato (Davies et al., 2000), hot pepper (Kang et al., 2001; Dorji et al., 2005), grapevines (dos Santos et al., 2003) and common bean (Wakrim et al., 2005).

CONCLUSIONS

In general, coffee seed beds covered with 3.00 – 5.00 thick mulch need to be watered at two days interval right after sowing seeds until seedlings emerge during the dry season. After emergence, by removing mulch and providing moderate overhead shade, the watering frequency could be reduced to twice a week until seedlings produce two to four pairs of true leaves. Then, watering at a week interval may result in vigorous growth and higher dry matter yield of coffee seedlings during the nursery period. Results of irrigation studies also showed significant reductions in shoot vegetative growth and TDM yield, and increases in root: shoot ratio and IWUE of coffee seedlings in the PRD treatment. Soil drying or deficit irrigation during the reproductive phase decreased marketable crop yield. On the other hand, it was observed that the overall raw and liquor or cup quality of coffee substantially improved without significant yield losses by deficit irrigation, particularly with the PRD practice. In addition, PRD was found to be an effective deficit irrigation practice (followed by NDI) to increase IWUE, thereby decreasing irrigation water requirement (by 50%) without substantial adverse effects on plant growth and development. On the other hand, it was observed that the difference between SFI and SDI was not significant for crop yield, but SFI resulted in significantly higher coffee yield as compared to that obtained from RF plots, and SDI exhibited 21% to 25% more yield than the RF treatment. Besides yield advantage, overall

quality of the crop (coffee beans) was substantially improved and the amount of irrigation water applied was considerably reduced by SDI compared to the SFI practice. Therefore, PRD and SDI could be practically advantageous, effective and feasible for coffee production, especially in areas where water is scarce and dry spells are prolonged.

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Early Screening of Arabica Coffee Genotypes for Drought Tolerance in Ethiopia

S. G. TESFAYE

Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, P.O.Box, 192, Jimma, Ethiopia

SUMMARY

In Ethiopia, coffee production is increasingly constrained by changes in local weather and global climate, which brought about erratic distribution of the seasonal rain fall and recurrent droughts. In order to address the problem, genotype screening for drought tolerance has been under taken in a rain shelter at Jima Agricultural Research Center (JARC). Accordingly, it was observed that there were significant differences among Arabica coffee (C*offea arabica* L.) genotypes for sensitivity to water deficit stress, rate of survival and recovery from drought and concentration of inorganic solutes (K, Ca and Mg) in leaves. Some of the cultivars, such as F-59 and *Geisha*, exhibited higher rate of survival and recovery, despite higher level of sensitivity to the imposed water stress treatment. This could be probably because of higher rate of leaf shed and, thus, maintenance of lower total leaf area and specific leaf Area (SLA) (higher leaf thickness) and higher root:shoot ratio in these cultivars. On the other hand, the rate of stress development and leaf shed was lower and survival and recovery rate, as well as total leaf area was higher in cultivar 74110, 8/85 and 74112, probably because of the increased concentration of inorganic solutes mainly K, Ca and Mg in their leaves and, hence, a more effective osmotic adjustment. Therefore, it appears that, besides measuring the rate of stress development and morphological changes, concentration of osmoregulators, such as compatible inorganic solutes, are also important parameters to be considered during early screening of coffee genotypes for drought tolerance.

INTRODUCTION

Changes in local weather and global climate brought about reduced amount and erratic distribution of the seasonal precipitation, which, in turn, resulted in frequent drought incidences in Ethiopia, as well as in most potential agricultural regions of the world. This situation necessitates the use of drought tolerant varieties in these areas.

Plants are frequently subjected to periods of water deficit stress, which ultimately leads to reduced growth and productivity by affecting various physiological and biochemical processes. However, they have evolved different strategies to cope with water deficits through avoidance or postponement of dehydration or stress tolerance (Pugnaire *et al*., 1999). There exist variations among species or between genotypes within a species for acquiring different physiological, morphological and biochemical strategies for survival and even maintenance of some growth and physiological processes under stressful conditions (Joshi, 1999). Hence, these adaptive responses could be used as selection criteria during screening genotypes for drought tolerance (Sanchez *et al*., 1998). It has been reported that decline in shoot growth and leaf area and increase in leaf thickness and root: shoot ratio with soil moisture depletion are believed to be among the important stress avoidance or tolerance mechanisms in plants (Abernethy and McManus, 1998). Because of their role in osmotic adjustment, the accumulation of compatible inorganic ions, such as potassium, in plant tissues under water

deficit condition has also been considered as one of the adaptive mechanisms in drought-tolerant genotypes (Sanchez *et al*., 1998; Pugnaire *et al*., 1999). Like other crop plants, Arabica coffee is sensitive to water stress and its growth and yield potential is greatly affected by seasonal drought. In fact, there exists a large diversity among the genotypes of Arabica coffee with regard to adaptation to different growth conditions in its center of origin, Ethiopia. However, the variability has not been well studied and documented in relation to drought tolerance.

This paper is, therefore, intended to review research works, which have been carried out in the past, and document their results, regarding differences between known Arabica coffee cultivars for sensitivity to water deficit stress and some physiological and morphological mechanisms associated with drought tolerance.

RESEARCH FINDINGS

Sensitivity to Water Stress

In an attempt to screen Arabica coffee (C*offea arabica* L.) genotypes for drought tolerance, an experiment was conducted at Jimma Agricultural Research Center. The experiment was laid down in a rain shelter using 16 released selections, three hybrid varieties, two lowland materials and three promising genotypes. The seedlings were subjected to two watering regimes (water-stressed by withholding irrigation and well-watered control) when they produced four pairs of true leaves.

The overall performance of coffee genotypes was evaluated based on differences in mean stress score values (SSV), percent plants wilting (PPW) at noon, percent plants recovering during the night time (PPR), mean days to complete wilting (MDCW), rate of leaf shed (RLS) and rate of survival (RS). Depending on their overall mean rank positions and based on significant differences for mean performance, the genotypes were identified in to three categories: sensitive, moderately sensitive and relatively tolerant to water deficit stress. Accordingly, cultivar F-59, 7395xF-59, J-19, 7454, 754 and 75227 were identified as more sensitive than 7487, 74110xF-59, 741, *Geisha*, J-21, 744, 741xF-59, 74158, 77/85, 7395, F-35 and 7440, while 74148, 74140, 74110, 74112, 74165 and 8/85 were found to be relatively tolerant to the imposed soil drying treatment (Tesfaye, 2005; Tesfaye *et al*., 2008). Most of the relatively tolerant genotypes are compact types with smaller leaf size, shorter internodes and flexible stems, while those more sensitive ones are known for their open branching naure, larger leaves and stiff stem (Tesfaye *et al*, 2008). Four coffee genotypes were randomly selected from each of the three categories for further study of the mechanism of drought tolerance.

Results of the study with the 12 selected coffee cultivars showed that the rate of stress development estimated by SSV and PPW at noon was significantly higher in F-59, *Geisha*, 75227, 7487 and 741xF-59, but it was lower in 74110, 8/85 and 74112. As a result, the latter group has exhibited significantly higher PPR and MDCW, the values of which were consistently lower for F-59, 75227, 741 and *Geisha*. On the other hand, RLS was significantly lower for 74110, 8/85 and 74112, but it was higher for F-59, J-21, 75227 and 741. However, cv. F-59 showed significantly higher RS and rate of recovery, as did 74110, 8/85, 74112, *Geisha* and 741xF-59. Higher RS and recovery of F-59 and *Geisha*, despite significantly higher rate of stress development, could be attributed to higher RLS and, thus, maintenance of lower total leaf area and higher root:shoot ratio (Tesfaye, 2005) (Table 1).

Similar results have been reported for different genotypes of both Arabica and Robusta coffees (Maestri *et al*., 1995). Such a leaf movement (rolling or folding of leaves), an adjustment of leaf angle or modification of leaf orientation or reduction in total leaf area to reduce the interception of solar radiation and, thus, decrease leaf temperature and water loss by transpiration is regarded as one of the drought avoidance mechanisms evolved in plants (Pugnaire *et al*., 1999; Carr, 2001).

On the other hand, SSV, PPW and RLS were lower and RS, recovery and total leaf area were higher in 74110, 8/85 and 74112, probably because of the increased concentration of inorganic solutes, mainly K, Ca and Mg in their leaves (Table 1) and, thus, a more effective osmotic adjustment (Tesfaye *et al*., 2008). Such a lower rate of stress development (less wilting symptom) and stay-green under water stress conditions as a result of maintenance of turgor by osmotic adjustment has also been used as an important criteria during screening crop genotypes for drought tolerance (Lilley and Fukai, 1994; Kitbamroong and Chantachume, 1992).

Morphological and Physiological Responses

Water stressed plants of cv. 74110, 74110xF-59 and J-21 showed higher leaf elongation rate (LER), which was significantly lower in *Geisha* and F-59. Similarly, total leaf area (TLA) was significantly higher in 74112, 75227, 74110 and 8/85, but lower in *Geisha*, 74158, F-59 and 741xF-59. Cultivar 74112 and 75227 also showed significantly higher total dry matter (TDM) yield, but the reduction in TDM due to water deficit stress was significantly higher in F-59, *Geisha*, 741, 7487 and 74158. Higher rate of reduction in TDM yield of these cultivars could be due to higher rate of reduction in TLA or maintenance of lower TLA, though the former three cultivars had higher root:shoot ratio (Table 1) and significantly lower specific leaf area (SLA) (higher leaf thickness) (data not given) (Tesfaye *et al*., 2008).

Leaf K, Ca and Mg Content

Results of the experiment also showed that the concentration of potassium (K), calcium (Ca) and magnesium (Mg) was significantly increased in leaves of water-stressed plants. Differences among the cultivars were also highly significant for accumulation of the inorganic ions. It was observed that cultivar 74110, 8/85 and 74112, followed by J-21, 741xF-59, 74110xF-59 and 7487, had higher, while 74158 and *Geisha* accumulated lower K by the end of the drought period. The concentrations of Ca and Mg were higher in the leaves of stressed 74110, 8/85 and 74112 plants. However, the accumulation of both Ca and Mg was lower in F-59, 74158 and *Geisha* (Table 1).

Table 1. Morphological responses and concentration of inorganic ions in leaves of water stressed plants of different Arabica coffee cultivars.

Cultivar	LER (cm day^{-1})	Reduction in TLA (%)	Reduction in TDM yield (%)	Root to Shoot ratio	Concentation of inorganic ions in leaves (g kg^{-1})			Rate of Recovery (%)
					K	*Ca*	*Mg*	
F-59	0.10cd	81.50b	58.00b	0.415ab	36.50c	12.00b	2.90d	71.10bc
75227	0.14bc	50.80de	42.20cd	0.370cd	37.00c	13.10ab	3.60b	54.80d
741	0.13bc	68.20c	52.10bc	0.355cd	37.50c	12.50b	3.50bc	58.20cd
7487	0.13bc	78.60ab	49.50bc	0.425ab	41.50bc	16.00a	3.90ab	65.00c
74158	0.15b	74.80bc	48.60bc	0.347cd	37.00c	12.50b	3.25cd	52.50d
74110xF-59	0.18a	58.20d	39.40d	0.408bc	42.65abc	17.00a	3.61b	66.80c
741xF-59	0.12c	81.60a	42.80cd	0.320d	42.20abc	13.70ab	3.68b	76.30ab
J-21	0.18a	57.50de	39.00d	0.406bc	44.00ab	12.80b	3.65b	66.80c
Geisha	0.08d	88.20a	68.80a	0.483a	37.00c	12.50b	3.00cd	83.40a
74110	0.20a	57.50de	45.00c	0.390bc	48.60a	16.85a	4.20a	78.00a
8/85	0.15b	50.60e	48.80bc	0.422ab	47.90a	16.25a	4.31a	80.20a
74112	0.11c	20.00f	25.60e	0.346cd	47.60a	15.80a	4.12a	70.20bc

Figures followed by same letters with in a column are not significantly different at P = 0.05.
Note: LER=leaf elongation rate; TLA= total leaf area, TDM=total dry matter yield, K=potassium, Ca=calcium, Mg= magnesium.

In agreement with these results, Alam (1999) has reported that drought-tolerant wheat varieties accumulated more K than did the more susceptible varieties. In general, because of their role in osmotic adjustment (OA) in crops subjected to water deficit, the accumulation of osmotically active solutes, such as sugars and inorganic ions (Pugnaire *et al*., 1999; Arndt *et al*., 2000; Patakas *et al*., 2002), has been used as a single parameter to measure physiological dryness in plants and screen varieties for drought tolerance (Sanchez *et al*., 1998). Hence, the observed differences among coffee cultivars in this study might be associated with variations in turgor maintenance by osmoregulation, probably as a result of differences in K, Ca and Mg accumulation. With regard to this, leaf K and Mg concentration was highly negatively correlated with stress score values (SSV) and cultivars (such as 74110, 8/85 and 74112) with higher concentration of these ions experienced lower rate of stress development (wilting) (Tesfaye, 2005).

In general, differences among genotypes for the rate of stress development have been attributed to variations in OA in leaves. In line with this, it has been reported that maintenance of turgor by OA may reduce the rate of leaf senescence (leaves stay green and delay wilting) (Joshi, 1999), extend the life time of active tissues and, thus, decrease abscission of leaves (Pugnaire *et al*., 1999) and increase plant survival during water stress periods (Maestri *et al*., 1995). The role of OA in water stress tolerance has been reported for different crops, including wheat (Alam, 1999), grapevines (Patakas *et al*., 2002) and different fruit tree species (Arndt *et al*., 2000). Hence, difference between the coffee cultivars for the rate of wilting, leaf fall and survival under water stress condition might be associated with the ability of genotypes to maintain their internal water status through OA. Besides OA, increases in root: shoot ratio and reductions in total leaf area (Table 1) might have also contributed to lower rate of stress development and leaf shedding and higher survival rate in some of the coffee cultivars tested in this study (Tesfaye, 2005).

Rate of Recovery

Among the cultivars, F-59, 741xF-59, *Geisha*, 74110 and 8/85 showed considerably higher rate of recovery in terms of production of new flushes or leaves. The rate of recovery two weeks after the commencement of rewatering was substantially lower in cultivar 74158, 741 and 75227 than in 7487, 74110xF-59, J-21 and 74112 (Table 1) (Tesfaye, 2005). Higher rate of survival and recovery in 74110, 8/85, 74112 and 741xF-59 might be associated with the enhanced rate of accumulation of inorganic ions or solutes (K, Ca and Mg) and, thus, effective osmoregulation (OA). Similarly, increases in root: shoot ratio (Table 1) might have also contributed to the higher rate of recovery in *Geisha*, 8/85, F-59 and 7487 (Tesfaye, 2005).

In line with this, it has been reported that OA through accumulation of compatible solutes such as inorganic ions may increase plant survival rate under water stress conditions (Maestri *et al*., 1995; Pugnaire *et al*., 1999). Besides, increased root growth and higher root: shoot ratio have also been observed in a number of cases to improve plant water status, delay the rate of stress development (leaf senescence) and increase survival rate under water stress conditions. Higher rate of recovery in cultivar 741xF-59, 74110 and 8/85 could also be related to the reduction in SLA, as there was a significant negative relationship between these variables. Similarly, higher rate of recovery in F-59 and *Geisha* could be associated with maintenance of lower leaf area and reduced SLA, while greater leaf retention capacity and higher SLA (lower leaf thickness) in cultivar 75227 might have led to lower rate of survival during the drought period and reduced recovery after rewatering. Reductions in leaf area and increases in leaf thickness or decreases in SLA as a result of water stress are believed to be among the important mechanisms of plant adaptation and survival under drought (Abernethy and McManus, 1998; Pugnaire *et al*., 1999). In contrast, lower rate of survival during water stress period and, thus, lower recovery rate after rewatering in cultivar 741 and 74158 might be attributed to ineffective OA due to lower level of inorganic solutes accumulation (Table 1), higher SLA and lower root: shoot ratio (Tesfaye, 2005).

CONCLUSIONS

There were obvious differences among Arabica coffee cultivars for sensitivity to water stress. Accordingly, coffee cultivars could be grouped in to three distinct categories (sensitive, moderately sensitive and relatively tolerant) based on clear and significant differences for mean rate of stress development as commonly used in other crop species. Some of the cultivars, such as 74110, 7112 and 8/85, consistently outsmarted the other genotypes and exhibited higher rate of recovery and relatively lower level of sensitivity to drought, probably because of accumulation of K, Ca and Mg ions in their leaves and, thus, a more effective OA. On the other hand, higher rate of survival and recovery despite higher level of sensitivity in cultivar F-59 and *Geisha* could be attributed to the higher rate of leaf fall due to severe wilting, maintenance of lower leaf area and higher root: shoot ratio and reduction in SLA, while greater leaf retention capacity and higher SLA might have contributed to lower rate of survival during the drought period and reduced recovery after rewatering in some cultivars, such as 75227. Similarly, lower rate of survival and recovery in cultivar 741 and 74158 might be attributed to the lower root: shoot ratio and ineffective OA due to lower level of inorganic solutes accumulation in plants. Therefore, it appears that accumulation of compatible solutes, root: shoot ratio, SLA and rate of survival and recovery should also be considered in addition to stress scoring during screening genotypes for drought tolerance.

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Yield Performance of Some Robusta Coffee (*Coffea Canephora* Pierre Ex Froehner) Clones under Different Shade Intensities in Ghana

G. J. ANIM-KWAPONG, E. ANIM-KWAPONG

Cocoa Research Institute of Ghana (CRIG), P.O. BOX 8, New Tafo-Akim,Ghana

SUMMARY

Coffee agroforest has beneficial features such as conservation of natural resources and stability of coffee production. At the landscape level, it mitigates impacts of climate change and promotes biodiversity conservation. However, intensive crop management under full sun has replaced coffee agroforest in several areas mainly as a result of low crop yields from excessive shading. Obtaining optimal coffee yields in coffee agroforest is essential to the maintenance of this cultivation method. A field trial was established at the CRIG substation, Bunso in 2001 to evaluate yield performance of ten Robusta coffee clones (sub plot) at four shade levels (main plot) in a split plot design experiment with four replicates. Stand basal area was used as surrogate for shade intensity. Secondary forest was selectively thinned to reach three different final stand basal areas of 6.0 m^2 ha^{-1} (low shade), 9.0 m^2 ha^{-1} (medium shade) and 12.0 m^2 ha^{-1} (heavy shade) at six years after thinning. A 'no shade' control treatment was attained by clear-felling portions of the secondary forest. The low, medium and heavy shade levels had, in addition, *Gliricidia sepium* planted at a spacing of 9m x 12 m, 9m x 9 m and 6m x 6m respectively. Plantain (*Musa spp*) was planted at 3m x 3m and the coffee was planted at 3m x 2m in all the treatments. The main factors and their interaction had significant (*P<0.001)* effect on four years cumulative clean coffee yield and bean weight. Across the clones, the 'medium shade' produce the best cumulative yield of 3,068 kg clean coffee ha^{-1}. Clone E139 under 'low shade' produced the best (4,459 kg clean coffee ha^{-1}) and clone 181 under 'no shade' produced the least (1,611 kg clean coffee ha^{-1}) cumulative yields. Clone E 90 recorded the best mean bean weight of 15.3 g 100^{-1} beans across the shade levels. Clone E 139 under 'heavy shade' had the highest bean weight (16.9 g 100^{-1} beans). The 'no shade' treatment across the clones recorded the least mean bean weight (13.04 g 100^{-1} beans). The significant clone x shade interaction shows that Robusta coffee production can be optimised in a coffee agroforest by matching coffee clones to appropriate shade levels.

INTRODUCTION

Coffee agroforest mimics the natural sub canopy environment of wild *Coffea* species in the tropical forest. This agro-ecosystem is apparently ideal for the cultivation of the crop since it reduces the extent of photo oxidative damage, a phenomenon frequently observed in coffee grown under full sun exposure in marginal zones, and ultimately increases crop life expectancy (DaMatta *et al*., 2007). Crop productivity is linked to the efficiency of the utilization of photosynthetic active radiation. Thus with intensive crop management including high external input, coffee will often produce much higher yields under full sun than under shade (Van der Vossen, 2005). Yield potential, competition for water, and pest/disease incidence are fundamental issues in the controversy over the use of shade in coffee. Despite the controversy, shade is invariably recommended for the establishment of coffee (Kumar and Tieszen, 1980). The major physiological benefits that coffee receives from shade trees can be

placed in two main categories both associated with reduced plant stress namely: (1) Amelioration of climatic and site conditions through (i) reduction of air and soil temperature extremes, (ii) reduction of wind speeds, (iii) buffering of humidity and soil moisture availability, and (iv) improvement or maintenance of soil fertility including erosion reduction; and (2) Reduction in the quantity and quality of transmitted light and hence avoidance of over-bearing and/or excessive vegetative growth (Beer *et al*., 1998).

The high dependence of coffee production on climate is recognised (Camargo *et al*., 2006). Consequently, climate change poses a great threat to coffee production. Smallholder coffee farmers operating in marginal zones without high external input could be most vulnerable to the projected impacts of climate change (International Trade Centre, 2011). Coffee agroforest is an appropriate agronomic technique for climate change impact adaptation, mitigation and environmental sustainability. To promote the adoption of coffee agroforestry systems and also sustain the practice, there is the need to enhance coffee yields in the system. Thus, the objective of this study was to identify Robusta coffee genotypes that could cope with higher shade intensity and remain productive at the same time.

MATERIALS AND METHODS

The field trial was conducted at Cocoa Research Institute of Ghana (CRIG) substation, Bunso. The substation is located at 06^{o} 17'N and 00^{o} 23' West, 230 m above sea level with 1460 mm mean annual rainfall, 67% relative humidity and 31-33^{o}C maximum and 19-21 ^{o}C minimum temperature. The trial was initiated in 2001 to evaluate yield performance of ten Robusta coffee clones (sub plot) at four shade levels (main plot) in a split plot design experiment with four replicates. Shade tree basal cover (stand basal area) was used as surrogate for shade intensity. The number of shade trees and their diameters (diameter at breast height, 1.3m) were recorded and used to determine shade tree basal cover following Philip (1994). Secondary forest was selectively thinned to reach three different final stand basal areas of 6.0 m^2 ha^{-1} (low shade), 9.0 m^2 ha^{-1} (medium shade) and 12.0 m^2 ha^{-1} (heavy shade) at six years after thinning (Table 1a and 1b). A 'no shade' control treatment was attained by clear-felling portions of the secondary forest. The low, medium and heavy shade levels had, in addition, *Gliricidia sepium* planted at a spacing of 9m x 12 m, 9m x 9 m and 6m x 6m, respectively. Two years after establishing the shade treatments, Plantain (*Musa spp*) was planted at 3m x 3m and the coffee was planted at 3m x 2m in all the treatments. The *G. sepium* and Plantain served as temporary shade. The coffee clones (Table 1b) were planted in line plots of 12 plants per clone per line plot. The coffee plants were cultivated on one to two stems and height controlled at 1.7 m.

Table 1 a. Characteristics of the different shade levels in the trial.

Shade level	Mean DBH of shade trees (cm)	Mean density of shade trees (trees ha-1)	Mean basal area of shade trees (m2 ha-1)
Heavy shade	33.54	137.00	12.10
Medium shade	32.00	120.00	9.20
Low shade	30.00	91.00	6.40
No shade	-	-	-

DBH = Diameter at Breast Height, 1.3m.

Table 1 b. Tree species from the selectively thinned secondary forest used as shade and the Robusta Coffee genotypes (clones) tested in the trial.

Shade tree species	N	Mean DBH of shade trees (cm)	Coffee clones tested	Origin of Coffee clones tested
Terminalia ivorensis	28	35.36	A101	Cote d'Ivoire
Macaranga barteri	14	26.46	A115	Cote d'Ivoire
Morinda lucida	6	30.73	A129	Cote d'Ivoire
Terminalia superba	5	31.02	B96	Cote d'Ivoire
Milicia excelsa	5	62.61	E90	Cote d'Ivoire
Albizia zygia	5	23.76	E138	Cote d'Ivoire
Cordia millenii	3	40.76	E139	Cote d'Ivoire
Omphalocarpum elatum	3	26.43	149	Togo
Entandrophragma angolense	2	19.56	181	Togo
Alstonia boonei	1	32.80	197	Togo
Petersianthus macrocarpus	1	43.95		
Albizia ferruginea	1	35.35		
Ficus exasperata	1	17.83		
Funtumia elastica	1	35.98		
Margaritaria discoidea	1	19.11		
Holarrhena floribunda	1	23.24		

N=Number of trees per species
DBH = Diameter at Breast Height, 1.3m.

Data collection and analysis

Although many characters of the whole experiment were examined, only the four years cumulative clean coffee yield and 100 bean weight are reported in this paper. Yield data recording and transformation of cherry weight to clean coffee yield per hectare per year was done following Anim-Kwapong *et al*. (2011). Data on the four years cumulative clean coffee yield and 100 bean weight were subjected to analysis of variance (ANOVA, General Linear Model) using the MINITAB release 12 statistical software. Significant treatment means were separated using the Standard Error of the Difference between Means (SED).

RESULTS AND DISCUSSION

The main factors (clones and shade) and their interaction had significant ($P<0.001$) effect on four years cumulative clean coffee yield and bean weight (Tables 2 and 3). Across the clones, the 'medium shade' produce the highest cumulative yield of 3,068 kg clean coffee ha^{-1}. Clone E139 under 'low shade' produced the highest (4,459 kg clean coffee ha^{-1}) and clone 181 under 'no shade' produced the least (1,611 kg clean coffee ha^{-1}) cumulative yields (Table 2). Excessive shading negatively affects Robusta coffee production as indicated by Paulo *et al*. (2001). This confirms the result of the present study which showed that Robusta coffee clones under heavy shade recorded the least four years cumulative yield of 2,604 kg clean coffee ha^{-1} (Table 2). However, clones E139, E90, A101, A129 and 149 under heavy shade recorded higher yields than the treatment (shade level) mean of 2,604 kg clean coffee ha^{-1}. Of these clones, E139 and 149 also recorded higher yields than the treatment means of all the other shade levels tested (Table 2). These clones thus have the potential to enhance coffee yields in appropriately managed coffee agroforests.

Table 2. Effect of Shade and Clonal variation on four years cumulative yield of Robusta Coffee at Bunso, Ghana.

Shade level					
	High shade	***Medium shade***	***Low shade***	***No shade***	
	Clean coffee yield (kg ha^{-1})				
Clones					**Clone mean**
E139	3581	3389	4459	3743	3793
E90	2967	3068	3432	2943	3103
A115	2120	3606	2523	2295	2636
A101	2692	3279	2547	2473	2748
B96	2358	2509	2485	2257	2402
181	2051	2078	1828	1611	1892
A129	2717	2950	2589	2600	2714
E138	2378	2727	3741	3021	2967
149	2708	4116	3221	2878	3231
197	2469	2926	2953	3309	2914
Shade level mean	2604	3068	2979	2712	

S.e.d for comparing two shade means = 51.0 (9df)
S.e.d for comparing two clone means = 76.3 (108df)
S.e.d for comparing two clones at a single shade level = 152.7 (108df)
S.e.d for comparing two differences between two clones for two shade levels = 215.9 (108df)
S.e.d for comparing two shade levels, either for the same clone or for different clones = 153.6 (no exact df).

Caramori *et al*. (1996) reported higher coffee yield under the shade of *Mimosa scabrella* planted at 250 tree ha^{-1}. This shade tree density was over and above the density of 137 trees ha^{-1} (heavy shade) used in the present trial. Baggio *et al*. (1997) observed no loss in coffee production under *Grevillea robusta* planted at 71 tree ha^{-1} in Brazil. Though these results may apparently support the present finding, it is noteworthy to mention the difficulty in comparing the productivity of coffee obtained under different conditions as indicated by Morais *et al*. (2006). This is because the results are influenced by several factors including shade tree species used, tree planting density, soil and climatic conditions, coffee genotype, crop spacing and many more (Morais *et al*., 2006).

Clone E 90 recorded the best mean bean weight of 15.3 g 100^{-1} beans across the shade levels. While clone E 139 under 'heavy shade' recorded the highest bean weight (16.9 g 100^{-1} beans). Generally, bean size was largest under heavy shade (Table 3). Muschler (2001) indicated that shaded coffee plants produced larger cherries due to slower maturation resulting in larger bean size. The underlying factors influencing coffee yield and quality under different microclimatic conditions have been adequately explained by DaMatta *et al*. (2007).

Table 3. Effect of Shade and Clonal variation on bean size of Robusta Coffee at Bunso, Ghana.

Shade level					
	High shade	***Medium shade***	***Low shade***	***No shade***	
	Bean weight (g)				
Clones					**Clone mean**
E139	16.89	16.21	14.22	12.56	14.97
E90	15.48	15.97	15.40	14.29	15.28
A115	14.37	13.76	14.57	12.26	13.74
A101	14.64	15.16	13.70	12.47	13.99
B96	14.62	14.40	13.77	12.72	13.87
181	13.76	13.86	13.13	12.84	13.40
A129	14.77	14.47	14.51	13.10	14.21
E138	15.10	15.11	14.15	13.64	14.50
149	14.38	15.95	13.87	13.73	14.48
197	15.02	13.18	13.85	12.81	13.72
Shade level mean	14.90	14.81	14.12	13.04	

S.e.d for comparing two shade means = 0.08 (9df)
S.e.d for comparing two clone means = 0.19 (108df)
S.e.d for comparing two clones at a single shade level = 0.39 (108df)
S.e.d for comparing two differences between two clones for two shade levels = 0.55 (108df)
S.e.d for comparing two shade levels, either for the same clone or for different clones = 0.37 (no exact df).

CONCLUSIONS

The significant shade x clone interaction implies that matching specific clones to appropriate shade intensities can be exploited to optimize Robusta coffee yields in agroforestry systems. There are significant trade-offs between short-term maximization of yields under full sun and long-term productivity with high values of environmental services associated with coffee agroforests. The identification and utilization of Robusta coffee genotypes which are productive under shade could mitigate some of the negative aspects of the trade-offs.

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Shikimic Acid Accumulation and Plant Injury in *Coffea Arabica* after Simulated Glyphosate Spray Drift Exposure

L. SCHRÜBBERS[1], J. C. SØRENSEN[1], B. E. VALVERDE[2,3], N. CEDERGREEN[1]

[1]University of Copenhagen, Faculty of Life Sciences, Department of Basic Sciences and Environment, Frederiksberg C, Denmark
[2]University of Copenhagen, Faculty of Life Sciences, Department of Agriculture and Ecology, Frederiksberg C, Denmark
[3]Investigación y Desarrollo en Agricultura Tropical S.A., Alajuela, Costa Rica

SUMMARY

Research is being conducted to evaluate the effect of a simulated spray drift exposure to glyphosate (the active ingredient in Roundup®) of coffee (*Coffea arabica*) plants. *In vivo* shikimic acid concentration is being assessed as a biomarker to confirm an unintended glyphosate exposure. To simulate spray drift, glyphosate was sprayed at doses ranging from 11 to 1080 g a.e. (acid equivalent)/ha with the highest dose corresponding to the recommended dose (RD). Estimated ED_{50} values (50% effect dose) were 3.5 – 50.9% of the RD depending on the endpoint (growth, damage, leaf area, presence of symptoms, leaf biomass). The best sampling time for shikimic acid as biomarker under the applied growing conditions was two weeks after exposure to glyphosate.

INTRODUCTION

Glyphosate is the world's most ubiquitously used herbicide. It is commonly used in coffee plantations to combat weeds that compete with the crop for resources such as light, water and nutrients. Glyphosate is a non-selective, broad spectrum herbicide thus possessing the risk of crop injury through unintended crop exposure from spray drift. Once taken up, glyphosate is translocated throughout the plant following primarily photosynthate transport patterns in the phloem from source to sink tissue. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which leads to, besides visible symptoms, an accumulation of shikimic acid in plant tissue. Low spray drift exposure has shown to decrease yield in various crops. Spray drift exposure can be as high as 16% of a regular dose. Coffee plants are negatively affected by sub-lethal doses, though references on shikimic acid levels have not been found. Two experiments have been carried out; 1.) Coffee plants (*Coffea arabica*) were sprayed with seven different doses in order to estimate ED_{50} values for a range of endpoints and 2.) Coffee plants were sprayed with three sub-lethal doses and their leaves subsequently sampled at four different times to determine the best sampling time to use shikimic acid as a biomarker for glyphosate exposure.

MATERIALS AND METHODS

Coffee plants cv Nana were grown under greenhouse conditions (day/night; 26/20 °C, 12 h/12 h). For the estimation of ED_{50} values plants 31.5 cm tall (13.5 % relative standard deviation, RSD) bearing 31 leaves per plant (24.1 % RSD) were used. Plants were 1.5 years old and had been grown under suboptimal spatial conditions that limited their development. Six weeks before spraying plants were repotted and thereby grown under better conditions. For both experiments glyphosate was sprayed "over-the-top" using a spraying cabinet

equipped with Hardi LD-02-110 hydraulic nozzles delivering 150 L/ha at 4 bar. All spray solutions contained 1% v/v Tween 20 (lowered to 0.1% Tween 20 for the shikimic acid accumulation study). The doses used were 0, 11, 28, 69, 173, 432 and 1080 g a.e./ha, corresponding to percent levels of 3 L Roundup®/ha of 0,1.0, 2.6, 6.4, 16, 40 and 100%, with 100% being equal to the recommended dose (RD). On the spraying day plants were marked on the main stem to be able to distinguish between damage of the complete plant ("total damage") including older plant parts and the damage solely to newly grown parts after glyphosate exposure ("damage of the new growth"). Eight weeks after spraying, total leaf area per plant, the degree of "damage", percentage of plants exhibiting visual glyphosate symptoms, new growth, and leaf biomass were assessed. Leaf area was measured using a LI-3100 Area Meter (LI-COR Inc, USA). Symptoms and damage (total or new growth only) were assessed visually in relation to the untreated controls. All three visual endpoints were determined in a blinded manner. Data was described with dose-response curve (DRC) models using the open-source statistical software, R and the complementary DRC-package. The basis for the DRCs was a 4-parameter log-logistic function (LL.4, equation 1) where *d* denotes the upper limit, and *c* is the lower limit. The parameter *e* is the dose at which the value of *d* - *c* is reduced by 50% (ED_{50}), and *b* is proportional to the slope around ED_{50}. For the growth and the plant damage data a 3 parameter log-logistic function (LL.3) was used where the lower limit was set to 0 compared to the LL.4. For the leaf area the lower limit was set to the level of untreated plants (n=7) at the day of spraying. For the leaf biomass the lower limit was determined in the same way whereas the upper limit was fixed at the biomass obtained by the untreated plants after 8 weeks. For the symptom appearance a 2 parameter log-logistic (LL.2), binomial function was used with the upper limit set to 100% and lower limit 0%.

$$y = \mathrm{c} + \frac{\mathrm{d} - \mathrm{c}}{1 + \exp\{b[\ln(x) - \ln(e)]\}} \quad (1)$$

For the shikimic acid accumulation experiment, plants seven months old, 16-36 cm tall at the 12-20 leaf stage were used. The plants were sprayed with glyphosate at 0, 86, and 432 g a.e./ha. The four youngest, fully developed leaves were sampled after 1, 2, 4 and 8 weeks. Shikimic acid was determined according to Petersen et al with slight modifications. Briefly; 250 mg freeze-dried leaf tissue was extracted 3 times with 3 mL boiling methanol (70%) using a homogenizer (Ultra Turrax T 25, Janke & Kunkel, Staufen, Germany). The solvent was evaporated to dryness in a heating block (40°C) and dissolved in 1 mL running buffer and analyzed by capillary electrophoresis (CE) and UV–light detection (Hewlett-Packard HP^{3D} CE, model G1600AX, Hewlett-Packard, Waldbronn, Germany). For statistical comparison SigmaPlot (12.3, Systat Software Inc, USA) was used.

RESULTS AND DISCUSSION

Glyphosate-treated plants began to develop slight symptoms one week after spraying and more clearly after two weeks. New leaves became narrow, bent and discolored. Old leaves already fully developed at the time of herbicide exposure in plants treated with the highest herbicide doses turned darker green and appeared less waxy and stiffer than normal. None of the plants died even at the full dose. With increasing doses, plant damage (total or plant parts grown after the exposure) and percentage of plants showing symptoms increased, while the leaf biomass, the leaf area, and the increase in height from the start of the experiment (new growth) decreased (Figure 1).

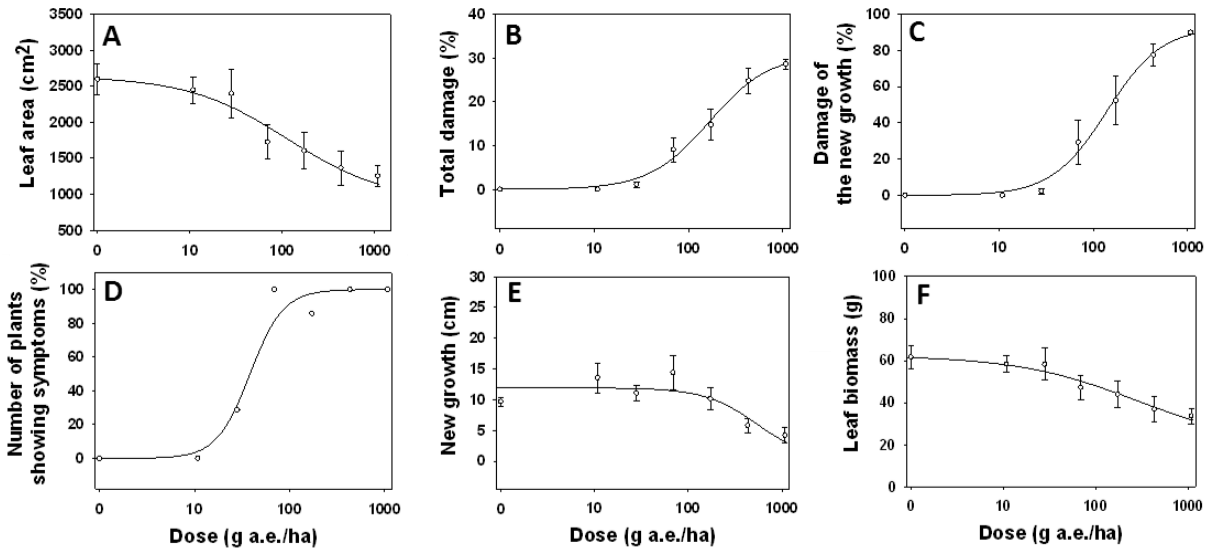

Figure 1. Response of coffee plants to increased doses of glyphosate at 8 weeks after foliage spray application. Different endpoints are plotted against dose in g a.i./ha. Data are given as mean ± SE (n = 7 for treated plants and n = 10 for controls). A: Leaf area; LL.4 with fixed lower limit to the level of untreated plants on the spray application day (903 cm^2), B: Total damage compared to control plants; LL.3 with fixed lower limit to 0%. C: Damage of the new growth compared to control plants; LL.3 with fixed lower limit to 0%. D: Percentage of plants with glyphosate symptoms per treatment; LL.2, binominal with upper limit fixed to 100% and lower limit fixed to 0%, E: New growth; LL.3 with fixed lower limit to 0 cm. F: Leaf biomass; LL.4 with fixed lower limit to the level of untreated plants on the spraying day (19.5 g) and fixed upper limit to the level of control plants (62.4 g) after 8 weeks.

The ED_{50} values differed depending on the measured variable from 3.5% of the RD for glyphosate symptom development to 51% for the increase in height at 8 weeks after treatment (Table 1).

Table 1. Estimated ED_{50} values for the different evaluation parameters.

Endpoint	**ED_{50} in g a.e./ha (SE)**	**ED_{50} in % of RD; 100% = 3 L Roundup®/ha**
Symptoms	38 (9)	3.5
Leaf area	113 (55)	10.5
Damage (new growth)	139 (35)	12.9
Damage (total)	166 (44)	15.4
Leaf biomass	280 (120)	25.9
New growth	550 (189)	50.9

SE = standard error.

How transferable the data is to the field is difficult to determine. In general plants are about 1 year old when transferred from the nursery to the field. The plants used in this study were 1.5 years old but smaller than regular. The formulation used was artificial and commercially formulated products might have a higher efficacy. Certainly, environmental factors are important as well. Nevertheless, the ED_{50} values for the symptoms, leaf area decrease and plant damage are in the range of a possible spray drift expose scenario (Table 1). In general, visual symptoms were the most sensitive parameter, while the actual growth in canopy height

was the least sensitive within the frame of the experiment. Similar results with respect to plant injury, leaf area decrease, and growth reduction were also observed in coffee by Franca et al but no ED_{50} values were estimated. Our findings justify further research on the unintended exposure of coffee plants with glyphosate, especially since it remains unknown if the yield is affected as well.

Shikimic acid slightly but non-significantly accumulated in coffee plants treated with either 8 and 40% glyphosate after one week but its concentration increased at two weeks remaining high until the fourth week in those plants that received the highest dose (Figure 2).

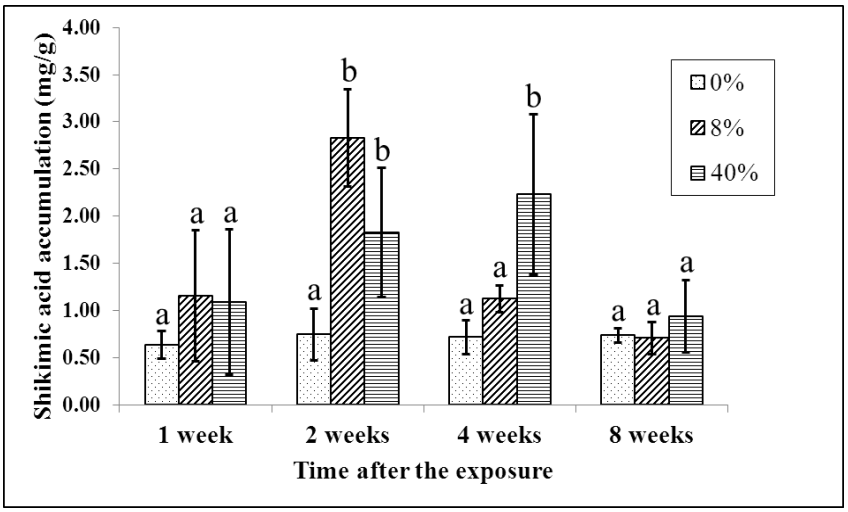

Figure 2. Shikimic acid accumulation at different time points. Different letters denote a significant difference at one sampling point. 3 different doses have been applied; 0% (controls), 8% (86 g a.e./ha) and 40% (432 g a.e./ha) of a RD.

After 8 weeks the shikimate concentration returned to levels similar to those of untreated plants. Interestingly, after two weeks there was no significant difference between the two doses of glyphosate even though the high dose was five times more than the low one. Neither was the total accumulated amount of shikimic acid higher for the 40% than for the 8% dose. It seems that under these growing conditions; the optimal sampling time to detect a possible increase in shikimic acid levels as a result of a glyphosate exposure is two weeks after spraying. These preliminary tests are the basis for the ongoing detailed evaluation of glyphosate drift effects in coffee.

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Injury Profiles in Coffee Are Dependent on Production Situations: Case Studies in Costa Rica

C. ALLINNE[1,2], M. BARQUERO-MIRANDA[3], A. ROMERO-GURDIÁN[2], S. SAVARY[5], J. AVELINO [1,2,4]

[1]Cirad
[2]CATIE
[3]ICAFE
[4]Promecafé
[5]INRA

SUMMARY

An injury profile is a combination of injury levels caused by different pests and diseases. A production situation is defined as the combination of factors (physical, biological and socio-economic) determining agricultural production. Linking injury profiles with production situations is a way (i) to identify factors influencing pests and diseases development and (ii) to explore management options to reduce crop losses due to pests and diseases in each specific production situation.

We used data from Avelino et al. (2007) where 141 coffee plots from Costa Rica were characterized by assessing 17 variables describing the topo-climatic conditions, the crop management, and the coffee plant characteristics. The incidence of 10 pests and diseases was also evaluated.

We demonstrated that injury profiles were determined by a combination of factors (topo-climatic conditions, physiological crop characteristics and cropping practices) in interaction (situation production). Specific management options can be identified to reduce incidence in each combination. We also show that existing trade-offs between yield, ecosystem services provided by shade and pests regulation. Trade-offs must be assessed to propose sustainable solutions.

INTRODUCTION

A recent study highlighted the need to quantify crop losses caused by pests and diseases in coffee and cacao crops, in order to better quantify the costs and values of ecosystem services provided by these tropical tree crops (Avelino et al., 2011). Agroforestry systems provide multiple ecosystem services whose economic value has been recognized by payment schemes particularly in Costa Rica with the establishment of the FONAFIFO fund. To be interesting, payment must be higher than the cost of implementation and management of agroforestry systems in comparison to conventional monocropping systems. Payment also must compensate eventual losses, especially those caused by pests and diseases which are frequently favoured by shaded conditions. There is therefore a need for quantifying losses caused by pests and diseases in agroforestry systems to support farmers' decisions and to better establish the amount of the economic incentives for the provision of ecosystem services. The first step in the economic loss quantification is the yield loss assessment caused by all the pathosystem.

Studying injury profiles is a way i) to quantify crop losses caused by pests and diseases, ii) to better quantify the costs and values of ecosystem services provided by coffee agroforestry systems, iii) to propose innovative pest management strategies and reduce the use of chemicals to control pests and diseases.

The objective of this study is first to link injury profiles to production situation typologies and second to identify management options to reduce crop losses due to pests and diseases in each specific production situation.

MATERIALS AND METHODS

Sampling and data collection

Data were obtained during two surveys (Avelino *et al.*, 2007,2009, 2012; Romero, 2010) performed in five regions: Turrialba (53), Western Valley (21), Central Valley (24), Tarrazu (24) and Coto Brus(19). The study area, the plot sampling methods and the plot descriptors have already been described in (Avelino *et al.*, 2007; Avelino *et al.*, 2009).

Production situation descriptors: Total of 141 production situations were describe using 17 variables: crop management practices description (density of coffee trees per hectare, number of coffee trees per hole, shade percentage, annual number of coffee trees pruning operations, annual number of shade pruning operations, annual number of fertilisations, annual number of weeding rounds, annual number of fungicide sprays), physiological crop characteristics description (height of the coffee tree, coffee tree age, number of fruiting nodes per plant, foliage density) and topoclimatic conditions (altitude, annual rainfall, slope inclination and soil description).

Pest and diseases descriptors

In each production situation, incidence of 10 pests and diseases was assessed: leaf rust (*Hemilia vastatrix),* leaf minor (*Leucoptera coffeicola*), brown eye spot disease (*Cercospora coffeicola*), thread blight (*Corticium coleroga),* American leaf spot disease (*Mycena citricolor*), dieback (*Colletotrichum coffeanum*), root-lesion nematode (*Pratylenchus coffeae*), root-knot nematode (*Meloidogyne exigua*), ceratocystis canker (*Ceratocystis fimbriata*), coffee blight (*Phoma costaricencis).*

Statistical data analysis

Synthetics variables were compute from factor analysis performed with the variables describing soil: silt, clay and sand proportion and chemical composition (pH and concentration of K, Ca, Mg, Al, P, Fe Cu, Mn, and Zn). The first three components were included as variable in the following analyses.

Typologies were built for topoclimatic data, crop characteristics data, cropping practices data and pests and diseases data using a hierarchical clustering. Associations between profiles were assessed by Chi-Squared test and represented by a Correspondence Factor Analysis (CFA). Indicator of potential yield was built (number of fruiting nodes per plant x plant density) and classified in four equal classes to be added in additional variable in the correspondence analysis. Location was also added as supplementary variables.

RESULTS AND DISCUSSION

Three types of soils were obtained from principal component analyses (figure 1). Four clusters were obtained by topoclimatic typology, three clusters were obtained for physiological crop characteristics typology, three clusters were obtained by crop practices typology and three injury profiles were obtained by pests and diseases typology.

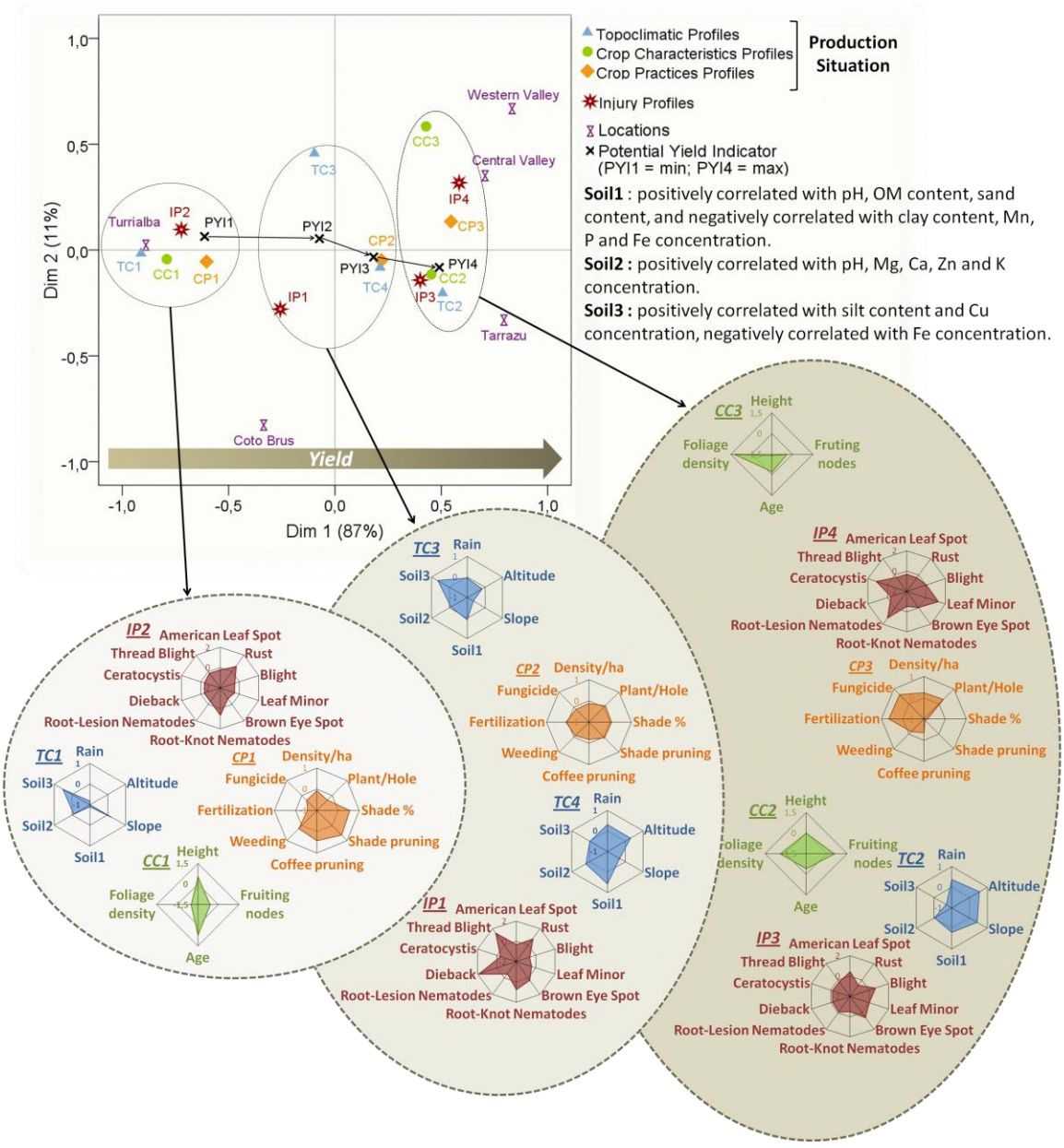

Figure 1. Correspondence Factor Analysis representing the associations between crop management practices profiles, physiological crop characteristics profiles and topoclimatic conditions profiles. Indicator of potential yield and location were added as supplementary variables on CFA.

Injury profiles are significantly related to production situations, i.e. topoclimatic conditions, crop characteristics, crop practices. Three type of combination of associations were observed (figure 1). The first combine low incidence injury profiles with extensive systems, aged plantations composed of tall trees with sparse foliage and few productive, and with by low altitude regions. This combination is mainly found in Turrialba region. In these situations

lower shade cover could be explored to reduce high leaf rust incidence due to shade cover in interaction with low altitude regions. But lower shade cover means reduce other ecosystem services provide by shade tree (carbon sequestration, biodiversity conservation, nutrient cycling, preserving soil from erosion). Balance of the system must be assessed before taking management decision.

The second combine high incidence injury profiles with conventional systems and with two types of topoclimatic conditions (TC3 and TC4). These production situations are present in each region.

The third combine two high incidence injury profiles with technified systems, very productive young plantations (CC2) and small trees with dense foliage (CC3), and with high rainfall, high altitude, and high steep regions. These are typical of highly productive coffee cropping system in altitude regions. In this combination, reducing injury levels could be considered in several ways. Indeed, strong pruning favors the transmission of Ceratocystis that could be controlled adopting specific measures during coffee pruning operations. Furthermore, systematic eradication of weed reduce habitat for leaf minor auxiliaries. Encourage or protect natural enemy populations already present in the crop ecosystem (developing living hedge or grass strip) could be a way to better control of leaf minor. Then, high density favours spread of root lesion nematode (by root proximity) as well as American leaf spot disease (by plant proximity and dense foliage). Reducing plant density could reduce incidence of these two pests.

Injury profiles are not strictly related to region. Each region comprises several production situations. Injury profiles are determined by a combination of factors in interaction (situation production). Specific management options can be identified to reduce incidence in each combination. This could have implications when ecosystem services are considered. Indeed, trade-offs between service of pest and disease regulation and services associated to shade (biodiversity, carbon sequestration) depend on the production situation. It means that all production situations are not equal in terms of costs and gains with respect to ecosystem services provision.

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Damage of Arabica Coffee Caused by Coffee White Stem Borer (*Xylotrechus Quadripes*) in Indonesia.

S. WIRYADIPUTRA, S. MAWARDI

Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java, Indonesia

SUMMARY

Coffee white stem borer (CWSB, *Xylotrechus quadripes*) is well known as a main coffee pest in India and causing significant losses of production on Arabica coffee. It is very important insect pest in Arabica coffee and difficult to control. The pest together with coffee berry borer as main causing agents for the decline of India's coffee exports in the 2011-2012 crop year as amount of 16.7 %. An estate of Arabica coffee in East Java, with acreage of around 1348 ha, has been infested with CWSB of about 206.2 ha or about 15,3 %, and total number of coffee trees infested is more than 2.61 million. Expanding and heavy infestation of CWSB in that estate is presumed to be in connection with the opening of shade trees during the last decade. As stated in several references that the beetles are active during bright and hot weather. Female beetles lay eggs in the cracks and crevices of the bark and under the loose scaly bark of the main stem and thick primaries, preferring the plants exposed to sun light. Preliminary trial with trapping using pheromone trap produced by PCI, India, revealed that the male beetles were attracted by the trap. Installation of five traps during three weeks, it has been caught 24 beetles of the pest. It was indicated that pest species found in East Java was the same as CWSB infested coffee in India. In the future, it needs deeper study of many aspects of the pest in Indonesian conditions.

INTRODUCTION

According to USDA report, India's coffee exports are expected to decline by 16.7 per cent to 2,40,000 tones in the 2011-12 crop year (October-September) due to lower production and tight carry-over stocks. Low production, especially caused by coffee pests such as the white stem borer and berry borer will be continued to affect coffee yields (Anonim, 2011). As a result, robusta variety has replaced arabica due to its lower susceptibility to diseases and higher productivity. Robusta is slowly replacing arabica as the latter's output is affected by a white stem borer attack on the crop (Business Standard, August 2011). In India, coffee white stem borer (*Xylotrechus quadripes* Chervr.)(Coleoptera: Cerambycidae) is the most serious pest of Arabica coffee. Hall *et al*.(?) reported that loss of production due to *X*.*quadripes* on Arabica was a capital loss caused by the need to uproot and replace infested plants, and it was estimated that the national loss due to this pest was 130 million rupees or about £ 2 million per year. Arabica coffee is the most preferred and principal host but it can also complete its life cycle in Robusta coffee (*Coffea canephora*), tree coffee (*Coffea exelsa*) and *Olea dioica*.

In Indonesia (Java), Le Pelley (1968) cited that *Xylotrechus javanicus* was known as also coffee white stem borer but did not attract much attention and was not great importance. It was synonymous wih *X. quadripes* according to Duport, despite not generally accepted. In last decade, an insect pest was found attacking Arabica coffee in Esat Java, and was determined belong to Coleoptera order and Cerambycidae family. Morphologically, it is very similar to the white stem borer attacking Arabica coffee in India. Infestation in several Arabica estates quite heavy and the planters worried very much since the coffee production

gradually decreased. This paper presents occurrence of the pest on Arabica coffee in Indonesia.

WHITE STEM BORER ON COFFEE IN INDONESIA

Identification of the pest

A symptom of Arabica coffee attacked by stem borer was as showed in Figure 1A. Arabica coffee trees attacked by the pest showed coffee trees are decline in growth, yellowing, and finally dying. The older attacked trees will be less productive and yielding more floats. On the damaged coffee stem showed externally visible ridges and exit holes of the adult beetle (Fig. 1B). Identification of the insect morphologically agrees with *Xylotrechus quadripes* Chevr. It is the same with the borer found in India and also matchs with identification conducted by Hayashi and Makihara (1981).

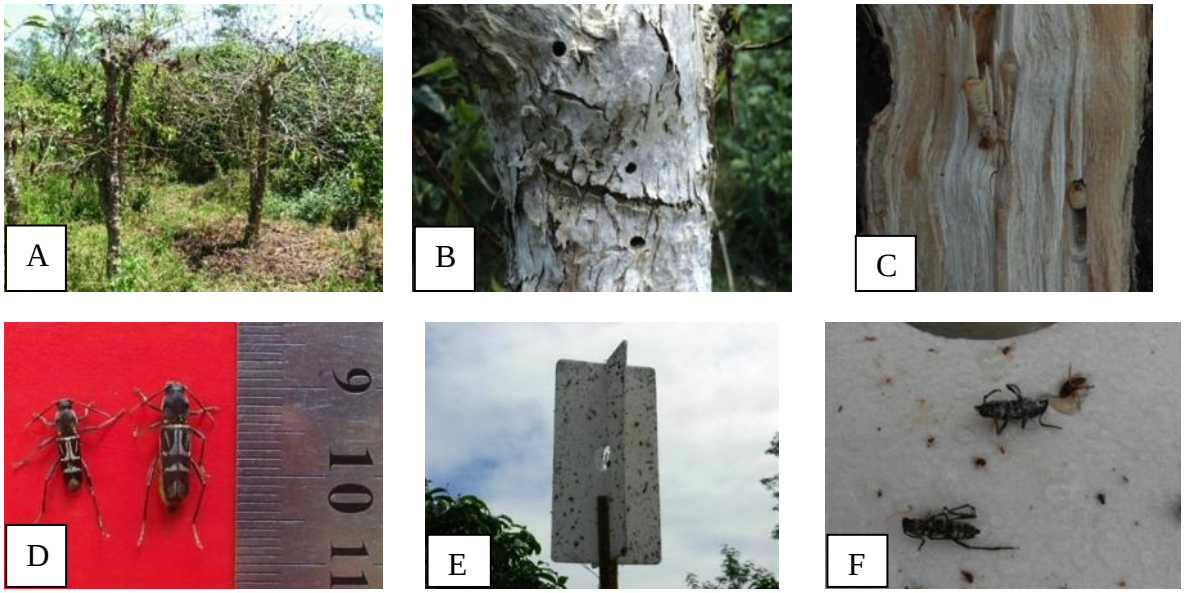

Figure 1. Arabica coffee attacked by white stem borer in East Java (A), coffee stem with ridge and exit holes of adult beetle (B), larva of the insect in the tunnels (C), male and female adult insects (D), pheromone trap installed in affected coffee estate (E), and adult insect captured in pheromone trap (F).

Initially, CWSB was not considered as important pest on Arabica coffee in some estates in East Java until about 1995s, as cited also by Le Pelley (1968). Formerly, cultural practice at these coffee areas was using two to three tier of shade trees, such as Albizzia (*Paraserianthes falcataria*), Dadap (*Erythrina* spp.) and Leucaena (*Leucaena* spp.), but in the end of 20th century cultural technique was changed by decreasing of shading trees in order to increase the productivity. As a result the microclimate also changes to drier condition and coffee plants to be in stress. Several problems of pests and diseases occurred after that, especially parasitic nematodes and CWSB. In India, beetles are active during bright and hot weather. Female beetles lay eggs in the cracks and crevices of the bark and under the loose scaly bark of the main stem and thick primaries, preferring the plants exposed to sun light.

Economic Importance of the pest in Indonesia

Arabica coffee areas affected by CWSB in East Java threaten sustainability the commodity and will impact on productivity in the future. Infestation of the pest at the moment is quite severe (Table 1).

Table 1. Infestation of coffee white stem borer on Arabica coffee in one estate in East Java, Indonesia.

Estate Division	Total coffee acreage (ha)	Infested coffee acreage (ha)	% Infested areas
Division 1	106.21	14.10	13.28
Division 2	345.58	60.83	17.60
Division 3	86.18	15.32	17.78
Division 4	79.36	9.45	11.91
Division 5	82.45	18.66	22.63
Division 6	55.72	6.16	11.06
Division 7	158.08	49.48	31.30
Division 8	231.03	22.94	9.93
Division 9	203.91	9.30	4.56
Total	1348.20	206.24	

It will also threaten Arabica coffee production in that areas, as shown in Figure 2. During six years, the production of Arabica coffee in this area decreased, despite of a fluctuation as a result of biannual bearing.

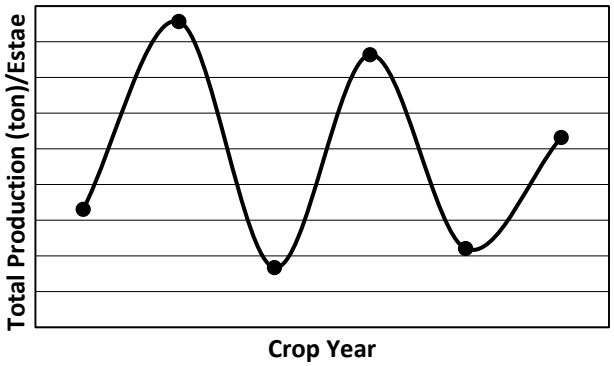

Figure 2. Production of Arabica coffee in an estate infested by white stem borer in East Java, Indonesia.

RECOMMENDATION FOR CONTROL MEASURES

Integrated pest management (IPM) for the pest in India has been developed in advance. It can be adopted in Indonesia to solve the problem. In the first step, cultural practice should be changed and back to cultural method practiced before 1990s. It means coffee plants must be shaded by shading trees because the pest prefers open coffee plantation than coffee shaded areas. It also has several advantages since coffee plantation will be more sustainable and more environmentally friendly. Pheromone trap has also been tried in the area infested and the result was very prospective to control the pest (Figure 1E and 1F). At the moment, estate

management practiced uprooting the heavily affected coffee trees and then replanting with new coffee seedling. This practice is very costly and has not controlled the pest effectively. The prospective methods for management of the pest are the application of biological control. It needs to explore and develop biological agents which are effective to control the pest. Vega *at al*. (2006) mentioned that entomopathogenic fungus, *Beauveria bassiana* was associated with low infestation (~ 2.5 %) of the pest in India.

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Profile of Rural Properties of an Association of Familiar Coffee Planters in Southern Minas Gerais, Brazil, in Relation to Good Agricultural Practices in Coffee (*Coffea Arabica* L.) Cultivation[1]

S. P. PEREIRA[2], B. T. ROSA[3], R. J. GUIMARÃES[4], C. M. G. AGUIAR[5], M. M. D. ROMANIELLO[4], E. E. SILVA[6]

[1]This work is part of a project funded by Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG and of the first author's thesis in progress at Universidade Federal de Lavras – UFLA
[2]Researcher at Instituto Agronômico de Campinas – IAC
[3]Student on a scholarship from FAPEMIG
[4]Teacher at UFLA
[5]Journalist at UFLA
[6]Manager at Incubacoop UFLA

SUMMARY

This study aimed at categorizing the rural properties of coffee planters of an association in Southern Minas Gerais - Brazil, according to the performance in relation to Good Agricultural Practices (BPAs) in coffee plantation. With the applied methodology, it was able to distinguish two different coffee planter groups, in accordance to their performance in relation to BPAs, in which coffee planters from Group 1 were better adapted than planters from Group 2.

INTRODUCTION

Brazilian agriculture and cattle breeding, especially coffee culture, has gone through structural transformations for the past four decades that resulted in a new insertion in the international socioeconomic scenario. It is true that there is an increasing preoccupation with the production of agro-foods, regarding socio-environmental criteria, among wholesale networks and consumers. Brazil is the biggest world provider of certified sustainable coffee, besides being the biggest world producer and exporter of commodities and second biggest coffee consumer.

Due to consuming market pressure, great corporations of the world coffee sector announced, about two years ago, the increase in certified sustainable coffee commercialization in their products portfolio. Some of these companies' goals are reasonable, some others not much, and the demand for certified sustainable coffee may overcome the offer. The Market notices that the certified coffee offer from other countries tends to stagnate and that Brazil shall be the greatest provider of these coffees for the world.

The certification models, regardless of the seal or appeal that they are provided with, consider social, environmental and economic dimensions, also known as Triple Bottom Line or Sustainability Tripod. Sustainability in coffee properties is narrowly connected to Good Agricultural Practices (BPAs) in coffee cultivation. It is about rationalization in coffee production, based on technique adoption criteria and procedures preconized by agro-cattle breeding research.

So that Brazil be kept as a leader in certified coffee production and exportation, and so that it can insert a larger number of coffee planters in this Market, private and public incentive policies for adaptation to BPAs are required. Programs of incentive to certification and adequacy to conduct codes in vogue are, in fact, Programs of Technical Assistance and Rural Extension (ATER), since there are no other performance forms, other than a close accompaniment of an extensionist, who will indicate the bottlenecks and the solutions for each property, based on BPAs precepts.

In this process, a good way to insert familiar agriculturists and decrease the costs of audits and inspections on properties has been the adoption of a group certification. This modality has been being applied as a facilitator to guarantee access to a differentiated market. Before searching for adequacy of coffee properties to conduct codes, it is necessary to get acquainted with the reality of such properties individually, or with the performance of a set of properties, being able to categorize them according to their adequacy to BPAs. This division of a group of coffee planters in clusters, or smaller groups, is necessary when it is intended to create private or public ATER policies and afterwards, make the certification in group viable.

Being study object to this research, AFASA - Association of Familiar Agriculturists of Santo Antonio do Amparo was born from a project named Coffee Force, funded by Hanns R. Neumann Stifung Foundation in Brazil, linked to Neumann Kaffee Gruppe. This research is justified by the fact that it proposes distinct actions of technical assistance and rural extension, aimed at different groups in the association.

The aim of the current study was to categorize rural coffee properties (AFASA) in the South of Minas Gerais State, according to their performance in relation to Good Agricultural Practices (BPAs) in coffee cultivation.

METHODOLOGY

To carry out this work, the following steps were used:

- The questionnaire was elaborated by a group of Agronomists and Technicians with wide experience in Coffee Culture in COCAPEC - Cooperative of Coffee Planters and Agro-Cattle Breeders, settled in Franca, SP. The questionnaire was made collaboratively and based on the main norms, conduct codes of certification programs and laws that are in vogue in countries that report to this agricultural issue. The questionnaire was applied and validated in 2008, in 251 properties in Alta Mogiana, by technicians from COCAPEC, and aided by Sebrae-SP.

- In this kind of research, the obtention of a representative sample is an important matter. The research was done with 32 coffee planters (total universe of members of AFASA back then) between May and June 2009. It allowed the adoption of a probabilistic sampling, reaching the whole population.

- The applied questionnaire was a survey type and had 158 questions that were analyzed by means of the statistical software SPSS (Statistical Package for the Social Sciences). The questionnaire comprises a survey of BPAs through a three point scale, in which the answers regarding adequacy could be: “yes”, “partially” or “no”, and also “not applicable”. The variables “not applicable”, which comprised over 50% of the properties, were later excluded from the analysis.

- Having the results of the 32 coffee planters' questionnaires, regarding BPAGs survey, a Cluster analysis was made. The aim of this analysis is to organize data within a determined structure, which allows making groups in which the elements will show similarities. The used methodology was hierarchical agglomerative cluster, which allows obtaining the total group by summing the subgroups. The agglomerative cluster was processed by Ward's Method, the most used methodology, for it combines individuals within clusters, in accordance with the lower increment criteria of total sum of Euclidian distance to the square inside the cluster.

- After the separation by cluster analysis, a discriminating analysis was made, presenting the identified variables that caused the biggest divergence or differentiated both groups of producers. Malholtra (2001) defines that the discriminating analysis or linear combinations divide the variables that best discriminate the categories of the dependent variable (groups).

It is important to highlight that, in this work, there is no hypothesis to be confirmed, but it is intended to gather elements with given similarity.

RESULTS AND DISCUSSION

Based on all variables, a cluster analysis was made on SPSS. According to Malhotra (2006) and Hair Jr. et al. (1995), Cluster is a technique in which there is no dependence among the variables. Thus, the analysis classifies the individuals in homogenous groups or conglomerates names Clusters. It is understood that groups created by this analysis are similar among them (within a minimum variance) and different from other clusters (between clusters, the variance is maximum).

In Figure 1, it is observed the dendogram, which is a specific type of diagram that organizes determined factors and variables. It results from a statistical of given data, applying a quantitative methods, which leads to groups and to their ascendant hierarchical organization. Namely, the diagram illustrates the arrangement of groups derived from the application of a Clustering algorithm.

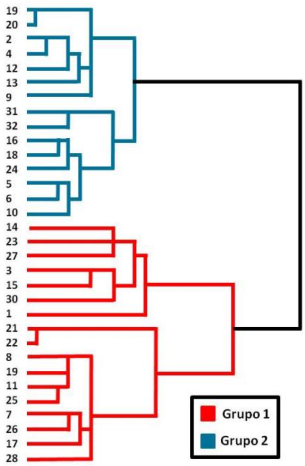

Figure 1. Division of AFASA coffee planters' population in two clusters (groups).

According to the representation, it can be clearly observed the division in two groups of coffee planters. Among the 32 interviewees, all of them participated in the division, being that

Group 1 comprises 17 coffee planters (53.13%) and Group 2 has 15 coffee planters (46.87%). The idea to divide the interviewees aims to analyze whether there is any difference between both groups' profiles, and thus, categorize them.

Table 1 shows the identified variables or questions that caused the greatest divergence between both groups. Out of twenty variables, 15 show the superiority of Group 1 over Group 2. In three variables, both groups showed similar performance and in two questions, Group 2 had better adequacy than Group 1.

Table 1. Variables that differed both groups of producers in accordance to their performance of Good Agricultural Practices.

DISCRIMINATING ANALYSIS		No	Partially	Yes	Not applicable
Do you have the registration/quality control of the coffee you sell?	*Group 1*	5,9%	23,5%	70,6%	
	Group 2	53,3%	40,0%	6,7%	
Do you have the registration/control of field operations?	*Group 1*	17,6%	23,5%	58,8%	
	Group 2	80,0%	6,7%	13,3%	
Do you use the internet?	*Group 1*	41,2%	11,8%	47,1%	
	Group 2	100,0%	0%	0%	
Are the phytosanitary products kept apart from food and fodder?	*Group 1*	0%	17,6%	82,4%	
	Group 2	40,0%	33,3%	26,7%	
Do you wash pulverizers / tractors / implements in an adequate place?	*Group 1*		17,6%	64,7%	17,6%
	Group 2		40,0%	53,3%	6,7%
Do you use adequate beaks, nozzles for each type of target?	*Group 1*		29,4%	70,6%	
	Group 2		86,7%	13,3%	
Do you have enough terrain for coffee production?	*Group 1*	11,8%	47,1%	41,2%	
	Group 2	13,3%	80,0%	6,7%	
Do you remove coffee from the terrain on both directions, several times a day?	*Group 1*		76,5%	23,5%	
	Group 2		73,3%	26,7%	
Do you use bags in good conditions? Clean, without scent and/or fungi?	*Group 1*			100%	
	Group 2		6,7%	93,3%	
Do you do or have you ever done PEPRO?	*Group 1*	64,7%		35,3%	
	Group 2	100,0%			
The control of invasive plants (weed) is duly made?	*Group 1*		11,8%	88,2%	
	Group 2		13,3%	86,7%	
Do you get rid of water used on machine and implement cleansing in a proper place?	*Group 1*	5,9%	11,8%	58,8%	23,5%
	Group 2		40,0%	33,3%	26,7%
Is the source of water used in pulverizations adequate?	*Group 1*	11,8%		88,2%	
	Group 2	6,7%	20,0%	73,3%	
Is the mechanized or back pulverizer in good conditions for applications?	*Group 1*		17,6%	82,4%	
	Group 2			100,0%	
Do you follow and register the number of pumps used in pulverization?	*Group 1*		23,5%	76,5%	
	Group 2	33,3%	40,0%	26,7%	
Do you calibrate the equipment before each application?	*Group 1*	41,2%	5,9%	52,9%	
	Group 2	100,0%			
Do you prioritize the beginning of harvest with less than 5% of green grains?	*Group 1*		41,2%	58,8%	
	Group 2		66,7%	33,3%	
Do you make furrows respecting the declivity of the terrain?	*Group 1*		23,5%	76,5%	
	Group 2		13,3%	80,0%	6,7%
Do you have all legal reservation areas delimited and registered?	*Group 1*	41,2%	5,9%	52,9%	
	Group 2	20,0%	26,7%	53,3%	
Do the workers have a proper place for meals?	*Group 1*	17,6%	23,5%	58,8%	
	Group 2		40,0%	60,0%	

The methodology used was able to distinguish two groups of coffee planters in accordance to their performance in relation to Good Agricultural Practices. The coffee planters in Group 1

showed better adequacy to BPAs than coffee planters from Group 2. Based on this data, it is possible to carry out the Technical Assistance and Rural Extension, in accordance to each group's technological and management needs.

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Coffee Yield Variations and their Relations to Rainfall Events in Nicaragua

L. LARA[1A] , J. HAGGAR[1], D. STOIAN[1], B. RAPIDEL[2]

[1]Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica
[2] CIRAD, UMR SYSTEM, Montpellier, France

SUMMARY

In order to predict the impacts of foreseen climate change on coffee production, one first step would be to check the impacts of past climate variations on coffee yields. We developed a survey in coffee zones in Nicaragua to compile the historical registers made by farmers on yields, rainfalls and temperature daily, management and blossoming date, and analyze their relationship to specific climate events. The farmers' perceptions on climate risks and actual damages were also investigated. A simple model was then developed, that links coffee phenology, rainfall effects on flowering and soil water balance. Coffee yield data were obtained from 23 farms, over a span ranging from 6 to 78 years. The Pacific Zone, and the most ancient coffee zone, presented the longest series of yields and rainfalls. Coffee yields are much more variable in this region than in the North Zone (variation coefficient 33% vs. 18%, resp.).Farmers' perceptions agreed with this finding, with much higher risks perceived in the Pacific Zone. Drought and rainfall excess alike were identified as causing the highest risks, temperature variations were not reported, possibly because they are much less easy to perceive than rainfall variations. The blossoming period was perceived, in both regions, as the most sensitive period, to drought as well as to rainfall excess. Drought events are perceived as more frequent. Very long series on blossoming dates and intensities allowed us to build and calibrate a model, based on rainfall and temperature, to estimate the rainfall during blossoming and the resulting yield loss. Rainfall over 40 mm during the blossoming could reduce the yield from 60%. Alternative practices are discussed that could mitigate the risks identified in the risk-prone Pacific zone.

INTRODUCTION

Climate change is expected to impact heavily on coffee production in the next decades. However, these expectations are based on chains of models with significant uncertainties. One first step to ascertain these impacts would be to check the impacts of climate variation on coffee yield in the past. However, this work is rarely done due to the extreme rarity of historical coffee yields records in Central America, particularly at the finer scales. At higher scales, where data are more currently recorded, yields can only be calculated as a ratio of production per area, both variables having their own uncertainties, resulting in even higher uncertainties on yields.

The analysis of statistical series to extract the particular effect of climate on crops is always blurred by various factors: the evolution of cultivation practices, triggered by technical progress, current or past commodity prices, and sectorial policies and laws; the bi-annual productivity oscillation that affects on most perennial crops, and is further complicated by the multiannual cycle of coffee pruning; the evolution of other production factors, like soil

[A]Present affiliation: Research Unit Sustainability and Global Change. University of Hamburg. Germany.

fertility or plantations age. The extraction of the climate effect from the scarce yields series is therefore easier at local scale, where these factors can be monitored and their effects removed from the signal.

Climate data also show information gaps in many countries in Central America: time gaps, when series are temporarily discontinued; space gaps, due to the combination of loose meteorological network and high spatial variability of certain meteorological variables, like rainfall or wind speed. Nevertheless, some individual farmers, or agricultural enterprises, for historical and personal reasons, keep long, unexpected records on the weather and productivity in their farms. Those farmers are usually among the richest ones, and some bias might exist when considering these records as representative of the whole sector. As long as we only address biophysical relationships between productivity and climate, these records are a very valuable tool.

This study aims at studying the effects of past variations of climate on coffee yields, based on local records. To this end, we set up a detailed survey looking for long term records for coffee yields and rainfall, the climatic variable that is measurable most easily in the field, and the perceptions of farmers on the climatic hazards affecting coffee production.

MATERIALS AND METHODS

The survey was developed on the coffee zones of the Pacific and North in Nicaragua. Coffee producers, agronomist, and companies related to coffee trade chain were interviewed. Each farm pre-identified was visited, in order to get historical registers of coffee yields and farm management, and any other historical observations. The farmers were interviewed about these registers, and their perceptions about the impact of climate on coffee productivity were recorded. Historical climate data were collected from coffee farms registers, local meteorological stations, and Grid-extractor database (Uribe 2011).

The registers of yield and management collected in the interviews were analyzed and carefully depurated. The longer yield registers were selected to the analysis. In some farms, for example, coffee was not harvested during some years in the midst of the coffee price crisis, and yields were recorded as zero. Total renovations of coffee plantations, abrupt changes in farm management were looked for and, if identified, excluded. To account for local differences and progressive improvement in coffee management, annual coffee productivities were divided by the running average over 10 years in the same farm. Rainfall data sets were equally selected and depurated, comparing each record with each other in the same region at various time scales to detect anomalities. The final records of yields and rainfall were matched based on closeness. Farmers were also asked for damages in coffee productivity related to climate, identifying the coffee stage most sensitive, the years that productivity was most affected, and their climatic causes, if any.

Simple models based on literature were developed to calculate water stress index (simple, FAO-based, water balance model), phenological phases, and to calculate the blossoming dates each year, based on water stress accumulation and a triggering rainfall.

RESULTS AND DISCUSSION

Longest registers of yield and rainfall were found in the Pacific Zone (since 1925). We found shorter registers in the North Zone, the oldest beginning in 1977 (Table 1).Coffee productivity varies more in the Pacific (VC 33%) than in the North (VC 18%).

Table 1. Yield and climatic registers found out at coffee farm level in each coffee zone.

Zone	Coffee Yield			Climatic variables		
	n	Years	Period	n	Years	Period
Pacific	16	6-78	1925-2010	7	8-84	1979-2010
North	16	4-33-	1977-2010	9 9[1]	2-11 2-36	2000-2010 1971-2007
Country	-			Grid-Extractor	30	1978-2008

[1]*Meteorological stations.*

The Pacific Zone presents the lowest historical mean of rainfall (1564 mm ± 321) and highest mean of air temperature (26 °C ± 0.5) than the North Zone (1917 mm ± 313; 22 °C ± 0.6). The variations on the coffee yield in the Pacific Zone could be related to adverse historical conditions. However, according to the interviews years of lowest yield are related with extreme climate events in both zones. In addition, observations on blossoming dates and intensity were obtained from various farms, the longest in the Pacific Zone covering 67 years, starting in 1936.

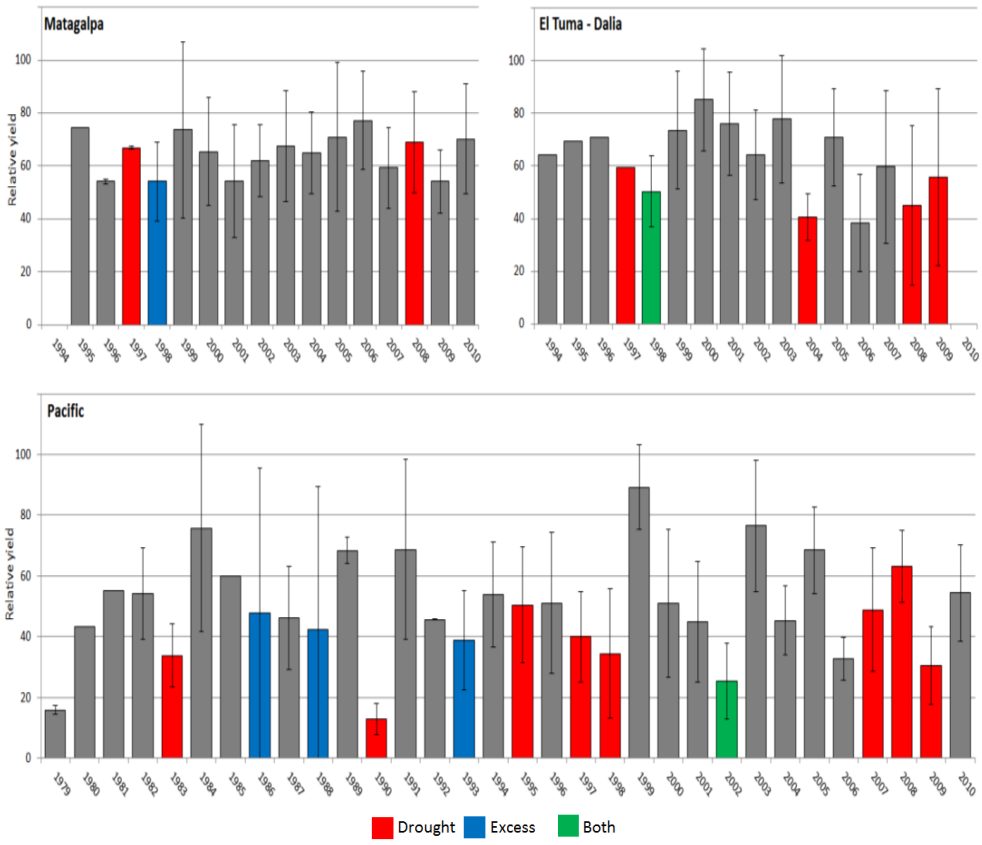

Figure 1. Yield relative of coffee farms and years identified by producers with affectations in the yield because drought, excess rainfall or both.

Most of the producers remembered the yield variations during the last 10 years or less. Only dramatic years were remembered earlier. The perceptions were improved where we could rely on written qualifying records, more frequent in the Pacific zone (figure 1). Excess rainfalls seem less frequent than drought events. Some years were consistently identified as bad climatic years (1997, 1998, 2008), but the causes can differ: 1998, was considered as a dry

year in the Pacific, a wet year in Matagalpa, and a year that suffered from both in La Dalia (Hurricane Mitch).

Table 2. Farmers' perception of climate hazards on coffee productivity.

Zone	Risks	Damage on phenological phases (%)			
		Blossoming	Pin head	Growth	Harvest
Pacific (n = 17)	Drought	29 (76)[1]	37 (41)	0	0
	Excess rainfall	43 (76)	0	0	23 (12)
North [2](n = 18)	Drought	11 (22)	27 (44)	3 (6)	3 (6)
	Excess rainfall	0	3 (6)	7 (39)	3 (6)

[1]( ) = % farmers. [2]North Zone: La Dalia, Matagalpa, Jinotega.

The farmers' perceptions of damages related to rainfall were assessed in severity (calculated for the year in which the event were mentioned) and agreement (% of farmers mentioning the same particular events) in Table 2. The stronger risk mentioned was excess rainfall during blossoming in the Pacific region. Over 40% of producers mentioned losses between 27 – 37 % by drought on pin head phase in both zones.

Being blossoming a very short period very sensitive to rainfall excess, a model was build on an Excel sheet, based on water stress, thermal time and rainfall occurrence. The model was able to simulate correctly the blossoming timing and intensity, as recorded in a coffee estate in the Pacific zone (figure 2, left). Rainfall on blossoming presented a negative and significant correlation with relative yields in the Pacific region (-0.46, p= 0.007).

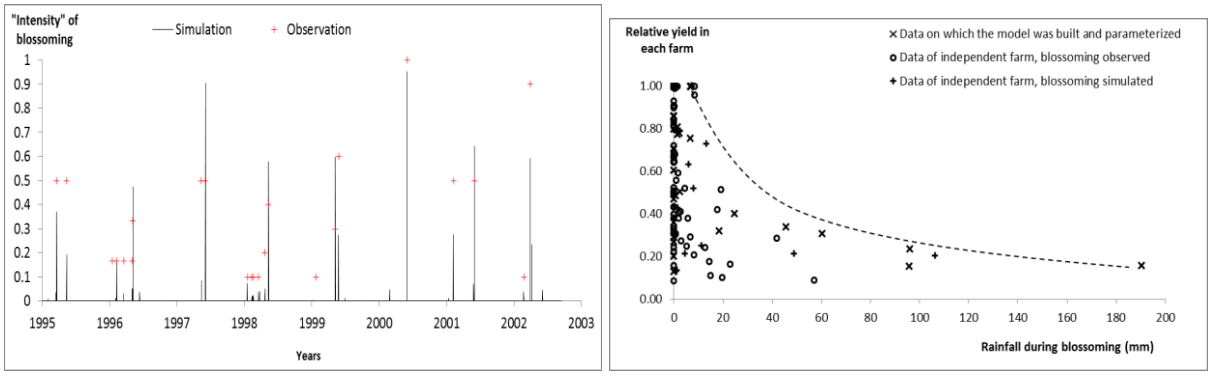

Figure 2. Left: Comparison between observations and blossoming simulations in a coffee farm in the Pacific. Right: Relation between coffee yield and rainfall on blossoming.

Some practices and strategies to reduce this particular risk can involve targeted irrigation (to trigger blossoming at dates where the risk of rainfall is very low), use of medium term climate forecasts. Drought events might be even more difficult to cope with, in a future climate where water will become a rare resource confronted to competitive uses. For all these non avoidable risks, financial tools like agricultural insurances could possibly be implemented, based on the sort of models we developed.

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Environmental Characterization of Coffee in the Environmental Protection Area of Coqueiral, Southern Region of the State of Minas Gerais, Brazil

C. G. SOUZA[1], R. A. T. BORÉM[1], L. M. T. CARVALHO[1], M. M. L. VOLPATO[2], H. M. R. ALVES[3], T. G. C. VIEIRA[2], L. ZANELLA[1]

[1]Universidade Federal de Lavras, CxP 3037, 37200-000, Lavras, MG, Brasil
[2]Empresa de Pesquisa Agropecuária de Minas Gerais, CxP 176, 37200-000, Lavras, MG, Brasil
[3]Empresa Brasileira de Pesquisa Agropecuária, CxP 40315, 70770-901, Brasília, DF, Brasil

SUMMARY

This study aimed to characterize the environment of existing coffee plantations in the Environmental Protection Area (APA) Coqueiral using geotechnologies. To survey the use and occupation of the land, satellite images from SPOT were used, a SPOTMAPS product, with a spatial resolution of 2.5 m. Topographic data from the IBGE and the SRTM radar were used to generate information on the topography and altimetry. The software SPRING was used to evaluate the spatial distribution of coffee in the landscape. The coffee in the APA occupies almost 12 % of the total area. A little over 83% of the coffee plantations are at altitudes ranging from 800 to 950 meters. About 10% of the coffee is at altitudes ranging from 950 to 1000 meters. At altitudes above 1000 meters only 1 % was occupied. As for terrain, 64.08% of the coffee crops were planted on steep hills (slope of 8-20%) and 23.05% on gentle hills (3-8% slope). It is found that the distribution of crops by soil classes occurs predominantly in Ultisols and Cambisols, accounting for about 85% of the total area, followed by Gleysols/Organosols with a little over 8% occupied by coffee plantations. The monitoring of the coffee plantations in this region is important, since this information provides the subsidies needed to maintain a competitive and sustainable coffee sector.

INTRODUCTION

Coffee makes up one of the most important sources of income for the Brazilian economy, as it plays an important role on the country's exports. The state of Minas Gerais stands out on the national scene as a major coffee producer in Brazil, with a market share of 50.99% of the total production of the country. Because it is an important crop of Minas Gerais, coffee has been historically influential in the use and occupation of the State. Despite being a perennial crop, coffee is dynamic, with cycles that rise and fall which influence, among other things, their relationship with the environment. The Brazilian coffee is currently going through a favourable time for both the national and the international scene, which has led the sector to seek improvements in production systems. These improvements are without question environmental issues, sustainability and competitiveness.

The areas occupied by coffee plantations in recent years have gone through changes with new planting, replanting and as well as abandonments and eradications, complicating the assessment of the current situation of the coffee plantations in Minas Gerais. The survey of these areas and the establishment of methodologies enabling the monitoring of this culture,

with periodic updating of this information, have become important for managing this agribusiness. Rational planning of any agricultural activity requires, first of all, knowledge of the environment in which this activity is inserted. For this, geographic information systems together with remote sensing tools are indispensable in assisting in the representation and in detailing the territorial space and can provide information for the conservation and sustainable management of land use and occupation.

Planning for sustainable use of natural resources requires knowledge and organization of updated information about the environment, and the basis of any environmental study is the characterization of the physiographic region of interest. The integrated analysis of the characteristics of the physical and socioeconomic aspects of the areas occupied by coffee plantations enables the rational planning of agricultural activities to be used there, but for this to happen, it is first necessary to have knowledge on the environment in which this activity is embedded.

In that sense, this study aimed to characterize the environment of existing coffee plantations in the Environmental Protection Area (APA) of Coqueiral by using geotechnologies.

MATERIALS AND METHODS

Study Area

The study area is located in the municipality of Coqueiral in the south of Minas Gerais, Brazil. It has an area of 6836.21 ha, located at the geographic coordinates 45° 19’37.5" and 45°26’16.3" W and 21°03’52.7" and 21°09’30.8" S. The microregion is situated in a terrain ranging from rolling to mountainous hills. The predominant soils in the area are shallow, with Ultisols and Inceptisols being the principle classes.The vegetation types are cerrado and semi deciduous forests, which are located in the Atlantic Forest biome.

Mapping and environmental characterization of coffee

The data relating to land use were extracted from SPOT 5 satellite image, with 2.5 m spatial resolution. The image was acquired with preprocessing radiometric and geometric patterns which were then orthorectified (SPOTMAP to form the basis for the realization of mapping occupation and land use). Only coffee areas were used for checking the spatial distribution of the culture in the APA.

The characterization of the terrain (altitude and slope) was generated from the digital elevation model (MNE) obtained from SRTM data. The model was interpolated from 90 to 30 meters, using the algorithm of bi-cubic interpolation from SPRING 5.1.5. The soil map of the area was prepared by the Department of Soil Science, Universidade Federal de Lavras. The slope classes and their gradients were: Nearly Level: 0 - 3%, Gently Sloping: 3 - 8%, Strongly Sloping: 8 - 20%, Moderately Steep: 20 - 45%, Steep: 45 - 75%, and Very Steep: > 75 %.
The thematic maps of the environmental characterization obtained where overlapped with the land use/land cover map. The procedure was performed using the software SPRING and the language program LEGAL.

RESULTS AND DISCUSSION

Through the interpretation of satellite imagery, it was possible to classify the area into nine types of land use, but the study in question used only the coffee area. The coffee in the APA makes up for about 12% of the total environmental protection area.

Environmental characterization of the coffee lands

From the results obtained it was observed that the land use class coffee is distributed in all classes of altitude, soil and slope observed in the study area.

Most of its surface (about 80%) is found at altitudes between 734-900 meters above sea level. Classes of higher elevations (900 to 1040m) account for about 20% of the area.

Slightly more than 83% of the coffee plantations are at altitudes ranging from 800 to 950 meters (Table 1), whereas, the majority are found at altitudes between 900-950 meters. About 10% of the coffee is at altitudes ranging from 950 to 1000 meters. At altitudes above 1000 meters, with the highest potential for production of quality coffee, only 1% was occupied.

Table 1. Distribution of coffee plantations by classes of altitude and slope.

Altitude	Area (%)	Class of terrain	Area (%)
<800	6,34	Nearly Level	3,71
800-850	26,43	Gently Sloping	23,24
850-900	24,87	Strongly Sloping	64,08
900-950	31,90	Moderately Steep	8,96
950-1000	10,15	Steep	0,09
>1000	0,31	Very Steep	0,00

In regards to terrain, 64.08% of the coffee plantations are in rolling areas and 23.05% in gently rolling terrain (Table 1). The agricultural areas with very steep slopes are limited, since in most cases the control of erosion is costly and may be uneconomical, althought with coffee the risk of erosion becomes smaller since it is a perennial crop. The terrain of the area is mostly composed by slopes of 8 to 20%, with over 50% of the total APA. These areas may be used for farming since an efficient management and control of soil erosion is undertaken. The APA does not have areas with steep slopes.

Regarding the distribution of crops by soil classes there is a predominance of occurrence of the classes Ultisol and Cambisols with about 85% of the total area, followed by the class Gleysol/Organosol with a little over 8% occupancy by coffee (Table 2).

Table 2. Distribution of coffee plantations by soil classes.

Soil Class	Area (%)
Rocky outcrop	0,20
Ultisol/Cambisol	43,34
Cambisols	40,11
Gleysol / Organosol	8,43
Fluvic	2,87
Udorthent	5,05

The geotechnology allowed the characterization of the coffee plantations of the APA, quantifying the occupation of coffee in the environmental units of altitude, topography and soil, showing that they are important and efficient tools, both in terms of time savings and resources.

The monitoring of the coffee plantations in this region is important, since these subsidies provide information needed to maintain a competitive and sustainable coffee sector. The environmental characterization can be considered essential to initiate the development of a sustainable management plan for the area.

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Agronomic Evaluation of Progenies Derived from Genetically Low Caffeine Arabica Coffee Genotypes

M. T. V. LOBATO[1,2], M. B. SILVAROLLA[1], R. SAATH[1,2], G. S. GIOMO[1], R. R. LEAL[1,3], I. J. SAKAUE[1,3], O. GUERREIRO-FILHO[1]

[1]Instituto Agronômico - IAC, Centro de Café, P.O. Box 28, 13012-970, Campinas, SP, Brasil
[2]Scholarship PNPD/CAPES, Brasil
[3]Scholarship CBPD/Café, Brasil

SUMMARY

Arabica cultivars presenting genetically low caffeine in beans are not yet available for commercial plantation. These cultivars will represent an important alternative for production of low caffeine coffee beans, as in addition to a differentiated cup quality, they attend the demands of consumers sensitive to this alkaloid, and, at the same time allow coffee producers to increase their income due to the possibility of adding value to the final product. This study aimed to identify plants that combine appropriate levels of productivity, high cup quality and beans with low levels of caffeine. Plants from F_2 and F_1BC_1 generations of crosses involving low caffeine mutants and elite cultivars, along with the clonal variety IAC 045125 (AC) and standards cultivars were evaluated during 2011/2012 crop years. Agronomic aspects such as production and reaction response to coffee leaf rust were evaluated, as well as determination of caffeine content in the beans. In general the progenies with the best behavior comparing to the average yield were those originated from backcrosses. Among ten F_2 progenies derived from crosses with short stature cultivars the production in nine of them was greater than IAC Ouro Verde cultivar. Also, plants with low caffeine content were observed among several F_2 progenies, but a higher frequency of low-caffeine plants was observed in backcrosses progenies. As expected clonal plants from the low caffeine mutant genotype, and as well as some descendent progenies showed low levels of caffeine in beans. These results indicate the possibility of either utilization of these genotypes in the development of new low caffeine clonal cultivars or carry-on with the breeding program to develop seed propagated cultivars.

INTRODUCTION

Arabica coffee (*Coffea arabica*) is the species most widely cultivated and consumed in the world, due mainly to its quality of drink. The popularity of coffee as a drink is due in large part to the stimulating effect of caffeine. However there are people who have a higher sensitivity to this alkaloid and that even so, enjoy the drink. To attend these consumers the industrially decaffeinated coffee was developed. Beyond caffeine a decaffeinated coffee can lost important substances for development of aroma and flavor, as they are water soluble, needing to be recovered at the end of the process. Another alternative would be to produce decaffeinated coffees by breeding process in which a coffee cultivar produce beans with low or no caffeine, being a natural product and free from solvent residues. Arabica varieties genetically deprived of caffeine in the beans are unique in the world and represent an important alternative for the production of coffee free of this alkaloid and with differentiated quality. In order to not only attend the demand for a coffee with naturally low caffeine levels, but also to supply the coffee grower with a cultivar option whose aggregated value came from

the field, the Agronomic Institute – IAC in Campinas carried on a line of research aiming to develop a coffee cultivar with low caffeine content in seeds. Among several possible strategies to reach this objective we decided to throw a genebank quite representative in accessions of *C. arabica*, received from Costa Rica and from Ethiopia, systematically seeking between this germplasm materials with reduced caffeine content in seeds. The most significant result of this work was the identification of three mutant plants, called AC1, AC2 and AC3, with reduced caffeine content in the endosperm, 0.07%, greatly reduced when compared to normal content for arabica coffee cultivars, which is 1.2%. This work aimed to evaluate F_1BC_1 and F_2 generations plants derived from the crossing of elite cultivars and mutants AC targeting the selection of plants combining adequate levels of productivity and quality of drink, as well as low levels of caffeine.

MATERIALS AND METHODS

About 400 plants of $F_2$and F_1BC_1 generations derived from crosses involving AC1 mutant with low caffeine content and elite cultivars were evaluated (2010/2011 and 2011/2012 crop years) in Experimental Center of Agronomic Institute, Center of Coffee ' Alcides Carvalho ', in Campinas-SP, Brazil. In the same field it was included standards cultivars like: IAC 81, IAC144, IAC Ouro Verde, IAC Obatã and Mundo Novo cultivars. Evaluations were made of the agronomic point of view including: each plant was harvested individually on cherry state during two years and its yield was weighted and the average yield of each progeny was calculated. The behavior of the plants of the studied progenies was evaluated for the reaction to coffee leaf rust, taking into account only the presence or absence of disease and by calculating the percentage of infected plants within each progeny. For the determination of caffeine % (db) from each plant of the progenies in study were collected samples of 30-50 ripe fruits, which after drying in an oven were peeled and the grains grinded in a mill and submitted to a methanolic extraction of caffeine. After centrifugation and filtration of extracts the separation and quantification of the compounds was done using a Shimadzu HPLC by means of a reverse phase C18 column and an UV detector at 272 nm wavelength. The caffeine concentration of samples in the study were calculated using calibration curves obtained from the injection of solutions of known caffeine concentration standards.

RESULTS AND DISCUSSION

In Table 1 are presented the average yield obtained in two crop years, the production range, percentage of plants with low caffeine content and reaction to coffee leaf rust of 25 F2 progenies, 18 progenies obtained by backcrosses (F1BC1) in addition to the standard cultivars IAC 81, IAC Ouro Verde, IAC Obatã, IAC 144, Mundo Novo and the clonal cultivar IAC 045125 (AC). It was found that, in a general way, progenies that showed better behavior in relation to the average yield were obtained from backcrosses (with IAC Obatã and IAC 144), highlighting the numbers 53, 54, 41, 48 and 49. Cultivar IAC Obatã showed the highest average yield in relation to the other standards. The F_2 progenies number 64, 65 and 57 had higher averages yields then the standards IAC 81, IAC 144 and IAC Ouro Verde. The F_1BC_1 progenies 43 and 44 presented the highest percentage of plants with low caffeine content. Among F_2 progenies that of number 18 showed the highest percentage of plants with reduced caffeine content in beans. Although several plants with low caffeine content were found in the F_2 generation offspring, increased frequency of plants with this characteristic was observed in progenies from backcrosses. The data of production ranges of each progeny showed great variability among plants of each progeny. It occurred from unproductive plants even plants with superior productions overcoming the best individual standard plants. It is expected that the inclusion of data from more two harvests (2013-2014) will allow a better yield average of progenies in study, as well as will allow a secure selection of individual plants with adequate

levels of production for generation advancement. As expected the cloned plants of AC1 (mutant with low caffeine content) and G1 (AC1) presented 100 % of plants analyzed with low caffeine content in grains, less than 0.1%, indicating the feasibility of using the cloning technique to the spread of promising genotypes that can become new cultivars in a near future. Although the resistance to coffee leaf rust is not the primordial goal of this work the qualitative data reaction to the pathogen were raised. It was found that progenies numbers 42, from backcross to AC, 39 (AC1 clone) and G1 (AC) showed lower percentage of plants with coffee leaf rust. It should be noted that these data should be confirmed in the next crop years where rust reaction will be evaluated associated on the levels of production of each plant.

Table 1. Average yield, % of plants with reduced caffeine content in grains, production range and reaction to coffee leaf rust in F_2 and F_1BC_1 progenies of coffee (*Coffea arabica*) evaluated in 2010/2011 and 2011/2012 crop years – Campinas-SP-Brazil.

Treatment	Identification	% plants	Average yield	Production range	Reaction
(progenies)	(generation)	with reduced caffeine content	(g)	(g)	to coffee leaf rust[1] (%)
1	F_1BC_1	25	531,13	(0 – 2173)	100
2	F_1BC_1	20	1027,50	(0 – 2315)	100
3	F_1BC_1	0	618,50	(0 – 2250)	100
5	F_1BC_1	0	342,50	(0 – 1935)	100
6	F_1BC_1	30	534,35	(0 – 1965)	100
7	F_1BC_1	0	758,00	(0 – 2040)	100
8	F_2	0	713,00	(0 – 2825)	100
9	F_2	14	891,50	(0 – 3260)	100
10	F_2	6	382,53	(0 – 1240)	100
11	F_2	0	1092,75	(0 – 4855)	100
13	F_2	5	890,60	(0 – 2670)	100
15	F_2	0	883,13	(0 – 3640)	100
16	F_2	0	797,88	(0 – 4530)	100
17	F_2	0	1017,13	(0 – 4050)	100
18	F_2	25	1208,70	(0 – 3980)	100
20	F_2	0	1172,00	(0 – 4595)	67
21	F_2	0	1411,50	(0 – 3410)	100
22	F_2	11	1493,05	(0 – 3910)	80
28	F_2	0	716,90	(0 – 3470)	58
29	F_2	22	1434,40	(0 – 4985)	100
39	AC1Clone	100	561,58	(0 – 1620)	0
41	F_1BC_1	0	2587,25	(0 – 7355)	70
42	F_1BC_1	33	1248,90	(0 – 5212)	40
43	F_1BC_1	38	848,20	(0 – 2478)	100
44	F_1BC_1	50	1015,40	(0 – 4520)	100
45	F_1BC_1	0	1935,50	(0 – 3960)	100
46	F_1BC_1	0	639,40	(0 – 5100)	100
47	F_2	20	1724,33	(0 – 3900)	100
48	F_1BC_1	0	2741,75	(440- 5640)	100
49	F_1BC_1	0	2504,50	(680 – 4635)	100
50	F_1BC_1	0	1780,83	(0 – 6240)	100
52	F_1BC_1	0	2465,40	(0 – 7560)	100
53	F_1BC_1	0	3084,63	(0 – 9220)	90
54	F_1BC_1	0	3044,83	(0 – 7960)	80
57	F_2	0	2045,33	(0 – 4900)	73
58	F_2	0	1395,50	(0 – 4170)	100
59	F_2	0	1267,00	(0 – 4585)	90
60	F_2	10	998,50	(0 – 3755)	80
61	F_2	8	1856,32	(0 – 5330)	76
62	F_2	14	1771,27	(0 – 4050)	93
63	F_2	7	1625,83	(0 – 4070)	100
64	F_2	10	2180,00	(0 – 5055)	100
65	F_2	7	2150,38	(0 – 6345)	100
66	F_2	0	1129,43	(0 – 3590)	100
67	IAC 81	0	962,23	(0 – 2455)	100
68	IAC Ouro Verde	0	1073,64	(115 – 2105)	100
69	IAC Obatã	0	2881,05	(190 – 6125)	100
70	IAC 144	0	558,93	(0 – 1565)	100
MN	Mundo Novo	0	272,32	(0 – 2045)	100
G1	AC1	100	195,89	(0 – 1605)	42

[1] *% of plants with coffee leaf rust in each progeny*

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Bananas in Coffee Agroforestry in Latin America: Assessing Ecological and Socio-Economic Benefits

C. H. STAVER[1], P. SILES[1], O. BUSTAMANTE, H. GARMING[2], N. CASTELLON[1], J. GARCIA[3]

[1]Bioversity International, Turrialba, Costa Rica
[2]Thünen Institute, Bundesallee 50, 38176 Braunschweig, Germany
[3]UNAN-León, León, Nicaragua

SUMMARY

Bananas are commonly grown by small coffee growers throughout Latin America in shaded coffee fields. Coffee technicians advise small coffee growers against planting bananas in their coffee fields. To assess the costs and benefits of bananas in shaded coffee we analyzed the results from a study in seven sites in Central and South America. Does the presence of banana reduce the availability of light, nutrients and water for coffee below crop requirements? Does banana increase soil pest problems for coffee? Do bananas increase labor costs for coffee production? Does labor invested in banana provide better returns than if the same labor were invested in coffee? Results showed that banana with coffee is useful for reducing coffee production costs through weed suppression, low cost, easy shade management with quick shade recovery and improved soil health and conservation. The income generated by banana covers household expenses and routine coffee management practices after coffee income has been spent. The major drawback from banana production in coffee is increased potassium use. The analysis points to the need for additional studies on the marginal returns to alternative investments in coffee or tree management, external inputs and diversification.

INTRODUCTION

Bananas are commonly grown by small coffee growers in shaded coffee fields throughout Latin America, occupying over a half million hectares in Mesoamerica, the Caribbean and South America. Coffee technicians, focusing on coffee modernization, advice small coffee growers to eliminate bananas from their coffee fields, proposing that bananas generate excess shade, compete for nutrients and water, increase nematode problems for coffee, and result in damage to coffee bushes during banana harvest. Given that the practice is widespread, further analysis is merited to orient programs with smallholders. Does the presence of banana reduce the availability of light, nutrients and water for coffee below crop requirements? Does banana increase soil pest problems for coffee? Do bananas increase labor costs for coffee production? Does labor invested in banana provide better returns than if the same labor were invested in coffee? Mixed cocoa agroforestry, for example, had greater productivity through economies of scope, since associated crops shared production costs and provided additional income.

MATERIALS AND METHODS

We analyzed the results from our grant focused on small farmer strategies to improve the productivity and value of banana in shaded coffee to assess the agroecological and socioeconomic costs and benefits of bananas in this system. In each of six sites in Honduras, Nicaragua, Costa Rica and Peru we surveyed 30 shaded coffee plots with banana to characterize coffee, banana and tree density and estimate light partitioning. In meetings with

farmer experimentation groups during 2010-2012, we collected and analyzed data on labor and input costs and income with farmers. Studies were also conducted on the effects of the presence of banana on nematodes in different combinations of coffee and trees.

RESULTS AND DISCUSSION

Do bananas reduce the availability of light, water and nutrients?

Coffee density was quite uniform across sites from 3500-5000 plants/ha, while banana ranged from 288 to more than 500 mats/ha and trees from 161 to more than 500/ha. Light reaching coffee in these multi-strata systems was generally below 50%, averaging between 29 and 49% in the different zones (Table 1). Visual estimates suggested that banana intercepted between 11 and 32% of total light for 5 zones with one zone at 50% interception. Light interception by the tree strata was more variable between 16 and 53%. In most plots sampled, coffee received less than adequate light for good production, but tree shade was often much higher than banana shade. In addition, tree pruning was infrequent and minimal in four sites.

Table 1. Light availability to crops in substrata (% open sun) and % substrata crops shaded by upper strata.

	Costa Rica	Honduras		Nicaragua		Peru
	Turrialba	Laureles	Tutule	Yasica	Monterrey	Selva Central
% light to coffee	46 ± 3	36 ± 4	34 ± 3	49 ± 6	41 ± 5	39 ± 2
% light to banana	68 ± 2	68 ± 3	84 ± 2	79 ± 4	52 ± 7	50 ± 2
% coffee plants with banana leaves above	61 ± 5	47 ± 5	64 ± 8	61 ± 6	60 ± 3	56 ± 4
% banana mats with trees above	38 ± 5	66 ± 4	54 ± 8	38 ± 6	55 ± 4	73 ± 7

Calculations of nutrient export in banana and coffee indicated that farmers had a positive balance for nitrogen, but were negative for potassium (Table 2). The production of 300 20 kg bunches/year/ha, the potential yield from bananas at a density representing about 15-20% light interception, increases potassium export by 30 kg/ha/year, equal to potassium in 11 hundredweights of green coffee.

Table 2. Nutrient balance for 4 growers – Nutrients (N, K) in coffee, banana and firewood taken off field were subtracted from nutrients added through organic and chemical fertilizer to yield balance for N and K.

Grower	Coffee sacks/ ha	bunches banana/ ha	Wood kg/ha	Nutrients export kg/ha				Nutrients applied kg/ha		Nutrient balance kg/ha	
				coffee		Banana					
				N	K	N	K	N	K	N	K
R T	30	160	0	87	87	5	17	120	30	28	(-74)
W T	6	200	500	17	17	6	21	52	0	27	(-39)
M A	26	90	800	75	75	3	10	102	18	22	(-67)
I R	23	120	350	67	67	4	13	77	23	6	(-57)

Water use studies were not conducted, but data on stomatal conductance showed higher rates for banana than coffee or legume trees (*Erythrina*, *Inga*) at field capacity. Banana is a water

conserving crop under water stress conditions. Stomata close when soil water drops to -0.10 to -0.20MPa, well above levels for coffee at -.50 to -1.0MPa.

Do bananas increase soil pest problems for coffee?

Banana and coffee suffer from several plant parasitic nematodes – *Meloidogyne, Pratylenchus* and *Heticotylenchus*. A study comparing different combinations of banana, coffee and trees showed that the presence of banana is not associated with higher plant parasitic nematodes in coffee. *Meloidogyne* nematodes in coffee roots, the most common genus found in the study, were lower in the presence of banana (Table 3). Nematode levels of these three genera in coffee and banana were linked significantly to different soil physical and chemical parameters, suggesting that different nematode species or subpopulations may be involved. This response may have been mediated through the presence of free living nematodes stimulated by greater organic matter inputs in the presence of bananas which in turn contributed to altered predator – prey ratios. Banana and tree plots had greater amounts of ground cover litter followed by coffee with bananas or trees and coffee alone (Table 3).

Table 3. Leaf litter and nematodes in different combinations of coffee, banana and trees in Monterrey, Nicaragua.

	Coffee – banana - legume	**Coffee – banana**	**Coffee – legume**	**Coffee**
Shade %	78 ± 2 b	64 ± 4 a	71 ± 3 b	0
Total leaf litter g/40 cm^2	125 ± 10 d	72 ± 7 b	83 ± 8 c	35 ± 5 a
Leaf litter banana g/40 cm^2	37 ± 4 a	45 ± 22 a	-	-
Leaf litter coffee g/40 cm^2	35 ± 5 a	28 ± 4 a	34 ± 4 a	33 ± 5 a
Leaf litter trees g/40 cm^2	53 ± 5 a	-	48 ± 4 a	-
Meloidogyne nematodes /100 gs coffee roots	2843 a	2586 a	3429 b	2914 b
Metabolic footprint ratio predators/objective prey	44 ± 8.6 a	18 ±8.4 b	29 ±8.2 ab	40 ± 8.2 a

Do bananas increase labor costs for coffee production?

While coffee technicians sometimes suggest that smallholders do not manage their banana, from zone to zone and farm to farm the average number of tall banana stems for fields in the different zones varies from 1.2 to 3/mat and total stems from 2.2 to 4.3/mat. Within zone variability is much higher with some growers as high as 6-7 total stems/mat, a level suggesting little mat management. The banana component absorbs between 15-40 work days/hectare which is approximately 12% of total production costs/ha. Deleafing and desuckering represent between 52-66% of the labor costs for banana, without including harvest costs. Practices such as deleafing and desuckering are often done during weeding which increases the total weeding cost. However, other farmers weed and then separately carry out banana mat management. Farmers agreed that the presence of banana reduces the cost of weeding compared to coffee without banana for numerous reasons (Table 4). Bananas provide additional crop residues which protect the soil and reduce weed growth. Bananas provide shade which is rapid to establish, rapid to recover after leaf pruning and stem cutting and easy to manage from the ground. Weeding makes up nearly 50% of labor used in coffee production, increasing the value of bananas in cost reduction.

Does labor invested in banana or coffee provide better returns?

The available data do not permit an analysis of marginal returns for redistributing labor among banana or coffee either at the field or the household level. Gross income from bananas ranged from $USD200-450 or the value of 1-3 hundredweights of green coffee, with minimal purchased input cost, about $10-12 gross return per day of labor. A day of labor in coffee generated 0.3 – 1.1 qq coffee depending on the zone with an additional investment of 0.2 – 0.6 qq fertilizer/qq coffee. Given the flexible labor scheduling of banana management throughout the year, the shifting labor from banana to coffee may not compensate the loss of banana income with increased coffee yields. In addition, the income from banana is monthly, taking on a greater importance, according to farmers, when coffee income has run out, 3-4 months after the harvest. Data on monthly banana sales indicated that sales were higher once coffee income was no longer available.

Table 4. banana production practices - Labor use and possible effects on coffee.

Banana production practices	Average person days/ha	Possible effects on coffee labor costs	Possible effects on coffee productivity
Planting/replanting	3	Reduce weeding costs	Banana spacing linked to shade % / distribution
Chopping down / cutting up harvested/fallen stems	4	Reduce weeding costs	Banana crop residues protect soil /improve structure
Desuckering	2	Ensure adequate banana spacing	Number stems/mat linked to shade %/distribution
Deleafing	8	Reduce weeding costs	Banana leaf number linked to shade %
Deflowering	3	None	None
Banana harvest	12	None	None
Total days/ha	32	Higher banana costs reduce coffee costs	Higher banana costs ensure coffee productivity

CONCLUSIONS

Banana intercropped with shaded coffee, as currently practiced by millions of smallholder coffee growers in Latin America, plays a useful role in reducing the costs of coffee production through weed suppression and providing a shade management practice which is low cost, easy and with a quick response time. The income generated by banana covers household expenses and routine coffee management practices after coffee income has been spent. Adequate tree pruning and appropriate banana spacing with timely desuckering and replanting of bananas should ensure adequate sunlight for coffee production. The major drawback from banana production in coffee fields is the increased demand for potassium, also an important element in coffee production. This analysis of bananas in coffee agroforestry points to the need for additional studies on the marginal returns to alternative investments in coffee or tree management, external inputs or diversification to guide farmer decision-making for greater returns, lower risks and improved household resilience.

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Effect of Shade on Yields of Selected Improved Hybrid *Coffea Arabica* Varieties in Tanzania

G. P. MARO, J. M. TERI, F. L. MAGINA, E. O. NKYA

Tanzania Coffee Research Institute (TaCRI), P.O.Box 3004, Moshi, Tanzania

SUMMARY

A study was conducted at TaCRI Lyamungu, Tanzania from 2007/08 to 2010/11 seasons to assess the effect of shade on yields of four improved hybrid Arabica coffee varieties. We used two blocks (*Albizzia*-shaded and unshaded) adjacent to each other, where two separate variety performance trials were set with 4 improved varieties N39-2, N39-4, N39-5 and N39-7 checked against KP423 commercial variety. The experimental design was RCBD with 3 replications. Yields of clean coffee were subjected to descriptive statistics and 2-way ANOVA under COSTAT Software. Yields over seasons were checked for response to biennial bearing.

All the varieties experienced biennial bearing except N39-2 which showed stable yields. The high years under shaded regime corresponded with the low years under unshaded regime and vice-versa. Regime (shade or open) showed to be very highly significant ($P<0.001$), seasons highly significant ($P<0.01$) and variety significant ($P<0.05$), while the interaction was not significant ($P>0.05$). The highest mean yield over the four seasons was obtained from N39-4 (1730 kg.ha^{-1}) while the lowest mean of 1260 kg.ha^{-1} was from N39-5. Yields without shade were about two times yields under shade, confirming the yield reduction effect of shade trees. The low oscillation between highs and lows under shade suggests that shade has mitigation effect on biennial bearing. Relative yield reduction due to shade was in the order N39-7 < N39-4 < N39-2 < N39-5 < KP423. This work has therefore revealed another positive attribute of the improved TaCRI varieties; their relative adaptability to shading regimes.

INTRODUCTION

From its origin, coffee is an understorey, shade loving plant (Da Matta, 2004), which was later adapted to unshaded culture for yield improvement and reduction of fungal infection. Both shaded and unshaded coffee cultures are practised in Tanzania, the former more common with smallholders and the latter with estates. The shaded coffee culture, despite yield reduction, is said to help in climate change mitigation (Maro and Teri, 2008; Maro *et al*, 2010) and biodiversity conservation (Priyadarshini *et al*, 2010). Shade is also said to help even out erratic yields caused by periodical overbearing; a phenomenon popularly known as biennial bearing (Kimemia and Kaminchia, 1994).

Although much is already known with respect to traditional varieties, more answers are needed, particularly for the newly released coffee varieties. A study was therefore conducted to assess the yield response of 4 selected new varieties when grown under shaded and unshaded condition; and check whether any of the two practices has effect on biennial bearing.

MATERIALS AND METHODS

The study was conducted at TaCRI Lyamungu, Tanzania from 2007-08 to 2010-11 seasons. Two blocks were selected adjacent to each other. The first had a sparse, pre-established shade of *Albizzia* trees while the second was unshaded. In each block, a separate variety performance trial was established in a Randomized Complete Block Design (4 new varieties N39-2, N39-4, N39-5 and N39-7 with KP423 commercial variety as a check, replicated 4 times). Yield data in kg of clean coffee per ha were subjected to descriptive statistics and a 2-way analysis of variance under COSTAT Software, with means separated by Tukey's HSD at 5% significance level. Relative yield reduction was calculated from the four year means using the relationship % reduction = ((Yopen - Yshade)/ Yopen) x 100.

RESULTS AND DISCUSSION

The variation in yield of the tested varieties under shaded and unshaded regimes is shown in Figure 1. We noted that, in both cases, all the varieties were affected by biennial bearing except N39-2 which experienced a steady yield increase. For all varieties that experienced this biennial yield shift, the high years under shaded regime corresponded with the low years under unshaded regime and vice-versa.

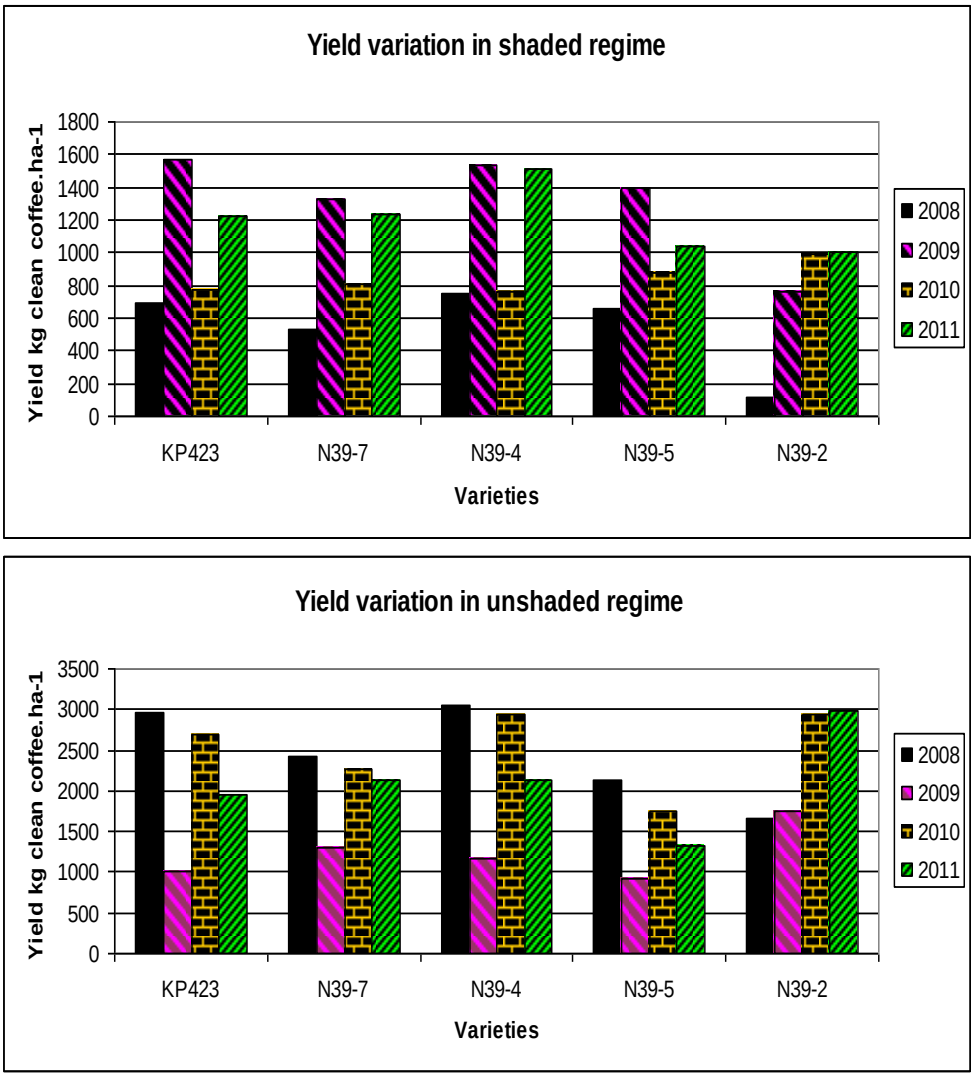

Figure 1. Seasonal yield variations in shaded regime (left), unshaded regime (right).

The Bartlett's test for homogeneity was significant with P <<0.05 and corrected X^2 of 76.44. The ANOVA is shown in Table 1, with regime (shade or open) very highly significant (P<0.001), seasons highly significant (P<0.01) and variety significant (P<0.05). The interaction between varieties and seasons was however not significant (P>0.05). The highest mean yield over the four seasons was obtained from N39-4 (1730 kg clean coffee.ha^{-1}) while the lowest mean of 1260 $kg.ha^{-1}$ was from N39-5. The rest of the varieties were not significantly different among themselves. Seasons 2010/11 gave the highest yields both in shaded and unshaded regimes, followed by 2011/12; and the two seasons did not differ significantly in yield (difference of 25 kg). The remaining seasons differed significantly (difference of 222 kg), with 2009/10 having the lowest mean yield of 1274 $kg.ha^{-1}$, followed by 2008/09 yield of 1496 $kg.ha^{-1}$.

Table 1. ANOVA for varieties and seasons.

Source	df	Type II SS	MS	F	P	Sign
Regime	1	47990204.45	47990204	156.70363	.0000	***
Main Effects						
Variety	4	3845114.954	961278.74	3.1388879	.0166	*
Season	3	4158124.357	1386041.5	4.5258764	.0046	**
Interaction						
Variety x season	12	6679873.756	556656.15	1.8176635	.0507	ns
Error	139	42568498.67	306248.19			
Total	159	105241816.2				
Model	20	62673317.51	3133665.9	10.232439	.0000	***

The overall means for all varieties showed seasonal variations as illustrated in Figure 2 (left), while mean reduction in yield due to shade is shown in Figure 2 (right). Unshaded plots yielded higher than plots under shade in all seasons except 2009/10 when yields were slightly lower. Yields without shade are about two times yields under shade, implying that one effect of shade trees is coffee yield reduction as also noted by Ayano, (2010) and Steiman et al (2008). The least shade effect on yield was noted in variety N39-7 (32.4% reduction) followed by N39-4, N39-2 and N39-5 (39.5, 42.5 and 43.0%). The old variety check was most affected with a reduction of 66.8% (Figure 2 right).

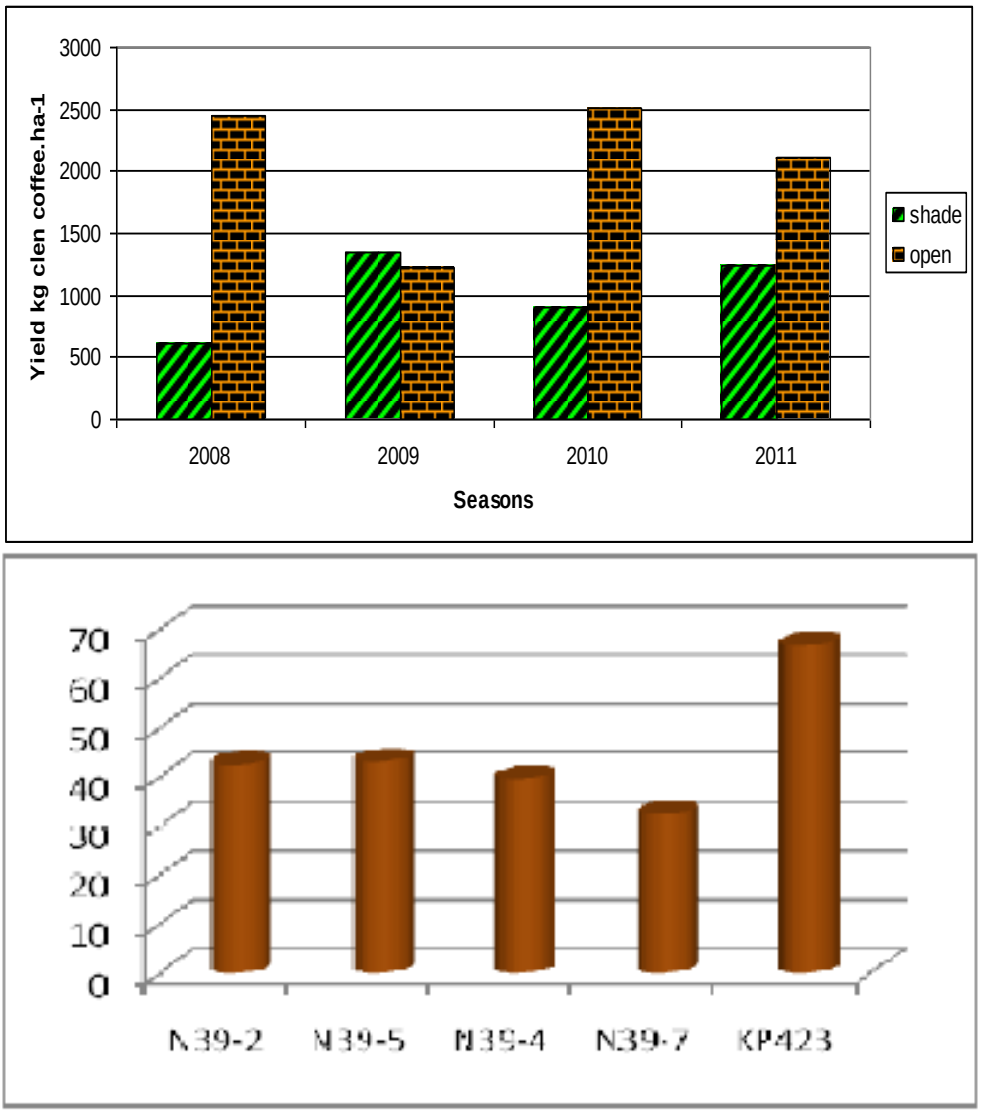

Figure 2. Overall mean yield variation per season (left), mean percent decrease in yield due to shade culture (right).

CONCLUSIONS

The results of this work suggest a difference in varietal performance under shaded and unshaded regimes. Variety N39-4 gave highest yields, while N39-2 showed more stable yields. The yield reducing property of shade was also proved, varying from 32-43% for the test varieties and as high as 66.8% for KP423. This work has therefore revealed another positive attribute of the improved TaCRI varieties; their relative adaptability to shading regimes.

ACKNOWLEDGEMENTS

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Efficacy of Fish Bean, *Tephrosia Vogelii* for the Management of Coffee Antestia Bugs, *Antestiopsis* Spp in Kilimanjaro Region, Tanzania

F. L. MAGINA[1], G. P. MARO[1], A. P. MAERERE[2], J. M. TERI[1]

[1]Tanzania Coffee Research Institute (TaCRI), P.O Box 3004, Moshi, Tanzania
[2]Sokoine University of Agriculture (SUA), P.O Box 3005, Morogoro, Tanzania

SUMMARY

A study was carried out on station at TaCRI, Lyamungu to assess the efficacy of Fish bean (*Tephrosia vogelii*) for management of coffee antestia bugs in Kilimanjaro Region. Fresh leaves of *T. vogelii* were collected in the field before flowering, pounded in a mortar with a pestle. Four dosages (800g/l, 1000g/l, 1200g/l and 1400g/l) of pounded leaves in one litre of water was made and left for 24 hours. The four treatments, plus Profenofos 720 EC (Selecron 720 EC) 1.5 milligram/litre of water and untreated (control) were applied to 20 antestia bugs contained in petri dishes, using one litre atomizer and kept in a temperature and relative humidity control chamber in the laboratory. The same treatments were tested under field conditions whereby 20 bugs were put in a mosquito net slid over coffee branches. The mortality rate of pest in both laboratory and field experiment were recorded at 24, 48 and 72 hours. Dosages of 1200 g/l of water and above showed no significance differences ($p \leq 0.05$) from the standard (Selecron 720 EC) in both the laboratory and field trials while lower dosages were significant ($p \leq 0.05$). The study therefore recommends *T. vogelii* rate of 1200g/l of water for use by small scale coffee farmers for management of antestia bugs at the medium altitude (1200-1600 m. a. s. l) as a cost effective and suitable alternatives to synthetic insecticides. We will continue research to compare the efficacy of different plant parts fresh and dry formulations and the effects of altitudinal gradients.

INTRODUCTION

Botanical insecticides are naturally occurring chemicals extracted from plants, which act on both behavioural and physiological processes in other plants or animals (Gonzalo, 2004). A number of botanicals are known to play part in pest management. Some may possess insecticidal properties, while others repel pests or discourage feeding or egg-laying activity, and have antifungal, antiviral, and antibacterial properties against pathogens (Isman, 2006; Varma and Dubey, 1998). One such botanical is fish-bean (*Tephrosia vogelii*).

One of the key coffee pests in East Africa is Antestia bug (*Antestiopsis* spp). It is a sucking insect that damages coffee flowers and berries (Le Pelley, 1968). Presence of a very small population (2-3 bugs/tree) in the coffee field can cause about 45% crop losses (Le Pelley, 1968). The insect may also carry spores of the fungus *Nematospora coryli* in its proboscis, and when these are introduced into the bean may cause a crop loss up to 90% of the green bean and also lower the quality (Le Pellay, 1968). The current control measure is by spraying with Profenofos (Selecron 720EC) (Magina, 2005); however, for reasons of consumer health, the world is moving from synthetic pesticides. Already some farmers in Tanzania are using botanicals for management of insect pests (Paul *et al.,* 2000); but dosages and specific target

pests are not yet known. A study was therefore carried out to evaluate the efficacy of *T. vogelii* against Antestia bugs in coffee.

MATERIALS AND METHODS

Collection of leaves and extract preparation

Leaves of Fish bean (*Tephrosia vogelii*) were collected from February to August, 2011, in the botanical garden at TaCRI Lyamungu (1268 m. a. s. l.), Tanzania. The collected leaves from un-flowered *T. vogelii* plants were ground in a mortar with a pestle, weighed to portions of 800 grams, 1000gms, 1200gms and 1400gms and mixed in one litre of sterilized water. The mixture was left to ferment for one day (24 hours), thereafter the extract was carefully filtered using clean cloth and later mixed with 1 gram of soap/1 litre of extract to save as sticking agent.

Antestia bugs collection

We hand-collected 720 adult antestia bugs of the same size from coffee fields and brought them into the laboratory one day before bioassay and field experiments. These were put in a cage fitted with a coffee branch having full grown green berries as feed.

Bioassay and field experiments

The work involved two experiments. The first one was bioassay and second field experiment. In bioassay experiment, extracts with 4 dosages of *T. vogelii* (800, 1000, 1200 and 1400 grams) were evaluated. A known effective pesticide used to control antestia bugs, Selecron 720 EC (Profenofos 720g/l) at a rate of 1.5 milligram/ 1 litre of water (standard) and untreated (control) were used as checks. The bioassay was arranged in a Complete Randomized Design (CRD) and field experiment in Complete Randomized Block Design (CRBD).

In the laboratory bugs were picked up from the insect rearing boxes by use of fine pipette connected to a rubber tube which is connected to a suction pump. The bugs were held at the dorsal scutellar region and one micro-litre of *Tephrosia* extract at different concentration and Selecron 720 EC were applied using micro applicator on the ventral thoracic region. The treated and untreated 20 bugs (replicated 6 times) were placed in the Petri-dishes of diameter 10 cm lined with filter paper and fed with fully grown green berries (20 berries per Petri dish) and then placed in the temperature and relative humidity control machine at room temperature. The same treatments were applied in the field conditions so as to prove the effectiveness. In this case the treated antestia were put in a mosquito net that slid over coffee branches and tied at both ends with sisal twine to prevent the bugs from escaping. Mortality rate were assessed at 24, 48 and 72 hours after application. The collected data on mortality rate of the pest were subjected to analysis of variance (ANOVA) using a COSTAT statistical package and means separated by the Student-Newman_Keuls (SNK).

RESULTS AND DISCUSSION

We observed an increase in efficacy with concentration. The last two treatments of *T. vogelii* (1200 and 1400 g/litre) did not show significant difference from the standard (Selecron 720 EC) for the mortality rate of adult antestia bug in both the laboratory and under field conditions at 72 hours. However, significant differences ($p \leq 0.05$) were observed between *T. vogelii* extracts at 800g and 1000g/litre of water and the standard in both cases (Figure 1).

The study revealed that the leaf extract of *T. vogelii* has an efficacy against the adult antestia bugs in line with Morris, (1999) who reported that the twigs and leaves of *T. vogelii* contains retenoid compound which kills and repels insect attacking various crops. Isman (2006) also reported that *T. vogelii* is being used for control pests of economic importance on lettuce and tomato in California.

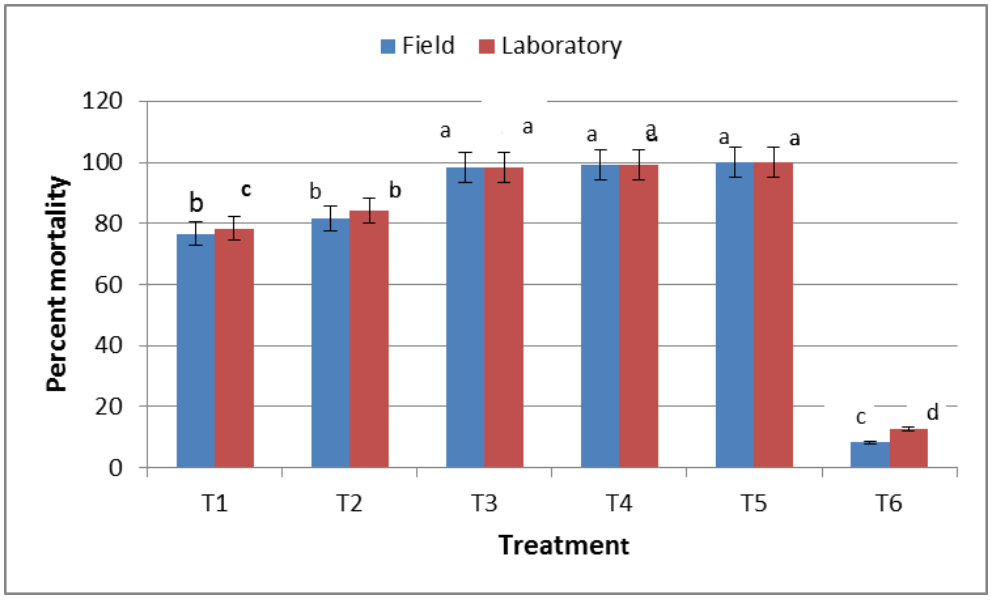

Figure 1. Percentage mortality rate of antestia bugs after treatment with Selecron and four dosages of *T. vogelii* extracts at 72 hours. Mean values with the same letters are not significantly different at P≥ 0.05 Student Newman- Keuls (SNK). T1 = 800 g of T. vogelii/1 Litre of water; T2 = 1000 g of T. vogelii/1 Litre of water; T3 = 1200 g of T. vogelii/1 Litre of water; T4 = 1400 g of T. vogelii/1 Litre of water; T5 = Selecron 1.5 mls/1 Litre of water (standard); T6 = Untreated (control).

Botanicals insecticides have long been used in various countries because they are reputed to pose little threat to the environment or human health, delay the development of resistance in pest populations, they only affect the target pest and closely related organisms, decompose quickly, and provide safe, residue-free food and have lower cost of production. Furthermore, botanical pesticides are unique because they can be produced easily by small scale farmers and small industries (Khater, 2012; Isman, 2006). However, precise timing and/or more frequent applications may be necessary.

CONCLUSIONS

It has been revealed that the leaf extract of *T. vogelii* possess insecticidal properties against antestia bugs. The *T. vogelii* rate at 1200g/litre is recommended to be used by coffee farmers for management of antestia bugs at the medium altitude (1200-1600 m. a. s. l.). However, further work is required to evaluate the efficacy of the botanical at different altitudes, different plant parts and fresh/dry formulations.

ACKNOWLEDGEMENTS

We are grateful to European Commission (EC, Tanzania), IPM CRSP in East Africa and Tanzania coffee growers for their financial support for this study. We are also grateful to TPRI National Herbarium of Tanzania, for identification of the plant sp.

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Dissemination of Technologies to Coffee Growers in Tanzania

J. MAGESA, I. MUSHI, G. SHAO, N. M. NG'HOMA, S. MDEMU, E. TARIMO, J. M. TERI

Tanzania Coffee Research Institute (TaCRI), P. O. Box 3004, Moshi, Tanzania

SUMMARY

Coffee is one of Tanzania's primary agricultural export crop produced 90% by smallholders and 10% large producers. The industry provides direct income to more than four hundred thousand farmer families and also benefits indirectly the livelihoods of over two and half million Tanzanians representing 6% of the Tanzania population estimated to be 40 million. Despite the benefits obtained from coffee, production levels have been below the potential. To address the existing problem of low production levels, the Tanzania Coffee Research Institute (TaCRI) has developed, tested, packaged and is currently promoting and disseminating appropriate technologies to coffee growers together with empowering coffee growers with sustainable technologies. This has been successfully done by conducting promotional activities including provision of village based training. For the last ten years, 313,051 coffee growers across the country have benefited from the training with 1,061 demonstration plots established on farm; 140,215 extension leaflets distributed to coffee growers and 89 open days conducted in coffee growing areas. The results show that there is increase in the number of coffee growers who adopt the new technologies, increased the area planted with hybrid varieties, increased coffee yield and improvement and the livelihoods of coffee growers. This paper outlines various dissemination strategies that TaCRI and coffee growers have been implementing to rejuvenate the coffee industry in Tanzania.

INTRODUCTION

Coffee provides direct income to more than four hundred thousand farmer families and benefits indirectly the livelihoods of over two and half million Tanzanians. About 90% of coffee is grown by small holders on average plots of 1-2 hectares; the remaining 10% is produced on large scale by large producers (coffee estates). Tanzanian coffee production levels are of 50,000 metric tons on average, which is much lower than that of many other coffee producing. To address the existing problem of low production levels, a number of interventions have been carried out including researching on disease resistant coffee varieties. For the past 10 years, Tanzania coffee research institute (TaCRI) has released 15 Arabica coffee varieties that are resistant to coffee berry disease (CBD) and coffee leaf rust (CLR); and 4 Robusta coffee varieties that are resistant to coffee wilt disease (CWD).The improved coffee varieties have confirmed to be high yielding, good cup quality equal to or better than that of the traditional varieties. More important is that they are cultivated without fungicides application thus cuts the costs of production by 30-50%. In addition to the release of new coffee varieties, TaCRI has packaged technologies for their multiplication including constraints to coffee productivity, quality and income of coffee growers.

TaCRI has established technology transfer and training programme that deals directly with coffee growers to ensure that appropriate technologies developed are effectively disseminated to the end users. This document summarizes dissemination strategies which have led to

increased adoption of technologies in terms of seedlings production, coffee productivity, quality and income of coffee growers.

DISSEMINATION STRATEGIES

Technology development, dissemination and utilization need interdisciplinary approach that involves researchers, extension agents and farmers (Orodho, 2012). The failure of a technology that is being disseminated to farmers is the result of poor participation of the end users in technology development and dissemination. Other authors (Magesa *et al.*, 2010; Wolf, 1995) have outlined a number of strategies that are used in disseminating technologies to the end users. These include study tours, field days, demonstration plots, public media, training courses, exchange visits, structured visits, agricultural shows and publications.

Packaging and distribution of extension leaflets

Research recommendations in the form of leaflets written in simple language to enable farmers read easily and follow have been packaged. For the last 10 years, 140,215 extension leaflets to promote coffee technologies on coffee agronomy and primary processing, seedlings multiplication and farmer groups' formation and management have been distributed of farmers.

Training of farmers

It has been documented that training courses are for creating and developing synergy and complicity between the participants, with the ultimate goal to raise the rural farmer's level of skills so that later on they become part of the decision making in managing their rural activities (Wolf, 1995).TaCRI has adopted participatory village based training approach as one of the dissemination strategies of technologies to coffee growers. About 647 coffee nurseries have been established with 313,051 coffee farmers trained in coffee agronomy, seedlings multiplication, and group formation and management with outstanding achievements (Figure 1).

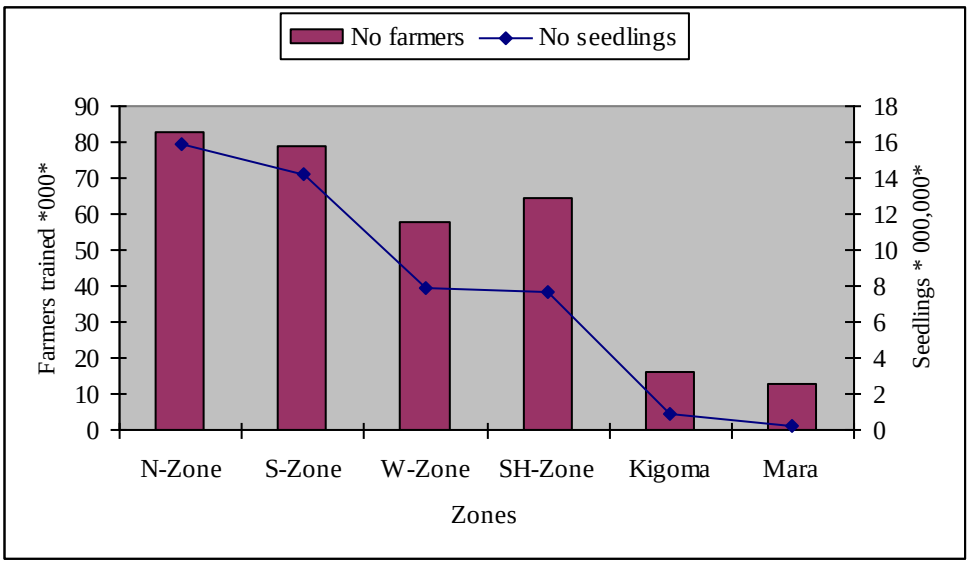

Figure 1. The number of coffee farmers trained and hybrid seedlings multiplication. N= Northern; S= Southern; SH= Southern highlands; W= Western.

Training of extension officers including farmer to farmer training

The involvement of extension officers including farmer to farmer extension who then works with farmers is important for successful dissemination of technologies (Magesa et al., 2010). TaCRI has trained 5,430 extension officers and 3,307 farmer promoters who are now participating in the dissemination of technologies to coffee growers in their respective districts, and there is increased adoption of technologies where such extension approaches have been used (Figure 2).

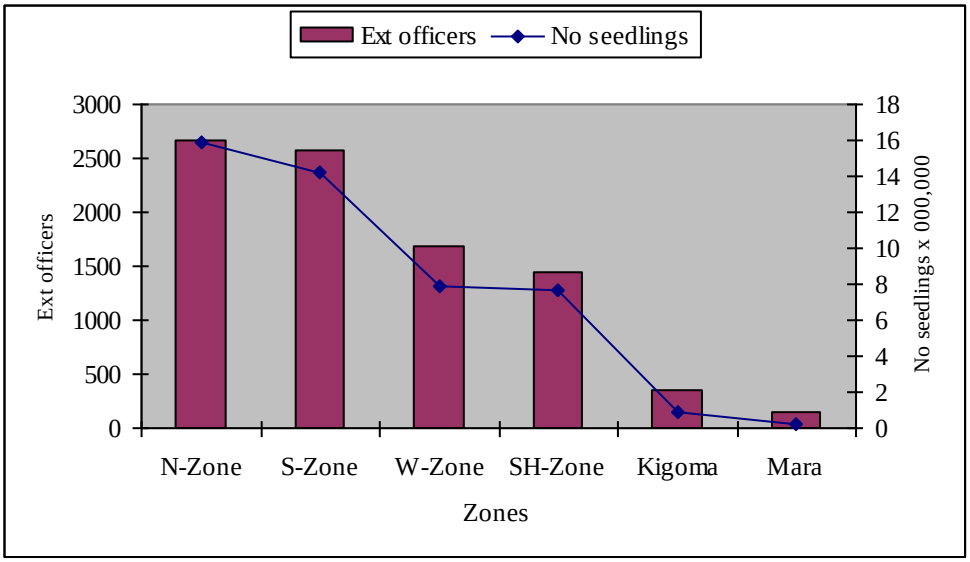

Figure 2. The number of extension officers trained and hybrid seedlings multiplication. N= Northern; S= Southern; SH= Southern highlands; W= Western; Ext= Extension.

Demonstration as learning plots on coffee technologies

On-farm demonstration plots are one of the effective means of promotion and dissemination of research technologies (Magesa *et al.*, 2010). TaCRI has established 1,016 demonstration plots on the performance of both improved and traditional coffee varieties in the coffee growing districts. Demonstration plots are now becoming centres for learning about the recommended research findings especially the performance of improved hybrid varieties and the application of good agricultural practices.

Exchange visits/study tours

They form part of strengthening partnerships and capacity building training whereby farmer groups or coffee growers conduct field visits to their fellow farmers with the aim of learning on certain technologies that is in need identified by the farmers in collaboration with extension officers. They focus in areas with impacts (successful clonal seedlings multiplication/increased yield/improved quality). The level of adoption has increased in areas where exchange visits/study tours have been conducted.

OTHER OUTSTANDING ACHIEVEMENTS

Number of farmer groups and area under improved coffee varieties

The area planted with hybrid coffee varieties has also increased in areas where a high number of farmer groups have been formed and participated in the multiplication of seedlings (Figure 3).

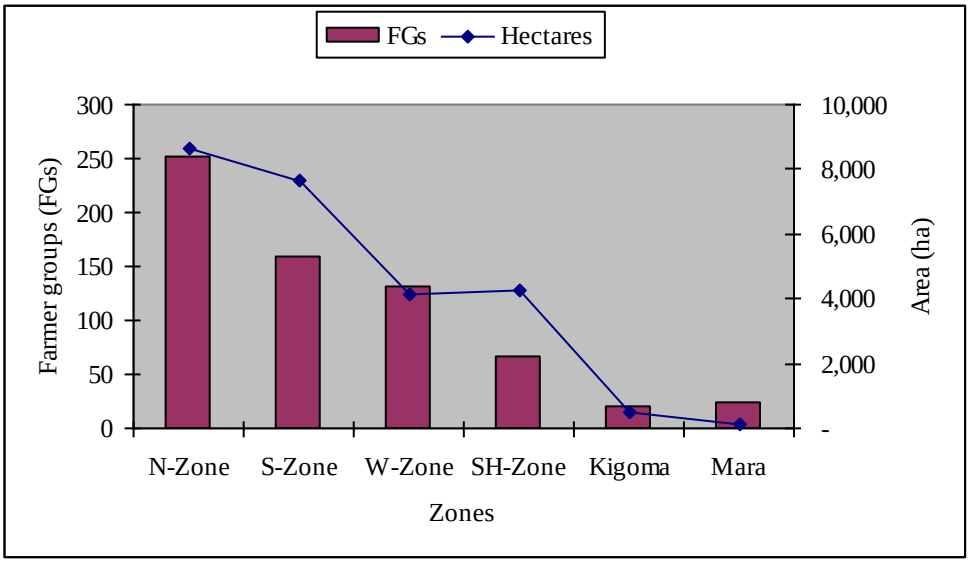

Figure 3. The number of farmer groups and the area planted with hybrid seedlings. N= Northern; S= Southern; SH= Southern highlands; W= Western; FGs= Farmer groups.

Yield, quality and livelihoods improvement

It has been reported that there are yield increase up to 2Kg/plant and improved quality classes up to 7-4 which farmers never realized before planting new varieties and adopting the good agricultural practices. The costs of production have also reduced as the new varieties are grown without fungicides application and hence the increased incomes and livelihood improvement of coffee growers have been noticed in some coffee growing areas.

CONCLUSIONS

This paper has summarized various disseminations strategies for increased adoption of coffee technologies to coffee growers. Therefore, multi approach strategy is important for successful dissemination of research recommendations to end users.

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We are grateful to the European Commission (EC, Tanzania) and Tanzania coffee growers for their financial support for this study. We are also grateful to the coffee growers who participated in the on-farm activities.

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Oyster Mushroom Farming Utilizing Primary Coffee Wastes: Preliminary Results from Northern Tanzania

J. A. MABAGALA, J. MAGESA, E. S. MBEGETE, J. M. TERI

Tanzania Coffee Research Institute (TaCRI), P.O.BOX 3004, Moshi, Tanzania

SUMMARY

Coffee farmers in Tanzania are producing over 200,000 tones of coffee wastes from their farms annually. These coffee wastes includes coffee pulps and coffee husks which are normally left to decay on top of the soil, disposed in rivers, streams or burnt ending up polluting the environment. Primary wastes from coffee farms can be economically used by growing oyster mushrooms, which in turn provides a complimentary source of family income to cushion the effect of fluctuating coffee prices. The study conducted in Northern Tanzania using basic principles developed by Zero Emission Research Initiative (ZERI), showed that on average a small-scale farmer produces 100 kilograms of wastes per year, which has a potential of producing 120 kg of fresh oyster mushrooms. The average price for a kg of fresh oyster mushrooms in local supermarkets and hotels around the study area is USD 4 which gives a farmer USD 480 per season from 100 kg of wastes produced. The harvested mushrooms could also be consumed by the house holds thus improving their nutritional and health. The spent mushroom substrates (SMS) can be used as organic fertilizers. Thus, with mushroom farming technology using primary coffee wastes, coffee farmers can complement their income from coffee to improve their food security and livelihoods.

INTRODUCTION

According to Tanzania coffee board, average coffee production as for the past five years (2004/05–2008/09) is 51,777 tons of clean coffee (http://www.coffeeboard.or.tz/tzcoffee_%20profile.php). In the coffee industry, only 9.5% of the weight of the fresh material used for the preparation of the beverage, and 90.5% left as residue (www.scizeri-nm.org/ZERI/mushrooms.asp). From the average coffee produced in Tanzania, more than 200,000 tones of coffee wastes resulting from wet and dry processing of cherries produced annually. Common method used to dispose off this material involves dumping it in water catchments areas ending up polluting natural water systems. Alternatively, this by-product piled nearby agricultural lands rendering it unavailable for agricultural production.

In coffee producing countries, it constitutes a source of severe contamination and a serious environmental problem thus, since the middle of the present century, efforts have been made to develop methods for its utilization as a raw material for the production of animal feeds, beverages, vinegar, biogas, caffeine, pectin, pectic enzymes, protein, and compost (R.Martinez and R. Jose, 1998).

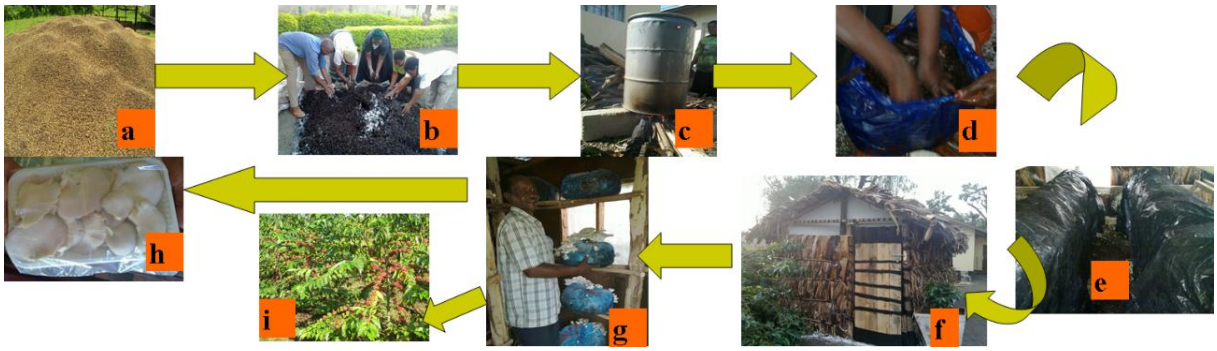
Primary wastes from coffee farms can be economically spent by growing oyster mushrooms (*Pleurotus ostreatus)*, which in turn provides a complimentary source of income to cushion the effect of fluctuating coffee prices in bad years when the coffee prices are much lower as coffee has notoriously unstable market. More over, income from mushroom farming can help

small scale coffee farmers have the capacity of purchasing some of the necessary inputs and equipments required to maintain their coffee farms.

The aim of the study was to train farmers to produce oyster mushrooms utilizing wastes from primary processing as a complimentary source of income to farmers in Northern Tanzania.

MATERIALS AND METHODS

Twelve individual farmers and three farmer groups (with 10 farmers each) were exposed to oyster mushroom farming technology using basic principles developed by Zero Emission Research Initiative (ZERI) [Chang S.T, 2007] and modified to fit the study environment. Coffee pulps from primary processing were fermented for one month to have odorless, physically and chemically homogenous substrate (Martinez-Carrera, D, 2000). Fermented coffee pulps were soaked in clean water for 48 hours then removed and placed on a wire mesh to drain until no drips when squeezed. Coffee pulps were mixed with lime at the ratio of 5 kilograms: 250 grams respectively and packed in 5 kilogram bags then subjected to steam sterilization for 2 hours. The bags with sterilized substrate were left to cool for 24 hours. Thereafter, 3 kilograms of substrates were mixed with 50 grams of spores, tightly packed in plastic bags of 5 kilograms and sealed. Mushroom spores were obtained from commercial producers. Bags packed with substrate and spores were placed in a dark room, after 25 days, a white growth, referred to as mycelium, filled the bags. Bags filled with any other colors such as green, red or black, were contaminated thus, discarded without opening. After the bags were fully filled with mycelium, they were randomly punctured 20 times with a bleach sterilized nail to allow air penetration. The bags were moved to a room with indirect light and enough air circulation and arranged on racks. Humidity was well maintained by misting the mushroom racks using hand sprayer. Mushrooms were harvested within 2 weeks after emerging out of the punctured holes and continued to be harvested for about 8 weeks.



Figures. (a) A pile of fresh coffee pulps. (b) Fermented pulps mixed with lime. (c) Steam sterelization process. (d) filling sterilized substrate & spawn in bags. (e) Filled bags at the dark room. (f) Mushroom growing structure made of dry banana sheath. (g) Mushroom ready for harvesting. (h) Fresh mushroom packed for sale. (i) Spent mushroom substrates sent back to the coffee farm as fertilizer.

RESULTS AND DISCUSSION

The study showed that, on average a small-scale farmer with 300 trees produces about 100 kilograms of coffee wastes (fresh weight) during primary processing. A bag with 5 kilograms of substrates from primary processing produced 6 kilograms of fresh mushroom by the end of the harvesting season, which ranged between 2 and 3 months. A farmer could therefore produce about 120 kilograms of fresh mushroom (based on fresh weight) per 100 kilograms of wastes from primary processing. On average, price per kilogram of fresh oyster mushroom

in local supermarkets and hotels around the study area was USD 4, which gives a farmer USD 480 per season from 100 kilograms of wastes produced. Part of the income obtained from the sale of mushrooms could be used by the farmers to purchase inputs to improve their coffee farms for the increased yield and quality. The family members could also consume small portion of the mushrooms produced by farmers as their important source of protein thus, improving their health and nutrition status. The sms obtained after harvesting mushroom was used by farmers as compost to their coffee farms based on the fact reported by Rinker *et al.,* 2004, referring to the study by Zheng *et al.,* 2002 that, *Pleurotus* compost contains high percentages of the three main primary nutrients (nitrogen, N; phosphorus, P or P_2O_5 ; and potassium, K or K_2O) as shown in table below.

Table 1. Analysis of the fertilizer value of compost from the edible *Pleurotus ostreatus.*

Material	**N%**	**P2O5%**	**K2O%**
Pleurotus compost	1.7	0.61	1.13
Human manure and urine	0.3	0.16	0.3
Pig manure	0.6	0.6	0.5
Cow manure	0.59	0.28	0.14

Source: Rinker et al. 2004.

CONCLUSIONS

Preliminary results show that, coffee wastes from primary processing can be important raw material for oyster mushrooms farming to complement income of coffee farmers obtained from direct sale of coffee thus improving their food security and livelihoods while protecting their surrounding environment.

AKNOWLEDGEMENTS

We are grateful to European Commission (EC, Tanzania) and Tanzania coffee growers for their financial support for this study. We are also grateful to the coffee growers who participated in on farm activities.

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Factors Affecting the Accelerated Multiplication of Seedlings of Improved Hybrid Coffee Varieties in Northern Tanzania

E. S. MBEGETE, J. A. MABAGALA, J. MAGESA, J. M. TERI

Tanzania Coffee Research Institute (TaCRI), P.O.Box 3004, Moshi, Tanzania

SUMMARY

A study was conducted in Northern Tanzania to assess factors affecting the accelerated multiplication of improved hybrid coffee seedlings by farmer groups. Factors examined were; number of mother plants, access to inputs, training and backstopping, rooting media, price of seedlings, women involving and water for irrigation. Data were collected from 50 farmer groups and other stakeholders by using structured questionnaires and focus group discussion. The data were analyzed by using statistical package for social science (SPSS) and system thinking approach. It was found that 37% of the improved coffee seedlings are produced by farmer groups while others TaCRI account 27%, Estate 21%, Cooperatives 8% district 6% and individual farmers 1%. Training and backstopping, inputs suppport, rooting media, price of seedlings, women involvement and group leadership affects the accelerated multiplication of improved hybrid coffee seedlings by farmer groups. Therefore more efforts or emphasis is required to overcome the mentioned factors so as to speed-up the seedling multiplication from farmer group in the study area.

INTRODUCTION

Tanzania Coffee Research Institute is responsible for coffee research and technology transfer to support the rejuvination and development of the coffee industry in Tanzinia to sustainable prosperity. Since become legally consitituted and operational in September 2001, TaCRI has listed a number of milestones passed by the institute in modernizing coffee farming through appropriate coffee research in the country. TaCRI, 2012; 2011: 2005 officially released 15 improved Arabica hybrid coffee seedlings with high yielding potential and resistant to coffee berry diseases (CBD) and coffee leaf rust (CLR) (TaCRI publicity booklets 2011). The challenge is to fulfill high demand of the imroved arabica hybrid seedlings varieties to farmers. Efforts have been done by TaCRI to multiply seedlings of the improved hybrid coffee varieties through clonal propagation and grafting by organizing a number of stakeholders (farmer groups, co-operatives, individual farmers, districts, and estate). Clonal multiplication with this not only multiplication and distribution of improved Arabica hybrids but also guarantees uniformity as it maintains the genetic make-up of the mother plants, which are usually heterozygous (Nzallawahe *et al.,* 2004; Beyl and Trigiano, 2008). Vegetative propagation offers the unique advantage of preserving all the characteristics of mother plants in their offspringTaCRI supports stakeholders with planting materials, input and technical advises. Working with farmer groups is more conducive to effective dissemination and adoption of technologies (Esber and Sthapit, 2000 and FARA, 2006) in Ethiopian for example where the extension staff working with groups the adoption of technologies have been much higher than extension working with individual farmers (Wolf. N Judith 1995). TaCRI is working with more than 800 smallholders farmer groups in Tanzania, with 251 farmer groups in northern zone with about 83,171 mother plants. This study highlights factors affecting the accelerated multiplication of improved hybrid coffee seedlings by farmer groups and suggests ways for improvement.

MATERIALS AND METHODS

The study employed structured questionnaire and focus group discussion to collect data from 50 farmer groups, which were randomly selected from the northern Tanzania coffee grown areas. The study interviewed leaders from each group. Data were analyzed using Statistical Package for Social Science (SPSS) and system thinking approach. Results were summarized in percentage form. Secondary data obtained in available books and on farmer groups' documents, were also included.The study focused on farmer groups (37%) who has high capacity of seedlings produced in northern Tanzania.

RESULTS AND DISCUSSION

Information extracted from the questioneare (Table 1) indicates that 16% of respondent were women and 84% male. Majority of the group leader have primary level of education at 70%, secondary 27% and 3% have informal education.

Table 1. Characteristics of respondant.

Description		Frequence	Percentage (%)
Sex	Male	42	84
	Female	8	16
Age	<41	15	30
	41-60	30	60
	>60	5	10
Education	Primary	35	70
	Secondary	14	27
	Others	2	3

It was revelled that 85% of the interviewed stalkehoders have an average of 250 mother plants, each producing 25 seedlings in a yea rper plant, and 15% produce an average of 34 seedlings in a yea/per plant.

Figure 1 shows 37% of the improved coffee seedlings are produced by farmer groups, while TaCRI on-station nursery account 27%, Estate 21%, Cooperatives 8%, district 6%and individual farmers 1%. From the observation farmer groups are the potential coffee seedlings multiplication.

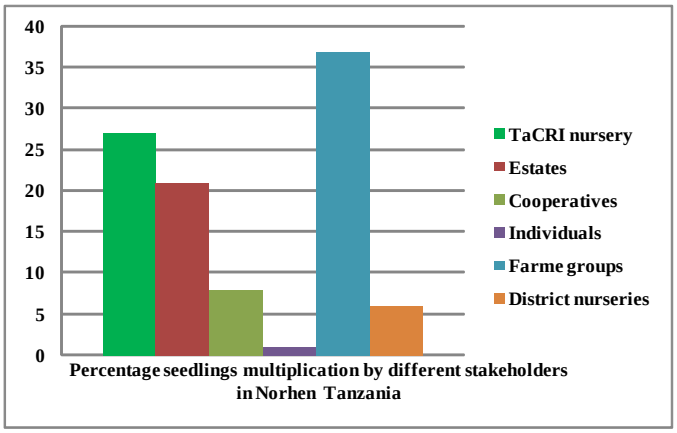


Figure 1. Percetage seedlings multiplication by differents stakehoders.

Figure 2 shows that, training and backstopping affects seedlings multiplication by 40% compared to other factors, such as inputs suppport 33%, group leadership 18% and women involvements 9%. Training and backstoping contibtes higher due to participatory approach as formerly documented by Esber, F.H (2000).

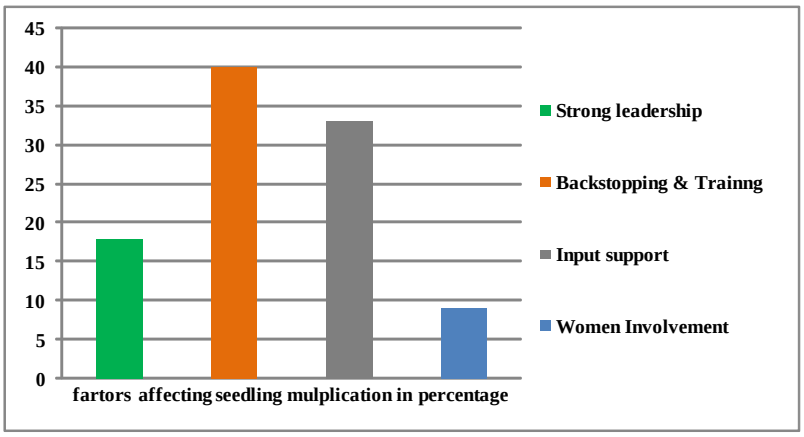
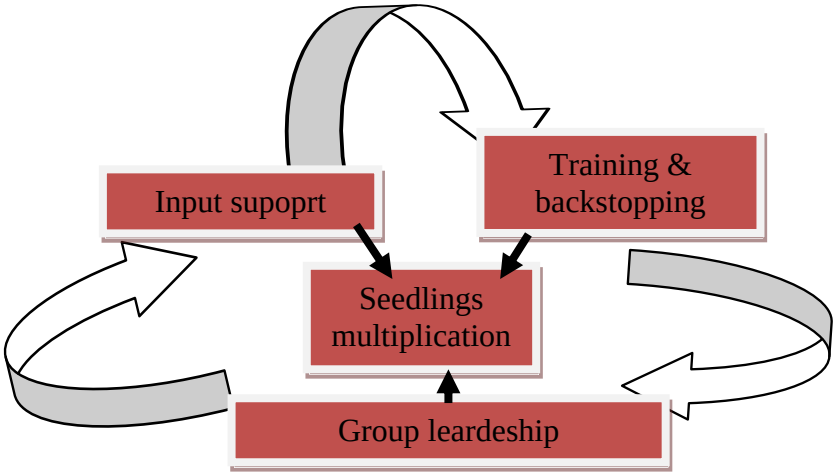


Figure 2. factors affecting farmer groups on seedlings mulplication.

Training and backstopping, group leadership, and input support were the major factors contributing to the success of seedlings multiplication. Out of this *successive cycle for sustainability of farmer groups* is developed.



Successive cycle for sustainability of farmer groups

Focus on group discussion revelled that seedlings produced are circulated to the members of the group for planting to their own farm, the remain they sell to others. The prices of improved coffee seedlings is or above breakeven point (approximately US $ 0.8 per seedling) the price motivate farmer groups on multiplication of improved seedlings. Areas to establish clonal mother garden, rooting media(forest soil & sand) and water for irrigating nuseries seems to be not a big challenge to most of the farmer groups.

CONCLUSIONS

The study indicate that backstopping and training, input support, and stronger leadership are the major factor to be considered on sustainability of coffee farmer groups in northen zone of Tanzania on clonal seedlings multiplication.

ACKNOWLEDGEMENTS

We are grateful European Commission (EC, Tanzania) and Tanzania coffee growers for their financial support for this study. We are also grateful to the coffee growers who participated in the on farm activities.

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The influence and implications of climate change and variability on *Coffea* arabica in the East African highlands: Mt. Kilimanjaro case study

A. C. W. CRAPARO[1], P. J. A. VAN ASTEN[2], P. LÄDERACH[3], L. T. P. JASSOGNE[2], S. W. GRAB[1]

[1]School of Geography, Archaeology and Environmental Studies, University of the Witwatersrand, P/Bag 3, WITS 2050, South Africa
[2]International Institute of Tropical Agriculture (IITA), P.O. Box 7878, Kampala, Uganda
[3]International Center for Tropical Agriculture (CIAT), Cali, Colombia

SUMMARY

The coffee growing regions on the slopes of Mt. Kilimanjaro have become progressively warmer and drier over the past 48 years. The minimum temperature is the predominant climatic variable which influences coffee yields over longer-term climatic change. In contrast, the amounts of rainfall and in particular the timing of rainfall at specific growth periods are the most important climatic variables governing coffee yields on the micro-climatic scale. The predicted increase in climate change (temperature) and variability (rainfall) in future may thus adversely affect yield and cause numerous sporadic and incomplete flowering periods, effectively decreasing the amount of fruit set.

INTRODUCTION

Coffea arabica is highly sensitive to climate change, with a particular vulnerability to changes in temperature and precipitation. (Labouisse *et al*., 2008; DaMatta *et al*., 2008; Schroth *et al*., 2009; AdapCC, 2010; Jaramillo *et al*., 2011). In accordance with other parts of Africa, warming has occurred over much of equatorial East Africa, and most particularly since 1970 (IPCC, 2007; Williams and Funk, 2011; Conway & Schipper, 2011; Kadi *et al*., 2011). Unpredictable rainfall, extended drought periods and extreme weather events are just a few influences which are evident in a number of coffee producing areas, not only in the region, but also throughout the world (Baker and Haggar, 2007; Lin *et al*., 2008; Schroth *et al*., 2009; ITC, 2010; AdapCC, 2010). Occurrences of pests and diseases are becoming more frequent, resulting in detrimental effects to coffee yield, which in turn is attributed to a loss of $US 500 million annually (Labouisse *et al*., 2008; Schroth *et al*., 2009; ITC, 2010; Jaramillo *et al*., 2011).

In order to develop effective adaptation and mitigation strategies, accurate knowledge of the arabica plants response mechanisms to climate variables within different agroecological zones of East Africa is required (lobell & Burke, 2008). The aim of this study is therefore to quantify the relationship between climate change and variability and the response of the coffee plant over space and time in East Africa.

MATERIALS AND METHODS

In order to investigate the climate and yield dynamics over varying agro-ecological zones, high resolution climate and yield data (37 years) were attained from 8 geographically distant coffee plantations on the slopes of Mt. Kilimanjaro and the Ngorongoro Crater, Tanzania. Further to this, long-term lower resolution climate and yield data (48 years) from the Tanzanian Meteorological Agency was used to determine the macro-scale aspects of the plant-climate relationship.

Multiple regression and Indicator/dummy variable analyses were used on these data sets. In concordance with the literature on coffee physiology and phenology, several bioclimatic indices of the phenologically-important stages were determined and used in the analysis. The variables consisted of; the annual mean minimum, maximum and mean temperature of the year prior to harvest, as well as the minimum, mean and maximum temperatures of each phenological phase. Precipitation variables included the annual total rainfall, the total rainfall during the dry season prior to harvest and the number of days with rainfall during the flowering period. Step-wise regressions, both forward and backward as well as regression subsets were used to select parameters for the model. Principal component analysis and the variance inflation factors (VIF) were used to identify and mitigate multicollinearity between the predictor variables. Climatic periods and variables which were found to be significant (p-value: <0.05) were then further investigated using indicator variables in order to determine subtleties in the relationships and boundaries. All model assumptions were met and verified using the GVLMA statistic and no transformations were needed. All statistical analyses were carried out using R software version 2.8.1.

RESULTS AND DISCUSSION

Macro-scale influence of climate change on coffee

Climate data from the coffee growing regions of Tanzania indicate a mean minimum temperature increase of 0.34°C/decade and a decrease in the mean rainfall of 30mm/decade over the past 48 years (1962-2010). Using all bioclimatic variables, the model selection and multiple linear regression reveals that the mean minimum temperature of the coffee growing regions of Tanzania is the most influential climatic parameter impacting on yield (p-value: 5.11e-09). The minimum temperatures alone account for approximately 52% of the variation in yield. As a result, an increase in the mean minimum temperature of 1°C results in an average loss of 98 Kg/Ha (Figure 1).

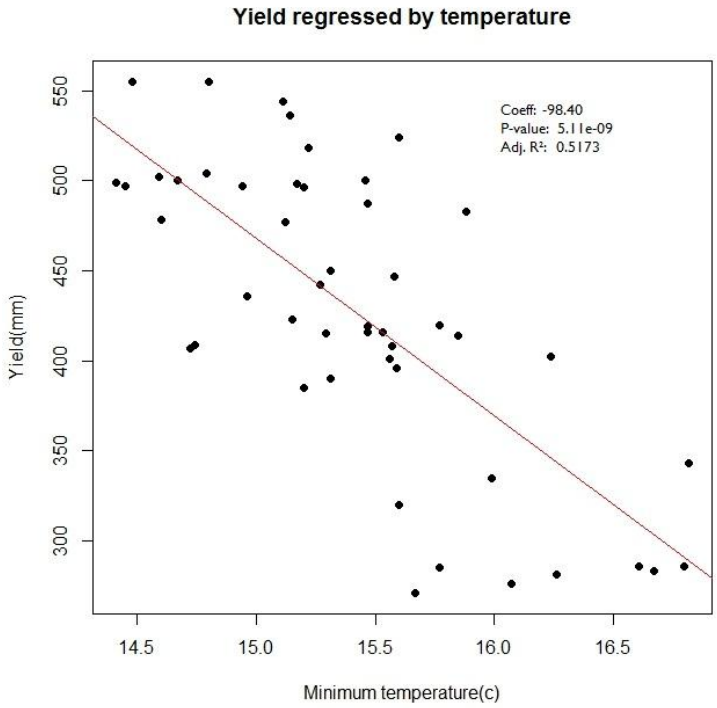

Figure 1. Yield regressed by minimum temperature.

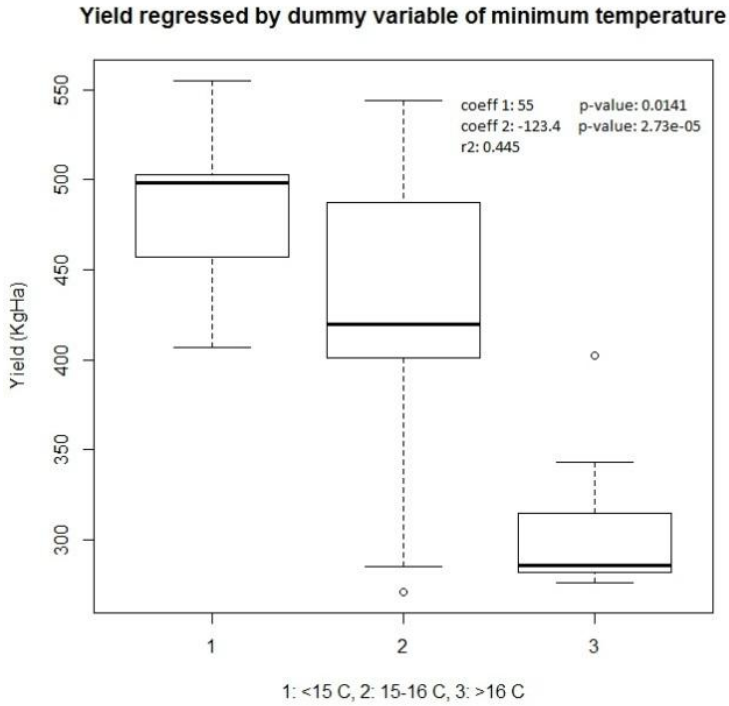

Figure 2. Dummy variables for minimum temperature.

Dummy variables were then created for the minimum temperatures. Three factors were contrasted between each other in order to gauge the relative importance and impact each category would have. The results indicated that the higher minimum temperatures (16-17°C) had the greatest negative impact with a loss of 125 Kg/Ha for every degree rise in temperature (p-value: 1.2e-06). On the contrary, minimum temperatures from the lower end of the

spectrum (14-15°C) had a minor positive influence with a gain in yield of 40.9 Kg/Ha (Figure 2).

Micro-scale Influence of climate change and variability on coffee

In contrast to the regional scale, the plantation-level analysis reveals that the rainfall variability is in fact the most significant climatic factor impacting on yields. The amount of rainfall during the dry season prior to flowering has a very significant inverse relationship with yield (0.00107), which is likely the result of the rainfall disrupting quiescence. An increase of 10mm of rainfall during this dry period would result in a loss of approximately 56 Kg/Ha (Figure 3). Dormancy during this dry period lasts between 2-4 months and is required by arabica to initiate flowering (Gay *et al.*, 2006; DaMatta *et al.*, 2008). In addition, low intensity rains during the latter part of flower bud development are thought to be responsible for several blossom periods of arabica. Fruit ripening is consequently unsynchronized, which therefore has serious consequences for management and production (DaMatta *et al.*, 2008). Dummy variable analysis using the number of days with rainfall during the flowering period in Tanzania (Sep-Feb) confirms this. Rainfall on approximately 20 days or less during this flowering period does not appear to have an adverse effect on yield and is in fact associated with an increase in yield of between 50Kg/Ha and 89.5 Kg/Ha depending on the site (p-value: 0.00344). On the contrary, once this boundary has been breached yield appears to be adversely affected. While not as significant in all plantations, rainfall on greater than 20 days is generally associated with a loss of 1.8 Kg/Ha (p-value: 0.0102). The high coefficient of variation for rainfall during the flowering period suggests that the variability and influence on yields may become a greater issue in future (Figure 4).

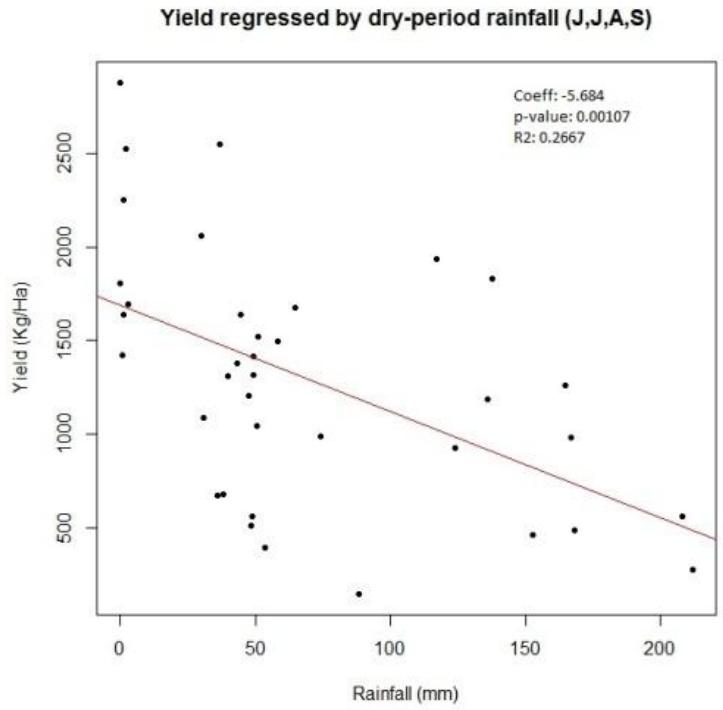

Figure 3. Yield regressed by rainfall during the dry period.

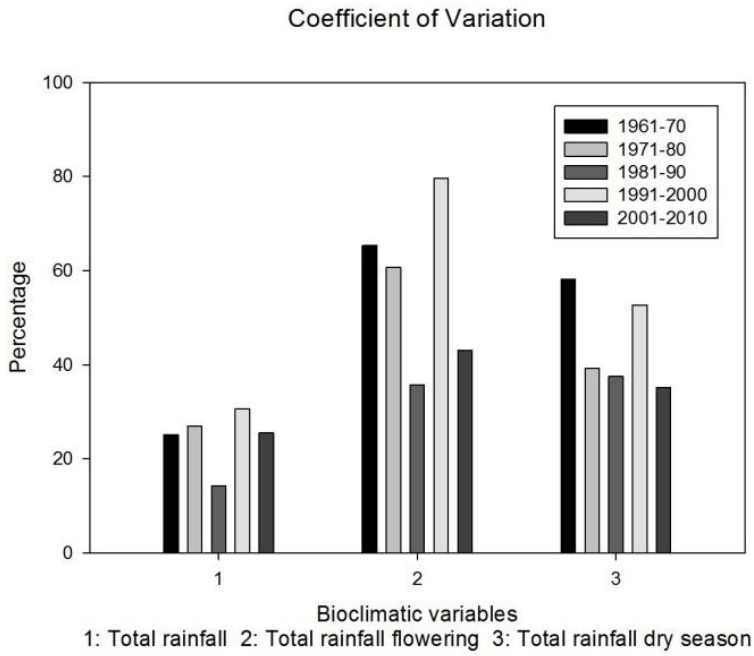

Figure 4. Coefficient of variation for bioclimatic variables of rainfall for past 5 decades.

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Landuse Composition and Configuration Affect Coffee Borer Distribution and Dispersla in Localized Farmscapes

F. A. J. DECLERCK[1,2], J. AVELINO[3,4], C. RIVERA[2], A. OLIVAS[2]

[1]Bioversity International, Turrialba, Costa Rica
[2]CATIE, Turrialba, Costa Rica
[3]IICA-PROMECAFE, Guatemala
[4]CIRAD UPR 106, Montpellier, France

SUMMARY

The coffee berry borer (*Hypothenemus hampei)* is a major pest of coffee in Central America. Recent studies demonstrate landscape context impacts distribution and dispersal of this pest where probability of successful dispersal events is higher across some agricultural land uses such as sugar cane and pasture, but limited by others, in particular forest. This impact of land use composition has been tested at local (Olivas et al. 2011), and landscape scales (Avelino et al. 2012). In this study, we used a small mixed-use landscape (550 x 500 m; 27.5 ha) to further test the impact of land use composition and arrangement on coffee borer densities and distribution. We placed 121 ethanol-based coffee borer traps (Brocap ®) at 50 m intervals throughout the sample grid plus an additional 32 traps located 10 m from adjacent traps along both diagonals of the study area for a total of 153 traps. We checked these traps every two weeks during a five-month period between February 2nd and July 7th 2010. Traps were located in seven different landuses, including: coffee (22%), pastures (22%); sugar cane field (18%), banana orchard (16%); pine plantation (10%), forest (9%); and annual crops (3%). We captured a total of 9984 dispersing female beetles, with the peak capture during the last two weeks of February. The majority of the coffee borers were found in the coffee plots as expected (89%). Eleven percent were found outside of coffee. The presence of coffee borers was significantly different in the alternate land uses with the lowest densities found in the forest (3%). Our results demonstrate that management of local landscape context can contribute to (but not replace) the control and management of the coffee boring beetle by limiting the movement of the organism and either increasing the effectiveness of field scale management intervention and/or reducing re-infestation rates.

INTRODUCTION

The coffee berry borer (CBB), *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae) is a major pest of coffee in Central America. Recent studies demonstrate landscape context impacts distribution and dispersal of this pest where probability of successful dispersal events is higher across some agricultural land uses such as sugar cane and pasture, but limited by forest (Avelino et al. 2012, Olivas et al., 2011). These studies have suggested a strong temporal signal to beetle movement, as well as fine scale movements on the order of <100's of meters. We explore the fine scale movement of the coffee berry borer in this study by establishing a trapping grid and monitoring changes in beetle densities and both time and space over a six month period.

MATERIALS AND METHODS

We used a mixed-use landscape near Jicotea, Costa Rica (9° 48' 48 N, 83° 32' 23 W) to conduct this study (Figure 1). Traps were located in seven different land uses, including: pasture (22%), forest (21%), coffee (20%), sugar cane (18%), pine plantation (10%), annual crops (5%) and banana orchard (4%). Coffee dominated the SW, SE, and NE corners of the grid, but was intersected by sugarcane, pasture, banana, pine plantations, and forests. The forest included a small riparian forest running NS at the top of the quadrant and a larger ridgetop forest running WE across the quadrant.

We placed 121 ethanol based Brocap ® traps on an 11 x 10 grid with 50 m distance between all traps shown in figure 1. We placed an additional 32 traps at 10 m distances from eight traps located along the diagonal (not shown). We checked the traps every two weeks recording both the trap location, land use and number of beetles captured. At this stage of the study we analyzed the number of beetles captured by trap in the various land uses during each sample period. We used krigging to test the spatial distribution of of borer and the impact of landscape context. Further analysis will be used to identify the response scale for beetle distribution across the landscape, and the fine scale effect of each of the eight land uses on beetle distribution.

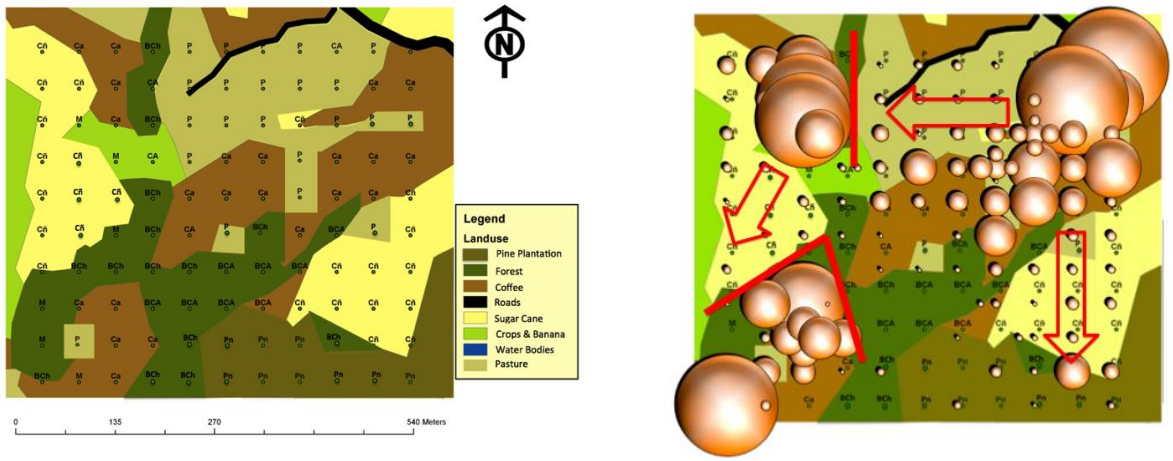

Figure 1. Landuse map of the 27.5 ha study area (500 x 550 m) with trap locations and landuse identified (left). Total beetle densities (right) as distributed across the landscape where bubble size indicates the relative beetle density.

RESULTS AND DISCUSSION

We captured a total of 9984 beetles during this six month study. Although the coffee points comprised only 22% of the study area, they contained 89% of the captures (figure 2) . Peak beetle dispersal occurred in early March with 1949 individuals captured in this landscape. Circles in the map above represent the total CBB density captured throughout the duration of the study. We found evidence of CBB movement between coffee parcels where the species was primarily found as well as into adjacent sugar cane, pasture, and pine plantations (arrows). However there was little evidence of beetle movement into and through forest (bars). Repeated sampling (Figure 3) showed a distinct movement event concentrated in late February/March accompanied by CBB emigration. This temporal pulse has been found in similar studies in the same landscape and is thought to be associated with the emergence and flight of female beetles in response to limited colonization sites (ripe berries) associated with the plants phenology and the harvest.

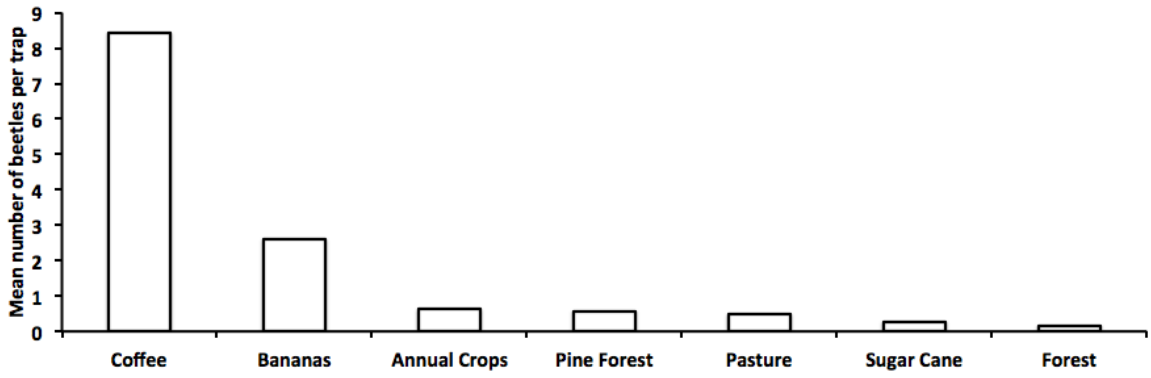

Figure 2. The mean number of beetles captures by trap day in each of the land uses. The majority of beetle captured outside of the coffee plots were found in banana plantations with additional captures found in the other agricultural land uses. Although forests comprised a significant portion of the landscape (21%) it contained one of the lowest beetle densities.

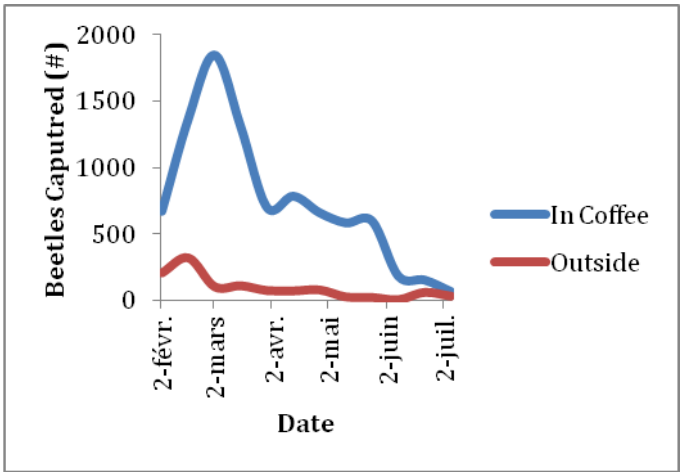

Figure 3. Total beetle captures through time both inside the coffee plots (22% of all traps) is compared to the number of beetles captured outside the coffee plots (78% of all traps). Captures inside and outside are correlated (R^2 = 0.30) though the peak captures outside coffee is advanced by two weeks compared to captures in coffee.

Krigging of the points (figure 4) showed strong spatial significant with the highest degree of semivariance running perpendicular to the forest patches and the lowest, running parallel suggesting a strong influence of the forest elements in directing beetle. Both the sill and the nugget of the semivariograms were at their highest the March movement events indicating a shift from very localized movements to landscape scale movement during this dispersal event.

Multiple studies now confirm that the land use composition and configuration adjacent to coffee parcels affects CBB spillover of and movement to adjacent coffee parcels. Barriers to dispersal are not absolute (yes or no situations) but rather that they are an effect of the larger landscape context. Movement of beetles is largely unrestricted across adjacent coffee parcels, but biological movement to coffee interspersed by alternate land uses will largely be a function of the land use and the distance that must be crossed

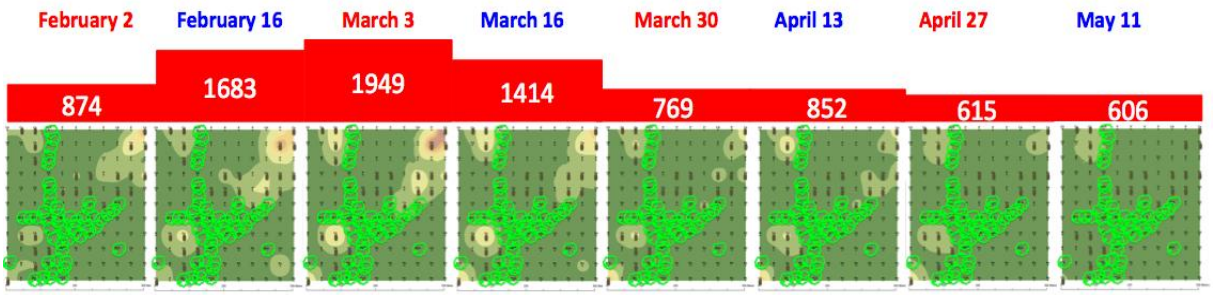

Figure 4. Interpolated beetle densities (krigged) throughout the study period. Actual beetle densities are indicated in the red bars above the figures. The distribution of the beetles was dynamic through time with beetles originating from the NE corner (February 2nd) of the plot, moving towards the centers (March 3 and 30), including across several of the agricultural land uses, and receding (May 11).

before reaching coffee. Different land uses have distinct dispersal thresholds that operate at relatively fine scales (10-100's of m) with free dispersal through coffee, moderate dispersal through pasture and sugar cane, and restricted dispersal through forests. Like forests, sugar cane can also serve as a barrier to movement, only at greater distances. The contribution of this fragmentation to coffee berry borer control is largely untested, but should increase the efficiency of control measures by limiting colonization events. We are currently testing the combined effects of dispersal limitation, and increased predator abundance on coffee berry borer control.

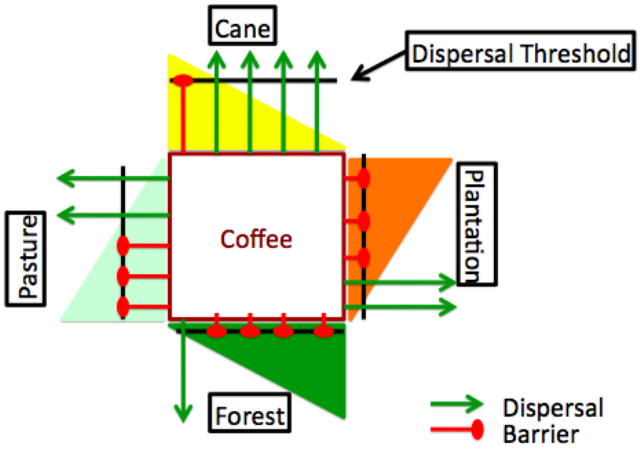

Figure 5. This study, and others (Olivas et al. 2010; Avelino et al. 2012), suggest that all land uses may be permeable to beetle movement, although the permeability is a function of the distance that the organism must traverse. Coffee offers the least resistance, and forest land uses the greatest. Sugar cane, and pastures offer intermediate resistances to movement.

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A Decision Support System for the Calculation of the Coffee Post-Harvest Costs (Pós-Café)

R. V. M. SANTOS[1], H. D. VIEIRA[1], F. M. BORÉM[2]

[1]Universidade Estadual Norte Fluminense
[2]Universidade Federal de Lavras

SUMMARY

This work presents the development of a decision support system to analyze the calculation of coffee processing costs along the after coffee harvest stage. The purposed system aims to facilitate producers, cooperatives and area consultants' decisions concerning to which processing should be applied to coffee.

INTRODUCTION

In a time when the market for special coffee is being expanded more and more and the consumers are becoming more demanding, increasing the quality of the drink is essential to supply market and increase the coffee grower's income. And the leading factor for this is the post-harvest.

The agronomic issues of coffee are already very well solved, however, at the moment when coffee is picked and routed to the coffee drying patio the main doubts come up. For the coffee producers is still very difficult to decide about which processing should be applied to coffee during the post-harvest stage (Figure 1).A lot of aspects must be considered in order to make these decisions easier.

In light of the above, it is purposed a development of a decision support system directed to the coffee post-harvest area. This program aggregates specialist knowledge in the area, not only to facilitate the decision concerning to the best cost-benefit processing to be applied, but it also clarifies the producers about the cost factors and critical points in this stage, before considered by them.

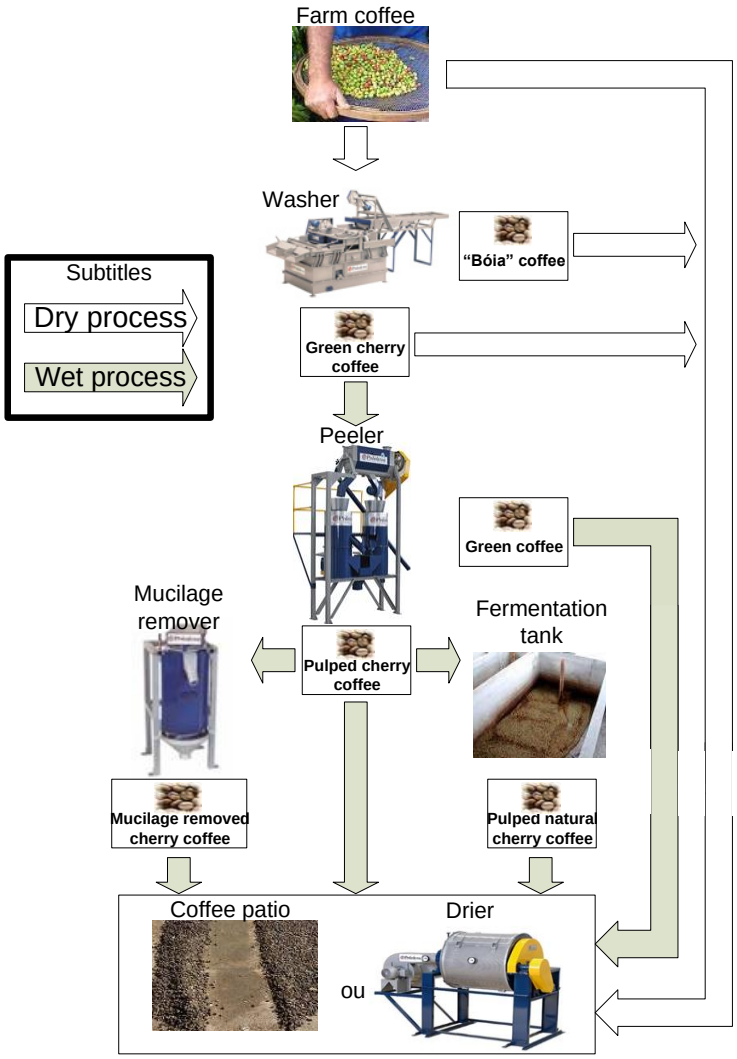

Figure 1. Coffee processing.

MATERIALS AND METHODS

Technology choice

The tools chosen for the application development will be show in this section.

Programming Language

The programming language chosen for the implementation was the Visual Basic.
The main reasons for this language choice were:

- Easy language: Considering the little experience of the developers.
- Support to various graphic patterns: necessary for the simulation results.

Modeling Language

For the modeling of the *Pós-café* system, the language UML (Unified Modeling Language) was used. It is a graphic language for visualization, specification, construction and documentation of artifacts for software complete system.

The modeling of the system was made using the program Rational Rose.

Data base

Access was chosen as database. That one, as its driver, does not need to be installed in the user's machine, making it simple the *Pós-café* installation.

Requirement specifications

All the knowledge in the *Pós-café* was initially represented in Excel sheets. The requirements were specified during meetings with specialists. At these meetings this initial knowledge was detailed and the doubts about formulas were solved.

During this process, the variables involved in the system were differed.

- Producer's entries: entry data related to the particularities of the coffee production from a producer in order to calculate the cost of a determined processing. Example: production of coffee in bags.

- Calculation index: it designates some special values, using in calculation formulas for the costs of the processing, which are results of researches developed in relation to coffee post-harvest. Example: the thickness of the cherry coffee which was pulped on the patio.

System Implementation

The implementation , of the *Pós-café* was made in Visual Basic, based on the modeling UML. In the result section the main functionality of the system will be detailed.

System Verification

During the development of the system two min kinds of tests were made:

- Made by the system developers: about the verifications made by the developers in order to confirm if the results obtained by the system corresponded to the requirement specifications.

- Made by the coffee producers: some producers, potential auxiliaries, received copies of the *Pós-café* with the intention for the ones to return suggestions, improvements and possible modifications in the system, in order to make the software to correspond as much as possible with the reality of coffee post-harvest.

RESULTS AND DISCUSSION

Processing Choice

The system makes possible for the user to choose two kinds of processing (Figure 2)

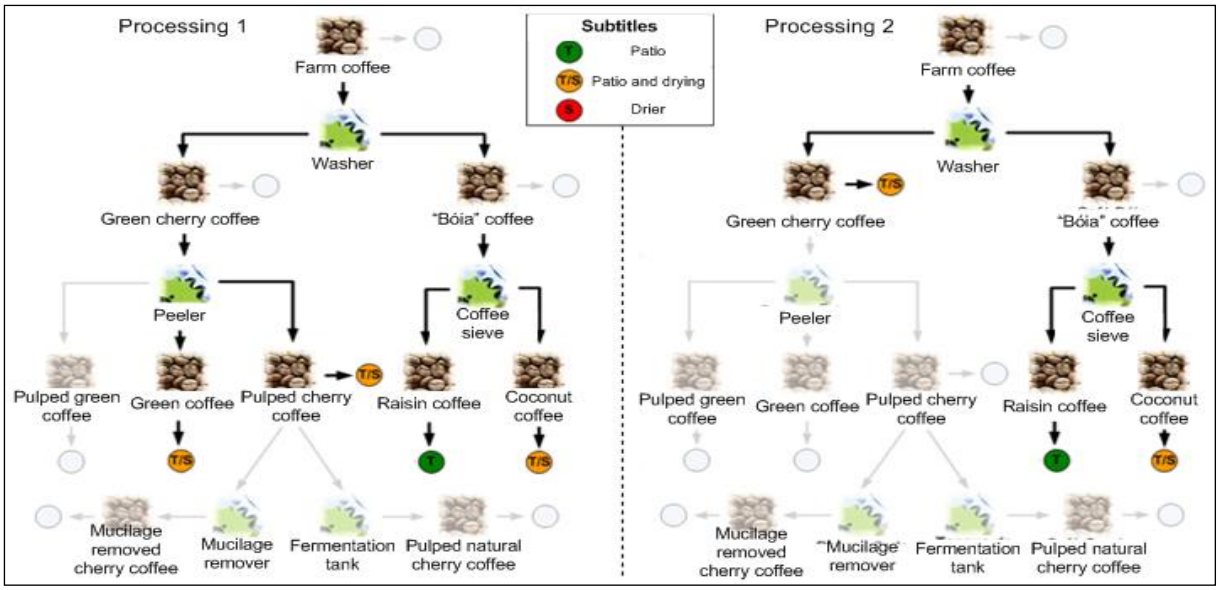

Figure 2. Processing Choice Screen.

Data Entry

After the processing choice, the user must provide data related to the production, according to some forms of data entry (Figure 3).

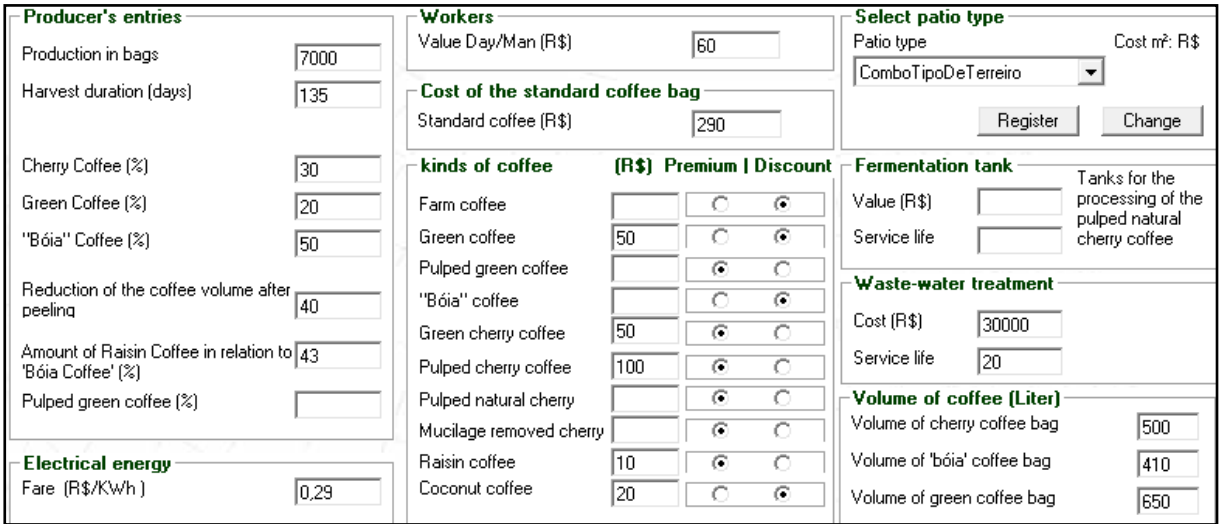

Figure 3. Data Entry Screen.

Reports

The Pós-café system will process the entry data and will return cost reports of the processing and of the net margin among them.

From the inform reports the producers will have his decision making facilitated concerning the processing to be applied in his production.

- Cost reports: presents the costs for bags, in Brazilian reais, separated by categories of electrical energy, workforce with the coffee processing (agricultural machine operators), workforce in the patio (workers working on the coffee revolving), total depreciation (depreciation of the patio and of the agricultural machines) and the cost of fuel (used in the coffee mechanical drying).

- Net margin report: presents the cash value, per coffee bag, that the producer will earn or lose when he chooses one kind of processing in relation to the other kind.

All the report mentioned before take into consideration the values of the percentage of cherry coffee, percentage of green coffee, premium and discount of the coffee bags informed in the data entries forms (Figure 3).

Figure 4 shows a graphic of net margin generated by the tool *Pós-café*.

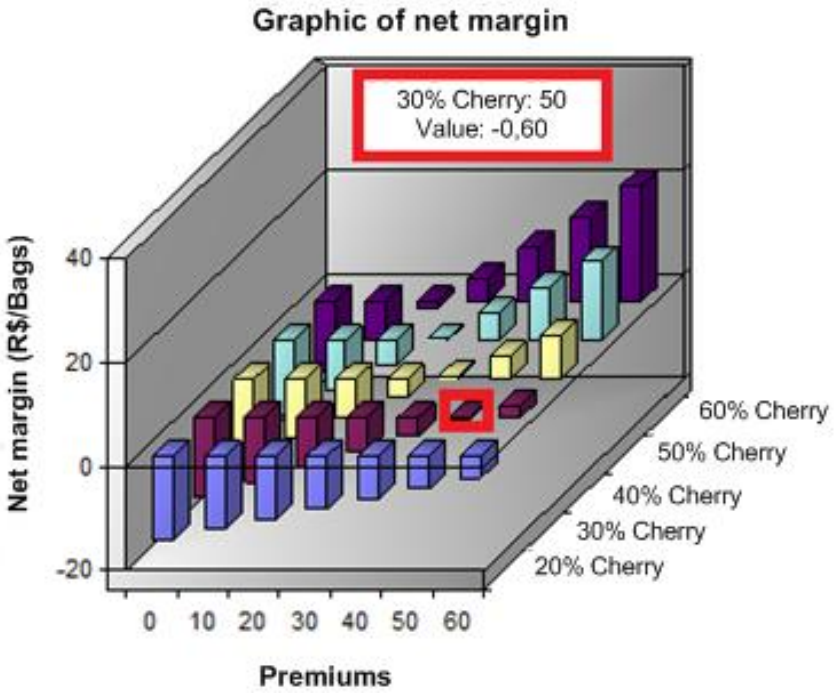

Figure 4. Graphic of the Coffee Post-harvest Net Margin.

Through the graphic of the net margin the producer can visualize other situations considering several premiums (value in Brazilian real referred to the difference between the bags of the kind of coffee with the biggest value of each processing) and percentages of cherry (percentage of cherry coffee).

Conjecturing about these topics the producer can identify in which moment one or other processing is financially viable or not. In the figure it is possible to observe highlighted the

point 30% of cherry and and a premium of R$50,00- difference between the bags with the biggest value of the processing one (bag of the pulped cherry coffee- R$ 390,00) and the processing two (bag of the green cherry coffee- R$340,00). Atthis point the net margin is approximately less sixty of real (-0,60). In other words, to choose the processing one (1) instead of the processing two (2), at these conditions, will make the producer not to gain sixty cents of real (-0,60).

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Density and Diversity of Nematodes in Coffee Agroforestry Systems Intercropped with Bananas and Legumes Shadow in Jinotega, Nicaragua

J. M. GARCÍA-SALAZAR[1], G. SOTO[2], H. FERRIS[3], F. CASANOVES[2], C. H. STAVER[4], J. AVELINO[2], J. CASTELLON[1]

[1]Universidad Nacional Autónoma de Nicaragua – León
[2]Centro Agronómico Tropical de Investigación y Enseñanza
[3]UC Davis
[4]Bioversity Internacional, Turrialba, Costa Rica

SUMMARY

Phytonematodes and soil nematodes population was studied in the presence of bananas, coffee and coffee trees in Jinotega, Nicaragua. The most densely populated phytonematode in the roots of banana and coffee was Meloidogyne J2, with higher concentration in the roots of coffee (3.429 individuals / 100 gr of coffee root). The genus Pratylenchus increased in coffee under CBL condition (1.186 individuals / 100 gr of coffee root), while decreases in bananas. Trophic groups identified in soil showed no difference between treatments. The largest number of bacteriophages in the treatment appeared CBL, bactephagos of cp2 group showed a higher number of individuals in C, the higher presence of micófagos was found in CB, predators were more numerous in CL and omnivores in C. Indices were calculated about soil web food where the ratio between predator and prey footprint showed statistical difference between treatment CB and treatments CBL, CL and C.

INTRODUCTION

Planting banana intercropped with coffee has been used by farmers in Nicaragua as an alternative temporary shade for coffee and for family consumption (Siles et al., 2010). Census of the coffee Sector from 2004 shows that the total national coffee production area with banana and timber trees is 77,228.94 ha, distributed 60,268.97 in the north and 16,959.97 in the Pacific (CENAGRO, 2004). However, some producers have been eliminating bananas from their coffee plantations because they believe it causes an increase in nematode populations. In this study, we analyzed the interactions in soil nematode populations and phytonematodes in coffee plantations with and without presence of banana for shade.

Numerous studies have covered the topic of phytonematodes in coffee (Villain et al., 2008; Avelino et al., 2009) and banana (Araya, 1995), but few studies have evaluated the interactions between both crops (Morales , 2001), noting that Pratylenchus found in coffee is not related with that found in bananas.

Various indices have been developed and implemented for assessing environmental quality and trophic structure in order to determine the potential functions and ecosystem services; these are founded on the relative abundance of structural and functional groups (Bongers 1990, Ferris et al.).

For this study four treatments were established with different densities of association of coffee, bananas and legumes. Data analysis was performed using descriptive statistics (graphical methods, measures of central tendency and dispersion), correlation and regression.

Conducting this research in the areas of higher coffee production in northern Nicaragua provides information on the relationship between infections by phytonematodes in the root system of coffee and bananas. This work answers the concerns of producers on the issue of phytonematodes and possible mechanisms or strategies to manage them.

MATERIALS AND METHODS

The study was conducted in the Community Monterrey located at 1038 meters above sea level, in the municipality of Jinotega, in the North Central Region of Nicaragua. In this region Bioversity International and UNAN – Leon University are working together in the project "Improving the production and marketing of banana trees in coffee plantations with small farmers, living soil, cultivar selection and marketing strategies".

In Monterrey are about 200 coffee producers of which 48 are members of the Cooperative Julio Osegueda. From this group we selected 28 producers who had coffee in partnership with Gros Michel bananas and legumes (guava, Inga sp.).

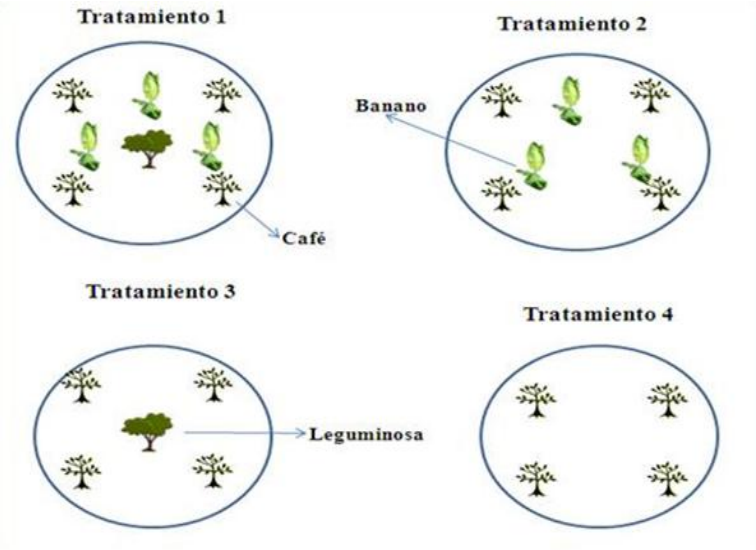

Figure 1. Treatments evaluated in community farms Monterrey.

The treatments evaluated were (Figure 1): High diversity CBL (Treatment 1), Diversity intermedia1 CB (Treatment 2), Diversity intermedia2 CL (Treatment 3), Low diversity C (Treatment 4).

We defined plots of 5 m diameter considering as sampling unit four coffee plants. To select the sampling sites the following criteria were used:

- Legume: One shady tree covering the study unit was selected.
- Bananas: at least three and maximum four plants in the sampling unit.
- Banana and coffee plants in production were sampled; this means plants over 4 years for coffee and over 6 months for banana, all of these close to start production.

- Coffee without shadow: areas with coffee without shadow (at least two years without shadow) of any type within 7 meters were selected and with any effect of banana mulch or legumes within five meters.

To analyze data from free-living nematodes indices of soil web food were used (Bongers, 1990), including the Enrichment Index, Structure Index and Change Index (Neher, Bongers, and Ferris, 2004).

The experimental design corresponded to a block design completely random with four treatments. Variance analyses were performed for the following variables: Percentage of fallen leaves, percentage of shade, percentage of weed, bulk density and fallen leaves weights, and Poisson (the significance test is the $\chi 2$ statistic) for the variables: number of phytonematodes in coffee and banana roots. The model used was: $Yij = \mu + \tau j + \varepsilon ij\ \beta i$ where: Yij = response variable, μ = overall mean, βi = Effect of i-th block (farm), τj = effect of the jth treatment (high density, intermediate density1, intermediate2 and low), εij = random error term and independent normal distributed with mean zero and constant variance.

Where statistical difference was evidenced multiple comparisons tests LsD Fisher was used. Triplot graphics were also obtained by the technique of Partial Least Squares (PLS).

RESULTS AND DISCUSSION

The phytonematode that represented higher population density in coffee roots (root 2.943 individuals / 100gr roots) and also banana roots (1,100 individuals / 100gr root) was *Meloidogyne* J2, with greater presence in the roots of coffee. The genus *Meloidogyne* J2 in coffee had higher population density in the treatments where bananas were not present (CL 3.429 and 2.914 individuals/100g root C). The genus *Pratylenchus* in bananas had higher population density in the CB treatment (543 individuals/100g root), while the J2 *Meloidogyne* treatment was higher in the CBL (1,257 individuals/100g root). The number of *Pratylenchus* in coffee has increased when it has CBL, while in bananas it decreases (Figure 2 and 3).

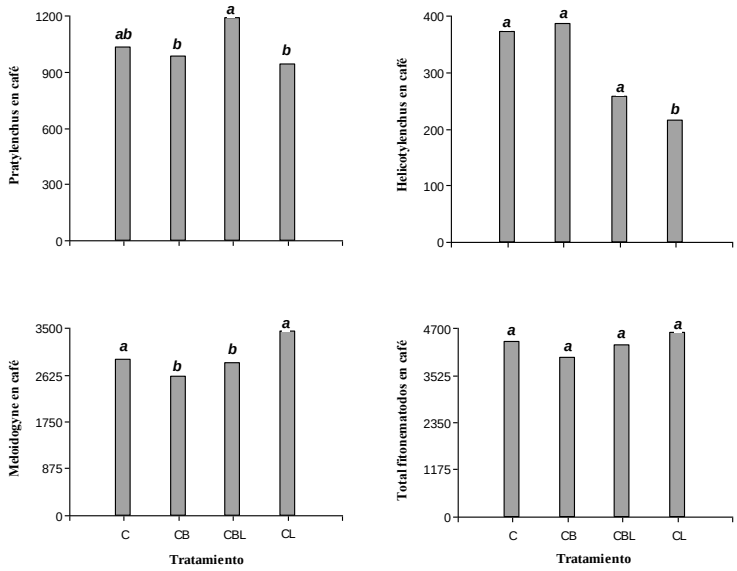

Figure 2. Average phytonematodes presented in 100 gr of coffee root treatments in coffee and banana plantations in Jinotega, Nicaragua. C: Treatment only Coffee, CB: Treatment coffee - banana, CBL: Treatment coffee-banana-legume, CL: Treatment coffee-legume. Different letters indicate differences between treatments.

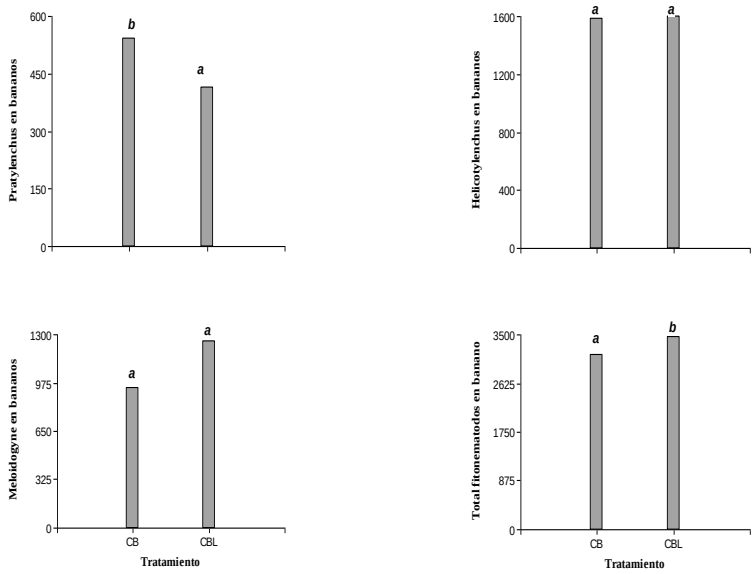

Figure 3. Average phytonematodes presented in 100 gr of banana root for treatments in coffee and banana plantations in Jinotega, Nicaragua. CB: Treatment coffee and banana, CBL: Treatment coffee-banana-legume. Different letters indicate differences between treatments.

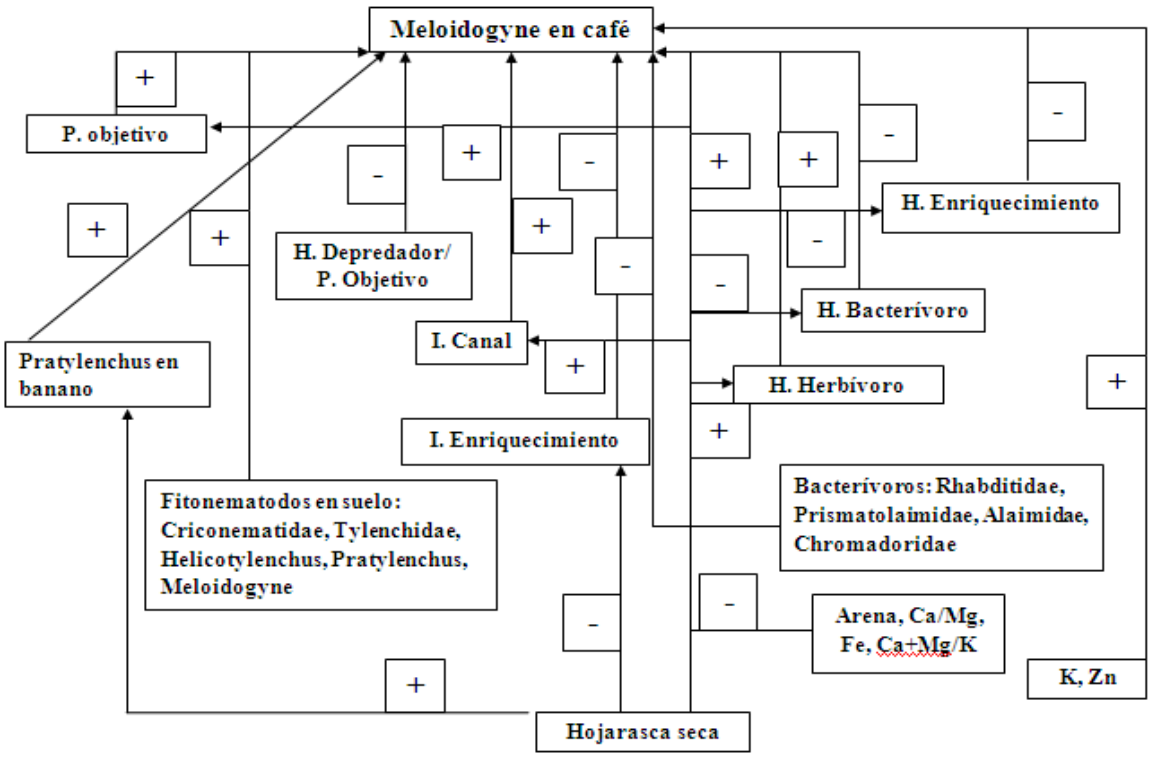

Figure 4. Relationship of different variables evaluated with Meloidogyne found 100g of coffee estate in Jinotega, Nicaragua.

In banana roots was found a significant positive relationship between root damage and the presence of the genus *Meloidogyne* and Helicotylenchus and the total phytonematodes in banana roots. The genus *Pratylenchus* in banana presented a significant negative relationship

with *Pratylenchus* in coffee and positive with *Meloidogyne* in coffee. The phytonematodes of coffee and bananas, despite being of the same gender, do not respond well to the physical and chemical characteristics of the soil, this could indicate that do not belong to the same species.

An effort was made to present in a single figure the observed correlations between the population of Meloidogyne in coffee, treatments and other variables quantified (Figure 4). The genus Meloidogyne J2 was positively related to the dry weight of the fallen leaves. However, the treatment that had a higher amount of dry fallen leaves was CBL and in that treatment the population was low. The genus Meloidogyne J2 also presented significant negative relationship with some of the indices of the soil web food as: Footprint Enrichment (nematodes classified as opportunistic enrichment, cp1) Bacterívoro Footprint (represents bacterívoros relative to the population of predators), Enrichment Index (measure bacterívoros fungivorous opportunistic and nematodes), and the ratio of the footprint Predator / Prey Objective (Predators / phytonematodes). Who in turn had negative significant relationship with the dry fallen leaves. According to Ferris et al. (sf) bacterívoros enrichment and footprints enrichment can be indicators of resource input in the soil web food, so you can say that the higher the amount of fallen leaves on the ground these footprints should increase, but this study found the opposite. It is possible that the increase in these footprints also depends as much rooted are in the soil, it means weeds or other crops and this variable was not measured in depth.

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Nitrogen Supply in the Consortium Between Coffee and Forage Plant

W. PEDROSA, A. L. S. VASCONCELOS, B. V. CARVALHO, P. P. C. TEIXEIRA, J. L. FAVARIN

Crop Science Department - "Luiz de Queiroz" College of Agriculture ESALQ/USP, Avenida Pádua Dias, 11, CEP 13.418-900, Piracicaba, SP, Brazil

SUMMARY

In the consortium of *Brachiaria* and coffee plants, the decomposition of the grass tillage under the coffee canopy contributes to the maintenance of soil fertility and improves the utilization of nitrogen (N). However, the means of application of nitrogen fertilizers is yet unknown, i.e., whether broadcast application should be performed on the forage, the coffee trees, or both. The experiment was performed under vegetation house conditions. Seedlings of *Coffea arabica* L. var. Mundo Novo IAC 379-19 were planted in 25-L vessels. The N dosage used was the same as that applied on the coffee crop (300 kg·ha^{-1}), corresponding to 2.88 g of N in each vessel. The N was applied in 2 ways: directly on the coffee trees or on the *Brachiaria* (*Brachiaria brizantha* var. Marandu). The forage plants were grown in 30 m^2 bags without N or in the presence of 150 and 300 kg·ha^{-1} N in the form of ammonium sulfate. In the experiments where N treatment was applied, the plants were grown in the presence or absence of the forage straw. The forage was not fertilized with N. The forage plants were fertilized with N 45 days before the plants were harvested. Next, 500 g of fresh forage material was wrapped in a nylon bag and placed on the vessels in which coffee plants were grown. Fresh and decomposed *Brachiaria* samples and coffee foliar tissue were collected for the determination of the total N and C/N ratio. The following factors were evaluated: dry mass, number of leaves, and leaf area of the coffee tree, and the amount of *Brachiaria* biomass in the decomposed state. The nutritional status of the coffee trees was not impaired by the application of N via biomass cycling or cycling of plant biomass forage fertilized with N. Coffee plants grown in the presence of residual forage fertilized with N showed greater dry matter and leaf number. On the other hand, the coffee plants grown under low N conditions showed higher C/N ratio and low leaf-N concentration.

INTRODUCTION

Nitrogen (N) is one of the nutrients applied in large quantities to full-sun grown coffee. In Brazil, about 300 kg·ha^{-1} N is used for growing coffee. Adoption of the consortium of coffee with *Brachiaria* might decrease N loss and reduce the need of nitrogen application because the N contained in the residual forage released by microbial decomposition can be efficiently used by coffee.

The amount of N in tropical soils is considerably lower, and measures for making the land fertile are necessary to obtain high yields. The amount and means of fertilizer application vary depending on the management of each crop, and the efficacy of N fertilizer depends on whether mineral or organic N is used.

The retention of plant residues on the soil surface promotes a gradual cycling of some nutrients, particularly N, benefiting plant nutrition. The decomposition of forage tissues is an

alternative source of N that can be absorbed when the forage tissue is placed under the canopy of coffee trees in the interrow, which has the highest density of roots. The amount and C/N ratio of organic matter influences the accumulation of organic matter, and the accumulation of organic matter is higher when nutrient cycling occurs. Loss of N is inevitable, but the extent of loss can be controlled if the process via which N loss occurs is known, i.e., volatilization or immobilization. Immobilization refers to a decrease in the available N content that persists in the soil. When fertilizer is applied to the soil, almost 70% of N is retrieved by the coffee plants. The rest remains in the soil in an organic immobilized form because negligible loss of less than 5% occurs through leaching. An efficient way to reduce N immobilization is by facilitating the gradual release of nutrients from plant residues.

The consortium between coffee and *Brachiaria* (*Brachiaria brizantha*) is a recent production system used to grow Brazilian coffee. In this system, mineral fertilization occurs partly via foraging, and the forage is then deposited via mechanical harvest under the canopy of coffee to facilitate a gradual release of N. However, whether this mode of application is effective is still unclear; hence, this study was conducted.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse at ESALQ-USP, Piracicaba, SP. In April 2010, the seedlings of *Coffea arabica* (L.) var. Mundo Novo IAC 379-379-19 IAC were planted in 25-L pots containing a mixture of 70% clay soil (425 $g \cdot kg^{-1}$ clay) and 30% sand. The chemical composition of the substrate at the beginning of the experiment was as follows: pH ($CaCl_2$) = 5.2 $mmol \cdot dm^3$, Ca = 37 $mmol \cdot dm^3$, Mg = 13 $mmol \cdot dm^3$, H + Al = 28 $mmol \cdot dm^3$, K = 4.7 $mmol \cdot dm^3$, SO_4-S = 17 $mg \cdot dm^3$, P-resin = 21 $mg \cdot dm^3$, organic matter = 17 $g \cdot cm^{-3}$, cation exchange capacity (CEC) = 83, V = 66%, and N = 1.05 $g \cdot kg^{-1}$. Nitrogen fertilization was performed as described in treatments, and other nutrients were added as recommended by Raij et al. . The experiment was performed in a complete randomized block design with 4 treatments replicated 6 times.

B. brizantha 'Marandu' was grown in 10-m^2 plots that were fertilized with 150 and 300 $kg \cdot ha^{-1}$ N; N was supplied only via mulching such that it contributed to 50% of the dose (150 $kg \cdot ha^{-1}$). The treatments are detailed in Table 1. The 300 $kg \cdot ha^{-1}$N in the form of ammonium sulfate corresponded to 2.88 g N per pot, regardless of the mode of application. Fertilization was conducted in November and December 2010 and January 2011.

At 30 days after the first application of fertilizer, 500 g of *Brachiaria* prunings were placed at the base of the coffee trees that were grown in vessels. The samples were placed in forage nylon mesh bags (mesh size, 4 mm^2; size, 30 × 30 cm) to avoid losses and allow monitoring of the decomposition residue. Simultaneously, the stem diameter, length and height of the branches of the plant, and N availability were evaluated in the treated plants. The *Brachiaria* plants were reaped, and further evaluations were performed at 50 and 70 days. After each harvest of *Brachiaria*, sub-samples were obtained from *Brachiaria* and coffee leaves, and the concentration of total-N was determined according to the Kjeldahl method.

At the end of the experiment, dry matter accumulation (g) in different parts of the coffee plants (leaves, branches, stems, and roots) was determined. Leaf number and leaf area were also evaluated using a leaf area meter (model LiCor 3100; LiCor, Nebraska, USA).

Treatment outcomes were analyzed using analysis of variance (ANOVA) and Tukey's test by F test at 5% probability by using the Statistical Analysis System (SAS) software for Windows 6.11.

RESULTS AND DISCUSSION

The nutritional status of coffee was not impaired by providing nutrient cycling from biomass of mulch fertilized with N (Table 1). The other application methods included half of the N content to the *Brachiaria* plants, half of the content to coffee trees, and 100% content to the *Brachiaria* plants without N supplementation to the coffee trees; the N losses using these methods were not different from those noted in the traditional mode (100% N supplementation to coffee without the forage residue). This suggested that the presence of biomass at a C/N concentration of less than 31 did not impair the nutritional status of coffee. Supplying the total dose of N to the coffee plants (100%) in the presence of plant residues without nitrogen treatment reduced leaf N concentration. This could be explained by the greater N immobilization by soil microorganisms, since the forage has higher C/N combined with higher C oxidative rates (Table 1).

Table 1. Concentrations of N in coffee trees and Brachiaria (Brac), C/N ratio of the biomass of forage, dry matter Brachiaria (DMB) in the decomposition vessel (g), leaf area (LA) and number of leaves (NL) at the end of the experiment.

Treatments	N(g kg^{-1})				DMB		C/N	LA		NL	
	Coffee		Brac		g						
N at coffee tree (100%), no mulch	34,9	a	--		---		---	8758,4	b	214,2	b
N at coffee tree (100%), much without N	29,5	b	14,5	c	106,6	a	31,0	8782,6	b	274,7	a
N at coffee tree (50%), mulch with N (50%)	32,6	a	22,0	b	83,5	b	20,5	9998,5	a	278,8	a
N only at the mulch (100%), without N at coffee tree	34,9	a	26,5	a	88,3	b	17,0	9562,4	ab	290,1	a
CV (%)	5,77		6,18		5,35		---	5,89		9,42	

Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

Plant residues having a C/N ratio greater than 23 are more resilient because of the necessity of immobilizing the microorganism portion of soil N since the amount of N present in the plant biomass is insufficient. This reduces nutrient availability for coffee tree.

The increase in the coffee leaf area in the presence of residues of forage can be partly explained by the greater number of leaves and the need to conserve soil moisture for longer. In the absence of forage residue, the soil dries faster, causing leaf senescence due to water stress.

The dry mass of leaves, branches, stems, and roots in the total waste of coffee trees that were forage fertilized with 150 and 300 kg·ha^{-1} N was higher than that of the total waste from coffee trees grown without *Brachiaria* biomass (Table 2). These data indicate that the efficiency of N use in the coffee trees that are supplemented with forage is higher than that in

the coffee trees grown with N without forage supplementation, as is in the conventional production system.

Table 2. Dry matter accumulation (g) at leaves (DML), branches (DMB), steam (DMS), roots (DMRt) e total (DMT) do cafeeiro, no final do experimento.

Treatments	DML		DMB		DMS		DMRt		DMT	
	g									
N at coffee tree (100%), no mulch	64,6	b	25,7	b	49,6	b	26,6	c	166,5	b
N at coffee tree (100%), much without N	85,2	a	38,2	a	49,2	b	30,8	cb	203,4	a
N at coffee tree (50%) , mulch with N (50%)	80,7	ab	37,5	a	62,6	a	35,8	ab	216,6	a
N only at the mulch (100%), without N at coffee tree	77,2	ab	37,2	a	57,3	a	40,6	a	212,3	a
CV (%)	15,02		19,17		17,98		14,61		10,46	

Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

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The Sustainability of Coffee-based Livelihoods: A Study of Social and Economic Change in Rural Indonesia

J. NEILSON[1]

School of Geosciences, University of Sydney – Australia

SUMMARY

Recent high coffee prices, due to a combination of rising demand in emerging markets and declining production outside of Brazil and Vietnam, have sparked concerns over the long-term supply of coffee beans. This paper evaluates the potential for expanding production from Indonesia – currently the world's third largest producer - to play a significant role in meeting predicted global demand. This research examines this possibility through a socio-economic assessment of coffee-based livelihoods in Indonesia. The research finds that coffee production retains an important function as a *de facto* social safety net for many impoverished rural households. However, coffee production is rarely considered a vehicle for genuine poverty alleviation. As a result, broader processes of industrialisation and economic development in Indonesia appear to be working against the possibility of Indonesia significantly increasing coffee production in the foreseeable future.

BACKGROUND: THE INDONESIAN COFFEE INDUSTRY

Indonesia has been a leading global coffee producer for centuries, and has been the world's third largest since 2008 due to declining production in Columbia. Export volumes, however, have not increased significantly in recent decades, and have tended to oscillate between 300,000 and 500,000 tonnes each year since 1990. An estimated 95 percent of total land planted with coffee is managed by smallholders in the outer islands (major Arabica producing regions are shown in Figure 1). These smallholders teeter either side of the poverty line depending on international commodity prices, and are provided with few institutional or government supports.

[1] I acknowledge the considerable assistance provided by Faila Hartatri (ICCRI) and Yayoi Fujita Lagerqvist (University of Sydney) in both data collection and analysis for this research.

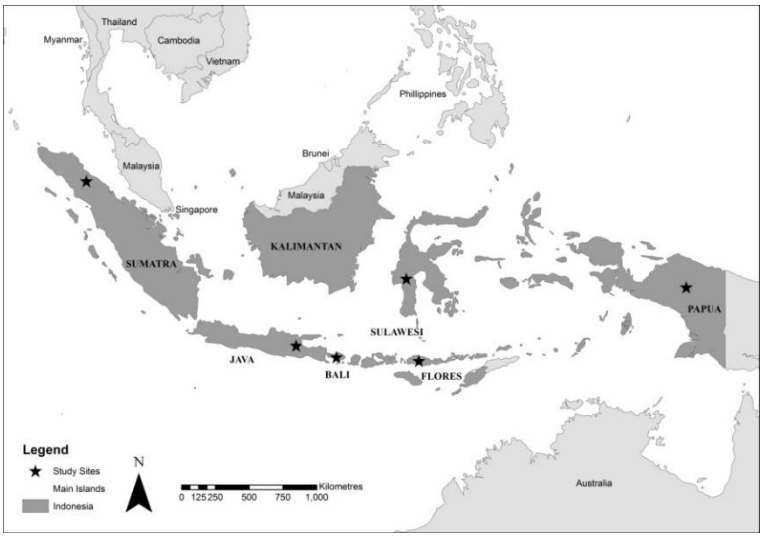

Figure 1. Research Sites - Major Arabica-producing regions in Indonesia (Map prepared by Karen Sowden, University of Sydney).

While we expect a delay between elevated prices and an output response from a tree crop such as coffee, Indonesian production is not responding to the relatively high prices seen since 2006 (Figure 2). Moreover, despite export prices more than tripling since the 'coffee crisis' days of 2001, the contribution of coffee exports to total export earnings in Indonesia has remained at the same low level of 0.5%. With production stagnant, this situation is due to the rising importance of other exports (manufacturing and resource commodities feeding the China boom). The disinterest in coffee can be contrasted with the increasing significance of palm oil exports over the same period. Under Indonesian conditions, coffee is a labour-intensive crop, and oil palm is capital-intensive, suggesting the nature of economic change now shaping rural Indonesia.

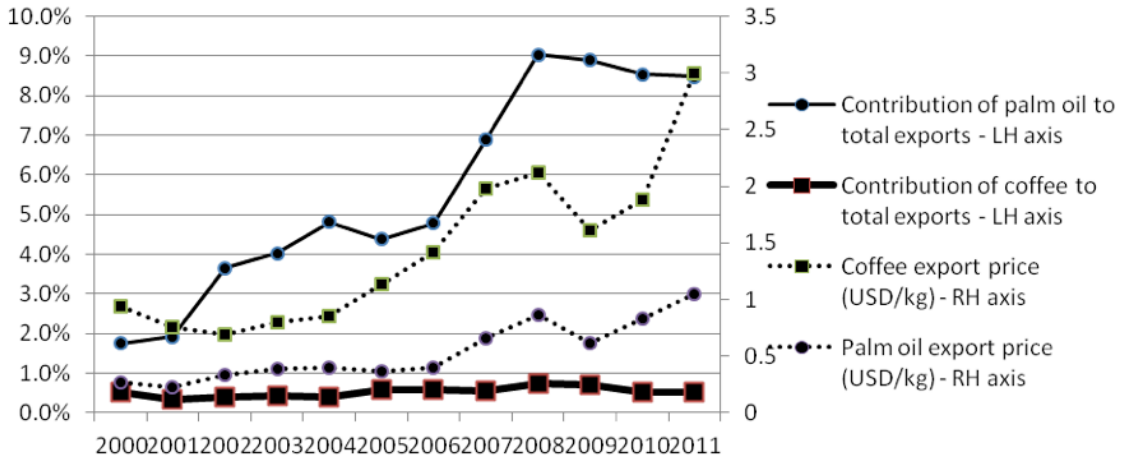

Figure 2. Contribution of coffee and palm oil to total Indonesian Export Earnings (Data Source: http://comtrade.un.org).

METHODS

An initial survey of household livelihood strategies was conducted across the islands of Sulawesi and Flores (n = 803) in 2008 (Figure 1). Survey respondents were located within sub-districts where coffee production was expected to be a relatively important source of locally-produced income, based on official production data obtained from the District-level Estate Crop Agencies. This household livelihood database was then supplemented with field visits to other major Arabica producing regions in Indonesia (Aceh, North Sumatra, Bali, Java and Papua), as shown in Figure 1, where a series of stakeholder interviews were performed with farmers, traders, NGOs and government officials. This field research was used to establish the role of coffee production within broader livelihood strategies and its relationship with perceived poverty alleviation pathways. This household level of analysis was then combined with a national-scale analysis of changing export patterns, industrial policy, and poverty reduction.

COFFEE LIVELIHOODS: A POVERTY ALLEVIATION PATHWAY?

The typical Indonesian coffee farmer maintains a small mixed plot of about a hectare, where coffee sales are just one component of a mixed livelihood portfolio. Coffee income is an important source of cash, with which farmers can purchase daily necessities. Farmers, however, are generally unwilling to invest scarce household resources to increase coffee productivity, but would rather invest in education and rural-urban migration (Table 1). Coffee cultivation is a fall-back strategy when other options have been unsuccessful, and few parents want their children to continue coffee farming. This follows broader trends in Southeast Asia, where rural livelihoods are increasingly divorced from land, and where poverty alleviation is de-linked from agriculture. Even at current high prices, Indonesian smallholders relying solely on coffee-derived income are subsisting barely above a $1.25 / day poverty line.

Table 1. Indicators of smallholder livelihood strategies in Indonesia (Source: field surveys).

Indicator	Results	Comments
Average (gross) annual coffee sales	$642/year	This is gross coffee income for the year. Costs are more difficult to estimate, and are strongly influenced by unpaid family labour input. This needs to be calculated based on opportunity costs. A conservative estimate would be that average net coffee income might be $350 / year, well below a $1.25/day poverty line.
Average contribution of coffee to gross household income	65%	This is gross coffee income and gross household income, and probably overestimates the importance of coffee to net household income.
Households that apply urea fertiliser to their coffee trees	22%	Urea fertiliser is subsidised by the government in Indonesia and costed (on average in 2008) $8 per 50kg bag. This is used here as an indicator of the households willingness to invest in their coffee farm.
Households that apply compost fertiliser to their coffee trees	12%	Compost is an indicator of the willingness of households to invest their own labour to increase farm productivity.
Households that receive remittances from family members living in urban areas (in Indonesia and abroad)	23%	Remittance income varied considerably between districts. The importance of rural-urban migration to achieving poverty alleviation was significant in the coffee communities.

PATTERNS OF ECONOMIC DEVELOPMENT IN INDONESIA

Indonesia has recovered well following the 1998 Asian Financial Crisis, with an average annual GDP growth rate of 5.5% between 2001 and 2011 (over a period that spanned the Global Financial Crisis of 2008). Rising per capita incomes in Indonesia have occurred alongside declining poverty levels (Figure 3). While some expansion of agricultural land has occurred in Indonesia (primarily for oil palm), the total area available for coffee is ultimately finite and there is increasing pressure from alternative landuse functions, including estate-scale plantations, domestic food production, and ecological services. Sustained coffee production also requires the allocation of adequate rural labour. Importantly, for the first time in 2010, more Indonesians were living in cities than in rural areas. Moreover, this inexorable trend towards an urbanised Indonesia is further encouraged through supportive industrial policies. Since 2009, Indonesian cocoa farmers have been subsidising the downstream processing industry by way of an export tax on raw cocoa beans, and the government has announced similar ambitions towards protecting an industrialised coffee sector. Both from the perspective of both rural producers and the state, there is little space for expanding coffee production in the modern Indonesia.

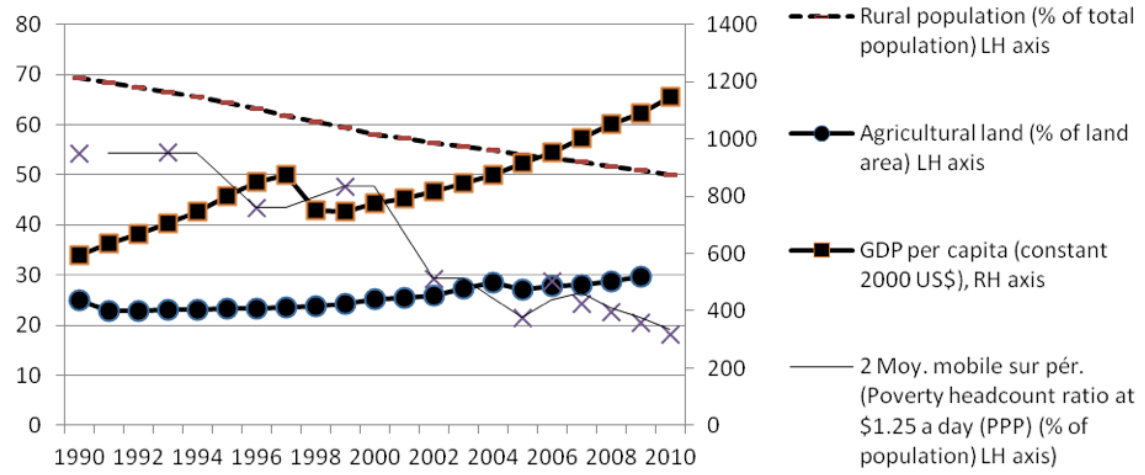

Figure 3. Indicators of Economic and Social change within Indonesia (Data: http://data.worldbank.org).

CONCLUSIONS

Coffee is a crop of last resort for smallholder Indonesian farmers, and fewer poor households are choosing coffee production as a viable pathway out of poverty. It seems that one of three conditions would need to be satisfied to significantly increase coffee production in Indonesia: i) a reversal of current trends towards industrialisation and economic opportunity; ii) prices increase sufficiently to stimulate renewed investment in coffee production (although this seems unlikely in Indonesia due to the presence of other constraining factors); or iii) downstream users begin investing in social facilities in coffee communities to change current attitudes towards the crop, possibly through enhanced value chain mechanisms that deliver real services to farmers. The sustainability of future global coffee supplies requires downstream actors within the global value chain to demonstrate a substantially heightened interest in facilitating socially attractive coffee-based livelihoods in origin countries.

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Estimate of Shading Hours in Function of Latitude, Spacing, and Stature of Agroforestry Plants in Coffee Plantations in Northeastern and Southeastern Brazil

P. C. LIMA[1], W. M. MOURA[1], C. F. M. CARVALHO[2], R. S. R. ANJOS[1], M. G. M. GONÇALVES[3]

[1]Institute of Agricultural Research of Minas Gerais, Vila Gianetti, 46/47, Campus da UFV. Viçosa, MG. CEP 36.570-000, Brazil
[2]Federal University of Viçosa, Departamento de Solos; Bolsista CBP&D Café/ EPAMIG
[3]University Federal de Viçosa/ Bolsista BIC Fapemig/ EPAMIG, Campus da UFV, Viçosa, MG., Brazil

SUMMARY

Afforestation of coffee plantation could contribute to the expansion of Brazilian coffee, making it possible in areas presenting solar radiation and high temperatures. Shading time depends on the latitude of the area, the disposition of the tree rows, the height of these trees and the spacing between them. In Brazil, shading of 25 to 50% of the surface is recommended, depending on the region. However, no recommendations are available on how to estimate the spacing between the many species of trees that ensure the appropriate shading of coffee trees, making it difficult to plan the implantation of SAFs. This work aims to propose a methodology to estimate shading hours in function of the latitude, spacing and height of plants in SAFs with coffee trees. Plants receiving no shading are called plants with N hours of direct insolation. The N value can be modeled in function of the latitude. Shading time can be estimated by the angle formed by the sun inclination and the height of the tree providing shade in a particular spacing on the soil surface. The application of this methodology allowed estimating spacing between trees in SAFs in two municipalities of two Brazilian regions (Northeast and Southeast).In the Northeastern region this study was carried out in the municipality of Guaramiranga, CE, latitude 4º 15' 48"S, and in the Southeastern region, in the municipality of Araponga, MG, latitude 20º 40' 00"S, where 25% of shading is recommended. The native tree species and fruit trees commonly used in each region were listed, grouped by height. The more adequate spacing in Guaramiranga, CE region varied from 5 to 20m among 5 to 25m tall trees. In the Araponga-MG region, the spacing varied from 10 to 50m among 5 to 25m tall trees.

INTRODUCTION

Because of the climatic conditions in the northeastern region of Brazil, such as those in the municipality of Guaramiranga, Maciço de Baturité,in the state of Ceará, coffee plantations must be implanted under a shading system to prevent the death of the plants due to excess of sunlight and lack of water during drought. In these Equator Line regions, where hot climate predominates, with a higher incidence of solar radiation, coffee plantation shading must be denser, comprising approximately 50% of the area. For the regions with tropical climate altitude in southern Brazil, such as that of the municipality of Araponga, Zona da Mata, in the state of Minas Gerais, the cover of the terrain is thin, with values close to 20%.

Shading is related with the height of trees and spacing between them. A practical definition of spacing between trees may be proposed by adapting the indication described by Resende et.al. for shading of agricultural properties. Calculation is made of the shading hours that trees would provide to the areas during the periods of full sun. Plants which do not receive any shading are considered as plants with N hours of direct insolation. The N value represents a maximum duration of daily insolation (day 15 of each month), in hours, of the months during full sun periods, with references indicating these values for each month of the year, such as the List Table.

To estimate the shading hours of a given plant, the angle inclination of the sun is calculated and this is divided by 15º (rotation of the Earth in 1 hour). Inclination A is obtained using the tangent, calculated in function of the height of the plants and distance between them.

The A value, sun height, can be calculated by the expression tgA = h/x, where h = plant height and x = plant spacing. For instance, if N=12.1 h, h = 6m and x = 4m; tgA = 1.5 and A = 56.3º. Under this circumstance, there will be a reduction of A/15 hour,i.e.: 56.3º/15º = 3.75 shading hours; i.e., N-3.75 = 8.35 insolation hours.

To estimate shading hours in situations presenting two plants of different sizes, the shading hours are calculated individually and the two results are added.

This work aimed to propose a methodology to estimate the shading hours in function of the latitude, spacing and height of the plants in SAFs containing coffee trees in two municipalities located in two Brazilian regions (northeast and southeast).

MATERIALS AND METHODS

This study was initiated by listing the native and fruit tree species commonly used in agro-forest systems in coffee plantations in the northeastern and southeastern regions in Brazil. These species are grouped by the following height ranges: up to 5, from 5 to 10, from 10 to 15, from 15 to 20, from 20 to 25, and from 25to 30 meters.

The northeastern region study was carried out in the municipality of Guaramiranga-CE, with latitude of 4º 15' 48"S, where 50% shading is recommended for the coffee trees; the southeastern region study was conducted in the municipality of Araponga-MG with 20º 40’ 00’’S latitude and recommendation of 25% shading.

The heights of the trees used ranged from 5; 10; 15; 20; and 25 meters. Based on the principle that h (opposite cathetus) = plant height, x adjacent cathetus) = distance between the trees, where tg A = h/x, with A = sun height in angles. The A value is estimated by transforming the tangent into an angle for each situation.

The A value found for each plant height was divided by 15, with the number of shading hours on the plant being obtained. Total insolation in each distance was obtained by subtracting the N value by the total number of shading hours.

RESULTS AND DISCUSSION

The tree species commonly used in the municipality of Guaramiranga are classified in 6 height ranges: up to 5 m: *Psidium guajava* (guava), *Malpighia punicifolia* (acerola), *Spondias purpúrea* (ciriguela);from 5 to 10 m: *Inga spp.*(ingá), *Cecropia adenopus* (torém), *Eugenia uniflora* (pitanga), *Achras zapota* (sapoti), *Spondias tuberosa* (umbu), *Talisia esculenta*

(pitomba), *Musa paradisíaca* (banana); from 10 to 15 m: *Pithecolobium polycephalum* (camunzé), *Erythrina velutina* (mulungu), *Licania tomentosa* (oiti), *Genipa americana* (jenipapo), *Syzygium malaccense* (red jambo); from15 to 20: *Syzygium jambos* (pink jambo), *Persea americana* (avocado); from 20 to 25: *Simarouba amara* (paraíba), *Spondias lutea* (cajá);and from 25 to 30 m: *Cedrela odorata* (cedrus), *Cordia trichotoma* (louro-freijó). The tree species commonly used in the municipality of Araponga are classified in 6 height ranges: up to 5 m: *Erytthrina speciosa* (mulungu); from 5 to 10: *Aegiphila sellowiana* (papagaio); *Bombax marginatum* (castanha-mineira), *Inga spp.* (ingá), *Musa paradisiaca* (banana), *Senna macranthera* (fedegoso), *Cecropia sp.* (embaúba); from10 to15 m: *Luehea grandiflora* (açoita-cavalo); from 15 to 20: *Persea americana* (avocado), *Zeyheria tuberculosa* (ipê-preto); and from 20 to 25: *Dalbergia nigra* (jacarandá-caviúna), *Spondias lútea* (cajá manga).

The results on the total of shading hours and insolation on the coffee trees are found in Tables 1 and 2. It was observed that the higher the tree heights and the shorter the distance between them, the higher will be the total number of shading hours and the lower the total number of hours of insolation on the coffee plantation.

The calculations allow recommending more adequate spacing in function of shading time of the coffee trees in SAFs under distinct conditions of insolation. The most adequate spacing for the region of Guaramiranga-CE varied from 5 to 20m in 5 to 25 m tall trees. In the region of Araponga- MG, spacing varied from 10to 50m among the 5 to 25m all trees.

The methodology used to estimate the shading hours presented in this study was found adequate. There is regional specificity for determining the calculation of shading hours, when maximum insolation in each latitude and the height of the tree species present are observed. The method was found to be efficient in establishing adequate spacing among the trees based on the shading time provided by them.

Table1. Estimate of the shading hours in function of latitude, spacing, and height of the trees in coffee plantation agro-forest systems in the municipality of Guaramiranga, located in the Maciço de Baturite-CE, Brazil.

N=12,2 (h)	Stature (m)		x	Tangent = h/x		Ângle (degrees)		A/15	B/15	A/15 + B/15	Total insolation (N- (A/15) - (B/15))
50% N	Tree 1	Tree 2	Spacing (m)	Tree 1 (A)	Tree 2 (B)	Tree 1 (A)	Tree 2 (B)	Shade Tree 1	Shade Tree 2	Total of Shade	Guaramiranga CE
6.1	25	25	20	1.25	1.25	51.34	51.34	3.42	3.42	6.85	5.35
6.1	20	25	20	1.00	1.25	45.00	51.34	3.00	3.42	6.42	5.78
6.1	15	25	20	0.75	1.25	36.87	51.34	2.46	3.42	5.88	6.32
6.1	10	25	15	0.67	1.67	33.69	59.04	2.25	3.94	6.18	6.02
6.1	5	25	10	0.50	2.50	26.57	68.20	1.77	4.55	6.32	5.88
6.1	20	20	20	1.00	1.00	45.00	45.00	3.00	3.00	6.00	6.20
6.1	15	20	15	1.00	1.33	45.00	53.13	3.00	3.54	6.54	5.66
6.1	10	20	15	0.67	1.33	33.69	53.13	2.25	3.54	5.79	6.41
6.1	5	20	10	0.50	2.00	26.57	63.43	1.77	4.23	6.00	6.20
6.1	15	15	15	1.00	1.00	45.00	45.00	3.00	3.00	6.00	6.20
6.1	10	15	10	1.00	1.50	45.00	56.31	3.00	3.75	6.75	5.45
6.1	5	15	10	0.50	1.50	26.57	56.31	1.77	3.75	5.52	6.68
6.1	10	10	10	1.00	1.00	45.00	45.00	3.00	3.00	6.00	6.20
6.1	5	10	5	1.00	2.00	45.00	63.43	3.00	4.23	7.23	4.97
6.1	5	5	5	1.00	1.00	45.00	45.00	3.00	3.00	6.00	6.20

Table2. Estimate of the shading hours in function of latitude, spacing, and height of the trees in coffee plantation agro-forest systems in the municipality of Araponga, located in the Zona da mata-MG, Brazil.

N = 13,2 (h)	Stature (m)		x	Tangent = h/x		Ângle (degrees)		A/15	B/15	A/15 + B/15	Total insolation (N-(A/15)-(B/15))
25% N	Tree 1	Tree 2	Spacing (m)	Tree 1 (A)	Tree 2 (B)	Tree 1 (A)	Tree 2 (B)	Shade Tree 1	Shade Tree 2	Total of Shade	Araponga MG
3.3	25	25	50	0.50	0.50	26.57	26.57	1.77	1.77	3.54	9.66
3.3	20	25	50	0.40	0.50	21.80	26.57	1.45	1.77	3.22	9.98
3.3	15	25	40	0.38	0.63	20.56	32.01	1.37	2.13	3.50	9.70
3.3	10	25	40	0.25	0.63	14.04	32.01	0.94	2.13	3.07	10.13
3.3	5	25	30	0.17	0.83	9.46	39.81	0.63	2.65	3.28	9.92
3.3	20	20	40	0.50	0.50	26.57	26.57	1.77	1.77	3.54	9.66
3.3	15	20	40	0.38	0.50	20.56	26.57	1.37	1.77	3.14	10.06
3.3	10	20	30	0.33	0.67	18.43	33.69	1.23	2.25	3.48	9.72
3.3	5	20	30	0.17	0.67	9.46	33.69	0.63	2.25	2.88	10.32
3.3	15	15	30	0.50	0.50	26.57	26.57	1.77	1.77	3.54	9.66
3.3	10	15	30	0.33	0.50	18.43	26.57	1.23	1.77	3.00	10.20
3.3	5	15	20	0.25	0.75	14.04	36.87	0.94	2.46	3.39	9.81
3.3	10	10	20	0.50	0.50	26.57	26.57	1.77	1.77	3.54	9.66
3.3	5	10	15	0.33	0.67	18.43	33.69	1.23	2.25	3.48	9.72
3.3	5	5	10	0.50	0.50	26.57	26.57	1.77	1.77	3.54	9.66

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Effect of Weed Control Methods in Coffee Interrows on Yield Coffee.

E. N. DE ALCÂNTARA[1], M. M. FERREIRA[2]

[1]EPAMIG Southern of Minas Gerais state, Brazil
[2]FERREIRA, M. M., Federal University of Lavras

SUMMARY

The coffee is very sensitive to weed competition for water, light and nutrients. The weed control represents on average 30% of the production cost. Because of this various weed control methods have been tested to find the best weed control method in the inter rows coffee plants. With this objective was implemented in September 1977, an experiment in randomized block design with seven treatments at inter rows: mower, disk harrow, rotary tiller, post-emergence herbicides (glyphosate) at 720g ai / ha herbicide Pre-emergence (ametryn simazin +) at 250 + 250 g ai / ha, hand weeding and no control as check, in three replications. The experimental area with 8% slope, and 2300 coffee plants IAC Catuaí 99 cultivars in 4.0 x 1.0 m spacing in Oxisol at the EPAMIG Experimental Station in São Sebastião do Paraíso, MG. Yields from 1978 to 2005 were grouped into biennia, as well as some annual productions, totalizing 15 repetitions. In 2006 this experiment was replanted, keeping the same inter rows spacing, but 0.70m among plants using the coffee cultivar Paradise, MGH 419, keeping the same treatments at inter row, using oxyfluorfen at 3.0 liters commercial product /ha as pre-emergence herbicide. Yields from 2008, 2009, 2010 and 2011 were also analyzed. After 34 years results show that the pre-emergence herbicide, gave the highest yield. The non weeded treatment had the lowest production. The use of the disk harrow, rotary hoe, manual weeding and post-emergence herbicide showed intermediate yields, because are methods that rely the timing and availability of weed control. The mower pulled by tractor, although widely used by farmers, presented yield next to the no weed control. This result has been attributed to excessive use of mower with moist soil in rainy occasions when weed control is repeated every 25 days, causing compression in the region of the tracing wheel tractor, where are most of the surface absorbing roots.

INTRODUCTION

The coffee is sensitive to weed competitions but they may cause yield reductions in from 55% to77%, BLANCO, OLIVEIRA and Pupo, 1982. With the rising weed control cost, several alternative methods have been introduced (SILVEIRA and Kurachi, 1981 and Muzilli, 1987), but almost none are studies on the impacts of different weed control methods on coffee soil quality in long term (LAL, 1993) and production.

In 1974, AVATRAMANI, suggested an integrated weed control with reduced cultivation and mulching formation. The significance of these trends was grounded in the existence of soil organic matter highlighted by STEVENSON in 1986, which affects all other attributes of soil quality, (Fernandes et al., 1997).

The soil organic matters level, according DUXBURY et al., 1989 depend strictly of soil management and vegetation cover. Therefore, the presence of organic matter in the soil is taken as the sustainability key, through their influence on different soil properties, whose

disability, and other factors, directly contributes to soil degradation and quality (Stevenson, 1986).

Therefore the effect of various weed control methods on soil chemical and physical parameters and production has been evaluated since September 1977. Some articles from this study (ALCÂNTARA, 1997, and ALCÂNTARA FERREIRA, 2000a, 2000b, ALCÂNTARA, and FERREIRA MERCER 2003 and NOBREGA, ALCÂNTARA and FERREIRA, 2006) show that the soil physical and chemical properties are affected by the weed control methods.

It has been demonstrated that the continued use of pre-emergence herbicide increased soil bulk density, promoted crusting formation, dispersed particles on the soil surface and that the use of rotary hoe formed a dense layer subsurface (ALCÂNTARA, 1997). It was also observed that the effects of the weed control methods were more expressive in the soil surface layer, and the soil quality is directly related to the organic matter content (ALCANTARA and FERREIRA, 2000a), without, however, see an increase in yield due to the increase in organic matter content.

It is further shown that the pre-emergence herbicide use for many years although diminish the organic matter content, did not affect the coffee yield, (ALCANTARA, FERREIRA and MERCER 2003).

Therefore, the specific objectives of this study are to show the weed control effects on inter rows of the coffee on yield after 35 years of experimentation.

MATERIALS AND METHODS

The experiment was installed in September 1977 at the EPAMIG Experimental Station in São Sebastião do Paraíso, MG, with coffee cultivar IAC Catuaí 99, planted in 1974, using a spacing 4 m between rows and 1 m between plants in an Oxisol, in an area with a 8% slope.

Was used a randomized block design with seven treatments (weed control methods) at between rows: mower, disk harrow, rotary hoes, post-emergence herbicides (glyphosate) at a dose of 720g ai / ha, pre-emergence herbicide (simazine+ametryn) at dose of 250 +250 g a.i / ha, hand weeding and without control treatment, at three replications.

The coffee planting rows were always kept clean by hand weeding or herbicides applications. The operation numbers per year, for maintenance of coffee rows free of weeds is in Table 1.

Table 1. Number of annual operations to control weeds, in the experiment in S.Sebastião Paraíso, MG, 2011.

Treatment at coffee inter rows	Number of operations per year
Mower	Five
Disk harrow	Three
Rotary tiller	Three
Post emergence herbicide	Three
Pre emergence herbicide	Two
Manual weeding	Five
No control (without weed control)	/

After each year or biennium the processed coffee yield, from the 1978 to 2005 period the yield in bags / ha, were grouped for analysis in 15 replications and seven treatments.

In 2006, this experiment was replanted with coffee cultivar Paraíso MGH 419 in the same area, keeping the same between lines spacing of 4 m, but using the spacing between plants in the row of 0.70 m . It was used a randomized block design, with the same seven methods (treatments) between the lines: mower, disk harrow, rotary tiller, post emergence herbicide (glyphosate applied at 720g of a.i./ha, pre emergence herbicide (oxyfluorfen) at 3,0 kg of commercial product/ha, a hand weeding and no control inter rows as check, using three replications.

RESULTS AND DISCUSSION

It was evident that weed control previously performed with pre emergence herbicide avoid the weed infestation e crop competition during all year.

Results shown that pre emergence herbicide use gives the highest coffee yield. The post emergence herbicide use and manual weeding also presented yields statistically equal to the last treatment in processed coffee bags. On the other side, also is shown that the no control treatment gave the lowest coffee yield, shown that this low yield was due to the weed competition of coffee inter rows, mainly during the driest period. It is interesting to note also that the mower use, very common in coffee crop, presented yield closest to no weed control treatment.

According to ARAUJO Jr. et al., 2007a, this effect is due to é explained by the load support capacity, which is a function of soil density and moisture content. According to this author, the load support capacity is reduced with the pressure of the tractor wheels on the soil moist affecting the coffee root system, and consequently the coffee yield, since the operations are intensified with frequent mower (at each 25 days) use during the rainy season, due to the higher growth of weeds. The weed management that presented greater mechanical resistance from 0 to 3cm depth, ARAUJO Jr. et al 2007b, as pre-emergence herbicide use showed also higher initial development and production.

The post emergence herbicide use and the manual weeding, although presented no statistical differences to pre-emergence herbicide (Table 2) showed the second highest yield. These results are explainable because the weed control depends upon the operability, i.e., climate and atmospheric conditions, for the post-emergence herbicide application, such anthropogenic factors, hand hoeing and other favorable conditions for operations, allowing weed competition occurrence during the time interval waiting for favorable conditions for operations.

Table 2, however, shows the yield difference between mower and pre emergence herbicide use greater than fourteen coffee bags/ha. This result confirms the previously obtained results.

Table 2. Yield average of processed coffee bags / ha from 1978 to 2005 of IAC Catuaí 99 coffee cultivar, under different weed control methods f rom 1978 to 2005. São Sebastião do Paraíso, MG.

Treatments	78/ 79	80/ 81	84/ 85	86/ 87	88/ 89	90/ 91	92/ 93	94/ 95	96	97	98/ 99	00	01	02	04/ 05	Média
Mower	22, 3a	32, 3a	31, 7a	36, 5a	28, 7a	22, 7a	10, 7a	26, 1a	20, 3a	41, 7ab	34, 0a	20, 9c	14, 4b	65, 3a	14, 0b	27, 8cd
Disk harrow	23, 6a	29, 6a	34, 0a	39, 8a	35, 4a	22, 5a	12, 7a	34, 5a	25, 0a	49, 3ab	24, 7a	28, 2c	17, 4ab	54, 8b	21, 9ab	30, 2bcd
Rot. tiller	25, 3a	34, 1a	32, 8a	38, 0a	30, 3a	25, 7a	11, 8a	25, 8a	21, 6a	47, 5ab	24, 7a	22, 3c	12, 4ab	57, 1ab	18, 7ab	28, 5bcd
PósE.herb.	22, 3a	33, 1a	35, 0a	36, 1a	35, 0a	23, 4a	16, 4a	29, 6a	18, 4a	45, 6ab	27, 3a	42, 9ab	18, 6a	55, 4ab	32, 5a	31, 4ab
PreE herb.	25, 6a	33, 8a	33, 0a	38, 6a	32, 8a	21, 7a	16, 6a	34, 1a	23, 8a	51, 7a	29, 1a	50, 1a	15, 9ab	65, 4a	33, 1a	33, 7a
Manual hoe	25, 6a	33, 4a	33, 1a	40, 8a	36, 2a	21, 3a	15, 8a	29, 9a	21, 7a	51, 6a	33, 3a	33, 1bc	14, 5ab	57, 5ab	17, 7b	31, 0abc
No control.	22, 3a	32, 4a	24, 9b	35, 0a	27, 3a	24, 1a	13, 1a	33, 3a	20, 9a	36, 1b	26, 7a	30, 3bc	11, 2ab	55, 9b	12, 9b	27, 1d
C.V. (%)	5,62	4,57	4,79	5,13	4,71	5,07	10,4	7,42	9,45	14,43	13,7	13,3	16,43	8,36	14,22	13,74

Averages followed by the same letters do not differ between them by Tukey test a 5%.

Table 3, shows however, the yield difference greater than fourteen coffee bags from the mower to preemergence herbicide use. This result confirms the previously results. The physical impediment caused by compression in addition to impaired production, as explained, may also be affecting other factors which should be object of further studies in the area of physical, chemical and microbiological soil.

Table 3. Yield average of processed coffee bags / ha from 2008 to 2011 coffee cultivar Paraíso MGH 419, under different weed control methods. São Sebastião do Paraíso, MG.

Manejo das entrelinhas	Número de sacas beneficiadas por há				
	2008	**2009**	**2010**	**2011**	**Média 4 anos**
Mower	8,0 bc	26,0 ab	25,22 bc	27,17 ab	21,6 bc
Disk harrow	11,7 bc	20,7 ab	30,33 b	20,40 b	20,8 bc
Rotary tiller	17,0 ab	25,7 ab	29,33 b	27,83 ab	24,9 b
Post emerg. herbicide	14,0 b	25,0 ab	29,33 b	28,60 ab	24,2 b
Pré emerg. herbicide	31,4 a	33,0 a	41,77a	31,60 ab	31,4 a
Manual weeding	17,7 ab	24,0 ab	30,33 b	36,67 a	27,2 ab
No control	4,0 c	19,0 b	21,11 c	25,36 ab	17,4 c
V.C (Variation coeficiente %)	11,41	9,8	12,46	8,42	9.83

Médias seguidas pelas mesmas letras não diferem entre si pelo teste Tukey a 5%.

CONCLUSIONS

Coffee interrows always infested with weeds (no weed control) presented always the lowest yield.

Coffees inter rows free of weed by pre-emergence herbicide, after 35 years showed the highest yield.

Treatment with the interrows kept free of weed by mower presented yield slightly higher than the control without weeding.

Treatments using mower, rotary tiller, hand weeding and post-emergence herbicide between the interrows, presented equal yields, higher only than the no weed control treatment, but lower than the pre-emergence herbicide weed control plot.

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Identification of Natural Enemies of the Coffee Berry Borer (*Hypothenemus hampei*) in Costa Rica

M. ROJAS, J. MORALES, C. DELGADO, R. MARÍN, L. C. TORRES-MURILLO

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica

SUMMARY

With the purpose of identifying the insects in the fruits infected with the coffee berry borer and their relation with the plague as potential agents for natural control, 15 coffee farms with different characteristics of height, weather, soil, variety, density, shadow and management were selected for sampling in the Turrialba region.

Samples were collected from coffee fruits that remained in the plant and on the ground in the 2009/2010 harvest, during March, April and May 2010, and from mature fruits in the 2010/2011 harvest, during August and September 2010. The coffee fruits samples were taken to the Phytoprotection Laboratory of CICAFE. Of all the fruits infected with the coffee berry borer, a representative number was dissected in order to find potential biological controllers. The others were stored in plastic boxes with a lid that allowed aeration, to keep them for a month with a temperature of 27°C (80.6°F) and 75 percent relative humidity. The fruits were kept hydrated and each week they were exposed to light to stimulate the emergence of potential biological controllers.

The main insects associated to the remaining coffee fruits infected with the coffee berry borer in the 09/10 harvest were *Lyctocoris* sp, *Solenopsis geminata* and *Blastobasis* sp, in the plant, together with pseudoscorpions on the ground. The number of insects, except for the pseudoscorpions, was higher in the coffee fruits left in the plant than those on the ground. In the fruits infected with the coffee berry borer at the beginning of the 10/11 harvest only *S. geminata* were found.

Preliminary lab tests assessed the predaceous capacity of the coleopterous pests *Cathartus quadricollis*, *Ahasverus advena* and *Lyctocoris* sp. The three insects showed high predaceous capacity in the biological states of the coffee berry borer, although the *Lyctocoris* sp showed higher voracity and was the only one predator of adult coffee berry borer. A single *Lyctocoris* sp. preyed upon over 40 eggs of coffee berry borer during 10 days; the *C. quadricollis* ate almost 40 and the *A. advena* ate over 33. During its nymphal stage (24 days) *Lyctocoris* sp. predated on average 119 eggs, 116 small larvae, 131 large larvae, 45 pupae or 39 adults of coffee berry borer.

INTRODUCTION

In the Turrialba region has been experiencing a significant reduction in the level of coffee berry borer attack, which could be related to the presence of insects that have adapted to the behavior of the pest, natural enemies becoming. The study of these insects can help to maintain and increase their populations in the field and even open the possibility of multiplication in laboratory to include as part of the integrated management.

In Colombia, Bustillo et al (2002) reported the presence of a parasitoid of coffee berry borer, probably the *Cryptoxilos* genus; seven genera of ants, two bugs (*Calliodes* and *Scoloposcelis*) and the *Cathartus quadricollis* beetle preying CBB on the field. In Mexico, Pérez-Lachaud and Hardy (1999) found a new species of parasitoid identified as *Cephalonomia hyalinipennis*. In Costa Rica, Varón et al (2004) reported that ants *Solenopsis geminata*, *Pheidole radoszkowskii* and *Crematogaster torosa*, have large capacities of predation CBB under laboratory conditions.

During 2007 and 2008, ICAFE released *Prorops nasuta* in Turrialba region. Parasitism levels reached 27% average four months after release (Rojas et al 2008). At the beginning of the crop 08/09 parasitoids were found in 78% of the lots, at 50 and 200 meters of the lot released parasitoids was observed in 44% and 33% of farms, values decreased due to the harvest (Rojas et al 2010).

MATERIALS AND METHODS

Sampling sites included 15 coffee plantations, ages 8 and 30, located between 630 and 1220 masl, with an annual average temperature of 21-22 °C and total annual rainfall between 2300 and 2600 mm. The plantation management considered differences between the lots, such as cropping system (full sunlight, shade of *Erythrina poeppigiana, Eucalyptus deglupta* or *Cordia alliodora*), insecticide application, release of *Prorops nasuta* in 2008, the coffee tree pruning (selective, rows or lots), among others.

The sample consisted of taking residual fruits of plant and ground of the harvest 2009/2010 during the months of March, April and May and ripe fruits of the harvest 2010/2011 during the months of August and September 2010. Samples were taken to the laboratory of Plant Protection of CICAFE. Some of the fruits were dissected for possible biological control and the other part was kept hydrated for a month in conditions of 27 °C and 75% RH to stimulate the emergence of potential biological control agents.

We studied the biological cycle and reproductive capacity of a predatory bug of CBB. Newly hatched nymphs were placed individually in Petri plates and multiwell plates with different immature stages of CBB (20-25 eggs, 20-25 small larvae, 20-25 large larvae, 5-10 pupae or 15 adults). The observations were made every three days and offered new stages of CBB according to the voracity observed on each plate.

RESULTS AND DISCUSSION

The main insects related to residual attacked fruits of the plant after harvest 2009/2010 were *P. nasuta, Lyctocoris* sp, *S. geminata* and *Blastobasis* sp (Table 1), adding also pseudoscorpions in the attacked fruits of the ground. The number of insects, except pseudoscorpions, was higher in the fruits of the plant than in the fruits of the ground. In attacked fruits of beginning of harvest 2010/2011, there were very low amounts of *P. nasuta* and *S. geminata.*

The *Lyctocoris* bug was found in dried fruits of the plant (53.3% of farms) and ground dried fruit (20% of farms), but not found in ripe fruits, indicating that prefers old fruit. *Blastobasis* sp was mainly abundant in fruits of the ground. On farms where released *P. nasuta*, the parasitoid was found well established in the ground dried fruit (62.5% of farms), but to a lesser extent in the ground dried fruits and ripe fruits of the harvest 2010/2011.

Table 1. Number of insects per sample related to dry attacked fruits of the plant. Turrialba Region, March to June 2010.

Farm/Lot	***P. nasuta***	***Lyctocoris* sp**	***S. geminata***	***Blastobasis* sp**
CATIE/Convencional	1	0	13	5
CATIE/Insecticida	0	0	0	0
CATIE/Orgánico	4	0	0	0
CATIE/Colección	43	2	7	0
Santa Rosa/Convencional	30	9	21	0
Santa Rosa/Parasitoides	8	6	0	1
Aquiares/Convencional	0	1	0	0
Aquiares/Parasitoides	0	6	0	0
Bienvenido Badilla	1	6	1	0
La Gloria/Convencional	0	1	0	2
La Gloria/Parasitoides	0	1	0	2
Zalmari	42	0	0	0
Chúcaras/Convencional	3	0	0	1
Chúcaras/Parasitoides	12	0	0	0
Hacienda Navarro	0	0	0	1

The characteristics of the lots did not show a clear relationship with the insect population, including the use of insecticide. The lot under organic management was not among the farms with greater abundance of insects. The number of insects found in attacked fruits may be more related to harvesting practices and more food for natural enemies.

Rojas and Morales (2011) reported the presence of *Cathartus quadricollis*, *Ahasuerus advena* and *Lyctocoris* sp. in attacked fruits from Turrialba, which in the laboratory showed their predatory activity on different stages of the coffee berry borer. Indicate that an adult of *Lyctocoris* sp, optionally can prey on 41 eggs, 21 pupae, 18 small larvae, 12 large larvae or 6 adults of CBB in 5-10 days.

Laboratory studies indicated that females of *Lyctocoris* sp. oviposit 121 eggs on average with 70% viability during 49 days, feeding CBB adults under conditions of 27 °C and 75% relative humidity. The egg takes about a week to incubate. The duration of nymphal development ranged from 22 to 24 days depending on the state of CBB offered as prey. On average the first moult observed occurred 3.6 days after hatching, the second at 9.2 days, and the third at 16.9 days.

Under multiwell plate, nymphs controlled on average 118 eggs, 101 small larvae, 63 large larvae, 45 pupae and 25 adults of CBB in a period of 21-22 days (Table 2). In conditions Petri dish each nymph controlled 119 eggs, 116 small larvae, 131 large larvae and 38 adults over a period of 22-24 days. It is notable that even the smaller nymphs managed effectively maintained and developed preying any stage of CBB.

While this behavior laboratory with high prey availability may differ greatly from the situation in the field, the study demonstrates the potential that this bug can have made an important contribution to biological control in coffee plantations.

Table 2. Number of stages of CBB controlled (consumed + dead) by *Lyctocoris* sp. under laboratory conditions in multiwell plates (27 °C and 75% RH), during the development of nymph to adult.

CBB stage	Stages ± d. e.	Days ± d. e.
Eggs	118,4±22,9 (n=5)	21,8±2,2
Small larvae	100,8±20,1 (n=5)	21,4±3,8
Large larvae	62,8±8,7 (n=5)	20,8±3,3
Pupae	45,4±12,1 (n=5)	21,6±4,3
Adult	24,8±6,9 (n=5)	21,2±2,9

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The Crespera Del Cafe in Costa Rica and its Association to *Xylella Fastidiosa*

M. BARQUERO-MIRANDA, A. ROBLES-CHAVES, N. ARRIETA-ESPINOZA

Costa Rica Coffee Institute, Coffee Research Center (CICAFE)

SUMMARY

In Costa Rica, the "Crespera del café" is a physiological disorder which symptoms are not always present in the entire plant. These symptoms are visible in the leaf deformation: bifurcation of the midribs, serrated edges, raised midribs, chlorotic spots and a coriaceous appearance that not always covers the entire leaf. There may be a proliferation of secondary axes in the upper third of the orthotropic axis with the shortening of internodes. It is also possible to notice a growth distortion forming a slightly spiral, while the plagiotropic growth affected is characterized by short internodes, particularly in those leaves with the disorder symptoms. During 2006-2010 several field studies were conducted in areas which presented the symptoms, and laboratory studies were made in the Phytopathology Laboratory of CICAFE, with the purpose of understanding the dynamics and spread of this disease, both in the plant and in the coffee plots, and the symptomatology relationship with or without Xylella fastidiosa. These studies were also intended to evaluate different antibiotics in the development of the symptoms through several consecutive years. The results from these studies reported that the X. fastidiosa bacterium was detected in 97,5 percent of the coffee plantations; however, the symptoms in the field do not match the proportion of the bacteria that is present. Moreover, the spread of the disease in the plant did not follow a specific pattern; most of the bacteria were concentrated in the plagiotropic tissues of the middle and upper stratum of the plant. Asymptomatic plants were positive in the DAS_ELISA test. No association was observed between the progress of the symptoms and the concentration of bacteria in the plant; the antibiotics did not reverse the symptoms or prevented the emergence of new ones.

INTRODUCTION

Xylella fastidiosa pathogen is mostly associated symptoms Crespera coffee in Costa Rica (Rodriguez et al, 2001). This bacterium lives in the xylem conduits of plants, blocking the passage of water and inorganic salts to the roots, stems and leaves. High production conditions, nutritional depletion and low water availability is directly related to the expression of symptoms Crespera in coffee plantations in Costa Rica. Due to lack of information about the distribution of bacteria in different growing regions of the country and its consistency or otherwise with Crespera symptoms in coffee. It was proposed to sample around the coffee area of the country and study the distribution of the bacteria in the plant, and to evaluate the effectiveness of various antibiotics on the progress of the symptoms of Crespera.

MATERIALS AND METHODS

Distribution in the coffee area

To evaluate the relationship between symptoms and the presence Crespera the bacterium *Xylella fastidiosa* in coffee trees, we sampled throughout the coffee regions of Costa Rica, during the years 2006 and 2007. The number of sample by region was equivalent to 0,4% of the area in hectares coffee. At each sampling site were taken 10 subsamples, each consisting of a pair of leaves. A total of 5000 sub-samples examined. Each sampling point was geo-referenced, also recording the latitudinal position. Samples were sent to the Research Center in Cellular and Molecular Biology (CIBCM) at the University of Costa Rica (UCR), for the analysis of the presence of the bacterium *X. fastidiosa* by ELISA. The realized sampling period from march to december. The results were analyzed by interpreting the proportion of sub-samples positive (presence of bacteria) in each coffee region studied.

Distribution in the coffee plant

During the years 2007 and 2008, were selected 10 plants with 6 different degrees of symptoms Crespera (0-5), in a plantation with the presence of symptoms. We collected three subsamples for each degree of symptom, composed of four leaf petioles located in strata: low, middle and top of each plant. Also petioles of leaves were selected, of the outer, middle and inner plagiotropic axis, as described above. The analysis was performed for comparison with the absorbance of the bacteria X. fastidiosa, obtained from DAS-ELISA assay.

Biological efficacy of antibiotics

The study was conducted in a farm located in Corralillo of Cartago. Were used 30 plants with Crespera symptoms by treatment, for a total of five treatments. We applied the following bactericides: Agrimycin WP 16,5 (2.5 kg / ha), Kasumin 2 SL (2 L / ha), Gent Agry-WP Plus 8 (1,6 kg / ha), Inside 2 SL (2 L / ha), and no treatment was included as a control product application. Applications were fortnightly from august to november 2009 and 2010. Plants were evaluated monthly, counting and healthy leaves Crespera symptoms.

RESULTS

It was determined that in all sites sampled one positive result for the presence of *Xylella fastidiosa*. In all sampling points analyzed, only 11 cases were negative for the presence of bacteria, which represented 2,5% of the total sample sampling general. In addition, there was no clear pattern of presence of bacteria, considering the latitudinal. The bacterium *X. fastidiosa* is present from 400 m up to altitudes above 1400 m.

As to the distribution of *Xylella fatidiosa* in the plant in Figure 1, shows as the highest concentration of the bacteria encounter in the middle and upper plants, with values between 1200 nm to 1400 nm. Conversely, the lower stratus reaches values lower than 600 nm absorbance.

The results of the evaluation of antibiotics in plants Crespera symptoms observed in Figure 2. The initial and final incidence of Crespera was statistically similar for each treatment, except for treatment with the product Agry-Gent, which showed a slight decrease in incidence between the first and the last assessment. However, the overall incidence ranged from 40 to 70%, values that are statistically equal treatment without application of products (60%).

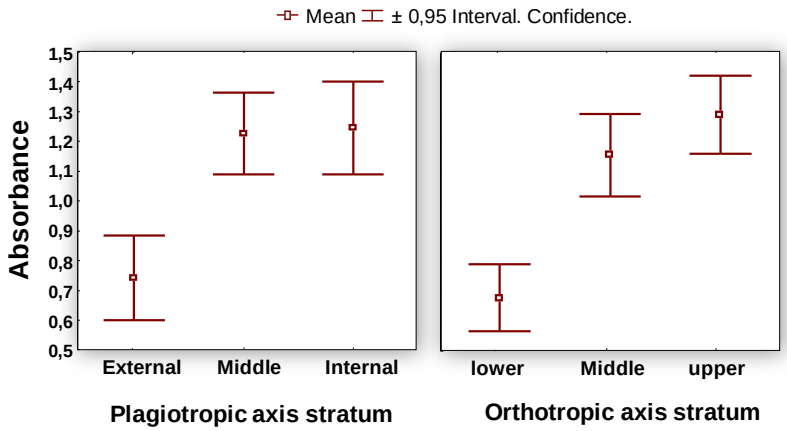

Figure 1. Average absorbance of *Xylella fastidiosa* for strata upper, middle and lower plants of coffee and three positions in plagiotropic axes: internal, middle and external.

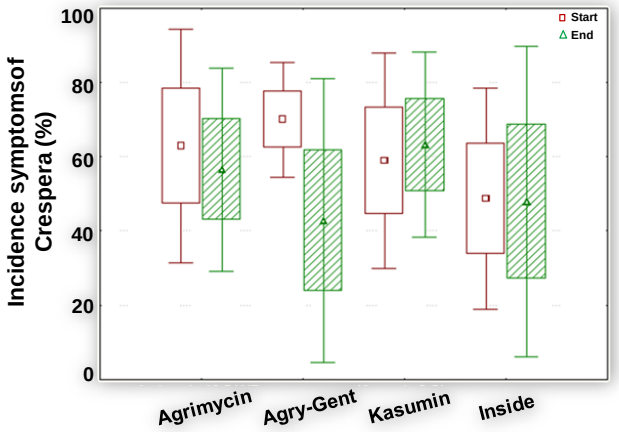

Figure 2. Comparison of effect between the first and last evaluation of Crespera diseased plants treated with different antibiotics.

DISCUSSION AND CONCLUSIONS

This research revealed that in the coffee area of Costa Rica, the bacterium *Xylella fastidiosa* was detected in 97,5% of the sampling sites. However, no direct relationship exists between the observed symptoms of Crespera field distribution and the presence of the bacterium and *X. fastidiosa* in coffee growing. Fournier (2007), diagnosed the presence of bacteria in plants and in healthy and diseased plants absorbance analysis established that the higher concentration of bacteria occurs in the middle and upper nodes oldest branches, however, not possible to relate the amount of bacteria with symptoms due to the absorbance between each set levels was similar disease.

The results obtained in this work demonstrated a greater concentration of *X. fastidiosa* in the tissues of the upper strata of the plant, as well as older petioles of plagiotropic axes. Similar results are reported by Krivanek and collaborators (2005), finding that greater mobilization and concentration of *X. fastidiosa* in the xylem of plants of Vitis sp., is towards the upper levels of the plant, while lower concentrations of bacteria were found at low point immediately to the point of inoculation. Oliveira and collaborators (2002) indicate that the highest concentrations of *X. fastidiosa* is in the petioles and central veins of citrus leaves with ages over 8 months, while in lower leaves at 2 months not found significant concentrations of bacteria.

The application of antibiotics tested in this study failed to stop progression of symptoms Crespera in coffee plants, perhaps due to degradation of the molecules and the and the difficulties of contact between the antibiotic and the bacteria in the xylem of plants, are very important factors that may mediate the results of this study.

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Epidemiology of *Mycena Citricolor* in Costa Rica

M. BARQUERO-MIRANDA, N. ARRIETA-ESPINOZA, A. ROBLES-CHAVES

Costa Rica Coffee Institute, Coffee Research Center (CICAFE)

SUMMARY

Mycena citricolor, pathogen causing the disease known as "Ojo de gallo" or also called American leaf spot of coffee, is the most serious coffee disease in Costa Rica, and it is responsible for considerable economic losses every year. This disease decreases yield performance and causes premature exhaustion in coffee plantations, particularly in areas in which the environmental conditions are favorable for the development of the *Mycena citricolor* pathogen. Considering the influence of climatic conditions on the gemmae formation of *M. citricolor* and their germination on coffee leaves, a study was conducted from 2008 to 2011, to examine the progress curve of the disease and variations in temperature, rainfall, relative humidity and hours of leaf moistening. Throughout the study, the climatic conditions were influenced by the natural phenomena ENSO which clearly had an effect on the progress of the disease in either a positive or a negative way. The continued influence of three weeks at least 14 h of leaf moisture, 400 mm of rainfall and 4 rain days / week with > 1 mm precipitation, determine the development of the Ojo de gallo disease in Poás of Alajuela with a prediction of four weeks.

INTRODUCTION

The epidemiological development *Mycena citricolor* is heavily influenced by the humidity. The daily rainfall distribution, the constant presence of cloudiness that reduces solar radiation and ultraviolet (UV) during the day, with low clouds in the morning and / or afternoon favor disease development. These conditions are conducive a film of water on the leaves as much about injuries caused by the pathogen, inducing the formation of heads or gems, responsible for maintaining and increasing the presence of the disease in coffee (Barquero, 1997).

The implementation of a suitable chemical control of the Ojo de gallo coffee disease should consider the precise knowledge of the climatic conditions and the effectiveness of cultural and chemical practices for the control. At the end of the 1990s, the ICAFE determined that the use of systemic fungicides: Silvacur or Atemi, mixed with the Validamicina-A (Cepex); I was able to achieve adequate control of the disease. Subsequent work carried out during the years 2006, 2007 and 2008 confirm the effectiveness of this mixture of fungicides on control of the Ojo de gallo, even started with levels of incidence of 20% (ICAFE 2008; ICAFE 2009). The success of this mix of fungicides is, significantly limits of the formation of gems in the lesions of the disease of present in crop plantation for a period of 40 days.

To maximize the potential to control chemical is essential to know the factors that initiate the development of the disease and how it evolves it. So this paper seeks to establish a basis of climate information that can be correlated with the increase in the incidence of the disease of the Ojo de gallo over several periods of rain.

MATERIALS AND METHODS

The field test was carried out at the farm La Rosalía, located in San Juan Norte in the region of San Pedro de Poás, province of Alajuela, located at 1450 meters above sea level with an average annual temperature of 18 °C, relative humidity of 90% and annual precipitation of 3300 mm. The developments of the disease were evaluated on the Catuaí variety, established at 2 m between rows, and 1 m between plants and without fungicide applications. The experimental plots are formed by three rows of 10 plants and the experimental unit formed of 10 plants. In each one of them is marked 1 branch from the middle stratum of the plant, oriented to the West side of the same. The total number of sheets, number of sick leaves, was assessed total number of injury, total number of injury sporulated and total number of gems. Evaluations were carried out every 7 days during the months of June to October of the period 2008-2011. In analyzing the data, we calculated the incidence, apparent infection rate and related injuries and increased total number of gemmaes formed, with different climate rate parameters at a weather station registered trademark Davis Instruments.

RESULTS

The calculation of the apparent infection rate (r) of the Ojo de gallo disease, determined the existence of two peak of disease progression. Figure 1, shows that the infection rate is higher in the months of june and september, which reached a rate of about 1%. However, it was also possible to observe values of 4,5 in the rate of infection in different years and seasons during the study period.

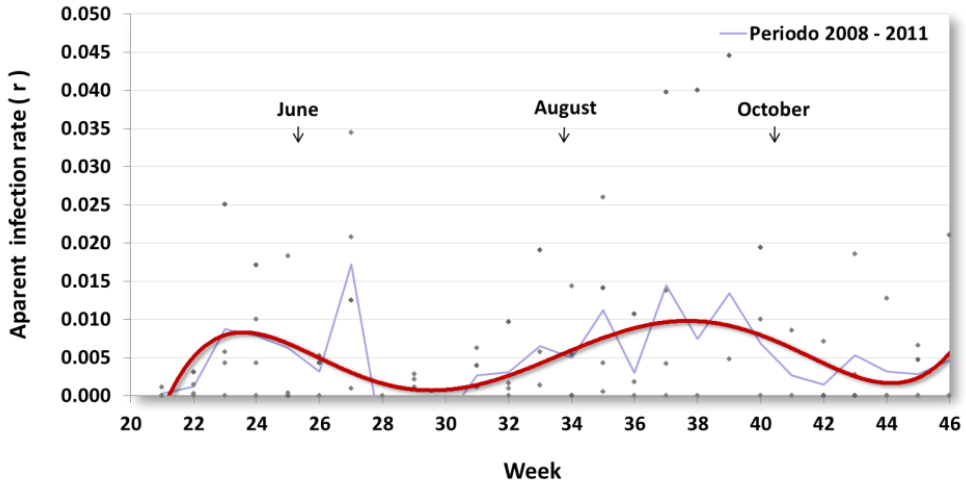

Figure 1. Apparent infection rate Ojo de gallo disease in San Juan de Poás, Alajuela. Period 2008 - 2011.

During the study period the data of climatic parameters such as precipitation, relative humidity, solar radiation, UV light and hours of wet leaf, varied significantly between years. In the years 2008 and 2010 are presented parameters favorable climate for the development of the Ojo de Gallo disease, compared to those presented for the years 2009 and 2011. These differences in climate influenced the differences found in the rate of increase of the disease over the initial incidence. During 2008 to 2011, the increase of the disease was 80, 69, 97, 60% respectively. The period of time where the disease is active was 28 weeks, mainly between weeks 19 to week number 46 of each year. Table 1 shows the average climatic parameters and the incidence of Ojo de gallo of four years of study, reported in the Rosalia farm in the San Juan Norte of Poás, Alajuela province.

Table 1. Average weekly incidence of Ojo de gallo disease and climatic records of San Juan Norte of Poás, Alajuela, Period from 2008 to 2011.

Week	T (°C)	RH (%)	Rain (mm)	Rain days > 1 mm	Solar Rad. (W/m²)	UV light	Wind speed (km/h)	Leaf moisture (h)	Incidence (%)
19	19	85	28	1	144	2	2	14	23
20	19	89	107	4	129	1	1	15	24
21	18	92	79	3	119	1	0	16	24
22	19	91	99	5	115	1	1	14	25
23	19	90	84	5	110	1	1	13	30
24	19	89	108	5	124	2	1	15	33
25	19	92	164	5	109	1	1	16	36
26	18	90	100	4	126	1	1	12	36
27	18	92	102	5	114	1	0	13	48
28	18	93	116	6	102	1	0	13	44
29	19	88	58	3	130	1	1	10	44
30	18	92	65	4	124	1	0	14	42
31	19	92	102	4	123	1	0	15	44
32	18	92	102	4	117	1	0	17	45
33	18	92	194	6	115	1	0	17	49
34	18	92	165	6	113	1	1	16	52
35	18	93	100	5	121	1	0	15	60
36	18	94	119	4	127	1	0	15	62
37	19	90	131	6	138	1	1	14	65
38	18	91	136	5	112	1	1	17	68
39	18	94	172	6	111	1	1	17	66
40	18	92	115	5	113	1	0	14	71
41	18	93	190	5	107	1	1	14	72
42	17	94	162	6	93	1	1	18	73
43	18	92	144	5	106	1	1	15	79
44	18	91	128	5	113	1	2	16	85
45	17	90	79	4	120	1	1	14	89
46	18	88	62	4	121	1	1	13	100

The relationship between weather parameters and development of Ojo de gallo disease is shown in table 2. The correlation analysis shows that the weather elements mainly explaining changes in the formation of gemmaes and increased of *M. citricolor* incidence is: the average 14 hours of leaf moisture, 400 mm of precipitation and average 4 days of rain> 1 mm accumulated for three consecutive week, Figure 2.

Table 2. Correlation of weather parameters and the incidence of Ojo de gallo in San Juan Norte de Poás, Alajuela, during the period 2008-2011.

Factor	**Mean**	**S. D.**	**r (x,y)**	**r^2**	**t**	**P < 0,05**
Solar Rad. (W/m²)	396,34	67,20	-0,55	0.35	-5.11	0.0004
UV Index	4,55	1,16	-0,58	0.30	-4.57	0.0010
Total rainfall (mm)	310,62	113,24	0,70	0.48	5,22	0.0000
Rain days > 1 mm (n)	4,16	1,32	0,67	0.45	4,99	0.0000
Leaf moisture (h)	14,31	1,46	0,60	0.36	4,08	0.0003

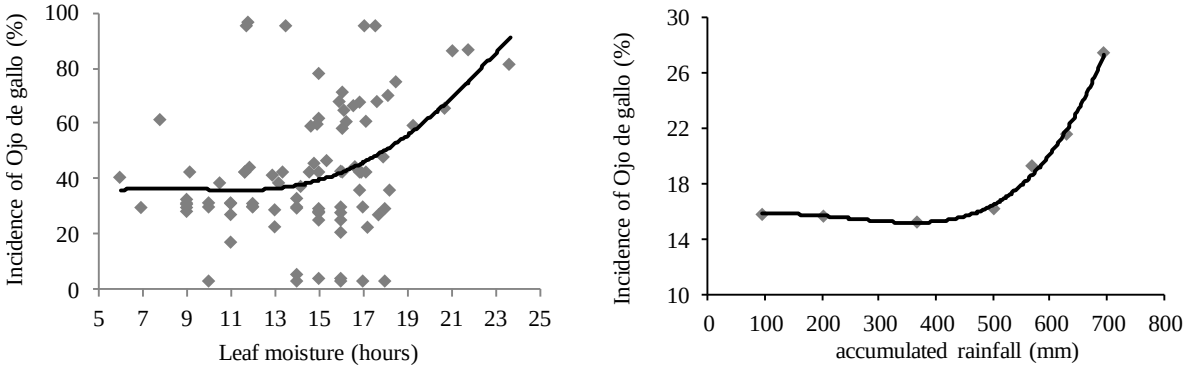

Figure 2. Influence of leaf surface moisture hours and cumulative rainfall in the increased incidence of Ojo de gallo disease. Poás of Alajuela, Costa Rica.

DISCUSSION AND CONCLUSIONS

The data obtained after four years of study allow to relate the development of "Ojo gallo" disease in coffee, according to variation in the prevailing weather conditions. The analysis of the climatic elements such as: leaf moisture, precipitation and number of rainy days; are key indicators to predict the increase or not of this disease with a notice of at least 4 weeks, after which time it is possible to observe a increases of "Ojo de gallo" lesions in leaf tissue of plants of coffee, if not there is a chemical control of the disease. The results of this study differ from those found by Vargas (2004), which indicated that the development of the disease was possible to predict only a few days of onset.

The information obtained through this study will be very important to establish a system for monitoring weather conditions in order to establish a alert system epidemic, which recommends the timely use of chemical control to minimize disease and even the definition of appropriate cultural conditions for the establishment of new plantations, according to the climatic conditions prevailing in different areas hit by the "Ojo de gallo". This will promote the efficient and rational use of agrochemicals and desirable agronomic management of the plantation, contributing to a more sustainable coffee production.

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Exploiting Genetic Diversity to Improve Coffee Quality

R. BORDIGNON[1], H. P. MEDINA-FILHO[2]

Centro de Café – Instituto Agronômico de Campinas
Avenida Barão de Itapura, 1481, Botafogo, 13020-902, Campinas, SP, Brazil
[1]Sponsored by Consórcio Pesquisa Café
[2]CNPq fellowship

SUMMARY

Presently 19 *Coffea* species are maintained in the germplasm collection of Instituto Agronômico, a fraction of the existing biodiversity. Breeding programs have being using much less. Exceptions are *C. canephora* source of nematodes and rust resistances, *C. liberica* source of SH_3 rust resistance allele and *C. racemosa* source of leaf miner tolerance. As to cup quality, general concept is that value exists only in *C. arabica*, but wide hybridization and recombination might generate new profiles and market opportunities. Such investigations are pursued in Campinas for agronomic, bean characteristics, cup profile, requisites hard to combine when breeding with exotic germplasm. Combinations potentially valuable are used in crosses and studied in more detail. Promising F_4 lines Catuaí x Glaucia from Ethiopia display uniform ripening and improved flavor. Crosses of Ibairi (Mokka) with Obatã, Tupi, Erecta, Nanico and *C. racemosa* backcross derivatives have been also studied for cup quality. Exotic profile is displayed by some S_3 lines of *C. arabica* x (*C. salvatrix* x *C. racemosa*)4n with unique black fruits. F_1 arabustas (*C. arabica* cvs. Ibairi and Obatã x *C canephora* 4n) have defective beans and intermediate quality but selections can be done in F_2. Genes of *C. eugenioides*, the evolutionary female genitor of *C. arabica* have been introgressed into cv. Obatã allowing selection for improved flavor, high yields and tropical conditions.

INTRODUCTION

Among the 124 recently reclassified *Coffea* species 19 are represented in the germplasm collection of Instituto Agronômico in Campinas. Though precious, it is a fraction of the biodiversity. Even less has been effectively exploited in breeding programs, exceptions being *C. canephora* source of nematodes and rust resistant genes, *C. liberica* donor of SH_3 rust resistance allele and *C. racemosa* origin of leaf miner tolerance. As to cup quality, attempts are hindered by the concept that little value exists outside the gene pool boundary of *C. arabica*. Nevertheless, exploratory investigations of available genetic diversity, is a straightforward strategy to access complex interactions of flavor profiles that could be expressed after wide hybridizations and ensuing genetic recombination. Unique profiles are open avenues for the evolution of market and opportunities of new consumer niches. In this line of investigation, many genetic recombinants have been generated, mostly backcrossed in different intensities to cultivars, planted in breeding plots for agronomic observations, bean characterization and beverage profile evaluations, notwithstanding that Campinas is not a specialty coffee producing region. Data and better knowledge have been obtained in different years, plots and generations of germplasm grown, harvested, processed and cup tested in same conditions at IAC in Campinas, thus conceivably comparable. Some with potential traits were used in crosses and generations advanced. As expected, the majority of derivative lines failed on agronomic performance, had severe bean defects or odd tasting beverages. Some with

potential and perspective of exploitation in the development of coffee cultivars with distinct cup profile are briefly reported here.

MATERIALS AND METHODS

Among many and diversified crosses and derivatives of IAC germplasm bank, some were studied in more detail. Glaucia have interest because is a very late uniform ripening Ethiopian accession with a quite different cup profile, mix of nuts, cereal and dark chocolate. Obatã, a high yielding cultivar is late and resistant to several rust races, both traits introgressed from *C. canephora*. Tupi is a sister line of Obatã with similar yields but ripens earlier. Erecta with upright branches was transferred to Catuaí background and has the potential for close spacing. Nanico is an extremely short stature line. Mokka gene of cv. Ibairi confers small rounded beans of very good quality. In addition, Ibairi plants are less attacked by leaf miner. *C. racemosa* and *C. salvatrix* are diploid leaf miner tolerant species bearing blackish fruits of unique, extremely different profiles. *C.eugenioides* is the diploid female genitor of *C. arabica* and the putative source of good flavor genes. *C. canephora*, the male genitor of *C. arabica* is a sturdy, high yielding cultivated species. Data were obtained in different years and plots in Campinas. Plots were conducted with commercial practices of fertilization, fruits were hand harvested and dry processed. Cup tests followed SCAA brewing standards with emphasis in global scores and descriptions of cleaned and graded samples.

RESULTS AND DISCUSSION

Crosses of Glaucia to several cultivars resulted in a number of hybrids and pedigree advanced lines. The most promising were F_4 lines Catuaí x Glaucia selected for and displaying uniform ripening. Cup analysis showed variable results but interestingly, a few sister lines consistently scored higher (83.5) as compared to Catuaí (74) and Obatã (76) with distinct citric orange lime notes resembling Harar coffees.

Other studied group comprised lines derived of crosses of Ibairi by Obatã, Tupi, Erecta, Nanico and derivatives of *C. racemosa* backcrossed to *C. arabica*. Many of them had improved cup profile, clearly Ibairi (Mokka) heritage. Descents of Erecta and Nanico were agronomically undesirable but F_1s of Obatã x Ibairi and reciprocals as well as Tupi x Ibairi were short stature, compact, resistant to rust and several hybrids had reduced attack of leaf miner [4]. Beverage quality is better than Obatã or Tupi, resembling Ibairi. Grains are of intermediate size, roundish, dark green. Exploitation of selected F_1 hybrids of them seems feasible. Of 10 Ibairi x Obatã and 28 Obatã x Ibairi F_2 progenies, 13 have shown good crops for three years (Figure 1 D) and scores 82 to 87.

Eight studied F_2 progenies of Ibairi x Tupi were all early maturing and scored 81 to 86 with varying but interesting fruity cup profiles, with orange acidity, nuts and dark chocolate notes. Most of these lines have quite uniform maturation (Figure 1C).

C. racemosa backcross derivatives had interesting fruity beverage but their hybrids with Ibairi were disappointing as to yield, beverage and grain characteristics.

Unparalleled characteristics were displayed by some early S_3 selections of *C. arabica* x (*C. salvatrix* x *C. racemosa*)4n. After two selection cycles, yields have improved, fruits are black at maturity (Figure 1F) that occurs very early. The outturn is higher than any other line, although beans are mostly peaberries. Beverage scored 84-85 with strong remarks of a sweet unique profile without astringency and a pleasant red wood winey taste with fruity, berry-like

aroma. Progenies have been advanced in order to better evaluate the agronomic and quality potential of such peculiar individuals.

F_1 arabustas (*C. arabica* x *C canephora* 4n) are invariably heterotic setting fruits abundantly, but commercial use of clones is precluded by the outcome of meiotic abnormalities, i.e., low outturn and defective beans. Flavor is intermediate between genitors but conspicuously segregate out in F_2 as vigor, fruit set and rated of normal beans. This had allowed identification of vigorous F_2 segregants of crosses of *C. canephora* 4n with Ibairi (Mokka) and Obatã (Figure 1E) cultivars with cup quality much closer to arabica that have to be studied as to rooting ability and biology of reproduction. F_2 cloned arabustas might be an option to robusta regions.

Figure 1. A. High yielding short stature F_2BC_2 progeny of *C. arabica* x (*C. eugenioides* 4n x *C. arabica*) and (B) their dense rosettes. C. Highly branched early uniform ripening F_2 *C. arabica* cv. Ibairi (Mokka) x cv. Tupi. D. F_2 plant of *C. arabica* cv. Ibairi (Mokka) x cv. Obatã. E. High yielding late F_2 *C. arabica* cv. Obatã x *C. canephora* 4n. F. Black fruited S_3 selection of *C. arabica* x (*C. salvatrix* x *C. racemosa*)4n.

For several years, detailed agronomic observation and repeated cup analysis of 126 Obatã backcrosses (BC_2) of *C. arabica* x (*C. eugenioides* 4n x *C. arabica*) has resulted in the

selection of 36 F_2BC_2 progenies. The agronomic evaluation and cup tests of these progenies have shown considerable progress in uniformity, overall yields and cup quality (Figure 1 A, B). Compared to Catuaí and Obatã that scored 74-76 with normal remarks, 5 good yielding progenies scored 70-80 but 16 scored 82-86 with relevant descriptions of chocolate-like and lemon grass aromas, orange-lemon acidity, salty-fruity taste, floral nuances, long lasting smooth aftertaste. Generation has been advanced with selection for plant and bean uniformity. Sister F_3BC_2 lines specially selected for lateness have been evaluated in the Amazonian Rondônia State and have shown similar trend of good yields and higher cup scores. Presently the F_4BC_2 progenies are being trued up.

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Green Bean Physical Characteristics of Promising Hybrids and Ethiopian Arabica Coffee Accessions in Brazil

G. S. GIOMO, M. B. SILVAROLLA, R. SAATH, A. DE C. IOBBI

Instituto Agronômico (IAC), P.O. Box 28, 13012-970, Campinas, SP, Brasil

SUMMARY

The green coffee beans size is a very important physical characteristic for coffee quality specification and coffee price determination in the specialty coffee market. In this study it was evaluated 44 *Coffea arabica* genotypes, including five Brazilian commercial varieties as control, four Ethiopian accessions and 35 advanced hybrids under selection at Instituto Agronômico de Campinas (IAC). The objective of this work was to evaluate the coffee bean size of these genotypes and to access the physical quality in order to identify the promising ones to improve the physical coffee quality in Brazil. The results showed that there are significant differences for green bean size among the evaluated genotypes. The F_1 hybrids showed large beans production higher than their parental genotypes and F_2 hybrids. Otherwise Ethiopian accessions showed large beans production lower than hybrids and control cultivars. The Obatã IAC1669-20 cultivar showed the highest potential for large beans production, both in isolated form or in different hybridization combinations with Ethiopian accession, constituting a very intriguing genotype to generate new varieties or new hybrids aiming to improve the coffee bean physical quality in Brazil.

INTRODUCTION

The narrow natural genetic variability encountered in original population of arabica coffee varieties derivate from Typica and Bourbon genotypes constitutes a limitative factor for breeding and selection of new coffee varieties with good physical quality. In order to increase the genetic variability and diversity for quality a long time ago it was introduced in Brazil diversified coffee types and accessions from Africa, India and Central America.

The bean size constitute the most important physical characteristic for green coffee grading and processing aiming to obtain homogeneous lots, but it is not yet totally elucidated the extend of bean size effects on beverage quality. Furthermore for some specialty coffee market the cup quality is considered more important than green bean physical attributes leading the coffee price determination. Since the bean size depends on the genotype and genotype × environmental interactions, recent studies have been done focused on the green bean size and shape in order to identify coffee varieties with better physical quality.

Considering the green bean size importance for coffee grading and for coffee price determination, this study was carried out to evaluate the green physical characteristics of several arabica coffee genotypes under selection at IAC's Breeding Program aiming to get reliable information about this aspect by genotype comparisons and to identify new possibilities to improve the physical coffee quality in Brazil.

MATERIALS AND METHODS

This study was carried out at Instituto Agronômico de Campinas (IAC) experimental station, in Campinas, São Paulo state, Brazil, in 2009/2010 crop year, where a total of forty four *Coffea arabica* L. genotypes were evaluated (Table 1). The Brazilian commercial varieties 'Mundo Novo', 'Catuaí Vermelho', 'Ouro Verde', 'Ouro Bronze', 'Bourbon Vermelho' and 'Obatã' were used as control for physical quality comparisons. Each one of these genotypes was represented by one or more plants and cherry samples were collected during the peak harvesting period of July-September. Ripe health fruits were harvested in bulk by hand from each plant and processed individually using dry processing procedures where the coffee berries were sun dried until the moisture content reached 11.5% (wb). After hulling the coffee beans were classified by size in several screens with circular perforations of 19, 18, 17, 16 and 15/64 inches and oblong perforations of 10, 11 and 12/64 x ¾ inches, using a randomized completely blocks statistical design with three replications. The sum of percentage of flat green beans retained over 19, 18 and 17 round screens constitute the large beans fraction (LB) and the sum of percentage of flat green beans retained over 19, 18, 17, 16 and 15 round screens and the percentage of peaberry beans retained over 12, 11 and 10 x ¾ oblong screens constitute the commercial beans fraction (CB).

Table 1. *Coffea arabica* L. genotypes evaluated in this study at IAC, Campinas, São Paulo, Brazil.

Genotype [(1)]	Genotype [(1)]
01 – F_1 Mundo Novo x Accession A (a)	23 – F_1 Accession C x Obatã IAC 1669-20
02 – F_1 Obatã IAC 1669-20 x Accession A	24 – F_1 Accession C x Catuaí Vermelho IAC 144
03 – F_1 Catuaí Vermelho IAC 81 x Accession A	25 – F_1 Diverse hybrid (b)
04 – F_1 Obatã IAC 1669-20 x Accession C (a)	26 – Catuaí Vermelho IAC144
05 – F_1 Catuaí Vermelho IAC 144 x Accession C	27 – Ouro Bronze
06 – Accession D	28 – Obatã IAC 1669-20
07 – F_2 Mundo Novo x Accession A	29 – F_1 Mundo Novo x Accession A (a)
08 – F_2 Accession A x Mundo Novo	30 – F_1 Mundo Novo x Accession A (b)
09 – Accession A	31 – F_1 Mundo Novo x Accession B (a)
10 – Accession C (self-pollinated)	32 – F_1 Mundo Novo x Accession A (c)
11 – F_2 Mundo Novo x Accession C (a)	33 – F_1 Mundo Novo x Accession C (a)
12 – F_2 Mundo Novo x Accession C (b)	34 – F_1 Mundo Novo x Accession A (b)
13 – F_1 Accession C x Mundo Novo	35 – F_1 Mundo Novo x Accession B (b)
14 – F_1 Accession B x Mundo Novo	36 – F_1 Mundo Novo x Accession B (c)
15 – F_1 Diverse hybrid (a)	37 – F_1 Mundo Novo x Accession C (b)
16 – F_2 Obatã IAC 1669-20 x Accession A	38 – F_1 Mundo Novo x Accession A (d)
17 – F_2 Ouro Verde x Accession A (a)	39 – F_1 Mundo Novo x Accession A (e)
18 – F_2 Ouro Verde x Accession A (b)	40 – F_1 Catuaí SH3 x Accession A
19 – F_2 Ouro Verde x Accession C	41 – Mundo Novo (a)
20 – F_2 Catuaí Vermelho IAC 144 x Accession C	42 – Mundo Novo (b)
21 – F_2 Obatã IAC 1669-20 x Accession C (b)	43 – Bourbon Vermelho (a)
22 – F_2 Catuaí Vermelho IAC 81 x Accession A	44 – Bourbon Vermelho (b)

(1) The small letter inside parentheses following the genotype's name indicates that there are several hybrids from the same parental or several progenies from the same coffee variety.

RESULTS AND DISCUSSION

The results of green bean grading are showed in table 2. It was observed significant effects of genotype on large coffee beans (LB) and commercial coffee beans (CB) percentages, indicating that there are genetic variability for bean size and possibility to select new coffee variety for better physical quality.

Table 2. Percentage of large beans (LB) and commercial beans (CB) of *Coffea arabica* genotypes evaluated at IAC, Campinas, São Paulo, Brazil.

Genotype	LB[(1)] %
02 – F_1 Obatã x Accession A	68.46a
04 – F_1 Obatã x Accession C (a)	61.48b
28 – Obatã IAC 1669-20	57.02c
43 – Bourbon Vermelho (a)	51.26d
37 – F_1 Mundo Novo x Accession C (b)	51.24d
42 – Mundo Novo (b)	45.40e
23 – F_1 Accession C x Obatã	45.37e
27 – Ouro Bronze IAC	42.40f
25 – F_1 Diverse hybrid (b)	41.75f
41 – Mundo Novo (a)	41.09f
40 – F_1 Catuaí SH3 x Accession A	40.40g
21 – F_2 Obatã x Accession C (b)	40.04g
39 – F_1 Mundo Novo x Accession A (e)	39.84g
38 – F_1 Mundo Novo x Accession A (d)	39.33g
26 – Catuaí Vermelho IAC 144	37.90h
44 – Bourbon Bermelho (b)	37.51h
05 – F_1 Catuaí IAC 144 x Accession C	37.02h
12 – F_2 Mundo Novo x Accession C (b)	35.01h
06 – Accession D	33.50i
14 – F_1 Accession B x Mundo Novo	33.46i
03 – F_1 Catuaí IAC 81 x Accession A	32.68i
07 – F_2 Mundo Novo x Accession A	32.17i
11 – F_2 Mundo Novo x Accession C (a)	31.98i
13 – F_1 Accession C x Mundo Novo	31.92i
01 – F_1 Mundo Novo x Accession A (a)	31.44i
36 – F_1 Mundo Novo x Accession B (c)	30.05j
24 – F_1 Accession C x Catuaí IAC 144	29.82j
18 – F_2 Ouro Verde x Accession A (b)	28.53j
16 – F_2 Obatã x Accession A	27.69k
20 – F_2 Catuaí IAC 144 x Accession C	26.56k
19 – F_2 Ouro Verde x Accession C	25.66k
30 – F_1 Mundo Novo x Accession A (b)	25.04k
33 – F_1 Mundo Novo x Accession C (a)	23.76l
31 – F_1 Mundo Novo x Accession B (a)	23.75l
17 – F_2 Ouro Verde x Accession A (a)	23.53l
32 – F_1 Mundo Novo x Accession A (c)	21.77l
34 – F_1 Mundo Novo x Accession A (b)	21.11l
22 – F_2 Catuaí IAC 81 x Accession A	16.35m
29 – F_1 Mundo Novo x Accession A (a)	15.69m
09 – Accession A	15.32m
35 – F_1 Mundo Novo x Accession B (b)	15.22m
08 – F_2 Accession A x Mundo Novo	11.60n
15 – F_1 Diverse hybrid (a)	6.94o
10 – Accession C (self-pollinated)	5.34o
Means	32.58
Fgenotype	175.31[3]
CV (%)	5.46

Genotype	CB[(2)] %
02 – F_1 Obatã x Accession A	96.49a
04 – F_1 Obatã x Accession C (a)	95.84a
28 – Obatã IAC 1669-20	95.54a
39 – F_1 Mundo Novo x Accession A (e)	95.36a
21 – F_2 Obatã x Accession C (b)	94.11a
23 – F_1 Accession C x Obatã	93.84a
27 – Ouro Bronze	92.55b
43 – Bourbon Vermelho (a)	91.70b
37 – F_1 Mundo Novo x Accession C (b)	91.56b
42 – Mundo Novo (b)	91.32b
01 – F_1 Mundo Novo x Accession A (a)	91.18b
44 – Bourbon Bermelho (b)	91.15b
03 – F_1 Catuaí IAC 81 x Accession A	91.09b
26 – Catuaí IAC 144	91.08b
38 – F_1 Mundo Novo x Accession A (d)	90.94b
25 – F_1 Diverse hybrid (b)	90.87b
14 – F_1 Accession B x Mundo Novo	90.77b
24 – F_1 Accession C x Catuaí IAC 144	90.66b
40 – F1 Catuaí SH3 x Accession A	90.57b
06 – Accession D	90.14b
41 – Mundo Novo (a)	89.58b
11 – F_2 Mundo Novo x Accession C (a)	89.49b
12 – F_2 Mundo Novo x Accession C (b)	89.18b
20 – F_2 Catuaí IAC 144 x Accession C	87.16c
36 – F_1 Mundo Novo x Accession B (c)	86.53c
19 – F_2 Ouro Verde x Accession C	86.04c
22 – F_2 Catuaí IAC 81 x Accession A	85.88c
13 – F_1 Accession C x Mundo Novo	85.22c
17 – F_2 Ouro Verde x Accession A (a)	85.19c
07 – F_2 Mundo Novo x Accession A	85.13c
16 – F_2 Obatã x Accession A	85.12c
30 – F_1 Mundo Novo x Accession A (b)	84.76c
18 – F_2 Ouro Verde x Accession A (b)	84.40d
05 – F_1 Catuaí IAC 144 x Accession C	83.65d
35 – F_1 Mundo Novo x Accession B (b)	83.50d
31 – F_1 Mundo Novo x Accession B (a)	83.02d
33 – F_1 Mundo Novo x Accession C (a)	82.93d
34 – F_1 Mundo Novo x Accession A (b)	80.90e
32 – F_1 Mundo Novo x Accession A (c)	80.58e
09 – Accession A	78.72f
29 – F_1 Mundo Novo x Accession A (a)	78.07f
08 – F_2 Accession A x Mundo Novo	67.84g
15 – F_1 Diverse hybrid (a)	64.95h
10 – Accession C (self-pollinated)	61.02i
Means	86.72
Fgenotype	84.19[3]
CV (%)	1.66

[(1)] Sum of percentage of flat green beans retained over 19, 18 and 17 round screen size;
[(2)] Sum of percentage of flat green beans retained over 19, 18, 17, 16 and 15 round screen size and peaberry beans retained over 12, 11 and 10 x ¾ oblong screen size
[3]Indicates significant genotype effects by F test at 1% of probability. Values following by the same small letter in the columns constitute homogeneous group by Scott-Knott test at 5% of probability.

Considering that all coffees were obtained in the same environmental conditions and by the same post harvest processing method, it is supposed that the differences in physical bean characteristics could be attributed to genetic effects. However, it is necessary to consider that according specific literature the better physical green bean quality could not present positive correlation with beverage quality.

Regarding the large green bean percentage it was observed that the genotypes were grouped into 15 distinctive classes, depicting the genotype 2 (F_1 Obatã IAC 1669-20 x Accession A), genotype 4 (F_1 Obatã IAC 1669-20 x Accession C) and genotype 28 (Obatã IAC1669-20) that showed the highest values, 68%, 61% and 57%, respectively.

Regarding commercial coffee beans (CB) it was observed the formation of seven distinctive groups, where the genotype 28 and its related hybrids (genotypes 2, 4, 39, 21 and 23) showed the highest values, up to 93% of commercial beans grade. In general, the F_1 hybrids showed better performance than the F_2 hybrids both for large beans and commercial beans production. There are significant differences for bean size among the evaluated genotypes and the Obatã IAC1669-20 cultivar showed the highest potential for large beans production, both in isolated form or in different hybridization combinations, constituting valorous genetic material to generate new varieties or new hybrids to improve the coffee bean physical quality in Brazil. For better understand complementary studies should be done to confirm these results.

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Effect of Different Shade Regimes on Coffee Quality

C. W. KATHURIMA, E. K. NJOROGE

Coffee Research Foundation. P.O. BOX 4, Ruiru, Kenya (00232)

SUMMARY

Current climate change patterns may cause more extreme and variable weather in the future, threatening agricultural productivity in many areas of the world. Use of shade trees in coffee farming systems may offer an effective coping mechanism to coffee in areas that suffer from climate extremes. Field trials were set up in a low altitude zone. A site was selected with a shade tree (*Ficus* spp.) casting shadow over coffee trees from 0 to 6 hours in a day in the morning and afternoon and some trees in full sun. Green coffee grade proportions, sensory and biochemical components were evaluated for the coffees from the different coffee trees. The coffee trees with grade AB at 80% and above were yielded under shade. Likewise, the trees which had the highest proportion of AA grade were under shade. The proportion of grade C was highest in the trees under full sun. The highest total sensory score which is an indicator of the sensory performance was obtained under full sun. No discernible trend was observed in the levels of biochemical components; sucrose, total chlorogenic acids (CGA), oil and trigonelline under shade or in full sun. However, highest caffeine contents were obtained in coffee under shade and lowest levels in coffee under full sun. The results are discussed in relation to possible impact of shade on coffee quality observing that shade/agro-forestry is one of the upcoming changes of coffee production systems to mitigate the climate change.

INTRODUCTION

Coffee is among the main export crops since its introduction by missionaries in the early 1900s' (Mwangi, 1983) and has remained an important commodity in Kenyan economy. Shade trees in coffee production provide several economic and ecological benefits. Shade trees, especially leguminous species, improve soil fertility, decrease soil erosion and nutrient leaching, reduce excessive solar irradiance and buffer large diurnal variations in air temperature and humidity that are detrimental to coffee physiology (Siles and Vaast, 2002). The crucial consideration for shade in coffee is to identify shade sources with minimal adverse effect on the coffee plant growth, yield and quality but provide all the other beneficial effects (Kimemia, 2004). The current study aimed at investigating the possible impact of shade on coffee grade proportions, cup quality and biochemical composition.

MATERIALS AND METHODS

Site selection

The study was conducted in Azania estate which is in upper midland 3 (UM3) coffee agro-ecological zone at Juja in Kenya. Juja is located at 1° 10' 60S and 37° 7' 0E at an altitude of 1416 meters above sea level. Twenty one trees (21) were selected along the path of the shadow and 8 control trees in full sun.

Processing

Cherry samples were picked separately from each tree and pulped using a disk-pulper during the 2010/11 main coffee season. They were wet processed (pulped, fermented and washed) and dried to final moisture content of 10.5 to 11% using standard recommended procedures (Mburu, 2004). The parchment was finally hulled and graded to seven grades based on size, shape and density. Grade AB was used as a representative grade for cupping and biochemical analysis.

Roasting and sensory evaluation

Roasting of green coffee was done to attain a medium roast using a Probat laboratory roaster within 24 hours of evaluation and coffee allowed to rest for at least eight hours. Samples were weighed out to the predetermined ratio of 8.25g per 150 ml of water. Sensory evaluation procedure described by Lingle (2001) was followed. Fragrance/aroma, flavor, aftertaste, acidity, body, balance and overall were assessed and scored together with three process control parameters (uniformity, clean cup and sweetness) by a panel of seven trained judges on a 10-point scale. All the sensory parameters (including the process control parameters) were added to constitute the total score which is a reflection of the broad quality performance of a particular coffee. This presents the total score as a key characteristic for evaluating the sensory quality performance.

Biochemical analysis

Caffeine, trigonelline and total chlorogenic acids (CGA) were extracted from green coffee powder by refluxing in distilled water. Caffeine, trigonelline and CGA were analysed using HPLC system (KNEUR) equipped with a Supel Co. Discovery column and a diode array detector at three wavelengths, 278nm for caffeine, 266nm for trigonelline and 324nm for CGA. Sucrose was extracted from green coffee powder using the method of Osborne and Voogt (1978) with modifications and analysed using a HPLC system (KNEUR) equipped with a Eurospher 100-5 NH2 column and a refractive index detector. Caffeine, trigonelline CGA and sucrose were identified by comparing the retention times of standards and their concentrations calculated from peak areas using calibration equations. Crude oil was analysed as outlined in the AOAC (1995).

RESULTS AND DISCUSSION

The coffee trees with grade AB at 80% and above were yielded under shade (Figure 1). Likewise the trees which had the highest proportion of AA grade were under shade. The proportion of grade C was highest in the trees under full sun. The trees under six hours of shade showed lower total sensory score than the trees under full sun (Figure 2). No discernable trend was observed in the levels of biochemical components; sucrose, total chlorogenic acids (CGA), oil and trigonelline of the coffee from the trees under shade and those in full sun (Figure 3). However, coffee from tree number 15 and 16 each under 6 hours of shade had the highest caffeine contents 1.44% and 1.38 % (dwb) respectively while tree number 27 under full sun had the lowest amount of caffeine 0.90% (dwb).

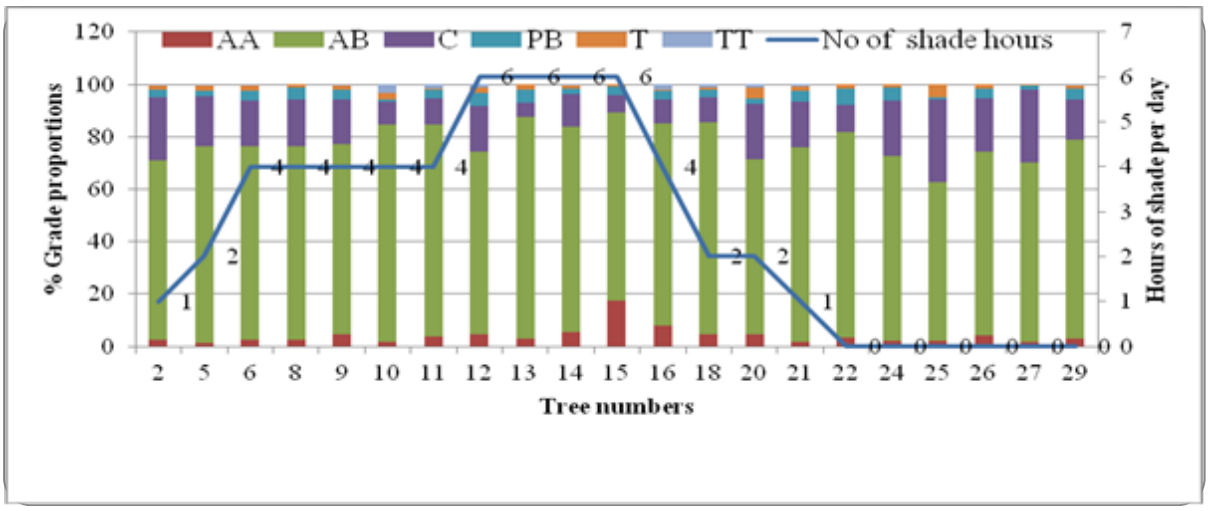

Figure 1. Grades of coffee under different shade regimes and in full sun.

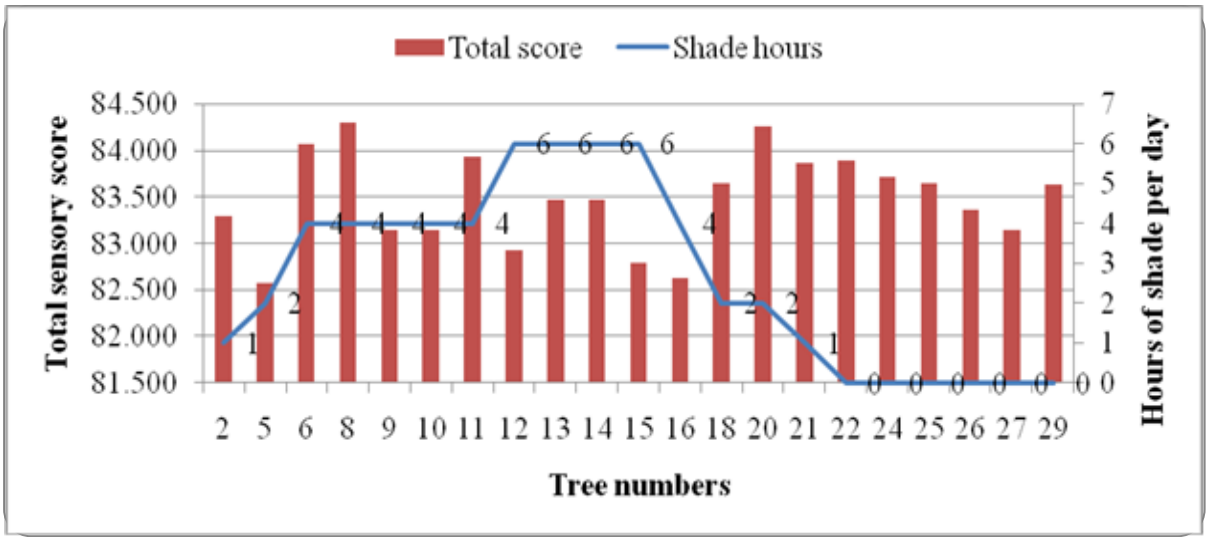

Figure 2. Total sensory score of coffee under different shade regimes and in full sun.

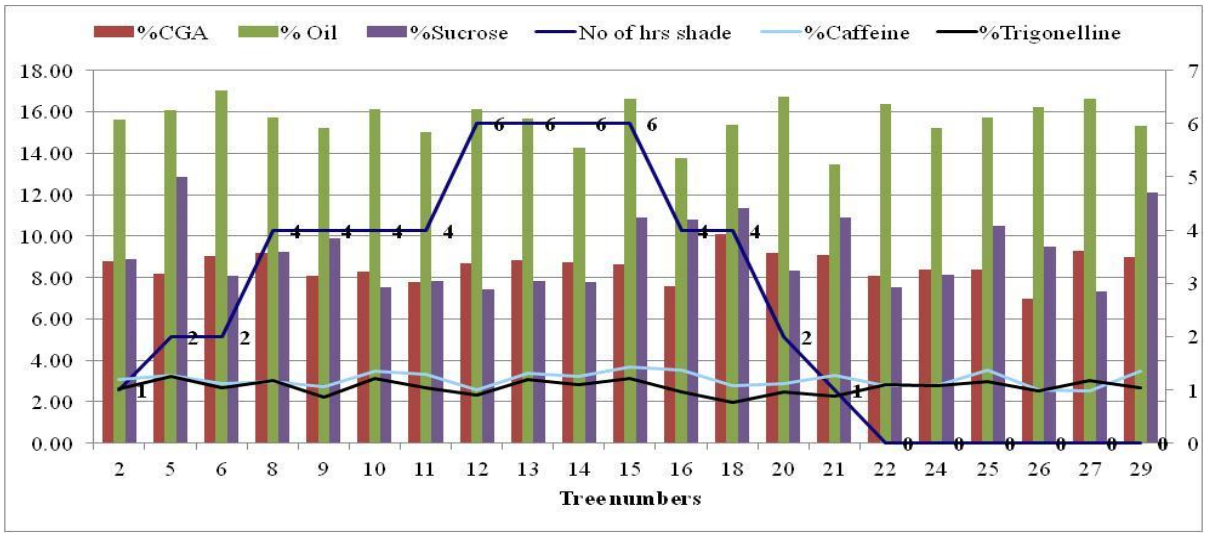

Figure 3. Biochemical components of coffee under different shade regimes and in full sun.

Shade resulted in larger coffee beans as reported in previous studies (Muschler, 2001; Youkhana and Idol, 2010) with differing explanations on the contribution of shade to the observations. As with the effect of shade on production, the influence of shade on organoleptic tributes is also controversial (Muschler, 2001, Vaast *et al*., 2006; Bote and Struik, 2011). In this study no clear gain was observed on the sensory quality parameters due to shade but the contribution of the shade to the increased premium grades (AA and AB) is important since these grades are highly valued in the coffee trade.

ACKNOWLEDGEMENTS

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Adaptability and Stability of Production of Coffee Cultivars in Organic Crops System in Minas Gerais, Brazil

W. M. MOURA[1], P. C. LIMA[1], V. S. LOPES[2], C. F. M. CARVALHO[2], C. A. SILVA[3], C. D. CRUZ[4]

[1]Researchers, Empresa de Pesquisa Agropecuária de Minas Gerais, Vila Gianetti, 46/47, Campus da UFV.Viçosa, MG. CEP 36.570-000, Brazil
[2]Researchers Scholarships, CBP&D-Café: EPAMIG/ UREZM
[3]Agronomist Student, UFV, Bolsista PIBIC FAPEMIG/ EPAMIG/UREZM
[4]Professor, University Federal de Viçosa (UFV), Campus da UFV, Viçosa, MG., Brazil

SUMMARY

The objective of this work was to evaluate the phenotypic adaptability and stability of coffee cultivars (*Coffea arabica*) looking for the identification of the most appropriate cultivars for the organic system in the State of Minas Gerais. The experiments were installed in randomized block design, with 30 genotypes (cultivars and lines) and three repetitions in locations in Minas Gerais (Araponga, Espera Feliz and Tombos) cosidering the crops 2005/2006, 2006/2007, 2007/2008 and 2008/2009 with a total of 12 enviroments. The Eberhart and Russel (1966) methodology was used to analyse the adaptability and stability of production. The cultivar Catucaí Vermelho 36/6 presented adapitability in favorable enviroment and high stability. The line H518 and the cultivar Oeiras MG 6851 were classifiqued as ideal for organic systems, because they presented high yield, adaptability and stability or predictability.

INTRODUCTION

The demand for healthier food produced in sustainable environmental systems has grown substantially in the world creating opportunities for commerce in several regions. In 2009, the coffee was the main culture produced in organic system, representing 22,5% (0.54 million hectares) of the cultivated area with permanent cultures in the world. The largest production areas are in Mexico, Ethiopia and Peru. In Brazil, there is no official data about the area and production of organic coffee but, in the season 2005/2006, the organic coffee production in the country was at least 180 thousand sacks, which represents 0.5% of total national production.

The organic coffee production, in Brazil, is ruled by Ministry of Agriculture and the use of high solubility manure and chemical products is forbidden. It is fundamental the improvement of researches looking for the development of appropriated technologies to this cropping system.

The demands for researches in this subject include the necessity of recommendation of appropriate cultivars. Thus, the objective of this work was to evaluate the phenotypic adaptability and stability of coffee cultivars (*Coffea arabica*) looking for the identification of the most appropriate cultivars for the organic system in the State of Minas Gerais.

MATERIALS AND METHODS

The experiments were installed in randomized block design, with 30 genotypes (cultivars and lines) and three repetitions. Each plot consisted of ten plants, in which,the spacing between plants and rows were 0.5 x 4.0 m for low-plant cultivars and 0.8 x 4.0 m for high-plants cultivars. The yield was evaluated (bags of green coffee.ha^{-1}) in four crops 2005/2006, 2006/2007, 2007/2008 and 2008/2009 in three locations in Minas Gerais (Araponga, Espera Feliz and Tombos) with a total of 12 environments. These locations were chosen because have different altitudes and soil and climatic conditions, besides the family tradition in coffee and organic system. A joint variance analysis involving the triple genotypes interaction x localities x years was done. The means were compared by test Scott-Knott. To estimate the parameters of adaptability and stability used the method of Eberhart and Russell (1966). In this method was considered general adaptability genotypes that present $\beta_{1i} = 1$; adaptability to environments favorable, those with $\beta_{1i} > 1$, and adaptability to environments unfavorable, those with $\beta_{1i} < 1$. The stability was related with the predictability of behavior given by the variance component deviations from the regression, $\sigma^2_{di} = 0$, being stable (predictableand) and when $\sigma^2_{di} \neq 0$, unstable (unpredictable). The statistical analyzes were realized used the computational program GENES developed in Federal University of Viçosa.

RESULTS AND DISCUSSION

The average productivity was approximately 28 bags of coffee / ha / year (Table 1), exceeding the yield observed in organic systems shaded and unshaded in the city of Valença - RJ. However, were similar to those found in the southern region of Minas in Poço Fundo and Machado. There is great variability among genotypes, and cultivars Sabia 708, H518, Catucaí Amarelo 24/137, IBC Palma 1, Catucaí Vermelho 36/6, Paraíso MG H419-1, Oeiras MG 6851, H514, Siriema 842 and Catuaí Vermelho IAC 15, had the highest yield. With the exception of the last cultivate other have genetic resistance to rust. The cultivars Icatu Vermelho IAC 4015, Mundo Novo IAC 379-19, Acaiá Cerrado MG 1474, Caturra Vermelho IAC 477 and Maragogipe were the least productive.

The Catuaí genotypes that are widely cultivated in the country due to high adaptability to different coffee regions, the cultivar Catuaí Vermelho IAC 15 showed adaptability ($\beta 1i = 1$), while the Catuai Amarelo IAC 62 cultivar, showed specific adaptability to unfavorable environments ($\beta 1i < 1$), however both cultivars were unstable ($\sigma 2di > 0$). Opposite results were observed for these cultivars showing high yield stability and adaptability to favorable only when evaluated in conventional systems in the southern region Minas Gerais. These differences are expected because of the diversity of environments and cropping systems used.

Among the ten most productive genotypes (Table 1), cultivars Catucaí Amarelo 24/137, IBC Palma 1, Paraíso MG H 419-1 and Catucaí Vermelho 36/6 showed specific adaptation to favorable environments ($\beta 1i > 1$), however only last cultivar showed high stability ($\sigma 2di = 0$). The cultivars Sabiá 708, Catuaí Vermelho IAC 15, Siriema 842 and lines H514 presented high adaptability ($\beta 1i = 1$), but low stability ($\sigma 2di > 0$). The line H518 and cultivar Oeiras MG 6851 were classified as ideal for organic systems, because they presented high yield and high adaptability ($\beta 1i = 1$) and high stability or predictability ($\sigma 2di = 0$).

Table 1. Estimate the parameters of the adaptability and phenotypic stability of the production of coffee cultivars using the method of Eberhart and Russell (1966), to productivity of coffee, in bags of 60 kg/ha/year.

Cultivars	Means ($ß_0$) [1/]	$ß_{1i}$ [2/]	σ^2_{di} [3/]	R^2(%)
Sabiá 708	42,64 A	1,18ns	78,12^B	63.69
H518	41,79 A	1,09ns	18,33ns	76.73
Catucaí Amarelo 24/137	40,89 A	1,52^B	49,28^B	79.73
IBC Palma 1	40,75 A	1,47^B	36,39^A	81.34
Catucaí Vermelho 36/6	38,47 A	1,83^B	-3,87ns	94.35
Paraiso MG H 419-1	37,34 A	1,46^B	33,86^A	81.84
Catuaí Vermelho IAC 15	34,41 B	1,21ns	67,64^B	67.05
Oeiras MG 6851	33,32 B	1,13ns	-18,75ns	93.31
H 514	32,39 B	1,19ns	61,86^B	67.55
Siriema 842	31,34 B	0,79ns	30,49^A	58.37
IBC Palma 2	30,25 C	0,53^B	08,92ns	48.89
Ouro Verde	29,87 C	0,65^A	128,62^B	26.48
Tupi IAC 1669-33	28,71 C	0,92ns	72,30^B	52.91
Obatã IAC 1669-20	27,90 C	1,19ns	50,63^B	70.49
Catucaí 785/15	27,08 C	0,96ns	05,18ns	77.46
Rubi MG 1192	27,04 C	1,02ns	02,40ns	80.80
Canário	26,19 C	0,55^B	11,22ns	49.01
Icatu Amarelo IAC 2944	26,13 C	0,88ns	37,04^A	61.00
Catuaí Amarelo IAC 62	26,04 C	0,39^B	59,00^B	18.99
Icatu Precoce IAC 3282	25,07 C	1,28ns	34,51^A	77.46
Catucaí Açu	24,89 C	1,12ns	146,04^B	49.13
Topázio MG 1190	24,85 C	0,96ns	67,12^B	56.29
Caturra Amarelo IAC 476	22,96 C	1,04ns	16,57ns	75.65
IAPAR 59	22,93 C	1,17ns	-2,91ns	86.89
Acauã	22,86 C	0,64^A	11,63ns	56.53
Icatu Vermelho IAC 4045	20,13 D	1,02ns	-19,09ns	92.12
Mundo Novo IAC 379-19	19,40 D	0,62^A	35,63^A	44.43
Caturra Vermelho IAC 477	16,80 D	0,82ns	23,20ns	63.18
Acaiá Cerrado MG 1474	16,66 D	0,74ns	72,79^B	42.26
Maragogipe	10,79 E	0,64^A	59,43^B	38.06
Média Geral	28,33			

[1/] Means with the same letters in the column are not statistically different by the test Scott-Knott at the level of 5% probability.
[2/] $H_o = ß_{1i} = 1$ and [3/] $H_o = \sigma^2_{di} = 0$: [ns] Not significant, [A] and [B] significant at 5 and 1% probability, respectively by Student´s t test.

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Performance of Tanzania Compact Hybrid Coffee Varieties Derived from Hybrid Seeds

D. L. MTENGA, D. L. KILAMBO, R. NGOMUO, J. M. TERI

Tanzania Coffee Research Institute (TaCRI), P.O. Box 3004 Moshi, Tanzania

SUMMARY

The main limitation for the accelerated multiplication and distribution of the new improved high yielding coffee hybrid varieties developed by the Tanzania Coffee Research Institute (TaCRI) that combine resistance to coffee berry disease (CBD) and coffee leaf rust (CLR) with good beverage quality is the slow multiplication rate that relies on vegetative multiplication using orthotropic shoot cuttings to produce clonal seedlings or top-worked (grafted) on rootstocks of the old varieties. TaCRI initiated a complimentary programme to produce hybrid seeds of the compact hybrid varieties in 2008 to accelerate the availability of the new varieties for the national coffee replanting programme. The production of the hybrid seeds is achieved by artificial manual pollination of preselected parent trees from the genotypes which meet all the pre-set selection criteria. On-farm trials (OFTs) established from these hybrid seeds are in their second year of production. We report here the performance of these materials in the OFTs.

INTRODUCTION

Coffee is one of Tanzania's primary agricultural export crop accounting for 24 % of traditional cash crops export earnings. Coffee earns the country an average of USD 100 million per annum (TCB and TaCRI 2010). The industry provides direct income to more than 400 000 farmer families and also benefits indirectly the livelihoods of over 2.5 million Tanzanians. The industry is faced with several production constraints. The most serious constraints include management of Coffee Berry disease (CBD) and Coffee Leaf Rust (CLR) disease in Arabica coffee, stagnated production, low productivity and poor crop husbandry. Tanzania Coffee Research Institute (TaCRI) had developed tall arabica hybrids which are resistant to both of the diseases with higher yields and good quality similar to the traditional varieties. These varieties are currently in farmer fields. Considering need for crop intensification and shrinking of suitable land for cultivation, the Institute recently developed compact hybrid varieties which are perfect for high density planting. They can also be distributed through hybrid seeds as a complimentary strategy to clones (Teri et al., 2011). The compact hybrids are the F1 between selected Catimor progenies and synthetic population of early generation selection of tall hybrids from backcross programme. These are resistant to both (CBD) and (CLR), high yielding and have excellent cup quality equal to the traditional Tanzanian varieties of N39 and KP423. Compact coffee hybrid seed is produced from selected Catimor progenies which are used as the mother trees on which seed is produced while the tall hybrids are used as pollen donors. The hybridization procedure is done manually by hand (van der Vossen 1985).

Planting of improved disease resistant compact coffee cultivars has several advantages when compared with traditional tall varieties. These cultivars have a more compact canopy and shorter branches, allowing higher planting densities under their own dense shade leading to increased production per unit area. The results of several studies revealed consistently

increased yield level with increasing population densities from 4,000 to 6,000 tree ha^{-1} (Yacob et al., 1996). Studies carried out in Ethiopia showed consistent and maximum yield at population density ranging between 7,062 and 10,000 trees ha^{-1}(Kufa et al., 2001). TaCRI's compact coffee varieties are now at on farm evaluation in farmers fields as a final stage for official release. The performance of these compact hybrids for the first two year is reported in this work.

MATERIALS AND METHODS

Sites for on farm evaluation were selected from all arabica coffee growing zones across the country. Materials used were the compact coffee hybrid lines which gave the best results on yield, diseases resistance and cup quality in the on station trials. The seedlings were raised from the hybrid seed obtained from the Institutes seed garden and they were managed at the nursery till when they were ready for field planting. At least three to four hybrids were provided to the selected farmers. These trials followed a completely randomized design taking into consideration the size and shape of the available land. The number of trees per hybrid was variable depending on the available seedlings at the time of planting but not less than five. Seedlings were planted by individuals of the selected site as recommended in coffee production. Data was recorded on establishment, stem girth, number of bearing primaries, clusters per primary and cluster size for the first and second year after planting. These characters were recorded on individual trees basis for the five trees per hybrid per site.

Table 1. List of Compact hybrid lines under on-farm evaluation.

Selection	Crosses
1	CVT_4
2	CVT_5
3	CVT_6
4	CVT_7
5	CVT_8
6	CVT_9
7	CVT_{10}
8	CVT_{11}
9	CVT_{12}

RESULTS AND DISCUSSION

Observation from the two zones on the performance of the nine compact hybrids derived from hybrid seeds shows almost similar performance. Presented data was obtained from two year old plants in the first crop year. Generally performance in the Northern zone is relatively high in almost all characters observed. This observation may be due to differences in management habits; while in the North they prefer intercropping with banana in the Southern zone they practice mono cropping. Findings from other studies (van Asten et al; 2011) showed that coffee banana intercrop improves coffee performance if planted at a recommended plant population of both crops.

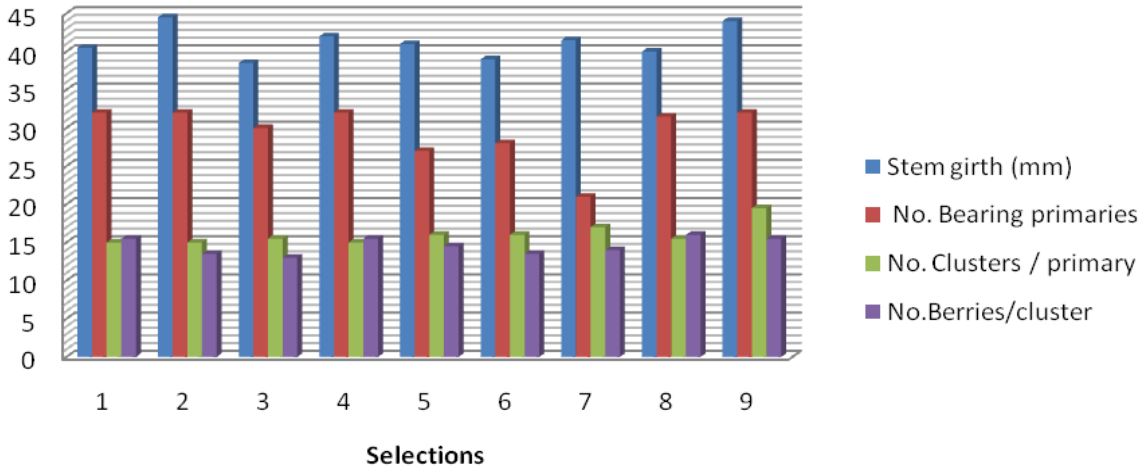

Figure 1. Northern zone sites growth and yield characters means

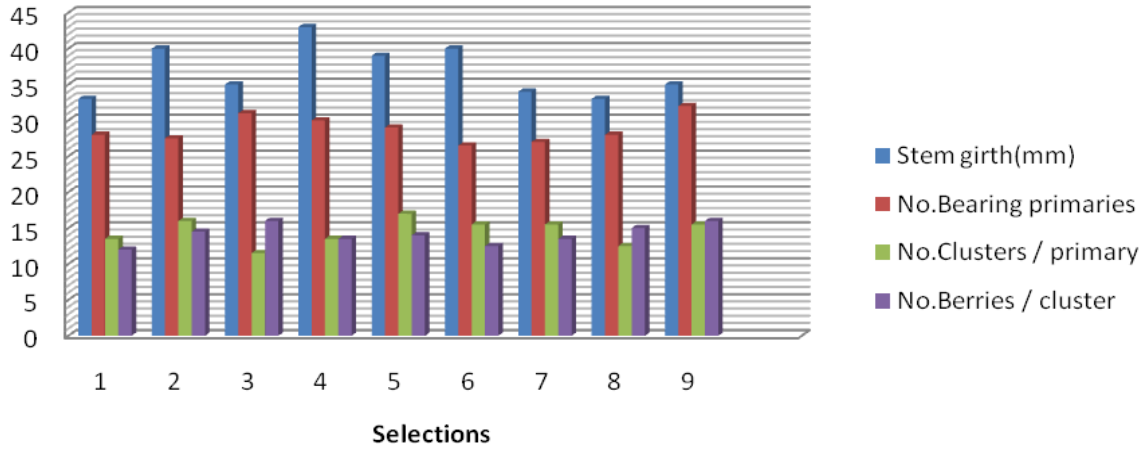

Figure 2. Southern zone sites growth and yield characters means

CONCLUSIONS

Performance of the compact hybrid varieties established from hybrid seeds is impressive. This confirms that production of hybrid seeds is a viable alternative to the much slower vegetative multiplication. We have experimented with clonal multiplication, grafting, somatic embryogenesis and hybrid seeds production; hybrid seeds could be the solution of the slow vegetative multiplication. We are collecting more data from these trials to identify the best performers and their management options in order to develop and recommend good agricultural practices (GAPs) for the compact varieties.

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Organic Coffee Production Model for Analysis of the Economic and Energetic Efficiency in the South Region of Minas Gerais State

P. H. N. TURCO[1], M. S. T. ESPERANCINI[2], O. C. BUENO[2], F. M. M. BLISKA[3], J. S. CAIADO[3]

[1]Secretaria de Agricultura e Abastecimento do Estado de São PAULO - APTA Regional
[2]UNESP – FCA – BOTUCATU
[3]Instituto Agronômico de Campinas

SUMMARY

The results indicate that also the revenues occur every year. Applying the equation estimates the economic efficiency we obtained the result of 1.21 indicating that the gross revenue exceeds operating costs by 21%, indicating an economically efficient system. In energy analysis, the results showed that the systems have positive energy balances.

INTRODUCTION

This paper aims to evaluate the economic efficiency of the organic coffee production system, through the total operational cost and gross income throughout the crop's lifespan. The energy consumption profile and energy efficiency will also be evaluated in this study. The energy analysis, as well as the economic analysis, contributes to the understanding of the production dynamics, particularly to the system's independency towards industrialized inputs, which provides an understanding of the production's degree of sustainability. With that we can evaluate the organic coffee production system's aim to reduce the environmental impact trough recycling practices of nutrients and organic matter.

MATERIALS AND METHODS

The data for the elaboration of the organic coffee's technical itinerary were collected among producers of the south region of the state of Minas Gerais, Brazil. This region was chosen for being one the most important organic coffee production areas in the country. Six organic coffee producers were interviewed. The criteria for the choice of farmers were: a) most of the production's income came from organic coffee; b) family agricrop; c) systematic data of the production that allowed the reconstruction of the technical itinerary.

Economic Efficiency

The economic efficiency is calculated trough the economic input and output, as shown in the formula:

$$Ee = \frac{GAR}{EAC} \times 100$$

- *Ee* = Economic Efficiency.
- GAR = Gross Annual Revenue.
- EAC = Equivalent Annual Cost.

The efficiency index is a non-dimensional number and it is possible to evaluate directly if a system is efficient by observing if its value is lesser or equal to 100.

The Equivalent Gross Annual Income (GAI) is obtained by Gross Annual Revenue (GAR), and it is updated to the year 0 from a predefined discount rate applied to the 20-year project life.

$$EGAR = \sum_{t=0}^{n} (GR \times (1+r)^{-t}) \times \left(\frac{(1+r)^t \times r}{(1+r)^t - 1} \right)$$

- EGAR = Equivalent Gross Annual Revenue (R$/ha).
- GR = Price (R$/bag) x Q (Productivity bag/ha).
- t = time that the crop usually lasts (in years).
- r = discount rate (per year, value).

Similarly, the costs were updated to the year 0 and were equally distributed to the 20 years that crop usually lasts:

$$EAC = \sum_{t=0}^{n} (OC \times (1+r)^{-t}) \times \left(\frac{(1+r)^t \times r}{(1+r)^t - 1} \right)$$

- EAC = Equivalent Annual Cost.
- OC = Operating Cost (or Total operating Cost).
- t = crop's lifespan (in years).
- r = discount rate (per year, value).

The costs were estimated for three different stages of the crop – implantation, conduction and production – based on the data provided by the selected farmers for the analyzed time period. These costs differ according to the stage of the crop. To calculate such costs estimation were made on the outlays with labor, seedlings, organic inputs, fuels and lubricants, machines and implements and micronutrients.

Energy Efficiency

The energy indexes used to evaluate the efficiency on coffee crop were as follows:

- Cultural efficiency = useful outputs / cultural inputs.
- Cultural productivity = product quantity / cultural inputs.

Product quantity = quantity of coffee grains produced per crop:

- Energy efficiency = Σ total energy / Σ non-renewable energy input, following Risoud's (1999) method.

The energy outlay was quantify based on the physical requirements of labor, fuels, lubricant oils, grease, organic input, soil correctives, seedlings, micronutrients, energy depreciation and the conversion into energetic units. The energy output was determined based on the production of grain coffee (green) and its conversion into energetic units. The energy outlays were grouped according to their origins as follows:

- Direct energy from biological origin: labor, seedlings, organic inputs and correctives.
- Direct energy from fossil origin: diesel, lubricant oil and grease.
- Indirect energy from industrial origin: machinery depreciation and implements and micronutrients.

When analyzing the labor force, the calculation of the energy invested by workers in the different operations of the technical itinerary followed the method proposed by Carvalho et al (1974), described by Bueno (2002) and used by Romero (2005). To estimate the energy content of the coffee seedlings we measured the gross calorific value (GCV) with a calorimetric pump, as per the ABNT-NBR/8.69 rule issued in 1997. Two seedlings of each variety (Mundo Novo and Catuaí Vermelho) were used for the measurements. After the separation of leafs, stem and root of the seedlings, each part was weighted separately and taken to the drying oven. Once dry they were grinded and prepared to be burned in the calorimetric pump. Afterwards we added the weights of all the plant parts.

The organic inputs and soil correctives conversions into energetic units were based on data available in the specialized literature. The same was done for the energy outlays of the direct energy from fossil origin, such as fuels, lubricant oils and grease. In the mechanized operations, the equation and the caloric coefficient used in the machine's and implement's energy depreciation were the same used by Comitre (1993), Bueno (2002) e Romero (2005). The micronutrients used in the organic coffee crop were potassium sulfate, copper hydroxide, organomineral fertilizers and magnesium sulfate. Because the specific values for these elements were not found in the literature, the copper sulfate caloric value (400 $kcal.kg^{-1}$ or 1,67 $MJ.kg^{-1}$) was used to all (FERRARO JUNIOR., 1999). The energy outlays were estimated for the crop's lifespan of 20 years and the production years were divided according to the coffee crop's biannual behavior (variation in the production levels between crops). To obtain the energy output coffee grain samples were dried in the drying oven. Afterwards the grains were grinned and prepared for burning in the calorimetric pump.

RESULTS AND DISCUSSION

The gross income data were obtained based on a productivity of 20 bags per hectare in 2009 prices. In the years of greater production the Gross Income was obtained by using an average production of 30.2 bags/hectare in 2009 prices. The costs for the crop's years of implantation, conduction and production were estimated. A yearly discount rate of 6%, equal to the 2010 savings account's return, was applied to the Gross Annual Income, as well as to the Annual Operation Costs. This rate was used because it is similar to the opportunity cost of the crop's immobilized capital.

The results show us that the gross revenue deducted, ie, the amount of gross revenue resulting from the organic coffee production, over 20 years, and updated to the present period, is equal toR$ 98,539.08. ha-1. Similarly, the updated operating costs represent the total of R$ 81,682.00. ha-1 per year. These results were applied to the discount factor, to obtain the Equivalent Annual Gross Revenue (EAGR) and Equivalent Annual Cost (EAC). The results for the EAGR and EAC were equal to R$ 4,926.95 ha-1 and R $ 4,084.09 ha-1, respectively. The calculation of economic efficiency indicates that the gross revenue exceeds operating costs by 21% pointing, therefore, to an economically efficient system.

The organic coffee production system's energy output was 27.732,00 $MJ.ha^{-1}$ in the years of smaller productivity and 41.875,32 $MJ.ha^{-1}$ in the greater production years. The coffee is sold in bags of 60 kg and it has an energy value of 1.386,60 MJ per bag. In the crop's lifespan the energy outputs were 626.465,88 $MJ.ha^{-1}$.

Figure 1 shows the types of energy utilized in the organic coffee growth, as well as its origins. The results show that the direct energy in much greater in energy stage of the crop, but in the harvest the direct energy from fossil origin becomes greater due to the consumption of fuels, both in the greater and lesser productivity years. The direct energy from biological origin in the form of labor is higher in every year. The figure also shows that in the crop's lifespan the energy outputs were approximately 6 times greater than the inputs meaning that for every energy unit introduced into the crop 6 units were produced for consumption.

As for the Energy Efficiency, the value was 7.4 units of non-renewable energy were applied to the organic coffee production system. The crop's total energy production was 5.54 kg/MJ. The analysis of the organic coffee showed the system's efficiency, both economic and energetic.

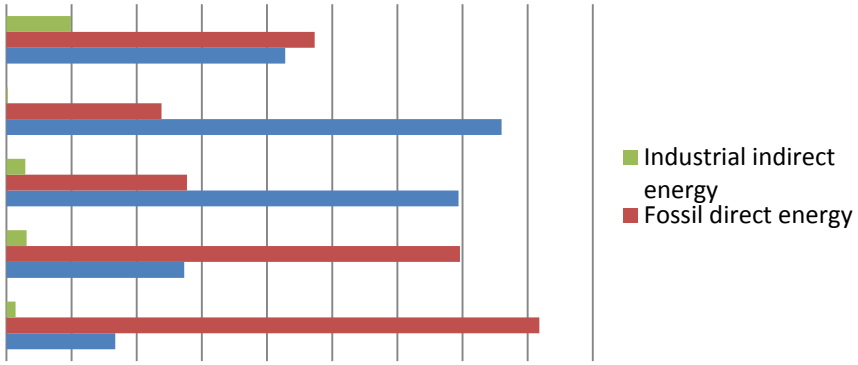

Figure 1. Energy participation (percentage) divided by origin.

The Gross Income is higher than the total production cost which means that the crop provides a positive net income. In this case study the farmers obtained an income 21% superior to the operational costs invested in the crop. As for the production costs, labor is responsible for the biggest part of those expenses.

In the 20 years of crop that were analyzed the energy production was 637.697 MJ per hectare. The total of utilized energy was 112.998 MJ per hectare, leading to the conclusion that the production is energetically efficient. The energy efficiency value was 7.4 units of non-renewable energy applied to the organic coffee production system. The cultural productivity index shows that one MJ inserted in the production resulted in 5.54 kg of processed coffee. The energy efficiency system resulted in greater expense of direct energy from biological origin in the implantation and conduction fazes. In the production fazes the greater energy source is the direct energy from fossil origin in both the larger and smaller productivity years.

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Arabica Selections with *Coffea eugenioides* and *C. canephora* Introgressions for Rondônia State in Brazilian Amazon[A]

H. P. MEDINA-FILHO[1,4], R. BORDIGNON[1], F. F. SOUZA[2], A. L. TEIXEIRA[3], J. M. DIOCLECIANO[3], G. O. FERRO[2]

[1]Centro de Café – Instituto Agronômico de Campinas, Avenida Barão de Itapura, 1481, 13020-902, Campinas, SP, Brazil
[2]Embrapa Semiárido, BR 428, Km 152, CP 23, 56302-970, Petrolina, PE, Brazil
[3]Embrapa Rondônia, BR 364, Km 5,5, CP 127, 76815-800, Porto Velho, RO, Brazil
[4]BCNPq fellowship

SUMMARY

Among 126 Obatã backcross hybrids (BC_2) with *C. arabica* x (*C. eugenioides* 4n x *C. arabica*) studied several years in Campinas, 24 were additionally selected for the eugenioides very late ripening, desirable by a cultivar for the tropical conditions of Rondônia State in the Amazon region of Brazil as a strategy to escape from rainy season during harvest. There, robustas thrive and arabicas are no longer grown due to a history of poor crops of low quality beans. Three F_3 lines of Catuaí x Glaucia were also selected for lateness. In addition, 11 F_2 Arabusta populations were also studied, been one of *C. arabica* cv. Ibairi x *C. canephora* 4n, one of its reciprocal cross and 9 of Obatã x *C. canephora* 4n. Ripening check cultivars were represented by two very late Obatã lines, late Catuaí Vermelho and Catuaí Amarelo, medium two Paraíso lines and Topázio and by early Oeiras and Bourbon Amarelo. Plots were set up in Ouro Preto do Oeste (Lat 10°45'S, Long 62°15'W, Alt 300m, Temp 25°C, Rainfall 2000mm, Aw Köppen). Vigor and general plant characteristics were observed during growth seasons, yields recorded for three years and cup quality evaluated in the second crop. Greater variability was observed among lines and much less among individuals within them, except Arabustas. Control cultivars showed all medium to early ripening and except one Obatã confirmed the known poor performance, yielding 10 to 24 clean 60 kg bags/ha, global quality 1 to 3 (out of 5), SCAA scores 40 to 62. Obatã lines yielded 25-32 bags/ha, global quality 2-3, SCAA scores 56-58. F_2 Arabustas displayed scanty berry set and no one was selected. F4 Catuaí x Glaucia lines did not come up late, yielded or cup tested well. The 24 F_2BC_2 Obatã x *C. eugenioides* backcrosses were the most promising. Many lines were remarkably late, several with impressive yields up to 45 bags/ha, global quality up to 4 and SCAA scores up to 80. Ninety-seven selected F_3BC_2 were advanced and the two profuse crops of this generation displayed 28 progenies ripening of 3-4 weeks later, attesting the adequacy of germplasm choice and selection procedures. Late and very late F_4BC_2 selections will be trued up also in neighboring Acre State.

INTRODUCTION

With 2 million bags per year, Rondônia State in the Amazon region ranks fifth in coffee production in Brazil. Cultivation is almost exclusive of *Coffea canephora* comprised approximately by 90% of Conilon and 10% of Robusta types. Frequently a mixture of both is cultivated with reduced technologies by small farmers. The crop is exported to Southern States from where arabica coffee is imported for local consumption in blends.

[A] Sponsored by Consórcio Pesquisa Café

Coffee was introduced in the State 40 years ago with Brazil's leading *C. arabica* Mundo Novo and Catuaí cultivars, nowadays replaced by *C. canephora*, due to a continuous history of low yields and poor quality. Coffee regions are located in tropical conditions, 9-13°S latitudes, altitudes <300m, precipitations >1500mm, temperatures >24°C, technically recognized apt only for the cultivation of robustas. Arabicas require milder, subtropical climates.

Besides of poor agronomic performance, even the latest arabica cultivars ripe too early, in February-March. Rain is still frequent, turning harvesting and drying difficult operations, yielding low quality grains and beverage. Thus, ideal arabicas should be very late and adapted to tropical conditions, attributes not found in current cultivars.

In the breeding program of IAC, the following germplasm deserved attention by their specific attributes or origins and were selected as the diversity putatively suitable to be studied in the aforementioned conditions of Rondônia.

At high latitudes of Southern States, *C. arabica* cv. Obatã has performed also very well in hot climates as far as irrigation is provided. Obatã is a rust resistant cultivar developed at IAC from a cross of *C. arabica* cv. Vila Sarchi and Timor Hybrid the later a natural cross derivative of *C. arabica* x *C. canephora*. Besides the rust resistance, *C. canephora* conceivably contributed to the lateness and high temperature adaptation genes of Obatã, since those characteristics are not observed in Vila Sarchi cultivar but are common to *C. canephora*. Campinas germplasm evaluations have revealed also that *C. arabica* Ethiopian accession Glaucia ripens later than Catuaí, a widely planted late cultivar. The same was true for several Arabustas, hybrids of *C. arabica* x *C. canephora* 4n that in Campinas ripens as late as the robustas and have cup quality superior to them. Hopefully genetic segregation of F_2 would provide genotypes with improved outturn, cup quality closer to arabicas, better adaptation to tropical conditions and the necessary easy vegetative propagation capacity of robustas.

As to cup quality, cv. Ibairi of *C. arabica* has shown superior beverage. Likewise, ongoing program exploits *C. eugenioides* as source of quality in introgressed *C. arabica* lines. This diploid species is very late in Campinas conditions and is regarded as the female genitor of *C. arabica* and the source of good cup quality genes provided *C. canephora* is the male genitor. Among Campinas high yielding hybrids and breeding lines with good cup quality, special selections were made for lateness. Field evaluations, local selections and ensuing progeny tests were effected in Rondônia.

Present investigation reports the results of this joint effort of Instituto Agronômico de Campinas and Embrapa Rondônia aiming at the development of arabica cultivars adapted to Rondônia and similar tropical states.

MATERIALS AND METHODS

Among 126 Obatã backcross hybrids (BC_2) with *C. arabica* x (*C. eugenioides* 4n x *C. arabica*) studied several years in Campinas as to stature, vigor, yield, maturation, bean characteristics and beverage profile, 24 were additionally selected for the *C. eugenioides* very late ripening. Three F_3 lines of Catuaí x Glaucia were also further selected for lateness. Ripening check cultivars were represented as follow: two very late Obatã IAC 1669-20 lines, one late Catuaí Vermelho IAC 15, one late Catuaí Amarelo IAC 62; two medium Paraíso lines, H419-10-6-2-10 and H419-10-6-2-3-27, one medium Topázio MG 1190; one early Oeiras MG 6851 and one early Bourbon Amarelo IAC J10. All are commercially grown in

Southern Brazil. This 36 items plot was set up in three replicated blocks, 10 plants per treatment and is referred as Plot IA. Plot IB comprised 11 F_2 Arabusta populations represented by over 30 plants of each cross. One was of *C. arabica* cv. Ibairi x *C. canephora* 4n, one of its reciprocal cross and 9 of Obatã x *C. canephora* 4n. Vigor and general plant characteristics were observed during growth seasons and yields were recorded for three years. Cup quality was evaluated in Campinas in the second crop samples.

Phenotypic plant selections were made on March 2008 originating F_3BC_2 evaluated in Augmented Block Design referred to as Plot II. In 2012, phenotypic selections were effected giving rise F_4BC_2. Plots I and II were set up in Ouro Preto do Oeste (Lat 10°45'S, Long 62°15'W, Alt 300m, Temp 25°C, Rainfall 2000mm, Aw Köppen), at 3 x 1m spacing.

RESULTS AND DISCUSSION

With reference to Plot IA, as expected, greater variability was observed among such genetically diverse lines assayed and much less among individuals within them. Decreased within lines variability was also expected on the basis of careful selection among progenies and their elite individuals selected in previous BC_2 Obatã backcross generation. Control cultivars showed all medium to early ripening and except one Obatã confirmed the known poor performance, yielding the equivalent of 10 to 24 clean 60 kg bags/ha, global quality 1 to 3 (out of 5), SCAA scores 40 to 62. Obatã lines yielded 25-32 bags/ha, global quality 2-3, SCAA scores 56-58. F4 Catuaí x Glaucia lines did not come up late, yielded or cup tested well, with 15 to 26 bags/ha, global cup scores 2, SCAA 52-56. Nevertheless, seven uniform ripening plants were selected for progeny tests. The 24 F_2BC_2 Obatã x *C. eugenioides* backcrosses were the foremost promising. Most lines were remarkably late, several with impressive yields up to 45 bags/ha, global quality up to 4 and SCAA scores up to 80. Ninety-seven F_3BC_2 were phenotypically selected to be progeny tested. These 97 selected lines in Rondônia traced back to 20 late Obatã BC_2 selected in Campinas.

As to Plot IB the observed results were not encouraging. Although late, F_2 Arabustas displayed scanty berry set despite the fact that most plants had quite luxuriant foliage, lavish blooming and pollinators were abundant. No one was selected for further investigations on grain characteristics and rooting ability. Such behavior was somewhat unexpected by the results of sister F_2 populations grown in Campinas, where some late segregants have shown good yields, improved outturn compared to F_1 and cup quality inferior to arabica but conspicuously better than robustas. This investigation attempts robusta type plants with improved beverage and certainly would not be truly comparable to arabicas.

Regarding to Plot II (Figure 1) that corresponds to the ensuing generation it comprised 120 items, being 16 controls, 7 F_5 Catuaí x Glaucia and 97 F_3BC_2 Obatã x *C. eugenioides* backcrosses. Among them, 69 were rated as medium-early maturation at the second crop year, and 35 as late, ripening 3-4 weeks after other germplasm and control ripening cvs. In 2012 grain characteristics of second year crop were quite acceptable, average screen 16.4 with over 70% screen 16 and above, with peaberries less than 16%. Results of cup analysis of dry processed samples varied from hard to soft but it can be surely improved by ameliorating processing.

Among the 35 late progenies, 28 were elected from which 98 single plant selections constitute the advanced F_6 and F_4BC_2 generations to be evaluated in Rondônia and neighboring Acre State. Considering the general uniformity within lines, the grain analysis and the two profuse crops of Plot II it is anticipated that selections in F_6 and F_4BC_2 are at final stage. The results

attest the germplasm choice and selection procedures. Hopefully they represent the forerunners of arabica return to Rondônia.

Figure 1. Above: F_3BC_2 selected line of *C. arabica* Obatã x *C. eugenioides* grown in Rondônia State. Below: Early and late ripening plants.

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Pursuing Green Coffee Geographic Origin Discrimination through Relations between Isotopes and Environmental Factors (Isogeocoffee Project)

C. RODRIGUES[1], R. MAIA[1], M. PIMPÃO[1], M. BRUNNER[2], G. BOWEN[3], P. HILDEBRANDT[4], J. COCHICHO-RAMALHO[5], L. D. GAUTZ[6], T. PROHASKA[2], C. MÁGUAS[1]

[1]University of Lisbon, Faculty of Sciences, Center for Environmental Biology, Stable Isotopes and Instrumental Analysis Facility (SIIAF), Ed. C2, Room 2.1.16, Campo Grande, 1749-016 Lisbon, Portugal
[2]University of Natural Resources and Life Sciences (BOKU-UFT) Department of Chemistry Research group of Analytical Chemistry - VIRIS Laboratory, Konrad Lorenz Strasse 24, A-3430 Tulln, Austria
[3]University of Purdue, Department of Earth and Atmospheric Sciences, Purdue Climate Change Research Center, 550 Stadium Mall Dr., Purdue University, West Lafayette, IN 47907, USA
[4]Technische Universität Berlin, Institut für Chemie, Sekr. PC14, Straße des 17. Juni 135, D-10623 Berlin, Germany
[5]Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal, Instituto de Investigação Científica Tropical (Eco-Bio/IICT), Quinta do Marquês, Av. da República, 2784-505 Oeiras, Portugal
[6]Department of Molecular Biosciences and Biosystems Engineering, 1955 EastWest Rd, Honolulu, HI 96822, USA

SUMMARY

In the present work, several spectroscopic techniques were applied for the discrimination of different types of coffee and of their geographical origin. In a first approach, a study on the application of Raman spectroscopy to the differentiation of coffee type (*Arabica versus Robusta*) was developed, based on the determination of kahweol content, wherein the results obtained allowed their discrimination. Then, isotope ratio mass spectrometry (IRMS) was applied for the determination of the isotopic composition of carbon (C), nitrogen (N) and oxygen (O) of the green coffee bean, allowing coffee differentiation at continental level. It was also shown that O was the fundamental element to achieve this differentiation, reflecting the hydrology of the coffee-producing regions. Subsequently, IRMS was combined with inductively coupled plasma mass spectrometry (ICP-MS), to determine the isotopic composition of strontium (Sr) in the coffee bean, in particular the ratio of the isotopes 87 and 86 ($^{87}Sr/^{86}Sr$). The results obtained demonstrated that the isotope ratios of Sr and O were promising for coffee authenticity, as these elements reflect the local geology and hydrology. However, in order to expand the understanding of how environmental factors determine the isotopic composition of the different elements on the green coffee bean, it was necessary to study a model region of production, in which Hawai'i was selected. This study performed at micro-scale, with coffees from different islands, allowed the detail analysis of how the various environmental factors prevailing in the location and time of coffee production, were reflected in the elemental isotopic composition of the coffee beans. This approach allowed the discrimination of coffees from the different islands of Hawai'i. It was also shown that the isotopic composition of O, Sr and S in the green coffee beans is related to known

environmental factors, namely the isotopic composition of the O of local precipitation ($\delta^{18}O_{prec}$), the distance to the coast, and the volcanic activity characteristic of that region. In addition, IRMS was applied to measure the isotopic composition of the caffeine molecule ($\delta^{18}O_{caff}$) previously extracted from the green coffee bean. As O isotopes of caffeine molecule originate from the metabolic water of plant tissues, where this metabolite is biosynthesized, this analytical approach constitutes an alternative for studies on the ecophysiology of the coffee plant.

INTRODUCTION

A growing number of consumers demanding diversity and distinctiveness in food and an increasing public concern over issues such as health and ecology have changed public confidence in conventional food production. Thus, standards and certifications nowadays represent a means of demonstrating quality and gain confidence of consumers with whom the producer does not have a direct relationship. In other words, certification is a way of communicating with consumers living outside the region of production. It is argued to have a number of benefits for consumers and producers, and responds to the growing demand that exists in western nations for foods that are produced in ethical, environmentally sustainable, and socially just ways. This is also reflected in scientific research with attempts to respond to the increasing demand for developing analytical tools to prove food authenticity and/or quality.

In the case of coffee, several attempts have been made to determine the origin of green and roasted coffee beans. Analytical methods such as gas chromatography-mass spectrometry (GC-MS) and near infrared spectroscopy (NIR spectroscopy) were applied for the determination of organic compounds such as fatty acids profiles, tocopherols and triglycerides. Stable isotope ratios of carbon, nitrogen and oxygen of specific compounds extracted from green coffee beans were studied with promising results. Krivan and co-authors demonstrated the potential of measuring elemental fingerprints in *Coffea arabica* coffee beans and quantified manganese (Mn) along with carbon (C), cobalt (Co), cesium (Cs), sodium (Na) and rubidium (Rb) in order to discriminate between green coffees from 8 different origins. That study was complemented by Anderson and Smith with the determination of the multi-element composition of roasted coffee beans from 8 different origins of Central and South America, Indonesia, and East Africa. Other authors studied variations in the boron (B) isotope composition of *Coffea arabica* beans, showing that the measured variation in B isotopic composition among different coffee beans is significant and can be related to differences between local growing conditions. Based on previous studies, Serra and co-authors determined the isotopic composition of C, N and B in green coffees from 19 different countries, showing that the isotopic composition of these three elements is a good indicator of geographical-dependent parameters, and therefore represents a useful tool to infer the region of production of green coffee. The authors suggested that the use of stable isotope ratios might be improved by the use of climatic data as an additional variable for the construction of a statistical model. These are important ‘preliminary’ studies although some of the authors acknowledge the relatively small number of authentic samples included in the studies. Recently, Techer and co-authors have characterized the strontium (Sr) isotopic composition of all components of a cultivation system *i.e.* plants, rocks, soils and water in the frame of an intensive coffee-growing project on the Réunion Island, East of Madagascar. These studies indicate that measuring elemental concentrations and isotopic variation in regional coffees is arguably the best analytical strategy for accurately verifying coffee geographical origin. This approach results from global variations of isotopes abundance of ‘light’ bio-elements and ‘heavy’ geo-elements.

MATERIALS AND METHODS

The materials and methods are according to the Journal of Agricultural and Food Chemistry.

RESULTS AND DISCUSSION

Many coffees from different geographical origins and of different types and grades are imported yearly by coffee roasting companies through a commercial chain that usually involves several intermediates. To ensure that coffees had not been adulterated, it is important to develop analytical tools for coffee bean type and geographical origin discrimination. In figure 1 it is shown how the different goals of this work were integrated in the different phases of the global coffee bean pathway, in a tentative to respond to important demands from the coffee industry.

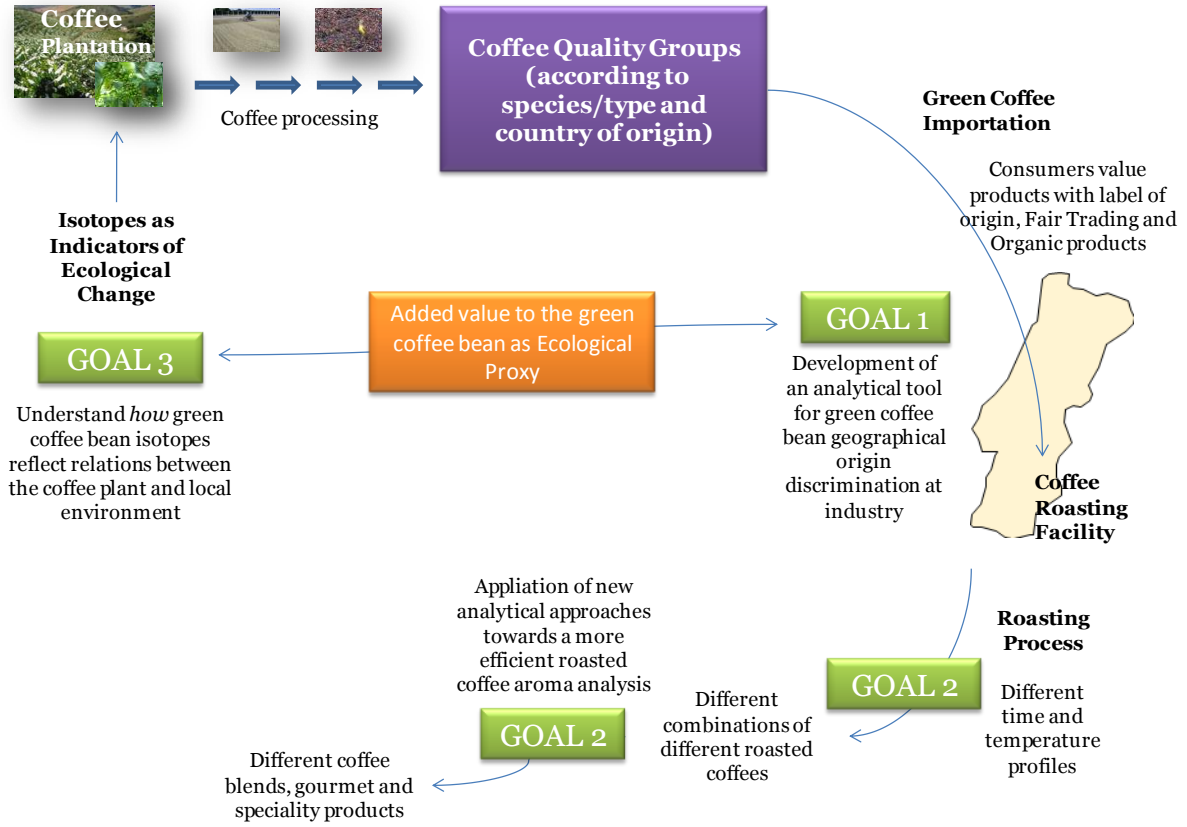

Figure 1. The main goals of this work.

For instance, visual inspection has been common practice to evaluate if a coffee is of *Arabica* or *Robusta* type, but it does not allow the safe detection of "contaminations" of *Arabica* beans by small amounts of *Robusta* beans. Consequently, developments of more objective methods that can be certified are desirable. The Raman spectroscopy approach presented in this work allowed the differentiation between *Arabica* and *Robusta* whole green coffee beans based on their kahweol content. A spectral kahweol index (σ_{KA}) proportional to the coffee bean kahweol content represented a good criterion to differentiate *Arabica* versus *Robusta* coffees (figure 2).

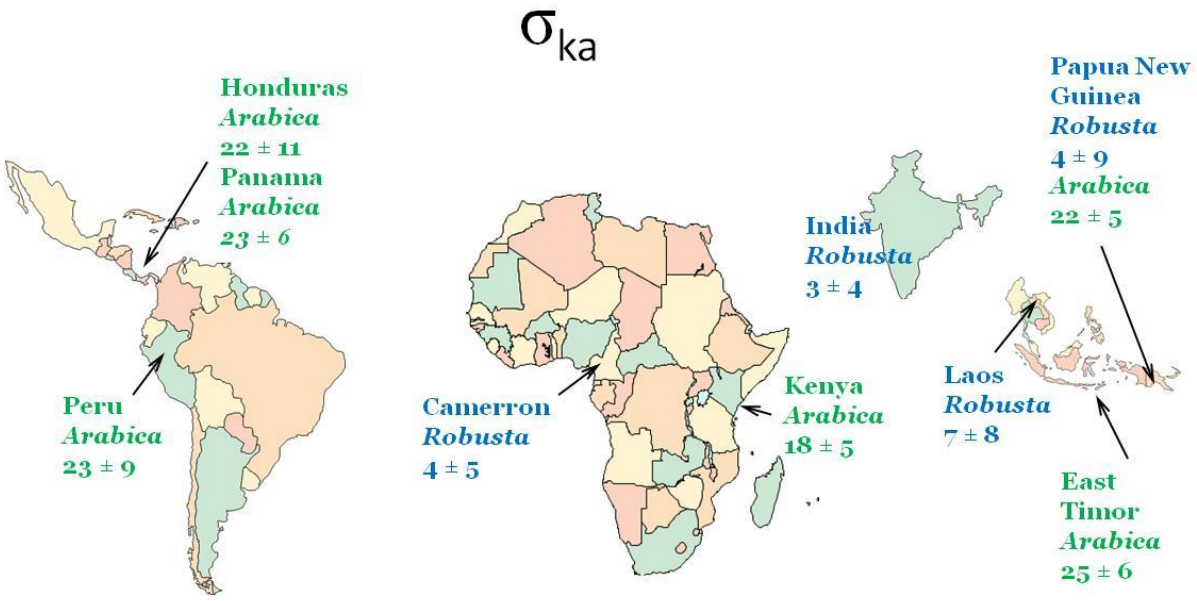

Figure 2. Spectral kahweol index (σ_{ka}) of *Arabica* and *Robusta* whole green coffee beans from different geographical origins (maps are not at scale).

This analytical approach showed several advantages by not relying on pattern-matching spectral techniques dependent on spectral differences between coffee samples, as in the case of NIR spectroscopy (results not shown). Moreover, it was shown that no mechanical or chemical processing of the coffee beans is necessary making this analytical alternative to *Arabica versus Robusta* differentiation less time-consuming and less costly compared to other analytical techniques. Besides the coffee type differentiation, the aim of this work was also to discriminate the geographical origin of the green coffee bean. The measurement of the isotope ratio of different elements in the coffee bean, namely C, N, O, Sr and S was performed in order to achieve this goal. Applications of stable isotope analysis in fields, *e.g.* chemistry, geochemistry, biogeochemistry and ecology had proven to be an extremely valuable and a powerful tool for indicating (sourcing), tracing, and recording various changes to the Earth's diverse terrestrial, aquatic, marine, and atmospheric systems. In this sense, variations in isotopic composition of coffee beans from different geographical origins with their own climate and geology were expected. The isotopic fingerprint of the coffee bean should be a resultant of plant variety, cultivation practices, processing, and, most important, of the relation between plant and local environment. C, N and O were the first elements to be studied in this work at a global scale. However, an overlapping of the mean coffee bean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values from different countries was observed and compromised the geographical origin discrimination at global scale. Nonetheless, the first results obtained allowed the discrimination between coffees from Africa and Asia (figure 3). The most relevant element to achieve this was oxygen. The values of coffee bean $\delta^{18}O$ separated the coffees originating from these two continents (figure 3), because $\delta^{18}O_{bean}$ reflects the $\delta^{18}O$ of local precipitation, and should also be influenced by plant evapotranspiration.

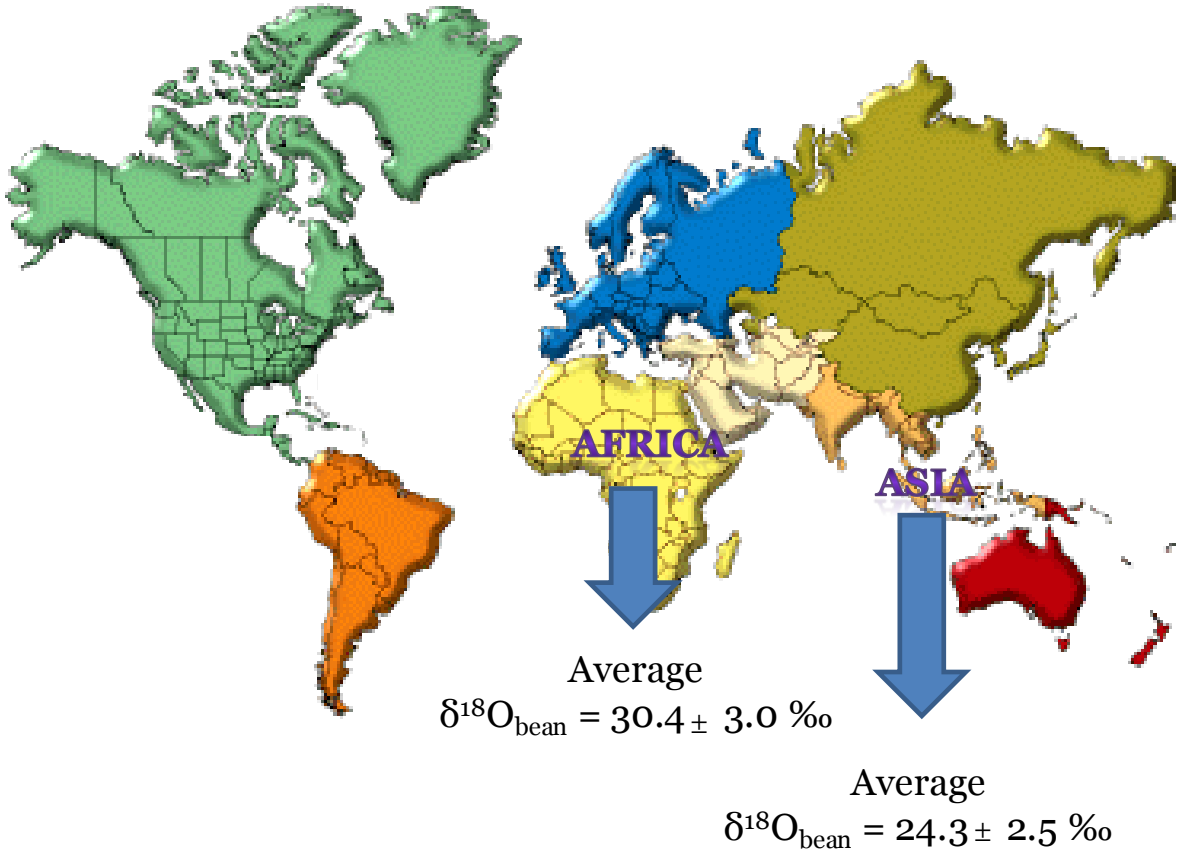

Figure3. Average $\delta^{18}O$ values of green coffee beans ($\delta^{18}O_{bean}$) originating from Africa and Asia.

At this stage, it was clear that to enhance the degree of geographical origin discrimination it was necessary to measure the isotopic composition of other element(s). Studies with food traceability indicated that the analysis of Sr isotope abundance ratios could improve the development of an analytical tool for coffee authenticity studies. Sr concentration and isotopic composition in plants are closely affected by the soil where they grow, formed by weathering of parent rock and depending on environment (*e.g.* fertilizer and moisture). By combining O and Sr isotope ratio analysis, it was possible to achieve a separation between selected origins and groups of provenances. Coffees from East Timor differentiated from all other origins solely based on their $^{87}Sr/^{86}Sr$. In South America, coffees from Brazil, Peru and Ecuador coffees were discriminated based on the principal component analysis of their $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ values. Coffees originating from the different islands (Papua New Guinea (PNG), Hawai'i, Indonesia, Jamaica and East Timor) also differentiated on the basis of their $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ values (figure 4). Some of these coffees are considered *gourmet* as is the case of the Hawaiian's and Jamaican's.

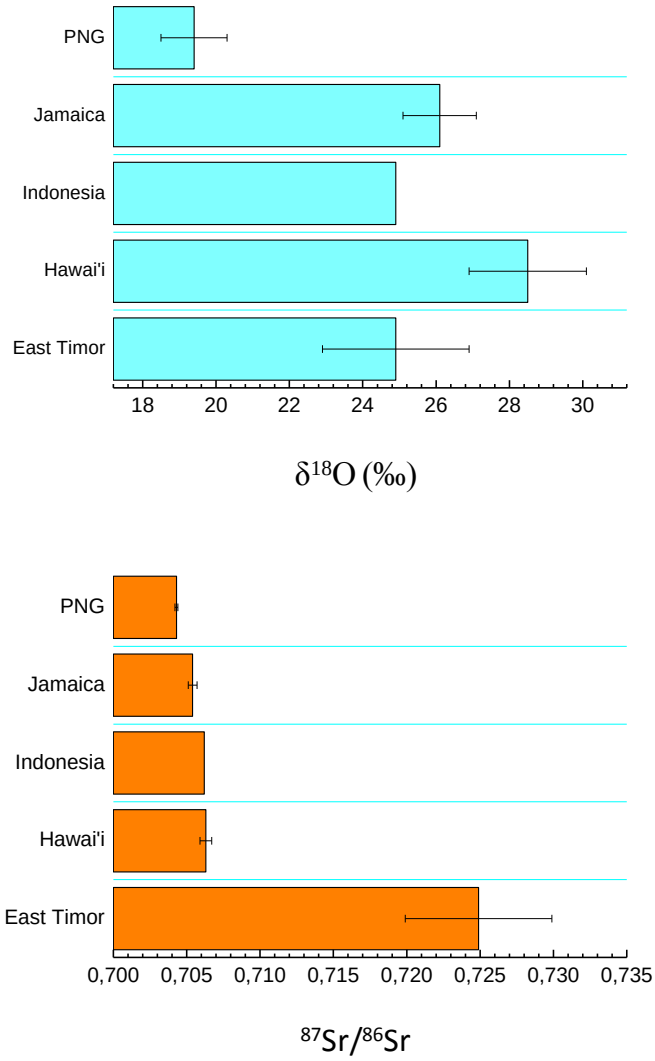

Figure 4. $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ of green coffee beans from different islands. The combination of $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ allows the discrimination of coffees from different islands.

In addition, the $^{87}Sr/^{86}Sr$ values measured in several origins, *e.g.* Hawai'i, Guatemala, Ecuador, Kenya, UR Tanzania, Zambia, Malawi and Rwanda were correspondent to the reported $^{87}Sr/^{86}Sr$ values for local parent rock type. In some cases where it was possible to access information on both Sr amount and isotope abundance ratio of parent rock, principal component analysis allowed the differentiation of coffees from UR Tanzania, Rwanda, Kenya, Hawai'i and East Timor (figure 5). The combined O and Sr isotope ratio measurement of green coffee bean presented itself as the best approach to discriminate the geographical origin of the green coffee bean. This series of analysis may, however, be reinforced by the measurement of the isotopic composition of other elements depending on the coffee-producing region under study.

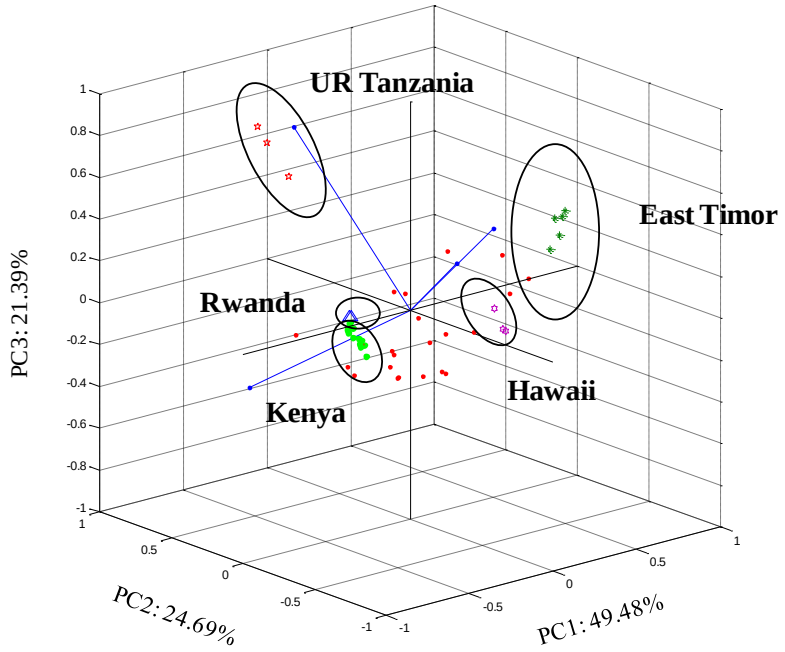

Figure 5. Scores on the three principal components explaining variability in Sr (ppm) and 87Sr/86Sr of green coffee beans and correspondent parent rock.

When working at global scale and with the 'bulk' bean isotope analysis, it is as considering a 'black box' where a multitude of climatic, geological and physiological processes are enclosed but not evident. For this reason, a scale-down to a smaller coffee-producing region (Hawai'i) was the next step in this work. In addition, multi element analysis and sulfur isotope ratio analysis complemented the series of analysis performed with the Hawaiian green coffee beans. The results showed that by combining 'light' (S, O, C and N) and 'heavy' (Sr) isotopes with multi element analysis it was possible to discriminate the Hawaiian coffee-producing regions. This case-study also constituted an opportunity to improve the understanding of the relationship between environmental variables and the green coffee bean isotopic composition. Again, the results obtained with the Hawaiian coffees confirmed that isotope ratio and multi element analysis constitutes the best analytical tool for coffee authenticity studies. In short, in order to apply this analytical approach, it is important to understand the geographical area under study. A characterization from the climatic and geological point of view should be performed at first place, and the most extensively possible. Also, information on cultivation methods, species and varieties/cultivars, and processing should be important to build the most extensive database. Next, a series of analyses encompassing a set of chemical elements may then be selected and tested to achieve the highest degree of provenance discrimination. The fact that, for a large number of coffees included in this work, information on exact geographical location and related environmental factors could be accessed, allowed the correlation between experimental results and data available on, *e.g.* altitude, latitude, $\delta^{18}O$ of precipitation, and, in some cases, Sr amount and isotope composition of parent rock. This represented the innovation of this work in relation to previous studies. It was showed that the $\delta^{18}O_{bean}$ reflects the local precipitation oxygen isotopic signature whether at global scale or, in the case of the Hawai'i. Significant positive correlations between the $\delta^{18}O$ values of coffee bean and of local precipitation were observed.

Higher values of $\delta^{18}O_{bean}$ were measured in coffees originated from regions where more enriched weighted annual $\delta^{18}O$ of precipitation are reported (Africa) (figure 6a). The O isotopes of plant water, the organic molecules that make up plant tissues and the gases produced during plant metabolism all record important aspects of a plant's growth environment and physiological activity *at various spatial and temporal scales* (figure 6b). Many isotope effects were observed, *e.g.* altitude effect on $\delta^{18}O_{bean}$ in the American continent, and in Africa, and islands. In Hawai'i, the influence of volcanic activity, tropical storms, of the distance to the coast and altitude were inferred from the isotope ratios measured in the Hawaiian coffee beans. These observations were supported by significant correlations between the green coffee bean isotopic composition and the various environmental factors. All this reflects the importance of the seed, the coffee bean, as a 'tool' to study climate and plant primary production spatial and temporal variations. Cellulose, which is an important component of the green coffee bean, has been the preferred material for $\delta^{18}O$ analyses for climatic and ecophysiological studies. However, its $\delta^{18}O$ has a mean 27 ‰ enrichment resultant of the carbonyl-water interaction during its biosynthesis. This biochemical fractionation may difficult the application of isotopic considerations in plant ecophysiological studies. In the present work, it was demonstrated that oxygen isotope ratio measurement of caffeine constitutes a viable alternative to cellulose or 'bulk' organic matter analysis, not only from the biochemical point of view but also in what refers to extraction method. In caffeine, the origin of the O isotopes is different comparing to plant carbohydrates (figure 7). That consists in the advantage of studying caffeine in comparison to cellulose, as biochemical fractionation does not theoretically determine caffeine's oxygen isotopic composition. This is supported by the observed similar magnitudes of $\delta^{18}O_{caff}$ and $\delta^{18}O_{prec}$, and by correlation between $\delta^{18}O_{bean}$ and $\delta^{18}O_{caff}$ in relation to $\delta^{18}O_{prec}$. Caffeine $\delta^{18}O$ measurement is important because it gives an approximate isotopic signal of plant leaf water, and may eventually aid in the estimation of plant transpiration on the basis of the ^{18}O enrichment at leaf or seed level, providing additional information is available on local isotopic composition of water vapor and soil water.

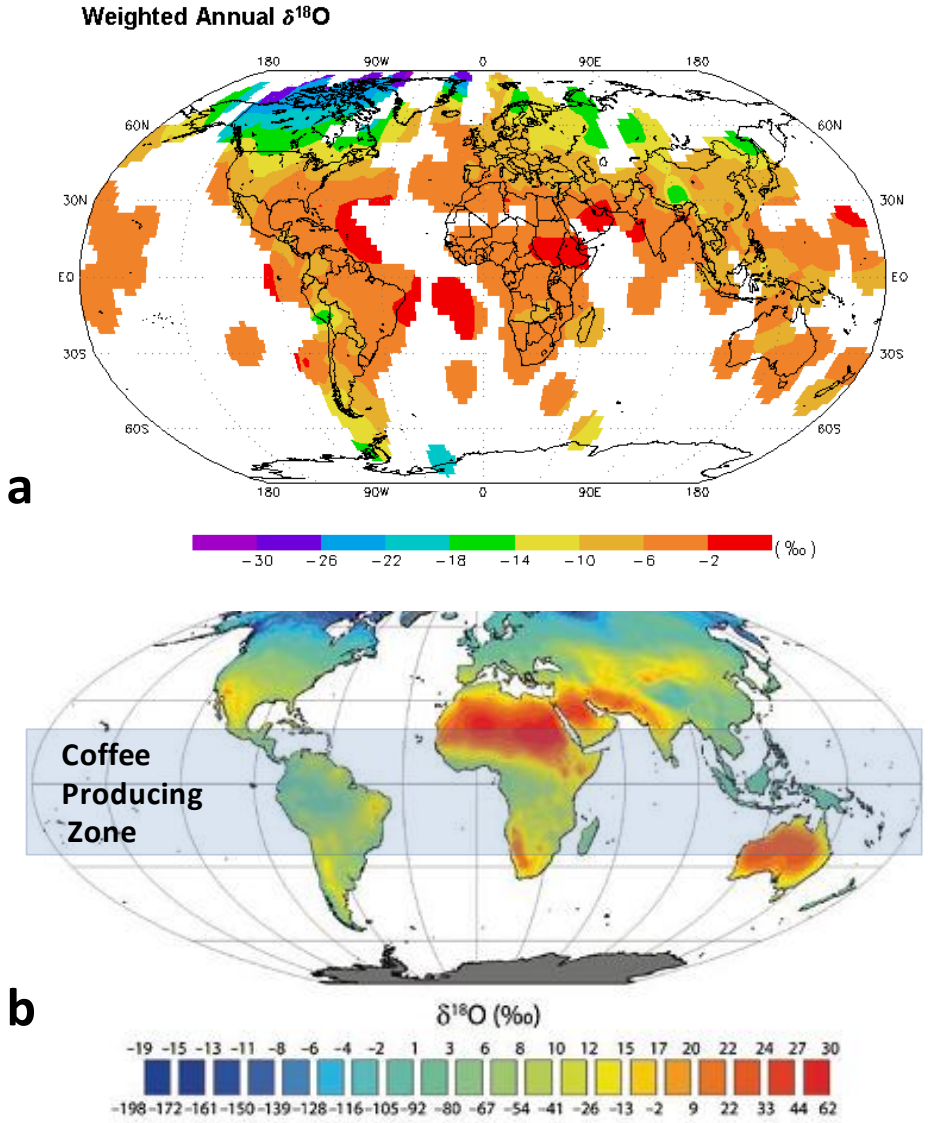

Figure 6. Global map of weighted annual $\delta^{18}O$ of precipitation (a) (IAEA, 2001) and of annual average leaf water $\delta^{18}O$ for the sites of evaporation within leaves (b) (adapted from Journal of Agricultural and Food Chemistry).

The caffeine isotope ratio analysis should work as an 'inversion method' to point trends in $\delta^{18}O_{prec}$ and in plant water $\delta^{18}O$, in different globe regions, thus, improving global precipitation and plant water $\delta^{18}O$ isoscapes. It will be fundamental to develop real time field experiments in order to relate the results from the coffee bean isotope ratio analysis with physiological responses from the plant. Additional isotope ratio analysis of hydrogen of the coffee bean and caffeine will be an important complement to this research.

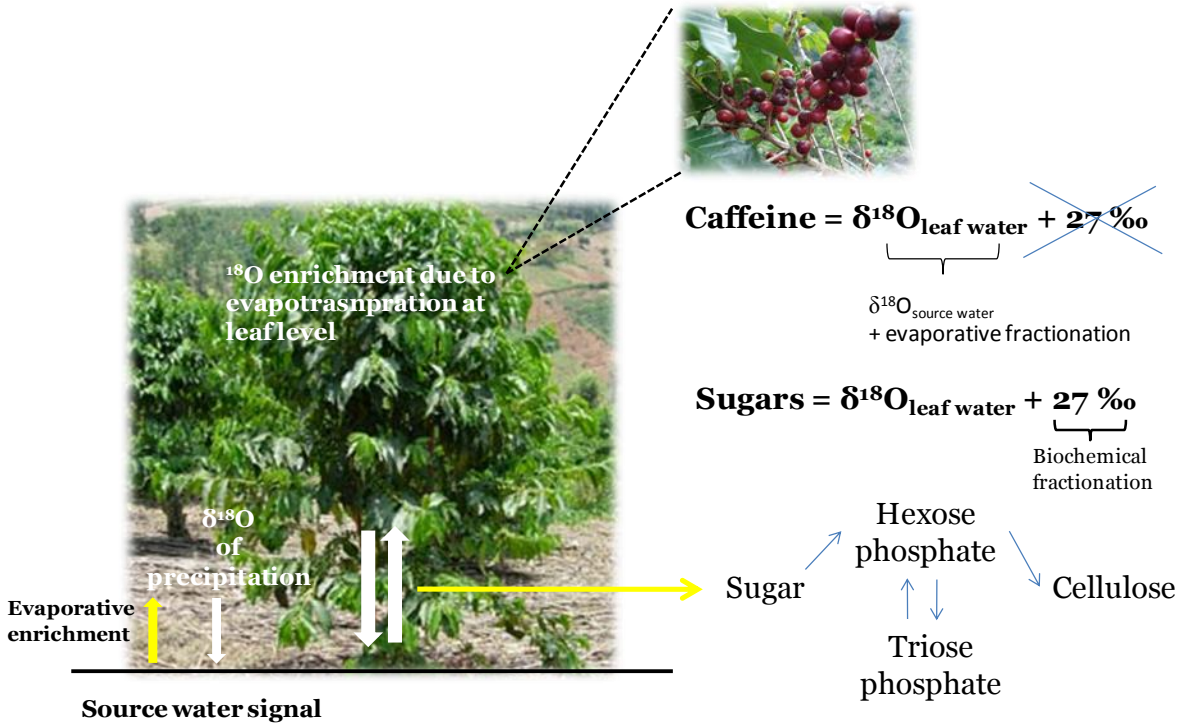

Figure 7. Diagram of a coffee plant and fruit showing the main fractionations of O isotopes determining cellulose and caffeine $\delta^{18}O$.

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Coffee Sustainability, the View from a Roaster

J. MELROSE[1], S. FOX[1], F. GUILMINEAU[1], A. STOCKWELL[1], M. HETT[2], G. O'GRADY[2], F. TRAMONTIN[2]

[1]Mondelēz International, Global Coffee Technology, Centre Ruscote Avenue, Banbury - OX16 2QU, UK[A]
[2]Mondelēz International, Lindbergh-Allee 1, 8152 Zurich, CH[A]

SUMMARY

There are four key drivers of sustainability in coffee from a roaster perspective: a) Sustainability issues affecting security of supplies such as climate change, land constraint, poverty etc.; b) Eco-efficiency opportunities in manufacturing, packaging etc. b) Consumers in Western markets look at sustainability as a factor in making their choices c) Corporate reputation concerns. Roasters, recognise their responsibilities both to farming communities and consumers – who rightly expect roasters to act on their behalf, not delegating the issue to consumer demand. All this has driven substantial business engagement among coffee roasters in the past decade, in particular in terms of sourcing standards and agriculture practices. This paper reviews these activities and the challenges for the future.

LIFE-CYCLE ANALYSIS

In recent years the carbon impact of products has been assessed by full life-cycle analysis (LCA). Another poster at this conference (PA139) gives an example of our work in this area and details of our approach. We and others Humbert *et al* (2009) have completed LCA for a wide range of coffee beverages. Figure 1 compares the carbon footprints of R&G and Soluble beverages from our study – we strongly argue that comparisons should be made per cup than by weight of product. Overall the soluble beverage has a lower footprint: the cost of manufacture is higher but this is more than offset by a reduced agricultural contribution, due to the higher yield per bean of the industrial extraction process. The contribution from the primary production and agriculture (green coffee) is the most significant part of the total footprint, varying from circa 40% in instant coffee systems (and On Demand system, not shown) to 80% of the footprint in R&G systems.

[A] About Mondelēz International: Mondelēz International, Inc. (NASDAQ: MDLZ) is a world leader in chocolate, biscuits, gum, candy, coffee and powdered beverages. The company comprises the global snacking and food brands of the former Kraft Foods Inc. following the spin-off of its North American grocery operations in October 2012. Mondelēz International's portfolio includes several billion-dollar brands such as *Cadbury* and *Milka* chocolate, *Jacobs* coffee, *LU, Nabisco* and *Oreo* biscuits, *Tang* powdered beverages and *Trident* gums. Mondelēz International has annual revenue of approximately $36 billion and operations in more than 80 countries. Visit www.mondelezinternational.com and www.facebook.com/mondelezinternational.

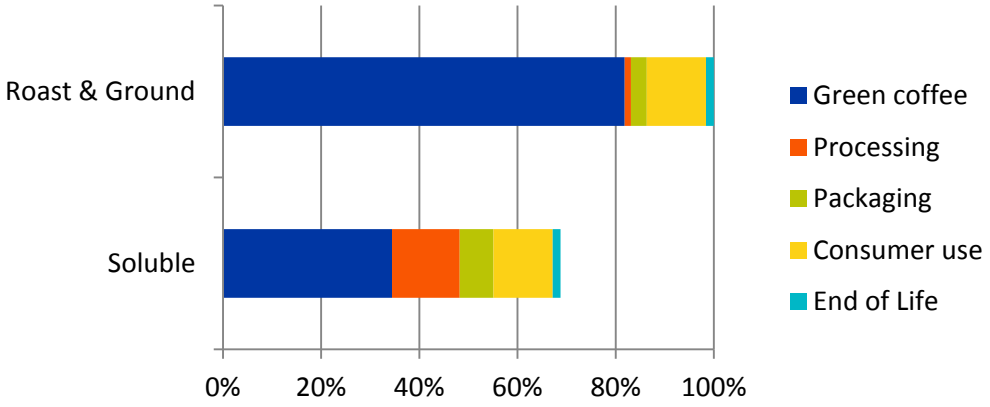


Figure 1. GWP for 100ml beverages at 1.2% brew solids brewed perfectly.

The consumer use included here is just boiling the water; if we include the addition of milk this can make a significant extra contribution to the carbon footprint. As the data of figure 1 shows, the production of soluble coffee is energy intensive, however we and other manufactures are pursuing significant publically announced targets to reduce energy usage in manufacturing. We are also focused on waste reduction: today, all 10 of the company's European coffee plants send zero waste to landfill. These are rolled up and reported in company aggregate figures.

The Green Coffee contribution is where there is both greatest uncertainty in the data and limited studies reported in the open literature, one is Coltro *et al* (2006), in limited geographies. Therefore we and other roasters are collaborating within the SAI (Sustainable Agricultural Initiative) to create some Product Category Rules for the assessment of carbon footprint of primary production and agriculture. At the moment LCA is used by us to inform and guide our own sustainability efforts with respect to Carbon and for voluntary communications. However we have supplied figures to some of our supermarket customers under commercial request and there is the possibility that future regulation (e.g. the possible French Grenelle Law) may require formal reporting of such Carbon figures - it is therefore vital that the paucity of agriculture data be improved.

SUSTAINABLE FARMING AND COFFEE

Today many small and large coffee companies are using sustainability claims in the market mostly through agriculture certification and verification schemes, Indeed Kraft Foods Europe (Now Mondelez International) had a pioneering role in the use of Rainforest Alliance certification, and there was significant gain in market share when it converted the UK Kenco soluble brand to RA. Mondelēz international is the biggest buyer of RA certificate coffee, and more recently we have announced that all EU coffees will be sustainably sourced[B] by 2015. Mondelēz International are heavily engaged in driving 4C verification and it continues to work with Rainforest Alliance.

Consumers in the developed world often look at certification and ethical/environmental label when purchasing coffee ;however there are a plethora of schemes; there are many initiatives to train and prepare smallholders for certification and verification and to increase productivity. Mondelēz International is involved in a number of schemes, to both drive its own supply of sustainable coffee and with other roasters to address pre-competitive

[B] "Sustainably sourced" defined as third-party certification or verification.

sustainability issues. In recent years the plethora of certification has been seen by some as confusing for producers and especially for smallholders. To relieve some of the complexity of multiple schemes, some national bodies (in Brazil and ASEAN) have started initiatives to set national standards. Training for improved agriculture practice includes components concerned with mitigation and adaption for possible climate change impact.

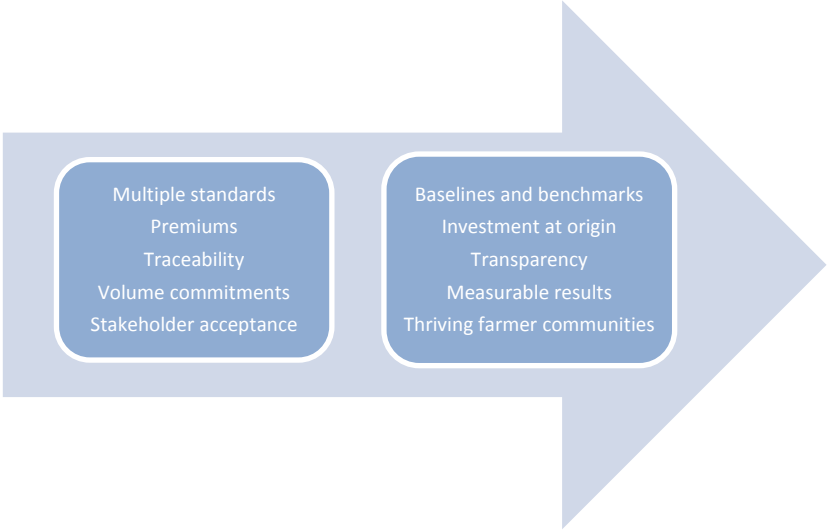
In general, for the coffee sector, the success in protecting the pleasure of coffee for future generations requires creation of a trusted value chain and transparent collaboration of agents within in it. Tracking activities and impact is vital to clear communications to consumers. What is most important is that our commitment to sustainable coffee production standards will result in increased investment in farming communities. With economic development in producing regions, there is a risk that the next generation will not find coffee farming a competitive livelihood or see it as a desirable lifestyle.

In a resource constrained world and a very competitive marketplace, the larger coffee roasters need to:

- secure supplies of quality raw materials, impacted by such things as changing climatic conditions, ageing farmer population, an ageing crop, urbanization etc.
- reduce the impact of farming on the environment
- support the farming communities we rely upon
- and to do this whilst generating growth and consumer preference for their brands.

On Oct 29th, Mondelēz International announced an expanded effort to make its coffee business more sustainable. “Coffee Made Happy” will invest a minimum of $200 million to empower one million coffee farming entrepreneurs by 2020. The program is designed to help the next generation of farmers -- inspiring, training and building their capacity to improve their livelihoods and attract new generations back to the small-scale farming sector. Coffee Made Happy plans to increase farmer productivity and the viability of small-scale coffee farming, strengthening agricultural practices and helping to build more sustainable coffee communities. Mondelēz International is already collaborating in sustainable agriculture with partners like Rainforest Alliance and 4C. By continuing these relationships and fostering new collaborations, Mondelēz International will boost existing commercial skills development programs in Vietnam, Peru and other important coffee markets.

To conclude, a vision of sustainability going forward is given below



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Preliminary report on the status and host plant utilization by the Black Coffee Twig Borer, *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae) in Uganda

G. H. KAGEZI[1], P. KUCEL[1], D. MUKASA[2], P. VAN ASTEN[2], P. C. MUSOLI[1], A. KANGIRE[1]

[1]Coffee Research Center (COREC)/National Crops Research Institute (NaCRRI)/National Agricultural Research Organization (NARO), P.O. Box 185 Mukono, Uganda
[2]International Institute of Tropical Agriculture (IITA), P.O. Box 7878 Kampala, Uganda

SUMMARY

The Black Coffee Twig Borer, *Xyalosandrus compactus* (Eichhoff) is a new but rapidly spreading pest of coffee and other plant species. However, knowledge of its pest status, damage and host plant species utilization in Uganda is still limited. To ascertain its spread and impact, a survey was conducted on 250 farms in 25 districts in the 5 major coffee growing regions of Uganda. At farm level, 12 coffee trees were randomly sampled along a diagonal transect and assessed for *X. compactus* infestation. In addition, host plant utilization by the pest was determined through farmers' interviews and direct search by researchers. Our data show that the pest is present in all the 5 districts (100%) sampled in central region *viz*:- Mukono, Luwero, Mityana, Mubende and Mpigi, and at least 50% of the districts in southwestern *viz*:- Bundibugyo, Kasese and Rubirizi. However, the beetle was not observed in northwestern (West Nile), northern and part of eastern (Mt. Elgon) regions. *X. compactus* prevalence (percentage of infested farms), incidence (percentage of infested trees) and damage (percentage of infested primary branches) were 58.1, 34.0 and 3.8% respectively in the central region whereas, 22.1, 7.7 and 0.8% respectively in the southwestern region. At district level, the highest prevalence (100%) was observed in Mukono and Luwero then Bundibugyo (62.5%), Mityana (50.0%), Rubirizi (40%) and Kasese (30%). Similarly, high incidence rates of 91.7, 73.3 and 44.8% were observed in Mukono, Luwero and Bundibugyo respectively. Likewise, high damage rates of 13.6, 5.2 and 4.8% were observed in Mukono, Luwero and Bundibugyo districts respectively. Further, our study identified and documented more than 30 plant species in 17 families as potential hosts for *X. compactus*. These include important commercial and food crops, forest, fruit and shade trees and shrubs. In conclusion, this study confirms earlier surveys and reports that the black coffee twig borer is fast and rapidly spreading away from its initial occurrence (southwestern Uganda) and epicenter (central Uganda) to new infestation areas, posing a big threat to coffee production in the country. Therefore, there is an urgent need to put in place comprehensive mitigation measures in order to prevent the pest from spreading to new areas and also to minimize its impact on coffee production within the already affected areas.

INTRODUCTION

The Black Coffee Twig Borer (thereafter referred to as BCTB), *Xyalosandrus compactus* Eichhoff is currently a major pest of coffee in Uganda, particularly in the central, southwestern and part of eastern regions. These regions happen to produce the bulk of Robusta coffee which contributes to about 80% of the Uganda coffee export volume. The

female beetle bores into the berry-bearing primary branches (twigs) causing them to wilt and eventually die after a few weeks. Thus, the infested twigs will not bear berries which will definitely result into loss of income. BCTB is a highly invasive and damaging pest that spreads far and wide over a short period of time. It is probably native to Southeast Asia**,** but currently distributed worldwide, particularly in tropical and subtropical countries. In Uganda, it was first reported in 1993 in the southwestern district of Bundibugyo and then in the neighboring districts of Rukungiri, Kanungu, Bushenyi and Rubirizi in 2002-4. In 2007/8, another serious outbreak of the pest was reported in Kayunga and Mukono districts of central Uganda. In the two districts, 38% of the farms were infested. Within the infested farms, 21.2% of the coffee trees had been attacked and 3.7% of their berry bearing branches killed. Since nearly all the Robusta coffee produced in Uganda is for export, this loss could be extrapolated into a 3.7% loss of the coffee export volume and value which may be translated into a financial loss of US$14.3 million earned in Financial Year 2007/2008. Further, the twig borer is highly polyphagous and has been reported to infest more than 224 plant species in about 62 families including those listed as threatened and endangered. In Uganda, although the pest was initially mainly reported on Robusta coffee, recent reports show that it also attacks Arabica coffee as well as a number of other plant species (Kagezi *et al*., unpublished data). Basing on this background, a survey was conducted in the 5 major coffee growing regions of Uganda with the aim of (1) determining the extent of spread of the pest and its impact on coffee production, and (2) documenting host plant species utilization for *X. compactus* in Uganda.

MATERIALS AND METHODS

Two hundred and fifty (250) households in 25 districts in the five principal coffee growing regions of Uganda who had been sampled in an earlier study by Piet van Asten *et al*. (Unpublished data) were surveyed in 2011/12. The districts included: - Luwero, Mityana, Mubende, Mpigi and Mukono (central), Ibanda, Rubirizi, Kasese, Bundibugyo, Kabarole and Kisoro (southwestern), Zombo, Nebbi, Arua, Maracha and Yumbe (West Nile), Lira, Oyam, Gulu and Amur (northern) and Kapchorwa, Sironko, Manafa, Mbale and Bududa (Mt. Elgon). On each farm, 12 coffee trees along a diagonal transect were sampled and the total number of primary branches (twigs) and those infested with BCTB were determined. BCTB prevalence (percentage of infested farms), incidence (percentage of infested trees) and damage (percentage of infested primary branches) were computed. In addition, host plant species for the twig borer were searched for through farmers' interviews and direct observation by the research team. These were identified by both their indigenous and taxonomic names. Data were analyzed using descriptive statistics including means and percentages.

RESULTS AND DISCUSSION

Our data clearly show that although BCTB is relatively a new pest in Uganda, it was confirmed in all the 5 districts (100%) surveyed in central Uganda (Mukono, Luwero, Mityana, Mubende and Mpigi) and 50% of the districts sampled in the southwestern region (Bundibugyo, Kasese and Rubirizi). Incidence and damage were 34 and 3.8% respectively in the central region and 7.7 and 0.8% respectively in southwestern region. These results agree and confirm several earlier surveys and reports. These reports show that the pest is fast and rapidly spreading from its place of initial incidence (Bundibugyo district) and its epicenter (Mukono and Kayunga districts) to several other new infestation areas across the county particularly in the central, southwestern and part of eastern region (Busoga sub-region). Incidentally, these regions produce the bulk of Robusta coffee (*Coffee canephora*) in Uganda which contributes to more than 80% of the coffee export volume in the country. At district level, Mukono and Luwero registered the highest prevalence level (100%). Bundibugyo,

Mityana and Rubirizi districts also had high prevalence rates of 62.5, 50 and 40% respectively. These data are in agreement with some reports which show high prevalence rates of the twig borer in some of the surveyed districts, including, Mukono/Kayunga and Mityana/Mubende (40-50%) and Luwero/Nakaseke (40-60%). It should be noted that these districts with high prevalence, incidence and damage levels happen to be located in the center of origin (southwestern region) and epicenter (central region) of the pest. However, the present survey was limited to only 25 districts out of the more than 80 coffee growing districts of Uganda. This calls for an urgent need to conduct a broader and more comprehensive countrywide survey to establish the status, impact and damage of the pest. This information is critical in formulating a national strategy to protect coffee plantations in free/threatened zone, halt further advance of the pest in frontline zone (the advance edge of the pest endemics) and eradicate the pest in already infested zone (endemic). The control strategies in the three zones definitely differ. For example, in endemic zone, phtosanitory measures followed by "blanket spaying" with chemicals may be the best option whereas in free/threatened zones, quarantine strategy whereby movement of plant materials from endemic zone is restricted may work best. Further, the study provided a preliminary inventory of host plant species range of *X. compactus* in Uganda. This adds vital information to the existing literature on host plant species utilization by the twig borer. Here we report >30 plant species belonging to 17 families as potential plant hosts for the twig borer including: - *Acanthus pubescens* Engl. (Acanthaceae), *Mangifera indica* L. (Anacardiaceae), *Crassocephalum crepidioides* (Benth.) S.Moore, *Tithonia diversifolia (*Hemsley) A. Gray, *Vernonia amygdalina* Delile, *V. auriculifera* Hiern (Asteraceae), *Markhamia lutea* (Benth.) K. Schum. (Bignoniaceae), *Maerua duchesnei* (De Wild.) F (Capparaceae), *Sapium ellipticum* Pax (Euphorbiaceae), *Sesbania sesban* (L) Merr., *Senna occidentalis* (L) Link., *Entada abyssinica* Steud., *Albizia coriaria* Oliv., *A. chinensis* (Osbeck) Mer, *A. zygia* (DC) Macbr., *Indigofera arrecta* A. Rich., *Leucaena leucocephala* (Lam.) De Wit, *Calliandra calothyrsus* Meissner and *Senna spectabilis* (DC.) Irwin & Barneby (Fabaceae), *Persea americana* Mill. (Lauraceae), *Ficus natalensis* Hochst., *Artocarpus heterophyllus* Lam. (Moraceae), *Eucalyptus* spp. (Myrtaceae), *Grevillea robusta* A. Cunn. ex R. Br. (Proteaceae), *Maesopsis eminii* Engl. (Rhamnaceae), *Coffea arabica* L. and *C. canephora* Pierree (Rubiaceae), *Solanaceous aethiopicum* L., *S. melongena* L., and *S. incanum* L. (Solanaceae), *Theobroma cacao* L. (Sterculiaceae), *Camellia sinensis* (L.) Kuntze (Theaceae) and *Grewia trichocarpa* A. Rich (Tiliaceae). Our findings are in agreement with several other researchers who have reported that the twig borer is a highly polyphagous pest infesting more than 224 plant species in 62 families including those listed as threatened and endangered. Incidentally, these host plant species recorded in this study are important food and commercial crops, forest, fruit (horticultural), shade and fodder trees, and, medicinal and ornamental plants. These plants are usually deliberately planted and/or maintained in or near coffee agro-systems by farmers. This may have a lot of management implications for the pest whereby farmers have to make a choice between protecting the coffee from BCTB infestation by eliminating the alternate hosts or maintaining the trees for various purposes. Secondly, most farmers in Uganda and elsewhere are currently relying on pruning and burning and/or burying of infested plant materials and/or removing them from the vicinity to control the beetle. However, these cultural methods require a thorough understanding and knowledge of the host plant range and utilization of the pest in question. Occurrence of alternative hosts may influence the ecological dynamics and biology of the pest and thus complicate cultural control strategies. Nevertheless, a more extensive search for more host plant species needs to be conducted particularly in the place of origin of the pest, its epicenter and the new infestation areas. This information is vital in designing management practices particularly phytosanitory (cultural) practices in order to reduce or eliminate the source of infestation.

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Biological and Molecular Characterization of Isolations of the Entomopathogenic Fungus *Beauveria Bassiana* (Balsamo) Vuillemin

F. ECHEVERRÍA-BEIRUTE[1], M. BARQUERO-MIRANDA[1], J. PERAZA[2]

[1] Coffee Research Center (CICAFE), Costa Rica Coffee Institute (ICAFE)
[2] Center for Biotechnology Investigation (CIB); Costa Rica Institute of Technology (ITCR)

SUMMARY

The Coffee Berry Borer (*Hypothenemus hampei*) is a plague of the Costa Rican plantations. As control strategy, the entomopathogenic mushroom *Beauveria bassiana* is used. Ten isolations from different areas of the country were evaluated biologically according to their Daily Rate of Radial Growth (DRRG), production of spores, resistance to ultraviolet light and aggressiveness to CBB. Also, they're molecularly characterized using 8 specific microsatellite primers. Single spore culture was made as base. The DRGG provide significant differences on three strains *(P=0,05)* and production of spores revealed that statistically *(P=0,01)*, they behave on two types: those of high and those of reduced spore's production. Germination of 30 minutes irradiated spores were higher than 82% in six isolations, where three reached 100%, evidencing the possibility of defense mechanisms. The aggressiveneness evaluation revealed a colonization of 100% before 9 days of the initial exposure in two strains. Regarding to the molecular characterization, seven primers showed high allelic variability, allowing the construction of a dendrogram. The isolated groups evidenced high diversity: all isolates are strains, few very related, two are very genetically distanced. No biological or geographical relationship could be correlated with any classified group.

INTRODUCTION

One of the mayor problems that afflict the coffee culture is the coffee berry borer (*Hypothenemus hampei* Ferrari), whom since the year 2000, overpass the containment barriers and enter to the country. It´s recommended control is based by an integrated management, the use of biological controllers, principally by the entomopathogenic fungi *Beauveria bassiana* (Bálsamo) Vuillemin, and at the last option with insecticides (Campos, 2001; Haraprasad, 2001; Mora & Avilés, 2003).

Natural populations of the fungus had been isolated, however it´s genetically relationship and potential use, are unknown. In this article, is exposed the molecular and biological characterization of the fungus, visualizating a better performance as biocontroller (Vélez *et al*, 2000).

Eight specific SSR´s markers for Beauveria bassiana were used and evaluated on 10 single-spore isolates, and evaluated it´s biological and pathogenic properties against CBB (McGuire et al., 2005; Enkerli et al., 2001; Rehner & Buckley, 2003; Wang et al., 2003; Estrada, 1997).

OBJECTIVES

- Evaluate the aggressiveness against CBB.
- Evaluate radial growth, esporulation level, UV light tolerance of isolates.
- Evaluate the PCR microsatellite technique for isolate characterization.

MATERIALS AND METHODS

Coffee beans with interior whitish spots of Coffee Berry Borer infected by *Beauveria bassiana*, were collected from different parts of the country. Spores were cultured in PDA media and serial dilutions where later prepared to isolate single spores of each isolate.

Cultured single-spores cultures where biologically evaluated by means of radial growth, tolerance to UV light exposure and aggressiveness against *H. hampei*. Also, each cultivated isolate was used for DNA isolation and specifically SSR markers, for genotype characterization.

Radial growth was evaluated by culturing 15 days discs of active growth cultures, in Petri dishes. UV tolerance was evaluated by generating a solution ($1x10^6$ spores/ml), varying exposure time from 1) 0 minutes (Control); 2) 10 min.; 3) 20 min, 4) 30 min, to a 254 nm radiation, 100 watt at 17 cm distance, by a Longwave UV Lamp (Black-Ray, model B-100A). Later, samples of the dilution were cultured in a PDA layer over a glass slide, and 24 hours later evaluated the germination percentage at 10X in microscope.

Aggressiveness to *H. hampei*, was established *in vitro* by cultivating 20 field disinfected CBB´s bathed in a 1 x 10^6 spores/ml solution of each isolation, and left in Petri dishes, during 12 days, with an every 3 day death evaluation.

For DNA isolation, three protocols where evaluated and optimized (Vélez *et a*l., 2000; Zambrano *et a*l., 2002; and Wizard (Promega, 2005). Eight SSR´s markers described by Rehner & Buckley (2003) where used for characterization.

RESULTS AND DISCUSSION

Numeric variances obtained for the biological characterization where analyzed and classified from "1" (lowest) to "5" (highest) for each parameter evaluated (Table 1).

Table 1. Categories resuming the principal biological results obtained.

Isolation	Esporulation level	Radial Growth	UV Tolerance	Aggressiveness
A03	4 [a]	2	1	3
Oro	1 [b]	3	1	1
Aco	4 [a]	3	5	2
TT	1 [b]	1	4	4
Co2	3 [a]	3	4	5
Sar	5 [a]	4	5	5
SMN	4 [a]	4	2	3
PZT	1 [b]	2	1	4
SCa	4 [a]	5	5	3
LH	5 [a]	4	3	5

[a / b] *For 99% accuracy, exists statistical differences.*

The molceular analysis showed the existance of variation in the obtained SSR´s, varying according to each isolate and primer sequece from 50 to 400 bp (Table 2).

Table 2. Number and fragment lengh of productos obtained with SSR primers.

Primer	Number	Approximated Length (bp)
1	0	-
2	2	200-50
3	5	400-50
4	4	200-50
5	9	200-50
6	5	400-200
7	5	400-150
8	5	400-150

The bands analysis permit the generation of a dendrogram with the vinculation rule of Complete Linkage and Squared Euclidean Distances measure (Figure 1). According to the phylogenetic data, exists genetic relationships between some isoltes and some geographical tendencies, however, non direct association between agressievness or biological characteristics.

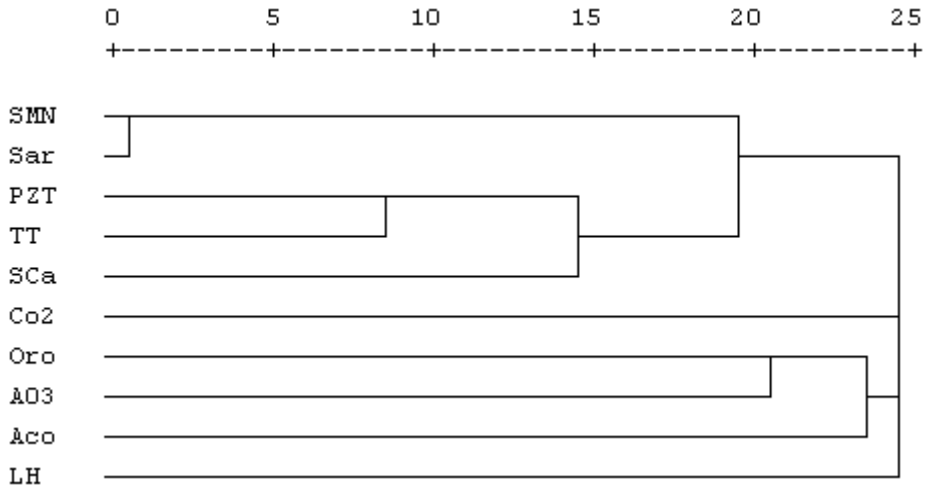

Figure 1. Dendrogram based on specifically microsatellite patterns of the molecular characterization of *Beauveria bassiana* isolates. SPSS 13.0.

CONCLUSIONS

- Potential isolations of *Beauveria bassiana* exists against CBB.
- Isolations showed different biological and molecular behavior, cataloging them as strains.
- An specific protocol for *Beauveria bassiana* molecular characterization was validated.
- Non geographical nor phylogenetic patterns where associated.

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Aclimatization of F1 Hybrids Reproduced *In Vitro*

F. ECHEVERRÍA-BEIRUTE, M. BARQUERO-MIRANDA

Coffee Research Center (CICAFE), Costa Rica Coffee Institute (ICAFE)

SUMMARY

The multiplication of F1 hybrid materials consists in the cloning of the donator plant by means of tissue culture. This process is performed in the laboratory until a level of development reach and then requires to be transferred from a highly controlled environment in the laboratory to an external, fluctuating environment. For this reason, as a preliminary development in the nursery, in vitro propagated plants must go through the acclimatization phase in a greenhouse. In order to evaluate different methodologies for adaptation of seedlings from a controlled in vitro environment to an ex vitro environment, different substrate mixtures were evaluated for planting seedlings in trays in a humid chamber system, under humidity and temperature conditions. The experiment was conducted in the greenhouse of the agricultural center of Centro Agrícola Cantonal, Grecia, Alajuela, Costa Rica, with in vitro material of the F1 Hybrid L13A44 variety, provided by the Biotechnology Laboratory of the Tropical Agricultural Research and Higher Education Center (CATIE), Turrialba, Cartago. The substrates used were Soil-Shrub, Rice Chaff (TBG, in Spanish), Soil-Organic Manure-Rice Chaff (TAG, in Spanish), Soil and Coconut Husk Fiber (TF in Spanish) and Compressed Peat (Jiffy®). The development of seedlings were evaluated for four weeks, calculating mortality and etiolated plants according to treatment. With the methodology used, there were 90 percent of acclimatized plants in three weeks. The highest mortality rate was observed in the coconut husk fiber substrate. The results showed a lower percentage of dead seedlings in Jiffy®, but a higher percentage of etiolated plants. In the study, it was found that the use of substrates such as Soil-Shrub-Rice Chaff and Jiffy® contributes to an adequate seedling development, as long as the entry of light is reduced in the early days, and substrate is moisture saturated.

INTRODUCTION

The tissue culture plays an important role in world´s agriculture due the opportunities of rapidly propagation of desired plants. The development of coffee hybrids with remarkable agronomical potential, quality, productivity and disease tolerance, gave an important gain in harvest and market, however, it´s extensive lapse of time required for genetically stabilization, limits the offer to producers. Because of that, the clonation of those plants by *in vitro* techniques protrudes as an important tool for research and in rapid in field use.

As final step for the propagation of the *in vitro* coffee, is the hardening *ex vitro*. This process involves the diminution of controlled conditions, in adaptation to light, humidity, temperature and nutrition.

The present article refers to the evaluation of different substrates during the acclimatization of selected plants obtained under *in vitro* conditions in Costa Rica.

OBJECTIVE

Evaluate the response of several substrates during the acclimatization of *in vitro* coffee plants.

MATERIALS AND METHODS

A plastically made tunnel was accondicionated inside a greenhouse in Alajuela city. The *in vitro* plants corresponding to the F1 Hybrid L13A44 were obtained from the Biotechnology Laboratory from CATIE, transported in flasks to the experimental location and freed 24 hours by opening their lid but keeping inside the container. A total of 2952 plantlets reproduced by indirect somatic embryogenesis were used.

Four combinations of substrates were evaluated: Peat Moss (Jiffy®) (#7, in 30 mm pellet); Soil + Coconut Fiber (1:1) (SF), Soil + Pulp Compost + Rice Husk (2:1:1) (SBG), and Soil + Organic Compound + Rice Husk (2:1:1) (SAG), all of them where previously heat sterilized with PRO-GROW (Model SS-15), during 24 hours at 170°C. The substrates where settled in plastic trays of 98 units (7x14): 1 ¼ high and 2 inches depth. Jiffy´s ® where located in their commercial support of 150 units capacity.

Once each plantlet was planted in one treatment substrate, where saturated inside the tunnel with water and leaved during 7 days, covered with 80/20 shade cloth, a daily temperature of 33°C and 20°C at night, with a relative humidity of 95 %. Later the shade was removed and modified the humidity to 85% through other seven days. The acclimatization phase concluded at third week eliminating the tunnel structure and implementing a 15 minutes irrigation, 3 times a day, with same temperature and a relative humidity of 80%. For a period of 45 days, each week was evaluated for every substrate treatment, the number of etiolated, death plants and contamination.

RESULTS AND DISCUSION

Survival percentage showed variation according to each treatment and week of evaluation; the second being high in the first two weeks, but decreasing at the last two evaluations. The lowest percentage was obtained by SAG, evidenced since the 4th week (Figure 1).

The highest survival´s response substrate for acclimatization was SBG, with 94%, followed by the Jiffy® with 91%, SF with 90% and SAG with 88%.

Despite of higher level of abnormal plants obtained (7,2%), dwarf (10,2%) and etiolated (4,5%), SBG was the best media for acclimatization, because it´s constitution gave a better consistency, keeping enough humidity but permitting an adequate flow, needed for adequate survival. For the Jiffy's® case, appeared to be capable as a good acclimatization support; however had the inconvenient that need more control of the water retention. The fiber substrate, showed a good retention of humidity and a plantlet response. The SAG substrate had some difficulties of water saturation, but with same survival response than SF.

No direct relationship was obtained between aclimatizated, abnormal, smaller or etiolated plants, principally due an additive effect, but not dependent to progress.

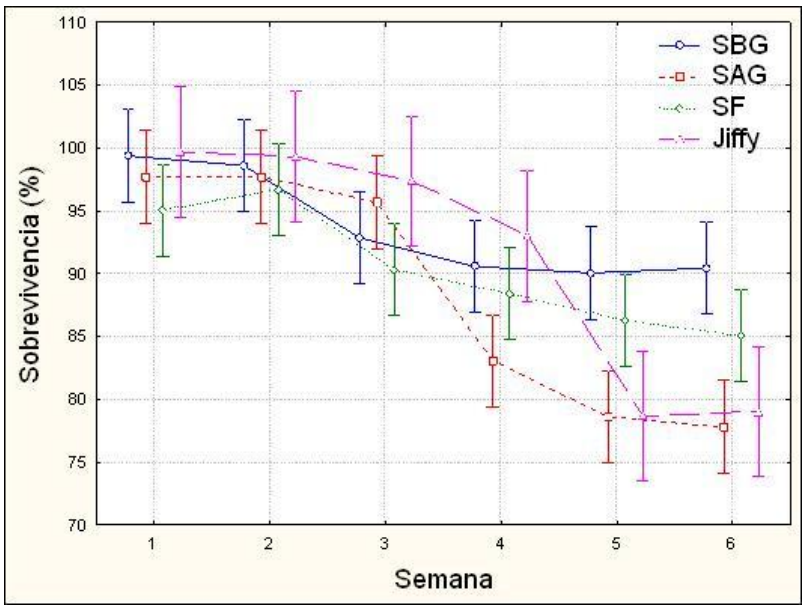

Figure 1. Percentage of survival plantlets according to each substrate treatment.

CONCLUSIONS

- Substrates used for acclimatization of in vitro coffee plants, could improve or affect the plants development.
- The study checked that the use of substrates like Soil, Pulp Compost, Rice Husk or Jiffy®, allow and adequate development of plantlets, dismiss of a graduated decrease of humidity and gas exchange.

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Phenology of Coffee Fruits in Relation to Coffee Berry Borer (*Hypothenemus hampei*) Attacks

M. ROJAS, A. GAMBOA, M. MORA, R. ALFARO, G. RAMÍREZ, J. ARAYA, P. MURILLO, L. RODRÍGUEZ, J. ARIAS, C. FALLAS, O. RODRÍGUEZ

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica

SUMMARY

The attacks of the coffee berry borer (*Hypothenemus hampei*) start 40-50 days after blooming, but generally, the insect reproduces until the coffee fruit has accumulated 20 percent of dry matter. The purpose of these studies was to calculate the number of days between blooming and the moment in which the coffee fruit reach 20 percent of the dry matter in 19 Costa Rican coffee areas.

Research was conducted during 2002, 2003, 2004 and 2006 in Pérez Zeledón (four locations), Buenos Aires de Puntarenas, Coto Brus, Cartago, Acosta, Tarrazú, Valverde Vega, Turrialba, Paraíso, Atenas, San Ramón, Poás, Tres Ríos, Puriscal, Grecia and León Cortés, in ranges from 550 to 1740 m.a.s.l. The fruit development was studied in multiple and one-time blooming. The fruit samples were collected from shoots previously marked in the plants selected every two weeks and were taken to the laboratory to determine their dry weigh.

The number of days between blooming and the moment when the fruits reach 20 percent of dry matter differed depending on the location and the prevailing weather conditions in each area. In general, it was found that the fruits accumulate dry matter faster in the lower areas, while more time is required in the higher areas for the coffee berry borer to reproduce inside the fruits. The results indicated that under the Costa Rican coffee growing conditions the coffee berry borer can start laying eggs 120 days after bloom (DAB) in the lower areas and up to 172 DAB in the higher areas. The pest management practices, such as applying *Beauveria bassiana* should be performed at least one month before the fruits accumulate 20 percent of dry matter.

INTRODUCTION

Attacks CBB (*Hypothenemus hampei*) in fruits starts from 40-50 days after flowering, but the insect reproduction occurs normally until the fruit has accumulated 20% of dry matter. Before this, the CBB can bite and leave the fruit or remain in the perforation channel for some time, waiting for it to be able to start oviposition (Table 2).

In Colombia the coffee berries reach 20% of dry weight between 120 and 150 days after blooming, depending on the production area (Arcila sf). In the Dominican Republic under conditions of 22 ° C, Camilo et al (2003) reported that the onset of reproduction of CBB in the fruit was presented to the 122 days after the main flowering.

The purpose of these studies was to calculate the number of days between blooming and the moment in which the coffee fruit reach 20 percent of the dry matter in 19 Costa Rican coffee areas.

MATERIALS AND METHODS

Investigations were conducted during the years 2002, 2003, 2004 and 2006 in Pérez Zeledón (four locations), Buenos Aires, Coto Brus, Cartago, Acosta, Tarrazú, Valverde Vega, Turrialba, Paraíso, Atenas, San Ramón, Poás, Tres Ríos, Puriscal, Grecia and León Cortés, in ranges from 550-1740 masl (Table 1). Was studied fruit development on condition of single and multiple flowering. Fruit samples were taken from previously marked nodes in the selected plants every two weeks and were taken to the laboratory to determine the dry weight.

RESULTS AND DISCUSSION

The days passed after blooming until the fruit reached 20% dry matter was variable, depending on location, influenced by the climatic conditions prevailing in each area. In general it was found that the fruit dry matter accumulates faster in the lower areas, while in the higher areas should take more time so that the coffee berry borer achieved multiply inside the fruit. The results indicated that under the conditions of Costa Rican coffee production, the CBB can begin egg laying from 120 days after flowering (DAF) in the downstream and up to 172 DAF in the areas of greatest height.

We found very little difference in the accumulation of 20% dry matter by comparing single and multiple blooms (Table 1). 80% of the general data of dry matter accumulation in the fruits according to the days elapsed after anthesis, conform to an equation that responds to the height (Figure 1), which is related to the temperature.

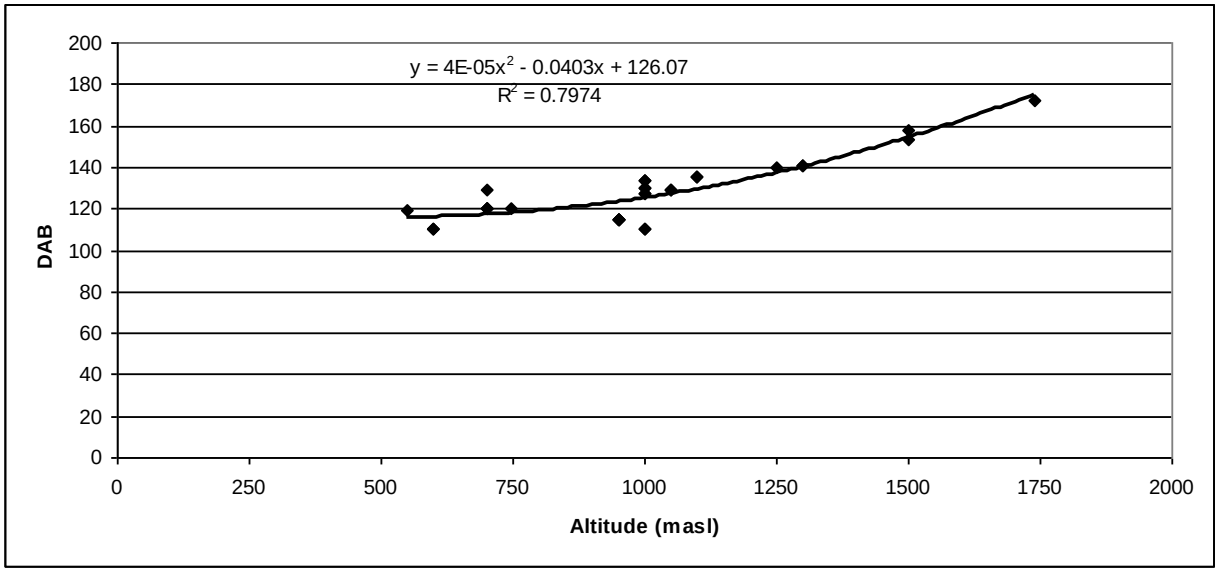

Figure 1. Curve of 20% accumulation of dry matter in fruits, depending on the height in 19 locations of Costa Rican coffee.

The activity of CBB on fruits was a function of the accumulation of dry matter (Table 2). Under conditions of 700 masl and single bloom in Pérez Zeledón, 20% dry matter was reached at 129 DAF and 11 days after larvae were already. Penados and Ochoa (1977) reported the same condition at 147 DAF, but Cure et al (1998) reported in Brazil the CBB waits until the onset of ripening to start laying.

In Colombia agree that the coffee fruit reaches 20% of dry weight between 110 and 140 days after blooming and CBB play starts on fruits of more than 120 days. They also showed that

oviposition occurs in less than 5 days in fruits of more than 150 days with a 27% dry weight, while taking up 90 days when exposed to fruits 60 days old (Bustillo 2006).

Table 1. Study sites, altitude and days after blooming for the accumulation of 20% of dry matter in fruits single and multiple flowering.

Study site	Altitude (masl)	20% dry matter (days after blooming)	
		One-time blooming	Multiple blooming
Buenos Aires	550		119
Turrialba	600	110	110
Pérez Zeledón	700		120
Pérez Zeledón	700	129	
Pérez Zeledón	746		120
Valverde Vega	950	115	115
Grecia	950	97	97
Acosta	1000	134	134
Coto Brus	1000	110	127
Atenas	1000	130	130
San Ramón	1050	129	129
Puriscal	1050		105
Paraíso	1100	135	135
Pérez Zeledón	1250		140
Desamparados	1300	141	141
Tarrazú	1500	158	153
León Cortés	1740		172

Table 2. Activity of the coffee berry borer, according to the percentage of dry matter accumulated in coffee fruits, variety Catuaí. San Isidro, Pérez Zeledón, 2004.

Days after blooming	Dry matter (%)	Observations
67	11,8	Drilling
81	13,7	Drilling
96	16,3	Drilling
111	18,1	Biting endosperm
124	19,3	Biting endosperm
140	22,2	Eggs and larvae
154	27,3	Eggs and larvae

The differences between growing regions on dry matter accumulation in the fruits and the subsequent penetration of the coffee berry borer, marks an important contrast management. In areas of lower altitude control practices such as *Beauveria bassiana* applications and/or insecticide should be made at least one month before the fruit accumulate 20% of dry matter, in order to prevent the insect manages to penetrate and affect the grain. Registration of flowering dates and their magnitudes provide practical information relevant to the farmer on the critical times to apply the most appropriate management practices, according to the level of pest attack and the position of the insect in the fruit.

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Releasing Assessment of *Prorops nasuta* to Control the Coffee Berry Borer in Turrialba, Costa Rica

M. ROJAS, J. OBANDO, C. DELGADO, G. GUERRERO

Instituto del Café de Costa Rica (ICAFE), Centro de Investigaciones en Café (CICAFE)
Apartado postal 37-1000 San José, Costa Rica

SUMMARY

The purpose of the study was to evaluate the establishment of *Prorops nasuta* and its control on the coffee berry borer in the coffee growing area of Turrialba, Costa Rica. The parasitoids were released in 20 farms (31.3 ha) between December 2007 and February 2008. At the beginning, the coffee berry borer total in different stages was between 9.8 and 14.2 individuals per fruit. The release relation was between 0.08 and 9.9 parasitoids per fruit infested with the coffee berry borer. Samples of fruits infected were dissected during the 2008 postharvest. The establishment and spread of the parasitoid were studied during the 2008/2009 harvest and the 2010 postharvest.

The average levels of parasitism after release reached 17, 28, 28 and 27 percent for the first, second, third and fourth months, respectively; with 6.0, 4.3, 4.1 and 4.3 stages of the parasitoid per parasitized fruit, respectively. A month after the release *P. nasuta* reduced the coffee berry borer population of eggs and adult insects in more than 80 percent and the first-instar larvae in 70 percent, in relation to the fruits with no parasites. At the beginning of the 08/09 harvest, there were parasitoids in 78 percent of the plots, and there were found parasitoids at 50 and 200 meters of the release plot in 44 and 33 percent of the farms, but the values decreased during the harvest.

In the 2010 postharvest, there were parasitoids in 62 percent of the release farms and in 71 percent of the sampled farms with no release, even at several kilometers from the release site. *P. nasuta* was found in 90 percent of the cases in the fruits in the plants and 40 percent in the fruits on the ground. It was concluded that the parasitoid was established and spread in the coffee plantations, providing good control of the coffee berry borer and showing its potential within the integrated pest management.

INTRODUCTION

Among the integrated management practices CBB worldwide, is included the release of the African parasitoids *Cephalonomia stephanoderis*, *Phymastichus coffea* and *Prorops nasuta*, which have been introduced to Latin America primarily for research and development of pilot projects. The adult of *Prorops nasuta* depredates adults, eggs and small larvae of coffee berry borer, mainly in mature and dry fruits. Also parasitizes larvae of the last instar and pupae, in which lays an egg. The *P. nasuta* larvae feed on the hemolymph of the immature of CBB, up to eliminate it and then spin a cocoon where they complete their development. According to Borbón (1991) the female of *P. nasuta* threaded from 8 to 20 eggs per fruit.

In field investigations, Trejo (2003) reported parasitism from 10% to 65% and mortality of CBB states of 95% in fruits parasitized by *C. stephanoderis* and *P. nasuta* in Honduras. Bacca

(1999) cited by Aristizábal (2003) mentions that the release of *P. nasuta* after harvest, reduced the population of CBB immediately, through predation and parasitism.

Borbón (2007) reported that inundative release of *P. nasuta* in Costa Rica in 2003 was obtained nearly 40% of control on average. He added that it was found in 93.3% of the lots which it was released and also dispersed up to 700 m of the lots which it was released, being found in 44.4% of sampled plots.

The purpose of the study was to evaluate the establishment of *Prorops nasuta* and its control on the coffee berry borer in the coffee growing area of Turrialba, Costa Rica.

MATERIALS AND METHODS

The parasitoids were released on 20 farms (31.3 ha) between December 2007 and February 2008. The sum of CBB stages initially ranged between 9.8 and 14.2 individuals per fruit and release ratio ranged from 0.08 to 9.9 wasps per attacked fruit (Table 1). During the post-harvest 2008, attacked fruits were dissected to determine parasitism and reproduction. During the 2008/2009 harvest and postharvest time of 2010, we evaluated the establishment and spread of the parasitoid in selected lots.

Table 1. Location of farms and conditions of release of parasitoids.

Farm	Location (District)	Stages CBB/fruit	Release ratio wasps/attacked fruit
San Joaquín	La Suiza, Turrialba	10,6	0,08
Puebla Real	La Suiza, Turrialba	11,7	0,23
La Isabel	La Isabel, Turrialba	11,7	9,93
Santa Rosa	Santa Rosa, Turrialba	9,8	0,59
CATIE Promecafé	Turrialba, Turrialba	11,5	0,21
Congo	Tucurrique, Jimémez	11,3	0,50
CATIE Cabiria	Turrialba, Turrialba	16,3	0,77
Colima	Santa Teresita, Turrialba	10,5	0,36
Celulosa	Pavones, Turrialba	12,3	0,64
Aquiares	S. Rosa, Turrialba	14,2	1,46
La Gloria	Juan Viñas, Jiménez	11,0	1,60
Papalico	Juan Viñas, Jiménez	10,0	1,24
San Hilario	Turrialba, Turrialba	10,5	1,72
Blanes	Turrialba, Turrialba	10,9	0,74
Belgravia	La Suiza, Turrialba	10,4	1,22
B. Badilla	Turrialba, Turrialba	13,0	3,17
Navarro	Dulce Nombre, Cartago	11,6	0,48
Las Chúcaras	Orosi, Paraíso	11,5	1,20
Zalmari	Cachí, Paraíso	13,2	0,86
Luis Montejo	Santiago, Paraíso	13,3	1,33

RESULTS AND DISCUSSION

The average parasitism after release reached 17%, 28%, 28% and 27% for the first, second, third and fourth months, respectively, with 6.0, 4.3, 4.1 and 4.3 of the parasitoid stages for fruit parasitized respectively (Figure 1A). A month after release *P. nasuta* was able to reduce the population of eggs and adults of CBB in 80% and first instar larvae in 70%, compared to

unparasitized fruit (data not shown). At the beginning of the crop 08/09 parasitoids were found in 78% of lots, 50 and 200 meters of the lot released parasitoids was observed in 44 and 33%% farm, but the values decreased during harvest (Figure 1B).

In the post-harvest period 2010 parasitoids were found in 62% of the farms where it was released and 71% of farms where it was not released. *P. nasuta* was found in 90% of cases in the fruits of the plant and in the 40% in the ground fruits (Table 2).

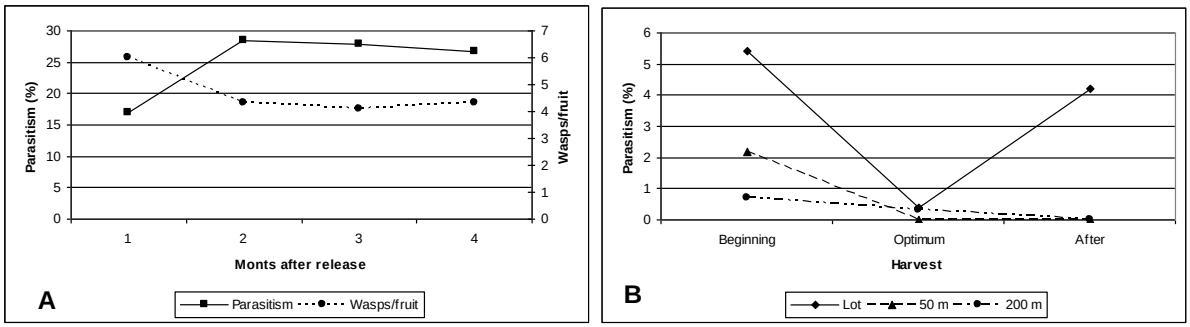

Figure 1. Percentage of parasitism and number of biological states of *P. nasuta* after release (A) and dispersal of the parasitoid during the harvest 2008/2009.

Table 2. Identification of *P. nasuta* in fruit samples taken from the plant and soil in the postharvest period 2010 (March, April, May and June), on selected farms.

Farm/Lot	Release	Identification of *P. nasuta*	
		Plant	Soil
CATIE 1	No	Yes	Yes
CATIE 2	No	No	No
CATIE 3	No	Yes	No
CATIE 4	No	Yes	No
Santa Rosa 1	Si	Yes	Yes
Santa Rosa 2	No	Yes	Yes
Aquiares 1	Si	No	No
Aquiares 2	No	No	No
B. Badilla	Si	Yes	No
Navarro	Si	No	No
Chúcaras 1	Si	Yes	No
Chúcaras 2	No	Ye	Yes
Zalmari	Si	Yes	No
La Gloria 1	Si	No	No
La Gloria 2	No	No	Yes

The condition of attacked fruits per hectare was quite variable between farms, allowing assess parasitoid behavior in different environments, heights and release ratio of wasps per attacked fruit. Was obvious the high number of states of *P. nasuta* per parasitized fruit, showing its good establishment in the coffee plantations in the region.

The evaluation about establishment and spread in the crop 08/09 in the 9 selected farms showed that CBB control was low, and was seen the effect of fruit harvesting, eliminating wasps. The amount of biological states per fruit parasitized also decreased, indicating that the harvest leaves little chance for the parasitoid to reproduce. Quintero et al (1998) indicate that

the harvest of ripe fruits every 15 days and precipitation are the main factors contributing to the decline of parasitism.

During the postharvest period 2010 *P. nasuta* was still present in a high percentage of lots which it was released, highlighting the ability of dispersion to other farms located even several kilometers from the release sites. However, to get results of impact on pest control must be made each year releases of wasps to minimize the effect of the harvest. In this aspect, inoculative release would be better adapted to our conditions than inundative releases.

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Genetic Characteristics of Populations of Robusta Coffee in the State of São Paulo, Brazil

J. C. MISTRO[1], L. C. FAZUOLI[1], M. T. BRAGHINI[1], E. S. MANTOVANI[1], G. S. GIOMO[1], R. VENCOVSKY[2], M. D. V. RESENDE[3]

[1]Centro de Café - Instituto Agronômico (IAC/APTA), Campinas, SP, Brasil
[2]Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ) – Universidade de São Paulo (USP) - Departamento de Genética, Piracicaba, SP, Brasil
[3]EMBRAPA Florestas, Colombo, PR, Brasil

SUMMARY

The current research was to estimate genetic parameters that help to understand the characteristics and the genetic potential of two populations of robusta coffee in the São Paulo State, Brazil. Two experiments were established, one obtained by sexual propagation (seedling progenies) and the other by asexual propagation (clones), following a randomized block design. There were four consecutive harvests, and the data assessed using linear mixed models (REML/BLUP). The clonal population showed greater potential for genetic improvement than the population consisting of progenies. The coefficients of residual variation in the seedling experiment were higher than in the clonal experiment, both in the analyzes for individual years and for all years combined.

INTRODUCTION

Many of the agronomical important traits present quantitative inheritance. This type of inheritance cannot be studied in the same way as the inheritance of qualitative traits. Quantitative inheritance produces a large number of phenotypes which makes it impossible to associate different genotypes to phenotypes. There is an area of genetics that studies quantitative variation, ie, quantitative genetics. Analyses of quantitative traits are based on the quantification of variation in populations. In this case, quantitative traits are to be described in terms of statistical parameters. The term parameter is used to denote the characteristics of a population, particularly its mean and variances for different traits. From these parameters, we can estimate others that will serve as basic knowledge of the genetic structure of the population and the potential for genetic improvement of the same, for the efficient selection of superior individuals based on their genotypic and not phenotypic values.

Among these parameters, we highlight the heritability, coefficient of genetic variation and selection index. Heritability can be estimated in two ways: the broad and narrow sense. The broad sense heritability can be defined as the ratio of genotypic variance by phenotypic variance, whereas the narrow sense heritability is the ratio of additive genetic variance to phenotypic variance. The narrow sense heritability is most useful in species with sexual propagation, since it quantifies the relative proportion of additive genetic variance that may be transmitted to the next generation. The broad-sense heritability assumes greater importance in species with asexual propagation, in which genotypes are fully inherited by the offspring [3].
This study aimed to estimate genetic parameters that help to understand the characteristics and the genetic potential of two populations of *Coffea canephora* (robusta) coffee in the State of São Paulo, Brazil.

MATERIALS AND METHODS

Two experiments were established, one with sexually propagated progenies (seedlings) and another with asexual propagated material (clones), of robusta coffee in Campinas (SP, Brazil), following randomized block designs. The treatments that comprise each of the experiments were selected from a population in Costa Rica and later in Mococa (SP, Brazil) and, therefore, these genotypes were in the third selection cycle.

The first experiment was composed of 28 clones, three replications and four plants per plot. The second experiment consisted of 30 seedling progenies, with four replications and eight plants per plot. Observations were carried out during four harvests (2008, 2009, 2010 and 2011) and the data were analyzed by SELEGEN. The following mixed model, in matrix format, was adopted:

$$Y = Xb + Zg + Wp + e$$

Where Y = vector of phenotypic observations on each character, X = incidence matrix for fixed effects (blocks and overall averages), b = vector of fixed effects, Z = incidence matrix for genotypic effects considered at random; g = vector of genotypic effects, W = incidence matrix for the effect of plots, p = plot effects vector and e = vector of residual random error effects.

Due to the imbalance of data and the four harvests being measured on the same experimental unit, the variance components and the components of means were obtained using the methodology of a linear mixed model REML/BLUP.

RESULTS AND DISCUSSION

Clonal experiment

Table 1 presents estimates of genetic parameters of samples taken between 2008 and 2011 on 28 clones of robusta coffee. The years of low yields (2008 and 2010) and high yields (2009 and 2011) were well characterized. We observed the presence of genetic variation in all four harvests, except for the combination of the 1st and 3rd harvests (low yields). It was unexpected that in 2010 (3rd crop) the yield suffered a drastic reduction, because yield is normally increasing up to the 5th harvest. The low yield was due to the occurrence of dry periods in 2010 undermined this trend, anticipating the biennial yield cycles, alternating between low and high yields.

The analyses of individual harvests had more significant statistical parameters than the joint analysis over more than one harvests. However, the breeder should be very careful, because these figures are probably inflated by the effect of years. Thus, despite the genetic parameter estimates were lower, it is preferable to perform the selection of the best clones based on average production of four harvests, which levels off the effect of years. One could also make a selection considering only the years 2009 and 2011. It was thus possible to combine high yields with high genetic variability, besides low coefficients of experimental variation. Both for the joint analysis of the four crops as for the 2nd and 4th, the coefficient of relative variation was above 1.0, a desirable condition for selection impact. The results estimated for the various genetic parameters, shown in Table 1, demonstrate that in the population under study exists the probability of success in further selection cycles.

Table 1. Estimates of genetic parameters related to harvests between 2008 and 2011 in the trial with *Coffea canephora* (robusta) clones.

Parameters[1]	Individual harvests				Combinations harvests[2]			
	2008	*2009*	*2010*	*2011*	*1ª, 2ª, 3ª, 4ª*	*2ª, 3ª,4ª*	*1ª, 3ª*	*2ª, 4ª*
V_g^2	0,57[B]	3,91[B]	1,76[B]	17,25[B]	2,04[B]	2,76[B]	0,33[ns]	6,30[B]
h_g^2	27,27	33,86	42,49	42,75	14,02	15,23	11,47	24,95
h_m^2	69,43	78,50	84,46	85,97	55,34	59,49	42,12	55,97
CV_g(%)	77,28	48,59	94,01	48,37	37,95	35,41	48,18	39,67
CV_e (%)	83,51	40,21	65,67	29,20	36,35	54,25	62,87	22,87
CV_r (%)	0,92	1,21	1,43	1,66	1,04	0,65	0,77	1,73
Mean (kg/plant)	0,98	4,07	1,41	8,59	3,76	4,69	1,19	6,33

[A] and [B] X^2 (chi-square) tabulated: significant at 5% (3.84) and 1% (6.63) probability, ns = not significant by the likelihood ratio test (LRT).

[1] V_g^2 = effect of clones; h_g^2 = broad-sense heritability; h_m^2 = heritability based on averages of clones; CV_g =coefficient of genotypic variation;CV_e = coefficient of variation residual; CV_r = coefficient of relative variation.

[2] 1st and 3rd harvests (2008 and 2010) = considered as low yielding years; 2nd and 4th harvests (2009 and 2011) = considered as high yielding years.

Seedling progenies experiment

The analysis of deviance showed the existence of genetic variability among progenies only for the fourth harvest, obtained in 2011, the year with highest yields (Table 2). Heritability in the narrow sense, based on the plot averages, was high this year, despite that the genetic variation coefficient was lower in 2011 than in 2010. Possibly the lower value of this coefficient (CV_g) must have been influenced by the higher average yields observed in 2011, whereas the lower overall averages in 2010 might have resulted in a higher value of CV_g. The coefficient of relative variation (CV_r), which disregards the influence of the trait means, was higher in 2011 than in 2010. In 2011 the coefficient of residual variation, or experimental variation, was the lowest of all years (35%). This is in contrast with higher genetic variance in 2010.

Table 2 - Estimates of genetic parameters related to harvests between 2008 and 2011 for the seedling experiment of *Coffea canephora* (robusta).

Parameters[1]	Individual harvests				Combinations harvests[2]			
	2008	*2009*	*2010*	*2011*	*1ª, 2ª, 3ª, 4ª*	*2ª, 3ª, 4ª*	*1ª, 3ª*	*2ª, 4ª*
V_a^2	0,04[ns]	0,76[ns]	0,53[ns]	7,10[A]	0,14[ns]	0,19[ns]	0,05[ns]	1,80[ns]
h_r^2	2,52	5,63	12,13	17,39	0,95	0,97	1,67	6,63
h_m^2	10,88	21,01	37,72	51,82	6,49	4,76	6,51	15,81
CV_g(%)	18,14	9,91	28,38	18,12	5,56	5,03	12,22	11,41
CV_e (%)	103,14	38,42	72,78	34,95	73,23	68,42	118,42	67,47
CV_r (%)	0,18	0,26	0,39	0,52	0,08	0,08	0,10	0,34
Mean (kg/plant)	0,56	4,39	1,29	7,35	3,40	4,34	0,93	5,87

[A] and [B] X^2 (chi-square) tabulated: significant at 5% (3.84) and 1% (6.63) probability, ns = not significant by the likelihood ratio test (LRT).

[1] V_a^2 = additive genetic effect; h_r^2 = heritability in the narrow sense; h_m^2 = heritability based on averages of clones; CV_g = coefficient of variation genotypic; CV_e = coefficient of variation residual; CV_r = coefficient of variation relative.

[2] 1st and 3rd harvests (2008 and 2010) = considered of low yields; 2nd and 4th harvests (2009 and 2011) = considered of high yields.

One way to increase the frequency of favorable alleles in this population, in the medium-term, would be the use of recurrent selection. The environmental variation coefficients in the experiment were higher than with the clonal population, both in the individual years and joint analyzes. All genetic parameters estimated in the seedling population were lower than in the clonal population, showing that the fixation of genotypic variation is faster through clonal selection than through seedling selection.

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The Mutation Laurina Requires Blue Light to Express Dwarfism

J. HOAREAU[1], A. BRUGEL[2], J. SALMONA[2], T. JOËT[3], M. NOIROT[2]

1 Université de la Réunion, 97415 St Denis, France
2 IRD, UMR PVBMT, Pôle de Protection des Plantes, 97410 St Pierre
3 IRD, UMR DIADE, Centre IRD de Montpellier, 34000 Montpellier

SUMMARY

Coffea arabica 'Laurina' is a natural mutant of Coffea arabica 'Bourbon' (B) and is commercially known as 'Bourbon pointu' (BP). The laurina mutation leads to pleiotropic effects, including semi-dwarfism. The aim of this study was to highlight the role of light on semi-dwarfism.

There was no longer a size difference between B and BP seedlings grown under dark conditions. There was also no difference in gene expression in darkness, but the main findings revealed overexpression of cryptochrome 1 in under daylight. Lastly, seedlings grown under blue light confirmed its role on hypocotyl semi-dwarfism.

INTRODUCTION

Coffee is the second agronomic crop in terms of international trade after palm oil. *Coffea arabica* L. (2n = 4x = 44) is preferentially cultivated in the highlands and characterized by its low bitterness, high aroma and low caffeine content - 1.1% dry matter basis (Ky et al. 2001). Its production represented 65% of world coffee production in 2005-2006. On the island of Réunion (France), a Premium coffee is currently developed from the variety BP, which corresponds to *Coffea arabica* 'Laurina' (Lécolier et al. 2009a). Understanding the origin of its better qualitative value is a key challenge for the genetic improvement of coffee trees.

In fact, *Coffea arabica* 'Laurina' is a natural mutant of *Coffea arabica* 'Bourbon' (B) (Chevallier 1947; Lecolier et al. 2009a). *Laurina* is a monolocus, recessive Mendelian mutation (Krug 1949; Krug and Carvahlo 1951; Krug et al. 1954) and characterized by pleiotropic effects: smaller internodes and leaves, Christmas tree shape (Fig. 1) (Lecolier et al. 2009b, 2009c), better resistance to cold and drought (Chevalier 1947) and lower caffeine content in seeds (Mazzafera et al. 1997). The first aim of the present study was to compare the seedling morphology of B and BP growing under daylight or darkness to determine whether daylight contributes to th emorphological difference between B and BP. As light is known to inhibit the developmental process of growth during photomorphogenesis, hypersensitivity to light might explain the semi-dwarfism in BP.

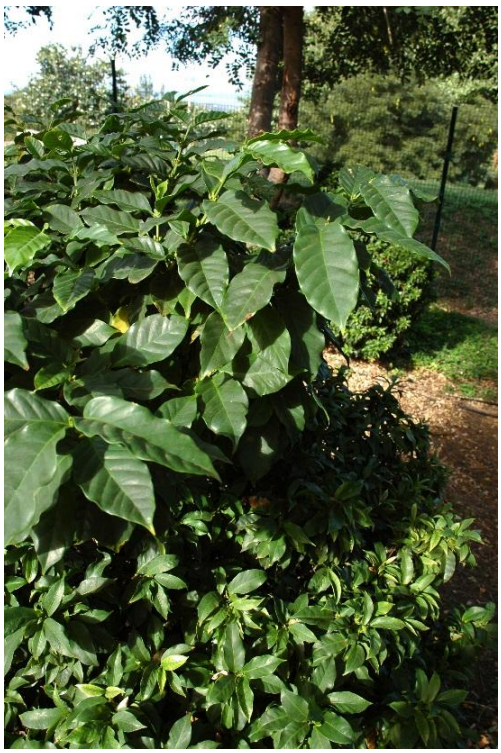

Figure 1. Bourbon pointu showing reversion to the Bourbon type.

Light responses during plant growth are regulated by phytochromes, cryptochromes and phototropines. Phytochromes allow plant responses to red or far-red light. The two other types of photoreceptors – cryptochromes and phototropines - are sensitive to blue light and UV-A. Nevertheless phototropines do no regulate hypocotyl length, i.e dwarfism (Ohgishi et al. 2004). Concerning cryptochromes, CRY1 also has a major impact on the hypocotyl length under blue light (Ahmad et al. 1995; Lin et al. 1996), whereas CRY2 has a weak effect (Mas et al. 2000). In summary, *PHYA, PHYB,* and *CRY1* are the most efficient genes for inducing hypocotyl growth inhibition under daylight conditions, and each of them could be overexpressed under daylight conditions, explaining the semi-dwarfism of BP. Four other genes, whose expression is known to be modified in the presence of light, were also added to the study. This is *COP1* (Constitutive photomorphogenic 1) whose expression is related to that of *CRY1* (Deng et al. 1991; Wang et al. 2001; Yang et al. 2001), *CAB1* (Chlorophyll A/B binding protein 1) (Cuming and Bennett 1981), *GI* (Gigantea), which is related to the circadian rhythm (Tseng et al. 2004), and *RPT2* (Root Phototropism 2) (Sakai et al. 2000).

As semi-dwarfism would depend on cell division or elongation, or both, the expression of four genes known to be implied in either of these processes or both were also studied. This included *CDKB2:2*, *CDC48D*, *EXPA8,* and *GPA1*, which code for a cyclin dependent kinase (Porceddu et al. 2001), a cell division control protein (Fröhlich et al. 1991), an alpha expansin (McQueen-Mason and Cosgrove 1995), and a G-protein α-subunit (Okamoto et al. 2001), respectively. Whether the first and main aim of the study was to describe the impact of daylight on seedling morphology, the second aim was to check whether the B and BP seedlings growing under darkness showed or not difference of expression for the above cited genes. For such comparison, gene expression was recorded on cotyledons on four sets of seedlings (B-light, B-darkness, BP-light and BP-darkness).

MATERIALS AND METHODS

Plant material and experimental design

Seeds of the two varieties B and BP were sown and grown in either daylight or dark conditions for 65 days. Each combination – B-light, B-darkness, BP-light and BP-darkness – was represented by two germination boxes. In each box, 28 seeds were sown in moistened vermiculite. The boxes were hermetically sealed after sowing. The boxes were wrapped in aluminum foil for the darkness treatments. The location of the eight boxes was randomly determined and changed weekly.

The experiment was carried out twice. The first experiment highlighted the effects of daylight on the seedling morphology. The second experiment was carried out for gene expression analysis. Lastly, a third experiment was carried out to determine whether blue or red light has a major effect on the hypocotyl semi-dwarfism. In this experiment, ten seeds of B and BP were sown in moistened vermiculite and grown in either blue light or red light conditions for 60 days.

Gene expression

The extraction of total RNA and the evaluation of its purity and integrity constituted the first phase. There were two RNA extractions per plant material combination (BP-light, BP-darkness, B-light, and B-darkness). The second step consisted to design primers and to verify the specificity of PCR products. In the next step, two independent reverse transcription reactions were performed for each available RNA sample. The four cDNA samples belonging to the same plant material combination (2 extractions x 2 reverse transcriptions) were pooled to further minimize the residual variance, leading to four cDNA samples (BP-light, BP-darkness, B-light, and B-darkness). Lastly, oolymerase chain reactions were carried out in an optical 96-well plate with an ABI PRISM® 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA), using SYBR® Green to monitor dsDNA synthesis.

In order to obtain an accurate internal normalizer, the median expression (Ct_m) of each combination was computed from a data table including 55 genes constitutively expressed in each of the four following combinations: B-light, B-darkness, BP-light and BP-darkness. ΔCt, which represents $Ct_{(target\ gene)}$ minus C_{tm}, was then computed for the 11 above-cited genes. Fold change values were then estimated from the equation $(1 + E)^{-\Delta Ct}$ (Ramakers et al. 2003; Czechowski et al. 2004), where E is the PCR efficiency estimation.

Statistical analysis

All results were analysed using the Statistica software package (6.1 version, 1997 for Microsoft Windows). Two statistical methods were carried out: one-way ANOVA and principal component analysis. One-way ANOVA was used to compare BP-light, BP-darkness, B-light, and B-darkness for hypocotyl length and cotyledon sizes. When the F test was significant ($p < 0.0001$), a Bonferroni comparison of means was carried out.

A principal component analysis (PCA; Varimax normalized) was applied to the gene expression data recorded on cotyledons to highlight the reduced number of factors explaining the generalized variance recorded between BP-light, BP-darkness, B-light, and B-darkness.

RESULTS AND DISCUSSION

BP and B seedlings were phenotypically different only under light conditions

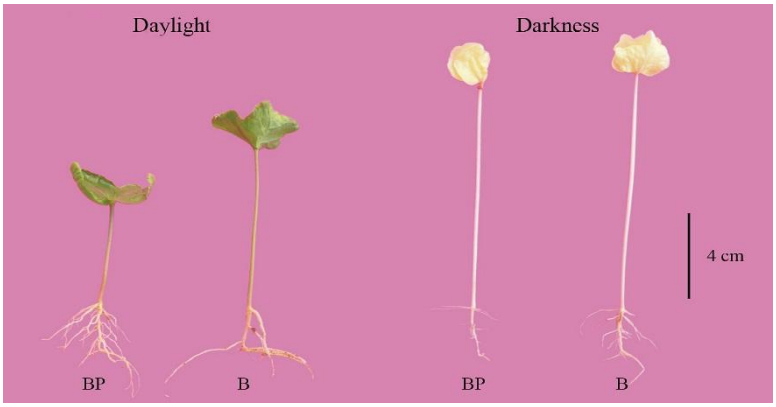

Figure 2. Bourbon and Bourbon Pointu seedlings under light (left) and dark (right) growing conditions.

Differences between BP and B appeared only under daylight conditions (Fig. 2). Under daylight, phenological differences mainly concerned hypocotyls and roots. Hypocotyl length was significantly lower in BP (4.5 cm vs 7.2 cm). Under darkness, the difference was not significant. BP also showed more secondary roots, without major changes in the main root length. In the case of cotyledons, visual differences between varieties seemed minor (Fig. 2). Nevertheless, their area decreased slightly in BP, with a ratio BP/B = 0.77.

As expected from the phenotype similarity, gene expression between B and BP did not vary under darkness conditions

The absence of morphological differences under dark growing conditions suggested that there was similar gene expression between B and BP. This hypothesis was checked on cotyledon transcriptomes and no significant difference was observed for the 11 studied genes.

In cotyledons, two principal components can be defined according to their expression in daylight compared to darkness conditions

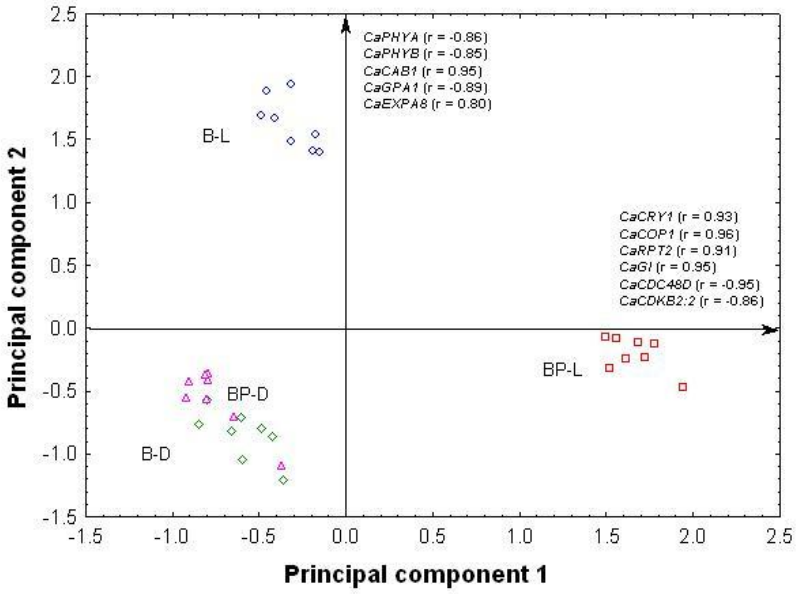

Figure 3. Principal component analysis using cotyledon-gene-expression data in B-light, B-darkness, BP-light, and BP-darkness.

A principal component analysis was carried out to summarize gene-expression variations in a minimum of explanatory factors. Two components explained 92% of the expression diversity. The first one was strongly and positively correlated with *CaCRY1 CaCOP1, CaRPT2, and CaGI*, while negatively correlated with *CaCDC48D* and *CaCDKB2:2* (Fig. 3). This component symbolized the effect of blue light perception on the transcriptome and this phenomenon was called blue light-dependent expression changes (BDEC). The second component was strongly and positively correlated with *CaCAB1* and *CaEXPA8*, while negatively with *CaPHYA, CaPHYB,* and *CaGPA1*. It represented the impact on the transcriptome of the red and far-red light perception by phytochromes A and B and this phenomenon was called red or far-red-dependent expression changes (RDEC).

In Figure 3, three points can be highlighted: 1/ B-darkness and BP-darkness were similar; and 2/ the effect of daylight on B cotyledons was related to PDEC. This would explain both greening and the cotyledon size increase due to light (Fig. 2); and 3/ the effect of daylight on expression in BP cotyledons was mainly summarized by CDEC. Figure 3 also indicates that the mutation would partly inhibit PDEC. Indeed, in the absence of inhibition on PDEC, BP-light seedlings would be located close to the point of coordinates x = 1.7, y = 1.7.

When comparing gene expression in hypocotyls of BP-light and B-light, only CaCDKB2:2 and CDC48D did not show differences. *CaEXPA8* showed a marked expression decrease in BP, which would be responsible for the hypocotyl dwarfism in the mutant. Lastly, the mutation had no effect on the expression of *CaCDKB2:2* and *CaCDC48D*. In hypocotyls, only cell elongation (expansins) were implied in their growth. The lower expression of *CaEXPA8* could explain hypocotyl dwarfism.

Checking *which type of the light — blue or red — has a major effect on hypocotyl dwarfism*

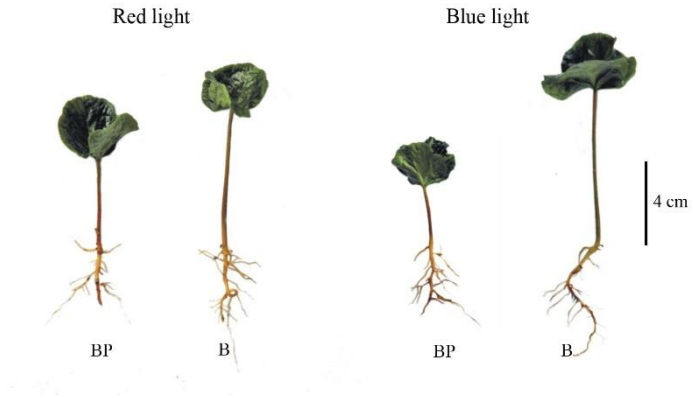

Figure 4. Bourbon and Bourbon Pointu seedlings under red light (left) and blue light (right) growing conditions.

After demonstrating an effect of daylight on the seedling morphology in BP and revealing a difference in expression between BP and B, we assessed the impact of red and blue light on seedling growth. Under red light conditions, hypocotyl length was 6.1 cm and 4.0 cm in B and BP, leading to a BP/B ratio = 0.66. Although growth inhibition was slightly higher than in daylight conditions, the impact of the mutation was similar (0.66 *vs* 0.63). Under blue light, hypocotyl length was 7.5 cm and 2.6 cm in B and BP, leading to a BP/B ratio = 0.34. Obviously, blue light showed a higher impact on hypocotyl dwarfism in BP (Fig. 4).

CONCLUSIONS

In summary, BP would be hypersensitive to blue light. At the gene expression level, *CaCRY1* was constitutively overexpressed in BP under daylight conditions in cotyledons and hypocotyls. This strongly suggests that blue-light detection by CaCRY1 would interact with the *laurina* mutation. Statistical correlations obtained between *CaCRY1* overexpression and *CaEXPA8* downregulation in hypocotyls also suggest that these features could have consequences on growth and could explain the semi-dwarfism of internodes and leaves. Further studies should be focused on phytohormones, which would be a link between blue-light detection and cell division and elongation.

ACKNOWLEDGEMENTS

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Genetic Diversity Assesment in Indonesian *Coffea canephora* Collection using SSR Markers

U. SUMIRAT[1], L. BELLANGER[2], V. L'ANTHOENE[2], S. MAWARDI[1], D. NUGROHO[1], PRIYONO[1], T. WAHYUDI[1], P. BROUN[2], C. LAMBOT[2], D. CROUZILLAT[2]

[1]Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java, Indonesia
[2]Nestle R&D Center, Tours, France

SUMMARY

Coffea canephora which is one of the two most important commercial coffee species is cultivated in Indonesia for more than 100 years. Planting materials collected were originated from various countries in Africa, mainly from two introduction phases in the beginning 20th century. Now, genetic material in the collection is mainly coming from breeding programs using the basic materials from previously introductions. This coffee collection is used in the breeding programs but its genetic diversity was not evaluated yet.

The genetic diversity of the Indonesian *C. canephora* collection was studied using 19 microsatellite (SSR) markers for 1886 accessions. However, due to possible gene introgressions of other *Coffea* species and also presence of duplicates, only 1382 accessions were finally kept for the analysis. Genetic structuration study identified three genetic groups in the collection. Robusta accessions controls included in this study have allowed the identification of two of these three groups as SG1 and SG2. The third genetic group identified appears to be unique with a low level of heterozygosity. According to ICCRI archives, this new group was originated from Republic Democratic Congo and closer to the location of group SG2. Principal Component Analysis clearly differentiates this group among the two others. The study will help the breeders to elaborate the best strategy to manage the genetic diversity according to the molecular and phenotypic data. Results will also be used to guide the breeding program of *C. canephora*.

INTRODUCTION

In many producing countries, the importance of coffee production is based on the diploid species, *Coffea canephora* Pierre ex A. Froehner. Breeding of this species was firstly done in Indonesia, soon after the successful introduction of a group of plants by the Dutch in 1900. Up to now, Indonesia is one of the countries having large coffee collections dominated by Robusta accessions. Even the field assessment on *Coffea* have been intensively used in the last two decades, their genetic diversity have not been assessed by the molecular tool which is an important key for optimum utilizations in the breeding programs. This study was aimed to analyze genetic diversity in Indonesian Robusta coffee collection using SSRS.

We believed that Indonesian coffee collection was covering wide geographic origin of African Robusta. Even if these accessions were mainly coming from breeding populations of *C. canephora*, however, according to some internal reports of ICCRI, there is an evidence that some wild accessions of *C. canephora* may be still present in the collection.

MATERIALS AND METHODS

Determination of genetic diversity in ICCRI's *C. canephora* collection was done involving 1886 accessions. The accessions are managed as working collection and maintenance of trees are according to standard coffee cultivation in Indonesia.

Nineteen microsatellite markers developed by Nestlé Tours were used to determine genetic diversity of the collection. DNA extractions were done on 10 mg of dried coffee leaf for each accession, following the protocol of DNeasy 96 Plant Kit of QIAGEN. For SSRs genotyping, PCR were done by mixing 2.5 µL of DNA elution with 19.5 µL of QIAGEN microsatellite PCR Kit.

The amplification program for PCR kit consisted of 5 min initial denaturation at 95° C, followed by 28 cycles covering 30 s denaturation at 95° C, 90 s annealing at 60° C, and 30 s extensions at 72° C. Final extension of 30 m at 60° C was then performed. In each PCR run, 6 DNA controls were added. Fluorescently labeled PCR products were analyzed on capillary electrophoresis (ABI Prism 3500xL, Applied Biosystems). All accessions carrying alleles outside *C. canephora* species were discarded, such as interspecific hybrid with *C. liberica*, *C. stenophylla* and *C. arabica*. Duplicated accessions were also discarded. Genetic diversity in the collection was analyzed using software STRUCTURE. The number of genetic groups is defined according to the method of Evanno et al., and then mapped using Principal Component Analysis (PCA). All alleles were used to estimate genetic parameters as follow: heterozygosity rate, allele number and polymorphic alleles number.

RESULTS AND DISCUSSION

One thousand and eight hundred eighty six accessions were sent to Nestle-Tours for assessment of the molecular genetic diversity, but 504 of them were discarded due to possible allele introgressions from other coffee species, and also presence of duplicates. 1382 accessions were finally used for the final assessment of their genetic diversity.

Structuration analysis combined with Evanno's method, show the detection of three groups in the ICCRI collection as shown in Figure 1. In this study, we can have the first report on the genetic diversity in the collection.

The first group contains the accessions developed from the populations introduced from Gabon Therefore this group is also containing the SG1 accessions and Conilon. In the Group 2, we identified BP409 clone which is previously identified as Congolese or SG2 member In the third group, we identified the accessions which were previously known as *C. laurentii*. However, Davis et al. has been considered this species as *C. canephora*. Cramer was reported that *C. laurentii* was introduced from Republic Democratic of Congo (RDC).

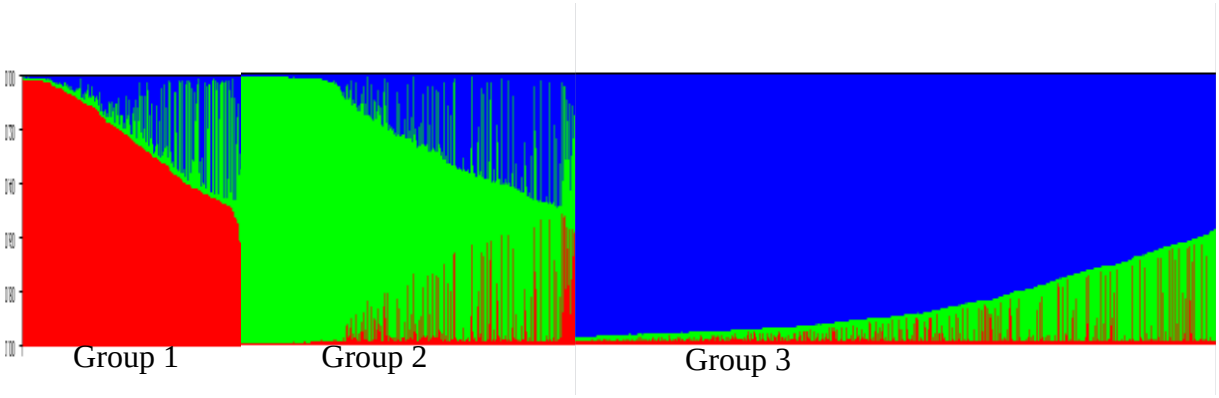

Figure 1. Characterization of three genetic groups in ICCRI C. canephora collection using Structure software.

Up to now, in the recent *C. canephora* structuration analysis a total of seven groups are identified in this coffee species. The group B (Central Africa) and C (Cameroon and Nana population) were characterized by Gomez et al., whereas Uganda group was reported by Musoli et al. The analysis by Cubry et al. reported also the existence of two groups in the Guinean genetic pool. Their works were also successful to differentiate the SG1 (Gabon) and SG2 (RDC). However, in our analysis we identified another different genetic group which is originated from RDC.

Our result, therefore, could be considered as the finding of new genetic group in *C. canephora*. To support this proposal, we selected non-admixture accessions coming from the three groups that have been previously identified in the structuration genetic analysis. There was 12, 28 and 166 non-admixture accessions taken from Group 1, Group 2 and Group 3, respectively. All accessions, including the non-admixture one, were then analyzed by Principal Component Analysis (Figure 2).

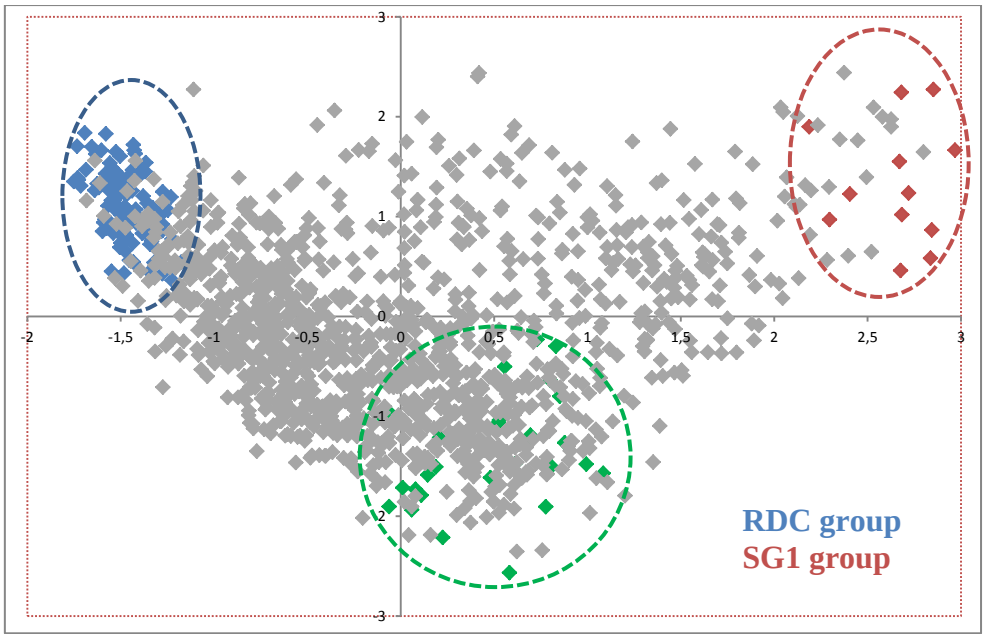

Figure 2. Genetic diversity of ICCRI Robusta collection based on PCA using 19 SSRs. Colored dots are non-admixture accessions identified by Structure analysis. Axis 1 and 2 explained, respectively, 13.4% and 7.5% of the total variance.

According to Figure 2, the three groups identified in ICCRI collection are well defined. However, genetic diversity inside the new putative group is the lowest one, even if the number of non-admixture accessions is the highest among others. Interestingly, this proposed

new group was characterized by a low heterozygosity rate, lowest allele number per group and lowest average polymorphic allele compared to the two other groups as shown in Table 1.

This result could be used for the establishement of a core collection. However, further analysis is required, involving the introduction of already known Robusta accessions from diverse genetic groups, in order to enrich the genetic diversity in this collection. This result has enlarged the genetic diversity in *C. canephora* and increase the possibility to elaborate new breeding programs. Furthermore, *C. canephora* due to its allogamous nature requires distantly genetic variability between parental clones to achieve high hybrid tree performances on valuable agronomic traits.

Table 1. Comparison of genetic parameters in the three groups in ICCRI collection.

Genetic Parameters	RDC	SG1	SG2
Average heterozygosity rate	31%	60%	55%
Number of allele per group	34	116	146
Number of specific allele per group	0	0	30
Number of polymorphic allele per group	1-4	3-11	4-14

ACKNOWLEDGMENTS

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Selection of Wild *Coffea Arabica* Accessions Resistant to *Meloidogyne Paranaensis*

B. J. R. FATOBENE[1], W. GONÇALVES[2], O. GUERREIRO-FILHO[2]

[1]PGIAC, Instituto Agronômico, Campinas, SP, Brazil (Fapesp Research Fellowships)
[2]Instituto Agronômico, Campinas, SP, Brazil

SUMMARY

Five wild *Coffea arabica* acessions from Ethiopia were evaluated aiming to select resistant genotypes to *Meloidogyne paranaensis*, one of the most destructive species of root-knot nematodes to coffee trees. Nematodes reproduction was measured in plants matrix clones as well in their progenies. The progenies performance was similar to the response of clones, indicating that the genetic resistance of the material was transferred to next generation.

INTRODUCTION

One way to assure the sustainability and competitiveness of the coffee production is to control consciously, regarding economic, social and environmental aspects, the pests and diseases that affect the crop, especially the nematodes of the genus *Meloidogyne*, since its eradication of contaminated areas is virtually impossible. Thus, the use of integrated management techniques against these parasites is the only viable way that can reduce the population of nematodes, or keep it at low levels preventing them to cause damage (Gonçalves et al., 2004).

Due to root-knot nematodes relevance as a limiting factor for world coffee production, Centro de Café 'Alcides Carvalho' of Instituto Agronômico has been deepening researches in order to obtain resistant cultivars, especially to *M. incognita* and *M. paranaensis*, which damage severely coffee trees, and may limit the economic maintenance of infested crops prejudicing implementation of new coffee plantations in soils contaminated by them (Gonçalves & Silvarolla, 2001). The usage of resistant cultivars has been considered the most efficient method among the various techniques of management of nematode parasites of coffee, being economically and environmentally safe.

Meloidogyne paranaensis, considered one of the most destructive species to coffee, is distributed in coffee plantations in Brazil and Guatemala, whose injuries are so severe as those caused by *M. incognita* (Campos & Villain, 2008), being all current cultivars of *C. arabica* susceptible to this parasite.

The objectives of this work were to obtain information about resistance of wild *C. arabica* accessions to *M. paranaensis*, in order to select resistant coffee trees for further use in hybridizations. This knowledge may to ease genetic introgression of this character to current cultivars, enabling in the future, to plant resistant ungrafted cultivars in areas where nematodes are a limiting factor for coffee production.

MATERIALS AND METHODS

Five wild accessions of *C. arabica*, belonging to S2 generation of Ethiopian original coffee trees and available in *Coffea* germplasm bank at Centro de Café of Instituto Agronômico were evaluated in controlled conditions regarding to *M. paranaensis* resistance.

Based on previous experiments, clones from plants matrix and their open pollinated progenies (S3) were evaluated. Seedlings with 3-4 pairs of leaves, grown in 500 ml pots containing a mixture of soil and sand (1:1) autoclaved, were inoculated with 5000 eggs and juveniles of *M. paranaensis* from the State of São Paulo, Brazil. Mundo Novo IAC 515-20 cultivar (MN) was used as susceptible control. The experiment was performed by a completely randomized experimental design, with 3-6 replications of each clone, and 10 plants per progeny.

At 120 days after inoculation the gall index (GI) was measured according to the scale proposed by Taylor & Sasser (1978) and the final population of nematodes in the roots of trees was quantified from extraction of eggs and juveniles (J2) according to Hussey & Baker (1973). Reproduction factor (RF) was estimated by the ratio between the initial and final population (RF = Pf / Pi) (Oostenbrink, 1966).

Was also assessed the percentage of population reduction (PR), calculated comparing the RF to the susceptible control, in accordance with the criterion established by Moura (1997), that consider plants immune (PR = 100%), highly resistant (PR = 99.9 a 95.1%) resistant (PR = 95, 0 to 90.1%), moderately resistant (PR = 90 to 75.1%) and susceptible (PR = 75.0 to 0%).

RESULTS AND DISCUSSION

The accessions IAC 2139, IAC 2200, IAC 2279 and IAC 2036 were classified as resistant according to the variables GI and RF, highlighting IAC 2139 and IAC 2279 with PR of 97% and 98% in clones and progenies, respectively (Table 1).

Accession IAC 2032 presented GI and RF above the control MN, offering great potential for multiplication of *M. paranaensis* and should be included in later experiments as susceptible control.

Table 1. Gall index (GI), reproduction factor (RF) and percentage of population reduction (PR) of plants matrix of wild *Coffea arabica* accessions and their progenies.

	Plants matrix clones[1]					**Progenies[2]**				
Accessions	GI	RF	Class	PR		GI	RF	Class	PR	Class
IAC 2139	2,0	0,04	R	97,6	AR	1,4	0,16	R	98,0	AR
IAC 2200	1,5	0,09	R	94,7	R	2,0	0,64	R	91,8	R
IAC 2279	1,2	0,05	R	97,0	AR	2,0	0,22	R	97,1	AR
IAC 2036	2,0	0,16	R	90,6	R	2,2	0,39	R	95,0	R
IAC 2032	3,6	3,52	S	-	-	4,0	13,8	S	-	-
MN	3,5	1,71	S	-	-	3,9	7,91	S	-	-

[1]The values given are the means of 3-6 clones per accession; [2]The values given are the means of 10 plants. per progeny. The variable PR is presented in percentage. AR = highly resistant; R = resistant; S = susceptible.

The performance of clones and their progenies was similar, showing that the genetic resistance to *M. paranaensis* was transferred to the next generation, as observed by Anzueto et al. (2001) that assessed the resistance of *C. arabica* Ethiopian accessions to *M. incognita*.

The results also confirmed the findings of Boisseau et al. (2009), suggesting that the resistance to *Meloidogyne* should be a relatively common feature and distributed between the *C. arabica* wild accessions and can be easily transferred to commercial cultivars through controlled crossings (Bertrand & Anthony, 2008).

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Aggressiveness of *Pseudomonas syringae* pv. *garcae* Strains in *Coffea arabia* cvs. Mundo Novo and Bourbon Amarelo

L. M. R. RODRIGUES[1], R. COMPARONI[2], I. M. G. ALMEIDA[2], F. R. A. PATRÍCIO[2], L. O. S. BERIAM[2], O. FILHO-GUERREIRO[1]

[1]Agronomic Institute of Campinas – SP, Brazil
[2]Biological Institute, Campinas – SP, Brazil

SUMMARY

This work was conducted to elucidate some aspects of the interaction between *Pseudomonas syringae* pv. *garcae* and coffee plants, hitherto unknown. Was evaluated the aggressiveness of 19 bacterial strains and its virulence in coffee seedlings cultivars Mundo Novo and Bourbon Amarelo in two experiments. In first experiment seedlings of coffee cultivar Mundo Novo were inoculated and the aggressiveness was evaluated at 21 DAI by measuring the diameter of lesions. Subsequently, seedlings of cultivar Bourbon Amarelo were inoculated and the evaluations carried out daily through observing the onset of symptoms, where it was possible to calculate the incubation and latency period of the pathogen, and also the aggressiveness of strains by measuring the lesions at 21 DAI. High variability in virulence of strains was observed, and no correlation between origin of strains and virulence was found. The incubation period of the mostly virulent strains in Bourbon Amarelo cultivar ranged from 5,63 to 7,68 DAI and latency period between 5 and 7 DAI.

INTRODUCTION

Bacterial halo blight of coffee caused by *Pseudomonas syringae* pv. *garcae* (Amaral et al.), has been frequently detected in Brazil in coffee plantations located mainly in the states of Parana, Sao Paulo and Minas Gerais, and in recent years has becoming a limiting factor of the production on rugged fields regions, high rainfall and constant wind, also in new or newly pruned crops (Sera et al.; Zoccoli et al.). The pathogen infects mainly coffee leaves causing death of the attacked tissue (Costa & Silva, reducing the photosynthetically active areas, and consequently a lesser energy to the physiological processes of the plant. When the bacterium colonizes branches and flowers, it causes directly losses of the production of coffee plants (Costa & Silva).

Due to the bacterial control difficulty studies have been conducted to support breeding programs, as well as the selection of resistant coffee to the bacterial halo blight, which may result in a decrease of number of pesticides application, and therefore the cost reduction of production, less environmental impact and exposure of workers to chemicals, reaching a better quality product to the final consumer.

Studies conducted by Moraes et al. and Mohan et al. by means of artificial inoculation with bacterial strains in coffee seedlings resulted in the selection of resistant sources of coffee to the bacterial halo blight. However, little information about this pathosystem is found, and studies related to the virulence of different strains are of great importance, since the use of more virulent strains will allow a more rigorous selection of resistance materials.

This study propose to assess the aggressiveness of *P. syringae* pv. *garcae* strains, and elucidate some aspects of host pathogen relationship, as well as knowing the most virulent strains of a bacterial collection, which could be used in breeding programs to selections of resistance sources to bacterial halo blight.

MATERIALS AND METHODS

This study included nineteen bacterial strains obtained from the Phytobacteria Culture Collection of the Biological Institute (IBSBF), which were listed in Table 1, from different coffee planting regions. For inoculation experiments bacterial strains were grown on Nutrient Agar culture medium (containing 0.5% peptone, 0.3% meat extract and 0.1% NaCl), and after 48h growth at 28 ° C suspended in saline (0.85% NaCl). Suspensions were standardized to approximately $3x10^8$ $UFC.mL^{-1}$ using Marc-Farland scale.

Coffee seedlings were inoculated by puncturing with hypodermic needle previously immersed in the bacterial suspensions in four points each leaf. Three leaves of the cultivar Mundo Novo IAC 376-4 seedlings with about five months age were inoculated with three replications and one plant per replication. Four pairs of leaves of the cultivar Bourbon Amarelo IAC J-30 seedlings in a similar stage of development were also inoculated by puncturing three plants with three replicates per strain. After inoculation plants were kept in a moist chamber for three days in a greenhouse with controlled irrigation. Also, in this experiment were tested a combination of some more virulent strains which were determined in previous experiments. Design of randomized blocks was adopted in the experiments.

The experiments were conducted during the months of January to March 2012. To evaluate the strains aggressiveness, measurements were made of the largest diameter of the lesions at 21 days after inoculation (DAI). In the experiment with the cultivar Bourbon Amarelo, assessments were made daily by observing the symptom development allowing to calculate the latency and incubation period of the strains used as well as the aggressiveness at 21 DAI.

RESULTS AND DISCUSSION

All strains were able to induce symptoms in at least one injury in both cultivars tested, and verified pathogenic variability among strains.

In the first experiment with the cultivar Mundo Novo IAC 376-4 the more virulent strains were IBSBF 75, 65, 1293, 2212, 1664, 1197, 2511, 2840 and 3024. Twenty-one days after inoculation these strains showed mean diameter around the point of inoculation ranging of 0.4 to 0.64 cm, while to some virulent strains, the average was 0.008 to 0.1 cm. The most virulent strains had a higher percentage of injuries evolving to symptom from 83.33% to 100%.

In the second experiment, latency and incubation period of strains were calculated through the beginning of the onset of symptoms, as well as the symptoms expression in young and mature leaves in seedlings of the cultivar IAC Bourbon Amarelo J-30. The most virulent strains were IBSBF 65, 75, 1197, 1293, 1372, 1664, 2511, 2840, 3024 and the mixture of strains 75, 1197, 1293 and 3024. The percentage of wounded tissues that developed symptoms on most virulent strains was similar to the previous experiment, between 80 and 100%, however, the average diameter observed for these strains were lower than those evaluated in Mundo Novo cultivar, from 0.3 to 0, 45 cm.

Regarding the age of the leaves, results obtained in this study corroborate with Oliveira & Romeiro, where the young leaves were more susceptible to the bacteria. Incubation period

most affected of leaves, ie young leaves, varied between 5.63 and 7.68 DAI to the more virulent strains. The latency period of these strains were 5 and 7 DAI. No correlation was observed between the geographical origin of the strain and virulence. Knowledge of the pathogenic diversity of different strains is of great importance, and should be considered in breeding programs because most virulent strains may eventually allow a more rigorous selection of plants.

Table 1. *P. syringae* pv. *garcae* strains utilized in this study.

Strain[1]	Origin	Strain[1]	Origin
65	Jaú - SP		
75	Piraju - SP	**2996**	Monte Santo de Minas - MG
248[P1]	Garça - SP	**2999**	Carmo de Minas - MG
1197	Campinas - SP	**3005**	Altinópolis - SP
1293	Guaxupé - MG	**3015**	Garça - SP
1372	Cristais Paulista - SP	**3019**	São Sebastião da Grama - MG
1664	Serra Negra - SP	**3022**	Bragança Paulista - SP
2212	Franca - SP	**3024**	Serra do Salitre - MG
2511	Patrocínio - MG	**3051**	Andradas - MG
2840	Caconde - SP	**3065**	Unaí - MG

[1] Strain identification - IBSBF (Instituto Biológico, Brazil); [2] Pathotype strain.

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Selection of Coffee Plants Resistant to Brown Eye Spot: Genetic Variability and Influence of the Nutritional Condition on the Expression of Resistance to the Pathogen[4]

M. S. SILVA[1], F. R. A. PATRÍCIO[3], M. T. BRAGHINI[1], D. SALOMÃO[3], M. P. MALUF[2], J. MARTINATI[1], L. C. FAZUOLI[1], O. GUERREIRO-FILHO[1]

[1]Instituto Agronômico de Campinas - IAC, SP, Brazil
[2]Embrapa Café, SP, Brazil
[3]Instituto Biológico, SP, Brazil
[4]Part of the first author D. Sc Thesis

SUMMARY

Brown eye spot is an important coffee disease, present in all regions where coffee is grown in Brazil. The productivity and quality of coffee beverage can be severely reduced by the disease, especially in the Cerrado region, where the coffee crop is expanding, and also in irrigated areas. The severity of the disease can be enhanced by N and K deficiencies. Resistant cultivars can be a valuable tool for the management of the disease, however few studies to evaluate sources of resistance to be employed in breeding programs of *Coffea* in Brazil are available. Little is known about the behavior of resistant and susceptible materials when submitted to N and K deficiencies. In the first part of this study the resistance to brown eye spot was evaluated in advanced progenies from the IAC Coffee Breeding Program, which included progenies IAC 5028 L95 C124 and IAC 5026 L95 C108 (F7 of crosses between the *C. arabica* cultivars Catuaí Vermelho IAC 81 and BA10, with resistance to coffee rust) and RC_1 H20406 (P_1 IAC 81 x F_1 H8105-7). Initially the best conditions for inoculations and assessment of the disease were determined. The concentration of 5×10^4 conidia of *C. coffeicola* mL^{-1} and seedlings containing seven pairs of leaves resulted in the highest levels of incidence and severity of the disease and were employed in the subsequent experiments. The progenies IAC 5028 L95 C124 and IAC 5026 L95 C108 showed less incidence and severity of the disease than the cultivars Tupi IAC 1669-33, Catuaí Vermelho IAC 144, Obatã 1669-20, Mundo Novo, Ouro Verde and Bourbon Amarelo IAC J19. The progeny RC_1 H20406 (P_1 IAC 81 x F_1 H8105-7) was also very resistant to brown eye spot. IAC 5028 L95 C124, the most resistant material of the previous experiments and Bourbon Amarelo, the very susceptible cultivar are being tested in hydroponic systems with solutions deficient or not in N and K to evaluate the molecular expression of defense genes involved in the resistance to brown eye spot and of genes involved in the transport and regulation of N and K ions.

INTRODUCTION

Brown eye spot, caused by *Cercospora coffeicola* (Berk. & Cooke), is an important disease of the coffee crop in Brazil, and present in all regions where coffee is grown, but more severe in the Cerrado region, where the coffee crop is expanding, in irrigated areas, and in crops with deficiencies in N and K. The disease causes lesions in leaves and berries where they can reduce the quality of coffee beverage. The disease is important in seedlings and young plants and the severity of brown eye spot can be enhanced by N and K deficiencies, or unbalance in the ratio of N and K in the coffee plants.

Resistant cultivars can be a valuable tool for the management of brown eye spot; however few studies evaluated sources of resistance to this disease, probably because the IAC Coffee Breading Program is focused on resistance to coffee rust, caused by *Hemileia vastatrix*, the most important disease of the crop in Brazil. Although it is well known that N and K are important nutrients involved in the plants defense mechanisms little is known about the behavior of resistant and susceptible materials of coffee inoculated with *C. coffeicola* when submitted to deficiencies of these nutrients.

Therefore the objective of this study is to identify sources of resistance to brown eye spot in coffee plants of *C. arabica* from the IAC Coffee Breeding Program, study the role of N and K in the expression of resistance and the expression of resistance genes from susceptible and resistant coffee plants to this disease.

MATERIALS AND METHODS

Initially one experiment was carried out to evaluate the size of the coffee seedlings and the quantity of inoculum to be used in the subsequent experiments. Seedlings of the cultivar Bourbon IAC J19 with 6 and 7 pairs of true leaves where inoculated with *C. coffeicola* spore suspensions of 5×10^3, 10^4, 5×10^4 e 10^5 conidia ml^{-1}.

In a subsequent experiment seedlings of coffee plants with 6 pairs of leaves of the progenies from the IAC Coffee Breeding Program, IAC 5028 L95 C124 and IAC 5026 L95 C108 (F7 of crosses between the *C. arabica* cultivars Catuaí Vermelho IAC 81 and BA10, with resistance to coffee rust) and RC_1 H20406 and H 20407 (obtained by crosses between F_1 H8105-7 and Catuaí Vermelho IAC 81 and BA10, respectively), H 8105-7 (obtained by crosses between Catuaí Vermelho IAC 81 x BA10) and the cultivars Bourbon Amarelo IAC J19, Ouro Verde IAC H5010-5 and Catuaí Vermelho IAC 144 were tested.

In a third experiment the progenies IAC 5028 L95 C124 and IAC 5026 L95 C108, RC_1 H20406 and H 20407 and the cultivars Bourbon Amarelo IAC J19, Catuaí IAC144, Obatã IAC 1669-20, Tupi IAC 1669-33 and Ouro Verde IAC H5010-5 were tested.

The seedlings were inoculated with a suspension of 5×10^4 conidia of *C. coffeicola* prepared with a mixture of five isolates obtained from different coffee producing areas in Brazil (IBLF 199, IBLF277, IBLF 280, IBLF 379 and IBLF 975).

The incidence and severity (based on a graded scale 1-5) of the disease were evaluated 30 to 60 days after the inoculations and the area under the progress curve for the incidence (AACPDI) and severity (AACPDS) of brown eye spot were estimated after four evaluations.

All experiments were carried out in a completely randomized design. The data were submitted to analysis of variance and the media were compared by the Tukey test at 5% of significance.

The most resistant material of the previous experiments, progeny IAC 5028 L95 C124, and the very susceptible cultivar Bourbon Amarelo IAC J19 are being tested in hydroponic systems with solutions deficient or not in N and K to evaluate the molecular expression of defense genes involved in the resistance to brown eye spot and of genes involved in the transport and regulation of N and K ions.

RESULTS AND DISCUSSION

In the first experiment it was shown that the there was no difference between the size of the plants, and the concentrations of 5 x 10^4 and 10^5 promoted adequate levels of disease in the leaves. In the second and third experiments the progenies IAC 5028 L95 C108 and IAC 5026 L95 C124 showed the lowest levels of incidence and severity of brown eye spot.

In the second experiment the plants of the progeny H20406 showed the higher levels of severity of the disease, and the cultivars Bourbon Amarelo IAC J19 and Ouro Verde IAC H5010-5 had the higher levels of severity of the disease.

In the present work as well as in previous studies no material showed complete resistance to brown eye spot. This study showed that the progenies IAC 5028 L95 C108 and IAC 5026 L95 C124 although they were selected for resistance to coffee rust, caused by *H. vastatrix*, also exhibited a relatively high resistance to brown eye spot, therefore they are promising materials and should be subjected to further studies in this pathosystem.

The studies in nutrient solutions deficient in N and K are still underway.

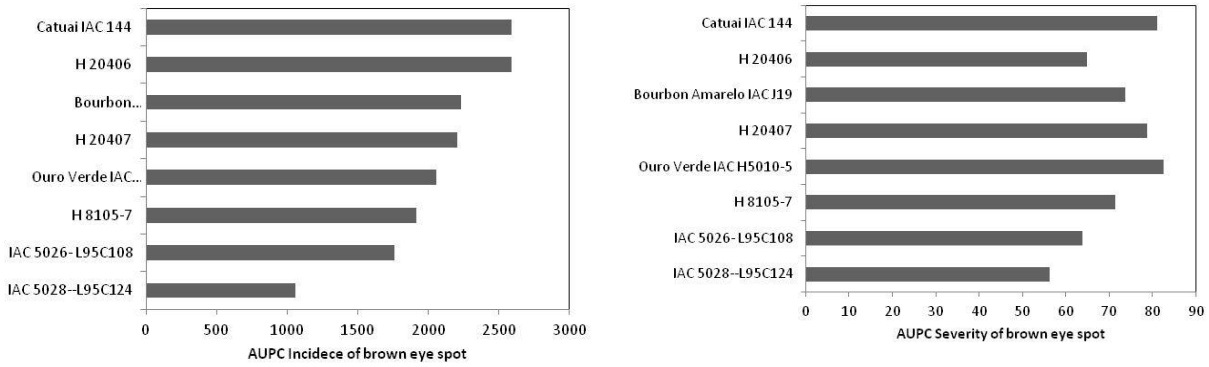

Figure 1. Resistance of coffee seedlings progenies and cultivars to brown eye spot, caused by *Cercospora coffeicola,* evaluated by the area under de progress curve (AUCP) of the incidence and severity of the disease in the second experiment.

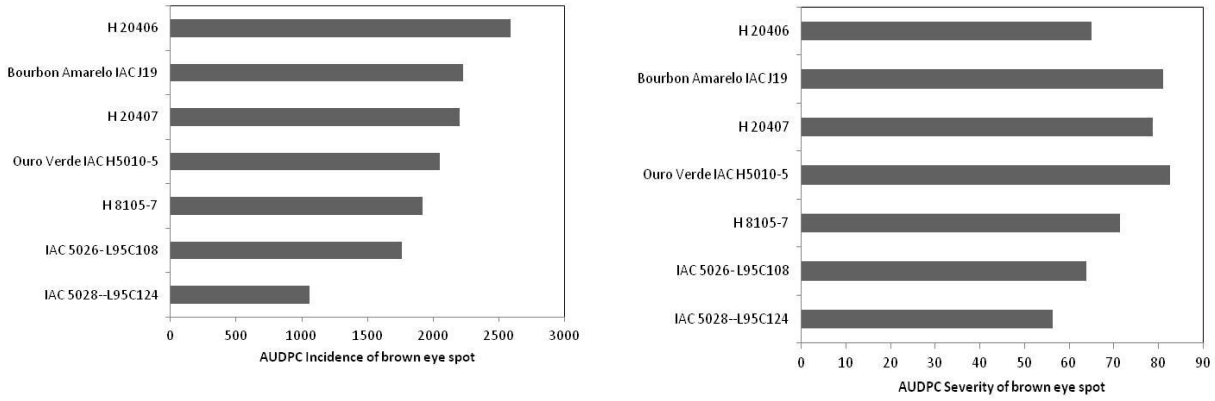

Figure 2. Resistance of coffee seedlings progenies and cultivars to brown eye spot, caused by *Cercospora coffeicola,* evaluated by the area under de progress curve (AUCP) of the incidence and severity of the disease in the third experiment.

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Characterization, Cloning and Sequencing of a Putative Metallothionein-Like Protein in *Coffea Arabica*

A. CHALFUN-JUNIOR, B. C. FERRARA-BARBOSA, S. S. CHAVES

Laboratório de Fisiologia Molecular de Plantas, Setor de Fisiologia Vegetal, Departamento de Biologia, Universidade Federal de Lavras Lavras, Brazil

SUMMARY

This study was addressed aiming at characterizing the putative metallothionein-like genes in coffee. The search for putative MT-like genes in the CAFEST database resulted in more than 1000 reads. After clustering, 27 contigs and 78 singlets were obtained. In the annotation process, this number decreased to 6 contigs and 1 singlet. The phylogenetic tree demonstrated a high similarity between the sequences found in the CAFEST and those from other species, the electronic Northern detected Methallothionein expression in different tissues, development stages and stress conditions. The result of the sequencing of the putative coffee Methallothionein suggests the existence of an intron in the coding sequence.

INTRODUCTION

Metallothioneins (MTs) are proteins of low molecular weight, part of an cysteine-rich superfamily. The cysteine domains have great affinity to metal ions, and have shown to associate more commonly to Zn^{2+} and Cu^{+}. When referring to plant MTs, it is preferred to use the term *MT-like* since most of them are inferred from DNA sequences derived from different species. The synthesis of MT can be induced by various agents such as hormones, growth factors, physical-chemical stress, under many other chemicals conditions, and in vivo or in cell cultures.

Although MTs are proteins known to be part in the processes of metal homeostasis and heavy metal detoxification, its precise function is still a subject of debate. Many recent researches have reported the existence of MTs in several different organisms such as cyanobacteria, plants and animals, which shows a high conservation of certain domains in this group of proteins and indicate its importance throughout the evolutionary pathway of many species. The effort to sequence coffee genome resulted in an EST (*Expressed Sequence Tags*) database – CAFEST. This allowed the search of putative genes of interest related to numerous physiological traits. It also made possible to determine the tissues where the genes of interest are expressed and the levels of its expression. Thus, this study aimed at characterizing the putative genes that encode metallothionein-like proteins in coffee (*Coffea arabica*).

MATERIALS AND METHODS

First, a search for MTs sequences was performed by keyword criteria at the NCBI database and obtained sequences were annotated. Then, a search using BLAST in the CAFEST (http://bioinfo04.ibi.unicamp.br) database was performed using as queries the sequences previously obtained, as well as keywords. Sequences showing reliable similarity (e-value <4.10) were deposited in the system for sequence management and manipulation − the GeneProject − and clustered using the CAP3 program. After clustering, the selected contigs

and singlets were annotated and compared to public protein databases to obtain more information about the probable proteins encoded by these sequences and eliminate false sequences. Only sequences involving the conserved domain were selected for subsequent analysis. Later, chosen sequences were also used as templates for a new search by CAFEST. For the phylogenetic analysis multiple alignments were performed comprising the protein coding sequence from the selected contigs and homologous sequences published in NCBI, and for the in silicio analysis of the spatial expression patterns, Electronic Northerns were designed. Through this, we calculated the appearance frequency of the contigs forming reads in each library. Subsequently, the data were normalized to give an accurate picture of the expression degree of candidate genes for each treatment and locus of the plant.

Based on the computational essays, two *contigs* were selected for further investigation. In order to isolate the putative coffee MTs genes, primers (table 1) were designed.

Table 1. Primers designed for fragment isolation.

Gene	Primer Sequence	Amplicon (pb)
CaMT19 F	5'CACCTTCTCACCATGTCGGACA	209
CaMT19 R	5'GCGTCAATTGTCACAGGTGCAG	
CaMT21 F	5'CACCAGAATGTCGTGCTGCG	248
CaMT21 R	5' CCTCATTTGCAGTTGCAGGGA	

The isolation was performed through PCR with both cDNA and DNA templates. The products of the amplifications were subjected to electrophoresis. Fragments obtained were eluted from the agarose gel and quantified. Isolated fragments were then ligated to the pCR II vector, using TOPO-TA kit (Invitrogen®) following manufacturer instructions. Recombinant vectors were inserted in E. coli, TOP10 strain chemically competent in Kan50 supplemented medium. Transformed colonies were submitted to a PCR, using an inoculum of each colony resistant to the selective medium to confirm the insertion and that the plasmid DNA was extracted from transformed colonies. Clones selected were sequenced with both M13 reverse and M13 (-20) forward primers. The resulting sequences were compared by BLASTX with those obtained previously in the *in silico* analysis as well as the ones deposited in the GenBank.

RESULTS AND DISCUSSION

The search for putative MT-like genes in the CAFEST database resulted in more than 1000 reads, after clustering, 27 contigs and 78 singlets were obtained. In the annotation process, this number decreased to 6 contigs and 1 singlet.

When submitted to a similarity search by blastp, the predicted proteins showed high identity levels with metallothioneins from coffee. One contig (CaMT21) in particular presented a complete identity, corroborating it stands for a coffee metallothionein (U11423.1) already presented in the GenBank. Hence, this contig CaMT21 was selected for further analyses.

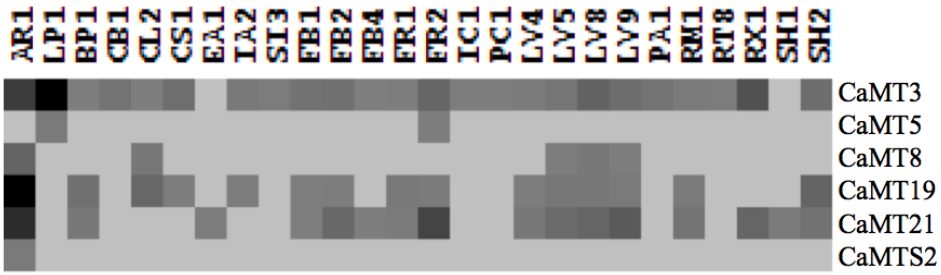

Figure 1. *In silico* expression profile of putative Metallothionein-like genes found in the database CAFEST. The normalized numbers of reads are represented in a gray scale, where zero or negative expression is represented by lighter colors and is gradually increased up to black, which represents the maximum degree of expression. Contigs and singlets are represented in rows and columns as libraries. The libraries, as defined by Vieira (2006) are: plantlets and leaves treated with arachidonic acid (AR1 and LP1); suspension cells treated with acibenzolar-S-methyl (BP1); suspension cells treated with acibenzolar-S-methyl and brassinoesteroids (CB1); hypocotyls treated with acibenzolar-S-methyl (CL2); suspension cells treated with NaCl (CS1), embryogenic calli (EA1 and IA2); germinating seeds (SI3); flower buds in different development stages (FB1, FB2 and FB4); flower buds + pinhead fruits + fruits at different stages (FR1, FR2); non embryogenic calli with and without 2,4 D (IC1, PC1); young leaves from orthotropic branch (LV4, LV5); mature leaves from plagiotropic branches (LV8, LV9); primary embryogenic calli (PA1); leaves infected with leaf miner and coffee leaf rust (RM1); suspension cells with stress from aluminum (RT8); stems infected with *Xylella spp*. (RX1); Water deficit stresses on field plants (pool of tissues) (SH2).

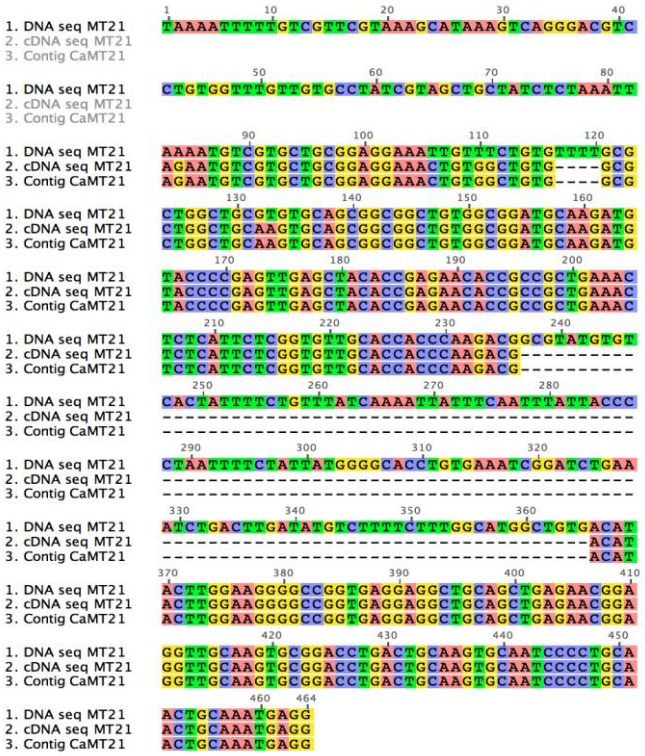

Figure 2. Alignment of the sequences obtained by DNA and cDNA clones and the contig selected in the *in silico* analysis.

The expression pattern accessed by the Electronic Northern (Figure 1) evidenced that the expression of the ESTs occurred more often in libraries of seedling and leaves treated with arachidonic acid. Thus, the *in silico* expression analysis fits with the proposed alternative function for plant MTs as protectants from oxidative damages, hence plants treated with exogenous Arachidonic Acid presented an increased expression of general stress-responsive genes. Nevertheless, the MTs showed a wide expression profile, being detected in many tissues, which indicates the probable involvement of coffee MTs in different pathways and phases of plant development. These results are consistent with the evidences of the involvement of MTs in the process of seed development and leaf senescence, in addition to its function in metal homeostasis and detoxification.

The amplification products obtained in the polymerase chain reaction resulted in fragments with the estimated sizes of 250bp with cDNA template and 750pb with DNA template, suggesting the existence of introns in the coding sequences. Thus, the alignment of the sequences obtained in sequencing for both DNA and cDNA templates of CaMT21 (Figure 2) shows the possible localization of this intron, corroborating with the results visualized in the electrophoresis gel. However, the sequence obtained by the DNA clone is smaller than expected (700pb), what could be explained by the low accuracy in sequencing borders of long fragments.

Zhou et al. (2006) reported the existence of one or more introns in the gene structure of eleven rice metallothionein-like genes. However, in rice, all type 2 metallothioneins presented two introns. On the other hand, five MT genes identified from the Arabidopsis genome also contain two exons interrupted by a single intron, which is the same pattern presented in our results.

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Heterosis and Drought Tolerance af F1 Hybrids between the Catuaí Vermelho Cultivar of *Coffea Arabica* and Introductions Geisha and Wush-Wush from Ethiopia

L. C. FAZUOLI, M. T. BRAGHINI, M. B. SILVAROLLA, O. GUERREIRO-FILHO

Centro de Café "Alcides Carvalho", Instituto Agronômico de Campinas (IAC/APTA), SP, BR.

SUMMARY

The Instituto Agronômico de Campinas received in 1953 the introductions IAC 1137 and IAC 1521, originating from Ethiopia and denominated Geisha and BE-5 (Wush-Wush) respectively. Some plants were selected in these introductions that were crossed in 1970 with "Catuaí Vermelho" of *Coffea arabica*. The F1 hybrids obtained were named IAC H8089 and IAC H8114, respectively, and were planted in experiment EP 131 in Campinas, SP, Brazil. The aim of this study was to evaluate the production and drought tolerance of coffee hybrids compared to the control "Catuaí Vermelho", which is a high yielding cultivar with tolerance to drought. In 16 harvests the average productivity of the F1 IAC H8089 and of the F1 IAC H8114 were 4.28 kg and 4.26 kg of cherries per tree/year, respectively whereas the control cultivar Catuaí Vermelho produced 3.02 kg per plant/year. Heterosis for yield of F1 IAC H8089 and IAC H8114, compared to "Catuaí Vermelho" was 41.72% and 41.06%, respectively.

The drought tolerance was measured in years of water stress in the field, by using an assessmernt scale with ten points (1-10) for turgidity index (IT), wherein 1 is a plant with withered leaves, and 10 a plant with turgid leaves. The range of variation averaged 3.5 to 6.3 for the hybrid IAC H8089, 3.0 to 6.7 for IAC H8114 to and 3.6 to 6.3 for "Catuaí Vermelho". The most productive IAC H8089-4 and IAC H8114-3 plants yielded 7.40 kg oand 8.17 kg coffee berries per year, whereas the best plant of "Catuaí Vermelho" yielded 4.36 kg. The average IT index assessed over 7 years in IAC H8089-4, IAC H8114-3 and in "Catuaí Vermelho" was 5.2, 5.0 and 4.6.

It was concluded that the hybrids showed substantial heterosis for yield, in relation to "Catuaí Vermelho" and that they displayed equal drought tolerance as "Catuaí Vermelho".

INTRODUCTION

The deployment of heterosis in arabica coffee hybrids has become possible with great advances in cloning techniques through tissue culture. It is important to consider that obtaining highly productive coffee genotypes with genes that confer resistance to diseases, pests and nematodes associated with drought tolerance and cup quality in a traditional breeding program is very time consuming. The aim of this study was to study F1 genotypes between arabica coffee varieties with high productivity, partial rust resistance, drought tolerance and good cup quality.

MATERIALS AND METHODS

The Instituto Agronômico de Campinas received from the U.S. Department of Agriculture, in 1953 the introduction IAC 1137, called Geisha, and IAC 1521, called Wush Wush or BE5, from CATIE (Costa Rica). The introduction Geisha originated in the region of Ethiopia called Geisha. Its leaves are leatherlike, concave and dark green. New leaves are green in color and the fruits are relatively large with late harvesting. This introduction has the SH1 gene which confers resistance to race II of coffee leaf rust (*Hemileia vastatrix*). Recently, in Panama and Costa Rica it was found that the Geisha introduction when grown in the highlands (1200 to 1400m) produces excellent cup quality. The IAC 1137-5 (Geisha) introduction was also considered as drought tolerant. Few details are available with regard to IAC 1521 (Wush-Wush). It has the SH1SH4 genes, therefore resistance to race 2 of coffee leaf rust. Its origin is also from Ethiopia.

The IAC selected a coffee plant in each of the two introductions in 1970 and crosses were made with Catuaí Vermelho of *Coffea arabica*. Hybrids obtained IAC H8089 (Catuaí Vermelho X Geisha) and IAC H8114 (Catuaí Vermelho X Wush Wush) and the control cultivar Catuaí Vermelho, were planted in the experiment EP 131 in Campinas-SP in 1972. Sixteen harvests were observed from a total of nine plants per entry (27 in total). The turgency index (TI) was also evaluated in six years during the dry season, using a scale of 1 to 10 with representing wilted plants and 10 plants with fully turgid leaves. In 2004 was made another assessment. Heterosis for yield was calculated as the percentage of yield relative to the control Catuaí Vermelho for both types of hybrids using the following formula:

$$H\ (\%) = (\text{yield F1}/\text{yield control} - 1)\ 100$$

RESULTS AND DISCUSSION

Estimation of heterosis for yield

The data obtained during the 16 harvests for the average of kg ripe coffee berries per plant and the heterosis calculated in comparison with the control cultivar Catuaí Vermelho, are shown in table 1. Table 1 shows that heterosis for hybrid F1 IAC H8089 is 41.72% and for IAC H8114 41.06%, which can be considered as very good. The range of variation in production is 2.83 to 7.40 kg for hybrid IAC H8089 and 1.04 to 8.17 kg for IAC H8114. The control Catuaí showed a range for yield of 1.40 to 4.36 kg. So coffee F1 hybrids with high yield were obtained in both genetic materials evaluated. Similar levels of yield heterosis have been observed by other authors like Fontes J. R. M and Fazuoli L. C., Braghini M. T., Mistro J. C., Petek M. R., Silvarolla M. B. The most productive F1 coffee trees, might be multiplied vegetatively (by cuttings or tissue culture).

Table 1. Average and range of production per plant per year observed over 16 harvests in kg of coffee berries and heterosis for three F1 hybrids in percentage compared to the control cultivar Catuaí Vermelho, in EP 131, Campinas/SP.

F1 hybrid	Average production per plant / year (kg)	Heterosis (%)	Variation (kg)
IAC H8089[1]	4,28	41,72	2,83-7,40
IAC H8114[2]	4,26	41,06	1,04-8,17
IAC H8142[3]	0,76	-	0,25-1,72
Catuaí Vermelho	3,02	-	1,40-4,36

[1]IAC H8089 = Catuaí Vermelho X Geisha; [2]IAC H8114 = Catuaí Vermelho X Wush-Wush; [3]IAC H8142 = IAC 1107 X IAC 1518 (no tolerant control).

Estimation of wilting tolerance (turgency)

The average data of six years of turgidity index, the range of variation of these indices and the indices of turgor evaluated in the dry season of 2004, are shown in table 2.

Table 2. Turgency Index for three F1 hybrids of *Coffea arabica* and for the control cultivar Catuaí Vermelho, in Campinas/SP, evaluated in six years and in 2004.

F1 hybrid	IT average	Variation turgency index (IT)	IT in 2004
IAC H8089[1]	5,2	3,5-5,9	7,6
IAC H8114[2]	5,0	3,0-6,7	7,6
IAC H8142[3]	3,1	2,2-4,4	5,2
Catuaí Vermelho	4,6	3,6-6,3	7,1

[1]IAC H8089 = Catuaí Vermelho X Geisha; [2]IAC H8114 = Catuaí Vermelho X Wush-Wush; [3]IAC H8142 = IAC 1107 X IAC 1518 (no tolerant control).

Table 2 shows the average indices of turgor are 5.2, 5.0,3,1 and 4.6 for IAC H8089, IAC H8114, IAC H8142 and Catuaí Vermelho, respectively. The range of variation of the mean ITs were 3.5 to 5.9; 3.0 to 6.7 and 3.6 to 6.3 for IAC H8089, IAC H8114 and Catuaí Vermelho, respectively. In 2004, these figures were 7.6 for both hybrids and 7.1 for the control cultivar Catuaí Vermelho. These results suggest drought tolerance in the hybrids Catuaí Vermelho X Geisha and Catuaí Vermelho X Wush-Wush at a similar level as the control cultivar Catuaí Vermelho. Other authors like Mazzafera, P., Carvalho, A. obtained similar results.

Rust resistance (*H. vastatrix*)

Both hybrids present are resistance to rust race II, inherited from the Ethiopian varieties. The control cultivar Catuaí Vermelho is susceptible to prevalent races in Brazil. In the experiment, with the presence of races III (v1v5) and X (v1v4v5) all the genetic materials become susceptible.

CONCLUSIONS

The best coffee trees in the hybrids IAC H8089 (Catuaí Vermelho X Geisha) and IAC H8114 (Catuaí Vermelho X-Wush Wush), which showed high heterosis for yield and good drought tolerance, should be multiplied through stem cuttings or tissue culture.

ACKNOWLEDGMENTS

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Somatic Embryogenesis in Hybrids of *Coffea Arabica*[A]

W. C. FREITAS[2], J. A. S. ALMEIDA[3], G. S. GIOMO

[2]Centro de análise e Pesquisa Tecnológica do Agronegócio do Café "Alcides Carvalho", Instituto Agronômico de Campinas, CP 28, 13.001-970. Campinas, SP, Brasil
[3]julietasa@iac.sp.gov.br

SUMMARY

The current coffee market has great demand for hybrids of *Coffea arabica* L. that due to the heterosis they may have special features, like production, resistance to biotic and abiotic factors or quality of drinking. But the multiplication of these hybrids should be vegetative because the plants from the germination of seeds lose the special characteristics due to the genetic segregation. These plants can be multiplicated by somatic embryogenesis. This process can occur by indirect via which consists of two phases, the callogenesis and the formation of embryos, or by via direct that occurs in one step. The objective of this study was to characterize the ability to direct and indirect somatic embryogenesis of genotypes of *C. arabica* with high quality beverage. To this study, it was used explants from leaves collected from adult plants of *C. arabica* hybrids H8105, H8427, H8089 and the cv Mundo Novo IAC 376-4, kept under field conditions. Each treatment consisted of 20 flasks, with two explants per each one, maintained in continuous dark at 25 °C and evaluated every 30 days to the presence of callus or embryogenic structures, staining of callus or embryogenic structures, the estimated size of callus or the embryogenic structures and number of somatic embryos. In the indirect pathway, explants of all genotypes formed callus. The calluses of cv Mundo Novo and genotypes H8105 and H8427 were larger than those of H8089, respectively, 7.0, 7.4, 7.2 and 5.2 mm in size. On the other hand, in the direct pathway, the cv Mundo Novo and the hybrids H8105 and H8089 had respectively 72.5, 63.2 and 83.3 % of explants with formation of embryogenic structures while H84273 had 56.3%.

INTRODUCTION

Currently, arabica hybrids have been cultivated with the objective of exploring the variability genetic resultant of the crossing. The hybrids of *Coffea arabica* may have special features, like production, resistance to biotic and abiotic factors or quality of drinking. But the multiplication of these hybrids should be vegetative because the plants from the germination of seeds lose the special characteristics due to the genetic segregation. The vegetative multiplication can be obtained by somatic embryogenesis since this process produces genetically identical plants to the mother plants.

Somatic embryogenesis allows for the formation of somatic embryos without gamete fusion. First, the somatic cells of the explant tissue des-differentiate from each other, leading to the formation of embryogenic cells. After, these embryogenic cells turn on to differentiation, which resulting in the somatic embryos. The somatic embryo is a bipolar structure presenting the same developmental stages as the zygotic embryo.

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Somatic embryos can be obtained by indirect somatic embryogenesis which consists of two phases, the callogenesis and the formation of embryos, or by via direct that occurs in one step. In the direct via, the embryos are formed in a single phase, without callogenesis.

The genotypes of *C. arabica* may have been broadly cultivated *in vitro* through indirect via, or direct way, whose embryos are formed directly from explants edge cells. So, the objective of this study was to verify the indirect and direct somatic embryogenesis capacity of three *C. arabica* hybrids and the cultivar Mundo Novo.

MATERIAL AND METHODS

To this study, it was used leaves collected from adult plants of the hybrids *Coffea arabica* H8105, H8427, H8089 and the cultivar Mundo Novo which were maintained under field. The leaves were first disinfested in a 2 % sodium hypochlorite solution for 20 minutes. Soon after, rectangular explants were extracted (1.5 x 2.0 cm). The explants were submitted to direct and indirect somatic embryogenesis induction media. The medium containing strength MS, with 5 g L^{-1} agar, pH 5.8, autoclaved for 20 minutes at 121 °C and 1.5 atm. The 25 mL medium was added in clear glass (100 mL). For indirect somatic embryogenesis, it was used two medium, to the induction of callus, the MS salts, 2.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 5 μM Kinetin. The calluses resulting were transferred to the embryogenesis induction medium, consisting of ½ MS salts with the addition of 0.5 μM 1-naphthalene acetic acid and 2.5 μM kinetin. For via direct, the medium consisted of ½ MS salts and the addition of 10 μM 2iP (N6-(2-isopentyl) adenine). The treatments comprised 20 flasks with two explants in each one, which were maintained in the absence of light, at 25^{o}C, and they were evaluated with respect to the explant with formation of callus or embryogenic structures, color of the callus or the embryogenic structures, estimated callus or embryogenic structures size and the number of embryos formed.

RESULTS AND DISCUSSION

The hybrids H8105, H8089, H8427 and cultivar Mundo Novo were evaluated for this somatic embryogenesis capacity. For the indirect somatic embryogenesis it was verified that explants of all genotypes presented capacity to this via, since they formed calluses (Figure 1, Figure 2A). After 90 days from the begin of the experiment, the hybrids showed more than 80 % of explants with formation of callus like the cv Mundo Novo. For the color of the callus, it was verified that the hybrids and the cv Mundo Novo reached the same results (Figure 2B), being that the callus did not reached elevated degree of oxidation. Another factor is the size of the callus, the hybrids H8105, H8427 and cv Mundo Novo had greater size than the H8089 in the end of phase of induction callus (Figure 2C). In the other hand, although the explants all genotypes had formed callus, it was verified that only H8105 and H8089 showed more formation of somatic embryos while the cv Mundo Novo and H8427 the results were less expressive (Figure 2D).

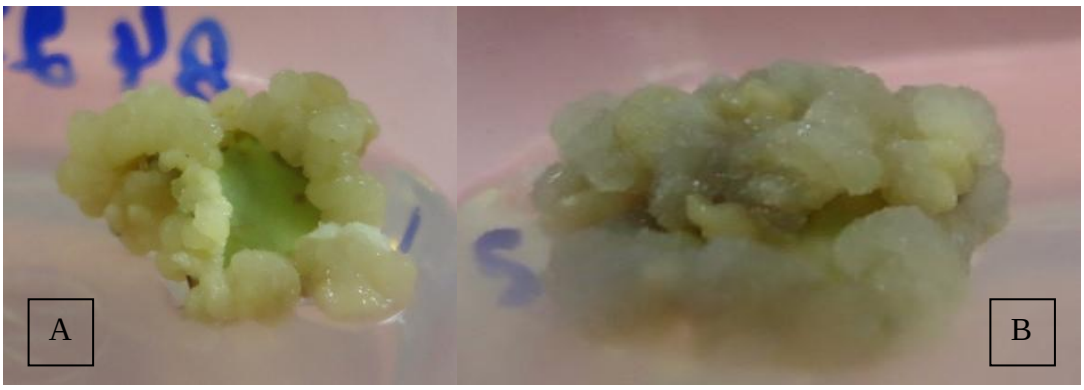

Figure 1. Callus of hybrids of *C. arabica* H8427 (A) and H8105.

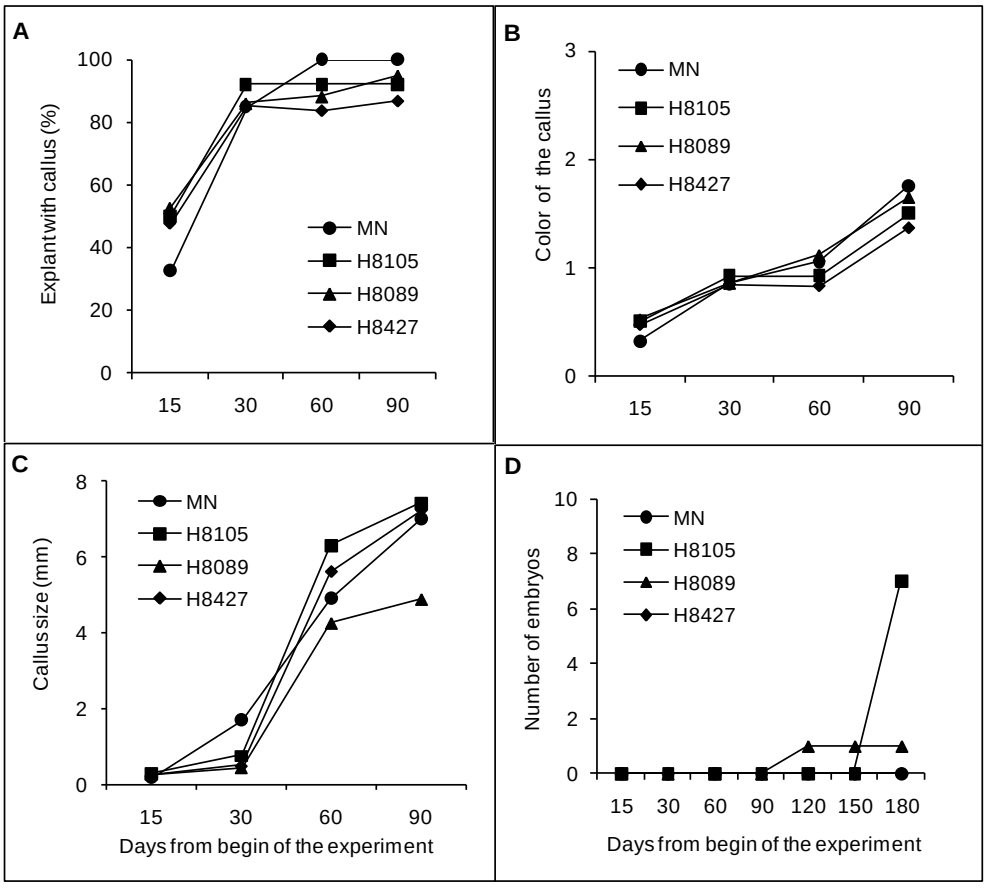

Figure 2. Indirect somatic embryogenesis on leaf explants of genotypes *C. arabica* in MS medium, maintained in absence of light and at 25 °C. *A*. Explant with presence of callus; *B*. Color of the callus: *1*. Clear; *2*. Intermediate; 3. Oxidated; *C*. Callus size and *D*. Number of somatic embryos.

The hybrid H8427 and cv Mundo Novo attained a higher percentage of explants with embryogenic structures than the H8105 and H8089 (Figure 3; Figure 4A). For the color of the embryogenic structures it was found that cv Mundo Novo, H8427 and H8105 reached a greater degree of oxidation while the H8089 had clear coloration (Figure 4B). The sizes of the embryogenic structures was different among the genotypes studied (Figure 4C). The cv Mundo Novo showed the largest mean, while the H8089 had reduced size. Besides, the H8427 and H8105 had similar sizes, which was about 2 mm for both. In the figure 2D it is

possible to see that the cv Mundo Novo and H8089 formed more number of embryos while H8105 and H8427 had reduced number (Figure 4D).

Figure 3. Direct somatic embryogenesis on explants of hybrids of *C. arabica:* A. Embryogenic structures of H8427 hybrid and (B) embryos of H8105 hybrid.

For the effective production of somatic embryos, in the final of the available it is possible to see that the majority hybrids can produce embryos by indirect and direct via. But until now, the results showed that the hybrid H8105 and H8089 had more formation of somatic embryos, respectivally by indirect and direct somatic embryogenesis. The results of this study indicate that arabica hybrids have ability to form embryos by indirect and direct somatic embryogenesis.

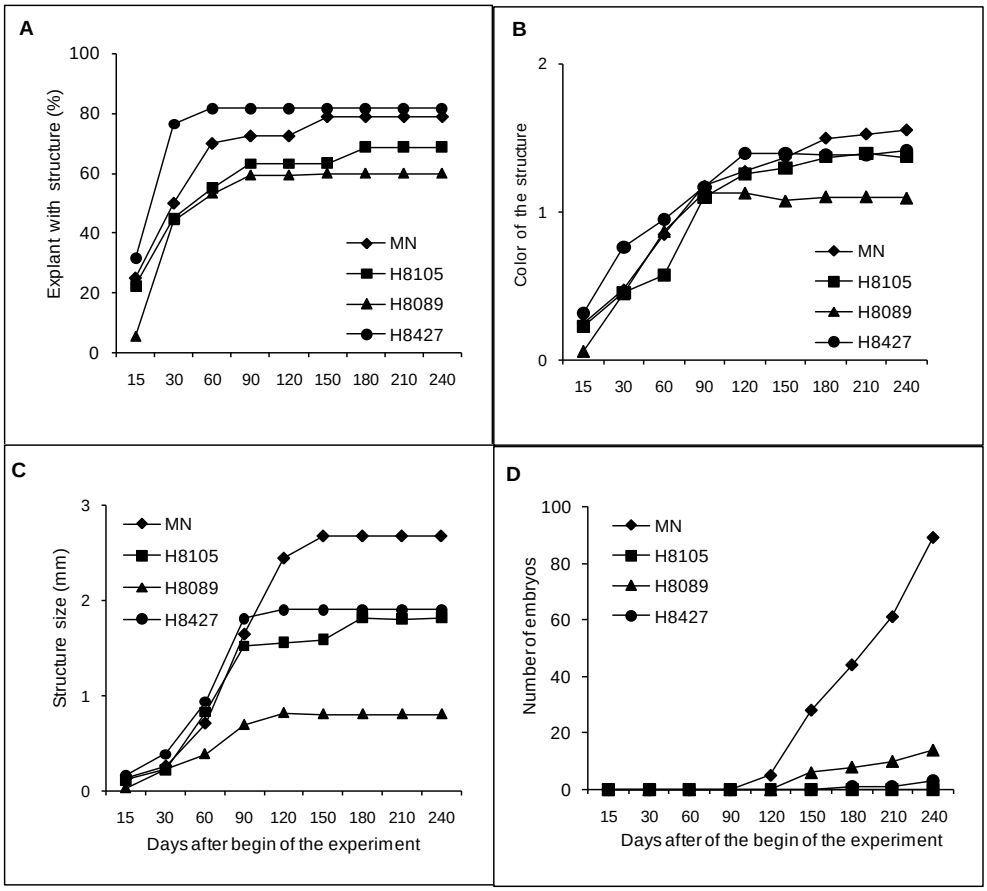

Figure 4. Direct somatic embryogenesis on leaf explants of hybrids and the cv MN *C. arabica* in half-strength MS medium, maintained in absence of light and at 25 ^{o}C. *A.* Explant with presence of embryogenic structures; *B.* Color of the embryogenic structures: *1.* Clear; *2.* Intermediate; *3.* Oxidated; *C.* Embryogenic structures size; *D.* Number of somatic embryos.

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Expression of bZIP19 under Control of the Zinc Deficiency responsive ZIP4 Promoter in Coffee (*Coffea arabica L.*)

A. H. HENRIQUES[1], A. CHALFUN-JUNIOR[1], M. AARTS[2]

[1]Universidade Federal de Lavras, Brazil
[2]Wageningen University, Netherlands

SUMMARY

Coffee is one of the most valuable agricultural export commodities. The expansion of coffee crops to less fertile soils has led to an increase of zinc deficiency issues, which compromises coffee growth and production. We showed that the expression increased of bZIP19 mediated by the promoter of the zinc deficiency responsive ZIP4 gene is a promising strategy in enhancing Arabidopsis zinc deficiency tolerance. The bZIP19 transcription factor is conserved in the plant kingdom, so the pZIP4:bZIP19 construction appears promising to test in coffee. Coffee seeds transformed with either pZIP4:bZIP19 or pDsRed-Root (control), by A. rhizogenes were grown in normal conditions for five months, then they were transferred to a solution containing a deficient or sufficient zinc supply. The results of this pilot project reinforce our previous findings. The pZIP4:bZIP19 transformed Coffee plants showed better adaptation to zinc deficiency, suggesting that this strategy may promote the development of zinc deficiency tolerant crops.

INTRODUCTION

Coffee is one of the most important primary products in world trade. This commodity is grown in around 80 countries, with Brazil being the largest coffee producer, followed by Colombia and Vietnam. It represents the key export product and cash, which in general causes a positive impact on the social and physical environment since this activity provides employment for hundreds of millions of people from production to final consumption.

The expansion of coffee crops to less fertile soils and the need to maximize production is leading to the use of larger amounts of corrective fertilizers that raise the pH of the soils producing mineral imbalance such as zinc deficiency, a micronutrient which is highly important in the coffee culture. These facts cause a widespread occurrence of zinc deficiency in most coffee crops, which affects plant performance in terms of growth, development, and yield.

The advance of coffee biotechnological techniques and the significant progress in understanding the regulators of zinc homeostasis in plants have offered new possibilities for the development of improved cultivars in terms of zinc deficiency tolerance. Recently, an important step towards unraveling the regulation of zinc homeostasis networks was made with the identification of two bZIP transcription factors, bZIP19 and bZIP23, which are essential for switching on the zinc deficiency response of *Arabidopsis thaliana*. Both genes are widely conserved in the plant kingdom, suggesting a conservation of their function. Previous experiments focused on the expression of the bZIP19 gene under control of the Arabidopsis zinc deficiency responsive ZIP4 promoter. This provided evidence that the increased expression control of bZIP19 is very effective in enhancing Arabidopsis zinc deficiency tolerance and may offer attractive advantages for crops in areas suffering from low zinc

bioavailability as well as contributing to enhanced yields and plant robustness. Therefore, this work was carried out with the objective to investigate if the overexpression of bZIP19 mediated by the ZIP4 promoter contributes to enhanced biomass yield and robustness of coffee crops under zinc shortage.

MATERIALS AND METHODS

Seed germination

Coffea arabica L. (Catuai Vermelho IAC 144) seeds without parchment were surface-sterilized by immersing the seeds in 5% HClO (w/v) bleach solution for 15 minutes then rinsed three times in sterile water and placed individually in Petri-dishes containing half-strength MS (Murashige and Skoog) medium and phytagel. Coffee seeds were kept for about 4 weeks in the dark at 28°C for full hypocotyls expansion.

Coffee transformation

As soon as the hypocotyls became fully expanded the root transformation procedure was carried out. Coffee seeds were cut above the hypocotyls-root boundary with a scalpel that was previously dipped in an Agrobacterium rhizogenes culture. This was collected from LB-agar plates with freshly grown A. rhizogenes cultures containing either the pZIP4:bZIP19 construct or the pDsRed-Root empty vector. Transformed seeds were grown in a climate chamber (16h/d at 22/20°C day/night temperature; 120 µmol photons $m^{-2}s^{-1}$; 50% relative humidity), for four weeks. After this time, the seedlings were transferred to pots containing half-strength MS (Murashige and Skoog) medium supplemented with tricarcilin (200 $mg.L^{-1}$) and continued growing for 10 days in the climate chamber. For the selection of transformed roots, seedlings were moved to new pots containing ½ MS medium added with tricarcilin (200 $mg.L^{-1}$) and hygromycin (25 $mg.L^{-1}$) for pZIP4:bZIP19 transformed roots or kanamycin pDsRed transformed roots and kept for 10 more days. Transformed roots were checked with markers by Stereo-macroscopy (pDsRed) or by PCR analysis (pZIP4:bZIP19).

PCR analysis

To confirm if the new formed roots were co-transformed with pZIP4:bZIP19 construct, DNA was extracted following a CTAB method as described by Doyle & Doyle (1991) and the pellet was re-suspended in 20 µL sterile water. For PCR reaction we used 4 µL of DNA, 0.5µL of 5 mM dNTPs, 2.5 µL of 10x Taq buffer, 0.25 µL Taq DNA polymerase, 1.25 µL of 10 pmol forward and reverse primers (Invitrogen) in a total volume of 25 µL. The experiment was carried out in an iCycler Thermal machine (Bio-Rad). The mixture was then subjected to 94.0°C for 5 min, followed by 29 cycles of 30 sec at 94.0°C, 30 sec at 54.0°C and 20 sec at 72.0°C. After the last cycle was completed, an additional 7 min at 72 °C elongation step was performed. The following primers were used, 5'TTCTCCCGGATGAGAGCGATGA3' and 3'GCTGATTCACCGCCCTAAGCCT5' getting the 150 pb amplification size. In order to confirm that the amplification results were not due to contamination of bacterial DNA a second PCR was performed, with the same thermal profile described above, which used primers for Coffea alpha tubulin (292 pb), 5'ATTGGTCAGGCCGGTATCCAGG3'and 3'AGATCGACGAACACGGCACG5'.

Zinc deficiency stress

The plants with roots co-transformed either with pZIP4:bZIP19 or pDsRed-Root (empty vector) constructs were transferred to pots containing a modified hydroponic solution and

placed in a climate chamber for 12h/d at 20/15 day/night temperature 250 µmol photons $m^{-2}s^{1}$ and 75% relative humidity. The hydroponic medium initially contained quarter-strength Hoagland's nutrient solution, which was gradually increased for 10 days, when it reached half-strength Hoagland nutrient solution. The hydroponic solution was maintained this way until coffee plants completed five months. After this time, a new hydroponic solution was prepared either with low zinc supply (0.05 µM ZnSO4) or optimal zinc supply (2 µM ZnSO4) to test the zinc deficiency tolerance of the pZIP4:bZIP19 transformed coffee plants. The pH buffer MES was included in the preparation of the all nutrient solutions and the pH was adjusted to 5.5. The plants were kept under this condition for about 30 days on which, during the first two weeks, the nutrient solution was refreshed once a week and thereafter twice a week.

RESULTS AND DISCUSSION

After 30 days being grown under zinc shortage both overexpressing bZIP19 plants and DSRed transformed plants (control) were not showing any visible phenotype zinc deficiency symptoms (Figure 1). However, the control plants were generally smaller than over expressing bZIP19 plants (Figure 1). Zabini et al. (2007) found zinc deficiency symptoms in coffee seedlings only after 10 months in hydroponic medium without zinc supply. Pedrosa also reports the appearance of deficiency symptoms in coffee seedlings under zinc shortage in a similar time (9 months). Thus most likely the absence of visual zinc deficiency symptoms was due to the short duration of the experiment.

When examined for biomass production, coffee plants grown on optimal zinc supply showed no significant difference in fresh weight (Figure1), but a difference was found for plants growing on zinc shortage. Under these conditions, pZIP4:bZIP19 transformed coffee plants showed significantly 27% more fresh weight than control plants and this accumulation was higher even than under optimal Zn, although not significant.

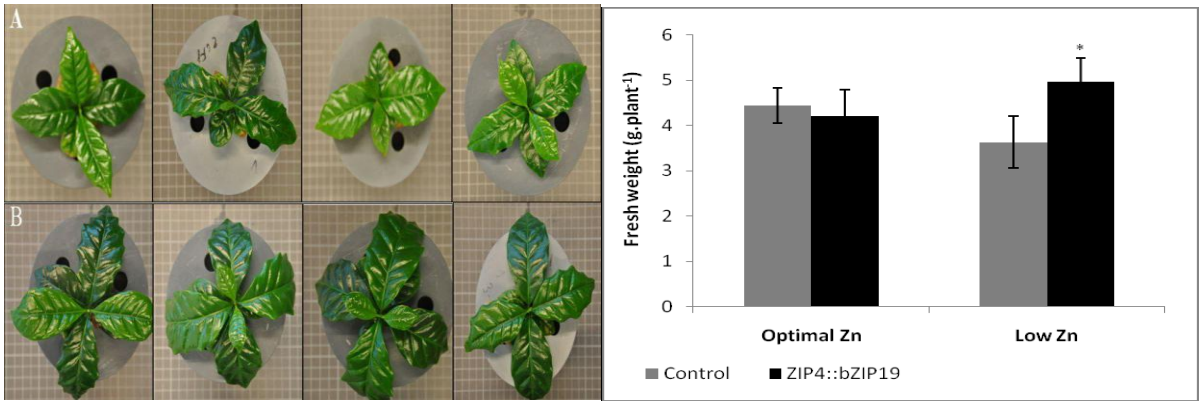

Figure 1. Visible phenotypes of of DsRed (control)(A) and pZIP4:bZIP19(B) plants, grown for 30 days on hydroponics medium to which 0.05 µM ZnSO4 has been added and fresh weight of pZIP4:bZIP19 and DsRed (control) plants, grown for 30 days on hydroponics medium either in low zinc supply (0.05 µM $ZnSO^4$) or optimal zinc supply (2 µM $ZnSO^4$). *Asterisks indicate values that are significantly different (P<0.05) from control.

Zinc is a vital micronutrient required in physiological and metabolic processes of coffee plants, although zinc deficiency is a problem frequently found in coffee regions. Plants facing zinc deficiency stress divert the destined growth energy to cope with the stress, causing reduction in biomass production. However, over expression of bZIP19 coffee plants did not

show decrease in fresh weight indicating that transcription factor bZIP19 is involved in regulating zinc deficiency response and can confer more zinc deficiency tolerance to coffee plants. So, even though this experiment was a pilot/small-scale project, the results reinforce our previous experiments performed with Arabidopsis which also showed that pZIP4:bZIP19 transformed plants to have a higher zinc deficiency tolerance. Therefore, based on these results and the fact that the transcription factor bZIP19 is conserved in the plant kingdom strongly suggested that strategies of expression bZIP19 gene under control of zinc deficiency responsive ZIP4 promoter may promote the development of a zinc deficiency tolerant crop, enabling plants growing without significant yield causes penalties in areas suffering from low zinc bioavailability, but better knowledge on physiological and molecular behavior of pZIP4:bZIP19 transformed plants in a wide range of crops is crucial to elucidate how these genes regulate the zinc deficiency response.

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Physiological, Biochemical and Molecular Responses of *Coffea* Spp. Towards Tolerance to Low Non-Freezing Temperatures

P. BATISTA-SANTOS[1], F. C. LIDON[2], F. L. PARTELLI[3], A. E. LEITÃO[1], A. S. FORTUNATO[1], P. SCOTTI-CAMPOS[4], I. P. PAIS[4], A. I. RIBEIRO[1], J. C. RAMALHO[1]

[1]Centro de Ambiente, Agricultura e Desenvolvimento (BioTrop), Instituto de Investigação Científica Tropical (IICT), Qta. Marquês, Av. República, 2784-505 Oeiras, Portugal
[2]DCTB, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal
[3]Dept. Ciências Agrárias e Biológicas, Centro Univ. Norte Espírito Santo, Univ. Federal Espírito Santo, Rodovia BR 101 Norte, Km. 60, Bairro Litorâneo, CEP 29932-540, São Mateus, ES, Brazil
[4]Instituto Nacional de Investigação Agrária e Veterinária, I. P. (INIAV), Qta Marquês, Av. República,, 2784-505 Oeiras, Portugal

SUMMARY

Low positive temperatures are of utmost importance to tropical plant species, namely *Coffea* spp., since they disturbs plant growth and metabolism, with impact on photosynthesis and yield. Coffee genotypes with contrasting cold sensitivity were used and determinations were performed along a slow cold imposition from 25/20 ºC (day/night) down to 13/8 ºC, after exposure to 4º C (chilling) and in the rewarming period thereafter. Cold exposure strongly affected net photosynthesis and chlorophyll *a* (Chl *a*) fluorescence parameters in all genotypes, although stomatal limitations were not detected. Some genotypes revealed lower leaf loss and less deleterious effects on photosynthetic capacity (A_{max}) and photosystem I (PSI) activity, as well as the reinforcements of PSII activity and of the antioxidative system. That was further related to qualitative changes in chloroplast membrane lipids and to the regulation of key genes expression. Considering a broader view that integrates the results from our group, this multidisciplinary approach points to useful criteria for cold tolerance selection in coffee plants.

INTRODUCTION

Low positive temperatures (chilling) are known to depress growth, photosynthetic performance and yield in *Coffea* spp. Despite the known sensitivity of the main producing coffee species, recent reports highlighted the existence of different degrees of cold sensitivity within the *Coffea* genus. It is widely recognized that the control of oxidative stress through the reinforcement of scavenging and detoxifying mechanisms is of crucial importance to plant tolerance and survival under cold conditions. It is also known that reactive oxygen species (ROS) accumulation provokes changes in the redox potential that are implicated in gene induction. Cold stress involves a tight regulation of expression of transcription factors and affects genes collectively called cold-regulated (COR) genes. Temperature induced changes in membrane fluidity might be the first signal in the perception and/or damage. Membrane fluidity is strongly influenced by the lipid molecular species composition, unsaturation degree and environmental temperature. Therefore, an integrated physiological, biochemical and molecular approach was used to evaluate cold impact and the triggering of mechanisms that

allow plants to cope with this environmental constraint, particularly in what concerns the photosynthetic pathway.

MATERIAL AND METHODS

Plant Material and Growth Conditions

The experiments were carried out as previously described, using 1.5 years old plants from the genotypes Icatu (IAC 2944 - *C. canephora* x *C. arabica*) and *C. dewevrei*. Potted plants were submitted successively to: 1) a gradual temperature decrease (0.5 ^{o}C day^{-1}) from 25/20 ^{o}C to 13/8 ^{o}C (day/night), to allow the expression of acclimation ability; 2) a 3 day chilling cycle (3x13/4 ^{o}C), where the plants were subjected to 4 ^{o}C during the night and in the first 4 h of the morning (thus, with light) and 13 ^{o}C throughout the rest of the diurnal period; 3) rewarming conditions (25/20 ºC) were applied for 7 days in order to allow plants to recover. Photoperiod was set to 12 h, RH to 65-70%, external CO_2 concentration to *ca* 380 µL L^{-1} and irradiance to *ca*. 750-850 µmol m^{-2} s^{-1}.

Visual evaluation of temperature impact on leaves

The percentage of damaged leaves (with visible necrotic injury in more than 50% of leaf area or those that shed) in relation to the number of leaves registered at the beginning of the experiment was determined in 8-10 plants.

Enzyme activities

Chloroplast Cu, Zn-superoxide dismutase (Cu, Zn-SOD- EC 1.15.1.1) was determined as in Plant Sci 135, 115–24.

Pigment analysis

β-carotene and chlorophylls were determined as in Braz J Plant Physiol 18: 55-81.

Gene expression studies

Based on coffee cDNA sequences, specific primers were designed in order to perform the mRNA expression studies by real time PCR as described in J Plant Physiol, 167: 333-342.

Statistical analysis

A two-way ANOVA ($P < 0.05$) was applied to evaluate differences between temperatures and between genotypes, followed by a Tukey test for mean comparison (95% confidence level).

RESULTS AND DISCUSSION

During cold exposure Icatu showed the lowest leaf loss, contrasting to *C. dewevrei*, giving the first (morphological) indication of a higher cold tolerance (Figure 1), since the loss of leaf area could be related with severe damages resulting from chilling exposure and with the sensitivity degree of *C. dewevrei* at metabolic level.

The detected impairments in net photosynthesis (P_n) (Figure 2) and fluorescence parameters probably integrate reversible (in Icatu) and irreversible mesophyll impairments, as reflected by the impacts in A_{max} (Figure 2). In fact, while the photosynthetic machinery of Icatu was

quite preserved, showing a maximal A_{max} drop of 19%, *C. dewevrei* suffered reductions of 47%, reflecting not readily reversible effects.

The involvement of oxidative stress often accompanies PSI photoinactivation *in vivo*. PSI photoinhibition may also be induced under decreased SOD activity, what happened in *C. dewevrei* upon chilling but not in Icatu (Figure 3). Furthermore, the gradual decrease of Cu, Zn-SOD activity in *C. dewevrei* suggests less efficient control of $O_2^{\bullet -}$ (and of $OH^{\bullet}$), which is consistent with its greater cold sensitivity reported previously. Impairments observed on PSI activity in *C. dewevrei* may be related to photoinhibition damage, as suggested by the parallel declines in the Chl *(a/b)* ratio and in β-carotene contents (Figure 4).

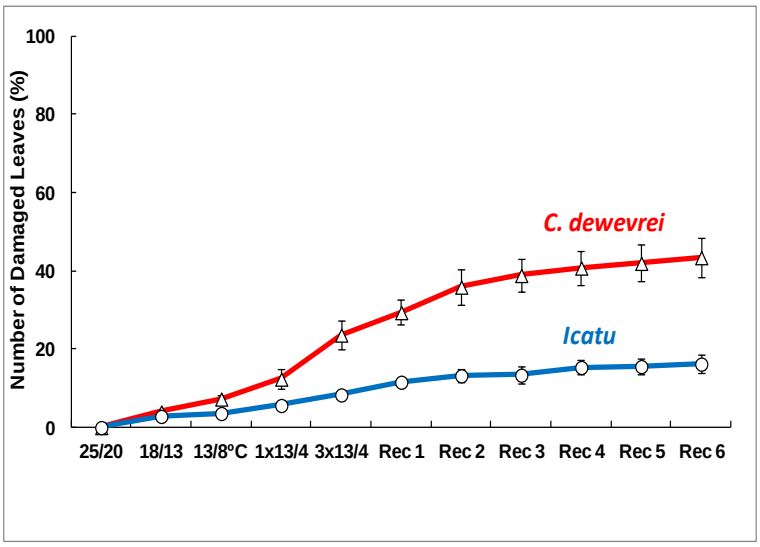

Figure 1. Variation of the number of damaged leaves (% per plant) by cold conditions in two *Coffea* spp. genotypes under the imposed experimental conditions.

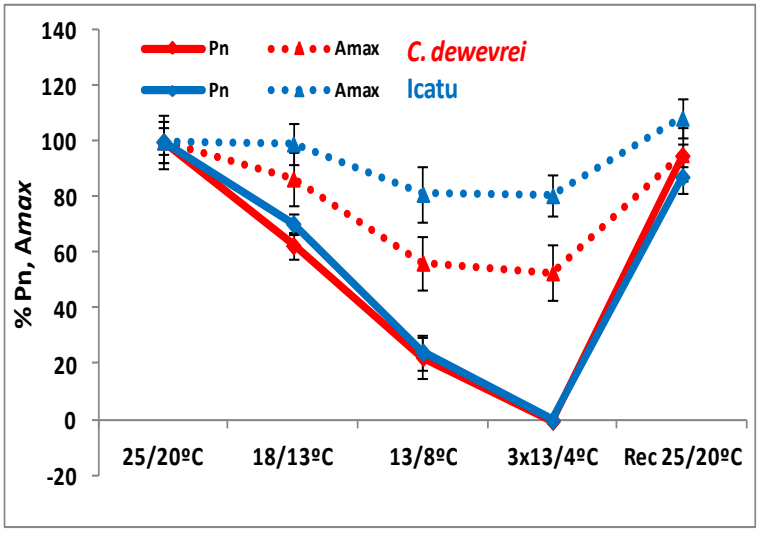

Figure 2. Changes in P_n and A_{max} (%), as compared to their respective control values, in two *Coffea* spp. genotypes under the imposed experimental conditions.

These changes were interpreted to reflect selective photobleaching of Chl *a* in PSI, associated with PSII inhibition, and impairments in the PSI complex, respectively. The higher β-carotene content in Icatu along the course of the experiment (Figure 4) could have contributed to

protect the PSs against photodamage, what agrees with the preservation of PSI activity in this genotype.

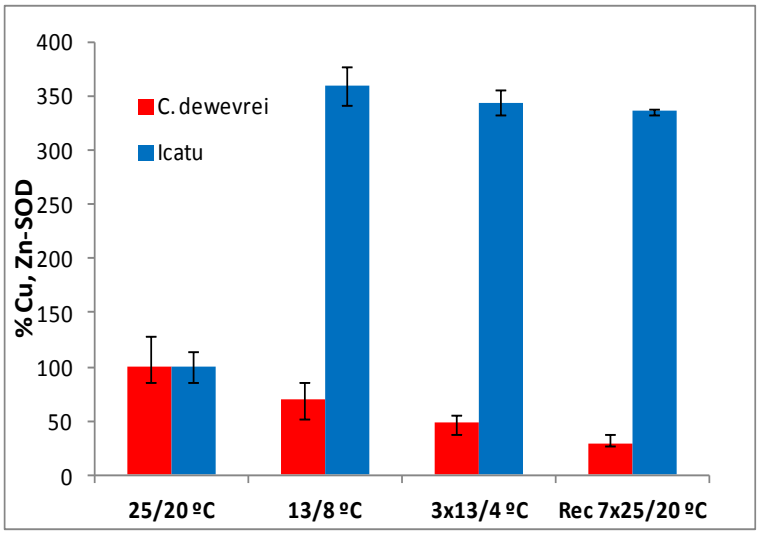

Figure 3. Changes in Cu, Zn-SOD (%), as compared to their respective control values, in two *Coffea* spp. genotypes under the imposed experimen tal conditions.

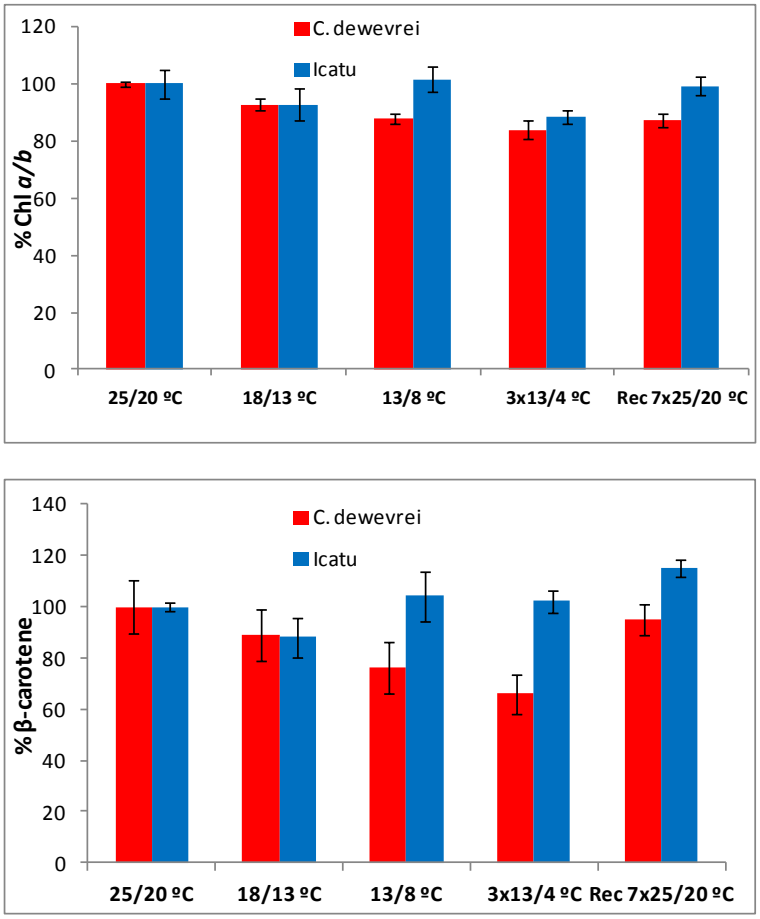

Figure 4. Changes in β-carotene and in Chl *a/b* (%), as compared to their respective control values, in two *Coffea* spp. genotypes under the imposed experimental conditions.

Phosphatidylglycerol (PG) is believed to be directly implicated in cold acclimation, through FAs unsaturation and specific roles of some fatty acids (FAs). After chilling exposure Icatu showed significantly higher linolenic acid (C18:3) and double bond index (DBI) values than

C. dewevrei (Figure 5). Furthermore, *C. dewevrei* presented the strongest reductions and the lowest values of *trans*-Δ^3-hexadecenoic acid (C16:1*t*), while Icatu displayed the lowest decreases in C16:1*t* and a full recovery by the end of the experiment (Figure 5). C16:1*t* was related to decreases of stress damage under excess of light energy and cold and to the process of replacement of damaged D1 protein. Additionally, a higher PG unsaturation also contributes to the renewal of photodamaged D1, reducing PSII photoinhibition and allowing a higher rate of the recovery from the photoinhibited state. In this way, the higher DBI value and lower C16:1*t* decreases could be related to the lower negative impact on the photosynthetic capacity upon chilling and to the prompt recovery of photosynthesis rate observed in Icatu, but not in *C. dewevrei*.

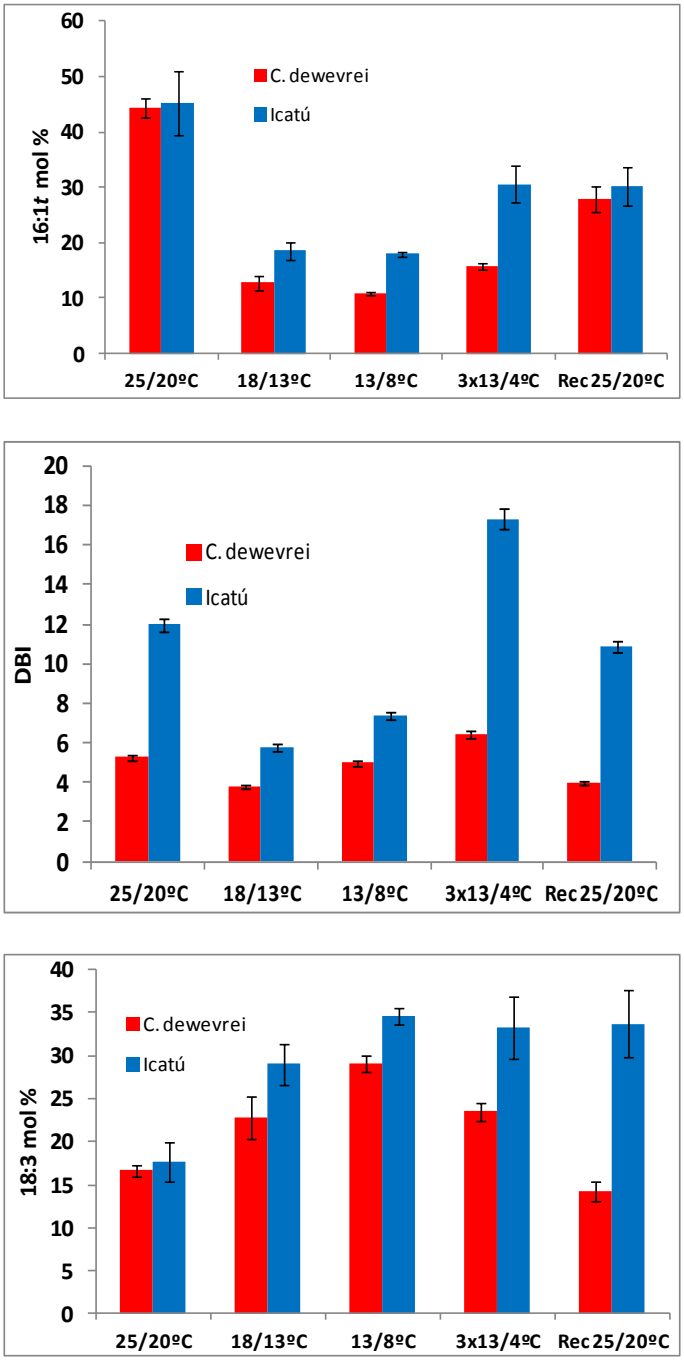

Figure 5. Fatty acid composition (mol %) and unsaturation degree (DBI) of phospholipid PG of chloroplast membranes in two *Coffea* spp. genotypes under the imposed experimental conditions.

Gene expression studies indicated increased mRNA levels of the gene encoding Chlorophyll a/b-binding 22kDa protein *(caCP22)* in Icatu, during cold imposition, while *C. dewevrei* showed strongly reduced values (Figure 6). Plant acclimation often involves an increased non-photochemical quenching (NPQ) capacity through abundance adjustments of the gene encoding the CP22 protein. That might be the case in Icatu since the calculated NPQ is probably underestimated due to overnight zeaxanthin (Zea) retention.

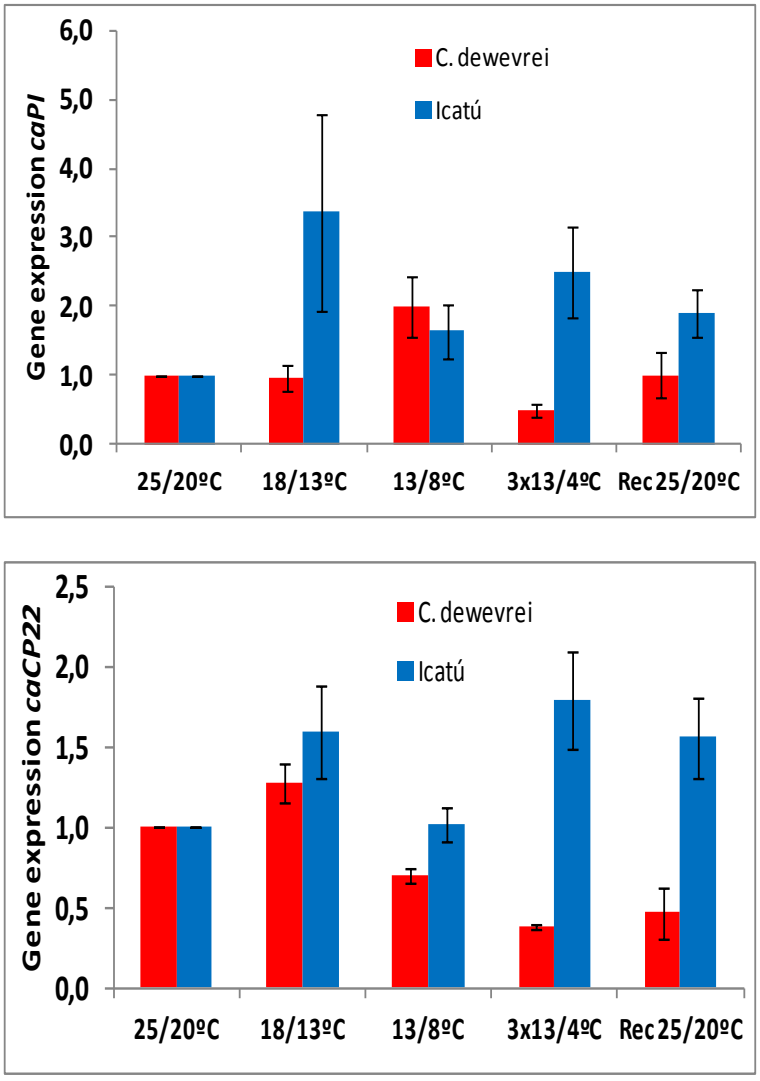

Figure 6. Real-Time PCR gene expression studies of *caCP22* and *caPI* in two *Coffea* spp. genotypes under the imposed experimental conditions.

Concerning the gene encoding the Photosystem I subunit protein (*caPI)*, *C. dewevrei* presented single transcript increments during cold imposition and reduced values (*ca*. 46%) after chilling (Figure 6). Icatu showed expression rises upon cold exposure, with a 2-fold increment (18/13 ºC). The low *caPI* expression in *C. dewevrei* upon chilling probably contributed to reduce the repair ability, resulting in a higher impact on PSI activity. By contrast, the assumed higher production of this protein in Icatu might have allowed PSI activity maintenance, since it is a cold sensitive key point in *Coffea* spp.

In conclusion, this multidisciplinary approach gave some insights concerning the differential photosynthetic cold tolerance in *Coffea* spp. with a better performance of Icatu. That was

achieved through the cooperation between photoprotection and repair mechanisms, which constitutes useful criteria for cold (and oxidative stress) tolerance selection in coffee plants.

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Evaluation of Coffee Progenies, BC_5 and BC_6 Generation According to its Resistance to the Leaf Miner and to the Rust

J. V. A. NONATO[1,2], A. P. MENDONÇA[1,2], O. GUERREIRO-FILHO[1]

[1]Centro de Café - Instituto Agronômico de Campinas (IAC), P. O. Box 28, 13012-970, Campinas, SP, Brazil
[2]Capes Research Fellowships

SUMMARY

Coffee culture is an important agricultural activity in Brazil. The country is responsible for about 25% of the international market. However, coffee culture efficiency can be affected by a great number of pests and diseases. Thus, it is important to increase this efficiency by selecting new cultivars resistant to the coffee leaf miner (*Leucoptera coffeella*) and to the rust (*Hemileia vastatrix*), which are considered the main phytosanitary problems of coffee culture. This study aimed to evaluate progenies from BC_5F_1 and BC_6F_1 generations, regarding leaf miner and rust resistance. For evaluations with the leaf miner, progenies were artificially infested, and for rust analysis, plants were evaluated at by natural infestation at field conditions. The H20049 progeny deserves special attention due to high resistance levels shown to the leaf miner and rust, an important fact for future selections aiming the development of new cultivars.

INTRODUCTION

Estimated brazilian production for 2012/13 coffee harvest (arabica and conilon) is of 50.48 million bags of processed product, in a planted area of 2.3 million hectares accounts for approximately 25% of the traded international volume. In this production, the species *Coffea arabica* is responsible for 37.9 million processed bags accounting for about 75% of production.

The productive potential of coffee can be affected by a number of pests and diseases, leaf miner and rust are the main phytosanitary problems of coffee culture. The *Leucoptera coffeella* (leaf-miner) is a major pest that attacks the coffee culture. Their caterpillars cause mines or lesions on leaves resulting in necrotic areas. The mined leaves fall, and depending on the intensity and the period of pest infestation, drastic defoliations occur, affecting fruit production. The coffee leaf rust is caused by the *Hemileia vastatrix* fungus and is the main culture disease in Brazil causing considerable losses to the production and coffee quality. The disease affects the underside of the leaves causing pale-yellow patches appearance, which in a few days develop to yellow-orange and pulverulent patches. These leaves generally fall and damage fruit production of mature plants.

The selection of new resistant coffee cultivars to pests and diseases is crucial to increase productivity and to reduce costs since the use of pesticides may not be effective, and besides degrade the environment. The coffee breeding program of the Instituto Agronômico de Campinas has been conducting the selection of resistant coffee to leaf miner through transferring genes from *Coffea racemosa* to *C. arabica* commercial cultivars. Allied to this, resistant plants to leaf miner selected in BC_4 and BC_5 generations were backcrossed with cultivars IAC 144 and Catuaí SH_3 aiming the selection of new cultivars with multiple

resistance to leaf miner and to rust. Therefore, this study aimed to evaluate progenies from BC_5F_1 and BC_6F_1 generations regarding to plant resistance to leaf miner and to rust.

MATERIAL AND METHODS

The progeny testing was planted in March 2003 at the Centro Experimental of Instituto Agronômico de Campinas. The experimental design used was randomized blocks consisting of six treatments and five replications with three plants per plot, totaling 90 plants. From the six progenies analyzed, four belong to the BC_6F_1 generation; two of them were obtained from the crossing of coffee trees resistant to leaf miner with cultivar Catuaí SH_3 and the two others from the crossing of insect resistant trees with the cultivar IAC 144. A fifth analyzed progeny belongs to BC_5F_1 generation. The cultivar Catuaí Vermelho IAC 99 (CV IAC 99) was used as control the suscetible.

Coffee resistance assessment

The plants were evaluated individually according to the following.

Resistance to leaf miner in the laboratory

Assessments were carried out from November/2011 to January/2012. To take measurements, leaves were collected from plants in the field, taken to the laboratory, washed and subjected to infestation by insects in breeding cages. Subsequently, discs were removed from the leaves just in places where eggs were laid. The discs were kept in plastic boxes with moistened foam. The disc assessment was performed seven days after infection, according to the type of reaction through grading scale already established, in which the disks were classified as: 1 = resistant to punctual lesions; 2 = moderately resistant to small filiforms type lesions; 3 = moderately susceptible to large and irregular filiform type lesions and; 4 = susceptible to large and rounded lesions.

Resistance to rust in the field

The assessment of field resistance to rust was performed in June/2012. The plants were assessed regarding type of reaction using a scale from 1 to 5 points: 1 point assigned to immune plants; 2 points awarded to the resistant plants; 3 points to moderately resistant plants; 4 points to moderately susceptible plants, and 5 points to susceptible plants.

The results presented in this paper are related to the average of assessments performed both for resistance to leaf miner as for rust resistance. The analysis of phenotypic segregation of plants for resistance to leaf miner and rust resistance was performed using the chi-square (X_2) using GENES program. Phenotypic proportions in population BC_5F_1 and BC_6F_1 for rust were analyzed for inheritance controlled by a dominant gene. For the leaf miner, phenotypic proportions in population BC_5F_1 and BC_6F_1 were analyzed for inheritance controlled by two complementary and dependents genes (3:1).

RESULTS AND DISCUSSION

The mean values of resistance and segregation evaluations, resistance and susceptibility of coffee trees to rust and leaf miner are shown in Table 1. Regarding resistance to the leaf miner, only Catuaí Vermelho IAC 99 and the progeny H20050 obtained grades above 3 points. The mean values related to rust show that progenies H20050 and H20049 are resistant, judging by the type of reaction. Two progenies resulted from crossings with Catuaí SH_3

which explains this possible resistance. As for multiple resistance, just the progeny H20049 showed resistance to rust and leaf miner.

Table 1. Mean of resistance profile and segregation of resistance (R) and susceptible (S) to leaf miner and rust of BC5 and BC6 progenies of coffee tree.

Progenies	Number of Plants	*Leucoptera coffeella*[1]						*Hemileia vastatrix*[2]						
		Resistance		*Susceptible*		*Mean*	X^{2A}	*Resistance*		*Susceptible*			*Mean (2012)*	X^{2A}
		1	*2*	*3*	*4*			*1*	*2*	*3*	*4*	*5*		
CV IAC 99	14	0	0	8	6	3.43	-	0	0	0	13	1	4.07	-
H20050	14	1	0	8	5	3.21	2.381	0	8	4	1	1	2.64	0.285
H20049	15	3	1	7	4	2.80	0.022	1	7	6	1	0	2.46	0.066
H20034	15	5	0	3	7	2.80	0.555	0	0	9	6	0	3.40	0.000
H20033	15	3	0	7	5	2.93	0.200	0	0	8	7	0	3.46	0.000
H20032	15	4	0	5	6	2.86	0.022	0	0	5	10	0	3.66	0.000

[1]Levels of resistance conferred to plants through scale of 1 to 4 points. 1 = resistant, 2 = moderately resistant, 3 = moderately susceptible and 4 = susceptible; [2]Levels of resistance conferred to plants through scale of 1 to 5 points. 1 = immune; 2 = resistant, 3 = moderately resistant, 4 = moderately susceptible and 5 = susceptible; [A]X^2 ($P<0.05$) = 3.84.

In the analysis of inheritance for leaf miner resistance, the progenies segregate at a proportion of 3 susceptible: 1 resistant. The results are in agreement with the studies conducted by Guerreiro-Filho O., Silvarolla M. B. and Eskes, A. B which concluded that two complementary genes and dominant (*Lm1* and *Lm2*) are responsible for resistance to pests.

The inheritance studies performed with progenies H20050 and H20049 in relation to rust resistance, show that inheritance is ruled by a dominant gene with 1 susceptible: 1 resistant segregation. Also considering these two progeny, it was also observed that the plant used as donor of resistance genes (Catuaí SH_3) is heterozygote. In relation to the other three progenies (H20034, H20033 and H20032), which are not derived from cross with Catuaí SH_3, there was no segregation. All these progenies were susceptible to rust. This result also shows that plants used as parents in these crosses are recessive and susceptible to the fungus.

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Performance of an Arabica Cultivar onto a Diverse *Coffea* Rootstock Germplasm[1]

H. P. MEDINA-FILHO[2], R. BORDIGNON[3]

[1]Sponsored by Consórcio Pesquisa Café
[2]CNPq fellowship
[3]Centro de Café – Instituto Agronômico de Campinas
Avenida Barão de Itapura, 1481, Botafogo, 13020-902, Campinas, SP, Brazil

SUMMARY

Many scion rootstock interactions are known in fruits, rubber trees and other cash crops. Little has been exploited in commercial coffee in Brazil except for grafts of *C. arabica* seedlings onto *C. canephora* cv. Apoatã to avoid nematode attacks. In order to study the influence of the genetic diversity of rootstocks on the canopy characteristics of *C. arabica* cv. Obatã, 509 control plants derived from seeds were compared to 446 plants of Obatã grafted at hypocotiledonary stage on 68 open pollinated progenies of *Coffea* germplasm comprising 15 related groups of accessions, mutants, hybrids or derivatives of *C. arabica*, *C. canephora*, *C. eugenioides*, *C. racemosa*, *C. salvatrix*, *C. kapakata*, *C. dewevrei*, *C. liberica* and *C. stenophylla*. Field evaluations were carried out from 2003 to 2012 as to vigor, yields, height, width, trunk circumference, wilt, ripening and cup quality in one or more cropping years. Greater variability was observed among grafted plants as compared to controls but the magnitude was not the same either among parameters or along years. The effects of some of the germplasm groups were evident from the second or third year becoming more intense by the end of assayed period. In 2003 average survival was 100% in control groups compared to 98.3% in the grafted ones, but in 2012, turned to 59.7% and 73.7% respectively, the latter having 6 groups varying from 81.6 to 100%. ANOVA identified 5 significantly higher and 6 wider canopies corresponding to graftings on *C. canephora* accessions, BC_1 *C. eugenioides* 4n x *C. arabica*, *C. salvatrix* hybrid derivatives and *C. dewevrei* 4n x *C arabica*. Throughout the years BC_1 *C. eugenioides* 4n x *C. arabica* had the strongest positive influence on vigor, but 3 accessions of *C. eugenioides* induced the weakest ones. Plants on specific progenies of Mokka and Maragogipe derivatives, Conilon, BC_1 *C. eugenioides* 4n x *C. arabica*, *C. arabica* x *C. dewevrei* 4n and *C. racemosa* x *C. arabica* BC_2 derivative were the most vigorous. In general, yields, cup quality and ripening did not show any consistent differences among Obatã controls and grafted groups. Within groups, variation reflected genetic variability among rootstock plants indicating that selections should be made on individual rootstock plant basis, previously secured by the novel procedure of rooting top hypocotiledonar cuttings of the grafted rootstocks.

INTRODUCTION

The coffee breeding program of Instituto Agronômico de Campinas, Brazil, is primarily focused in the development of cultivars of *Coffea arabica* aiming at, among others, high yields, vigor and tolerance to abiotic stress. As the result of such long run project, expressive cultivars with wide acceptance in Brazil and abroad have been released. If not all, the majority of selected characteristics have been evaluated in the canopies or their component parts. However, their growth and general performance are, at undetermined extent, reflex of the attributes of the root system. Except for the program for nematode resistance, little emphasis

has been put on the specific selection for root system mainly regarding to possible influences and interactions with grafted canopies. In such situations different canopies and rootstocks are forced to interact and constitute a single entity to the crop's benefit. This is well known in fruit and rubber trees where improved agronomic performance and quality are commercially exploited in grafted orchards.

Studies on coffee grafting had begun long ago. Commercially, G. van Riemsdijk farmer of Java, Indonesia, developed for *C. canephora* a graft technique aiming at control of nematodes, multiplication of hybrids, increase of yields and uniformity. It was initially little accepted due to high costs and low demands of farmers and market for uniform Robustas. Latter, the development of stable cultivars grown from seeds and efficient propagation of clones by cuttings lowered further the interest for grafting *C. canephora*. In *C. arabica*, grafted seedlings to control nematodes became commercially used after Reina developed the technique in Guatemala and Moraes and Franco preconized in Brazil the hypocotiledonary grafts of *C. arabica* onto *C. canephora*. It became widely used in nematode infested regions after the release of resistant cv. Apoatã of *C. canephora*. In nematode free soils scions of *C. arabica* have shown differences in height, leaf area and nutrient content when grafted on two progenies of *C. canephora*, one *C. congensis* and a few *C. arabica* cultivars.

In the present investigation *C. arabica* cv. Obatã was grafted onto a wide rootstock germplasm of *Coffea* species and derivatives. The influence of the genetic variability of rootstocks on the scions was studied by comparing grafted with non-grafted normal plants derived from seeds.

MATERIALS AND METHODS

Rootstocks seeds were obtained by open-pollination and sown in sand nursery beds. After germination when the cotyledons were still enclosed in the parchment coats, seedlings were pulled out, hypocotyledonary cleft grafts performed with *C. arabica* cv. Obatã IAC 1669-20 scions and immediately transplanted to 50% shaded nursery trays under continuous mist. Top cotyledonary cuts of rootstock seedlings were also transplanted to trays in order to clone and preserve individual genotypes used as rootstocks, a procedure that revealed to be easy and efficient. In this investigation 509 control plants derived from Obatã seeds were compared to 446 Obatã plants grafted onto 68 open pollinated progenies of *Coffea* germplasm comprising 15 related groups of accessions, mutants, hybrids or derivatives of *C. arabica, C. canephora, C. eugenioides, C. racemosa, C. salvatrix, C. kapakata, C. dewevrei, C. liberica* and *C. Stenophylla* (Table 1). Year old grafted and non-grafted Obatã control seedlings were transplanted to the field in 2002, in nematode free, low fertility typic Haplustox soil in Campinas. Spacing was 3,80 x 1,80m with grafted and controls plants in double alternated rows analyzed in randomized design with variable number of replicated plants per progeny. In order to study the influence of the genetic diversity of rootstocks on the canopy characteristics of Obatã, evaluations were performed in one or more crop years until 2012 for several parameters. Vigor and general performance were estimated by plant measurements of height (h), width (w) and trunk circumference and calculation of canopy volume ($\pi R^2 h$), shape (h/w), efficiency (yield/volume) as well as scores assigned (higher more intense) to yield, leafiness, vigor, wilting, ripening and dry processed SCAA global cup quality. ANOVA, means, standard deviations, coefficients of variation and mean comparisons with control non-grafted Obatã (Dunnett, 95% of probability) were performed with Minitab Statistical Software.

Table 1. Groups of rootstock germplasm, number of progenies in each group and number of grafted Obatã plants transplanted to the field.

Group	Rootstock germplasm	Nº of progenies	Nº of plants
1	*C. arabica* control, non-grafted Obatã	6	509
2	*C. arabica* mutants and hybrids	10	79
3	*C. canephora* accesses of Conilon	11	54
4	*C. canephora* accesses of Robusta	3	19
5	*C. canephora* progenies of cv. Apoatã	10	85
6	*C. canephora* var. Laurentii	1	2
7	*C. canephora* 4n x *C. arabica* Arabustas	4	17
8	*C. eugenioides* accesses	3	5
9	*C. eugenioides* 4n x *C. arabica* BC_1 progenies	3	14
10	*C. eugenioides* 4n x *C. Arabica* BC_2 progenies	3	20
11	*C. racemosa* x *C. Arabica* derivatives and BC_3 progenies	8	64
12	*C. salvatrix* hybrid derivatives	2	19
13	*C. kapakata* hybrid	1	1
14	*C. arabica* x *C. dewevrei* 4n derivatives	4	49
15	*C. liberica* and hybrids	4	16
16	*C. dewevrei* x *C. stenophylla*	2	2

RESULTS AND DISCUSSION

Since the first years it was observed greater variability among grafted plants as compared to controls (Table 2), evidencing the influence of the rootstocks or their interactions on the Obatã scion. For eight parameters after three years in the field, grafted plants showed an average of 45% more variation than non-grafted ones. The magnitude of variation was not the same either among parameters or along years, but the effects of some of the germplasm groups were evident from the second or third year becoming more intense by the end of assayed period.

Table 2. Coefficients of variation and percent relation to group 1 (control). Data of 3 years.

Group	Yield	Height	Diameter	Shape	Volume	Canopy Efficiency	Trunk Circumference	Drought Tolerance	Group Mean
1	38,5	12,6	11,9	13,8	27,3	41,6	9,2	17,3	21,53
2	42,2	11,9	8,2	11,2	23,7	34,7	9,2	18,8	19,99
3	56,2	18,6	26,7	50,8	44,5	93,8	20,0	29,1	42,48
4	60,0	26,0	8,9	25,5	34,4	100,7	8,0	12,5	34,51
5	44,5	13,6	17,5	32,5	30,9	92,4	14,0	20,7	33,27
6	35,2	10,9	6,9	3,9	24,6	11,2	3,6	5,2	12,70
7	40,6	8,3	8,9	7,5	23,5	44,8	11,3	10,3	19,40
8	139,5	24,6	46,9	74,5	80,8	141,0	28,0	23,8	68,65
9	46,1	12,5	6,2	8,5	23,3	46,7	10,4	6,0	19,98
10	40,3	10,4	10,9	10,9	27,0	33,8	9,4	21,5	20,54
11	49,1	16,3	19,8	43,6	40,1	51,4	19,5	22,4	32,74
12	41,9	8,7	4,3	8,1	14,3	35,4	8,5	8,2	16,17
13	-	-	-	-	-	-	-	-	-
14	49,1	17,9	17,3	48,0	38,3	111,4	13,9	20,4	39,55
15	48,0	17,2	9,2	13,0	33,1	41,1	9,4	20,1	23,90
16	114,5	23,6	20,9	2,6	61,6	81,8	14,9	12,1	41,51
Mean	57,60	15,75	15,20	24,35	35,72	65,75	12,87	16,51	**30,38**
%	+49,8	+24,9	+27,8	+76,4	+30,8	+58,0	+39,3	-5,0	**+45,45**

As observed in Table 3 yield, drought tolerance, ripening and cup quality despite their major interest, were not favorably influenced by any rootstock group. Height, width, trunk diameter and canopy volume were more affected as indicated by their significant mean compared to non-grafted control.

ANOVA for 2012 height and width identified 5 significantly higher and 6 wider groups corresponding to *C. canephora* accessions, BC_1 *C. eugenioides* 4n x *C. arabica*, *C. salvatrix* hybrid derivatives and *C. dewevrei* 4n x *C arabica*. Throughout the years BC_1 *C. eugenioides* 4n x *C. arabica* had the strongest positive influence on vigor but surprisingly, 3 accessions of *C. eugenioides* induced the weakest canopies of all groups. Within group analysis, some specific progenies of Mokka and Maragogipe derivatives, Conilon, BC_1 *C. eugenioides* 4n x *C. arabica*, *C. arabica* x *C. dewevrei* 4n and *C. racemosa* x *C. arabica* BC_2 derivative induced the most vigorous canopies.

Although the above analysis of assayed germplasm revealed a few trends for groups, more detailed analysis within groups and their progenies displayed considerable variation, some standing out, with conspicuous favorable effect on general vigor and yield of specific plants (Figure 1). As scions of all plants had the same Obatã genotype, observed variability should conceivably be ascribed to correspondent variability of rootstocks genotypes, reasoning further reinforced by the uncontrolled autocrossed origin of rootstock seeds.

Table 3. Group means of yield scores for 6 years, height, width, trunk diameter, volume and cup quality in 2008, drought tolerance and ripening in 3 years. Means in bold numbers are statistically different (Dunnett, 95%) from control group 1.

Group	Yield	Height	Width	Trunk diameter	Volume	Drought tolerance	Ripening	Cup quality
1	4.2	129.2	122.6	21.2	1.65	7.3	3.7	65.4
2	**3.5**	136.6	128.9	21.6	1.91	7.5	3.7	67.9
3	4.3	**150.0**	**137.8**	21.0	**2.40**	7.3	3.9	68.1
4	3.9	**156.7**	137.3	21.0	**2.46**	7.6	3.9	65.9
5	3.9	**151.2**	**136.1**	20.9	**2.42**	7.3	3.7	67.2
6	4.1	115.0	120.0	20.5	1.28	5.6	4.0	63.5
7	3.8	144.7	135.3	**23.7**	2.20	7.7	3.9	68.3
8	**1.9**	107.5	97.5	**16.0**	1.00	6.1	3.7	67.5
9	3.9	**158.6**	136.4	20.9	**2.54**	7.0	3.9	65.0
10	4.8	140.0	134.1	21.5	2.17	7.4	3.8	68.8
11	3.9	134.0	129.0	20.4	1.96	**6.8**	3.8	67.4
12	4.8	144.7	134.1	21.2	2.27	7.0	3.9	68.0
13	2.2	140.0	130.0	20.0	1.86	6.2	4.5	65.0
14	**3.4**	124.7	114.0	**19.7**	1.64	**6.6**	3.9	65.7
15	**2.3**	126.1	113.8	20.3	1.63	**6.3**	4.4	67.9
16	3.5	145.0	140.0	20.0	2.34	6.7	4.2	78.5

Missing plants were also recorded. In 2003 average survival was 100% in control group compared to 98.3% in the grafted ones. In 2006 this was 99.6% and 95.8%, in 2008, 95.1% and 91.4% but in 2012, after quite climate and cultural unfavorable conditions, shifted to 59.7% and 73.7% respectively, with 6 germplasm groups varying from 81.6 to 100% survival (Table 4). Certainly, some rootstocks impressed evident rusticity to the Obatã canopy reflected by both increased survival and vigor of plants. This was the case of groups 3, 4, 5, 9 and 12 for height and 2, 3, 4, 5, 9 and 14 for width, statistically significant at 5% level with special remarks to groups 2, 5, 9 and 14 in which reasonable number of individual plants were assayed and showed survival rates of 87.4, 82.4, 92.9 and 81.6, respectively.

Table 4. Number of plants transplanted to the filed in 2003, count in 2012 and their correspondent percentage survival in 2003, 2006, 2008, 2009 and 2012. For group 1, mean correspond to the percent average of 6 subgroups of non-grafted Obatã control plants and for groups 2 to 16 to the average of Obatã grafted onto all groups of assayed germplasm.

	2003	2012	2003		2006		2008		2009		2012	
Group	**Nº**	**Nº**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**	**%**	**Mean**
1	509	304	100.0	100.0	99.6	99.6	95.1	95.1	89.6	89.6	59.7	59.7
2	79	69	100.0		100.0		100.0		98.7		87.4	
3	54	39	94.4		87.0		75.9		75.9		72.2	
4	19	14	94.7		78.9		78.9		78.9		73.7	
5	85	70	98.8		90.6		88.2		87.0		82.4	
6	2	2	100.0		100.0		100.0		100.0		100.0	
7	17	15	100.0		100.0		100.0		100.0		88.2	
8	5	2	100.0		100.0		80.0		80.0		40.0	
9	14	13	100.0	98.3	100.0	95.8	100.0	91.4	100.0	90.2	92.9	73.7
10	20	14	100.0		100.0		85.0		85.0		70.0	
11	64	48	98.4		98.4		96.9		92.2		75.0	
12	19	14	94.7		94.7		89.5		84.2		73.7	
13	1	0	100.0		100.0		100.0		100.0		0	
14	49	40	100.0		100.0		95.9		89.8		81.6	
15	16	11	93.7		87.5		81.2		81.2		68.8	
16	2	2	100.0		100.0		100.0		100.0		100.0	

Within groups and their different progenies, variation reflects genetic variability of rootstock plants, as expected on basis of their hybrid nature, open pollination and outcrossed reproduction. This fact precludes obtaining identical genotypes by harvesting back original plants. However, selection of favorable rootstock genotypes would be most efficiently done on the basis of individual plants that, nevertheless, do not exist as such anymore but just as rootstocks. In order to circumvent this drawback, one could consider rescuing selected rootstocks after cutting back their canopies but this would be certainly hindered by the known absence of buds in the hypocotyl. The solution for this was worked out, a novel strategy consisting of cloning the tops of rootstock seedlings on occasion of the grafting operation. This procedure is easily performed and was successfully done provided young hypocotyledonary cuttings rooted promptly. Individual selections thus secured, form the future base population for an arabica rootstock breeding program.

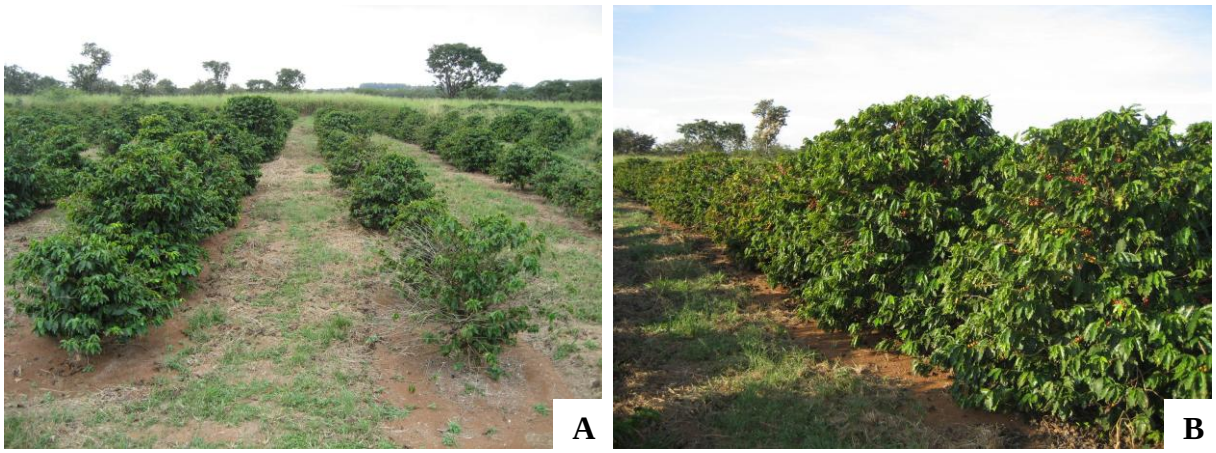

Figure 1. A - Vigor and morphological variations in canopies of cv. Obatã grafted (left) and non-grafted (right). B - Variation in vigor of grafted Obatã plants due to genetic variability of rootstocks.

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Phylogenetic Analysis of *Hemileia Vastatrix* and Related Taxa Using a Genome-Scale Approach

S. N. DIOGO[1,2], A. VIEIRA[1], P. TALHINHAS[1], H. G. AZINHEIRA[1], S. M. DO CÉU[1], D. FERNANDEZ[3], S. DUPLESSIS[4], O. S. PAULO[2], D. BATISTA[1]

[1]CIFC/IICT - Centro de Investigação das Ferrugens do Cafeeiro/Instituto de Investigação Científica Tropical, Quinta do Marquês, 2784-505 Oeiras, Portugal
[2]Computational Biology and Population Genomics group, Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[3]Institut de Recherche pour le Développement, UMR 186 IRD/CIRAD-UM2 Résistance des Plantes aux Bioagresseurs, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France
[4]Institut National de la Recherche Agronomique UMR 1136 INRA/Université de Lorraine Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France

SUMMARY

For more than a century, Coffee Leaf Rust caused by the biotrophic fungus *Hemileia vastatrix* (Berkeley & Broome), has increasingly stood out as one of the major factors hampering Arabica coffee production. Since its first historical outburst in the 19th century in Sri Lanka, this disease has rapidly spread worldwide and currently occurs in nearly all the regions of the world where coffee is grown. Despite its widespread distribution and negative economic impact, little is known about the pathogen's evolutionary origin and phylogenetic placement in the fungal tree of life. Attaining this knowledge, however, would provide fundamental insights on the evolutionary context in which *H. vastatrix* emerged, which in turn could have important implications to understand the evolution of pathogenicity in this fungus and in other rusts as well. With this in mind, we are undertaking a genome-scale approach on 32 fungal species to allow *H. vastatrix* and other rust fungi to be placed in the fungal tree of life with unprecedented details. The complete proteomes of three Pucciniomycotina species as well as of 11 other Basidiomycetes and nine Ascomycetes, approximately 250 000 publicly available EST sequences from several Pucciniomycotina species and transcriptome data recently obtained for *H. vastatrix* were used. A high quality matrix that includes orthologs, co-orthologs and recent paralogs was prepared with a sophisticated orthology detection strategy. On a first approach, we were able to identify at least 378 single-copy orthologs that were used for the phylogenetic analyses. Providing robust and resolved phylogenetic relationships will lay the ground for the identification of genes or gene families that are exclusive to *H. vastatrix* as well as genes of rapid evolution and/or under positive selection, which would be prime targets for functional studies aiming at disease control and prevention. This information will ultimately contribute significantly to advance our knowledge on *H. vastatrix*'s pathogenicity.

INTRODUCTION

Hemileia vastatrix (Berkeley & Broome), the causal agent of Coffee Leaf Rust, is a biotrophic fungus considered to be one of the major burdens to Arabica coffee production world-wide. However, despite the relevance of its devastating impact on the sustainable production of coffee crops, very little has been investigated on the evolutionary origin and phylogenetic

relationships of H. vastatrix with its related taxa. Hemileia vastatrix, which belongs to the Basidiomycota phylum of fungi, order Pucciniales (rust fungi), has only been included in a few phylogenetic studies of the rust fungi that employ a small number of loci. Following major revisions that rejected the original.

hypothesis that the evolutionary primitive rust fungi were those occurring in ferns, these phylogenetic studies consistently place H. vastatrix as the one of the most primitive lineages of the Pucciniales. Although these previous studies were important in placing H. vastatrix in a phylogenetic context relatively to other rusts, the continuous generation of genomic data for several fungal species in the last few years is creating unprecedented opportunities of going beyond and studying, for instance, the evolutionary drivers leading to the formation of pathogenic species. Since genomes hold a vast and intricate record of their carriers' evolutionary history, it is now becoming possible to harness this informative potential and go beyond the phylogenetic reconstruction of species. By employing hundreds or thousands of loci in a comparative framework across multiple species, large regions of the genome can be screened for signatures of natural selection in specific historical periods of target species or group of species with greater detail and robustness. These data can also be integrated with information from the fossil record to produce precise and accurate time-calibrated phylogenies, in which the phenotypic evolution of key pathogenic traits can be mapped and correlated with available environmental data from past geologic eras. However, due to the relatively recent availability of such genomic resources and scalable computational methods to analyze this amount of data, such integrative effort is still lacking.

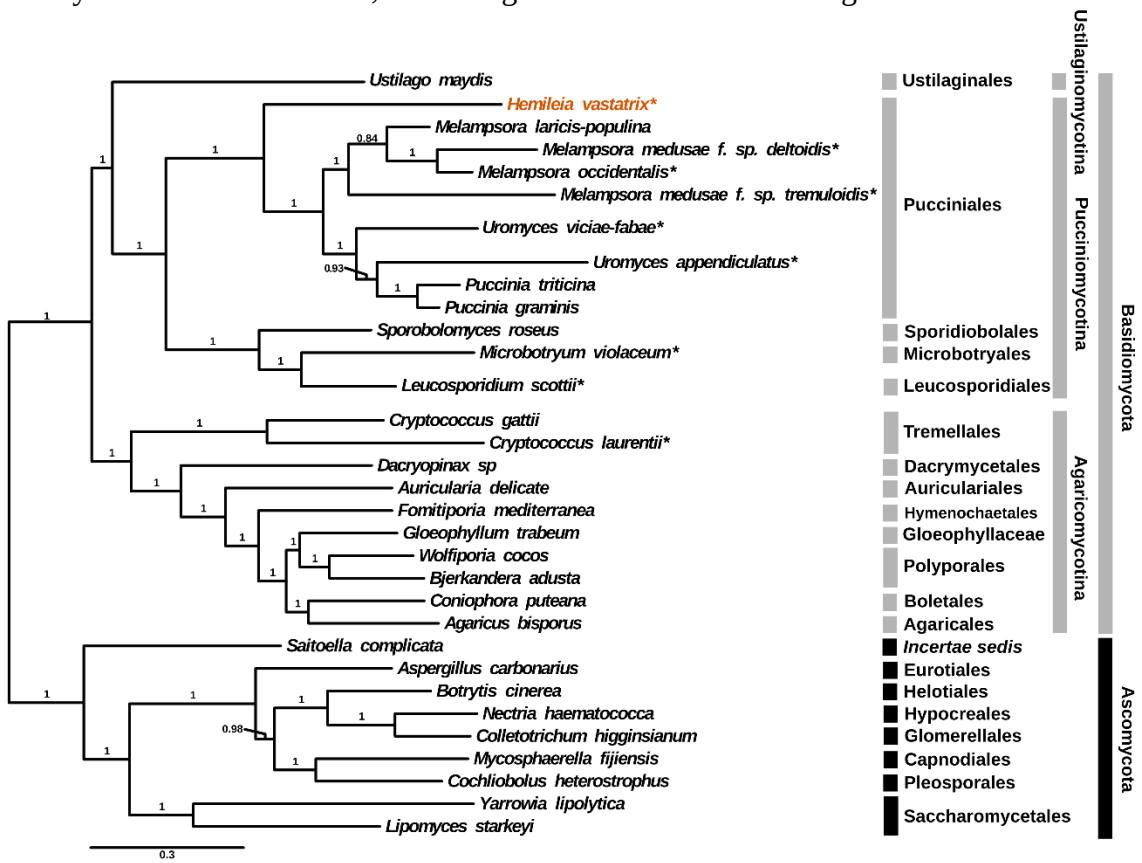

Figure 1. Maximum Likelihood tree estimate of the 378 gene data set for 32 fungal taxa using RAxML. Values above branches represent bootstrap values from 250 replicates. Taxa with an asterisk (*) appended are represented by EST data, otherwise they are represented by a complete genome. On the right, the first set of grey bars contains the taxonomic order while the second contains the sub-phylum.

In this work we report the first preliminary results of a phylogenomics project aiming at an integrative approach including a wider range of fungal species, here mainly centered in *H. vastatrix* and the rust fungi.

MATERIALS AND METHODS

Sequence data from 23 complete genomes, including the comprehensive analyses of rust fungi genomes and 9 EST databases were collected from multiple public sources and from *H. vastatrix*'s transcriptome sequencing project. Putative orthology relationships were first assessed using the deduced proteomes of the 23 complete genomes (171,987 proteins) using a Markov Clustering algorithm (MCL). Selected clusters were used as core orthologs to be complemented with EST data. Only those clusters that contained sequences of *H. vastatrix* were selected. The resulting clusters containing both genomic and EST protein data were then aligned with MAFFT v6.903b. To better explore our data set, two approaches were followed for the phylogenomic analyses. First, a Maximum Likelihood (ML) tree estimation was performed with RAxML using the rapid bootstrap algorithm ("-f a -x"), the PROTCATWAG substitution model and 250 bootstrap replicates. Second, a phylogenomic network was constructed with SplitsTree 4 using the NeighbourNet algorithm in order to visualize potential conflicting signals in the data.

RESULTS AND DISCUSSION

From the orthology assessment strategy employed in this work, 378 clusters (single-copy orthologs) were identified after the initial processing of the MCL output, completion with EST data and subsequent selection of clusters that would maximize the presence of *H. vastatrix* in the data matrix. This data matrix was remarkably complete for the complete genome taxa (average of 148,371 aligned sites) and patchy for the EST taxa (average of 9,161 aligned sites; 17,504 aligned sites for *H. vastatrix*). Even though the proportion of aligned data for EST taxa relatively low compared to complete genome taxa, it still represents a large absolute number of aligned sites for a group of taxa that is commonly analysed with a few loci.

Our ML tree reconstruction resulted in an exceptionally resolved and well supported phylogeny, particularly within the Pucciniales, compared to other recent attempts (Figure 1). These results support the traditional view that *H. vastatrix* occupies a basal position within the order and also highlight the polyphyletic relationships of the *Uromyces* genus. In addition, it is also noteworthy that the relationships recovered by the ML approach among the three sub-phyla Ustilaginomycotina, Pucciniomycotina, Agaricomycotina, which were not resolved until very recently already challenge the current view by placing the Ustilaginomycotina as a sister group of the Pucciniomycotina instead of the Agaricomycotina. Using the alternative approach of the NeighbourNet algorithm implemented in SplitsTree, the same general pattern is recovered with two additional notes (Figure 2). First, there are considerably more possible splits within the Pucciniales, which could indicate a source of genuine conflicting phylogenetic signal in our data or simply reflect the higher proportion of missing data for this group with the inclusion of EST data. Second, the position of the Ustilaginomycotina is again ambiguous in relation to the other two sub-phyla. Solving these problems will most likely require that addition of more high quality data from further loci and taxa and the application of additional filtering and quality control steps on the data matrix. Establishing a robust and well resolved phylogeny will then ensure the first and most important step in our project, by laying the basis from which further analyses can be undertaken such as divergence time

estimation, detection of natural selection and mapping of pathogenic traits or evolution of gene families on the evolutionary history of the rust fungi and *H. vastatrix*.

Figure 2. Neighbour-net based on the 378 gene data set for 32 fungal species. All supraclass and most supraordinal1 relationships are clearly defined by wide bosses, indicating limited conflict in the data. However, there is a substantial conflicting signal within the Pucciniales and there is uncertainty on the position of the Ustilaginomycotina relative to the Pucciniomycotina and Agaricomycotina.

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The Karyotype of *Hemileia Vastatrix*, the Causal Agent of Coffee Leaf Rust

S. TAVARES[1,2], A. I. OPINIÃO[2], A. LOUREIRO[1], H. G. AZINHEIRA[1], M. C. SILVA[1], P. TALHINHAS[1], R. ABRANCHES[2]

[1]Centro de Investigação das Ferrugens do Cafeeiro/Instituto de Investigação Científica Tropical, Oeiras, Portugal
[2]Instituto de Tecnologia Química e Biológica/ Universidade Nova de Lisboa, Oeiras, Portugal

SUMMARY

Hemileia vastatrix Berkeley and Broome causes the most important disease of Arabica coffee, coffee leaf rust. Although various aspects of *H. vastatrix* biology have been unveiled at the cytological, genetic and transcriptomic levels, little is known on its chromosome composition. In other fungal plant pathogens, the analysis of the karyotype revealed the association of pathogenicity factors with supernumerary chromosomes. Therefore, in this work the karyotype of *H. vastatrix* was investigated with the purpose of better understanding the fungus biology, namely by the identification of polymorphisms in the chromosome content of two distinct physiological races. Two complementary techniques were elected to unravel *H. vastatrix* chromosome number: a cytological one based on the application of the germ tube burst method (GTBM) to isolate metaphase nuclei in germinating urediniospores, followed by DAPI staining of DNA; and an electrophoretic one based on the pulse field gel electrophoresis (PFGE) of germinating urediniospore samples. In addition, the rDNA loci in *H. vastatrix* interphase nuclei were visualized by fluorescence in situ hybridization (FISH). The observation of the nucleolus was possible using fungal ribosomal probes. The implementation of these techniques led to the estimation of six to 10 chromosomes for *H. vastatrix*, enabling the study of its karyotype, which may contribute to link virulence spectra to the putative polymorphisms detected, and also to localize genes related to pathogenicity.

INTRODUCTION

Coffee leaf rust caused by the fungus *Hemileia vastatrix* Berkeley & Broome is one of the most destructive diseases of coffee, causing premature leaf fall, yield loss and even death of the tree in severe attacks. *Hemileia vastatrix* is an obligate biotrophic fungus studied at CIFC (Centro de Investigação das Ferrugens do Cafeeiro, Portugal) for more than 50 years, allowing a collection of cytological, transcriptomic, proteomic and genetic data, however the karyotype of *H. vastatrix* was not address until now.

The identification of polymorphisms associated with variations in the chromosome size or number (karyotype) in phytopathogenic fungi has revealed that supernumerary chromosomes (not ordinary) contain functional genes essential to the onset of a disease. *H. vastatrix* isolates of physiological races II and VI were elected to conduct the study of their karyotype. Two techniques were performed: a cytological one, using the germ tube burst method GTBM, and electrophoretical one, using Pulse-Field Gel Electrophoresis, PFGE.

MATERIALS AND METHODS

Fungal Material

Hemileia vastatrix urediniospores from two different races (race II - isolate 1065 and race VI - isolate 71 from the CIFC/IICT collection) were germinated on artificial surfaces as previously described and collected at different time points of incubation (12 to 24 h) with the purpose of identifying metaphase nuclei.

Germ tube burst method (GTBM)

The germinated samples were spread on a slide and air dried. Slides were immersed in a fixative mixture (99% methanol/ glacial acetic acid = 22:3, vol/vol) during 30 min. at room temperature. The slides were flame-dried to remove the mixture and stained with DAPI (1 µg/ml). The slides were mounted with an antifade mounting media (Vectashield, Vector Laboratories) and observed under UV light with a Leica fluorescence microscope.

Fluorescence in situ hibridization (FISH)

FISH was performed on fungal material that was previously treated by the GTBM. The ribosomal probe pABM1 was used following the protocol by Castilho & Heslop-Harrison. The probe was labelled with biotin by nick translation and detected with extravidin-Cy3 (red fluorescence). Chromosomes were stained with DAPI (1 µg/ml) and the slides were observed with a Leica fluorescence microscope. Deconvolution was done with Metamorph 7.6 and images were edited.

Pulse-Field Gel Electrophoresis

Intact chromosomal DNA was obtained following the protocol developed by McCluskey *et al*. Electrophoresis was performed with a Bio-Rad CHEF DR-II system using 0.5x TBE buffer cooled to 14 °C. To resolve medium-small size chromosomes, we selected the following parameters: a 1% agarose gel run for 24 hours at 3 V/cm with a pulse varying from 120 to 150 seconds, with a 120° angle. Gels were stained with ethidium bromide.

RESULTS AND DISCUSSION

Cytological Karyotype

In GTBM, nuclei were released from the germ tube of *H. vastatrix* and spread on the surface of the microscope slide (Figure 1A, B and C). Several mitotic stages were identified in the spreads, from prophase to telophase, however only metaphase nuclei (Figure 1 D, E, F and G) with highly condensed and individualized chromosomes are useful to establish the karyotype. A low frequency of metaphase nuclei was observed due to the asynchrony of *H. vastatrix* spore germination. Nevertheless, the metaphase nuclei observed permitted an identification of six to 10 clear chromosomes (Figure 1 D, E, F and G). This variation is common in cytological karyotyping of many organisms considering the difficulties in obtaining intact metaphase nuclei.

Identification of rDNA sites by FISH was performed on fungal material previously treated by the GTBM, using the ribosomal probe pABM1 (Taga *et al.*, 2003) and following the protocol developed by Castilho & Heslop-Harrison. In both isolates, rDNA was detected within the nucleolus domain (red signal) without a defined shape, which could indicate that it is in a

decondensed state (Figure 1 H, I and J). The application of FISH on material from the GTBM will enable visualization of specific chromosomes and genes permitting more detailed karyotypes, namely low copy number sequences candidates to virulence genes in the genome of *H. vastatrix*. These candidate genes will become available from ongoing large-scale transcriptomic characterization (Talhinhas et al. unpublished).

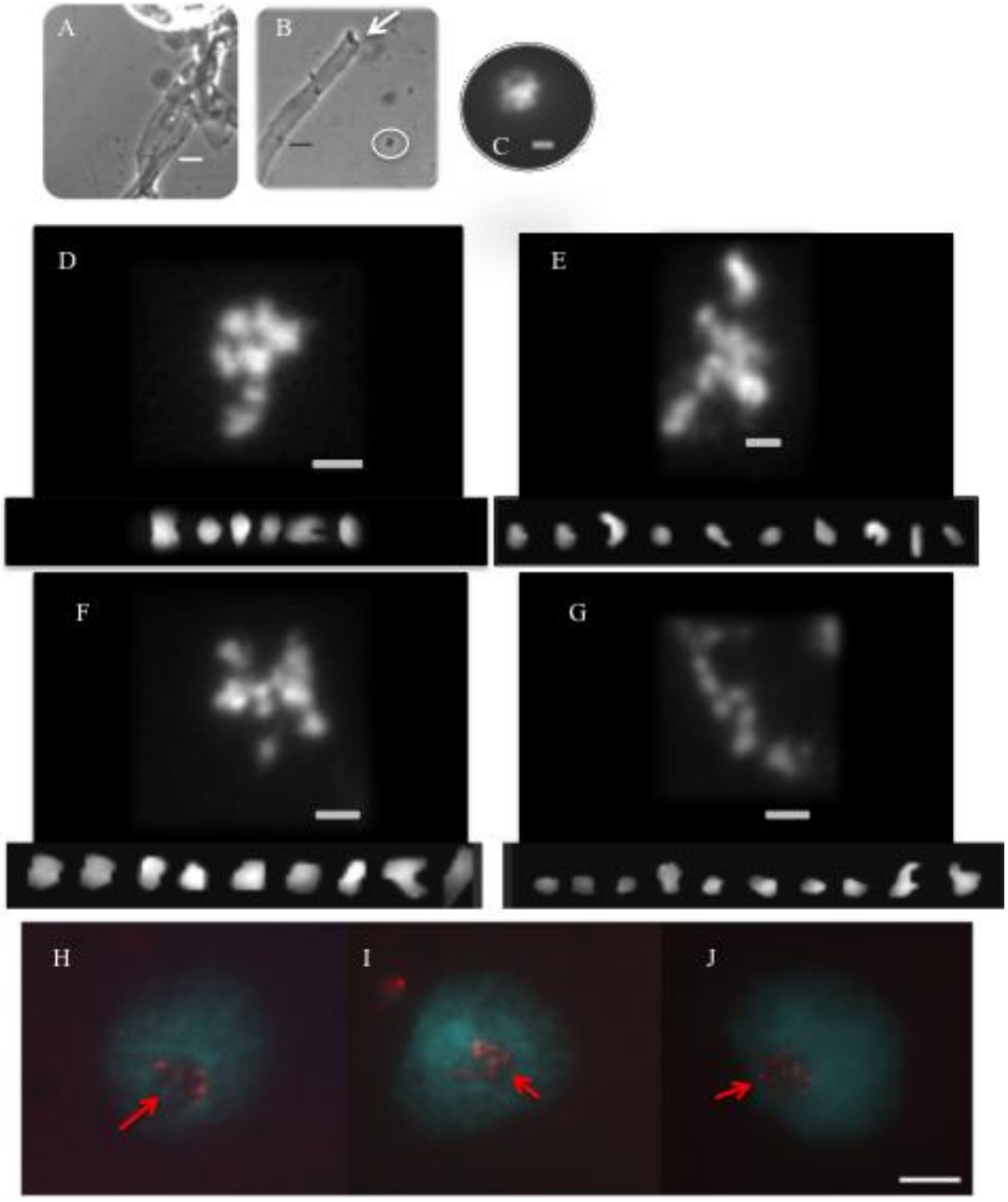

Figure 1. A and B, Burst of the *Hemileia vastatrix* germ tubes (white arrow) and the spread of nuclei (white circle) on the microscope slides. C, Detail of condensed nuclei stained with DAPI dye. D, E, F and G, Cytological karyotypes of *H. vastatrix* isolates 71 (D, E and F) and 1065 (G) by the germ tube burst method. The upper part in each shows DAPI-stained chromosomes as they were on a slide, and the lower part is the size-based alignment. H, I and J, Identification of rDNA sites by FISH using the pABM1 probe on interphase nuclei of a burst *Hemileia vastatrix* germ tube. The red signal (arrow) shows the rDNA sites in the nucleolus of isolate 1065 (H) and isolate 71 (I,J) DNA stained with DAPI. Scale bars: A, 3 µm, B, 6 µm, C, D, E, F and G, 1 µm, H, I, and J, 5 µm.

Electrophoretic Karyotype

The success of PFGE depends almost entirely on obtaining intact chromosomal DNA in an appropriate concentration. We used a protocol to resolve small-medium size chromosomes, however the DNA concentration was low and did not permit to resolve chromosomes above 1.1 Mb, since a smear can be observed in the corresponding area of the agarose gel. Two bands can be identified on the top of the gel (Figure 2, white square) that, compared with the molecular-weight marker from *Saccharomyces cerevisiae*, are between 2.2 and 1.6 Mb. Both isolates exhibit the same pattern on the PFGE, which is not surprising because polymorphisms in the number of chromosomes are more frequent in small size chromosomes.

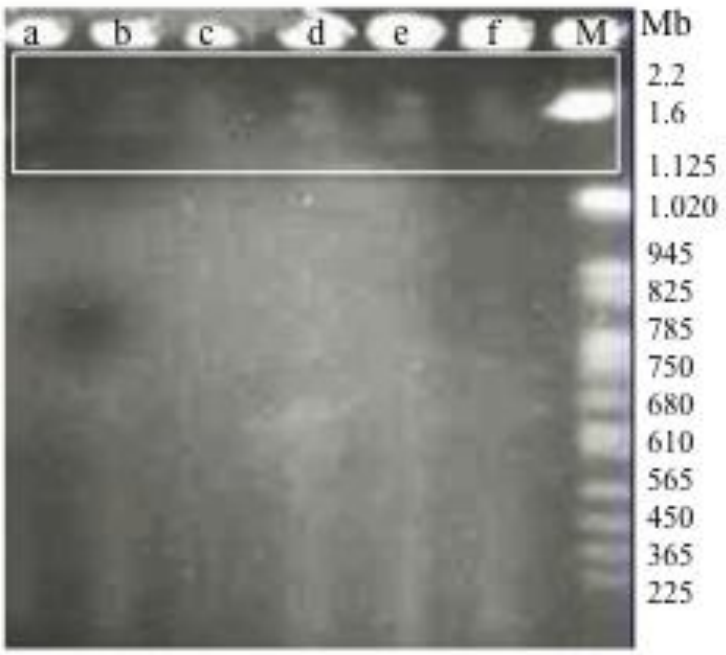

Figure 2. Separation of small-medium size chromosomes of *Hemileia vastatrix* by PFGE, of isolates 71 (a, b and c) and 1065 (d, e and f). Gel stained with ethidium bromide. The electrophoresis conditions are described in material and methods. Mb- Mega-base pairs of *Saccharomyces cerevisiae* chromosomes.

Preliminary results obtained from the two approaches, cytological and electrophoretical, on the karyotype of *H. vastatrix* indicate a number of chromosomes between 6 and 10, although more samples of metaphases are essential and a better resolution of PFGE is also mandatory, especially for chromosomes above 1.1 MB. Interestingly, two larger chromosomes are consistently found in the cytological karyotyping analysis, a fact that coincides with the observation of two distinct larger bands on the electrophoretic karyotyping analysis.

ACKNOWLEDGMENTS

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Integrated Cytologic and Proteomic Analysis of *Coffea Arabica* – *Hemileia Vastatrix* Interactions

L. GUERRA-GUIMARÃES[1], A. VIEIRA[1], I. CHAVES[2], V. QUEIROZ[1,3], C. PINHEIRO[2], J. RENAUT[4], L. SILVA[1], L. ZAMBOLIM[5], C. RICARDO[2], M. D. C. SILVA[1]

[1]CIFC/BioTrop/IICT, Oeiras, Portugal
[2]ITQB/UNL, Oeiras, Portugal
[3]UFES, Alegre, Brazil
[4]CRP - Gabriel Lippmann, Luxembourg
[5]UFV, Viçosa; Brazil

SUMMARY

Coffee leaf rust, caused by the fungus *Hemileia vastatrix* Berk & Br., is the most widespread disease of *Coffea arabica* L. cultivars. Coffee – rust interactions are governed by the gene-for-gene relationship, being the resistance conditioned at least by nine major dominant genes (S_H1-S_H9) singly or associated. A cytologic and proteomic approach was used to study *C. arabica – H. vastatrix* compatible and incompatible interactions. In the incompatible interactions the first leaf cytological changes corresponded to hypersensitive host cell death (HR), observed in more than 50% of infection sites, 48h after inoculation. Protein multiplex two-dimensional fluorescence differential gel electrophoresis (Refraction-2D^{TM}), was performed with 30 µg of leaf protein in 13cm gel strips with immobilized pH 4-7 gradient. Differentially expressed polypeptides were found in the ranges of 4.8 to 5.6 pI values and 45 to 60 kDa MW. The identification of the proteins by matrix assisted laser desorption/ionization time of flight-mass spectrometry (MALDI – TOF/TOF MS) followed by homology search in several NCBI databases provided insights into the molecular characteristics of proteins specific for each of the interactions. With this methodology we found proteins that are potential candidates for resistance markers and will be further validated.

INTRODUCTION

Coffee leaf rust, caused by the fungus *Hemileia vastatrix* Berk & Br., is the most widespread disease of *Coffea arabica* L. cultivars, causing serious damage. Although fungicides can provide adequate control, breeding for resistance is environmentally and economically the most appropriate and sustainable strategy to fight this disease.

The resistance of coffee to *H. vastatrix* is predominantly post-haustorial (the fungus ceases its growth at different stages of the infection, but more frequently after the formation of the first haustorium), and is associated with rapid host cell death (hypersensitive reaction – HR). The early increase of activity of oxidative enzymes such as lipoxygenases (LOX), peroxidases (POD) and superoxide dismutases (SOD) as well as other enzymes (e.g. phenylalanine ammonia-lyase and chitinases) have also been detected during the early expression of resistance in some coffee genotypes [1,2,3,4]. In this study an integrative cytological and proteomic approach was used to identify apoplastic proteins that are potential candidate markers for resistance. To this purpose we used *C. arabica* genotype S4 Agaro inoculated with two different rust races to establish a compatible (susceptible) and incompatible

(resistant) interactions. At least 9 protein spots (associated with signal pathways, stress and defense and proteolysis) showed increased expression in the resistant sample, comparatively to control or susceptible samples.

MATERIALS AND METHODS

Biological material

Coffee plants (*C. arabica* L.) of the genotype S_H4S_H5 (S4 Agaro) grown in greenhouse conditions were inoculated with fresh urediospores of *H. vastatrix*, races II (v_5) and XV ($v_{4,5}$) establishing an incompatible (resistant) and a compatible (susceptible) interaction, respectively. The infected leaves were collected at different time points after inoculation.

Light microscope observation of fresh tissues

Pre-penetration fungal growth stages (germinated urediospores and appressoria formation over stomata) were visualized on leaf pieces, as previously described. For time course studies of fungal growth and plant cell responses, cross sections of infected leaf fragments, cut with a freezing microtome, were submitted to blue lactophenol staining and an epifluorescence test. Observations were made with a microscope Leica DM-2500 microscope equipped with a mercury bulb HB 100W, u.v. light and blue light.

Protein extraction

Protein from intercellular fluid (IF) of *C. arabica* leaves (healthy and inoculated) were obtained. The IF fraction was desalted and concentrated on centrifugal filter Vivaspin2 (Sartorius) followed by purification in PD SpinTrap G-25 column (GE Healthcare). Protein content was measured using a modified Bradford assay.

Proteomics analysis

Multiplex two-dimensional fluorescence differential gel electrophoresis analysis was performed using Refraction-2D™ labelling kit (DyeAGNOSTICS). Fluorescent protein labelling was performed using G-Dyes (G-100, G-200, G-300) according to the standard workflow of DyeAGNOSTICS. Three biological replicates were prepared for each sample. Each sample was dye-swap labelled, to avoid artefacts due to preferential labelling and the internal standard was created by pooling an aliquot of all biological samples. Protein samples (30 µg per sample) were run in 13 cm long IPG strips, pH 4–7 (GE Healthcare). IEF was performed using the Ettan IPGphor (GEHealthcare) for a total of 33000 Vh at 20 ºC and a maximum current setting of 50 µA per strip. After IEF, the SDS-PAGE was run using the Hoefer SE 600 Ruby apparatus (GE Healthcare) at 10 mA for 15 min and then at 20 mA at 20 ºC until the bromophenol blue dye front had run off the gel. After electrophoresis, Ref2D gels were visualized and scanned at 700V using the FLA-5100 Fluorescent Image Analyzer (Fuji Film). The image gel analysis was carried out using the Progenesis Samespot 2D software. The differentially expressed spots (relative to the control) were isolated from the gel and the proteins identified by matrix assisted laser desorption/ionization time of flight-mass spectrometry (MALDI – TOF/TOF MS), followed by homology search in several NCBI databases.

RESULTS AND DISCUSSION

The microscopic analyses, 24h after inoculation, revealed that the percentages of germinated urediospores (70-75%) and of appressoria formed on stomata (54-56%) were similar in both resistant and susceptible samples. The majority (70-80%) of appressoria gave rise to penetration hyphae and in the susceptible the fungus pursued its growth without apparent inhibition, contrarily to what occurred in the resistant sample. The first sign of resistance (detected 24h after the inoculation), was cytologically expressed by the hypersensitive plant cell death (HR), as monitored by cell autofluorescence and/or browning. This response began to be observed in only the guard cells or in both guard and subsidiary cells at the infection sites where the fungus reached the stage of appressorium or penetration hypha (Figure 1). Death of subsidiary and mesophyll cells invaded by a haustorium was observed from 48hpost-inoculation. At this time, more that 50% of infection sites exhibited dead cells. The HR, the common expression of resistance of gene-for-gene interactions, has been associated with many events, including activation of defence genes, production of reactive oxygen species (ROS), increase in activity of oxidizing enzymes and the synthesis of pathogenesis-related proteins (PR proteins) [2,3,4,10,11]. The multiplex technology (Ref2D) allows the relative assessment of differences in protein levels between two or more samples.

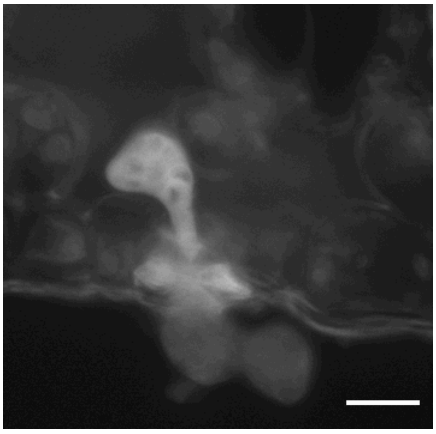

Figure 1. Resistant plant, light microscope observation, epifluorescence test (blue light). Infection site showing autofluorescence of guard cells (arrows) associated with a penetration hypha (h), 24h after inoculation; Ap= appressorium (bar = 10 µm).

However, this technology remains a challenging approach in non-model plants, since problems can arise at several points; particularly, artifacts related to inappropriate saturation labeling of protein spots. An additional problem we faced was the incomplete labeling of proteins of lower molecular weight (MW). Due to Ref2D technological specificities and the characteristics of the sub-cellular fraction (intercellular fluid), only a minimal fraction of the total cell proteome could be visualized. At 48h after infection it was possible to detect differentially expressed spots (pI from 4.8 to 5.6; MW from 45 to 60 kDa; Figure 2).

Multivariate analysis (principal components analysis, PCA) revealed a clear separation of control (healthy leaves), susceptible and resistant samples along the 1st axis, which explains 87.1% of the total variance (data not shown). The susceptible sample is closer to the control than to the resistant one. Considering all samples, a total of nine spots with a fold change threshold of 2.1 were selected as differentially expressed. The spots 1-5 were identified as different isoforms of aspartic proteinase nepenthesin-2 and the spots 6-9 as germin-like proteins, showing a higher expression in the resistant than in the control or susceptible samples (Figure 2).

The aspartic proteases superfamily is widely distributed in all living organisms and is one of the most important superfamilies of proteolytic enzymes. They are expressed in several plant organs and in the digestive fluids of carnivorous plants, being implicated in protein processing and/or degradation, in plant senescence, stress response, programmed cell death, and reproduction. Aspartic proteinase nepenthesin-2, has been associated to protein catabolism, folding, sorting and degradation and recently with rice resistance to sheath blight disease. The germin and germin-like proteins (GLP) family is a large and considerably heterogeneous group of proteins, expressed in several plant organs and developmental stages, and in response to a number of abiotic and biotic stresses. Three different enzymatic activities, oxalate oxidase (OxO), superoxide dismutase (SOD) and ADP-glucose pyrophosphatase or phosphodiesterase (AGPPase), have been associated with these proteins. Grapevine VvGLP3 is specifically induced in epidermal cells in response to powdery mildew infection; a proportion of the VvGLP3 protein is targeted to the extracellular space and shown to catalyze superoxide dismutation. These data suggest a potential role for VvGLP3 in the penetration-based defense response against powdery mildew infection. In *Brassica napus*, GLP are likely to participate in the *Sclerotiorum sclerotiorum* induced apoplastic formation of H_2O_2 and may act in concert with NADPH oxidases and peroxidases, enzymes known to execute the apoplastic oxidative burst in response to pathogen stress. The apoplastic localization of these proteins in combination with the H_2O_2 generating SOD activity offers a role in cell-wall fortification through the cross-linking of proteins and carbohydrates. Taken together, the cytological and the proteomic data reinforce the involvement of the apoplastic SOD activity and the enhanced cross-linking of cell wall components in the establishment of coffee resistance response. Further, this is the first time that proteinases, the aspartic proteinase nepenthesin-2, were referred as being associated with HR of coffee to leaf rust. Both enzymes are potential candidates for resistance markers and will be further validated.

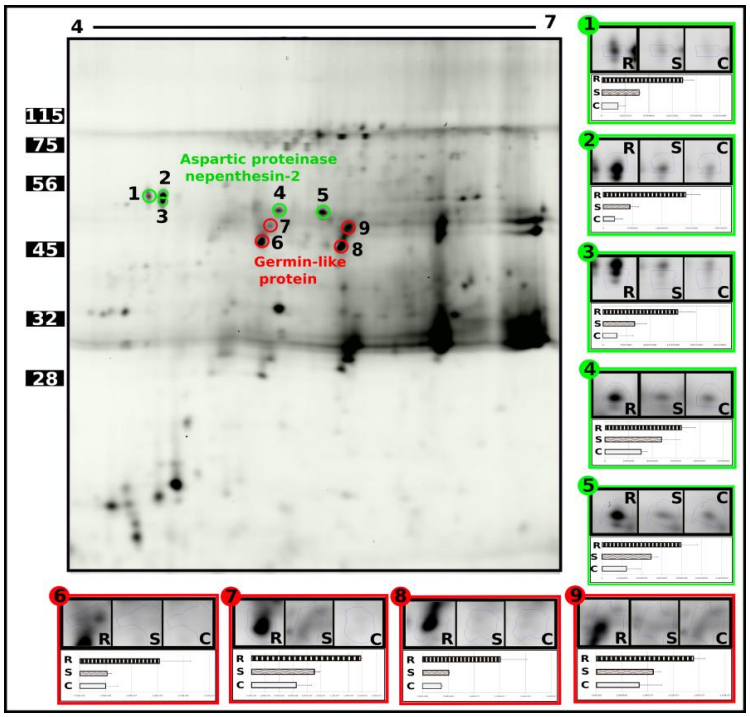

Figure 2. Representative proteomics Refraction-2D analysis of the apoplast fraction from *C. arabica* leaves. Circled gel spots evidence intense protein expression, due to *H. vastatrix* infection. Spots 1-5 represent the aspartic proteinase nepenthesin-2 proteins and spots 6-9 the germin-like proteins. Insert boxes show detailed changes in protein accumulation for the different samples: R-resistant, S-susceptible and C-control.

ACKNOWLEDGEMENT

This work was supported by Portuguese Funds through FCT (Fundação para a Ciência e a Tecnologia), under the project PTDC/AGR-GPL/109990/2009 and the strategic project PEst-OE/EQB/LA0004/2011 and by a Portuguese-Brazil collaborative project also funded by FCT and by CAPES (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil).

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Phenotyping and Genotyping Genetic Resources of *Coffea Arabica* at Iapar. FAO Collection

P. CHARMETANT[1], R. FERREIRA[2], G. ANDRADE[2], M. A. DOS SANTOS[2], P. MARRACCINI[1], T. LEROY[1], D. POT[1], F. DE BELLIS[1], T. SERA[2], L. F. PROTASIO-PEREIRA[3]

[1]Cirad, UMR AGAP, Avenue d'Agropolis, F 34398 Montpellier, France
[2]IAPAR www.iapar.br
[3]EMBRAPA café www.embrapa.br

SUMMARY

The genetic diversity of C. arabica in Ethiopia, its region of origin, was explored. Its use for C. arabica breeding, though limited to a few F1 crosses with cultivars, proved to be efficient in terms of genetic gain regarding important coffee agronomic characteristics such as vigor, adaptability and quality. A collection derived from the 1964-65 FAO survey in Ethiopia was established at IAPAR in Londrina, PR, Brazil in 1976. Genotyping was undertaken using SSR markers that were polymorphic within these accessions. Preliminary results regarding the relation between genetic and phenotypic diversity are presented. Self pollinated progenies of 179 plants representing 115 Ethiopian accessions were established at IAPAR, from 2009 to 2012. 47 of them were also established at CPAC, DF. Various growth parameters were measured.

INTRODUCTION

Outside Ethiopia one of the main sources of genetic diversity for *Coffea arabica* L. comes from a 1964-65 survey by FAO in the main coffee regions. Seed samples[1] were taken from wild or plantation trees, whether single trees, or "representative" of various locations, or random samples (on markets). A second survey by IFCC/ORSTOM focused mainly on single, putatively wild trees. These accessions were planted at Centro Agronómico Tropical de Investigación y Enseñanza (CATIE, Costa Rica). Their use has proved to be a good source of genetic gain for breeding programs in Central America. Various tools have been used to explore their genetic diversity. Indeed, in most coffee growing countries, one bottleneck for *C. arabica* breeding –outside interspecific hybridization – is the very narrow genetic diversity of most cultivars. This study aims at exploring the genetic and phenotypic variability of 130 accessions established at IAPAR (Parana, Brazil) in 1976, with open pollinated seeds from the CATIE collection. A preliminary selection of 13 polymorphic Single Sequence Repeat (SSR) markers was made in 2010 on 16 accessions from Ethiopia. Thanks to the "pools" methodology, 52 polymorphic markers were identified out of 307 tested (126 monomorphic and 129 that did not amplify). In this paper we report on two aspects of this work: genetic homogeneity of the accessions, and variation in response to drought.

[1] "Accession" = progeny and descendants issued from one seed sample in Ethiopia; "genotype" = single plant within a progeny.

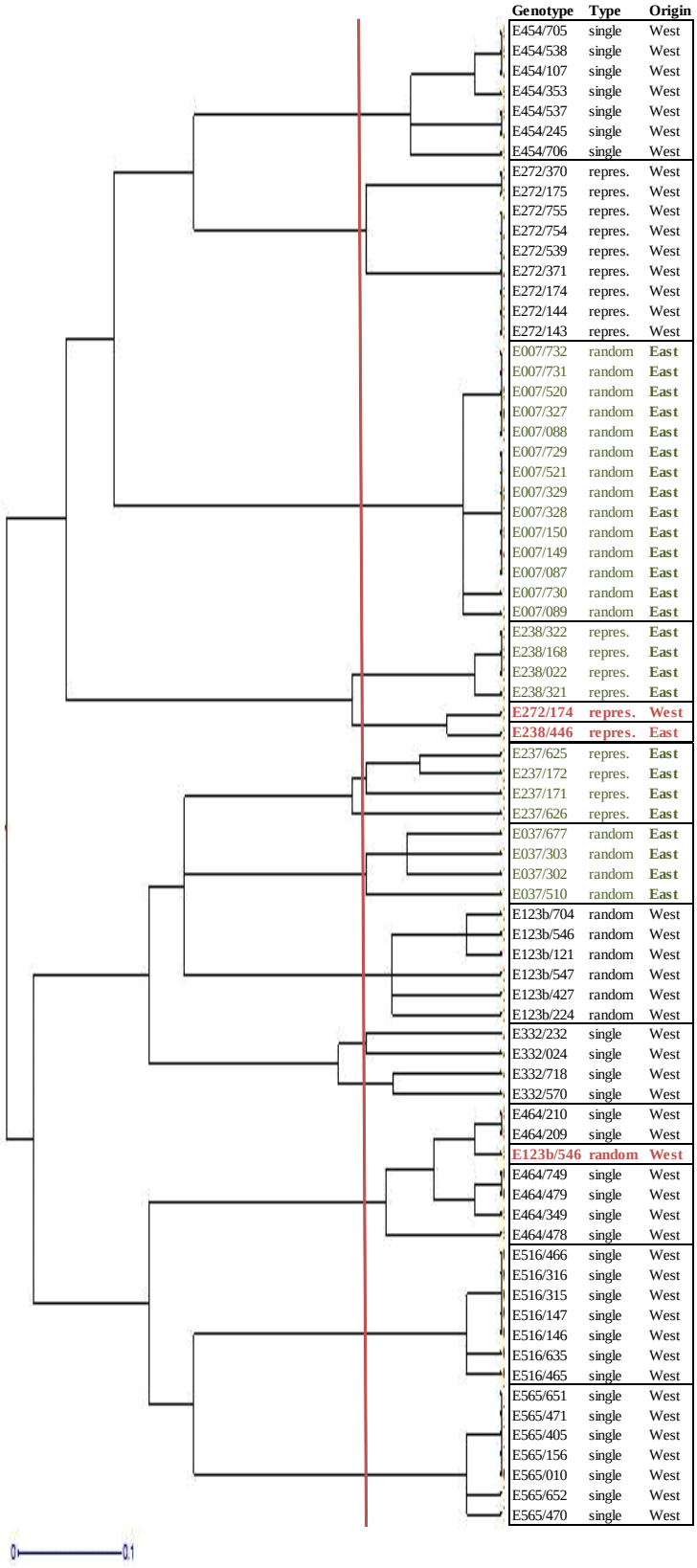

Figure 1. Dendrogram of genotypic diversity (dissimilarity, WPGMA construction) within 11 accessions from the "FAO" collection at IAPAR. In red:"outliers". Type = type of accession (single tree, representative sample, random sample). Origin = side of Rift Valley in Ethiopia.

MATERIALS AND METHODS

Genotyping was performed for 11 Ethiopian accessions represented by 75 genotypes. Five originated from a single tree in Ethiopia, two from representative samples, and four from random samples. Ten polymorphic SSR markers that showed more than 2 alleles for this population were tested (Table 1); electrophoresis was performed on polyacrylamide gels. We used DARwin 5.0 to analyze allelic data and to build a dissimilarity tree.

Phenotypic diversity

Selfings of plants were established in two locations, Londrina (PR) IAPAR and Planaltina (DF) CPAC, under two irrigation regimes, between 2010 and 2012. Growth parameters were measured since planting, and drought symptoms were recorded and the end of dry spells of at least 4 months in the field. Stem Diameter increase in a predominantly dry period gives a synthetic evaluation of tolerance to water stress. A progeny trial established at CPAC in 2010 with 66 progenies and 7 control cultivars is given as an example. Stem girth was measured in 2011-2012 at the beginning and at the end of the rainy season, and after the dry period.

Table 1. List of 10 SSR markers used to analyze genotypic homogeneity of 11 Ethiopian accessions, and Allelic diversity across 13 Wild Ethiopian accessions.

Marker name	International code[1]	Repeat type	No. of alleles	PIC[2]
258	AJ250258	(CA)3 / (CA)3 / (CA)18	4	0.62
260	AJ250260	(CT)9 / (CA)8 / (CT)4 / (CA)5	5	0.67
350	AM231550	(GT)8	3	0.56
414	AM408638	(CA)9	4	0.62
501	AM231576	(TG)8	5	0.58
503	AM408715	(AC)9 (CCTtt)3	3	0.51
509	AM408721	(CT)10 (CA)15	4	0.59
514	AM408726	(AC)8	3	0.57
755	AJ308755	(CA)20	4	0.73
772	AJ308772	(TC)8(AC)11(TC)10	3	0.47

[1]*Markers provided by Cirad, http://tropgenedb.cirad.fr/tropgene/JSP/interface.jsp?module=COFFEE;*
[2]*Polymorphism Information Content (PIC) values.*

RESULTS AND DISCUSSION

Genetic erosion

Out of 171 accessions imported from CATIE (Costa Rica) in 1975,147 only are still represented in 2012,16 of them by a single plant. Previous work2 showed that not only vigorous, well adapted accessions make good genitors for hybridization with traditional varieties. Thus it is important to preserve the whole diversity still available. Some seed progenies obtained from selfings are also entirely lost after two years.

Homogeneity of accessions, and genetic diversity

The dissimilarity tree (Figure 1) shows the presence of ‘outliers” within some of the accessions (in red, Figure 1), however most accessions are genetically homogeneous

regardless of the original type of sampling, as observed with RAPD markers. The global structure of genetic diversity is confirmed, with a separation between accessions from the East of the Rift Valley in Ethiopia (close to cultivars Typica and Bourbon), and those from the West, as seen in many genetic studies.

Phenotyping

Field observations confirmed the presence of "outliers" within heterogeneous accessions. We noted also that accession E007 may not be the one described in the survey report1! At IAPAR most progenies were hit by frost in 2011, which caused a very high heterogeneity between and within accessions. At CPAC and under controlled conditions response to drought already shows clear differences between the progenies that were first planted (Table 2, Figure 2).

Table 2. ANOVA; Relative increase of Stem Girth of 79 selfed progenies from IAPAR's FAO Ethiopian collection between dry and wet seasons. CPAC (DF, Brazil), 2011 – 2O12.

Source	DDL	Sum of Squares	Mean Square	F	Pr > F
Progenies	78	5.804	0.074	2.396	< 0.0001
Error	166	5.154	0.031		
Total	244	10.958			

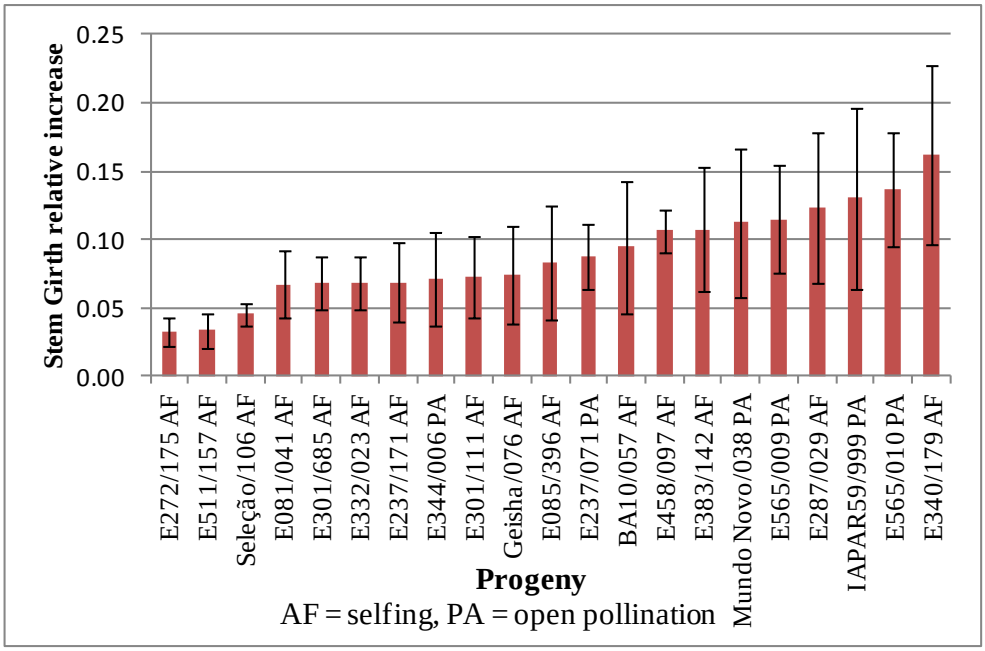

Figure 2. Stem diameter relative increase between dry and wet seasons of 21 selfed progenies from IAPAR's FAO Ethiopian collection. CPAC (DF, Brazil), 09/2011 – 09/2O12.

PERSPECTIVES

Genotyping at IAPAR is now speeding up; a structured image of the whole collection will soon be available. Hybridizations are being made between accessions representative of the

diversity with two commercial varieties; the hybrid progenies will give us an insight of the relation between genetic distance, complementary characteristics, and hybrids' value.

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Identification and Analysis of Polymorphisms in the Promoter Region of the Gene *DREB1A* from Contrasting Haplotypes of *Coffea Canephora*

G. S. C. ALVES[1,2], L. P. FREIRE[2], N. G. VIEIRA[2], P. MARRACCINI[2,3], L. V. PAIVA[1], A. C. ANDRADE[2]

[1]University of Lavras, Lavras, MG, BRA
[2]Embrapa Genetic Resources and Biotechnology, Brasilia, DF, BRA
[3]CIRAD UMR AGAP, Montpellier, FRA

SUMMARY

The aim of the present study was to identify and analyze the polymorphisms within the promoter region of the gene *DREB1A* in two clones of *C. canephora*, clones 14 (drought tolerant) and 22 (drought susceptible), but also in other accessions of *Coffea* genus (*C. eugenioides* and *C. arabica*). This allowed the identification of *cis*-regulatory elements and also of polymorphisms in the promoter region of the gene *DREB1A*. For example, the presence of *cis*-regulatory elements from transcription factors belonging to ABA–dependent pathway suggested the possibility of cross-talk between the two networks (ABA–independent and –dependent) response upon the regulation process of the *DREB1A* gene. The polymorphisms permitted the identification of different haplotypes among those genotypes and the allelic distribution of these loci. These polymorphisms may indicate the involvement of different haplotypes in the genetic control of drought tolerance.

INTRODUCTION

Although some physiological studies have resulted in a better understanding of the mechanisms involved in drought tolerance in coffee, information is still missing about the metabolic and molecular mechanisms controlling the response of coffee to drought. Unlike resistance to biotic stresses, the genetic responses to abiotic stresses are multigenic, and thus more complex and difficult to control. Due to its ability to interact with multiple genes, transcription factors (TFs) are a class of protein important for understanding the mechanisms of response to abiotic stress. The TFs interacts with *cis*-regulatory elements members in promoter regions of many genes responsive to abiotic stress, and therefore co-regulates genes related to adaptation to drought. In this regard, recent studies performed in our laboratory identified several candidate genes showing differential expression in leaves of contrasting clones of *Coffea canephora* var conilon for drought tolerance. Among these results, transcription factor gene *DREB1A* showed differential expression in the leaves of the clones 14 (drought tolerant) and 22 (drought susceptible) subjected or not to water stress. The objective of this study was to identify and isolate the promoter region of the gene *DREB1A* in different genotypes of the *Coffea* genus in order to analyze the characteristics of this promoter, and therewith try to elucidate the influence of its regulation in adaptation to drought.

MATERIALS AND METHODS

Plant material

The plant material used in this study was obtained from a selection of genotypes of the genus *Coffea*. The genotypes were chosen in order to obtain the best genotypic variability coverage of coffee. The selection of genotypes was based on criteria such as: genomics resources; diversity of geographical origin; morphological and agronomical difference. A total of 42 genotypes were used in this study (Table 1). DNA was extracted from 100 mg of pulverized leaf tissues according to the method devised by SAGHAI-MAROOF with some modifications. The amplicons were cloned and sequenced.

Table 1. Different genotypes used for the analyses and their descriptions.

Genotype	Species	Genotype	Species	Genotype	Species
1. Acaiá 47119	*C. arabica*	15. E123A	*C. arabica*	29. Palma 02	*C. arabica*
2. Bourbon	*C. arabica*	16. E123B	*C. arabica*	30. Psilanthus	*C. bengalensis*
3. C1007	*C. canephora*	17. E238	*C. arabica*	31. Purpurenses	*C. arabica*
4. C2011	*C. canephora*	18. E464	*C. arabica*	32. Racemosa	*C. racemosa*
5. C3001	*C. canephora*	19. E516	*C. arabica*	33. RH3 Rubi	*C. arabica*
6. C4001	*C. canephora*	20. Eugenioides	*C. eugenioides*	34. RH3 IAPAR59	*C. arabica*
7. C4001 D.P.	*C. canephora*	21. E237	*C. arabica*	35. Rubi	*C. arabica*
8. Canephora (G21)	*C. canephora*	22. G2020	*C. canephora*	36. Sabiá	*C. arabica*
9. Catuai 25	*C. arabica*	23. Guatemalense Baixo	*C. arabica*	37. San Bernardo	*C. arabica*
10. Catuai 144	*C. arabica*	24. Guatemalense Alto	*C. arabica*	38. San Ramon Baixo	*C. arabica*
11. Clone 14	*C. canephora*	25. IAPAR 59	*C. arabica*	39. Tupi	*C. arabica*
12. Clone 22	*C. canephora*	26. Ikatú Colombiano	*C. arabica*	40. Typica	*C. arabica*
13. E007	*C. arabica*	27. Mundo novo	*C. arabica*	41. UW002	*C. canephora*
14. E017	*C. arabica*	28. Obatã	*C. arabica*	42. UW099	*C. canephora*

Search of polymorphisms and *cis*-regulatory DNA motifs

The *DREB1A* promoter regions were amplified by PCR reactions using different sets of primer pairs (data not shown) - architected from the analysis of the promoter sequence *DREB1A* of *C. canephora*. After being amplified, these fragments were cloned, double-strand sequenced with the universal primers M13 forward/reverse, internal primers *DREBS2F* and *DREBS1R*, using the BigDye Terminator Kit v3.1 chemistry (ABI 3130xl Genetic Analyzer, Applied Biosystems). For each genotype, 12 clones were sequenced and a total of 48 sequences were aligned and analyzed for searching polymorphisms. The sequences were then clustered and DNA polymorphisms (SNPs: Single Nucleotide Polymorphisms and INDELs: insertions/deletions) were detected by careful analyses of their DNA electropherograms and by making multiple sequence alignments of *DREB1A* allelic locus of coffee genotypes using the SeqMan program (DNAStar). The same sequences were also used for searching *cis*-regulatory DNA motifs in the databases of regulatory elements Plant CARE ("Plant *cis*-acting regulatory element") and PLACE ("Plant *cis*-acting regulatory DNA elements").

RESULTS AND DISCUSSION

The reference sequence obtained from *C. canephora* has 1588 base pairs and includes the *DREB1A* gene region from -1451 to +137, thus including the first 45 amino acid residues of the protein. The S1 amplicon covers from base pair position -1438 to - 601 of the promoter region. The S2 amplicon amplifies the region - 843 to + 109, covering 843 bp of the promoter region plus 109 bp of the coding region.

A

Primer	Amplicon (bp)
DrebS1F [a]	837
DrebS1R [a]	
DrebS2F [b]	952
DrebS2R [b]	
DrebS1F [c]	1560
DrebS2R [c]	

B

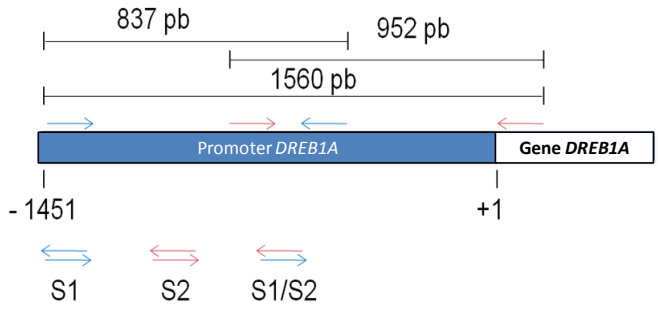

Figure 1. The primer pairs used to amplify of the *DREB1A* promoter regions from the different genotypes of *Coffea* are given (A). (a), (b) and (c) corresponded to primer pairs used. (B): Schematic representation of the *DREB1A* promoter region. The primer combinations used are localized as well as the length of amplicons obtained for each primer pair.

The S1/S2 amplicon covers the *DREB1A* gene from base pair position - 1438 to + 109. The primer pairs *DREBS1F/R*, *DREBS2F/R* and *DREBS1F*/*DREBS2R* amplified sequences of approximately 800, 900 and 1500 bp, respectively (Figure 1), consistent with the expected amplicons for target sequences, S1 (837 bp), S2 (952 bp) and S1/S2 (1547 bp). Analyses of the promoter sequences of *DREB1A* gene allowed the identification of polymorphisms, INDELs (insertion and deletion) and SNPs, in the different genotypes analyzed (Figure 2A and B). Two alleles of each genotype were selected and the sequences were aligned. The allele alignment revealed a pattern of polymorphisms creating a haplotype distribution. The genotypes of *C. canephora*, clone 14 and 22 showed different allelic distribution for *DREB1A* gene locus (Figure 2C).

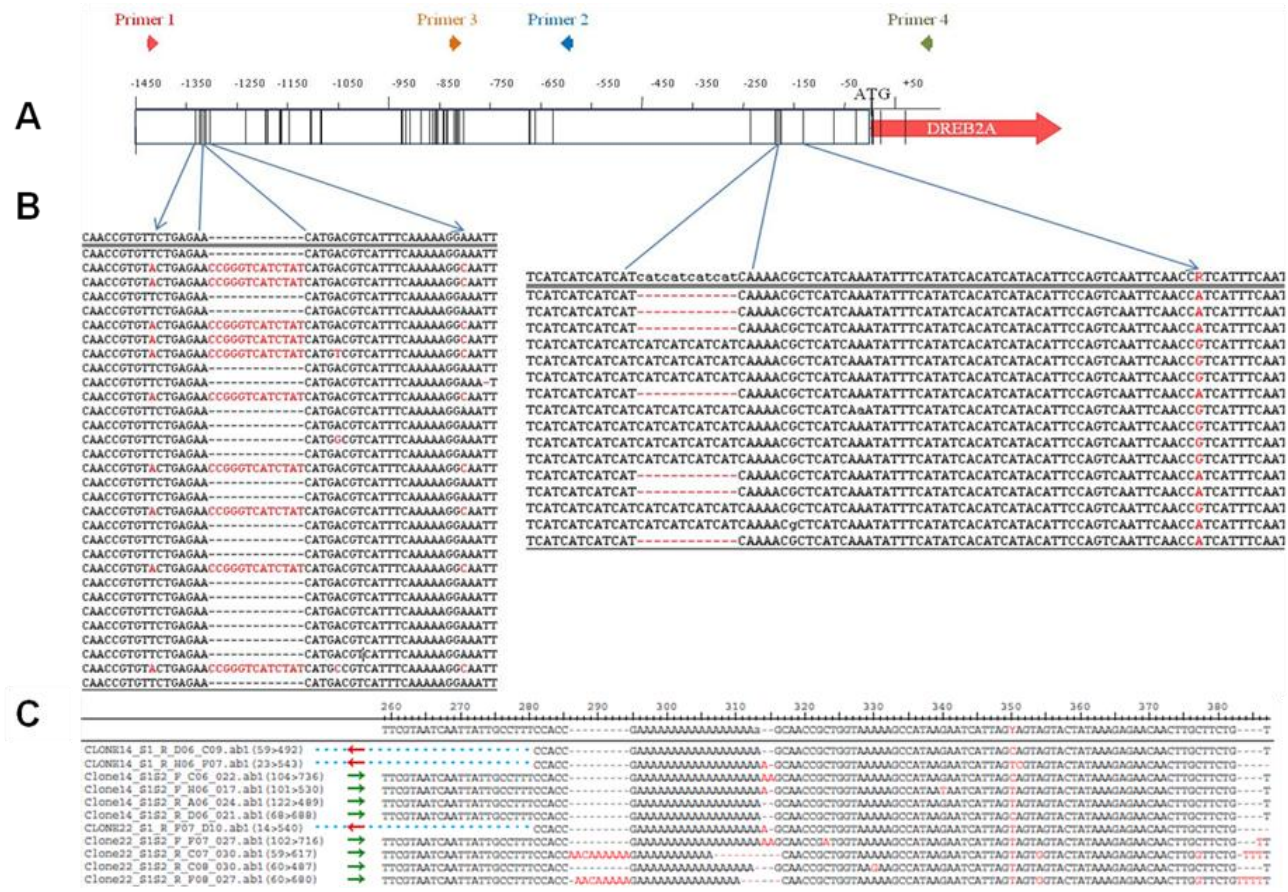

Figure 2. Nucleic alignment of *DREB1A* promoter regions amplified from different coffee genotypes. A schematic representation of polymorphims found in the promoter region is presented (A). Sequence alignments permitted to identify INDEL and SNP polymorphisms (marked in red) in two different regions (-1350 [left] and -200 [right]) of the promoter (B). Example of INDELs found in the *DREB1A* promoters of clones 14 (drought-tolerant) and 22 (drought-susceptible) of *C. canephora* (C).

The analysis of the pormoter sequence in the databases of cis-regulatory motifs PLACE and PlantCARE enabled the identification of regulatory elements characteristic of the response to water stress, as ERF / DRE (Dehydration Responsive Element), ERE (Ethylene Responsive Element), ABRE (ABA Responsive Element), ERD1 (Early Responsive to Dehydration 1), among others (Figure 3A). Cis-regulatory DNA motifs from both ABA-independent and -depended pathway were identified, indicating a possible cross-talk in the drought regulation pathways. Some of the INDELs and SNPs identified between clone 14 and 22 overlapped these cis-regulatory DNA motifs.

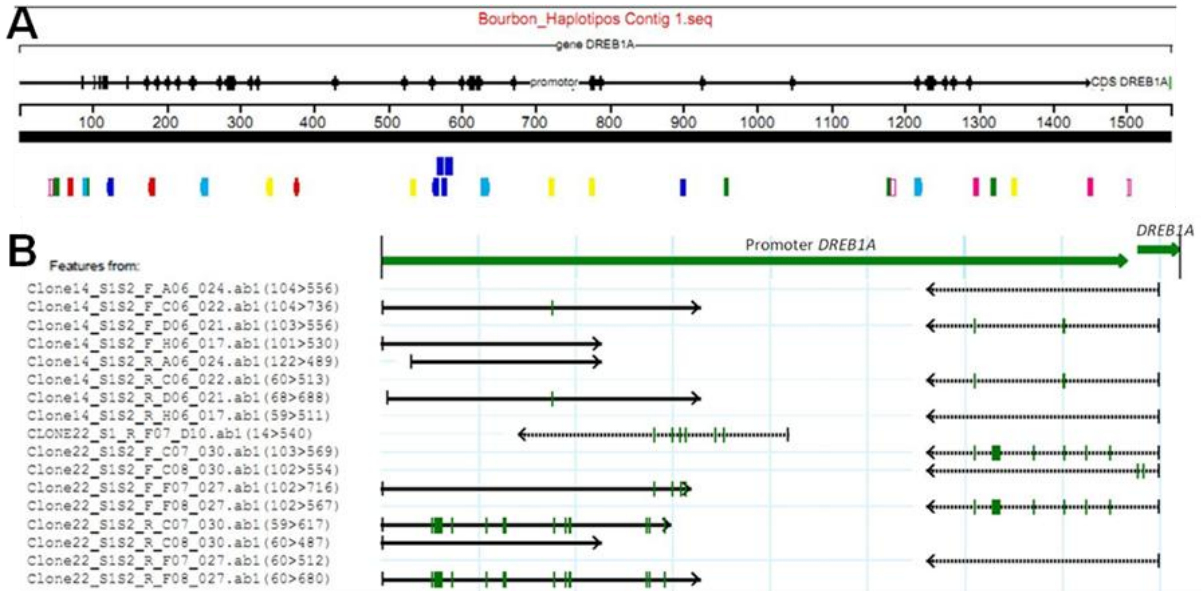

Figure 3. Analyses of the *cis*-regulatory DNA motifs. Motifs: MYC/MYB (green boxes); DOF4 (yellow boxes), TATA BOX (light blue boxes) and ERE (dark blue boxes) (A). More than 15 INDELs and SNPs were found by comparing the *DREB1A* promoters of clones 14 and 22 of *C. canephora* (B).

The presence of polymorphisms (particularly INDELs) in the *DREB1A* promoter regions of the clone 14 and clone 22 of *C. canephora* (Figure 3B), could explain the differential expression of this gene previously observed in the leaves of these two clones. In order to test this possibility, transformation vectors including different segments of the *DREB1A* promoters of the clones 14 and 22 were cloned into the binary vector pBI101 and are currently under study in transgenic citrus.

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Defense Gene Expression Induced by a Plant Extract Formulation and Phosphites in Coffee Seedlings Against *Hemileia vastatrix*

T. C. T. VALENTE, A. C. A. MONTEIRO, V. F. PEREIRA, P. M. RIBEIRO-JUNIOR, M. L. V. RESENDE

Departamento de Fitopatologia – Universidade Federal de Lavras
Campus Universitário, 37200-000 Lavras, MG, Brasil

SUMMARY

Coffee leaf rust, caused by *Hemileia vastatrix*, is one of the most destructive diseases of coffee worldwide. Our research group is conducting studies aiming at controlling this disease by activating defense mechanisms inherent to the coffee plant. Analysis of defense-related gene expression and activity of defense enzymes, in response to treatments with elicitors are important tools for studying the mechanisms of induced resistance. In this work we sprayed coffee seedlings with NEFID (coffee-leaf extract formulation), manganese phosphite and the association NEFID + copper phosphite + manganese phosphite and characterized levels of quantitative expression of two defense genes, *CAT* and *PAL*, which encode catalase and phenylalanine ammonia lyase, respectively. Activities of these enzymes were also evaluated, in a time course before and after inoculation with *Hemileia vastatrix*. *CAT* and *PAL* genes were induced in coffee seedlings by all treatments, exhibiting widely varied expression profiles according to the elicitor sprayed and the period analyzed. Before inoculation with *H. vastatrix,* the highest levels of expression of *CAT* and *PAL* genes were found in plants sprayed with NEFID. After inoculation we observed variations among the expression of these genes, the analyzed elicitors and times, but the highest levels of expression for both genes were found in plants treated with manganese phosphite or NEFID + copper phosphite + manganese phosphite. In general, PAL activity showed a different profile in relation to CAT activity, so that there was less activity of the enzyme PAL for the majority of the time courses analyzed.

Financial Support: CNPq, FAPEMIG e INCT do CAFÉ.

INTRODUCTION

The rust (*Hemileia vastatrix*) is one of the most destructive diseases of coffee and can cause major yield losses. We are currently using products based on plant extracts and nutrients to control plant diseases. Certain plant extracts, rich in bioactive substances, can act as inducers of resistance against diseases. Products based on nutrients, such as phosphites, have dual mode of action on disease control, acting directly on pathogens and also indirectly, by inducing plant defense responses.

Thus, the promising prospect of disease control through the activation of defense mechanisms has stimulated intensive research aimed at clarifying the biochemical and molecular aspects involved in this process.

The objectives of the current study was to verify the effect of products based on extracts of coffee leaves and phosphites on the expression of genes coding for catalase (CAT) and phenylalanine ammonia lyase (PAL) and the activity of these enzymes in coffee seedlings susceptible to *Hemileia vastatrix.*

MATERIAL AND METHODS

Coffee seedlings cultivar Mundo Novo (Cultivar MG-379-19) were sprayed with coffee leaf extract (NEFID), manganese phosphite (5.0 mL L^{-1}), or NEFID + copper phosphite (2.5 mL L^{-1}) + manganese phosphite (2.5 ml L^{-1}). Control plants were sprayed with water only. The inoculation with *H. vastatrix* was performed 168 hours after spraying. Leaves were collected for gene expression analysis, 12, 24, 72, 180, 192, 216 and 240 hours after treatment application. Leaves for biochemical analysis were collected 12, 24, 48, 72, 168, 180, 192, 216, 240 and 336 hours after product application.

The total RNA was extracted from plant tissue using the protocol Pine Tree adapted. Total RNA samples were treated with TURBO ™ DNase I (Ambion) and the cDNA synthesis kit 'SuperScript ® III First-Strand Synthesis Supermix' (Invitrogen) was used. Primers were designed using the program Primer3, suitable for real-time quantitative PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as constitutive control.

Regarding enzymatic analyzes, CAT and PAL were extracted with sodium acetate buffer 50 mM pH 5.2, 1mM EDTA, 1mM β-mercaptoethanol. Total protein was measured according to Bradford method. Catalase activity was determined using the method of Havir & McHale and the activity of phenylalanine ammonia-lyase was measured according to Mori et al. The mean enzyme activities at each sampling time, when significant by F test, were compared by Tukey test ($p < 0.05$) using SAS software V 9.0.

RESULTS AND DISCUSSION

CAT and *PAL* genes were induced in coffee by all treatments, with very different expression profiles in relation to the inducer used and the period of analysis. The highest levels of expression of these genes occurred in plants treated with NEFID. Plants treated with this extract showed higher levels of *CAT* gene expression at 72, 192 and 216 hours after spraying (HAS) (Figure 1A). *PAL* gene expression was higher in coffee seedlings treated with NEFID at 72 and 216 HAS (Figure 1C). *CAT* gene expression was significantly higher 24 hours after inoculation (HAI) for seedlings treated with NEFID or NEFID + Mn phosphite + copper phosfite (Figure 1B). There was a significant increase in levels of transcripts of *PAL* gene for NEFID and manganese phosphite, 48 HAI. In the period from 72 HAI, only plants treated with the combination NEFID + Mn phosphite + copper phosfite showed increased level of *PAL* gene expression; other treatments showed a reduction in this expression pattern (Figure 1D).

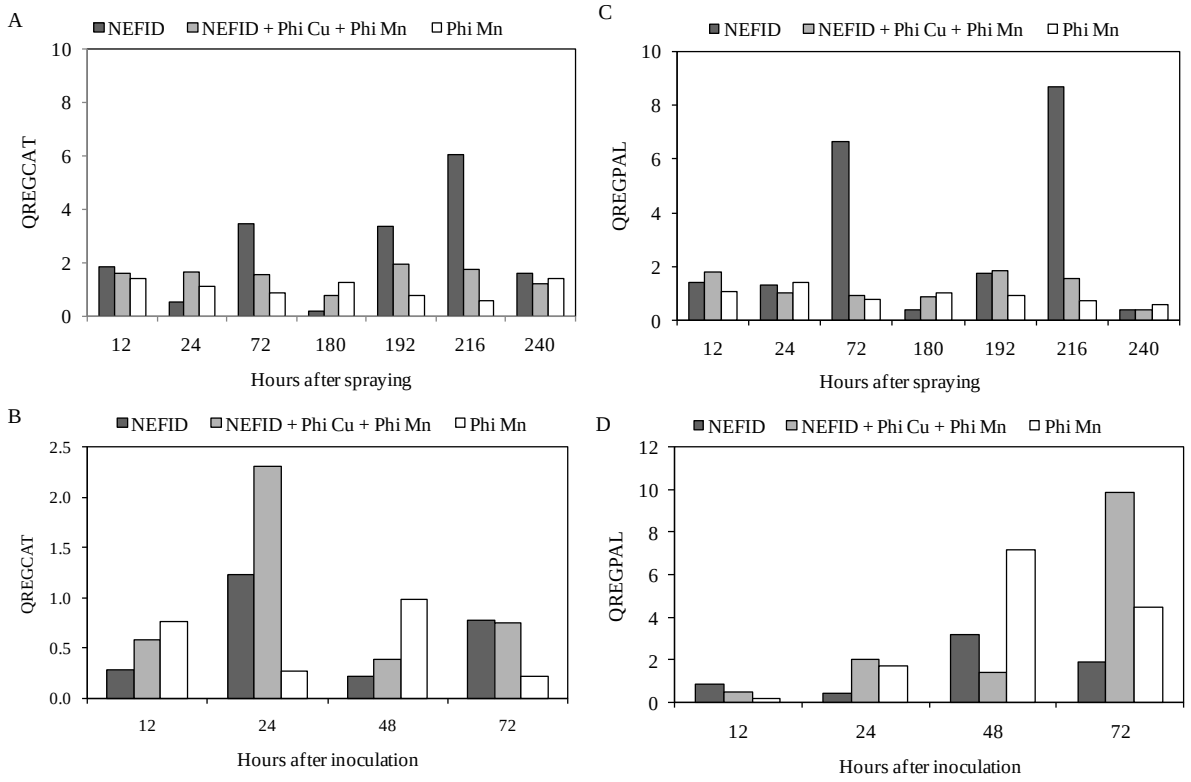

Figure 1. Quantitative relative real-time expression (qRT-PCR) of *CAT* gene after spraying with formulations (A) and after inoculation with *H. vastatrix* (B), and *PAL* gene expression after spraying (C) and after inoculation with *H. vastatrix* (D). Columns represent the average induction of transcripts related to non-inoculated controls in the same period.

The activity of catalase (CAT) and phenylalanine ammonia lyase (PAL) were quite variable in relation to the elicitor formulation and the period analyzed. In plants treated with NEFID, CAT activity was higher at 12, 24, 72, 168 and 336 HAS, compared to the control. Plants treated with manganese phosphite showed higher CAT activities at 24 and 48 HAS (Figure 2 A).

In the period of 216 HAS, CAT activity increased due to NEFID+ manganese phosphite + copper phosphite spraying, showing activity twice as high compared to other treatments (Figure 2A). PAL activity in plants treated with NEFID was higher at 24, 72, 168, 180 and 336 HAS, compared to the control. Plants treated with manganese phosphite showed maximum activity of PAL at 72 HAS, being twice as high compared to other treatments. At 192 HAS, all treatments showed increased activity of PAL, but only plants treated with the combination NEFID+ manganese phosphite + copper phosphite showed a significant increase compared to other treatments (Figure 2C).

After inoculation, the plants treated with manganese phosphite alone, exhibited higher CAT activity compared to the control, at times of 12 and 168 HAI (Figure 2B). The PAL enzyme activity in periods of 12, 24 and 48 HAI was higher compared to control for all treatments. However, in subsequent periods, all treatments had lower activity of this enzyme in relation to the control (Figure 2D).

Spraying with NEFID increased CAT activity possibly by the fact that this extract of infected coffee leaves has elicitors that mimics pathogen attack. These elicitors are recognized by the

coffee plants, which activate their defense responses, such as the increase in the activity of defense enzymes.

From the data presented here, it was observed that the results of enzymatic tests did not correlate with gene expression. Thus, we can infer that there are several isoforms of enzymes CAT and PAL. However, primers were designed for a specific isoform of each target gene and the enzymatic activity can detect multiple isoforms, ie, several gene products, which are represented here.

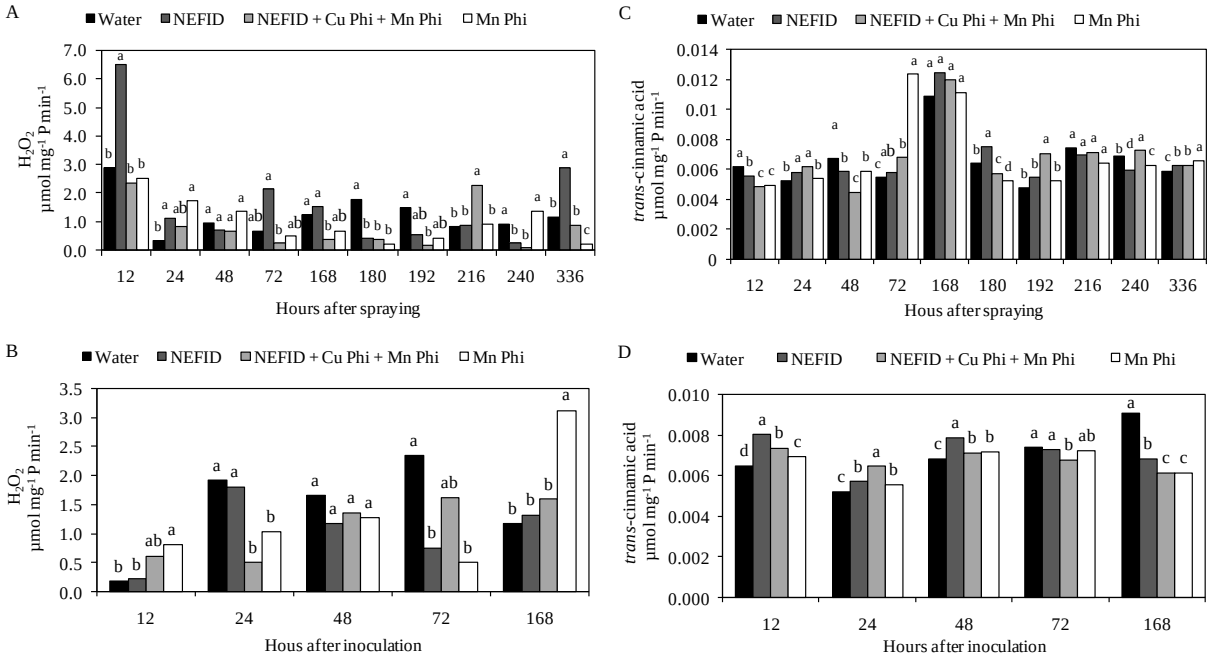

Figure 2. Activity of catalase (CAT) (mol of hydrogen peroxide oxidized per mg protein per minute) (A) after spraying with formulations (A) and after inoculation with *H. vastatrix* (B), and phenylalanine ammonia lyase (PAL) (mol of trans-cinnamic acid per mg protein per minute) (C) and after inoculation with *H. vastatrix* (D). Columns with same letter in each evaluation period did not differ by Tukey's test ($p < 0.05$).

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Arabica Genetic Mapping using SSR Markers in relationship with High Density Robusta Map.

M. RIGOREAU, V. L'ANTHOENE, N. MAYER, C. LAMBOT, D. CROUZILLAT

Nestlé R&D Center, Tours, France

SUMMARY

Two main coffee species are cultivated worldwide ranking among the most valuable agricultural exports from tropical countries. *Coffea arabica* (2n=4x=44) is an allotetraploid containing two genomes originated from the diploid wild coffee species, *Coffea canephora* and *Coffea eugenioides*. Arabica cultivars are characterized by a low genetic diversity due to limited accession diffusion in producing countries even if a larger genetic diversity was found in Ethiopia the center of origin of this coffee species. In contrast, *C. canephora* is a diploid allogamous species (2n=2x=22) with considerable variability.

Recent genomic and genetic data were at the origin of a high density Robusta map that has been used to initiate the Arabica mapping using the same set of SSR markers. These highly polymorphic markers can be easily used to map and characterize independently the two diploids genomes included in Arabica. A large number of these SSR markers has been obtained through 454 DNA Arabica sequencing. A total of 22500 microsatellites was detected including di to hexa-nucleotide patterns with a minimum of five tandem repeats. The di-nucleotide pattern SSRs were more frequent than others especially GA_n (36%), AT_n (32%), GT_n (14%) and GC_n (1%).

A F2 segregating population (138 individuals) was obtained from a cross between two Ethiopian Arabica Ar8 and Ar36B. The genetic map obtained so far include 356 loci distributed over 73 linkage groups. Its length is 2685 cM which represents approximately 65% of the final estimated size of Arabica map. Up to now, the Robusta and Arabica genetic maps show co-linear organization of the shared loci. But the two genetic maps obtained have already displayed significant differences, in the majority of cases, the genetic distances between loci are more important in Arabica than in Robusta.

The Arabica genetic map could be used to detect QTLs associated with agronomical, biochemical and technologic traits of interest for the breeders. The genetic and genomic ressouces developped for this project will be of interest for any further arabica genome sequencing project.

INTRODUCTION

Coffee is one of the most important beverage crops worldwide; it production is growing over 80 countries in the tropical and sub-tropical areas. Approximately 124 species, including recent *Psilanthus* species introductions, belongs to *Coffea* genus. The most important commercially species are *Coffea arabica* L., known as arabica coffee, and *Coffea canephora*, also named robusta coffee. *C. arabica* is an allopolyploid autogamous species (2n=4x=44) most likely resulting from hybridization event between *C. eugenioides* and *C. canephora* coffee diploid species (2n=2x=22).

A limited genetic diversity is observed among arabica cultivars due to a limited accessions number used for the world propagation of this crop species. However, wild arabica genotypes, show a higher level of polymorphism. But, only interspecific hybridizations with *C. canephora* are used to introduce genetic diversity in arabica breeding program for agronomical trait of interest. The resulting arabica hybrids (Catimor) have introgressed robusta genome areas leading to coffee rust resistance.

Up to now, genetic linkage maps available for arabica species were obtained from crosses involving Catimor in order to facilitate the detection of DNA polymorphism. The molecular markers previously used for these studies were mainly AFLPs which are considered as poorly informative to differentiate the two diploid sub-genomes at the origin of the arabica.

Our study is based on a segregating population from two wild arabica accessions and the use of SSR markers able to differentiate each of the two arabica sub-genomes. These data allow also a direct comparison with the international high density robusta genetic map and provide a data base for further genetic and genomic analyses.

MATERIALS AND METHODS

Two wild arabica accessions (Ar8 and Ar36B) were used from Ethiopian FAO prospection. One F1 plant was selected from the cross between these two parents. After selfing a F2 population of 138 individuals was obtained and planted in Ecuador in 2004.

The SSRs used in this study were coming from various genomic libraries including 454 sequencing. These microsatellites were selected according to their DNA polymorphism and their ability to differentiate the two diploid sub-genomes (*C. canephora* and *C. eugenioides*) in arabica.

PCR reactions were performed on each selected locus of microsatellite by using M-13 labelled primers. It was carried out by using the kits of Applied Biosystem "AmpliTaq Gold® PCR Master Mix". The amplification products were visualized using ABI PRISM® 3500 Genetic Analyzer.

The heterozygous markers segregating were used to construct the F2 genetic map. The genetic map was performed by using JoinMap® software 4.1 with Kosambi's mapping function with a LOD threshold of 5.

The diploid origin of each segregating locus is identified according to *C. canephora* and *C. eugenioides* DNA controls included in the study.

RESULTS AND DISCUSSION

Due to a low DNA polymorphism detected in arabica only 5% of the SSRs used were suitable for this genetic mapping analysis even if the two parental accessions selected are representative of wild Ethiopian Arabica diversity. In order to obtain more SSRs we used high throughput SSR identification with 454 genome sequencing. Up to five millions reads, with a mean length of 407 bases, were obtained representing 2 X arabica genome coverage. A total of 22500 microsatellites with at least five tandem repeats were characterised. The di-nucleotide pattern SSRs were more frequent than others especially GA_n (36%), AT_n (32%), GT_n (14%) and GC_n (1%).

Table 1. Main Arabica genetic mapping characteristics on the eleven linkage groups (A to K) on both *C.canephora* (R) and *C. eugenioides* (E) sub-genomes. The unidentified sub-genomes are coded: U.

Linkage	Arabica (R)			Arabica (E)			Arabica (U)			Arabica Total		
group	Loci	Groups	Size (cM)	Loci	Groups	Size (cM)	Loci	Groups	Size (cM)	Loci	Groups	Size (cM)
A	4	2	20	9	2	58	0	0	0	13	4	78
B	33	7	216	18	5	195	8	3	55	59	15	466
C	12	2	148	15	1	116	0	0	0	27	3	264
D	15	2	119	9	3	14	2	1	2	26	6	135
E	18	3	168	16	3	119	3	1	27	37	7	314
F	37	4	232	3	2	35	2	0	0	42	6	267
G	12	3	171	6	1	48	2	1	3	20	5	222
H	23	3	204	4	2	7	0	0	0	27	5	211
I	17	4	68	14	1	64	0	0	0	31	5	132
J	5	1	55	6	2	104	0	0	0	11	3	159
K	34	4	225	17	4	120	0	0	0	51	8	345
U							12	6	92	12	6	92
Total	210	35	1626	117	26	880	29	12	179	356	73	2685

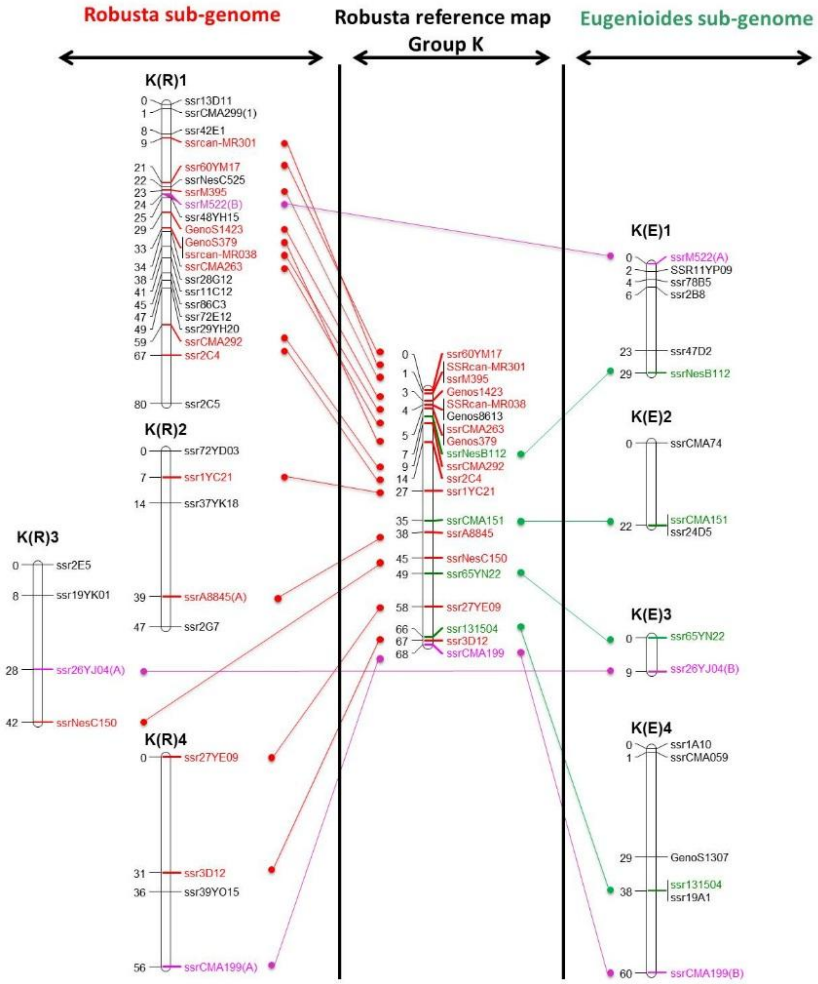

Figure 1. Arabica linkage group K map in comparison with Robusta reference group K. The two Arabica sub-genome maps (Robusta and Eugenioides) of the linkage group K are, respectively, on the right and on the left of the robusta reference map. The shared loci for Robusta and Eugenioides are indicated, respectively, in red and green. The common loci between the two arabica sub-genomes are in purple.

Up to now, a total of 396 segregating SSRs were genotyped and 356 loci were mapped on 73 linkage groups covering 2685 cM. Using a reference set of *C. canephora* and *C. eugenioides* accessions it has been possible to determine the origin of the diploid genome for a large majority of the segregating alleles in the arabica progeny. A majority of loci (210) are mapped on the sub-genome *C. canephora* in comparison with those mapped on *C. eugenioides* (117). A remaining set of 29 loci were not yet assigned to any of the two diploid genome (Table1). The lowest arabica mapping rate observed in the eugenioides sub-genome could be due to a lower polymorphism level in comparison with robusta species. This genetic bottleneck has been occurred either at the arabica species creation with a limited number of *C. eugenioides* individuals at the origin of the interspecific hybrid plant species or due to a lower genetic polymorphism of this diploid coffee species.

A high level of map co-linearity is observed between arabica and high density robusta genetic maps. But the genetic map size is higher for arabica than robusta. As example, the linkage group K (Figure 1) shows that the loci distances are at least 2 to 3 times higher for each of the two arabica sub-genomes in comparison with the Robusta reference map. The final estimated arabica map size is about 4150 cM. This main genetic difference between arabica and robusta genetic maps could originate from the two coffee pollination systems. For the autogamous arabica species, the pairing of highly homologous chromosomes could result in a higher rate of recombination in comparison with the allogamous robusta species with heterozygous chromosomes.

A large number of gaps are still remaining in this arabica genetic map and the locations of these gaps are usually correlated to low genetic marker density in the robusta genetic map. Using this robusta genetic map and the genome sequence of this plant species it is now possible to get access to the genome areas not yet mapped and to search for new targeted microsatellite markers.

In parallel to this approach, the arabica genome sequencing is also providing new genomic resources to create an interface between the genetic data and the genome sequence for the identification of genes responsible for key agronomic and quality traits.

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QTL Detection on Robusta using Single and Multi-Parent Mapping Populations in different Locations.

V. L'ANTHOENE[1], M. RIGOREAU[1], F. LEFEVRE-PAUTIGNY[2], C. LAMBOT[1], J. HUSSON[1], D. CROUZILLAT[1]

[1]Nestlé R&D Center, Tours, France
[2]Nestlé Nespresso, Lausanne, Switzerland

SUMMARY

A quantitative trait locus (QTL) analysis design for single and multi-parent populations was carried in coffee (*Coffea canephora*) which is a diploid allogamous perennial species with a high heterozygosity rate.

This study was performed on two progenies (FRT58 X FRT51) and (FRT67 X FRT51) located in two locations (Ecuador and Thailand), each of these two segregating populations having approximately 200 progenies. Two parents belongs to the Congolese group (FRT51 and FRT67) since FRT58 belongs to the Guinean group. These three parental genotypes were selected in a Robusta collection based on their genetic background and their phenotypic characteristics, especially their agronomic and sensory performances. Genetic maps of these populations were established using SSR markers allowing the detection of numerous QTLs related to agronomic, biochemical, technological and also sensory characteristics. This quantitative analysis showed that the favorable alleles are mainly carried out by the parents FRT58 and FRT67.

In Ecuador, a connected factorial mating trial including seven parents was genotyped using 84 SSRs to produce a consensus genetic map used for QTL detection. A total of 11 progenies of 32 individuals each were phenotyped individually using field data and biochemical composition predicted by NIR. The MCQTL software allows the QTL mapping in multi-cross design with additive and dominance models. For 19 quantitative traits studied, most of the QTLs have been identified using the additive model and few QTLs with dominance effects were detected. This multi-population analysis reveals several major QTL affecting biochemical bean composition with potential influence on the cup quality.

The comparison of the QTL detection between single and multiple progenies approaches reveals that the across-family analysis is efficient due to the interconnected families. Favorable alleles were detected in all parents involved in the multi-family populations. This approach involving several parents is promising for an application on polycross progenies, traditionally used in breeding programs.

INTRODUCTION

Robusta coffee is one of the only two commercial *Coffea* species and contributes to 30% of the world coffee production. Despite socio-economic importance of coffee sector for producing countries, few scientific studies are conducted on this perennial plant, especially for breeding purpose.

A research program has been established to evaluate the quantitative genetic determinism for agronomic, biochemical, sensory and technological traits in order to assist the management of breeding program. Most QTL studies are carried out on bi-parental populations which offer the possibility of whole genome scanning with a limited number of markers due to high linkage disequilibrium. However, these populations have a limited variability with a low breeding interest. Recently, it has been proposed to build crossing designs composed of bi-parental populations connected by common parents. The advantage of these connected multi-parental populations is to extend the genetic diversity within the cross design and to compare the alleles of different parents within a single model. The powerful of the multi-population design for QTL mapping was previously shown on different studies on oil palm and rye grass.

The main objective of this study was to compare these two QTL detection methods on Robusta coffee species.

MATERIALS AND METHODS

Seven robusta accessions were selected from a Nestlé collection to create the mapping populations. Two bi-parental populations of approximately 200 progenies resulted from the cross between the male parent FRT51 and the female parents FRT58 and FRT67. A multi-cross design based on the cross between three male parents (FRT51, FRT55 and FRT60) and four female parents (FRT9, FRT49, FRT58 and FRT67) was represented by 11 populations of 32 progenies each. These two experimental designs (bi-parental and multi-parental populations) were located in Ecuador and Thailand. Each population was phenotyped from 2006 to 2011 using field data and biochemical bean composition predicted by NIR.

Microsatellite markers from various genomic libraries were used to construct the genetic maps. PCR reactions were performed by using M-13 labelled primers with the kit of Applied Biosystem "AmpliTaq Gold® PCR Master Mix". The amplification products were detected using ABI PRISM® 3500 Genetic Analyzer. The genetic map was performed using JoinMap® software 4.0 with Kosambi's mapping function with a LOD threshold of 3.

For QTL mapping two methods were used. The first one detect QTL for each bi-parental population and was carried out using an interval mapping approach with MapQTL® software 6.0 using a LOD threshold of 4.3 determined by permutation tests. A second method was used to detect QTL in the 11 connected populations of the factorial mating and in the two bi-parental populations connected by the share parent FRT51 using a single model. The multi-populations connected analysis was carried out using the additive and dominance models of the MCQTL software. The significant threshold of 3.3 was determined by iterations tests on each trait.

RESULTS AND DISCUSSION

The quantitative analysis of the two bi-parental populations (FRT58xFRT51 and FRT67xFRT51) in Ecuador led to the identification of 125 QTLs controlling traits of interest for the breeders (Figure 1). The variance explained by the QTLs ranged from 7.3 to 38.3 %. The favourable alleles were mainly carried out by the two parents FRT58 and FRT67.

A total of 46 QTLs were identified for the parents of the multi-cross design planted in Ecuador using the additive and dominance model of MCQTL (Table 1). Their variance ranged from 8.3% to 34.4%. Few dominance effects were identified suggesting that most of the genetic value is determined by additive effects (72%). The multi-population connected analysis allowed a global comparison of the seven parental allelic effects at each QTL. The

majority of the favourable alleles are originating from the four parents FRT58, FRT67, FRT9 and FRT55. These results lead to the possibility of increasing the frequency of favourable alleles using MAS starting from these four cultivated accessions.

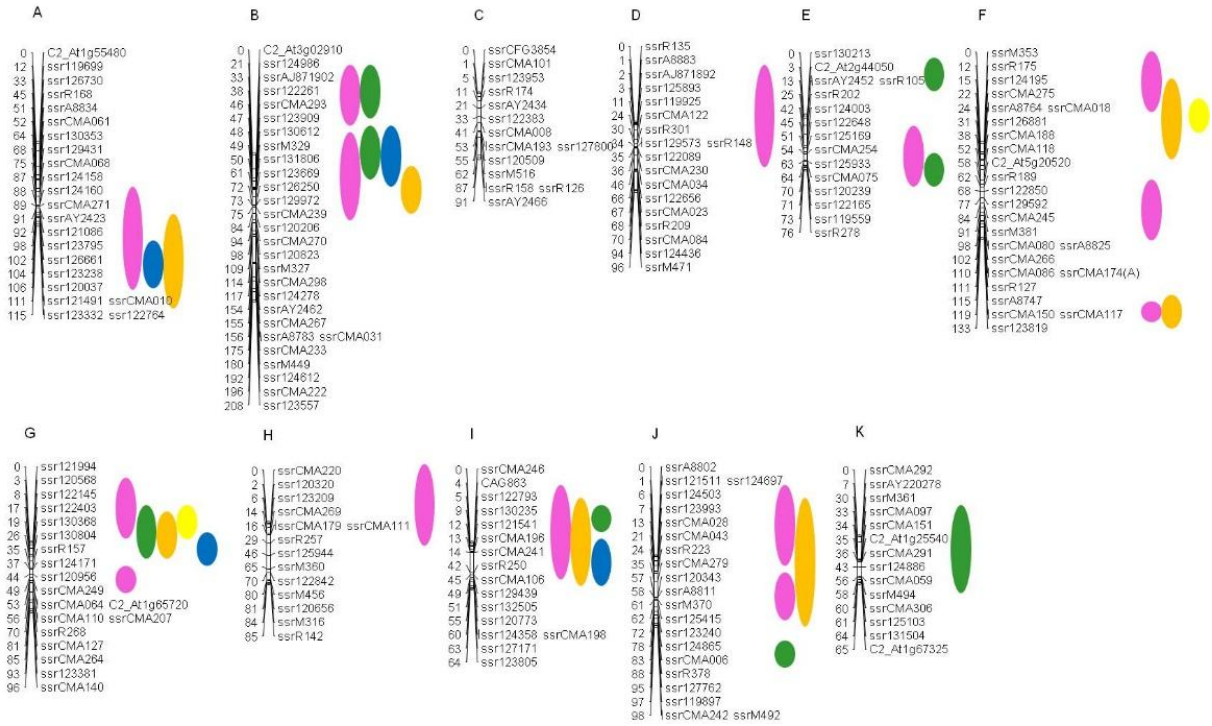

Figure 1. Summary of QTL mapping in the population FRT58xFRT51 located in Ecuador. The QTLs linked to biochemical, sensory, technologic, agronomic and yield traits are highlighted, respectively, in pink, blue, orange, green and yellow.

Table 1. Examples of QTLs detected in the multi-cross design with additive model using MCQTL software. For each parent, the significant effect at 5%, 1% and 1 ‰ are, respectively, indicated in yellow, orange and red.

									Additive Effects						
Trait	Type of trait	Linkage Group	Marker	Location (cM) Kosambi	Test	R^2	Global R^2	Confidence of interval	FRT49	FRT55	FRT60	FRT58	FRT51	FRT67	FRT9
Titrable Acidity bean content	Biochemical	F	123819	110	4.0	9.2	9.2	48-113	0.000	0.856	0.259	**-1.992**	-1.060	**8.063**	-0.606
Caffeine bean content	Biochemical	A	124158	89	5.1	11.5		82-100	0.000	0.011	-0.031	**-0.115**	-0.017	-0.007	**0.058**
	Biochemical	E	125169	33	6.8	14.9	31.8	13-39	-0.042	-0.007	-0.040	**-0.093**	**0.077**	**0.109**	-0.035
	Biochemical	J	123993	4	4.5	11.2		0-11	**0.086**	-0.033	0.031	**0.112**	0.035	-0.001	0.020
Oxalic acid bean content	Biochemical	A	119699	16	5.2	14.0		8-25	0.000	-0.001	0.005	**-0.020**	0.007	-0.001	0.001
	Biochemical	A	123238	108	5.0	13.5	36.1	99-113	0.000	-0.002	-0.002	**-0.019**	0.002	0.000	0.000
	Biochemical	E	125933	42	4.7	13.8		36-47	0.002	0.000	**0.008**	**0.015**	-0.005	0.004	**-0.006**
	Biochemical	F	CMA018	0	3.9	11.3		0-6	0.000	**-0.005**	0.002	**-0.016**	0.000	-0.001	0.001
Trigonelline bean content	Biochemical	B	123909	31	8.1	16.6	22.9	20-39	0.004	-0.004	-0.004	**-0.030**	**0.017**	**-0.025**	**-0.039**
	Biochemical	G	122145	12	3.4	9.0		0-29	**0.019**	-0.001	0.000	0.010	-0.001	**-0.025**	**-0.022**
Weight of 300 beans	Technological	E	125933	44	4.0	9.3	17.2	37-47	0.458	1.084	-0.913	-1.214	-0.896	**-2.075**	**4.716**
	Technological	F	CMA080	86	4.3	9.2		78-94	0.000	0.412	-0.137	-1.236	0.589	**-6.619**	**-5.264**
Caracoli beans	Technological	D	A8883	2	4.4	9.7	9.7	0-12	2.582	**7.888**	1.333	**-2.030**	**-3.020**	0.345	-0.363
Bean density	Technological	B	123909	37	3.0	8.3	18.3	23-48	0.000	**-0.004**	0.000	**0.003**	-0.002	**0.003**	**0.003**
	Technological	H	125944	58	5.1	11.9		43-68	-0.002	**0.004**	**-0.003**	0.002	**-0.003**	0.001	**0.005**

To compare the two QTL detection methods (single or multi-parent populations), a quantitative analysis on the two bi-parental populations connected by the common parent FRT51 was also carried out using the MCQTL software.

The multi-population connected analysis allowed the detection of 229 QTLs (Table 2). This multi-population analysis led to the detection of the majority of the 125 QTLs found in the previous analysis using single population design and a new additional set of 135 QTLs.

The multi-parental populations study combines the advantage of wider parental diversity and powerful QTL detection. This approach is promising for an application on polycross progenies, traditionally used in breeding programs. This could lead to a marker-assisted breeding strategy based on the hybrid creation cumulating key QTLs from different sources.

Table 2. Comparison of two approaches (single and connected populations) for the QTL analysis of bi-parental populations (FRT58 x FRT51) and (FRT67 x FRT51). The quantitative analyses of the two bi-parental populations were carried out with MapQTL and MCQTL softwares.

	Single Population Approach (MapQTL)				Connected Population Approach (MCQTL)	
Trait	Number of QTLs detected from the population FRT58xFRT51	Number of QTLs detected from the population FRT67xFRT51	Number of common QTLs between the two populations	Total number of QTLs	Total number of QTLs	Number of additionnal QTLs detected with the Connected Population Approach
Agronomic	14	2	0	16	56	42
Biochemical	46	21	5	62	97	51
Technological	21	10	0	31	53	31
Sensory	5	7	0	12	14	5
Yield	3	2	1	4	9	6
	89	42	6	**125**	**229**	**135**

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Early Selection for Drought Resistance in Coffee

C. LAMBOT, N. RUTA, S. GAYOT, V. L'ANTHOENE, D. CROUZILLAT

Centre de Recherche et Développement Nestlé, Tours, FRANCE

SUMMARY

Global warming with increased temperature and limited rainfall will generate more drought incidences in this century. In the future, it will strongly affect coffee production as coffee trees have significant water requirements for their growth and development. The environmental changes will limit suitable areas for coffee production. Our breeding activity aims to select Arabica plants at early stage for their drought resistance and investigate on their physiology parameters related to stress. Seeds were collected on a progeny derived from wild accessions and plantlets were screened under water-stressed conditions in the greenhouse using an hydroponic system. Water stress was induced by adding polyethylene glycol to the nutrient solution; PEG is a polymer that has long been used in research to mimic water-stressed conditions. Results are showing that a combination of high values for three parameters (stomatal conductance (Gs), leaf greenness (SPAD value), maximum quantum yield of PSII primary photochemistry (Fv/Fm)) is highly correlated with the capacity of plantlets to cope with drought. A number of plants has been selected based on drought tolerant score and other physiological parameters. Further investigation for their drought tolerance will take place in the field in order to confirm results obtained in the greenhouse. Output from physiological measurements will be used to identify genetic loci related to these traits. DNA markers related to drought tolerance will be used in breeding program to accelerate the process of selection.

INTRODUCTION

Global warming with increased temperature and limited rainfall will generate more frequent and/or intense drought stress in this century. In the future, it will strongly affect coffee production as coffee trees have significant water requirements for their growth and development. These environmental changes will limit suitable areas for coffee production. One of the solutions to easily control plant water potential is to use solutions of known concentration of osmoticum. For example, high molecular weight polyethylene glycol (PEG) has been used for a long time to simulate drought stress in plants. PEG is a non-penetrating osmotic agent, lowering the water potential in a way comparable to soil drying. PEG-induced drought selection has been successfully used in several species including *Medicago sativa* (Dragiiska, 1996), *Nicotiana tabacum* (Heyser and Nabors, 1981), *Zea mays L.* (Mohammadkhani, 2008), *Solanum tuberosum* (Al-Sharari, 2004).

MATERIALS AND METHODS

Plantlets derived from seeds collected on F2 Arabica progenies were used in the experiment. The seeds conformity was controlled through molecular biology by comparison of segregating markers identified on the parental accessions.

Seeds were collected on 137 F2 plants and for each of them 3 plantlets were introduced in the experiment in comparison with the 2 parental accessions and 4 control genotypes (Typica,

SL28, Caturra and Bourbon). Plantlets were transferred to hydroponic systems at the stage of 3 to 5 pairs of leaves. Three identical hydroponics systems have been used in the experiment, each were made up of 8 tanks with a capacity of 15 plants and approximately containing 20 litres of nutrient solution. The experimental design was a Complete Randomized Design with a total of 143 genotypes in 3 replications. PEG 8000 at the final concentration of 250g/L was progressively added in the nutrient solution to avoid plant shock. Pumps were used to sufficiently oxygenate the PEG solution and avoid anoxia risks.

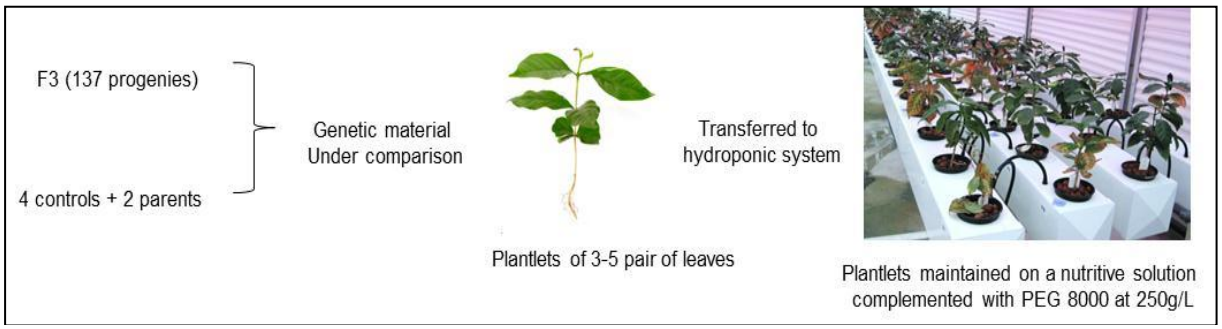

Figure 1. Genetic material and plantlets size used for the experiment on hydroponic system.

Physiological and vegetative parameters were observed during the experiment. Traits recorded and the equipment used for the data recording are listed in table 1. A drought tolerance score (RR to SS) was elaborated to evaluate the level of plant resistance to the drought stress (figure 2).

Table 1. List of parameters and equipment used for observation on coffee plantlets.

Type	Parameters	Instrument	Indication
Photosynthetic Traits	Photosynthetic rate (A)	LI-64000 XT	The capacity to photosynthesize
	Stomatal conductance (gs)	LI-64000 XT	The rate of CO2 flow through stomata
	Chlorophyll fluorescence (Fv/Fm)	LI-64000 XT	The stress level of plant (~0,79-0,85 for healthy plant)
Classic Physiological Traits	Drought score	-	Occurrence of drought symptoms
	Chlorophyll content (SPAD value)	SPAD 502	Relative amount of chlorophyll
	Relative water potential (RWC)	-	Capacity of leaf to retain water
	Total leaf area	LI-3000C	Leaf area
	Leaf area increment rate (LAIR)	LI-3000C	Increase rate of leaf area
	Shoot dry weight (SDW)	-	Accumulation of photosynthates in shoot
	Root dry weight (RDW)	-	Accumulation of photosynthates in root
	Biomass	-	Accumulation of photosynthates in plants
	Root to shoot dryweight ratio (S:R ratio)	-	Photosynthate partitioning
	Plant height (PH)	-	shoot morphology
	Root lenght (RL)	-	shoot morphology

RR — Highly tolerant | R — Tolerant | M — Intermediate | S — Sensitive | SS — Highly sensitive

Figure 2. Drought tolerance score.

RESULTS AND DISCUSSION

The main results obtained for the photosynthetic, physiological and vegetative traits are listed in tables 2 and 3 with the corresponding statistical analyses.

Tables 2 and 3. Statistical analyses (ANOVA) for the main parameters.

		Fv/Fm		Gs		A		SPAD	
		WW[1]	**WS[1]**	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**
Controls	**Ar8**	0.801	0.619	29	-1	3.25	1.49	52.48	59.53
	Ar36B	0.804	0.661	32	2	3.00	2.13	50.00	61.83
	CCCA10	0.803	0.748	51	3	3.57	1.15	51.37	60.53
	CCCA12	0.798	0.763	22	6	2.00	1.05	50.85	46.73
	Bourbon	0.800	0.763	30	3	4.80	1.52	55.61	54.03
	Caturra	0.792	0.680	34	4	4.00	1.34	56.80	56.56
Progenies	**Min**	0.750	0.002	-30	-119	1.00	-0.23	28.25	1.70
	Max	0.819	0.809	90	47	5.00	3.77	62.65	70.35
	Average	0.801	0.732	26	-2	2.07	1.16	52.02	51.12
Family (p-value)		0.973	**0**	**0.0001**	0.9731	0.0546	0.9731	0.8183	**0**
System (p-value)		0.1505	0.8345	**0.0001**	**0**	0.994	**0**	0.8136	0.4256

[1] *WW : we watered ; WS : water stressed.*

		Shootlength	Root length	Root:Shoot	Biomass	LA increment rate	RWC
Controls	**Ar8**	19.88	15.13	0.19	2.83	2.02	49.05
	Ar36B	17.50	15.88	0.45	3.73	2.73	54.48
	CCCA10	16.42	14.50	0.26	2.33	2.35	55.91
	CCCA12	18.83	11.00	0.20	0.79	4.43	44.11
	Bourbon	16.63	14.25	0.20	2.28	2.23	55.67
	Caturra	15.43	14.93	0.22	2.19	2.02	43.86
Progenies	**Min**	9.00	6.00	0.04	0.42	0.00	8.51
	Max	32.00	24.00	0.60	8.48	5.94	85.96
	Average	15.24	13.48	0.25	2.30	1.94	50.64
Family (p-value)		0.2104	**0**	**0**	0.0837	**0**	**0**
System (p-value)		0.0519	0.6823	0.3111	0.4542	**0.0003**	**0.001**

The statistical analyses is leading to the conclusion that 3 parameters are efficient and promising to discriminate the plants for their tolerance to water stress when maintained in the hydroponic system : Fv/Fm , SPAD and RWC can be associated to the drought tolerance score to select coffee plantlets with better stress tolerance.

Water stress was induced by adding PEG to the nutrient solution of coffee plantlets maintained on hydroponic system. Results are showing that maintaining a healthy photosynthetic apparatus (high values for Fv/Fm and leaf greeness) and high relative water content (RWC) is highly correlated with the capacity of plantlets to cope with drought. A number of plants was selected to further investigate in field conditions on their drought tolerance.

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DNA Traceability for Variety Purity in Nespresso Product

E. MOREL[1], L. BELLANGER[1], F. LEFEBVRE-PAUTIGNY[2], C. LAMBOT[1], D. CROUZILLAT[1]

[1]Nestlé R&D Center, Tours, France
[2]Nestlé Nespresso, Lausanne, Switzerland

SUMMARY

The coffee market is regularly developing finished products based on a single variety. Some of them are well recognized by the consumer for example Maragogype, Moka, Blue Mountain or Bourbon. The development of this product category affords some similarities with the wine market which is organized by grape varieties in many countries and could bring opportunities for premium product differentiation. Nevertheless, the green coffee market is not organized by varieties but mainly by species (Robusta versus Arabica) and by producing countries. The distinction of coffee varieties at the green coffee stage is almost impossible using physical or chemical analytical tools. A DNA method was developed to allow the identification of varieties through the value chain, from the field to the finished product. The method is applied on routine basis to guarantee the purity and authenticity of raw material used by Nespresso.The quality control test was recently applied and fine-tuned using green bean batches from farms in Southern Brazil, which grow red and yellow bourbon varieties. This Arabica blend is at the origin for the sensory specificity of "Dulsão do Brazil" capsule. Genetic diversity studies were performed among these farms using a set of eight microsatellite markers (SSRs) selected for their ability to discriminate the Bourbon origins. The DNA tool helped guide the farm selection. The same procedure could be applied to other Arabica varieties according to requirements and claims of the final coffee product. The method is being improved to increase the capacity of sample analysis and decrease its cost. In addition, a new technology is under test based on high throughput 454 DNA sequencing. This assay will provided higher reliability and accuracy both for genetic diversity studies and quality control tests.

INTRODUCTION

The commercial world coffee production is based on two species, *Coffea arabica* (70%) and *Coffea canephora* (30%). The best cup quality is associated with the species *C. arabica*. The quality may varied in the coffee batches selected by Nespresso according to several factors such as environment, harvest, post-harvest processes and the Arabica varieties used. R&D Nestle Tours and Nespresso focus on coffee origin using DNA traceability. Part of our R&D activity is dedicated to the development of DNA markers in order to identify and trace the raw material. Our laboratory develops methods to verify the authenticity of the plant ingredients used in food products. These protocols are used to identify the raw materials to provide consumers with products corresponding to the actual claims of purity and origin. These protocols are used on a routine basis to detect the presence of variety adulteration at the gates of the factory. This approach provides to consumers a product with a high quality that meets the criteria of purity and origin claimed by the manufacturer.

MATERIALS AND METHODS

Reference Material

Green coffee beans samples used in this study were provided by Nespresso. A main part of them are coming from commercial batches from Brazil.

DNA extraction of green coffee seeds

90 green beans per sample are individually and finely cut, pieces of seeds are collected in collection micro tubes. The DNA extraction was performed with the Qiagen kit (DNeasy 96 Plant) following the recommendations of the supplier.

DNA extraction from commercial bean batches

For each batch, a sample of 1000 coffee beans is grinding. The DNA extraction was performed according.

Selection of SSR markers

Thirty microsatellites were used to build a genetic database on Arabica strategic varieties already identified by Nespresso (Bourbon Red and amarello, Kona …). They have been selected according to their rate of polymorphism in C. arabica varieties.

Microsatellite analysis on 3500 xL Genetic Analyzer

PCR Amplified DNA products were separated using capillary electrophoresis according to their sizes and to the fluorescent labelling. Experimental data were analysed using GeneMapper® software (Applied Biosystems).

High Resolution Melting (HRM)

HRM is a post PCR method able to detect the genetic variations (SNPs) in PCR amplicons. The amplification cycles were followed by a high resolution melting step.

Genotyping on Arabica varieties using Roche 454 sequencing

Roche protocol for DNA amplicon was used to perform this sequencing.

RESULTS AND DISCUSSION

The first step in this workflow was the creation of an Arabica database on a representative strategic set of varieties and origins. For example, some high-quality coffees are genotyped to ensure the authenticity and origin of the raw material used. Nespresso capsules as "Dulsão Do Brasil" are made from a single variety of Arabica coffee (Bourbon). This test is part of the quality control chain implemented on key raw material. This QC is effective on the purchase chain developed by Nespresso to secure the supply of the specific coffee "pure origin". This database is already used to enlarge the purchase clusters in Brazil to accredit new farms with the variety request.

DNA testing established are routinely used to control the Bourbon variety batches to detect and assess the rate of possible adulterations by other varieties of Arabica. Early approaches to

trace DNA are introduced from the use of microsatellites markers directly issued from the genotyping. The traceability analysis is built thanks to a large database of microsatellite markers (SSRs) that discriminates Arabica cultivars which are usually characterized by a low genetic diversity. Targeted varieties are identified by a unique and specific set of microsatellites. These selected SSRs will be used for the assessment of the desired variety and the rejection of batches when none expected Arabica cultivars are detected in the commercial sample. The tool can be used to check raw material entering in the factory but also to identify the coffee farms producing the desirable or un-desirable varieties. The traceability technology was improved through close collaboration with suppliers, who want to guarantee a constant supply using recommended Arabica cultivars. This PCR based technology is used to determine the genetic polymorphism in *C. arabica* varieties and, therefore, can be used in quality control especially for variety authentication. Due to the sensitivity and specificity of the method, the quality control test is becoming also a reality in processed foods. (Figure 1).

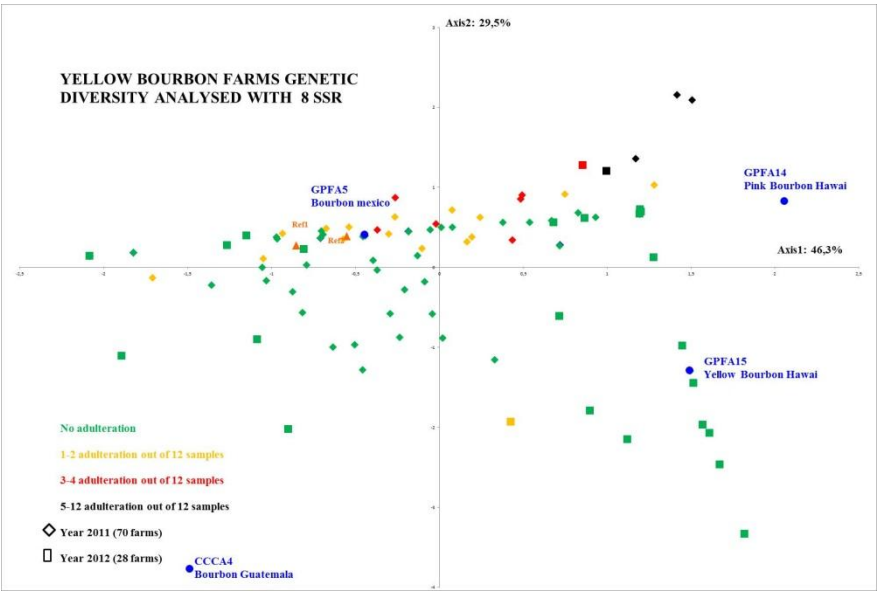
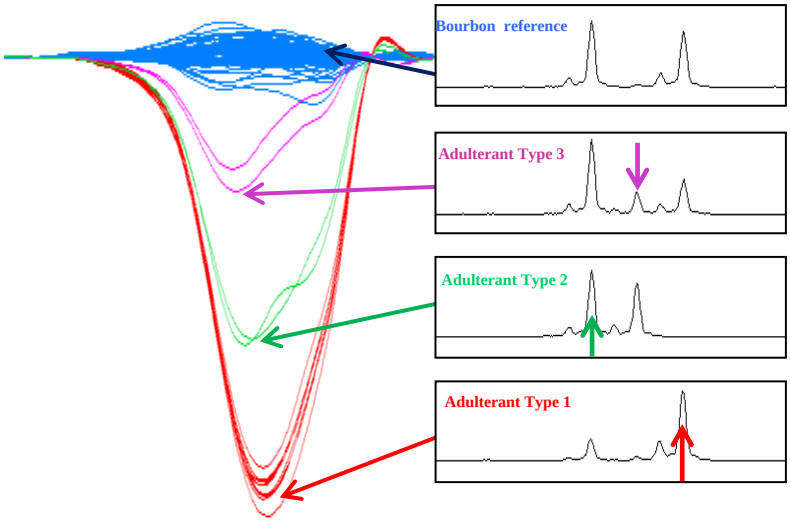


Figure 1. Principal Component Analysis using 8 SSR markers. 65 farms were tested. This figure shows the diversity found in different farms tested for the Quality Control developed for Nespresso. This test manages the selection of coffee farms producing the coffee variety claimed by Nespresso.

However, SSR technology has some weaknesses, such as slow analysis, costs and especially the low representation area of the Arabica genome studied. A major technological breakthrough has enabled the transfer of this method to the use of a new technology, HRM, which is also used in quality control in grape and olive origin. HRM allows gaining speed and accuracy of analysis. HRM is a technology that can detect with high sensitivity the slightest variation in a studied sequence. This high specificity led to the detection of new adulterants in bourbon arabica variety analysed for Nespresso. This method has allowed us to create, to develop and to use a specific DNA traceability test in routine. It enables to assess and to maintain the quality of coffee lots purchased by Nespresso among different suppliers. This confidence in the quality of batches gives to consumers a guarantee to the quality of the coffee consumed each day. HRM provides access to the genetic characterization and differentiation of varieties of Arabica with a high level of reliability using rapid tests and cheap (Figure 2).

New systems or platforms developed for high-throughput sequencing DNA have been also introduced in the QC workflow. The use of these technologies provides a much larger amount

of genetic information about the origin and characterization of a given Arabica variety. This technology also allows to better manage the quantitative aspect, essential in DNA traceability tests developed by our laboratory especially for the determination of the amount of adulterants in batches analyzed. Instead of studying few specific polymorphism of a given sequence, NSG technology can manage several thousand SNPs characteristics of a given variety and therefore be much more informative on a whole genome view. In the future, the genomic data accumulated by this type of technology could be used on a global quality control system to assess either adulteration and purity rate in a commercial Coffee batches. NSG authorizes the exhaustive detection of variants and will study the whole genome of Arabica varieties. It will be possible to record the main commercial Arabica varieties origins in relation to a reference Arabica core sequence and record the different polymorphisms (SNPs, DIPs…) present in these varieties. One of perspective related to the use of this technology is to create new tools for traceability more efficient, easy and cheap, combining in a single analysis all genomic information useful to be managed for QC in Arabica varieties.



CAAGGGTAAAACTGTCCAAGAAGGAAAGTGGGGGAAATGTTTGTCGTGATCATCAATGTCGTGATCGGGAGCTTTTCTGGCAAATTCCTGCCTT Reference
CAAGGGTAAAACTGTCCAAGAAGGAAAGTGGGGGAAATGTTTGTCGCGATCATCAATGTCGTGATCGGGAGCTTTTCTGG CAAATTCCTGCCTT Adulterant 1
CAAGGGTAAAACTGTCCAAGAAGGAAAGTGGGGGAAATGTTTGTCGCGATCATCAATGTCGTGATCGGGAGCTTTTCTGG CAAATTCCTGCCTT Adulterant 2
CAAGGGTAAAACTGTCCAAGAAGGAAAGTGGGGGAAATGTTTGTCGTGATCATCAATGTCGTGATCGGGAGCTTTTCTGG CAAATTCCTGCCTT Adulterant 3

ATTCACTCATTAATTTCCAATGTAACGTTCTAAATC*TCACACACACACACACACAC*----------GCAGAGAGCACCGCCAATTCTTCCTATAAAA_ Reference
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ATTCACTCATTAATTTCCAATGTAACGTTCTAAATC*TCACACACACACACACAC*----------GCAGAGAGCACCGCCAATTCTTCCTATAAAA Adulterant 3

Figure 2. HRM technology. HRM analysis used in routine test in order to detect adulteration in coffee batches. This technology gave a reliable and fast tool in routine quality control. DNA sequencing of 3 adulterants and reference detected by HRM is given below.

In conclusion, DNA markers are increasingly used by the food industry in the field of traceability of raw materials. This allows offering to consumers a better guarantee on the *Arabica* supply chain.

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Mass Propagation of Coffee Plantlets to Increase the Sustainability of Robusta Coffee Production within the Nescafé Plan

P. BROUN[1], B. TERRIER[1], C. LAMBOT[1], J. P. DUCOS[1], D. BRETON[1], J. ROJAS[2], C. GARCIA-MARTINEZ[3], L. C. NAVARRO[3]

[1]Centre de Recherche et Développement Nestlé, Tours, France
[2]Grupo Nestlé México, Mexico City, Mexico
[3]Agromod S.A. de C. V., Chiapas, Mexico

SUMMARY

Improving the sustainability of coffee production requires the widespread adoption of good farming practices, as well as maximizing the production potential of coffee orchards.

The Nescafé Plan was launched by Nestlé in 2010, with the aim of increasing the sustainability of coffee from farm to cup. A major focus of the Plan is on sustainable coffee farming and the improvement of farmer income. An important part of our approach is to provide plantlets to farmers with high field yield and quality potential. Technical assistance is also given to farmers to help them realize the expected yield and quality benefits. Buying stations close to production areas also give the farmers the option to benefit from a shortened value chain and a transparent buying system that rewards quality.

The production of coffee trees on a large scale is a challenge that is met through complementary approaches, according to local constraints and expertise. This includes an accelerated propagation method *in vitro*, which has been developed and validated by Nestlé over the past 20 years. The plantlets produced are either destined directly to farmers or used for the establishment of clonal gardens.

In Mexico, Nestlé has partnered with Agromod, a company specialist of *in vitro* plant production. The transfer of our accelerated propagation technology allows the production of millions of embryos and plantlets *in situ*, thereby shortening the distance between the laboratory and the farmer.

INTRODUCTION

Robusta coffee production is levelling off or declining in several producing countries in the face of technical and economic challenges: declining farm productivity, economic pressure on coffee farming, strong market price fluctuations and competing crops with higher profitability. Farm productivity is strongly dependent on the use of appropriate farm practices and also on the production potential of coffee trees. The ageing of coffee orchards, together with poor genetics strongly limit this potential.

A major focus of the Nescafé Plan, launched in 2010, is on agriculture. It builds on years of Plant Science expertise and has the objective to improve coffee farmers' income through technical assistance and the distribution of higher yielding coffee plantlets, in order to increase yields and coffee quality.

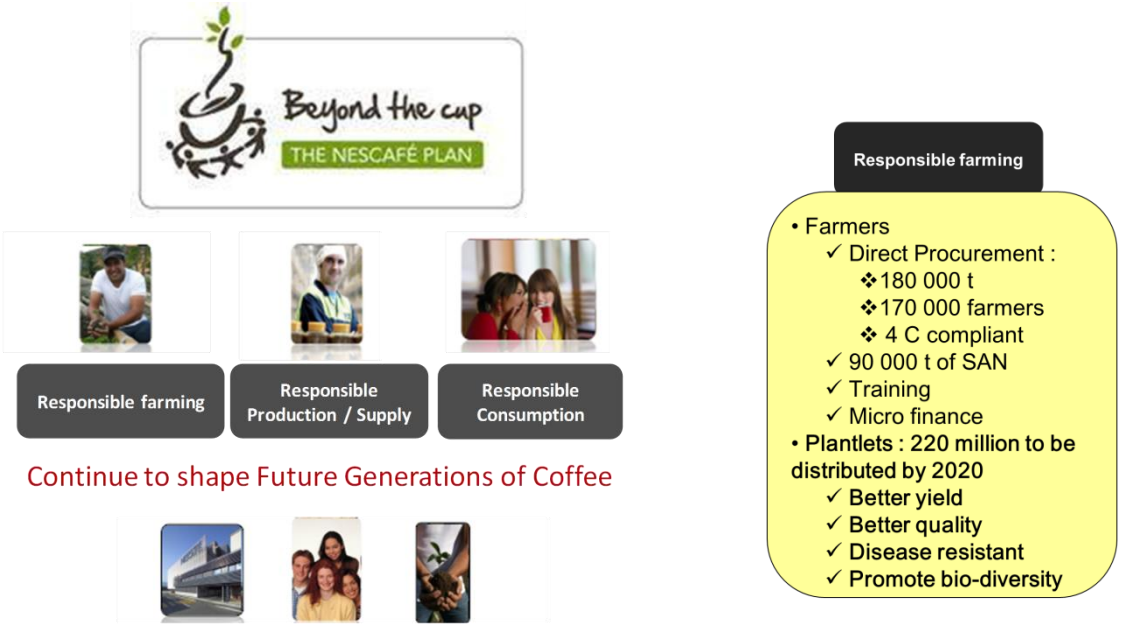

Figure 1. The Nescafé Plan promotes sustainable coffee production and the provision of high-yielding trees to farmers.

RESULTS AND CONCLUSIONS

We are taking a balanced tree propagation approach that is adapted to the type of genetic material that is locally available, as well as to local expertise and infrastructure. This includes seed, rooted cuttings, grafted plants and accelerated propagation *in vitro*.

The propagation approach is a two-step process. First, coffee trees are validated locally to guarantee a better performance to farmers. In a second step, mass propagation of coffee plantlets is performed.

Accelerated propagation, which builds on our expertise of somatic embryogenesis, allows to rapidly make available millions of trees from limited material. This method has been optimized to limit *in vitro* steps; today, a single operator can produce up to 1.5 million embryos in a year. The average embryo to plantlet conversion rate varies country to country and can reach 80% in the best cases. Under these conditions, the method allows the production of millions of embryos from limited material within 2 years at a cost that is competitive with conventional techniques.

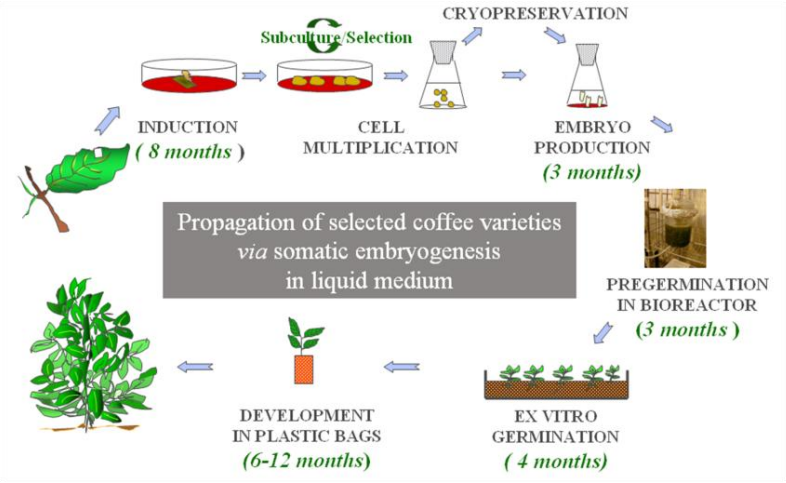

Figure 2. Accelerated Propagation, which is based on somatic embyogenesis, is a major component of ourRobusta propagation strategy: within 2 years, millions of plants can be produced from limited starting materials.

The technique is currently applied to a pipeline of validated varieties, bred in our experimental stations or in partner national institutes. The selection process includes the evaluation of the varieties for several key characteristics including yield, disease resistance, bean size and cup quality.

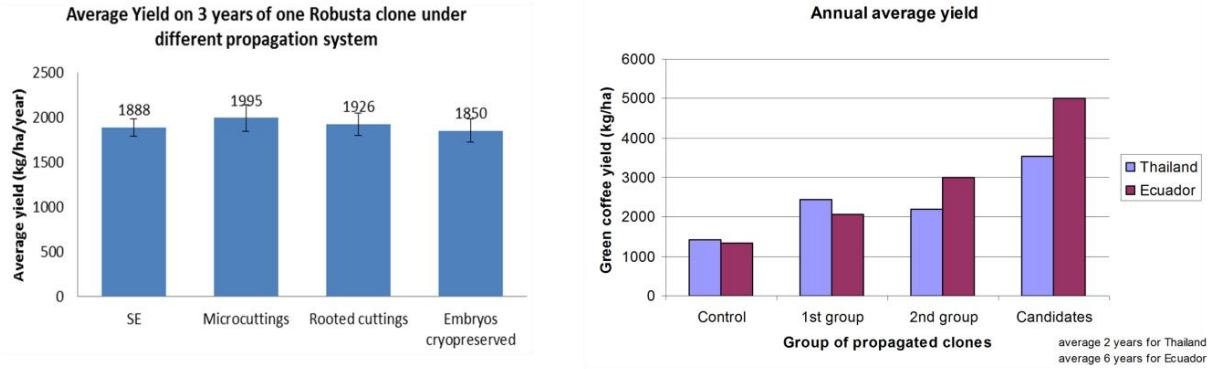

Figure 3. Accelerated propagation produces trees that are true to type; our propagation approach leverages on our breeding program to deliver increacing field performance.

Nestlé is actively transferring its propagation know-how to producing countries, for example to our partner Agromod in Mexico, which is building a large propagation capacity. Beyond Mexico, the accelerated propagation technique has been transferred to several National Institutes, for example in Vietnam, Indonesia, Thailand and Uganda. Technology transfer is also underway to our facility in Abidjan.

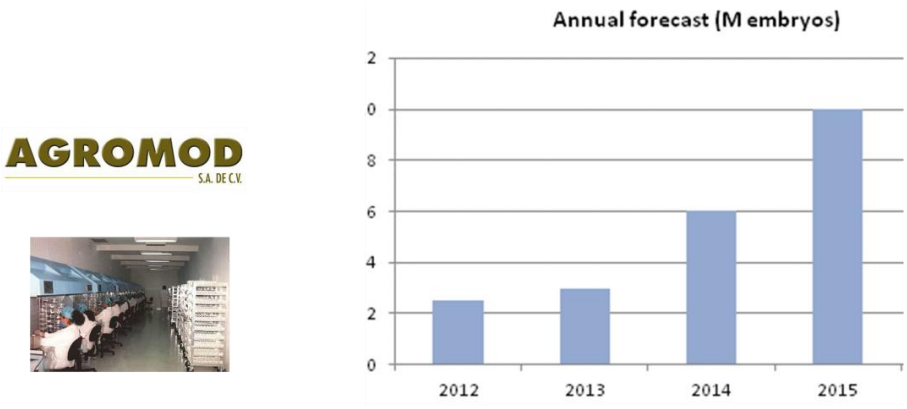

Figure 4. In order to produce closer to farmers, Nestlé is transferring the Accelerated Propagation technologies to producing countries. Agromod, our partner in Mexico, is developing the capacity to produce 10 million embryos per year.

Accelerated propagation is mostly applied to the multiplication of Robusta clones but will also be applicable to our Arabica hybrids, as we are progressing towards the Nescafé Plan goal of distributing 220 million trees by 2020.

The first results of our propagation program indicate clear benefits to our growers. This, however, necessitates the sustained use of good farming practices, which we are advocating through our technical assistance program.

While the challenge is clear, our first positive results indicate that the Nescafé Plan approach has a strong potential to help improve the sustainability of coffee production for the years to come.

Lipid Transfer Proteins in Coffee: Isolation of a Coffea Orthologs, Coffea arabica Homeologs, Expression during Coffee Fruit Development and Promoter Analysis in Transgenic Tobacco Plants

M. G. COTTA[1], L. M. G. BARROS[2], J. D. DE ALMEIDA[2], R. H. SANTANA[3], E. A. BARBOSA[2], G. S. C. ALVES[1], L. V. PAIVA[1], M. CARNEIRO[2], A. C. ANDRADE[2], P. MARRACCINI[2,4]

[1]University of Lavras, Lavras, MG, BRA
[2]Embrapa Genetic Resources and Biotechnology, Brasilia, DF, BRA
[3]University of Brasilia, Brasilia, DF, BRA
[4]CIRAD UMR AGAP, Montpellier, FRA

SUMMARY

Our study aims (i) to identify different coffee *nsLTPs* gene homeologs corresponding to the *Coffea arabica* (*Ca*) ancestor sub-genomes: *C. canephora* (*Cc*) and *C. eugenioides* (*Ce*); (ii) to evaluate the expression of these genes during the bean development and (iii) to characterize a grain-specific *nsLTP* promoter. We cloned three nsLTP-encoding cDNA highly expressed in coffee fruits. By RT-qPCR assays, the expression of each *nsLTP* genes carried by the *Cc* and *Ce* sub-genomes of *Ca* was also analyzed. The results highlighted the preferential expression of these genes in endosperm at 120 and 180 days after flowering (DAF). A *nsLTP* promoter region (1.2 kb) was also isolated and analyzed by 5' deletions for the ability to control the expression of the *uidA* reporter gene in transgenic *Nicotiana tabacum* plants. The GUS histochemical assays showed that 1.2 kb, 1.0 kb and 0.8 kb fragments of the *nsLTP* promoter directed the *uidA* expression to all tested plant organs with different activity levels while low or null expression was detected in roots. However, the 0.4kb fragment led to the expression of the *uidA* gene only in seeds, fruit and floral buds.

INTRODUCTION

Lipid-transfer proteins (LTPs) are characterized by their ability to bind fatty acids and to transfer in vitro lipids (e.g. phospholipids, cholesterol) between membranes. Because LTPs can associate with various phospholipids with broad specificity, these proteins are referred as nsLTPs for non-specific lipid transfer proteins. nsLTP have been purified from various plant tissues and are characterized by small molecular weights (usually ≈ 6.5 to 10 kDa) and basic isoelectric points (pI) ranging between 8.8 and 12. In addition to their role in mediating phospholipid transfer, nsLTP may also be involved in other biological functions as in plant defense activity against fungal and bacterial pathogens. Despite the fact that coffee is one of the most important agricultural commodities, basic knowledge is missing regarding many aspects of this crop particularly regarding lipid metabolism during bean development. Our study aims to evaluate the expression of nsLTP-encoding genes during the bean development and to study the nsLTP promoter in transgenic tobacco by analyzing its ability to control the expression of the *uidA* reporter gene.

MATERIALS AND METHODS

Plant material

Eight-year-old plants of *C. arabica* cv. IAPAR59 (I59) were cultivated under field conditions at the experimental station of the Embrapa Cerrado (Planaltina- DF, Brazil) under full-sun condition. Fruits were collected regularly (every 4 weeks) after flowering up to complete maturation (210 days after flowering [DAF], harvest 2006/2007). After harvesting, the fruits were immediately frozen in liquid nitrogen and stored at -80°C. Tobacco (*N. tabacum* L. cv. Xanthi XHFD8) was grown *in vitro* on solid MS medium.

Isolation of nsLTP-*encoding* cDNA and gene sequences

The *CaLTP1a*, *CaLTP2* and *CaLTP3a* cDNA sequences (Figure 1) were obtained by PCR using as a template 10 ng of a cDNA from entire fruits of *C. arabica* cv. I59 harvested at different time (30 to 210 DAF) of maturation. The PCR reaction was performed using the primer combinations LTP-F2/LTP-R2, LTP-F1/LTP-R2 and LTP-F1/LTP-R1 and *Taq* Platinum DNA polymerase under the following conditions: 94°C, 2min; followed by 40 cycles of 94°C, 30s; Ta 60°C, 30s; and 72°C, 3min. For each PCR reaction, the fragments were double-strand sequenced with the universal primers and the BigDye Terminator Kit v3.1 chemistry (ABI 3130*xl* Genetic Analyzer, Applied Biosystems).

RNA isolation and RT-qPCR assays

Total RNA was extracted from separated fruit tissues (perisperm and endosperm) as previously described. RT-qPCR assays were carried as previously described [4]. Gene expression levels were normalized (SDS 2.1 software) with the expression of reference gene ubiquitin (*UBI:* SGN-U347154) (Figure 2).

Cloning of nsLTP promoter

PCR reactions were carried out using 5 ng of genomic DNA of *C. arabica* cv. Catuaí Amarelo with forward primers (F1-pBI, F2-pBI, F3-pBI, F4-pBI) and reverse primer R1pBI and *Pfu* DNA polymerase, under the following conditions: 94°C, 1min; followed by 30 cycles of 94°C, 30s; 51°C, 30s and 72°C, 3 min. A *HindIII* restriction site was included in the 5'-end of all forward primers and a *BamHI* site in the 5'-end the reverse primer. PCR products were double-digested with *HindIII* and *BamHI* and further ligated into pBI121 (Clontech) vector previously cut by the same enzymes. Following ligation, the resulting vectors were obtained: pCaLTP-S (F4-pBI/R1-pBI: 451 bp), pCaLTP-M1 (F3-pBI/R1-pBI: 827bp), pCaLTP-M2 (F2-pBI/R1-pBI: 1047bp) and pCaLTP-L (F1-pBI/R1-pBI: 1252bp). For all these constructs, *LPT* promoter fragments were sequenced (Figure 3).

Genetic transformation of tobacco plants and histochemical GUS assays

The pCaLTP vectors (as well as the positive vector pBI121) were introduced separately into the disarmed strain of *A. tumefaciens* and transferred by genetic transformation in *N. tabacum*. After transformation, T0 transformants were regenerated for each construct and analyzed by histochemical GUS assay. Samples of different organs were incubated in 1mM X-gluc (5-bromo-4-choloro-3-indolyl-β-glucuronic acid) solution at 37°C overnight for blue color development and then treated by ethanol 70% to remove chlorophylls (Table 1).

RESULTS AND DISCUSSION

Nucleic alignments showed that *CaLTP1a* and *CaLTP2* cDNA sequences diverged by only 2 bases in the 5' UTR corresponding to the primer LTP-F1 and -F2 regions. The *CaLTP3a* cDNA was characterized by 15-bp changes in the nsLTP-coding regions compared to the LTP1 and LTP2-coding regions. In addition, the *LTP3* sequence also diverged from *LTP1-LTP2* by an insertion/deletion of 13bp in their 3' UTR regions (Figure 1). By sequencing corresponding genes of these cDNAs from *C. arabica* and *C. canephora* (data not shown), we deduced that *CaLTP1a* and *CaLTP2* corresponded to *nsLTP* genes carried by the *C. eugenioides* sub-genome of *C. arabica* (hereafter called *Ce*) and that *CaLTP3a* was carried by the *C. canephora* sub-genome of *C. arabica* (called *Cc*).

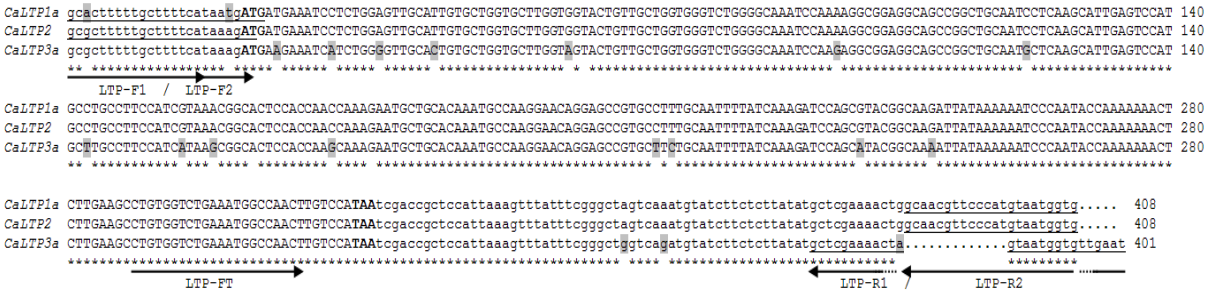

Figure 1. Alignment of coffee nsLTP-encoding cDNA sequences from *C. arabica.* The *CaLTP1a* was amplified with the primer pair LTP-F2/LTP-R2, the *CaLTP2* with LTP-F1/LTP-R2 and *CaLTP3a* with LTP-F1/LTP-R1. For each sequence, the nsLTP-coding sequences is in upper case, the 5' and 3' UTR regions in lower case and the start and stop codons in bold. The stars below the alignments indicate identical bases and the nucleotides are numbered on each lane (right). Nucleotides divergent between the sequences are boxed in grey. Horizontal arrows indicate primers used to amplify *LTP* cDNAs and to perform qPCR experiments.

Primer pairs LTP-FT/LTP-R1 and LTP-FT/LTP-R2 respectively specific for *Cc* and *Ce nsLTP* homeologous genes, were used in RT-qPCR experiments to analyze their expression individually in perisperm and endosperm fruit tissues of *C. arabica* cv. I59 harvested at regular stages of maturation. In perisperm, *Cc* and *Ce nsLTP* genes were expressed at 90 DAF but not at 60 DAF (Figure 2).

Figure 2

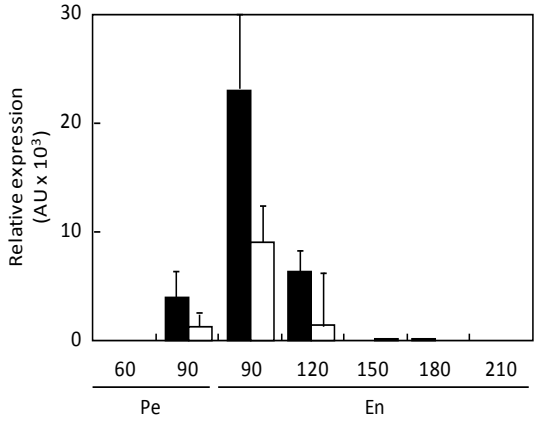

Figure 3

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CATCCTAAAATACATTCGAAAAACATTTAATTCAAACTGACATTTACTGTAAAAATTGTAACATATATCG   70
      F1pBI
CAACAACAACAAATCAAGGGCTTGTTTGGATAGCCATTTTCATCGAAAAATGACCTTGTTTTCCGTGAAC  140
ACATTCCCCTATCACCTTTTTACCTCACATACATCAAATCGCTACAGTAATTTTCCAACGAAAAATCCAT  210
GAAAAATGCAATCCAAACATAACCCAAAGTTTTCGGAAAAAGCATCTTCGAAAGTATCTAATCTACGCTG  280
      F2pBI
AGGCGTAATTTTGTACTCTCTGTACTAGTTAGTTTATGGATTTTAATCTTCGCCAAAAATATAAAAGCAT  350
TTCGTTTAATTAGAAACAGTAACAAACTGATAATTGGAGTGTGAGTTTTTTCTAGAAAAAAAATTAAAAT  420
ATTTTCCAAGACATTATTAATGATCTTTTTATTTCACGGATATCATATTGTTACGGTATATTTTTAACAA  490
          F3pBI
AATTACAATCTGAATGGGAAATTTTGCTTAAAGATTAACTCCTACTTCTCAAAACATTGATAACGTCCAT  560
TGTTGATTCACCCATGAGGATTAAGGAAAATTTTGCTTAAAAATTAACTCCAATTTCCTTAAAGATTAGC  630
TCCCACTTCTCAAAACTTGGATAACGTCCGTTGCTGATTCAGTCACGAGGATTAAGGAAAATTTTGCTTA  700
AAGATTAACGACTGCATCTCAAACTTGGATAACGTCCGTTACTGGGTCACTCATGAGGATTAATTAGCTT  770
TTGGCTTTTTGTGTTTCTGGATAAAGATTAACTCCCACTTCTCAAAACTTGGATAACGTCCGTTGCTGAT  840
                                F4pBI
TCAATCATGAGGATTAATTAGCTTCTGGCTTTTTTTGTGTTTCTGGGAAATTTTGCTTAAAGATTAACTT  910
CCACTTCTCAAAACTTGGATAACGTCCGTTGCTGATTCACTCATGAGGATTAATTAGCTTCTGGCTTTTT  980
GTGTTTCGGGACGTTTTTTCTTTTGTTTTTTCCCGGTGATTTGTTGGAAAGCAATTACTCTGCTTTGTAT 1050
CTTTCTCATTTTTGGCCGAACAAGTGAATGGGACACTACGCGTTATTGGCCCTCTTATTCACTGATTCAT 1120
GAGATCCTCGAGAGCCAATGCCCGCTATCTACAACTATAAATGCACTAATTAGCAGAGCAAAATTTTCAG 1190
GAAACAGTCGAAGCCATCTACAGAATTTCATTTAACTTTCTCTTCTGCACTTTTTGCTTTTC          1252
                                                          R1pBI
```

Figure 2. Expression nsLTP-encoding genes during coffee fruit development analyzed by RT-qPCR. Expression of *Cc* (black isobars) and *Ce* (white isobars) *nsLTP* genes was analyzed using LTP-FT/LTP-R1 and LTP-FT/LTP-R2 primer pairs, respectively, in perisperm [Pe] and endosperm [En] from fruits of *C. arabica* cv. I59. For each tissue, days after flowering (DAF) are indicated. The relative expression was measured using the *UBI* as a reference gene. The 60 DAF-perisperm samples was used as internal calibrator. Values are the mean of at least three technical repetitions ±SD.

Figure 3. Complete sequence of the nsLTP promoter region. Nucleotide numbers are indicated for each lane. The TATA box is double underlined. The primers used to construct pCaLTP vectors are boxed in grey.

In the endosperm, expression of both *Cc* and *Ce nsLTP* genes was high at 90 DAF and decreased at 120 DAF to be undetectable in the latest stages of maturation. It is worth noting that in perisperm and endosperm, *Cc* expression was always higher (3 to 4 fold) than expression of *Ce nsLTP* genes.

The four constructions of the nsLTP promoter were introduced separately into *N. tabacum*. Several T0 transformants were regenerated and used to perform histochemical assays by checking β-glucuronidase (GUS) activities in different tissues (Table). In tobacco plants transformed by pCaLTP-S (shorter fragment of nsLTP promoter), staining was observed in isolated mature seeds, but not in immature seeds, leaves, roots, petals and other flower tissues. In tobacco plants transformed by pCaLTP-M1, GUS activity was detected in placental (inner) tissue of fruits but also weakly in seeds. Slight staining also occurred in leaves but not in roots and flowers organs. In tobacco transformed by pCaLTP-M2, GUS staining was detected in leaves and also in isolated seeds but not in the capsule and flowers organs. In tobacco transformed by pCaLTP-L, GUS activity was observed in immature seeds and placental tissue of the capsule, in mature seeds as well as pistil style and weakly in leaves but not in roots, petals and stamens. As a positive control, GUS activity was highly detected in all tissues of plants transformed by the pBI121 vector. One the other hand, all tissues of untransformed tobacco plants remain unstained (negative control).

Table 1. The table summarizes the results of GUS histochemical staining obtained for each pCaLTP constructs. In transgenic tobacco plants. (2) unripe capsule (3) isolated seeds.(-) no expression, (+) low, (++) medium, (+++) high and (++++) very high GUS staining. The vectors are pCaLTP-S (F4-pBI/R1-pBI: 451 bp), pCaLTP-M1 (F3-pBI/R1-pBI: 827bp), pCaLTP-M2 (F2-pBI/R1-pBI: 1047bp) and pCaLTP-L (F1-pBI/R1-pBI: 1252bp).

	root	leaf	fruit[2]	bean[3]	stamen	petal	pistil
pCaLTP-S	-	-	-	++	-	-	-
pCaLTP-M1	-	++	++	+	-	-	-
pCaLTP-M2	-	++	-	+	-	-	-
pCaLTP-L	-	+	+	+	-	-	+
pBI121	+++	++++	++++	++++	+++	+++	++++
NT	-	-	-	-	-	-	-

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Impacts of Nanotechnology in the Brazilian Coffee Industry

F. M. M. BLISKA, C. L. R. VEGRO, P. R. MARTINS, C. FACCHINI

Centro de Café 'Alcides Carvalho', Instituto Agronômico, Av. Barão de Itapura, 1421, Campinas, Brazil, Postal Box 28, 13012-970

SUMMARY

Nanoscience and nanotechnology have been appointed as the basis for a new revolution, but this time not only industrial, but a revolution in the nature of the human civilization itself. The development of new materials, as well as changes in manufacturing platforms, will result in social, environmental and economic ever experienced. The exponential growth of investments in research in this area, especially in the central countries, shows the immense interest in the use of those technologies, although it is still only an infant sector. On the other hand, growing distrust of many sectors of public opinion, which tend, as has happened with genetically modified foods, to reject these innovations with a character so disruptor. Therefore, it is important to develop research to provide the public debate on nanotechnology, in order to engage segments of society. Because, just as occurred with biotechnology, there is a broader understanding of the likely benefits and risks of technologies derived from nanoscience, going beyond the information presented by the media, which are guided generally by the sensationalism that the subject entails. The overall goal of this research is to identify the social, environmental and economic impacts, actual and potential, arising from the introduction of nanotechnology in the coffee industry in Brazil. In this paper we present the method developed to evaluate those indicators, and to establish a reference for public policies on employment, labor, income, education, professional training and health; to support the regional development programs; and to indicate corrective and preventive actions.

INTRODUCTION

Nanotechnology is the manipulation of matter at the atomic and molecular scales. It has often been suggested as the basis of a new scientific revolution. The largest investments in this area are concentrated in the central countries. The global market for products and processes that include nanotechnology components may reach a value of $ 1 trillion over the next 10 years. The establishment of the network BrasilNano, in 2004 and the launch of the National Nanotechnology Program, in 2005, among others, are the landmarks of the initiatives in this branch of knowledge in Brazil. Statistics indicated that by 2009, 258 researchers, 77 institutions of research and development (R & D) and 13 companies were active in this area of knowledge, produced 991 scientific technical articles and generated 97 patents in Brazil. Research led by FIRJAN found that in 2010 the Brazilian market for products based on nanotechnologies originally developed in the country, recorded approximately R $ 115 million in business. The ultimate aim of this study is to identify opportunities for innovation in nanotechnology and its risks, for the production and processing chain of coffee in Brazil, about the social, environmental and economic aspects. This article deals the first phase of this study: the development of the method to analyze those impacts.

MATERIALS AND METHODS

Delphi technique is used to identify key opportunities for the application of nanotechnologies in different segments of the coffee industry, identify and rank the main impacts of its use and describes the most prominent contributions that potentially could result from this science. This technique allows a qualitative approach, based on expert opinion, through a structured questionnaire. Agents specializing in nanotechnology from coffee production and industrialization chain represent the experts, in this study. Delphi consists in the submission by repeated rounds of questionnaires, in which the set of experts, anonymous to each other after the first round, receives a summary of the responses of the other participants, setting up exchange of information, to ensure consideration minority ideas, and facilitate freedom of expression and consensus building.

Therefore, we ask the cooperation of experts to answer the attached questionnaire, subdivided into two sets of information: 1) Processes and Services for the coffee chain, according to the condition of developing innovation (adopted or in research and development) and processes, services and potential nanotechnology products to the coffee chain; and 2) Risks involved in the development and use of nanotechnology and the need for regulation, of the impacts of these innovations in the production and processing of coffee in Brazil.

RESULTS AND DISCUSSION

Preliminary remarks points that the discussions based on products already developed and those that are being studied are more concerned about the "economic impacts" once Brazilian public opinion are not aware on the theme. In this case, the federal regulation plays an important role on the process. The point is that experts on regulation still do not have a consensus opinion on the subject. We hope that this study contribute to the diagnosis date, of the development of nanotechnology products in the coffee chain in Brazil, and to evaluate the environmental, social and economic impacts of these products. It is also expected to offer subsidies to the productive sector that allow or encourage the implementation or the (re) formulation of public policies focused on information Questionnaire of Impacts Evaluation.

INSTRUCTIONS

A - Process and service ADOPTED or process in RESEARCH and DEVELOPMENT

A.1 - Innovations in nanotechnology for application in coffee plantations

The respondent should be noted in Table A1, items about which he has knowledge about nanotechnology innovations that are being used in coffee plantations.

A.2 - Innovations in nanotechnology for application to other agents in the supply chain

The respondent should mark in Table A2, the items that have knowledge about nanotechnology innovations, which are being employed by economic agents in the coffee business from the gate of the production units, i.e. everything that does not relate to agriculture in itself: machinery and equipment, including irrigation; agroindustrial units beneficiation; industries of roasting and grinding and solubilization; food industry (sweets, biscuits); utilization of industrial by-products (sludge, bark and straw), packaging industry and other industries as : pharmaceuticals (caffeine extraction) and cosmetics.

B – POTENTIAL processes and services

The respondent should list in Table B, nanotechnological processes and services that considers possible to be developed for different segments of the coffee industry in the next 30 years, and have POTENTIAL usage, respectively within the next 10 or 30 years.

C - RISK adoption of nanotechnology products and processes

Possibly science is the most complex, powerful and influential of contemporary institutions. Over the centuries scientific knowledge has gone through transformations, reaching the current stage the hyper-complex techno-science, which seeks equip all dimensions of life.

The exchange of more complex and holistic approach, full of interactions tangible and intangible, for the deterministic scientific reductionism, can narrow the field of relevant concerns about the risks that innovations may occur.

It is known that nano-scale components have new properties of matter that can possibly adduce yet unknown risks. Thus, this item seeks to ascertain how the theme of inherent risks (as an intervention over the world), since the incremental nanotechnology to break nanotechnology is being studied and the validity and / or interest in the adoption of the precautionary principle.

The respondent must select the scale of impacts presented in Table C, the intensity of the real or potential risks of the use of nanotechnologies in the coffee business, presented in the same framework, the items C1 to C9. And in C10, he should add, based on their knowledge, additional examples of real or potential risks arising from the use of nanotechnologies in the coffee business.

D - REGULATORY requirements, the diffusion of nanotechnology products and processes

The respondent must select the scale of importance shown in Table D, the degree of their agreement with the statements of items D1 to D7, concerning the regulation of the production and generation of nanotechnology products and processes in the coffee business. And he should add in D8, based on their knowledge, additional comments on these regulations.

E - Additional Comments: the respondent may express opinions about the topics covered in the questionnaire as a whole.

A1	Process and service adopted or process in research and development for coffee company
1	Improve the product (changes in the chemical or nutritional composition)
2	Promote changes in plant genomes (nanobiotechnology)
3	Analyzes the expression and regulation of genes of plants
4	Act directly on circulating hormones and antibiotics produced by plants (eliminating sprays)
5	Destined to soil management (desalination, removal of heavy metals)
6	For diagnosis of pests and diseases in plants incidents
7	Aimed at improving the efficiency of the use of fertilizers and agrochemicals (nanoencapsulation)
8	Intended for water management
9	For bioprocess (fermentation, production of enzymes, amino acids, vitamins, alcohols)
10	To post harvest (storage, transport logistics)

11	To monitor the identity and quality of coffees (certificates of origin, gourmet)
12	For precision agriculture (sensors, chips, nanotransmissores)
13	Others

A2	**Innovations in nanotechnology for application to other agents in the supply chain**
1	Use of electronic tongue or nose
2	To improve the roasting, grinding and solubilization (nano computing, increasing the accuracy of processes)
3	For the purification of water (waste treatment and water reuse)
4	Oriented to the slow release of nutraceutical compounds
5	Microencapsulation of additives in processed coffees (Toasted, Roasted and Ground and Soluble)
6	Deodorization (e.g. antimicrobial and antifungal)
7	Nano antimicrobial agents or adhesives in packaging lines (release of chemicals)
8	Packages with nanomaterials mechanically stronger, better heat and which indicate the conditions of consumption
9	Others

B	**Potential nanotechnological processes and services for the coffee and the other actors in the production chain**		
	Tipo de aplicação da inovação	*Adoption within the next 10 years*	*Adoption within the next 30 years*
1		( )	( )
...		( )	( )

C	**Real or potential risks of nanotechnologies in the coffee business**
1	Nanoparticles aggregate to the product / process cause toxicity in plants, animals and humans (by inhalation, ingestion, skin penetration)
2	Nanoparticles aggregated to product / process be inducing mutagenic processes or affect the process of DNA replication in humans and animals (by inhalation, ingestion, skin penetration)
3	Risks to the health and safety of workers in the handling of products containing nanometeriais (mixtures of grout defensive nanoencapsulation nanoparticles)
4	Risk of contamination of the environment with waste nanoparticles and nanotechnology
5	Tools and laboratory methods available for risk assessment of nanoparticles are sufficient for measures them
6	There is transparency in research and development of nanotechnology innovations and products for the market (military weaponry, absence of labeling, disclosure confident about their safety)
7	There is risk of nanotechnologies promote a breakdown of relevant industries in the country's productive matrix (bottom-up path, reduced wealth, unemployment, increased external dependence)
8	The field of nanoscience techniques (public or private) can extend socioeconomic inequality between nations.
9	Further examples:

D	**Regulation of production and generation of nanotechnology products and processes in the coffee business**
1	The current legislation is sufficient to control / monitor initiatives in nanoscience (direct consumer, environmental, labor and civil liability)
2	The legislation concerning the intellectual property right is in proper mechanism to the demands of nanotechnology development
3	International standardization proposed by Technical Committee ISO / TC 229 - Nanotechnologies is necessary and sufficient to allow safe advancement of this scientific field
4	There is enough structure to regulation in nanosciences, nanotechnologies, nanomaterials and nanodevices
5	The precautionary principle should be understood as: a) the adoption of proactive actions to protect the health of people and ecosystems, and b) the absence of evidence can not be taken as evidence of absence
6	Financing structures and appropriations to public research in nanosciences and development of nanomaterials should be managed by collegiate bodies composed of representatives of civil society
7	Accounting for liabilities incurred with respect to the production of nanomaterials, nanoparticles and nanodevices (ecotoxicity, negative energy balance, costs for collection and treatment of waste, damage to human health) is inadequate or partial.
8	Other examples:

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Do Roasting Conditions Affect Consumer Liking For Coffee Beverages?

R. DELIZA[1], J. C. SÁ FERREIRA[1], C. T. G. B. MATTOS[1], G. ARES[2], A. FARAH[3]

[1]Embrapa Agroindústria de Alimentos, Av. das Américas, 29501 CEP 23020-470 Rio de Janeiro – RJ, Brazil
[2]Facultad de Química, Universidad de la República, Montevideo, Uruguay
[3]Instituto de Nutrição, UFRJ, Rio de Janeiro – RJ, Brazil

SUMMARY

This study aimed at investigating the effect of roasting coffee conditions (temperature gradient and time) on consumer liking of beverages, taking into account individual preferences. Brazilian good quality green Arabica coffee beans were roasted by varying the roasting temperature gradient and time in a semi-fluidized bed roaster (three speed conditions: slow, medium and fast), and the colour (moderately light and dark), yielding six different types of beans, which were ground for preparing the coffee beverages. Fifty-seven coffee drinkers participated in the study in which the liking for the beverages was evaluated. Results from Preference Mapping showed that the first dimension separated samples by roasting colour, and moderately light coffee beverages were more liked by consumers, regardless of the roasting speed conditions. Three consumers segments were identified, with different coffee sample appreciation. However, most of them (segments 2 and 3, 67% of participants) didn't like dark coffee roasted in all speed conditions. The present results demonstrate that consumers were able to discriminate coffees roasted at different conditions, and that preferences varied considerably among them. Characterizing segments may be a valuable tool in order to produce targeted products for specific types of consumers. Further studies should also investigate more thoroughly the reasons for a determinate roasting degree preference.

INTRODUCTION

Brazil is the first coffee producer and the second world market consumer of the beverage with potential for an increasing consumption in the near future. Coffee consumers are becoming more demanding in terms of beverage quality, and, as a consequence, the industry has to optimize coffee processing to deliver products that meet consumer expectations, in addition to being healthy. Coffee roasting is a relevant step for the development of coffee flavour and aroma due to numerous chemical changes during the process and formation of compounds which are related to beverage quality. Nevertheless, few studies have been published focusing on the effect of roasting conditions on sensory attributes or even on consumer acceptance. A study using consumers developed by Mendes, Menezes & Silva investigated the optimization of roasting conditions of Robusta coffee using a laboratory scale electric rotary drum roaster, PROBAT-WERKE type RE 1, and suggested the optimum range for roasting as 22-28 min at a temperature of 225-230° C. No studies of this kind have been performed for Arabica coffee. As roasting has a considerable effect on the beverage sensory characteristics, it will impact on consumer liking. Therefore, investigating consumer liking is a matter of recognized importance in the development and optimization of coffee beverages because it plays an important role when one chooses and buys such product. This study aimed at investigating the effect of coffee roasting conditions on consumer liking of Arabica coffee beverages, taking into account individual preferences.

MATERIALS AND METHODS

Brazilian good quality green Arabica coffee beans were roasted in a plant scale semi-fluidized bed roaster (Lilla, OPUS 500 third generation, São Paulo, Brazil) by varying the roasting speed condition (slow, medium, and fast) and colour (moderately light and dark), yielding six different roasted coffee beans. Samples were ground in a discos grinder (Gourmet M-50, LEOGAP Ind. e Com. de Máquinas, Curitiba, PR, Brasil, grid # 6). Coffee beverages were prepared at 10% (weight/volume), i.e. 100 g coffee powder with 1 L mineral water in electrical coffee makers, and kept up to 20 min at 68˚ ± 2˚C; after this time they were discharged. Although it has been previously reported that some brewed coffee sensory attributes changed after 45 min, we decided to avoid any possible alteration, and use a fresher beverage.

Fifty-seven coffee consumers were invited to participate. They were employees or trainees at Embrapa Food Technology, Rio de Janeiro, Brazil and consumed at least one cup of black coffee a day. They evaluated samples regarding acceptance using 9-point hedonic scales varying from 1: "disliked extremely" to 9: "liked extremely". Samples were monadically presented in 50mL porcelain cups coded with three digit numbers, presented at 68°±1°C. They were evaluated in sensory booths under white light. Samples presentation order was balanced to prevent carry over effects, and water and biscuits were provided to participants as a cleanser between samples. Data were analyzed using ANOVA, Preference Mapping, and Cluster Analysis in XLSTAT (2011). Demographic and frequency of consumption data were also collected.

RESULTS AND DISCUSSION

The mean preference scores for the evaluated coffee beverages can be seen in Figure 1.

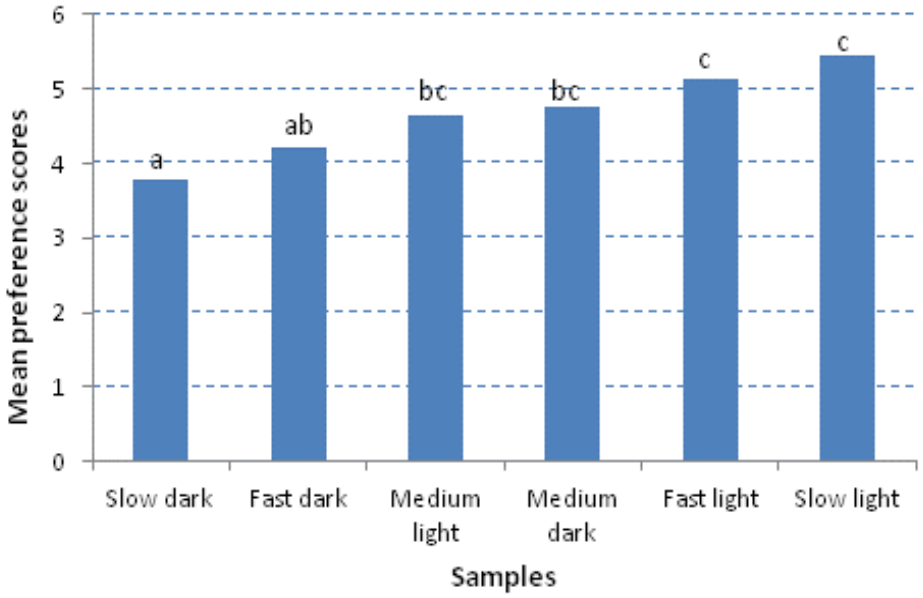

Figure 1. Means preference scores for the six coffee beverages. Evaluated in 9-point hedonic scales, varying from 1: disliked extremely to 9: liked extremely. Different letters imply in significant difference between samples ($p<0.05$).

On average, most evaluated coffee beverages received low liking scores, possibly because participants were not used to gourmet coffees. Samples "Slow light" and "Fast light" were

preferred by consumers. Looking at individual data, it was possible to observe higher scores, revealing that many people liked some of the beverages. This result demonstrated the need for analysing data using multivariate analysis. Therefore, Preference Mapping was used and results can be seen in Figure 2.

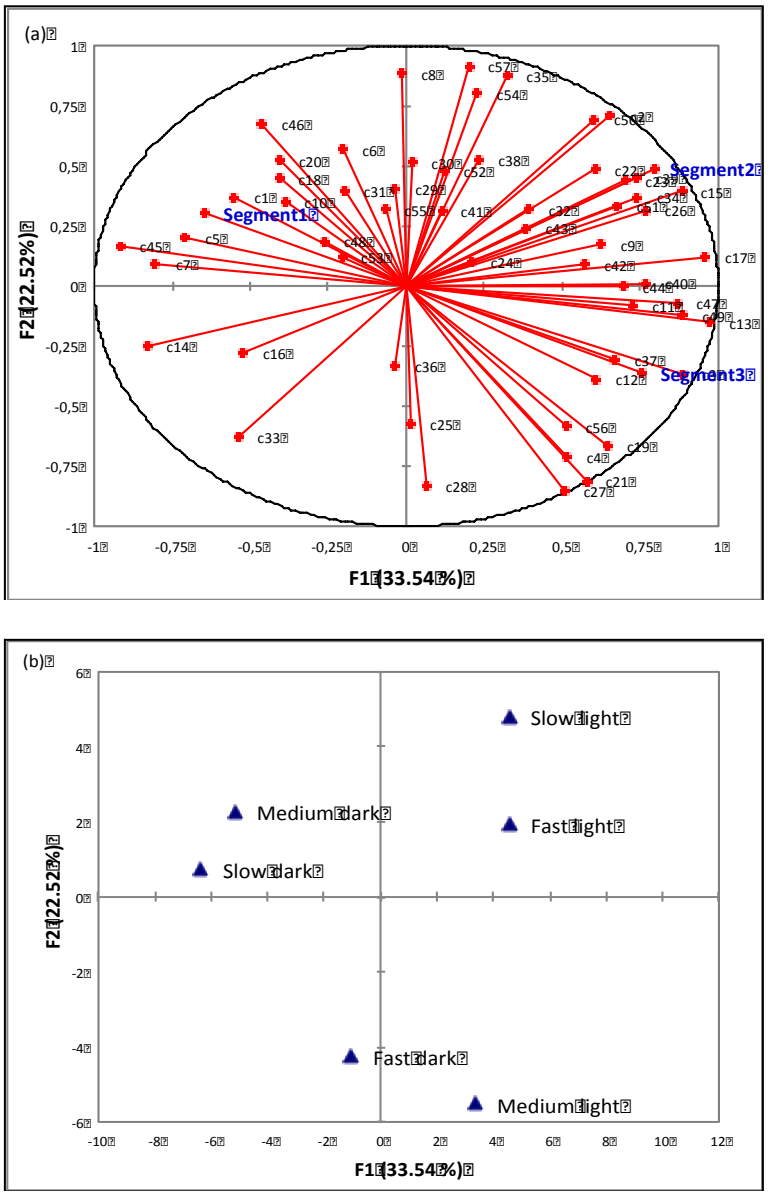

Figure 2. Preference mapping results for six beverages obtained from ground roast beans roasted at different conditions: (a) Consumers position, and the three identified segments; (b) Samples positions.

The first three dimensions of the PCA accounted for by 73.3% of the variance. The first dimension separated samples by roasting colour, and lighter coffee beverages were more liked by consumers, regardless of the speed conditions. Three segments of consumers were identified with different appreciation of samples. However, most of them (segments 2 and 3, 67% of participants) didn't like dark roasting in all speed conditions. Table 1 shows the mean liking scores by segment.

Table 1. Mean preference[1] scores (n=57) for six coffee beverages made from ground beans roasted at different conditions for the three segments of consumers.

	Segment 1 (n=19)	Segment 2 (n=25)	Segment 3 (n=13)
Fast light	4.1^{bcde}	6.5^{g}	3.9^{bcde}
Medium light	4.3^{cde}	4.9^{e}	4.7^{de}
Slow light	4.9^{ef}	6.1^{fg}	4.9^{ef}
Fast dark	5.0^{ef}	3.1^{abc}	5.1^{ef}
Medium dark	7.0^{g}	4.0^{bcde}	2.8^{ab}
Slow dark	4.9^{e}	3.7^{abcd}	2.4^{a}

[1]*Evaluated in 9-point hedonic scales, varying from 1: disliked extremely to 9: liked extremely. Different letters imply in significant difference among samples ($p<0.05$).*

Results from Table 1 allow us to conclude that roasting conditions (temperature and time) played a role on consumer's liking of the evaluated Arabica coffee beverages. Participants from segment 1 (n=19) preferred the medium dark beverage, and those in segment 2 (n=25) gave higher scores for the moderately light (fast and slow speed) samples. The latter didn't like the dark beverages regardless of the speed condition. On the other hand, no clear effect of coffee roasting conditions was observed for the segment 3 (n=13). People in this segment didn't really like any beverage, and only showed a slight appreciation for the fast dark beverage, suggesting that those consumers either do not drink coffee very often or are drinkers of bad quality brews.

Although further analyses should be carried out to look at the socio demographic variables and coffee consumption patterns of each segment, the segmentation of the present results demonstrate that consumers were able to discriminate coffees roasted at different roasting speeds. The characterization of segments may provide valuable information to industry regarding specific market niches allowing the development of marketing strategies to meet the adequate coffee and consumer.

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24ème Colloque Scientifique International sur le Café
San José (Costa Rica), 12 – 16 novembre 2012

List of participants

AUSTRALIA	
PUNYA-IN, SUTTHIDA	UNSW,AUSTRALIA PUNYAIN.S@GMAIL.COM
SRZEDNICKI,GEORGE	UNIVERSITY OF NSW G.SRZEDNICKI@UNSW.EDU.AU
NEILSON, JEFFREY	UNIVERSITY OF SYDNEY JEFFREY.NEILSON@SYDNEY.EDU.AU
MARSH,TONY	UNIVERSITY OF SYDNEY TONYMARSH@OZEMAIL.COM.AU
HILTON, BRIAN	WORLD VISION AUSTRALIA BRIAN.HILTON@WORLDVISION.COM.AU

AUSTRIA	
RIEDEL, ANNETT	UNIVERSITY OF VIENNA ANNETT.RIEDEL@UNIVIE.AC.AT

BELGIUM	
BOECKX, PASCAL	GHENT UNIVERSITY PASCAL.BOECKX@UGENT.BE

BRAZIL	
CAMARGO, GISELE ANNE	APTA-ITAL CAMARGO@ITAL.SP.GOV.BR
GUERREIRO FILHO, OLIVEIRO	IAC CAFÉ OLIVEIRO@IAC.SP.GOV.BR
REBELLES REIS, PAULO	EPAMIG SUL DE MINAS/ECOCENTRO PAULO.REBELLES@EPAMIG.UFLA.BR
DOS SANTOS, EDER RIBEIRO	COOPERATIVA REGIONAL DE CAFEICULTORES EM GUAXUPE CARMEN@COOXUPESANTOS.COM.BR
FERNANDES, LUIZ	SYNGENTA CROP PROTECTION LUIZ.FERNANDES@SYNGENTA.COM
LEITE, CLAUDIA	NESPRESSO CLAUDIA.LEITE@NESPRESSO.COM
PIMENTEL, GUSTAVO DUARTE	STATE UNIVERSITY OF CAMPINAS (UNICAMP) GUPIMENTEL@YAHOO.COM.BR
MACHADO DE OLIVEIRA, WALBER	FAZENDA SANTA RITA OLIVEIRAWALBER@HOTMAIL.COM
MARIN, FERNANDA	STOLLER DO BRASIL LTDA FERNANDABENTO@STOLLER.COM.BR
GASTALDO, RENATO	STOLLER DO BRASIL LTDA GASTALDO@STOLLER.COM.BR
DOS SANTOS, BORBA	OXIQUI_MICA AGROCIENCIA RAFAELBORBA.RP@HOTMAIL.COM
PARTELLI, FABIO LUIZ	UNIVERSIDADE FEDERAL DO ESPIRITO SANTO - UFES PARTELLI@YAHOO.COM.BR
DUTRA, MARCOS ROBERTO	SYNGENTA PROTECAO DE CULTIVOS LTDA MARCOS.DUTRA@SYNGENTA.COM
GONTIJO, IVONEY	UFES IVONEYGONTIJO@YAHOO.COM.BR
ARRUDA, NEUSA PETEIRA	INSTITUTO FEDERAL DO RIO DE JANEIRO NEUSA.ARRUDA@IFRJ.EDU.BR

LIVRAMENTO, KALYNKA	UFLA KALYNKAGABRIELLA@YAHOO.COM.BR
SALLES TREVISAN, MARIA TERESA	UNIVERSIDADE FEDERAL DO CEARA; IWCA-BRAZIL TREVISAN@UFC.BR
GHINI, RAQUEL	EMBRAPA ENVIRONMENT RAQUEL@CNPMA.EMBRAPA.BR
MAZZAFERA, PAULO	UNICAMP PMAZZA@UNICAMP.BR
FARAH, ADRIANA	UNIVERSIDADE FEDERAL DO RIO DE JANEIRO AFARAH@NUTRICAO.UFRJ.BR
NOGUEIRA TURCO, PATRICIA HELENA	APTA PATRICIA.TURCO@APTA.SP.GOV.BR
BOREM, ROSANGELA ALVES	FEDERAL UNIVERSITY OF LAVRAS TRISTAO@DBI.UFLA.BR
ALVES, JOSE DONIZETI	UNIVERSIDADE FEDERAL DE LAVRAS JDALVES@DBI.UFLA.BR
DELIZA, ROSIRES	EMBRAPA FOOD TECHNOLOGY RODELIZA@CTAA.EMBRAPA.BR
GOULART, PATRICIA DE FATIMA	CENTRO UNIVERSITARIO DE LAVRAS PAT.GOULART@HOTMAIL.COM
BLISKA, FLAVIA	INSTITUTO AGRONOMICO BLISKA@IAC.SP.GOV.BR
CERRI, CARLOS	UNIVERSITY OF SAO PAULO CERRI@CENA.USP.BR
SILVEIRA, JANE MARIA	APTA JANE@APTA.SP.GOV.BR
MEDINA FILHO, HERCULANO	INSTITUTO AGRONOMICO DE CAMPINAS MEDINA@IAC.SP.GOV.BR
BORDIGNON, RITA	INSTITUTO AGRONOMICO DE CAMPINAS RITA@IAC.SP.GOV.BR
DECARO, SERGIO	UNIVERSIDADE ESTADUAL PAULISTA SERGIOTDECARO@YAHOO.COM.BR
FERREIRA, MARCELO DA COSTA	UNESP - CAMPUS DE JABOTICABAL MDACOSTA@FCAV.UNESP.BR

LASMAR, OLINTO	FCAV/UNESP LASMAR84@YAHOO.COM.BR
SILVA, ROGERIO	EPAMIG ROGERIOSILVA@EPAMIG.UFLA.BR
ALCANTARA, ELIFAS	EPAMIG ELIFAS@EPAMIG.UFLA.BR
MONTEIRO DA SILVA, ORLANDO	UNIVERSIDADE FEDERAL DE VICOSA ODASILVA@UFV.BR
TEIXEIRA FERNANDES, ANDRE LUIS	UBERABA UNIVERSITY ANDRE.FERNANDES@UNIUBE.BR
VENZON, MADELAINE	EPAMIG VENZON@EPAMIG.UFV.BR
DAMATTA, FABIO	UNIVERSIDADE FEDERAL DE VICOSA FDAMATTA@UFV.BR
CHALFUN-JUNIOR, ANTONIO	UFLA CHALFUNJUNIOR@DBI.UFLA.BR
BOREM, FLAVIO	UNIVERSIDADE FEDERAL DE LAVRAS FLAVIOBOREM@DEG.UFLA.BR
MOURA, WALDENIA DE MELO	EPAMIG WALDENIA@EPAMIG.UFV.BR
DE LIMA, PAULO CESAR	EPAMIG PLIMA@EPAMIG.UFV.BR
CASANOVA TRETO, PEDRO	UNIVERSIDADE FEDERAL DE VICOSA PCASANOVA2000@GMAIL.COM
VIEIRA, HENRIQUE	UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE HENRIQUE@UENF.BR
CHARMETANT, PIERRE	CIRAD PIERRE.CHARMETANT@CIRAD.FR
PEREIRA, ROSEMARY	UNIVERSIDADE FEDERAL DE LAVRAS ROSEGFAP@DCA.UFLA.BR
PALLINI, ANGELO	FEDERAL UNIVERSITY OF VICOSA - UFV PALLINI@UFV.BR
ZAMBOLIM, LAERCIO	UNIVERSIDADE FEDERAL DE VICOSA - UFV ZAMBOLIM@UFV.BR

RIBEIRO, JULIANO	IFES - FEDERAL INSTITUTE OF ESPIRITO SANTO JULIANORIBEIRO@IFES.EDU.BR
BARRETO DA SILVA, MARCELO	UNIVERSIDADE FEDERAL DO ESPIRITO SANTO - UFES BARRETOFITO@UOL.COM.BR
TEIXEIRA, ALDIR	EXPERIMENTAL AGRICOLA DO BRASIL ALDIR.TEIXEIRA@ILLY.COM
FRANCA, ADRIANA	UNIVERSIDADE FEDERAL DE MINAS GERAIS DRISFRANCA@GMAIL.COM
PAIVA, RENATO	UNIVERSIDADE FEDERAL DE LAVRAS - UFLA RENPAIVA@DBI.UFLA.BR
PEREIRA PARREIRAS, SERGIO	INSTITUTO AGRONOMICO DE CAMPINAS - UFLA PEREIRACAFES@YAHOO.COM.BR
GOOD KITZBERGER, CINTIA SORANE	IAPAR - AGRICULTURAL INSTITUTE OF PARANA CINTIASORANE@YAHOO.COM.BR
SANTORO, PATRICIA	IAPAR - AGRICULTURAL INSTITUTE OF PARANA PATRICIASANTORO@IAPAR.BR
SERA, TUMORO	IAPAR - AGRICULTURAL INSTITUTE OF PARANA TSERA@IAPAR.BR
SERA, GUSTAVO	IAPAR - AGRICULTURAL INSTITUTE OF PARANA GUSTAVOSERA@IAPAR.BR
MENEGUIM, ANA MARIA	IAPAR - AGRICULTURAL INSTITUTE OF PARANA MENEGUIM@IAPAR.BR
SAGGION BERIAM, LUIS OTAVIO	INSTITUTO AGRONOMICO DE CAMPINAS BERIAM@BIOLOGICO.SP.GOV.BR
OLIVEIRA, LEANDRO	UNIVERSIDADE FEDERAL DE MINAS GERAIS LEANDRO.OLIVEIRA31@YAHOO.COM.BR
PAIVA, LUCIANO VILELA	UFLA LUCIANO@DQI.UFLA.BR
DOMINGUEZ, DOUGLAS	IAPAR - INSTITUTO AGRONOMICO DO PARANA DOUG@IAPAR.BR
ANDRADE, ALLAN	EMBRAPA ALAN.ANDRADE@EMBRAPA.BR
MARRACCINI, PIERRE	CIRAD UMR AGAP MARRACCINI@CIRAD.FR

VIEIRA, NATALIA GOMES	UFLA NATALIAEMBRAPA@YAHOO.COM.BR
COSTA, TATIANA SANTOS	UFLA TATIANA_ITASE@YAHOO.COM.BR
CARNEIRO FERNANDA	UCB FEARCA14@GMAIL.COM
LANI, JOSE ANTONIO	INCAPER JALANI@INCAPER.ES.GOV.BR
FERRAO, ROMARIO GAVA	INCAPER ROMARIO@INCAPER.ES.GOV.BR
PREZOTTI, LUIZ CARLOS	INCAPER PREZOTTI@INCAPER.ES.GOV.BR
OLIVEIRA, GABRIEL HENRIQUE	INSTITUTO FEDERAL DE BRASILIA GABRIEL.OLIVEIRA@IFB.EDU.BR
RAMOS ALVES, HELENA MARIA	EMBRAPA HELENA@EPAMIG.UFLA.BR
DE CARVALHO, CARLOS HENRIQUE	EMBRAPA CARLOS.EMBRAPA@UOL.COM.BR
TEIXEIRA CAIXETA, EVELINE	EMBRAPA CARLOS.EMBRAPA@UOL.COM.BR
CHQUILOFF VIEIRA , TATIANA GROSSI	EPAMIG PEREIRACAFES@YAHOO.COM.BR
RODRIGUES VIEIRA, ANTONIO	EPAMIG PEREIRACAFES@YAHOO.COM.BR
FAZUOLI, LUIZ CARLOS	CENTRO DE CAFE "ALCIDES CARVALHO" FAZUOLI@IAC.SP.GOV.BR
THOMAZIELLO, ROBERTO ANTONIO	CENTRO DE CAFE "ALCIDES CARVALHO" RTHOM@IAC.SP.GOV.BR
TOMA BRAGHINI, MASAKO	INSTITUTO AGRONOMICO DE CAMPINAS MAKO@IAC.SP.GOV.BR
SAATH, RENI	INSTITUTO AGRONOMICO DE CAMPINAS CAFE@IAC.SP.GOV.BR
SILVA GIOMO, GERSON	INSTITUTO AGRONOMICO DE CAMPINAS GSGIOMO@IAC.SP.GOV.BR

MORAIS OLIVEIRA, EDNA	EMBRAPA FOOD TECHNOLOGY EDNA@CTAA.EMBRAPA.BR
MOREIRA DA SILVA, FABIO	UFLA FAMSILVA@PRPG.UFLA.BR
ARAUJO DE LIMA, EDRIANA	UCB
VEIGA FRANCO DA ROSA, STELLA	EMBRAPA STTELA.ROSA@EMBRAPA.BR
SILVA DE ALMEIDA, JULIETA ANDREA	IAC JULIETASA@IAC.SP.GOV.BR
FERREIRA, JOAO	UNIVERSIDAD FEDERAL DE VICOSA JOAO.MARINHO@UFV.BR
AKIN, DEMET	ISTANBUL BILIM UNIVERSITY DEMETAKIN2000@YAHOO.COM
DE OLIVEIRA, CLEBER MOCEDO	UNIVERSIDAD FEDERAL DE VICOSA OLIVEIRAMACEDOCLEBER@GMAIL.COM
COSTA, HUGO ANDRADE	UNIVERSIDADE DE ESTADUAL SUDOESTE DA BAHIA

CAMEROON	
LEMA, ANDRÉ	CCIC NGABALEMA@YAHOO.FR

CANADA	
DYCK, KURT	SWISS WATER DECAFFEINATED COFFEE CO. KDYCK@SWISSWATER.COM

COLOMBIA	
SAMPER GARTNER, LUIS FERNANDO	FNC LUISFERNANDO.SAMPER@CAFEDECOLOMBIA.COM
QUINTERO. MONICA	COLCAFE MQUINTERO@COLCAFE.COM.CO
TABORDA, NELSON	COLCAFE NTABORDA@COLCAFE.COM.CO
OVALLE, ORIANA	CIAT ORIANA.OVALLE@GMAIL.COM
MOLINA VINASCO, DIAN MARIA	CENICAFE - FNC DIANA.MOLINA@CAFEDECOLOMBIA.COM
SALAZAR GUTIERREZ, LUIS FERNANDO	CENICAFE - FNC LUISFERNANDO.SALAZAR@CAFEDECOLOMBIA.COM
HENAO, LEONARDO	C.I CAFE DE SANTA BARBARA LHENAO@SBESTATECOFFEE.COM
LIEBIG, THERESA INES	CIAT | DAPA THERESA.LIEBIG@GMX.DE
BUNN, CHRISTIAN	CIAT | DAPA CHRISTIAN.BUNN@HU-BERLIN.DE
SCHUIT, PASCALE	CIAT PASCALE.SCHUIT@GMAIL.COM
GAITAN, ALVARO	CENICAFE - FNC ALVARO.GAITAN@CAFEDECOLOMBIA.COM
GONGORA, CARMENZA	CENICAFE - FNC CARMENZA.GONGORA@CAFEDECOLOMBIA.COM
CRISTANCHO, MARCO	CENICAFE - FNC MARCO.CRISTANCHO@CAFEDECOLOMBIA.COM
BENAVIDES, PABLO	CENICAFE - FNC PABLO.BENAVIDES@CAFEDECOLOMBIA.COM
POSADA, HUVER	CENICAFE - FNC HUVER.POSADA@CAFEDECOLOMBIA.COM
RIVILLAS OSORIO, CARLOS ALBERTO	CENICAFE - FNC CARLOS.RIVILLAS@CAFEDECOLOMBIA.COM

ARANA RENGIFO, VICTORIA	UNIVERSIDAD DEL VALLE VICTORIA.ARANA@CORREOUNIVALLE.EDU.CO
WIST, JULIEN	UNIVERSIDAD DEL VALLE VICTORIA.ARANA@CORREOUNIVALLE.EDU.CO

COSTA RICA	
MENA, FRANCISCO	EXCLUSIVE COFFEES S.A. FMENA@EXCLUSIVECOFFEECR.COM
CARRANZA HERNANDEZ, ARMANDO	COOPEATENAS R. L. ACARRANZA@COOPEATENAS.COM
MORA, ORLANDO	STARBUCKS COFFEE OMORAALF@STARBUCKS.COM
VEGA RODRIGUEZ, JOSE ANTONIO	COOPRONARANJO R.L. ANTONIO@COOPRONARANJORL.COM
HERNANDEZ PEREZ, EMILIO	COOPRONARANJO R.L. EMILIO.HERNANDEZ@COOPRONARANJORL.COM
CHAVES, XINIA	MAG XCHAVES@MAG.GO.CR
DE MELO VIRGINIO FILHO, ELIAS	CATIE ELIASDEM@CATIE.AC.CR
VALDEZ, MARTA	CENIBIOT MVALDEZ@CONARE.AC.CR
MATA GARBANZO, WILLIAM	ICAFE WMATA@ICAFE.CR
ROJAS CASTRO, HENRY	ICAFE HROJAS@ICAFE.CR
GAMBOA BARBOZA, ADRIAN	ICAFE AGAMBOA@ICAFE.CR
MARTINEZ GUILLEN, ADOLFO	ICAFE JOBANDO@ICAFE.CR
ARIAS VARGAS, JOSE EDUARDO	ICAFE JARIAS@ICAFE.CR

RODRIGUEZ ULATE, RICARDO	ICAFE RRODRIGUEZ@ICAFE.CR
BARQUERO MIRANDA, MIGUEL	ICAFE MBARQUERO@ICAFE.CR
ROJAS BARRANTES, MAINOR	ICAFE MROJAS@ICAFE.CR
ECHEVERRIA BEIRUTE, FABIAN	ICAFE FECHEVERRIA@ICAFE.CR
CHAVES ARIAS, VICTOR	ICAFE VCHAVES@ICAFE.CR
RODRIGUEZ SALAZAR, ALBONI	ICAFE ARODRIGUEZ@ICAFE.CR
CHACON ARAYA, ROLANDO	ICAFE RCHACON@ICAFE.CR
FERNANDEZ CASTRO, AARON	ICAFE AFERNANDEZ@ICAFE.CR
RAMIREZ ROJAS, JORGE	ICAFE JRAMIREZ@ICAFE.CR
ROBLES CHAVES, ALEJANDRA	ICAFE AROBLES@ICAFE.CR
ARRIETA ESPINOZA, NOEL	ICAFE NARRIETA@ICAFE.CR
ROJAS ROJAS, EDGAR	ICAFE EGROJAS@ICAFE.CR
FERNANDEZ, IVAN	CAFE DE ALTURA DE SAN RAMON ESP. S. A. CAFEDEALTURA@ICE.CO.CR
QUIROS, MANUEL ANTONIO	CAFÉ DE ALTURA DE SAN RAMON ESP. S. A. CAFEDEALTURA@ICE.CO.CR
ZAMORA, LUIS	MINISTERIO DE AGRICULTURA LZAMORA@MAG.GO.CR
BATALLA, CARLOS	TERRA BELLA CARBATALLA@GMAIL.COM
RODRIGUEZ, CARLOS MARIO	STARBUCKS COFFEE CO. CRODRIGU@STARBUCKS.COM

RODRIGUEZ, HELGA	ICAFE HELGA.RODRIGUEZ@GMAIL.COM
NAVARRO CECILIANO, JOSE LUIS	FAMILIA NAVARRO CECILIANO JLNAVARROC@HOTMAIL.ES
BANSBACH, STEPHAN	EL CUMINATE S.A LSANCHEZ@VOLCAFE.CO.CR
GONZALEZ, JOSE	EL CUMINATE S.A LSANCHEZ@VOLCAFE.CO.CR
GARCIA, ANDRES	EL CUMINATE S.A LSANCHEZ@VOLCAFE.CO.CR
MORA BOLAÑOS, JORGE	INTA JORMORA@INTA.GO.CR
VASQUEZ, ROLANDO	AGROINDUSTRIAL LAS MARIAS ROLANDOVASQUEZR@GMAIL.COM
VASQUEZ VARGAS, JOSE ANGEL	COOPEPALMARES INFO@COOPEPALMARES.COM
GONZALEZ, MIGUEL ANGEL	COOPRONARANJO R.L. MIGUELGONZALEZ2000@YAHOO.COM
CORRALES, BERNAL	COOPRONARANJO R.L. BCORRALES@BYG.CO.CR
VASQUEZ, NELLY	CATIE NVASQUEZ@CATIE.AC.CR
CARVAJA ORLICH, MANRIQUE	COOPERATIVA VICTORIA MCARVAJAL@COOPEVICTORIA.COM
MONTOYA OBANDO, PAUL	COOPERATIVA VICTORIA PMONTOYA@COOPEVICTORIA.COM
ALFARO VASQUEZ, SERGIO	COOPERATIVA VICTORIA SALFARO@COOPEVICTORIA.COM
ALPIZAR, EDGARDO	ECOM EALPIZAR@ECOMTRADING.COM
PONCON, ERIC	ECOM MEGONZALEZ@ECOMTRADING.COM
SONI, NEETA	ECOM MEGONZALEZ@ECOMTRADING.COM

ALFARO, MINOR	KAFFA CAFÉ KAFFACAFE@GMAIL.COM
CHACON, ADRIANA	INDEPENDIENTE ADRICHACON83@GMAIL.COM
PAEZ, MARCO	CRISTAL VITRO MARCO.PAEZ@CRISTALVITRO.COM
BADILLA, NESTOR	COOPEAGRI R.L NBADILLA@COOPEAGRI.CO.CR
OLIVARES, CARLOS	COOPEAGRI R.L COLIVARES@COOPEAGRI.CO.CR
COTO, RAUL	PLATO INDUSTRIES LTDA. JAROTO@RACSA.CO.CR
FALLA, RICARDO	BENEFICIO LAS CHUCARAS RICARDO@CHUCARAS.CO.CR
COTO, JAVIER	HDA. URASCA RICARDO@CHUCARAS.CO.CR
ELIZONDO CUBERO, RANDALL	ENLACE AGROPECAURIO S.A. RELIZONDO@GRUPOENLASA.COM
MATA NARANJO, ROBERTO	COOPEDOTA R.L. RMATA@COOPEDOTA.COM
ZUÑIGA, MARTIN	CROPLIFE LATIN AMERICA RARODRIGUEZ@INTERAMERICANA.CO.CR
BOGANTES, SARA	BENEFICIADORA SANTA EDUVIGUES SBOGANTES@BSE.CR
VARGAS, RODRIGO	BENEFICIADORA SANTA EDUVIGUES BSE@BSE.CR
RODRIGUEZ, RAQUEL	CROPLIFE RARODRIGUEZ@INTERAMERICANA.CO.CR
MATARRITA, LUIS	CROPLIFE RARORODRIGUEZ@INTERAMERICANA.CO.CR
ALVARADO, ROSSIBETH	BAYER SEBASTIAN.FOURNIER@BAYER.COM
FOURNIER, SEBASTIAN	BAYER SEBASTIAN.FOURNIER@BAYER.COM

BORGE. MANUEL	CAFETALERA TORREALBA MANUEL_BORGE@ME.COM
GUTIERREZ ZAMORA, GILBERTO	COMITE ORGANIZADOR LOCAL
VARGAS, GUIDO	COMITEORGANIZADOR LOCAL
MONTENEGRO, DIEGO	IICA
FLORES NEGRINI, FRANCISCO	ICAFE
HERNANDEZ JOHANSSON, ADRIAN	ICAFE
ROJAS BORBON, ALBA NIDIA	ICAFE
RAMIREZ HERRERA, FRANCISCO	ICAFE
HIDALGO ROMAN, JORGE MARIO	ICAFE
GARBANZO ELIZONDO, MIGUEL	ICAFE
VARGAS ROJAS, ANACEDIN	ICAFE
MONGE FALLAS, JUAN LUIS	ICAFE
VILLALOBOS, VICTOR	INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
PETERS SEEVERS, RONALD	ICAFE
GUZMAN ALVAREZ, JOSE ANTONIO	CICAFE AFERNANDEZ@ICAFE.CR
TORRES MURILLO, LUCIANO	CICAFE MPORRAS@ICAFE.CR
CASTRO, FRANCISCO	CAFE DE ALTURA DE SAN RAMON ESP. S. A. CAFEDEALTURA@ICE.CO.CR

GUTIERREZ ESQUIVEL, LUIS GILBERTO	ICAFE
PAEZ APONTE, OSWALDO JOSE	ABOPAC OSWALDO.PAEZ@ABOPAC.COM
MENA, GRACE	DELICAFE GMENA@DELI-CAFE.COM
ESQUIVEL, LAURA	CAMARA DE EXPORTADORES EXPCAFE@ICE.CO.CR
SAENZ PUCCI, IRENE	CAMARA TOSTADORES DE CAFÉ DIRECCIONEJECUTIVA@TOSTADORESCOSTARICA.COM
CALDAS, FERNANDO	FCA INGENIEROS CONSULTORES FPCALDAS1@YAHOO.COM
CANET, GUILLERMO	PROMECAFE
RODRIGUEZ ARCE, ALLAN	MAKHTESHIM AGAN COSTA RICA ALLAN.RODRIGUEZ@MACO.CO.CR
NUÑEZ HERNANDEZ, CHRISTIAN	CONSULTOR CRISTHIANNH@GMAIL.COM
GUERRERO LIZANO, GIOVANNI	NESPRESSO GIOVANNI.GUERRERO@CR.NESTLE.COM
ROMAN CORRALES, JUAN DIEGO	NESPRESSO JUAN.ROMAN@CR.NESTLE.COM
MORA, RODOLFO E.	HACIENDA TOBOSI RMORA@HACIENDATOBOSI.COM
GUTIERREZ ROJAS, TOMAS	ECOM TGUTIERREZ@ECOMTRADING.COM
HERNANDO, JOSE MANUEL	ICAFE DIRECCIONEJECUTIVA@TOSTADORESCOSTARICA.COM
MATAMOROS ROJAS, EDGARDO	ECOM EMATAMOROS@ECOMTRADING.COM
ORTIZ CALVO, ROBERTO	ECOM RORTIZ@ECOMTRADING.COM
BORJAS MARADIAGA, CARLOS	BORJAS MARADIAGA TICOROMANAS@YAHOO.COM

MOYA MASIS, REBECA	APPETIT REBECA_MM23@HOTMAIL.COM
VALVERDE, GIOVANNA	MAG MBORBON@MAG.GO.CR
ARREA SIERMANN, MARIO	SYNGENTA MARIO.ARREA@SYNGENTA.COM
ALVARADOJUAN RAMON	EXCLUSIVE COFFEES FMENA@EXCLUSIVECOFFEECR.COM
VIÑAS MENESES, MARIA	FUNDEVI / UCR MARIANELA.BAEZA@UCR.AC.CR
CUBERO-CASTILLO, ELBA	UNIVERSIDAD DE COSTA RICA ELBA.CUBERO@UCR.AC.CR
GRANADOS MONTERO, MARIA	UNIVERSIDAD DE COSTA RICA MARIA.GRANADOSMONTERO@UCR.AC.CR
ROUPSARD, OLIVIER	CIRAD-CATIE ROUPSARD@CIRAD.FR
CHARBONNIER, FABIEN	CIRAD FABIEN.CHARBONNIER@CIRAD.FR
BLANCO MENESES , MONICA	UNIVERSIDAD DE COSTA RICA MONICA.BLANCOMENESES@CR.AC.CR
URENA RETANA, NATALIA	EARTHWATCH INSTITUE NURENA@EARTHWATCH.ORG
AVELINO, JACQUES	CIRAD / CATIE / IICA JAVELINO@CATIE.AC.CR
CLEMENTINE, ALLINNE	CIRAD CLEMENTINE.ALLINNE@CIRAD.FR
LEITE, GILSON JOSE	UNESP/FCAV COMPRAS@FUNEP.ORG.BR
ROWE WILLIAMS, HARVEY	ABOCOL-FERTITEC HROWE@FERTITEC.CR
SANCHO BARRANTES, ELLEN	UNIVERSIDAD NACIONAL DE COSTA RICA-CIENCIAS AGRARIAS ESANCHO@UNA.AC.CR
AGUILAR, FRANCISCO	CONSEJO NACIONAL DE RECTORES AGUILAR.FRAN@GMAIL.COM

MORA VILLALOBOS, JOSE ANIBAL	CENIBIOT AMORA@CONARE.AC.CR
GUZMAN, LYANA	COOPERATIVA DE PRODUCTORES DE LECHE DOS PINOS R.L. LGUZMAN@DOSPINOS.COM
MORA BRENES, BERNARDO	AGROROSAYLA BEMORAB@GMAIL.COM
SILES, PABLO	BIOVERSITY INTERNATIONAL P.SILES@CGIAR.ORG
UMAÑA FIGUEROA, GABRIEL	MAG MBORBON@MAG.GO.CR
FUENTES DE LA O, RAFAEL	MAG MBORBON@MAG.GO.CR
HERNANDEZ, LUIS FERNANDO	MAG MBORBON@MAG.GO.CR
AZOFEIFA, FREDDY	MAG MBORBON@MAG.GO.CR
MORA SEGURA, NORMAN	MAG MBORBON@MAG.GO.CR
GONZALEZ, LUIS FERNANDO	MAG MBORBON@MAG.GO.CR
GOMEZ MENDEZ, MANUEL	MAG MBORBON@MAG.GO.CR
CASTRO RETANA, LUAN JOSE	MAG MBORBON@MAG.GO.CR
GARCIA, ANDRES	MAG MBORBON@MAG.GO.CR
ROJAS CASTRO, ROY	MAG MBORBON@MAG.GO.CR
DIAZ, CARLOS	MAG MBORBON@MAG.GO.CR
MONTENEGRO, JOHNNY	INTA MBORBON@MAG.GO.CR
SOLORZANO ARROYO, ARTURO	INTA MBORBON@MAG.GO.CR

ALPIZAR OSES, LUIS	INTA MBORBON@MAG.GO.CR
DIAZ PORRAS, ALEX	MAG MBORBON@MAG.GO.CR
RODRIGUEZ MURILLO, WARNER	MAG MBORBON@MAG.GO.CR
DELGADO VALVERDE, LUIS ANGEL	MAG MBORBON@MAG.GO.CR

DENMARK

BUDOLFSEN, GITTE	NOVOZYMES GIBU@NOVOZYMES.COM
RASMUSSEN, LOUISE	NOVOZYMES LOUR@NOVOZYMES.COM
SCHRUEBBERS, LARS	UNIVERSITY OF COPENHAGEN SCHRUEBBERS@LIFE.KU.DK

EL SALVADOR

COLOCHO ORTEGA, JOSE LUIS	BANDEIRANTE S.A. COLOCHORTEGA@YAHOO.COM
MAGAÑA ESCOBAR, JORGE ATILIO	COOP DE CAFETALEROS"LOS AUSOLES" JOATIMAGANAE@HOTMAIL.COM
ZALDAÑA, RAUL	PROMECAFE DULCE.OBIN@IICA.INT
VELASQUEZ ZARCO, ERNESTO	CONSEJO SALVADOREÑO DEL CAFÉ YMENENDEZ@CONSEJOCAFE.ORG.SV
MEJIA PALACIOS, RODRIGO	CONSEJO SALVADOREÑO DEL CAFÉ YMENENDEZ@CONSEJOCAFE.ORG.SV
FUENTES ROMERO, SALVADOR	FONDO DE EMERGENCIA DEL CAFÉ MGRANADOS@CONSEJOCAFE.ORG.SV

URRUTIA CASTRO, ENRIQUE ALFREDO	CONSEJO SALVADOREÑO DEL CAFÉ YMENENDEZ@CONSEJOCAFE.ORG.SV

ETHIOPIA	
ADUGNA, GIRMA	JIMMA UNIVERSITY GIRMA.ADUGNA@YAHOO.COM
BELLACHEW, BAYETTA	AFRICAN COFFEE RESEARCH NETWORK (ACRN) BBELLACHEW@YAHOO.COM

FINLAND	
KOULLIAS, KARLA	GUSTAV PAULIG LTD KARLA.KOULLIAS@PAULIG.COM

FRANCE	
BETRAND, BENOIT	CIRAD BENOIT.BERTRAND@CIRAD.FR
BROUN, PIERRE	NESTLE PIERRE.BROUN@RDTO.NESTLE.COM
LAMBOT, CHARLES	NESTLE R&D TOURS CHARLES.LAMBOT@RDTO.NESTLE.COM
RITCHIE, KAREN	INSERM KAREN.RITCHIE@INSERM.FR
LASHERMES, PHILIPPE	IRD PHILIPPE.LASHERMES@IRD.FR
CHARRIER, ANDRÉ	ASIC HACHARRIER@ORANGE.FR
CROUZILLAT, DOMINIQUE	CENTRE R&D NESTLE DOMINIQUE.CROUZILLAT@RDTO.NESTLE.COM

MCCARTHY, JAMES	NESTLE RESEARCH AND DEVELOPMENT JAMES.MCCARTHY@RDTO.NESTLE.COM
VAAST, PHILIPPE	CIRAD/ICRAF PHILIPPE.VAAST@CIRAD.FR
RAPIDEL, BRUNO	CIRAD BRUNO.RAPIDEL@CIRAD.FR
DAUZAT, JEAN	CIRAD JEAN.DAUZAT@CIRAD.FR
RAZAFINARIVO, JOSIANE	INSTITUT DE RECHERCHE POUR LE DEVELOPPEMENT NORORAZA@YAHOO.FR
CILAS, CHRISTIAN	CIRAD CILAS@CIRAD.FR
DE KOCHKO, ALEXANDRE	IRD ALEXANDRE.DEKOCHKO@IRD.FR
BERTHIOT, LAURENT	CIRAD LAURENT.BERTHIOT@CIRAD.FR
MEILE, JEAN-CHRISTOPHE	CIRAD MEILE@CIRAD.FR
AGUILLAR, PHILIP	CIRAD PHILIP.AGUILAR@CIRAD.FR
RIBEYRE, FABIENNE	CIRAD FABIENNE.RIBEYRE@CIRAD.FR
NOIROT, MICHEL	IRD

GERMANY	
HINDORF, HOLGER	BONN UNIVERSITY H.HINDORF@UNI-BONN.DE
BYTOF, GERHARD	TCHIBO GMBH GERHARD.BYTOF@TCHIBO.DE
LANTZ, INGO	TCHIBO GMBH INGO.LANTZ@TCHIBO.DE

GUENTHER, HELMUT	MONDELEZ INTERNATIONAL HGUENTHER@KRAFTFOODS.COM
BONGERS, SANDRA	PROBAT-WERKE S.BONGERS@PROBAT.COM
ZIMMERMANN, RALF	JMSC (HMGU/UNIVERSITY OF ROSTOCK) RALF.ZIMMERMANN@GSF.DE

GHANA	
ANIM-KWAPONG, ESTHER	GHANA COCOA BOARD EKWAPONG06@YAHOO.COM
KOFI OPPONG, FRANCIS	COCOA RESEARCH INSTITUTE OF GHANA EKWAPONG06@YAHOO.COM
MWINYOYE ANCHIRINAH, VINCENT	GHANA COCOA BOARD EKWAPONG06@YAHOO.COM

GUATEMALA	
PAIZ, LEONEL	NESTLE GUATEMALA LEONEL.PAIZ@GT.NESTLE.COM
PEREZ ANZUETO, DANIEL	ILLY CAFÉ DFPA25@HOTMAIL.COM
NIJ, ELMER	EFICO CENTRAL AMERICA S.A. TRADE@EFICOCENTRAM.COM
CUCHET, ENAUD	EFICO CENTRAL AMERICA S.A. TRADE@EFICOCENTRAM.COM
GARCIA, ARMANDO	PROMECAFE DULCE.OBIN@IICA.INT
OBIN, DULCE	PROMECAFE DULCE.OBIN@IICA.INT
LOPEZ DE LEON, EDGAR	ANACAFE INFO@ANACAFE.ORG

CAMPOS ALMENGOR, OSCAR	ANACAFE INFO@ANACAFE.ORG
ZAVALA BUECHSEL, JOSE ANGEL	ANACAFE INFO@ANACAFE.ORG
PEÑATE MUNGUIA, LUIS MOISES	ANACAFE INFO@ANACAFE.ORG
QUIÑONEZ, MANUEL OSWALDO	ANACAFE INFO@ANACAFE.ORG
ANZUETO, FRANCISCO	ANACAFE INFO@ANACAFE.ORG
AGUIRRE ROMERO, MARCO ANTONIO	ANACAFE INFO@ANACAFE.ORG
VASQUEZ, MAINOR	ANACAFE INFO@ANACAFE.ORG
PASCUAL, LUIS FELIPE	ANACAFE INFO@ANACAFE.ORG
PAPPA SANTOS, FLORENCIO	ANACAFE INFO@ANACAFE.ORG
DUARTE, MARCO TULIO	ANACAFE INFO@ANACAFE.ORG
LOPEZ, JAIME	ANACAFE INFO@ANACAFE.ORG
ALVARADO, JESUS	ANACAFE INFO@ANACAFE.ORG
LEAL QUEJ, ERMENEJILDO	ANACAFE INFO@ANACAFE.ORG

HONDURAS	
ORDOÑEZ, MARIO ADOLFO	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
CASTILLO, FRANCISCO	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN

ZUNIGA, VILMA	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
MENESES, NESTOR	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
GAMBOA, HAROLD	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
PINEDA, ARNOLD	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
MATUTE, NAPOLEON	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
HERNANDEZ, CRISTHIAM	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
SANTOS, GLENDA	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
NUÑEZ, RUBEN	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
CASTILLO, JHONY	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
MATAMOROS, ROMAN	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
OYUELA, ADAN	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN
MERLO, ALFONSO	INSTITUTO HONDUREÑO DEL CAFE NMENESES@IHCAFE.HN

INDIA	
GIRIDHAR, PARVATAM	CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE PARVATAMG@YAHOO.COM
KUYYALIL, SUREKKA	KANNUR UNIVERSITY, KERALA SKUYYALIL@YAHOO.COM
SURYA PRAKASH, NAYANI	DIVISIONAL HEAD, PLANT BREEDING & GENETICS NAYANIRAO@GMAIL.COM

INDONESIA	
MAWARDI, SURIP	ICCRI SURIP.MAWARDI@GMAIL.COM
SHOLEH, SUTRISNO	PTPN 12 SUTRISNO.PTPN12@GMAIL.COM
IRELAND	
WATERS, DEBORAH	UNIVERSITY COLLEGE CORK D.WATERS@UCC.IE
ISRAEL	
KANNER, JOSEPH	HEBREW UNIVERSITY JKANNER@BEZEQINT.NET
COHEN, ZION	STRAUSS COFFEE ZION.COHEN@STRAUSS-GROUP.COM
ITALY	
PELLEGRINO, GLORIA	L. LAVAZZA S.P.A. G.PELLEGRINO@LAVAZZA.IT
ILLY, ANDREA	ILLYCAFFE ANDREA.ILLY@ILLY.COM
ILLY, ANNA	ILLYCAFFE' SPA UFFVIAGGI@ILLY.COM
NAVARINI, LUCIANO	ILLYCAFFE' SPA UFFVIAGGI@ILLY.COM
LONZARICH, VALENTINA	ILLYCAFFE' SPA UFFVIAGGI@ILLY.COM

TURELLO, LUCA	ILLYCAFFE SPA UFFVIAGGI@ILLY.COM
SUGGI LIVERANI, FURIO	ILLYCAFFE' SPA UFFVIAGGI@ILLY.COM
PETRACCO, MARINO	ILLYCAFFE SPA UFFVIAGGI@ILLY.COM
CRISAFULLI, PAOLA	FONDAZIONE ERNESTO ILLY ANDREA.APPELWICK@FONDAZIONEILLY.ORG
BUROLO, ANGELICA	ILLYCAFFE SPA UFFVIAGGI@ILLY.COM
FAINA, MORENO	ILLYCAFFE' SPA UFFVIAGGI@ILLY.COM
ETTAMI, PIETRO GIORGIO	TME GIORGIO.ETTAMI@TMEITALY.COM
SEVERINI, CARLA	UNIVERSITA DEGLI STUDI DI FOGGIA C.SEVERINI@UNIFG.IT

JAPAN	
AKIYAMA, MASAYUKI	MORINAGA MILK INDUSTRY CO., LTD. M_AKIYAM@MORINAGAMILK.CO.JP
WATANABE, RYOSUKE	MORINAGA MILK INDUSTRY CO., LTD. RYO-WATANABE@MORINAGAMILK.CO.JP
IWAI, KAZUYA	UCC UESHIMA COFFEE CO.LTD. KAZUYA-IWAI@UCC.CO.JP
NARITA, YUSAKU	UCC UESHIMA COFFEE CO., LTD. YUUSAKU-NARITA@UCC.CO.JP
NAGATA, KOJI	UCC UESHIMA COFFEE CO.,LTD. KOJI-NAGATA@UCC.CO.JP
WATANABE, TAKUYA	KAO CORPORATION WATANABE.TAKUYA@KAO.CO.JP
AINO, KAZUYOSHI	KEY COFFEE INC. KAZUYOSHI_AINO@KEYCOFFEE.CO.JP

SETA, HARUMICHI	SUNTORY BUSINESS EXPERT CO.LTD HARUMICHI_SETA@SUNTORY.CO.JP
NAKAHARA, KOICHI	SUNTORY BUSINESS EXPERT LTD. KOICHI_NAKAHARA@SUNTORY.CO.JP
CHIKAMA, AKIRO	KAO CORPORATION 308309@KAO.CO.JP
KASAI, NAOYA	OSAKA PREFECTURE UNIVERSITY KASAI@BIOCHEM.OSAKAFU-U.AC.JP

KENYA

GICHIMU, BERNARD	COFFEE RESEARCH FOUNDATION WACIKUBM@GMAIL.COM
JARAMILLO, JULIANA	ICIPE JJAR2001@YHOO.COM
GICHURU, ELIJAH	COFFEE RESEARCH FOUNDATION EKGICHURU@YAHOO.COM
M'RIBU, KABURU	COFFEE RESEARCH FOUNDATION KMRIBU@YAHOO.COM
KIMEMIA, JOSEPH	COFFEE RESEARCH FOUNDATION KANGARAK@YAHOO.COM
NGAMAU, KAMAU	COFFEE RESEARCH FOUNDATION K_NGAMAU@YAHOO.COM

MEXICO

AMECA CASTILLO, EVA	AMECAFE EVA.AMECA@AMECAFE.ORG.MX
VENEGAS FLORES, JULIETA	AMECAFE JULIETA.VENEGAS@AMECAFE.ORG.MX
MARTINEZ, ARACELLI	AMECAFE ARACELI.MARTINEZ@AMECAFE.ORG.MX

GUZMAN, ENRIQUE	LABORATORIOS AGROENZYMAS INFO@AGROENZYMAS.COM
OSUNA, LUZ MARIA	CUMBRE LATINOAMERICANA DEL CAFÉ LUZMA@GRUPOAMCMEXICO.COM

NETHERLANDS	
DE VOS, RIC	WAGENINGEN UNIVERSITY RIC.DEVOS@WUR.NL
VAN DER VOSSEN, HERBERT	ASIC HERBERT.VANDERVOSSEN@QUICKNET.NL
DA SILVA HOINKIS, HERBERT ANDRAE	D.E. MASTER BLENDERS 1753 ANDRE.HOINKIS@GMAIL.COM
BIESTERVELD, STEVEN	D.E. MASTER BLENDERS 1753 STEVEN.BIESTERVELD@DEMB.COM
HAYWOOD, KIERAN	DE MASTER BLENDERS 1753 KIERAN.HAYWOOD@DEMB.COM
DIELEMAN, RUDI	WAGENINGEN UR RUDI.DIELEMAN@WUR.NL
BELALCAZAR, ANDRES	WAGENINGEN UR ANDRES.BELALCAZAR@WUR.NL

NICARAGUA	
BLANDON BLANDON, FRANCISCO	AGROPECUARIA SANTA MAURA, S.A. CHAVES2006@TURBONETT.COM.NI
ZELAYA BLANDON, DONALD	ESCUELA DEL CAFE DE JINOTEGA CHAVES2006@TURBONETT.COM.NI
FLORES, KARFEN	SMS-ECOM KAFLORES@ECOMTRADING.COM
ZELAYA, CARLOS	CIAT C.R.ZELAYA@CGIAR.ORG

LADERACH, PETER	CIAT | DAPA P.LADERACH@CGIAR.ORG
GARCIA SALAZAR, JULIA	UNIVERSIDAD AUTONOMA DE NICARAGUA JULGAR262@CT.UNANLEON.EDU.NI

PANAMA	
KOYNER, RICARDO	CAFE KOTOWA KOTOWAFARMS@CWPANAMA.NET
SERRACIN, FRANCISCO	PROMECAFE RMORA@DELI-CAFE.COM

PERU	
VARGAS CHAVEZ, PABLO	CAC LA FLORIDA ROCIOLEON@LAFLORIDAPERU.COM
QUISPE PENA, JOSE LUIS	APROSELVA CESARR@CAFE-PERU.COM
QUISPE PENA, BENJAMIN	CAFE PERU SAC BENJAMIN3206@HOTMAIL.COM
CORNEJO HERRERA, JOSE MANUEL	LOUIS DREYFUS COMMODITIES PERU JMCH_7@HOTMAIL.COM

PHILIPPINES	
ONG, DENNIS	MARGAJA MARGAJA_RDC@YAHOO.COM
CALINGIN, MARIA ROSARIO	MARGAJA MARIACALINGIN@YAHOO.COM
BELONIO, HARRY	MARGAJA HBBELONIO@GMAIL.COM

GONZAGA, NELDA	MISAMIS ORIENTAL NRUBA@YAHOO.COM

PORTUGAL	
MAGUAS, CRISTINA	UNIVERSIDADE DE LISBOA CMHANSON@FC.UL.PT
VARZEA, VITOR	CENTRO DE INVESTIGACAO DAS FERRUGENS DO CAFEEIRO VITORVARZEA@CLIX.PT
SILVA, MARIA DO CEU	INSTITUTO DE INVESTIGACAO CIENTIFICA TROPICAL MARIACEUDASILVA@GMAIL.COM
LOUREIRO, ANDREIA	INSTITUTO DE INVESTIGACAO CIENTIFICA TROPICAL ANDREIASOFIALOUREIRO@GMAIL.COM
RAMALHO, JOSE	IICT COCHICHOR@MAIL.TELEPAC.PT
COSTA, JOAO	INVITED SPEAKER JNCOSTA@FM.UL.PT

SOUTH AFRICA	
CRAPARO, ALESSANDRO	UNIVERSITY OF WITWATERSRAND SANDRO@MAILNET.CO.ZA

SWITZERLAND	
BLANC, MAURICE	ASIC COFFEE-SCIENCE@ASIC-CAFE.ORG
FOLMER, BRITTA	NESTLE NESPRESSO SA BRITTA.FOLMER@NESPRESSO.COM
RODRIGUEZ, ALEXIS	NESTLE NESPRESSO SA ALEXIS.RODRIGUEZ@NESPRESSO.COM

LEFEBVRE, FLORENT	NESPRESSO FLORENT.LEFEBVRE-PAUTIGNY@NESPRESSO.COM
GLABASNIA, SARNE	NESTLE ARNE.GLABASNIA@RDOR.NESTLE.COM
GLOESS, ALEXIA	ZHAW ALEXIA.GLOESS@ZHAW.CH
YERETZIAN, CHAHAN	ZHAW YERE@ZHAW.CH

TANZANIA	
NGOMUO, ROBERT	TACRI RNGOMUO@YAHOO.COM
MAGESA, JEREMIAH	TACRI JEREMIAH.MAGESA@TACRI.ORG
GODSTEVEN, MARO	TACRI GODSTEVEN.MARO@TACRI.ORG
MAGINA, FREDRICK	TACRI FREDRICK.MAGINA@TACRI.ORG
KIWELU, LEONARD	TACRI LEONARD.KIWELU@TACRI.ORG
MABAGALA, JOHN	TACRI JOHN.MABAGALA@TACRI.ORG
SYLVANUS, ESAU	TACRI ESAU.SYLVANUS@TACRI.ORG
HAMADI, ALMASI	TACRI ALMASI.HAMAD@TACRI.ORG
MTENGA, DAMIAN	TACRI DAMIAN.MTENGA@TACRI.ORG
NG'HOMA, NYABISI	TACRI NYABISI.NGHOMA@TACRI.ORG
KILAMBO, DEUSDEDIT	TACRI DEUSDEDIT.KILAMBO@TACRI.ORG

TERI, JAMES	TACRI JAMES.TERI@TACRI.ORG

UGANDA	
MUSOLI, PASCAL	NARO-COREC MUSOLI_PASCAL@YAHOO.CO.UK
CHESANG BHATIA, FRANCIS	UGANDA COFFEE DEVELOPMENT AUTHORITY CHESANG@UGANDACOFFEE.ORG
KAGEZI, GODFREY	COFFEE RESEARCH CENTER (COREC) GODKAGEZI@YAHOO.CO.UK
JASSOGNE, LAURENCE	IITA LJASSOGNE@GMAIL.COM

UNITED KINGDOM	
MELROSE, JOHN	MONDELEZ INTERNATIONAL JMELROSE@KRAFTFOODS.COM
STOCKWELL, AMY	MONDELEZ INTERNATIONAL ASTOCKWELL@KRAFTFOODS.COM
GUILMINEAU, FABIEN	MONDELEZ INTERNATIONAL FGUILMINEAU@KRAFTFOODS.COM
BAKER, PETER	CABI P.BAKER@CABI.ORG
HARGRAVES, NIGEL	MARS DRINKS NIGEL.HARGRAVES@EFFEM.COM
KINGSLEY, ANDREW	COFFEE & COCOA INTERNATIONAL ANDREW@SIEMEX.BIZ
FISK, IAN	UNIVERSITY OF NOTTINGHAM IAN.FISK@NOTTINGHAM.AC.UK
HAGGAR, JEREMY	UNIVERSITY OF GREENWICH J.P.HAGGAR@GRE.AC.UK

UNITED STATES	
SORTWELL, DANIEL	D.S. TECHNICAL SERVICES, INC. DRS@USA.NET
NEMZER, BORIS	FUTURECEUTICALS, INC. BNEMZER@VANDRUNEN.COM
COUGHLIN, JAMES	COUGHLIN & ASSOCIATES JRCOUGHLIN@COX.NET
JONES, LAWRENCE	HUNTINGTON MEDICAL RESEARCH INSTITUTES JONESLW@HMRI.ORG
GINN, BRENT	THE JM SMUCKER CO BRENT.GINN@JMSMUCKER.COM
HOWELL, JESS	THE JM SMUCKER CO JESS.HOWELL@JMSMUCKER.COM
KIRKPATRICK, STEVE	THE JM SMUCKER CO STEVE.KIRKPATRICK@JMSMUCKER.COM
BLADYKA, EMMA	SCAA EBLADYKA@SCAA.ORG
ZELLER, BARY	KRAFT FOODS BZELLER@KRAFTFOODS.COM
STOUFFER, SCOTT	PROBAT BURNS SSTOUFFER@PROBATBURNS.COM
KRIEG, RICAHRD	THE KRIEG GROUP RMKRIEG@GMAIL.COM
WELSH, DOUGH	PEET'S COFFEE AND TEA LCOOK@PEETS.COM
TAYLOR, LAUNTIA	PROBAT BURNS RGADSON@PROBATBURNS.COM
SCHMIDT, KARL	PROBAT BURNS RGADSON@PROBATBURNS.COM
WATTS, GEOFF	INTELLIGENTSIA COFFEE WATTS@INTELLIGENTSIACOFFEE.COM
MIRANDA, NELLY	CAFETO COFFEE COMPANY NELLYM@CAFETO.COM

BORLAND, CAROL	NESTLE PRODUCT TECHNOLOGY CENTER CAROL.BORLAND@RD.NESTLE.COM
LEONARD, EMERSON	STIR EMERSON@STIRTC.COM
LOCKWOOD JR., ROB	TEA & COFFEE R.LOCKWOOD@TEAANCOFFEEE.NET
FACENDA, VANESSA	TEA & COFFEE V.FACENDA@TEAANDCOFFEE.NET
SMALL, MILES	COFFEE TALK MILES@COFFEETALK.COM
PRENTICE, ASHLEY	COFFEE TALK ASHLEY@COFFEETALK.COM
NORTHROP, RACHEL	WIN MAGAZINE NORTHROP.RACHEL@GMAIL.COM
AKAI, KANJI	KAO HEALTH AND NUTRITION LLC KANJI.AKAI@KAOHN.COM
SCHULTZ, HAYLEY	KRAFT FOODS HAYLEY.SCHULTZ@KRAFTFOODS.COM
SCHILLING, TIMOTHY	WORLD COFFEE RESEARCH YANET.RODRIGUEZ@AGNET.TAMU.EDU
ROMAN, JOHANNA	WORLD COFFEE RESEARCH YANET.RODRIGUEZ@AGNET.TAMU.EDU
GRECO, ELSIE	UNIVERSITY OF HAWAII AT MANOA EBURBANO@HAWAII.EDU
GAUTZ, LOREN	MBBE, CTAHR, UHMANOA LGAUTZ@HAWAII.EDU
STEIMAN, SHAWN	COFFEA CONSULTING STEIMAN@COFFEACONSULTING.COM
NELSON, MICHAEL	LUND UNIVERSITY MNNELSONM@GMAIL.COM
NAGAI, CHIFUMI	HAWAII AGRICULTURE RESEARCH CENTER CNAGAI@HARC-HSPA.COM
PETIARD, VINCENT	WORLD COFFEE RESEARCH- TEXAS A&M UNIVERSITY YANET.RODRIGUEZ@AG.TAMU.EDU

KRIEG, JUDITH	UNIVERSITY OF MARYLAND, USA JCKRIEG2@GMAIL.COM

VENEZUELA	
TORRES, ANGEL	INIA-TACHIRA ATORRES@INIA.GOB.VE
SALAZAR, WILMER	UNELLEZ SWILMER_JOHAN@HOTMAIL.COM

VIETNAM	
TRUONG HONG, KIM	AGROINFO TRUONGHONGKIM@GMAIL.COM