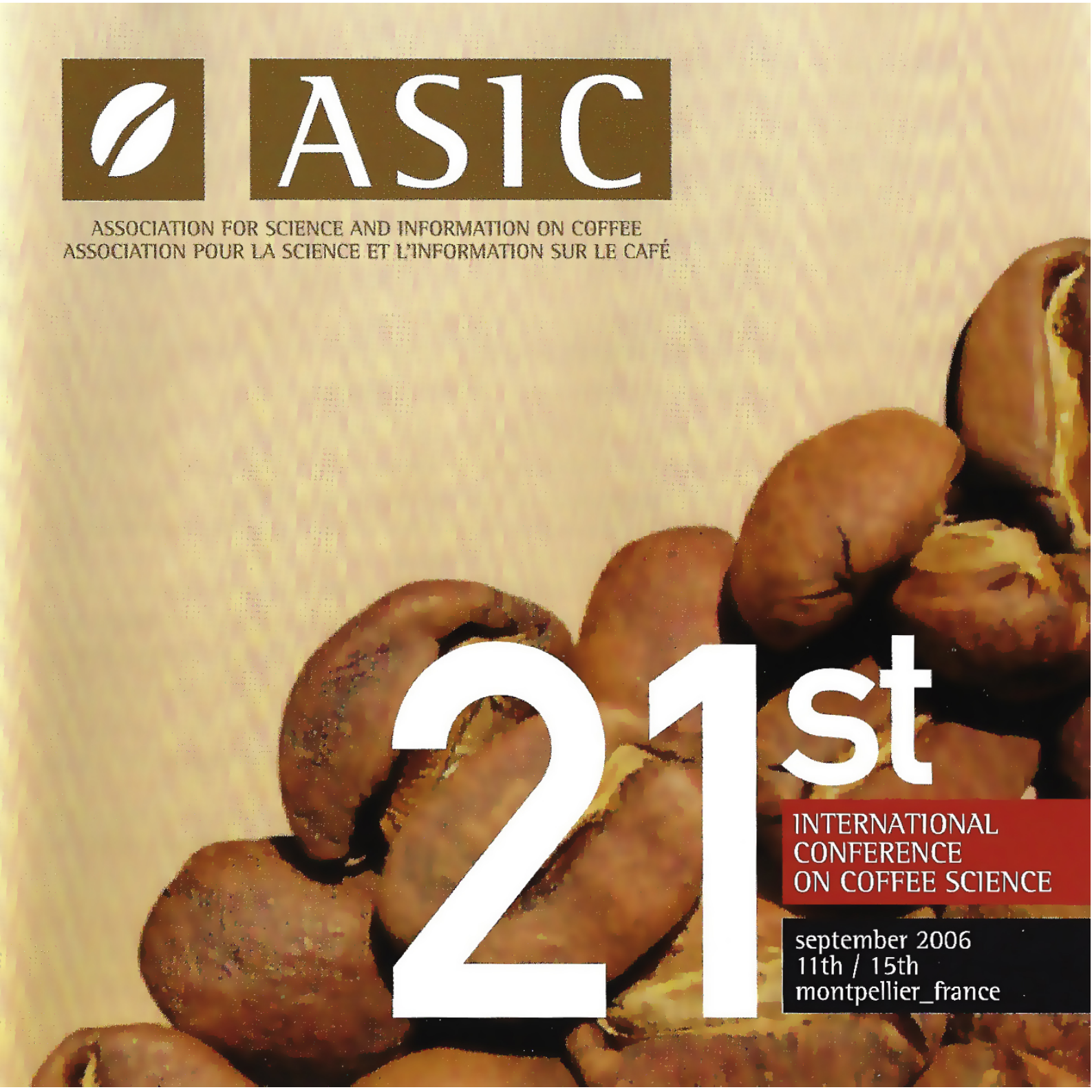

ASIC
ASSOCIATION FOR SCIENCE AND INFORMATION ON COFFEE
ASSOCIATION POUR LA SCIENCE ET L'INFORMATION SUR LE CAFÉ
21st
INTERNATIONAL
CONFERENCE
ON COFFEE SCIENCE
september 2006
11th / 15th
montpellier_france

VOLUME 2

21st International Conference on Coffee Science
Montpellier, 10th – 15th September 2006

21 ème Colloque scientifique international sur le café
Montpellier, 10 – 15 septembre 2006

Table of Contents

VOLUME 2

Diversity and Breeding / Diversité et sélection

Communications

- Development and Characterization of a True F2 Population for Genetic and QTL Mapping Arabica
  C. Nagai, M. R. Jones, A. E. Byers, D. J. Adamski, R. Ming

- Genetic Diversity of Commercial Coffee (*C. arabica* L.) from America, India and Africa Assessed by Simple Sequence Repeats (SSR)
  P. Tornincasa, R. Dreos, B. De Nardi, E. Asquini, J. Devasia, M. K. Mishra, L. Del Terra, P. Crisafulli, A. Pallavicini, G. Graziosi

- Characterisation and Genetic Mapping of a Gene Confering Resistance to Coffee Berry Disease (*Colletotrichum kahawae*) in Arabica Coffee (*Coffea arabica* L.)
  E. K. Gichuru, C. O. Agwanda, M. C. Combes, E. W. Mutitu, E. C. K. Ngugi, B. Bertrand, P. Lashermes

- State-of-the-Art of Developing Arabica Coffee Cultivars with Durable Resistance to Coffee Berry Disease (*Colletotrichum kahawae*)
  H. van der Vossen

- Genetic Diversity of Wild *Coffea arabica* in Ethiopia: Analyses Based on Plastid, ISSR and Microsatellites Markers
  K. G. Tesfaye, K. Govers, T. Oljira, E. Bekele, T. Borsch

- *In situ* Conservation and Use of the Wild Populations of *Coffea arabica* Coffee in the Montane Rainforests of Ethiopia
  M. Denich, F. Gatzweiler

- Arabica Coffee (*Coffea arabica* L.) Local Landrace Development Strategy in its Center of Origin and Diversity
  B. Bellachew, J. P. Labouisse

- *Coffea* spp. and *Coffea canephora* Diversity Evaluated with Microsatellites and SNPs. Lessons from Comparative Analysis
  T. Leroy, P. Cubry, N. Durand, M. Dufour, F. de Bellis, I. Jourdan, L. G. Vieira, P. Musoli, P. Aluka, P. Marraccini, D. Pot

- Fighting against Coffee Wilt Disease: Uganda Wild *C. canephora* Genetic Diversity and its Usefulness
  P. Musoli, P. Aluka, P. Cubry, M. Dufour, F. de Bellis, S. Nakendo, A. Nabaggala, J. Ogwang, D. Kyetere, T. Leroy, D. Bieysse, A. Charrier

- Breeding for Durable Rust Resistance in Coffee (*Coffea arabica*) – Field Performance of Some Tetraploid Inter-specific Hybrid Derivatives in India
  N. Surya Prakash, V.V.K.M. Laxmi, P. Sailaja, A.F.S.M. Reddy, M.S. Sreenivasan, K. Bhaskar Reddy, P.K. Vinodkumar, C.S. Srinivasan, A. Santaram, N. Ramamurthy, Jayarama

- Investigation of Natural Interspecific Hybrids from New Caledonia: a Very Promising Source of Genetic Diversity for *C. arabica* Breeding
  L. Mahe, D. Le Pierrès, M. C.Combes, V. Varzea, P. Lashermes

- New Coffee (*Coffea* L.) Species from Cameroon Brings Original Characters for Breeding
  F. Anthony, M. Noirot, E. Couturon, P. Stoffellen

- Phylogenetic Relationships in *Coffea* (Rubiaceae) Inferred from Sequence Data and Morphology
  A. P. Davis, O. Maurin, M. Chester, E. F. Mvungi, M. Fay

Posters

- Antioxidant Metabolism of Coffee Cell Suspension Cultures in Response to Cadmium
  R. A. Gomez-Junior, C. Moldes, F. S. Delite, G. B. Pompeu, P. L. Gratao, P. Mazzafera, P. J. Lea, R. A. Azevedo

- Synchronization of Coffee Somatic Embryos using Absisic Acid (ABA)
  J. W. Kahia, S. H. Mantell, E. W. Kahang

- Somatic Embryogenesis in Genotypes of *Coffea arabica* with Reduced Caffeine Content
  J. A. S. Almeida, M. B. Silvarolla, M. T. Braghinni, L. C. Fazuoli

- Transformation of *Coffea arabica* for Root-knot Nematode Resistance Using Cysteine and Serine Proteinase Inhibitors
  R. Cabos, B. Sipes, D. Schmitt, H. Atkinson, C. Nagai

- Coffee Seed Cryoperservation: Current Research Progress
  M. T. S. Eira, V. S. Ribeiro, L. C. Fazuoli, O. Guerreiro-Filho, C. Walters

- Mechanisms of Seed Ageing in Coffee
  S. Dussert, A. Laffargue, M. Davey, S. Doulbeau, H. Etienne

- Effects of the *Laurina* Mutation on the Shoot Apex in *C. Arabica* cv. Laurina
  A. Lecolier, J. L. Verdeil, H. Chrestin, M. Noirot

- Analysis of Dwarfism at the Microscopic level in *Coffea arabica* cv. Laurina
  A. Lecolier, J. L. Verdeil, H. Chrestin, M. Noirot

- Genetic Diversity within *Coffea canephora* Germplasm Maintained in RCI Using SSR Markers. Results and Future Prospects
  P. Cubry, P. Musoli, H. Legnate, P. Aluka, M. Dufour, D. Pot, F. de Bellis, T. Leroy

- Genetic Diversity in *Coffea* Genus Using Microsatellite Loci
  P. Cubry, D. Pot, F. de Bellis, P. Musoli, P. Aluka, H. Legnate, M. Dufour, T. Leroy

- Biochemical and Nucleotide Variability in the Diterpene Metabolism for *Coffea canephora* Genotypes from Uganda
  N. Durand, F. de Bellis, I. Jourdan, J-C. Manez, P. Aluka, L. G. Vieira, J. Ogwang, D. Pot, P. Marraccini, T. Leroy, B. Guyot

- Collection and *ex situ* Conservation of Coffee Landraces in Ethiopia – The Example of Harerge
  J. P. Labouisse, B. Bellachew,C. Hamelin, S. Kotecha, B. Bertrand

- Chemical Characterization of *Coffea arabica* Accessions from Instituto Agronômico Germplasm Collection
  T. J. G. Salva, V. B. de Lima, G. Martin, A. Sanvido, M. B. Silvarolla, L. C. Fazuoli, O. Guerreiro-Filho

- Tree Shape Modification Induced by the *Laurina* Mutation in *Coffea arabica*
  A. Lecolier, J. L. Verdeil, H. Chrestin, M. Noirot

- Variability and Association of Agronomic Characters in the Hararge Coffee Germplasm Accessions
  M. Kebede, B. Bellachew

- Diversity in the South Ethiopena Coffee (*Coffea arabica* L.)
  M. Kebede, B. Bellachew, S. Seifu

- Variability and Interrelationships between Coffee (*Coffea arabica* L.) Seedling Characters and their Implication in Selection
  S. Seifu, B. Bellachew

- Genetic Erosion – A Threat to the Conservation and Sustainable Utilisation of Ethiopian Coffee (C*offea arabica* L.) Germplasm
  S. Seifu, B. Bellachew

- Physiochemical Evaluation of Green Bean of Some Coffee *canephora* Varieties in Nigeria
  S. O. Ogunwolu, S. S. Omolaja

- Seedling Emergence and Growth Response of Wild Arabica Coffee Accessions in Southwest Ethiopia
  T. Kufa, J. Burkhardt

- Early Bearing Stage Evaluation of *Coffea arabica* L. Germplasm for Yield and Morphological Characters
  E. Habte, B. Bellachew, H. Singh

- Inheritance of Caffeine in Interspecific Hybrids of *Coffea arabica* x *Coffea canephora*
  R. H. G. Priolli, M. Möller, M. I. Zucchi, L. C. S. Ramos, P. Mazzafera, W. J. Siqueira, C. A. Colombo

- TUPI RN IAC 1669-13: A Coffee Cultivar Resistant to *Hemileia vastatrix* and *Meloidogyne exigua* Nematode
  L. C. Fazuoli, W. Gonçalves, M.Toma-Braghini, M. B. Silvarolla

- Genetic Basis of Spcies Differenciation between *Coffea liberica* Hiern and *C. canephora* Pierre: Analysis of Interspecific Cross
  A. N'Diaye, M. Noirot, S. Hamon, V. Poncet

- Progress in Developing Coffee Berry Disease (*Colletotrichum kahawae*) Resistant Compact Hybrid Varieties (*Coffea arabica*) in Tanzania
  D. J. Mtenga, D. L. Kilambo, J. M. Teri, L. Masumbuko

Ecophysiology

Communications

- Carbon Allocation between Growth and Production in Fruit Trees
  M. Génard, J. Dauzat, N. Franck, N. Franck, F. Lescourret, N. Moitrier, P. Vaast, G. Vercambre

- Coffee a Shade-adapted Plant: Implications on its Carbon Balance and Consequences on Coffee Yield and Quality in Agroforestry Systems
  N. Franck, P. Vaast, J. Dauzat

- Different Drought Adaptation Strategies of *Coffea arabica* Populations along Rainfall Gradient in Ethiopia
  J. Burckhardt, T. Kufa, A. Beining, H. E. Goldbach, M. Fetene

- Using Virtual Plants for up Scaling Carbon Assimilation from the Leave to the Canopy Level. Application to Coffee Agroforestery Systems
  J. Dauzat, N. Franck, P. Vaast

- Nitrogen Dynamics and Nnitrate Leaching in *Coffea arabica* Systems in Costa-Rica According to Site Conditions, Fertilization and Shade Management
  J.-M. Harmand, V. Chaves, H. Avila, P. Cannavo, L. Dionisio, G. Crouzet, B. Zeller, P. Vaast, R. Oliver, E. Dambrine

- An Integrative Approach to Study Environmental Stress Tolerance in *Coffea* spp. – From Leaf to Gene
  J. C. Ramalho, F. C. Lidon, A. E. Leitao, P. Eichler, P. Santos, I. P. Pais, P. S. Campos, A. P. Rodrigues, M. M. Chaves, M. P. Branco, A. I. Ribeiro

Posters

- Hydraulic Conductance of Wild Arabica Coffee Populations in Montane Rainforests of Ethiopia
  T. Kufa, J. Burkhardt

- Carbon Sequestration in Coffee Agroforestry Plantations of Central America
  J.-M. Harmand, K. Hergoualch, S. de Miguel, B. Dzib, P. Siles, P. Vaast

- Photosynthesis and Water use of Wild *Coffea arabica* Populations along Climatic Gradient *in situ* and under Stress in an *ex situ* Experiment
  A. Beining, J. Burkhardt

- Evaluation of Different Germination Media on Pre-germination Performance of Selected Clones of Robusta Coffee (*Coffea canephora*) in Nigeria
  E. A. Adeyemi, R. R. Ipinmoroti

- Accelrated Composting of Coffee Processing: an Organic Option for Soil Fertility Management in the Coffee Based Cropping Sytem of Southwestern Ethiopia
  S. Endris

- Response of Arabica Coffee Seedlings to Lime and Phosphorous: I. Shoot and Root Growth
  A. Netsere, H. Gebrekidan

- Response of Arabica Coffee Seedlings to Lime and Phosphorous: II. Dry Matter Production and Distribution
  A. Netsere, H. Gebrekidan

- Yield Response of Forest Arabica Coffee to Ridges and Rejuvenation Methods
  A. Netsere, T. Shimber, T. Kufa, E. Taye, W. Gebreselassie

- The Nutrient Status of Long Term Fertilized Soils of Coffee Plantations in Southwestern Ethiopia
  T. Kebede, Z. Mikru

- The Potential Use of a Silicon Source as a Component of an Ecological Management of Coffee Plants
  J. C. Martinati, R. Harakava, S. D. Guzzo, S. M. Tsai

Agronomy

Communications

- Geographical Analyses to Explore Interactions between Inherent Coffee Quality and Production Environment
  P. Laederach, P. Vaast, T. Oberthuer, R. O'Brien, L. Estrada, D. Leonel, A. Nelson

- Agrometeorological Model for Monitoring and Predicting Coffee (*Coffea arabica* L.) Productivity in São Paulo State, Brazil
  M. B. P. Camargo, M. A. Santos, M. J. Pedro, J. I. Fahl

- Bird Diversity on Coffee Farms in Central Colombia
  P. Baker, J. Botero, G. Lentijo

- *Coffea arabica* Clones from F1 Hybrids in Central America
  P. Charmetant, H. Etienne, R. Santacreo, B. Cisneros, F. Anzueto, S. Gil, E. Alpizar, B. Bertrand

- Variability in Yield and Yield Components of Wild Arabica Coffee Populations in the Montane Rainforests of Ethiopia
  T. Kufa, J. Burkhardt

- First Industrial Massive Propagation of *Coffea canephora* through Somatic Embryogenesis Organized in Thailand
  S. Siripong, J.-P. Ducos, C. Lambot, T. Kunasol, Y. Kasinkasaempong, P. Chantanumat, V. Pétiard

- The Potential of Participatory Extension Approaches in Coffee Rejuvenation - Experiences from Northern Tanzania
  M. H. Temu, T. Nzallawahe, S. Mdemu, J. M. Teri

- Integrated Sustainable Development of *Coffea arabica* in Vietnam
  Nguyen Tu Hai

Posters

- Yield Response of Arabica Coffee to Spacing and Training Methods in Southwestern Ethiopia
  E. Taye, A. Netsere, T. Shimber, T. Kufa, F. Asfaw

- Intercropping of Robusta Coffee Trees with Leguminous Plant: Effects on Physical and Chemical Figures of Marketable Coffee and Soil
  K. N'Goran, J. G. Nemlin, K. Amani, K. Laco

- Emergence and Growth of Arabica Coffee Seedlings as Influenced by Some Pre-sowing Seed Treatments
  T. Kufa, A. Yilma

- Effect of Inorganic and Organic Nutrient Sources on Growth, Dry Matter Yield and P-Uptake of Coffee (*Coffea canephora* L.) Seedlings
  R. R. Ipinmoroti, L. A. Abedowale, M. O. Ogunlade, G. O. Iremiren, G. O. Adeoye

- Influence of an Albizzia Shade Tree on Soil Chemical Properties, Coffee Plant Growth and Yields in Kenya
  J. K. Kimemia

- Evaluation of Berry Yield from Clonal and Seedling Materials of *Coffea canephora* Pierre ex Froehner in Different Geometry and Clonal Combinations
  A. O. Famaye, A. A. Oloyede, K. Ayegboyin

- Effect of Organic Fertilizers on Nutrient Uptake by *Coffea canephora* Seedlings
  M. A. Daniel, A. O. Obi

- Effect of Amended Growth Media on the Production of *Coffea canephora* Seedlings in the Nursery
  E. A. Adeyemi, M. A. Daniel

- Physiological Response of Coffee to Different Shading Regimes
  S. R. Steiman, T. W. Idol, H. C. Bittenbender, L. D. Gautz, M. C. Jackson

- Shade Coffee in Hawaii - The Impact of Light on Coffee Quality
  S. R. Steiman, T. W. Idol, H. C. Bittenbender, L. D. Gautz, M. C. Jackson

- Risk Management in Coffee Production in Kenya with Uncertain Rainfall
  J. N. Mutua

- Intercropping *Coffea canephora* Pierre ex Froehner with Food Crops at Established Stage in Nigeria
  A. A. Oloyede, A. O. Famaye

- Analysis of Coffee Output Variations as Correlate of Weather Variables in Nigeria
  R. A. Sanusi, M. O. Adejumo, T. R. Shittu

- Technology Transfer and Adoption of Coffee Coppicing in Kogi State, Nigeria
  A. Agbongiarhuoyi, O. O. Oduwole, R. A. Sanusi

- Preliminary Assessment of Smallholder Coffee Value Chains in Specific Regions of Kenya, Uganda and Rwanda
  A. T. Aithal, F. Pinard

- Analysis of Coffee Consumption in Nigeria
  R. A. Sanusi, J. O. Lawal, T. R. Shittu

- Prospects for Promotion of Local Coffee Consumption in Context of Increment of Manufactured Value Added Product in Africa
  J. R. Onzima

Pathology & Plant Protection / Maladies et protection des plantes

Communications

- Rationalizing Control of Coffee Tree Diseases
  J. Avelino

- Disease Situtation in Wild *Coffea Arabica* of Ethiopia with Emphasis on the Coffee Leaf Rust, *Hemileia vastatrix*
  H. Hindorf, A. Zeru

- Tracheomycosis (*Gibberella xylarioides*) - A Menace to the World Coffee Production: Evidence by Cross Inoculation of Historic and Current Strains of the Pathogen
  G. Adugna, J. Flood, H. Hindorf, D. Biesse, S. Sarah, M. Rutherford

- Characterization of *Colletotrichum kahawae* Diversity
  A. Loureiro, V. Varzea, L. Guerra-Guimaraes, A. Ribeiro, M. C. Silva, B. Bertrand

- Antagonistic Interaction between *Epicoccum nigrum* and *Colletotrichum kahawae*, the Causal Agent of Coffee Berry Disease
  L. Guerra-Guimaraes, H. G. Azinheira, A. C. Martins, M. C. Silva, E. K. Guichuru, V. Varzea, B. Bertrand

- (S)-2-Hydroxy-3-Decanone as Sex Pheromone for Monitoring and Control of Coffee White Stem Borer (X*ylotrechus quadripes*)
  Jayarama, M. V. d'Souza, D. R. Hall

Posters

- Genetic Variability of H*ypothenemus hampei* (Ferrari) in Colombia and Development of Molecular Markers
  P. Benavides, J. J. Stuart, F. E. Vega, J. Romero-Severson, A. E. Bustillo, L. Navarro, L. M. Constantino, F. E. Acevedo

- Histochemical Differences in *Coffea* Genotypes Resistant and Susceptible to the Coffee Leaf Miner
  D. A. Ramiro, O. Guerreiro-Filho

- Biodiversity of Root Knot Nematodes (*Melanoidogyne* spp) on Coffee in Central America
  L. Villain, J.-L. Sarah, A. Hernandez, P. Charmetant, B. Bertrand, F. Anthony, P. Topart, P. Lashermes, F. Anzueto, R. M. D. G. Carneiro

- Esterase Activity and Adhesion during the Early Stages of *Hemileia vastatrix* Differentiation
  H. G. Azinheira, L. Guerra-Guimaraes, M. C. Silva, V. Varzea, C. Ricardo

- Characterization of Histological Effects of *Meloidogyne incognita* Infection in Resistant and Susceptible *Coffea arabica* Genotype
  E. V. S. A. Barros, P. M. Costa, A. C. M. M. Gomes, R. Falcao, V. F. Ribeiro, M. T. S. Eira, A. A. Pereira, M. Nicole, D. Fernandez, M. F. G. de SA, R. M. D. G. Carneiro

- Preliminary Studies on Sources of Resistance in *Coffea arabica* L. to Coffee Blotch Miner, *Leucoptera caffeina* Washbourn
  E. Mendesil, C. Abdeta

- The Development Biology, Morphometrics and Damage Assessment of *Epicampoptera strandi* Bryk, Sub sp. *Glauca* Hmps. (Lepidoptera: Drepanidae) on Robusta Coffee in Nigeria
  F. A. Okelana, J. C. Anikwe

- Studies on Nematodes in Coffee Soils of Nigeria: Survey of Plant Parasitic Nematodes Associated with Coffee
  O. A. Fademi, S. B. Orisajo

- Impact des Facteurs Environnementaux sur les Populations de Scolytes (*Hypothenemus hampei* Ferrari) (Coleoptera: Scolytidae)
  K. Wegbe, C. Cilas, C. Alauzet, B. Decazy

- Reaction of *Coffea arabica G*enotypes to *Meloidogyne* spp.
  R. M. D. G. Carneiro, E. V. S. A. Barros, W. Gonsalves, A. A. Pereira

- Preferential Oviposition of Leaf-miner (*Leucoptera coffeella*) for Erecta Coffee Genotype
  H. P. Medina-Filho, O. Guerreiro-Filho, D. A. Ramiro, R. Bordignon

- The Reduced Infestation of Leaf Miner in Mokka Variety of Coffee and the Implications on its Origin and Breeding
  R. Bordignon, O. Guerreiro-Filho, D. A. Ramiro, H. P. Medina-Filho

- Life Tables of *Hypothenemus hampei* (Ferrari) on Three Coffee Accessions
  J. V. Romero, H. G. Cortina

- Population Density of *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae) and Its Effect on Coffee Berry Borer in Táchira, Venezuela
  A. Torres, Y. A. Amador

- Screening Populations of Arabica Coffee for Molecular Markers Linked to Coffee Berry Disease Resistance
  C.O. Omondi, F. Pinard

- Assessment of Coffee Berry Disease Using Artificial Laboratory Berry Inoculation Method
  F. Pinard, C. O. Omondi

- Progress towards Searching for Durable Resistance to *Fusarium* Wilt (*Fusarium xylarioides*) in *Coffea canephora* Germplasm in Tanzania
  D. L. Kilambo, N. M. Ng'Homa, D. J. Mtenga, J. M. Teri, T. Nzallawahe, R. Mike, L. I. Masumbuko

- Variability on the Insecticidal Protein Concentration within Clones of Transformed *Coffea canephora* Observed in a Field Experiment
  B. Perthuis, T. Leroy, M. Dufour, J. M. Vassal, J. L. Pradon, C. Fenouillet, V. Condina, S. Laurent

- Effective Application of Chemical Strategy against Coffee Berry Borer, *Hypothenemus hampei,* Infestation in Kenya
  H. M. Mugo

- Toxicity and Ovicidal Activity of Some Botanicals against Antestia Bug (*Antestiopsis intricata*) (Ghesquière & Carayon)
  E. Mendesil, C. Abdeta

- Efficacy of Copper Sprays in the Management of Coffee Leaf Rust in Kenya
  G.M. Kairu

- Biological Control of *Meloidogyne incognita* on Coffee Plants using the Isolate P10 of *Pasteuria penetrans*
  R. M. D. G. Carneiro, L. F. G. Mesquita, P. A. S. Cirotto, F. C. Mota, M. R. A. Almeida

- Evaluation of Different Alcohols in Trapping Adult Coffee Berry Borers (*Hypothenemus hampei* Ferrari)
  F. L. Magina, D. F. Mbowe, D. F. Chipungaelo, J. M. Teri

- The soil Fertility Status of Coffee Growing Areas of Tanzania
  G. P. Maro, H. Monyo, E. Nkya, J. M. Teri

- Determining the Effectiveness of Various Lures for Trapping the Black Twig Borer, *Xylosandrus compactus*, in Coffee
  E. Burbano, M. Wright, V. Easton-Smith

- Strategies for Coffee Wilt Disease Management in Robusta Coffee in Kagera Region, Tanzania
  N. M. Ng'homa, D. L. Kilambo, L. Masumbuko, J. M. Teri, S. T. Nzalawahe, M. P. L. Kaiza

---

List of participants

Development and Characterization of a True F2 Population for Genetic and QTL Mapping in Arabica

C. NAGAI[1], M. R. JONES[1], A. E. BYERS[1], D. J. ADAMSKI[1], R. MING[1,2]

[1]Hawaii Agriculture Research Center, Aiea, HI, USA.
[2]University of Illinois at Urbana-Champaign, Dept of Plant Biology, IL.USA

SUMMARY

Arabica coffee is a perennial tetraploid species, and developing a mapping population is time consuming and costly. Limited genetic variation within *C. arabica* also delayed genetic map construction of this commercially important species. A segregating mapping population of Arabica coffee (*Coffea arabica* L.) was developed from a cross between the two varieties, Tall Mokka (MA2-7) and Catimor (T 5175-7-1). Tall Mokka and Catimor are distinctively different in cupping quality as well as leaf, bean, and tree morphology, including branch angle, sub-branching numbers, leaf length and width, cherry and bean size and weight. Difference in cupping quality of the two parents was confirmed by a professional coffee cuppers. An F2 population was generated from two F1 plants, H00-20-25 and H00-20-41, each producing 75 trees. This F2 population was planted at HARC Kunia Station on Oahu in 2003. Phenotypic variations were detected and recorded on tree height and width, branch angle, leaf characteristics, cherry weight, and green bean weight. Cherry/green bean characteristics showed normal distribution. No difference in distribution patterns was found between two sub-populations originated from two F1 trees. The variations of the true F2 population were larger than those of the pseudo F2 population as expected. Additional 200 F2 seedlings were obtained, and are established in the field in 2006. A preliminary genetic linkage map was constructed using 797 polymorphic markers generated from 699 pairs of AFLP markers on the F2- population of Arabica coffee. This map is being used for mapping of quantitative trait loci controlling source and sink traits.

INTRODUCTION

Coffee is one of the most important beverage crops in the world and Hawaii is the only state in the United States where coffee is grown commercially. Coffee industry is economically important ranking as the 5th top crop commodity in Hawaii. Kona, on the island of Hawaii, produces one of the highest valued coffee internationally, while new and unique coffee are sought for other regions in Hawaii. Coffee breeding and selection program designed to develop unique coffee cultivars with high quality for Hawaii was initiated in 1997 (Nagai et al., 2004). Disease resistance and mechanical harvestability including synchronized flowering have been important goals for cultivar improvement for Hawaii Coffee Growers though no rust disease exists in Hawaii.

The narrow genetic diversity detected among Arabica coffee cultivars (Steiger et al., 2002) limits such efforts of new cultivar development. Linkage maps have been constructed using molecular marker in many crops including *C. canephora* (Paillard et al., 1996; Lashermes et al., 2001). Quantitative trait loci (QTL) controlling quality related traits have been mapped in backcross populations derived from interspecific crosses between diploid species of *Coffea* (Ky et al., 2000; Coulibaly et al., 2003). Marker-assisted selection allows screening of large

number of progeny at the seedling stage to expedite the coffee improvement program (Lashermes et al., 1997).

It's more difficult to develop a mapping population of Arabica coffee than many annual crops such as maize and tomato due to coffee's long generation time and limited genetic variation. Linkage and QTL maps of Arabica coffee will be invaluable for breeders whose breeding goals include Arabica specific traits such as cupping quality.

We developed a segregating F1 (pseudo F2) population of Arabica, (Tall Mokka x Catimor) and the first Arabica genetic map was constructed using a pseudo F2 population with 61 plants and amplified fragment length polymorphism (AFLP) markers (Pearl et al., 2004). We report here the development of a true F2 mapping population and characterization of its phenotypic characteristics including tree, leaf, and cherry/bean morphology. A preliminary linkage map of F2 was obtained to be used for mapping quantitative trait loci (QTL) controlling commercially important traits in coffee.

MATERIALS AND METHODS

Development of Mapping Population

A segregating F1 and F2 mapping population of Arabica coffee (*Coffea arabica* L.) was developed from a cross between the two varieties, Tall Mokka (MA2-7) and Catimor (T 5175-7-1 in 2000. Catimor, T5175-7, is a variety developed at Promecafe in Central America from a hybrid between *C. arabica* and *C. canephora* and backcrossed to *C. arabica,* Catuai (Osorto, 1991)**.** We obtained seeds of T5175-7 from Promecafe.

Table 1. Difference in cherry and tree characteristics of the two parents of an F_2 mapping population of *C. arabica*.

Traits	Catimor T5175-7-1	Tall Mokka MA2-7
Tree width**	80.6 ± 2.3 cm	64.8 ± 5.4 cm
Leaf size/shape**	Large Length:15.5 ± 0.2 cm Width: 7.7 ± 0.2 cm	Small Length: 11.4 ± 0.3 Width: 3.7± 0.2 cm
Branch angle**	More plagiotropic 64.0°±2.6	More orthotropic 46.6° ± 3.9
Secondary Branching	+ Primary branches	+++ Secondary and tertiary branches
Yield estimate[(2)] **	High 1.78 ± 0.03 kg	Low 0.93 ± 0.06 kg
Cherry/bean: size/shape 100 Cherry weight** Bean weight[(3)] **	Large , oblong 192.2 ± 6.8 g 27.9 ± 1.0 g	Small, round 122.2 ± 5.5 g 24.5 ± 0.2 g

*[(1)]2004 harvest at HARC Kunia field M., M. Alves unpublished Data (2005). [(2)]Yield was estimated by collecting all the cherries from 5 branches of 3 trees. [(3)]Form 100 Cherries with parchment. [(4)]Vander Vossen (2001). **Significant at P=0.01.*

Catimor trees typically have large, round leaves, resistance to rust, high cherry yield and large size beans, but known to have lower cupping quality. Mokka, a mutant of variety Bourbon, was developed in Brazil and has small, thin leaves, small beans, rust susceptible, and excellent cupping quality. Tall Mokka trees were imported to the University of Hawaii Collection in 1950s by Hamilton (University of Hawaii unpublished information) and later it was propagated on Maui for commercial use. MA2-7, Tall Mokka, is taller and more vigorous than the original mokka mutant (H. P. Medina-Filho, personal communication). No difference

was found in tree height between the two parents. Tall Mokka and Catimor are distinctively different in cupping quality as confirmed by professional coffee cuppers. The results of cupping tests on coffee beans harvested in 2004 showed Mokka Hybrid as "almost premium coffee with sweet/mellow flavor" while Catimor as "no premium quality with bland/astringent flavor" (M. Alves, Coffee Lab International). Difference in various traits in both parents was summarized in Table 1.

A total of 130 plants of the F1 population (pseudo F2) was planted in 2001 at HARC Kunia substation, Oahu together with selfed progenies of both parents. A true F2 population was generated in 2002 from two F1 plants, 00-20-25 and 00-20-41, each producing 75 trees. This F2 population was planted at HARC's Kunia Substation, Oahu, Hawaii in November 2003. Additional F_2 seeds of the same two F1 treeswere obtained and 125 seedlings will be planted in the field in September 2006.

Phenotypic Data Collection

Phenotypic data of F1 and F2 were collected for tree and leaf morphology and cherry and bean characteristics from 2002 to 2006. Cherry size and weight and green bean weight of F_2 individuals were collected in 2005 and 2006.

Linkage Mapping

Sixty-one plants of F2 sub-population H00-20-25 were used for linkage mapping.

Genomic DNA isolation and digestion was conducted as described previously (Pearl et al., 2004). Pre-amplification was performed as detailed by Vos et al. (1995), and selective amplification was completed according to the protocol of Qiagen (Valencia, CA). Combinations of thirty infrared dye labeled *Eco*R I primers and thirty-six non-labeled *Mse* I primers were used to screen the population. Selective amplification of each primer combination was run on a Li-Cor IR2 Automated DNA Sequencer.

Data analysis and map construction

AFLP polymorphic markers were manually scored as numerical data corresponding to dominant parental type. For Tall Mokka dominant markers, presence of a band was scored as a "5" and absence of a band was scored as "3." Catimor dominant markers were scored as "4" for the presence of a band and "1" for the absence of a band. Map construction was carried out using JointMap 3.0 with Haldane's function and a LOD threshold of 3.0.

RESULTS AND DISCUSSION

Tree and leaf morphology

Visual variation among F2 trees were large and distinct (Figure 1). Phenotypic data of the F2 plants confirmed that the variation among F2 trees in tree height and width, branch angle, and leaf characteristics. Both Catimor and Tall Mokka leaf morphology and their intermediaries were found in a typical F2 population (Figure 2c, d). Leaf length and width were measured for each tree in the population. F2 mean was between Tall Mokka and Catimor (Figure 2a, b). Variation in tree height and width were also observed (data not shown).

Figure 1. Variation in tree morphology among F2 trees.

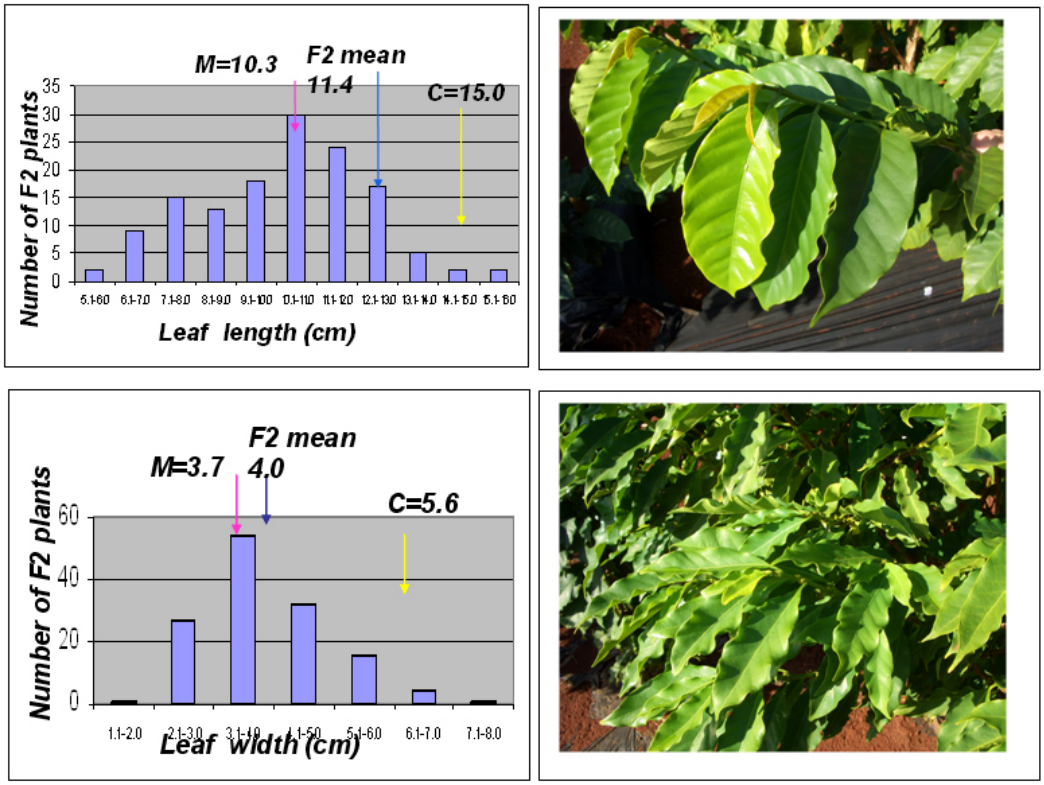


Figure 2. a,b: Distribution of leaf length (cm) and width (cm) in F2 population of Tall Mokka x Catimor. c,d: Catimor type leaves (top) and Tall Mokka type leaves (bottom).

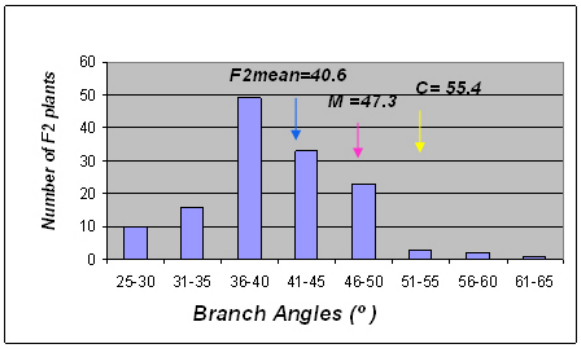


Figure 3. Branch angles of F2 population of Tall Mokka x Catimor.

Cherry and bean characteristics

Cherry and green bean characteristics data are collected in multiple years at one location. For F1 (pseudo F2) cherries were harvested in 2003-2005, while data collection was completed for 2005 (1st year) in this report. Cherry weight (100 cherry) and green bean weight (from 100 cherries) were collected twice (September and December) in 2005. Cherry/green bean weight showed normal distribution (Figure 4). Cherries will be harvested in 2006 as 2nd year data. No difference in distribution patterns was found between two sub-populations originated from two F1 trees (H00-20-25F2 and H00-20-41F2) (data not shown). The variation of 100 cherry weight in the true F2 population (mean = 138.5 g CV = 12.8% n = 106)) was larger than that of the pseudo F2 population (mean = 165.5 g CV = 10.6% n = 59) as expected (Figure 5). Difference in green bean weight variation in two populations were also larger in true F2 (mean = 20.2 g CV = 16.2%, n = 106) than in pseudo F2 (mean = 21.2 g CV = 11.4%, n = 59). These quantitative data on this F2 population will be used to map QTLs.

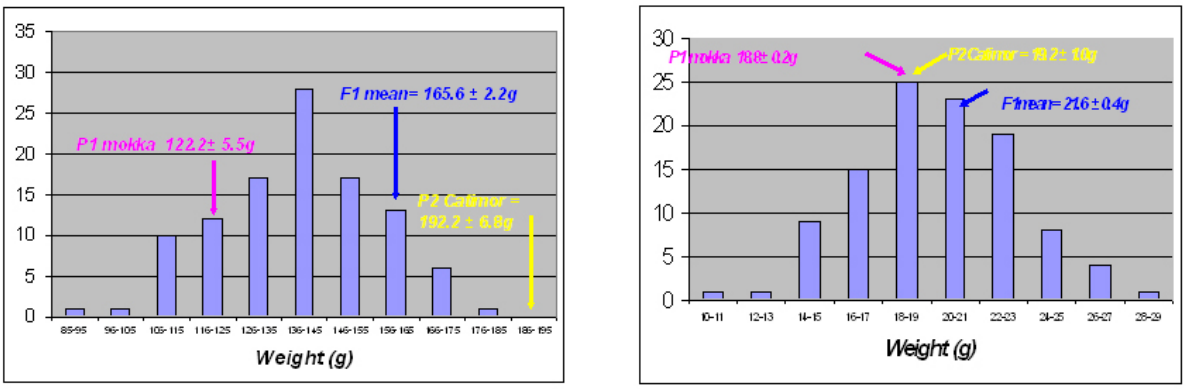
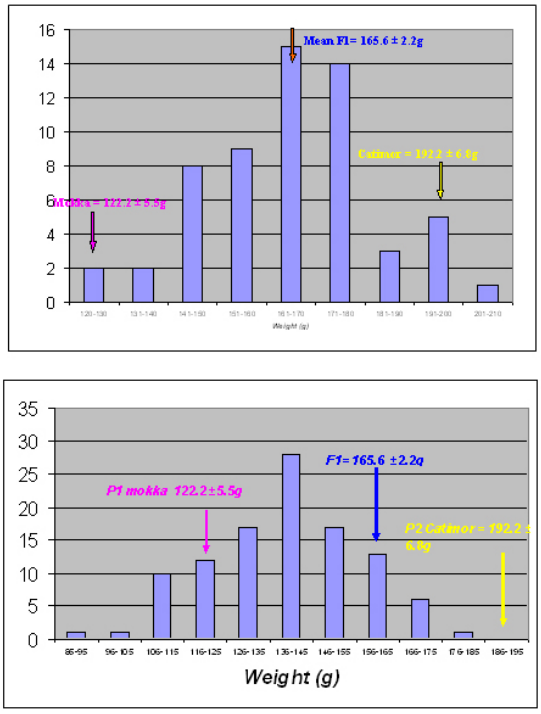


Figure 4. Distribution of 100 cherry weight (g) and green bean weight from 100 cherries in F2 population of Tall Mokka x Catimor.



Mean= 165.6 (g)
CV= 10.6%
n=59

Mean= 165.6 (g)
CV= 10.6%
n=59

Figure 5. Comparison of F1 (pseudoF2) and F2 population for cherry weight distribution.

Linkage Mapping

A preliminary linkage map was constructed using 61 plants of F2 sub-population of H00-20-25. A total of 797 polymorphic markers was generated from 699 pairs of AFLP primers and 511 of them were mapped on forty linkage groups. Among the mapped markers, 263 (51.4%) were originated from Catimor; 245 (47.9%) were from Mokka; and 3 (0.05%) were co-dominant. The total length of linkage map was 1,042.4 cM, and average genetic distance between adjacent markers was 2.0 cM. Additional polymorphic markers are being generated for construction of a high density linkage map. QTL mapping for the traits characterized will be carried out after this linkage map is completed.

ACKNOWLEDGEMENTS

We thank Josie Buenafe, Nellie Rosete, Mark Pascua and Gail Uruu for technical assistance, Dr. Qingyi Yu for supervising molecular biology work, and Lance Stanto for maintenance of the mapping population.

This work was supported by USDA-ARS Cooperative Agreement (No. CA58-5320-3-460) with Hawaii Agriculture Research Center.

REFERENCES

Coulibaly, I., B. Revol, M. Noirot, V. Poncet, M. Lorieux, C. Carasco-Lacombe, J. Minier, M. Dufour, and P. Hamon, 2003b. AFLP and SSR polymorphism in a *Coffea* interspecific backcross progeny [(*C. heterocalyx* x *C. canephora*) x *C. canephora*]. Theor. Appl. Genet. 107:1148-1155.

Ky, C.-L., P. Barre, M. Lorieux, P. Trouslot, S. Akaffou, J. Louarn, A. Charrier, H. Hamon, and M. Noirot, 2000. Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.). Theor. Appl. Genet. 101:669-676.

Lashermes P, M.C.Combs, N.S.Prakash, P. Trouslot, M. Lorieux, A, Charrier, 2001. Genetic linkage map of Coffea canephora: effect of segregation rate in male and female meioses. Genome 44:589-596

Paillard, M., P. Lasherme, and V. Petiard. 1996. Construction of a molecular linkage map in coffee. Theor Appl Genet 93:41-47

Pearl, H.M, C. Nagai, P.H. Moore, D.L. Steiger, R.V. Osgood, R. Ming. 2004. Construction of a genetic map for arabica coffee. Thoer. Appl. Genet. 108:829-835.

Nagai, C., R.V. Osgood, C. Cavaletto, H.C. Bittenbender, K. Weaver, J. Clayton, M. Jackson, R. Loero and R. Ming. 2004. Breeding and selection of coffee cultivars for Hawaii with high cupping quality using Mokka hybrids. Proceedings (CD-RAM). 20th ASIC International Conference on Coffee Science, Bangalore, India.

Osorto, J.J. 1991. Coffee variety and selection program in Central American countries. 451-459. Proceedings of 14th ASIC International Conference on Coffee Science, San Francisco.

Steiger, D.L., C. Nagai, P.H. Moore, P.H. Morden, R.V. Osgood, and R. Ming. 2002. AFLP analysis of genetic diversity within and among coffea arabica cultivars. Thoer. Appl. Genet. 108:209-215.

Van der Vossen, H.. 2001. Coffee breeding practices. In: Clarke, R.J. and Vitzthum O.G. (eds.) Coffee Recent Development- Agronomy1. Blackwell Science Ltd., London, pp.184-201.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23**:4407-4414

Genetic Diversity of Commercial Coffee (*C. arabica* L.) from America, India and Africa Assessed by Simple Sequence Repeats (SSRs)

P. TORNINCASA[1], R. DREOS[1], B. DE NARDI[1], E. ASQUINI[1], J. DEVASIA[2], M. K. MISHRA[2], L. DEL TERRA[1], P. CRISAFULLI[1], A. PALLAVICINI[1], G. GRAZIOSI[1]

[1]Dept. of Biology, University of Trieste, Piazzale Valmaura, 9 –34100– Trieste, Italy
[2]Research Dept., Coffee Board, Bangalore – 560001 – Karnataka, India

SUMMARY

Forty five accessions of commercial *Coffea arabica* collected form America, India and Africa and ninety six accessions belonging to four native Ethiopian populations of commercial coffee were analysed using twelve highly polymorphic microsatellites markers. The data was analysed and a phenetic tree was constructed using PHYLIP software. The microsatellites could differentiate almost all the cultivars. The tree revealed clustering of the individual populations. Wide genetic variability was obtained in native Ethiopian samples compared to the other commercial varieties. The study proved that: (1) the commercial varieties are rather homogeneous; (2) the Indian and American commercial varieties differ between them; (3) the genetic diversity within and between the Ethiopian populations is enormous.

INTRODUCTION

One of the major hurdles of *Coffea arabica* breeding programmes is the selection of parental lines purely based on quality and agronomic traits (Maluf et al., 2005). *C. arabica* is the only tetraploid (2n = 44) and self-fertile species and most of the presently cultivated commercial varieties are derived from a small number of inbred plant populations (Van der Vossen, 1985; Lashermes et al., 1996). These aspects confer a very low genetic diversity of commercial varieties of *C. arabica*. As a result, it is difficult to distinguish different arabica cultivars from each other. The low genetic variability in commercial varieties has also resulted in poor adaptability and high susceptibility of the arabica cultivars to many pests and diseases (Bertrand et al., 1997).

Widening the genetic base of *C. arabica* has direct relevance in improving the genetic traits, resistance to various pests and diseases and also the bean quality. The genetic base of a crop species can be defined as the extent of availability of genetically heterogeneous plant population. South Western highlands of Ethiopia is reported to be the place of origin of *C. arabica*. A greater genetic diversity of a species is generally found in its native place of origin. (Sylvain, 1958; Strenge, 1956; Meyer, 1965).

In recent years, molecular markers have been used for plant germplasm characterisation and cultivar identification. Some of the most commonly used molecular markers are RAPD, AFLP and Microsatellites. Studies reported on the genetic diversity of the Ethiopian populations used RAPD, ISSR and ISTR to analyse the variability in the population (Aga et al., 2003; Aga et al., 2005; Aga and Bryngelsson, 2005). The results of these studies are however not comparable to each other due to the diversity among markers employed and moreover only Ethiopian samples have been analysed. The current study analyses the genetic

variability using microsatellites, also known as SSRs (Simple Sequence Repeats). When compared to other markers, SSR have some advantages in that they are abundant, uniformly and randomly distributed in the euchromatin of eukaryotic genomes (Tautz and Renz, 1984; Wang et al., 1994). They are inherited in a co-dominant Mendelian manner and are stable and highly polymorphic. In addition, SSR markers are analytically simple and the results are also reproducible between different laboratories. Microsatellites markers have been developed and utilized for cultivar and germplasm characterisation in many crop species *viz.,* rice (Chakravarthi and Naravaneni, 2006), potato (Ashkenazi et al., 2001), soybean (Priolli et al., 2002) wheat (Manifesto et al., 2001) and coffee (Lashermes et al., 2000; Rovelli et al.; 2000, Baruah et al., 2003).

In the present study, *Coffea arabica* accessions from many coffee producing countries around the world were collected. For the first time, a global comparative analysis of the genetic variability was carried out using same molecular markers and experimental conditions.

MATERIAL AND METHODS

Plant material

Thirty two commercial coffee cultivars, thirteen inter-specific hybrids and twenty four plants each of four Ethiopian populations of *C. arabica* were used in the study (Table 1). The commercial coffee cultivars and inter specific hybrids were collected from America, India and Africa. Of the four Ethiopian populations, three were collected from farms in Agaro region and one population was from Yirga ch'efè region. Twelve highly polymorphic microsatellites were used to analyse the variability of the lines.

DNA extraction and purification

Genomic DNA was extracted from fresh young leaves or lyophilized leaves following the CTAB techniques (Murray and Thompson, 1980) and DNA was extracted from seeds following the protocol of Chang et al. (1993). The latter protocol, which was setup to extract RNA, was employed for extraction of DNA by substituting the LiCl with 0,6 vol of isopropanol for the DNA precipitation.

Construction and enrichment of the genomic library

A genomic library was constructed from DNA of *C. arabica* var. Bourbon Tekisic. The polymorphisms were assessed on 17 and 41 plants of F_1 and F_2 population (Sarchimor T5296t5 x Etiopica-6) respectively and on 96 plants of F_2 population (Caturra x Ethiopica-30).

The library was enriched in $(ACA)_{15}$, $(AGA)_{15}$, $(CAT)_{15}$, $(CTA)_{15}$ $(GA)_{20}$, $(CA)_{20}$. The basic approach reported by Rafalski et al. (1996) and Morgante et al. (1998) was adopted for microsatellites enrichment with some modifications as follows: Genomic DNA digestion was carried out with *HaeIII*, *AluI* and *RsaI* (NEB); DNA fragment selection was made between 500 and 800 bp from agarose gel; fragment ligation was made to an adapter containing an *EcoRV* site; magnetic beads conjugated with biotin-streptavidin oligonucleotides (Promega™) was used in the enrichment process; PCR amplification of the library was carried out by priming the adapters and the PCR purification by the Nucleobond AX® (Macherey-Nagel) kit; DNA ligation was made in the *EcoRV* site of pZErO®-1 (Invitrogen) vector; the transformation of DH10B electrocompetent cells was made with plasmid vector and the library was screened to identify the positive clones.

Table 1. *C. arabica* cultivar and interspecific hybrids (in bold) used for genotyping.

Plant	Origin	Plant	Origin
Aramosa	Brazil, Patrocinio	Catuai yellow	Guatemala
Bourbon VD	Brazil, Patrocinio	Bourbon red	Guatemala
Catuai 99	Brazil, Patrocinio	Bourbon tekisic	Guatemala
Catuai 144	Brazil, Patrocinio	Ethiopica wild 218	Brazil, Campinas, IAC
Caturra 239	Brazil, Patrocinio	Ethiopica wild 217	Brazil, Campinas, IAC
Laurina	Brazil, Patrocinio	Ethiopica-30	France, Montpellier, IRD
Laurina purpuracens	Brazil, Patrocinio	Sarchimor T5296t5	France, Montpellier, IRD
Laurina 26 rev	Brazil, Patrocinio	Caturra	France, Montpellier, IRD
Maragogype	Brazil, Patrocinio	Tafarifela Sln 4	India, Karnataka Chikmagalur
Mundo Novo	Brazil, Patrocinio	Sln. 3	India, Karnataka Chikmagalur
Mundo Novo acaià	Brazil, Patrocinio	Sln. 5A	India, Karnataka Chikmagalur
Pacas	Brazil, Patrocinio	Sln. 5B	India, Karnataka Chikmagalur
Icatu-56	Brazil, Patrocinio	Sln. 7	India, Karnataka Chikmagalur
Etypica	Brazil, Patrocinio	Sln. 9	India, Karnataka Chikmagalur
Tupi	Brazil, Patrocinio	Sln. 12	India, Karnataka Chikmagalur
Catuai	Brazil	KP 423	Africa Tanzania, Mufindi Estate
Laurina 2	Brazil, Minas Gerais Sao Sebastiao Paraiso	POP F6	Africa Tanzania, Mufindi Estate
Catimor	Brazil, Londrina, Paranà	K7	Africa Kenya, Mchana Estate Ruiru
Iapar 59	Brazil, Londrina, Paranà	SL 28	Africa Kenya, Mchana Estate Ruiru
Bourbon Yellow	Brazil, Campinas, IAC	SL 34	Africa Kenya, Mchana Estate Ruiru
Catuai red	Costa Rica, CATIE	Agaro-1 (1-24)	Agaro, Ethiopia
Ibrido di Timor	Costa Rica, CATIE	Agaro-2 (1-24)	Agaro, Ethiopia
Sarchimor 227	Costa Rica, CATIE	Agaro-3 (1-24)	Agaro, Ethiopia
Typica 221	Costa Rica, CATIE	Yirga (1-24)	Yirga ch'efè, Ethiopia
Ethiopica-6 T16695	Costa Rica CICAFE-1 Trial		

Primer design

Pairs of primers were designed on the regions flanking the microsatellites. The online software PRIMER3 (Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA) was used choosing a T_m of 60 °C. The constant tail KS (5'-

TCGAGGTCGACGGTATC-3') was added to the 5' end of each Forward primer. Primers were synthesized by MWG Biotech.

Amplification and analysis of the microsatellites

The microsatellites were amplified with touchdown PCR as described by Hecker and Roux (1996). A three primer system described by Mettulio et al. (1999) was used. The amplification conditions were: denaturation for 4 min at 96 °C, denaturation for 35s at 94 °C, annealing for 40s at decreasing temperature from 59 °C to 54 °C, elongation for 1 min at 72 °C and 32 cycles of denaturation for 30s at 94 °C, annealing for 30s at 54 °C and elongation for 35s at 72 °C (30 min for last cycle).

The amplified fragments were run on 6% acrylamide gel in an automatic sequencer ABI 373A. The allele identification was performed by the Perkin Elmer GENESCAN 672 software (1993).

Diversity evaluation

The data on polymorphic alleles obtained after detailed screening of the acrylamide gel was analysed using MICROSATELLITE ANALYSER (MSA) (Dieringer and Schlötterer, 2003) choosing the following options: Number of Nucleotides for Alleles Sizes, Repeat Length of two, Distances as $(\delta\mu)^2$ adjusting it for small sample size, using Taxon-Specific alleles for Statistics and specifying a Bootstrap value of mean of 1000. MSA provides input files as distance matrix(es) of genetic distances compatible to PHYLIP (Felsenstein, 1989), the software used to create trees. NEIGHBOR and DRAW TREE softwares of PHYLIP were used to make the final rooted phenetic trees of the populations and lines involved in the study.

RESULTS AND DISCUSSION

About 1,700 clones of the microsatellite enriched genomic library were picked up and about 800 of them were sequenced. After assembling to eliminate the redundant sequences, about 400 unique sequences were obtained, 287 of which were suitable for primer design. 97 primers gave a PCR amplification product. The analysis carried out on the F_1 and F_2 populations of two crosses, Sarchimor x Etiopica-6 and Caturra x Ethiopica-30 revealed 30 polymorphic microsatellites. Twelve of these microsatellites, SAT-11-A03, SAT-09-B05, SAT-09-C08, SAT-02-G09, SAT-03-A06b, SAT-03-A06a, SAT-13-B01, SAT-12-B05, SAT-10-A06, SAT-10-E02, SAT-11-E05, SAT-03-E10 were found to be highly polymorphic and were selected for the study. The details of the microsatellites are freely available in the coffee genome website *www.coffeedna.net* developed by the Laboratory of Genetics, University of Trieste, Italy.

The phenetic tree of the 32 commercial coffee cultivars, 13 inter-specific hybrids and 24 plants each of 4 Ethiopian populations used in the study is shown in Figure 1. The phenetic tree of the different groups of populations used in the study is shown in Figure 2. The dendrogram of the varieties analysed (Figure 1) revealed a clear clustering of the various populations used in the study according to their origin. The commercial varieties were clearly demarcated form the Ethiopian populations of Yirga ch'efè and Agaro regions (Figure 2). Among the commercial varieties analysed, the African varieties from Kenya and Tanzania were found to be very close to the American. This probably could be due to the origin of commercial cultivars from very few accessions which were introduced originally in that region and also due to continuous breeding programmes within a narrow genetic base (Maluf et. al., 2005). Interestingly, three commercial varieties such as Catimor, IAPAR 59 and Tupi

were not integrated with the commercial varieties but clustered in between Agaro population. However these plants were quite distinct from the Ethiopian population as observed in Figure 1. In fact all the three varieties were developed at CIFC (Centro de Investigação das Ferrugens do Cafeeiro), Portugal involving Hybrido de Timor in their origin. The difference between these three plants is that while Catimor involves Caturra as one of the parent, both Tupi and IAPAR 59 involve Villa Sarchi in their origin. Although they were quite close to each other, yet they are differentiated by their genetic base as revealed by SSR markers.

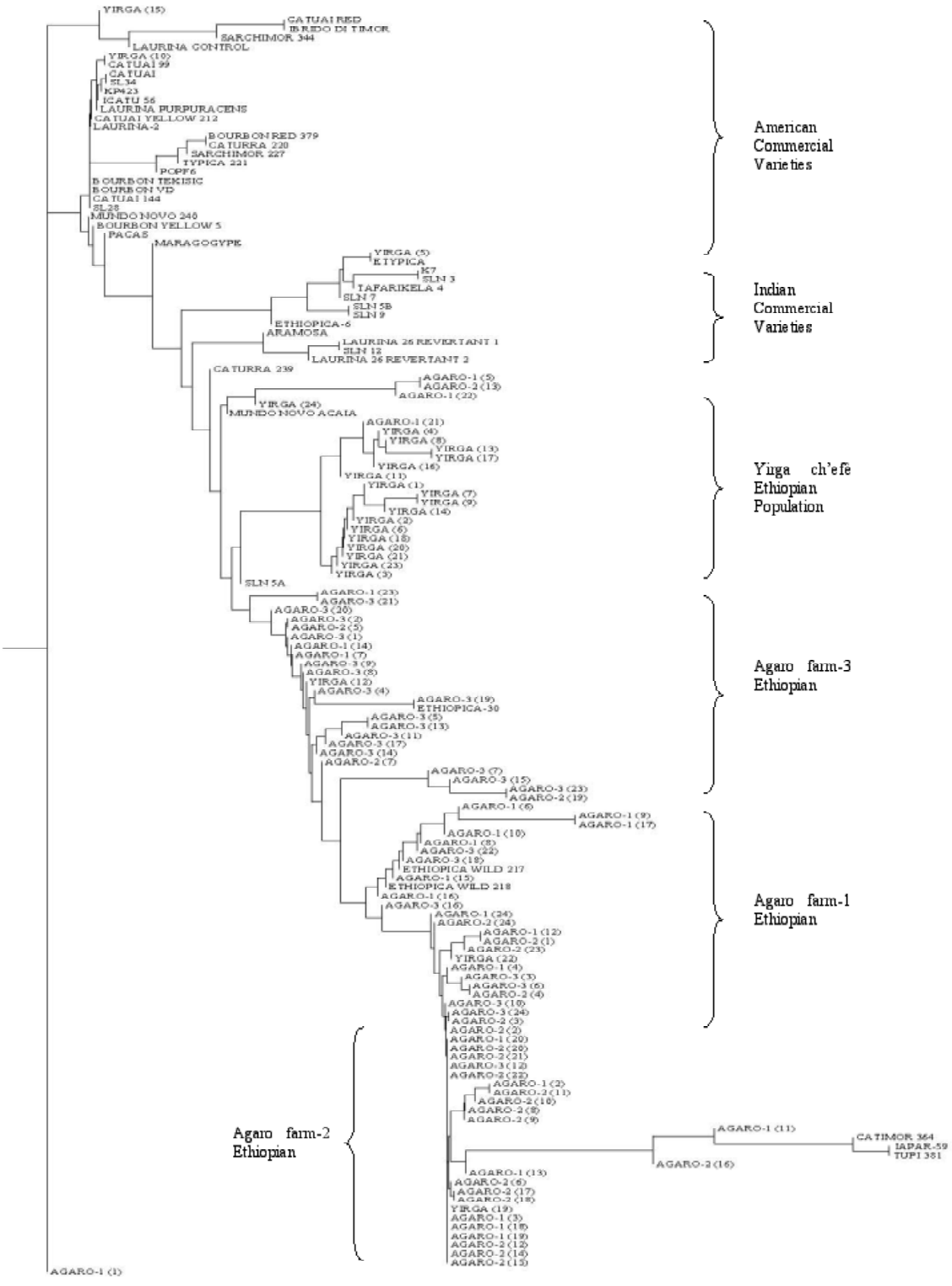


Figure 1. Phenetic tree of 45 accessions of commercial Coffea arabica and 96 accessions of four native Ethiopian populations.

The Indian commercial varieties formed a separate cluster and separated from the American and African commercial cultivars. This situation could be explained by the fact that most of the Indian cultivars used in the present study were derived from natural interspecific hybrids involving *C. liberica* / *C. canephora* lineages. The prevalence of SH_3 gene in Indian arabica (Sln.3) is believed to be originated from *C. liberica* and similarly Sln.5 involves Devamachy which is also a putative robusta-arabica hybrid. Among the Indian varieties, Sln.5A was far apart from the other cultivars. This could be due to the origin of this cultivar involving Rume Sudan which is an African variety and known to be genetically diverse from the cultivated arabicas (Agwanda et al., 1997). The position of Sln.5A in the tree near to Ethiopian arabicas justifies our contention.

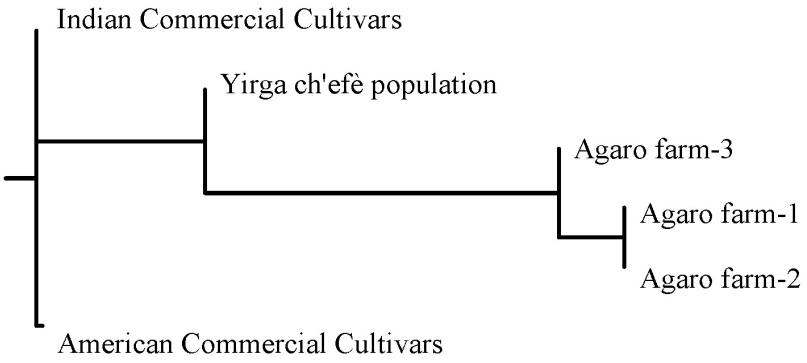


Figure 2. Phenetic tree of population groups involved in the study.

The commercial Ethiopian arabica presents an interesting situation. Of the four Ethiopian populations studied, three were from Agaro Region (South West of Addis Abeba) and one was from Yirga ch'efè Region (South of Addis Abeba). SSR markers clearly separated the Yirga and Agaro populations thereby indicating the existence of genetic variability among these populations. Since Yirga and Agaro represent two different regions separated by a distance of about 250 Km, genetic diversity in *C. arabica* in these places presumably reflect independent evolutionary events in these populations.

The three populations of Agaro, which were selected from farms located very close to each other, did not make clear independent clusters but intermixed with each other (Figure 1). Nevertheless, high variation in these three population was evident. Since all these populations were very close to each other, hybridization could have taken place, which might have resulted in close affinity between some of these plants. As observed in the phenetic tree, about 12.5% of the plants in Agaro population exhibit significant genetic divergence.

About 30% of the Yirga ch'efè population was scattered all along the dendogram indicating wide allelic diversity among the Yirga ch'efè population. The remaining 70% clustered together forming a single group with high internal diversity.

CONCLUSIONS

In the present global comparative genetic analysis of arabica populations comprising both commercial varieties and commercial Ethiopian germplasm using SSR markers, two important inferences could be drawn. First, the study has clearly demonstrated the existence of wide genetic variability among the Ethiopian arabicas. This makes Ethiopia a good source of valuable breeding material for *C. arabica.* Secondly, the common commercial varieties exhibited a low genetic diversity, a situation not favourable for arabica coffee. The main requirement of any plant improvement programme is a population with high genetic

variability. The present study has pointed out the place where such variability could be found. The study also confirmed that microsatellites markers are powerful tool to differentiate various populations and to study genetic variation within a population.

ACKNOWLEDGEMENTS

We thank immensely Mr. Patrizio Brusoni of M/s Hesse, Trieste, Italy and Mr. Gigi Micheli of M/s Illy Café, Trieste, Italy for giving the material from Ethiopia for the research study. We wish to place on records the valuable support of Dr. Simeone Dal Monego from Laboratory of Genetics, Trieste, on coffee informatics.

REFERENCES

Aga E., Bryngelsson T., Bekele E., Salomon B. (2003) Genetic diversity of forest arabica coffee (*C. arabica* L.) in Ethiopia as revealed by random amplified polymorphic DNA (RAPD) analysis. *Hereditas* 138: p. 36-46.

Aga E., Bekele E., Bryngelsson T., (2005) Inter-simple sequence repeat (ISSR) variation in forest coffee trees (*C. arabica* L) populations from Ethiopia. *Genetica* 124: p. 213-221.

Aga E., Bryngelsson T., (2005) Inverse sequence-tagged repeat (ISTR) analysis of genetic variability in forest coffee (*C. arabica* L) from Ethiopia. *Genetic resources and crop evolution* (in press).

Agwanda C.O., Lashermes P., Trouslot P., Combes M-C., Charrier A. (1997) Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee. *Euphytica*, Volume 97, 2, p. 241-248(8).

Ashkenazi V., Chani E., Lavi U., Levy D., Hillel J., Veilleux R.E. (2001) Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. *Genome* 44: 50-62.

Baruah A, Hendre PS, Rajkumar R, Rajendrakumar P, Aggarwal K (2003) Isolation and characterization of nine microsatellite markers from *Coffea arabica* L. showing wide cross-species amplifications. *Mol. Ecol. Notes* 3: 647-650.

Bertrand B., Aguilar G., Bompard E., Rafinon A., Anthony F. (1997) Comportement agronomique et résistance aux principaux déprédateurs des lignées de Sarchimor et Catimor au Costa Rica. *Plantation, Recherche, Développement*, 5: 312-321.

Chang S., Puryear J., Cairney J. (1993) A simple and efficient method for isolating RNA from pine trees. *Plant Biology Reporter* 11: 113-116.

Chakravarthi B.K., Naravaneni R. (2006) SSR marker based DNA fingerprinting and diversity study in rice (Oryza sativa L.). *African Journal of Biotechnology*, 5(9), p. 684-688.

Dieringer D., Schlötterer C. (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* 3 (1), p. 167-169.

Felsenstein, J. (1989) PHYLIP - Phylogeny Inference Package (Version 3.2). *Cladistics* 5: 164-166.

Hecker K.H., Roux K.H. (1996) High and low annealing temperature increase both specificity and yield in touchdown and stepdown PCR. *Biotechniques* 20: 478-485.

Lashermes P., Trouslot P., Anthony F., Combes M.C., Charrier A. (1996) Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. *Euphitica*, 87: 59-64.

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S., Graziosi G., Trouslot P., Anthony F. (2000) Molecular analysis of introgressive breeding in coffee (*C. arabica* L.). *Theor. Appl. Gen*. 100: 139-146.

Maluf M.P., Slvestrini M., Ruggeiro L.M., Filho O.G., Colombo C.A.(2005) Genetic Diversity of Cultivated *Coffea arabica* inbred lines assessed by RAPD, AFLP and SSR marker systems. *Sci. Agric.*, v. 62, n.4, p366-373

Manifesto M.M., Schlatter A.R., Hopp H.E., Suarez E.Y., Dubcobsky J. (2001) Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop sci*. 41: 682-690.

Mettulo R., Rovelli P., Anthony F., Anzueto F., Lashermes P., Graziosi G. (1999) Polymorphic microsatellites in *Coffea arabica*. *ASIC, 18e colloque, Helsinki*.

Meyer G.F. (1965) Notes on wild *Coffea arabica* from Southwestern Ethiopia, with some historical considerations. *Econ. Bot*. 19: 136-151.

Morgante M., Pfeiffer A. Jurman A., Paglia G., Olivieri A.M. (1998) Microsatellite markers in plants. In: Karp A., Isaac P.G., Ingram D.S. (eds) Molecular tools for screening biodiversity. Plants and animals. *Chapman and Hall*, p. 206-208, 288-296.

Murray M.G., Thompson W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res*. 8: 4321-4325.

Priolli R.H.G., Mendes C.T., Arantes N.E., Contel E.P.B. (2002) Characterisation of Brazilian soybean cultivars using microsatellite markers. *Gen and Molec. Biol*. 25,2: 185-193.

Rafalski J.A., Vogel L.M., Morgante M., Powell W., André C. Tingey S.V. (1996) Generating and using DNA markers in plants. In: Biren B., Lai E. (eds) Nonmammalian genomic analysis: a practical guide. *Academic press, London New York*, p. 75-134.

Rovelli P., Mettulio R., Anthony F., Anzueto F., Lashermes P., Graziosi G. (2000) Microsatellites in *Coffea arabica* L. In: International seminar on biotechnology in the coffee agroindustry, 3. Londrina. *Coffee Biotechnology and quality*; proceedings. Dordrecht: Kluwer Academic Publishers p. 123-133.

Strenge H. (1956) Wild coffee in Kaffa Province in Ethiopia. *Tropical Agriculture Trinidad*, 33: 297-301.

Sylvain P.G. (1958) Some observations on *Coffea arabica* L. in Ethiopia. *Turrialba* 5, 37-53.

Tautz D., Renz M. (1984) Simple sequences are ubiquitous components of eukaryotic genomes. *Nucleic acids Res*., 12: 4127-4138.

Van der Vossen H.A.M. (1985) Coffee selection and breeding. In: Coffee: botany, biochemistry and production of beans and beverage (eds Clifford M.N. and Willson K.C.). *Croom Helm, London and Sydney*, p. 48-97.

Wang Z., Weber J.L., Zhong G., Tanksley S.D. (1994) Survey of plant short tandem DNA repeats. *Theor. Appl. Gen.*, 88: 1-6.

Characterisation and Genetic Mapping of a Gene Conferring Resistance to Coffee Berry Disease (*Colletotrichum kahawae*) in Arabica Coffee (*Coffea arabica* L.)

E. K. GICHURU[1], C. O. AGWANDA[1]*, M-C. COMBES[2], E. W. MUTITU[3], E. C. K. NGUGI[3], B. BERTRAND[4], P. LASHERMES[2]

[1]Coffee Research Foundation (CRF), P O Box 4, Ruiru 00232, Kenya
[2]Institut de Recherche pour le Développement (IRD), UMR DGPC, Résistance des Plantes, 911, Av. de Agropolis, BP 64501, F-34394, Montpellier, France
[3]University of Nairobi, P O Box 29053, Nairobi, Kenya
[4]Centre de coopération Internationale en Recherche Agronomique pour le Développement, (CIRAD-CP). UMR-DGPC, IRD, 911 Av. de Agropolis, BP 64501, F-34394, Montpellier, France
*Current address: Coffee Research Network (CORNET), CAB International – Africa Regional Centre, P. O. Box 633-00621, Nairobi, Kenya

SUMAMRY

Coffee Berry Disease (CBD) which is an anthracnose of coffee berries caused by *Colletotrichum kahawae* Waller and Bridge, is a major problem in Arabica coffee (Coffea arabica L.) producing countries in Africa. Breeding for resistance to this disease has thus been a major priority to these countries. One of the sources of resistance to this disease is genomic material of *Coffea canephora* Pierre introgressed into *C. arabica* genome. This introgression occured via a natural cross between *C. arabica* and *C. canephora*, referred to as Hibrido de Timor (or Timor hybrid). In order to decipher the genetic base of the resistance gene, an F2 mapping population of a cross between Catimor as the donor and SL28 as the susceptible parent was established. Catimor is a *C. arabica* cultivar derived from Hibrido de Timor by crossing to Caturra, while SL28 is a susceptible commercial variety in Kenya. The population was screened for resistance to the disease by a two-step strategy which facilitated both verification of segregation and availability of counter-checking plant materials during the study. The first screening step was carried out 6 weeks after germination on half of the population by hypocotyls inoculation method. The second screening for CBD reaction was done after 1 year by young seedlings inoculation method on the other half of the population (Group 2) and resistant plants obtained during the hypocotyls inoculation step (Group 1). Microsatellite and Amplified Fragment Length polymorphism (AFLP) markers were used to analyse up to 95 segregating plants screened only by the shoot tips method, and 27 plants screened by both methods and identified as resistant. The use of the two screening methods helped to counter the disadvantages of each while providing a comparative verification system involving the two phenotypic classification and molecular markers. The results obtained by the molecular markers enabled the identification and mapping of a simply inherited major resistance gene, designated *Ck*-1, which is likely to be the one reported by van der Vossen and Walyaro (1980). The gene was located onto a mapped *C. canephora* chromosomal fragment introgressed into *C. arabica* genome. The significance of the results and further research needs in this line are discussed.

INTRODUCTION

Arabica coffee (*Coffea arabica* L.) accounts for about 75% of the total world coffee production and the rest is mainly Robusta coffee (*Coffea canephora* Pierre). Production of *C. arabica* is constrained by several diseases whose control is mainly by use of chemicals which account for up to 30% of the total costs of production (Masaba and Waller, 1992). One of the major coffee diseases of Arabica coffee in Africa is Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* and it can cause more than 50% crop loss (Griffiths et al., 1971; van der Graaff, 1978). Breeding of cultivars resistant of this disease is therefore a priority objective in many programmes. Efficiency of such breeding can be highly improved by molecular breeding tools. *Coffea arabica* is the only tetraploid species (2n = 4x = 44) of the genus *Coffea* and is characterised by very low genetic diversity, which is attributed to its allotetraploid origin, selfing reproductive nature and recent speciation (Lashermes et al., 1999). Introduction of desirable agronomic traits from other *Coffea* species is therefore desirable and one avenue is by use of a natural inter-specific hybrid called Hibrido de Timor (HdT) or Timor hybrid (Bettencourt, 1973). Progenies of this hybrid constitute a source of resistance to various pests including CBD, coffee leaf rust and nematodes. Based on inheritance studies, van der Vossen and Walyaro (1980) proposed the existence of one locus (*T*) for CBD resistance in Hibrido de Timor. Advanced inbred lines of a cultivar (cv) from a cross between Hibrido de Timor and *C. arabica* cv Caturra, (referred to as cv Catimor) are used in Kenya as donors of resistance to both CBD and leaf rust. The objective of this study was to identify molecular markers of this resistance which can assist in breeding programmes.

In the absence of a mature mapping population suitable for field evaluation for CBD resistance, a method for identifying phenotypes of young F_2 individuals was required. Two methods described by Van der Vossen et al. (1976) presented the disadvantages that inoculation of hypocotyls eliminate susceptible plants while inoculation of shoot tips would require selection of only seedlings with young shoot tips of 1-2 cm and the tests can only be done when environmental conditions are suitable. It was therefore a challenge within this study to screen and extract DNA from the entire mapping population.

MATERIALS AND METHODS

An F_2 population between cultivars Catimor (resistant) and SL28 (susceptible) was developed by selfing an F_1 tree. The F_2 berries were harvested in two lots (two weeks apart) as they ripened. Seeds were also harvested from the susceptible cv Caturra from the same field for use as susceptible checks for success of infection. F_2 seeds of different harvest dates were germinated separately as different lots of the same population. After germination, each F_2 seed lot and cv Caturra was divided into halves. One half (Group 1) was retained in the laboratory for hypocotyls inoculations while the other half (Group 2) was transferred directly to a nursery without screening at hypocotyls stage.

Group 1 seedlings were inoculated by the hypocotyls inoculation method of Van der Vossen et al. (1976) using a single-spore isolate of *C. kahawae*. Resistant seedlings obtained by this method by routine practice at Coffee Research Foundation (CRF), Kenya (Classes 1-4) were transferred to the nursery to provide a resistant sub-population in subsequent studies. These seedlings together with those of Group 2 and second half of cv Caturra were maintained in the nursery for one year without fungicide application. One week before inoculation, leaves were sampled from all the F_2 seedlings, lyophilized and stored for extraction of genomic DNA later the method of Diniz et al. (2005). The seedlings were transferred to a laboratory and inoculated by spraying their top parts (up to the third node) to run off and kept under a black polythene sheet for 48hr at room temperature with humidification. The seedlings were then

transferred into a cooled incubation room (18 ± 2 °C) for three weeks after which they were transferred back to the nursery and symptoms scored after two more weeks and a five class scale (Classes 0-4) was described.

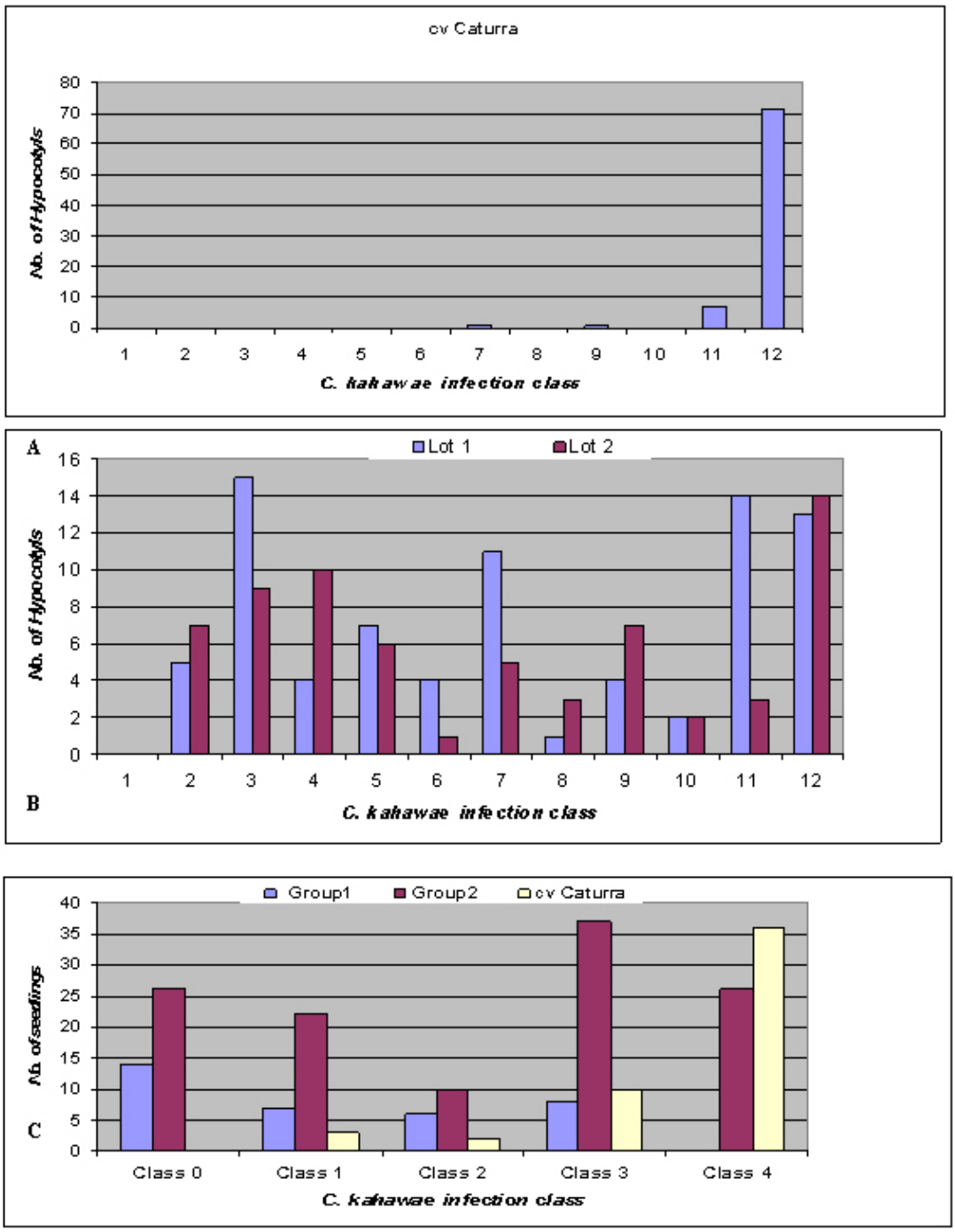


Figure 1. Bar graph presentations of the results of screening various genotypes and stages of coffee seedlings by inoculation with *C. kahawae*.: (A) susceptible cv Caturra, (B) Hypocotyl inoculation scores of the F_2 population (cv Catimor line 88 x cv SL28) (Lots 1 and 2 refer to first and second harvest) and (C) Young seedlings shoot tips inoculation scores of Groups 1 and 2 and cv Caturra (Susceptible). The higher the infection class, the higher the susceptibility.

Priority candidate markers for CBD resistance were identified by screening thirty one (31) AFLP primers combinations and thirty (30) microsatelittes against three accessions of CBD

resistant Hibrido de Timor derivatives (T5296, Catimor 127 and Catimor 88) the susceptible cvs SL28 and Caturra and five F_2 plants by the methods of Lashermes et al. (2000) and Combes et al. (2000). The primers were chosen to maximise polymorphism in relation to introgressed *C. canephora* fragments (Lashermes et al., 2000) and to identify markers of mapped fragments (Ansaldi, 2003). Seven microsatellites were consequently identified as candidate markers and they were analysed in twenty-seven (27) Group 1 plants and ninety-five (95) Group 2 plants (comprising of 47 resistant, 18 susceptible and 30 intermediate category plants). A mapped *C. canephora* chromosomal fragment linked to the resistance was identified and it was further analysed in 47 plants (including all Group 1 plants) with selected AFLP markers to delimit location of the gene.

RESULTS

Phenotypic screening of the F_2 population for CBD resistance

Seedling hypocotyls

The success of infection in the hypocotyls screening method was evaluated by comparison with its severity in the susceptible check cv Caturra. Majority (97.5% of 80 seedlings) of cv Caturra seedlings were in the highly susceptible classes 11 and 12 (Figure 1A) with a mean score of 11.8, and therefore was highly successful. On the other hand, the F_2 population segregated into all the classes, except class 1 (Figure 1B). The distribution patterns of the seedlings were similar between the seed lots ($\chi^2 = 1.05$; $p = 0.300$) with mean infection grades of 7.6 and 6.5 respectively and a population mean grade 7.1 for the first lot, second lot and overall population respectively. By comparing the results of infection on the F_2 population with those of the susceptible cultivar, seedlings in classes 11 and 12 were considered to be susceptible and the rest of the seedlings were considered to express some form or degree of resistance. This gave a ratio of 103:44 (resistant to susceptible) which fitted a 3:1 ratio with Chi-square value of 1.907 ($p = 0.167$). However, only seedlings within Classes 1 to 4 were preserved for use as resistant subpopulation (Group 1) in subsequent studies so as to enable direct comparison with observations of breeding programmes at Coffee Research Foundation, Kenya. Susceptible seedlings dried rapidly without a chance of obtaining DNA from them, and they were therefore not available for molecular studies.

Young seedlings

Subsets of the F_2 population i.e. Group 1 (resistant after screening hypocotyls inoculation method) and Group 2 (which were not screened at hypocotyls stage), were evaluated for CBD resistance after one year by inoculation with the same *C. kahawae* isolate as used during hypocotyls inoculation tests. Before inoculations, leaves were sampled from all seedlings thus ensuring availability of DNA for later studies from all the seedlings even if they died during the screening process. Visual symptoms of infection on the young seedlings were scored five weeks after inoculation with the CBD pathogen, and they varied from no visible signs of infection to complete death of the seedlings. The infection processes observed during the entire screening period were used to develop a five class scoring system where Class 0 had no observable symptoms, classes 1 and 2 had limited infections on the upper young parts mostly resulting into scabs, class 3 ranged from larger black lesions to girdling of nodes while class 4 was characterised by rapidly expanding active lesions causing death of the top parts of the seedlings or complete seedling death. The first three (classes 0-2) were considered to be resistant and the fifth class (Class 4) to be susceptible. Plants in Class 3 could not be clearly categorised into resistant or susceptible categories hence retained as intermediate. The distribution of the assessed seedlings into the various classes is presented in Figure 1C. There

were 5 plants (9.8%) of cv Caturra in classes 0-2 and were regarded as failed infection while three plants (10.3%) of Group 1 were classified as susceptible although they were resistant in hypocotyls screening test. These plants were of low vigour as either small or with very thin stems. These results indicated the likelihood of having some misclassified plants into both resistant and susceptible categories.

Identification of molecular markers for CBD resistance

Screening of 31 AFLP primer combinations and 30 microsatelittes identified 16 AFLP and 7 microsatellite candidate markers which were common in resistant Catimor and Sarchimor accessions (derivatives of Hibrido de Timor) and segregating in the F_2 population. Some of these candidate markers are mapped onto three *C. canephora* chromosomal fragments introgressed into *C. arabica* i.e. T2, T3 and T4 (Ansaldi, 2003). Six of the candidate microsatellite markers displayed segregation patterns conforming to Mendelian inheritance in the 95 Group 2 plants while one (Sat 11) was skewed in favour of the HdT derived allele mapped onto fragment T3. One microsatelitte (Sat 207) which is mapped onto the introgressed chromosomal fragment T2 was observed to be linked to CBD resistance (Table 1). AFLP analysis confirmed the same results and excluded the fragment T4 as a candidate because it segregated randomly in all categories (Table 2). Further analysis by AFLP markers of the T2 fragment showed that Sat 207 is on one side of the resistance gene with marker in the middle of the fragment were present in all Group 1 plants but recombination further on reduced the presence of the markers (Table 2). The distance between the identified delimiting markers (Sat 207 to AFLP-36) is 26.3 cM (Ansaldi, 2003). From the plants selected for AFLP analysis, one plant lacking all the markers of the T2 fragment was observed to have been classified as resistant while two with the markers were classified as susceptible and were all considered as misclassifications.

Table 1. Summary of the occurrence of a microsatellite marker of a *C. canephora* chromosomal fragment T2 introgressed into *C. arabica,* after analysis of a F_2 population between cvs Catimor line 88 and SL28.

Sat 207 (Introgressed *C. canephora* chromosomal fragment **T2**)							
			T2/T2	T2/0	0/0	Total	χ^2
Group 1	Resistant	Observed	10	15	2	27	4.941*
		Expected	6.75	13.5	6.75		
Group 2	TOTAL	Observed	21	48	26	95	0.536 (ns)
		Expected	23.75	47.5	23.75		
	Resistant	Observed	13	29	5	47	5.576*
		Expected	11.75	23	11.75		
	Susceptible	Observed	0	5	13	18	22.338***
		Expected	4.5	9	4.5		
	Others	Observed	8	14	8	30	0.133 (ns)
		Expected	7.5	15	7.5		

*ns: not significant . *, **, *** significant at p=0.1, 0.05 and 0.01 respectively.*

Table 2. Percent incidence of markers of three *C. canephora* chromosomal fragments introgressed into *C. arabica* in F_2 seedlings (Catimor x SL28) screened by inoculation of hypocotyls with *C. kahawae* (Group 1) and in a sub-population un-screened at this stage (Group 2).

		C. canephora chromosomal fragments introgessed into *C. arabica*							
		T2 markers					T3 markers		T4
	CBD Reaction	Sat 207	AFLP-22	AFLP-33	AFLP-33	AFLP-36	Sat 11	AFLP-23	AFLP-17
Group 1	R	92.60	100.00	100.00	100.00	96.30	81.48	83.30	69.85
Total Group 2*	All	72.63	nt	nt	nt	nt	90.32	nt	70.23

**Includes seedlings in the uncertain class 3. nt- not tested.*

DISCUSSION

One of the challenges of this study was to screen the mapping population for CBD resistance at an early stage, and yet be able to obtain DNA from all plants irrespective of their susceptibility to infection by *C. kahawae*. The combination of the hypocotyls and a modification of shoot tips inoculation methods (van der Vossen et al., 1976) enabled us to achieve this. There are different opinions on scaling and analysis of data generated by hypocotyls inoculation method (Van der Vossen et al., 1976; van der Graaff, 1978, 1982; Dancer, 1986; Owour and Agwanda, 1990). In our case, we made a cut-off between presence and absence of resistance at Class 10, in regard to the distribution of seedlings of the susceptible control cv Caturra, though 2 plants out of 80 were outside these brackets. The results fitted a 3:1 ratio (resistant:susceptible) expected for a major gene control. However, to obtain a resistant sub-population from the F_2 population, this method was adopted as routinely used in breeding programmes at CRF and only seedlings of Classes 1 to 4 were retained so as to gain from past experiences' confidence.

The distribution of markers of the introgressed *C. canephora* chromosomal fragment T2 was found to be linked to CBD resistance. The intermediate category (Class 3) was not biased for or against this marker indicating that other factors determined the occurrence of this class. Molecular analysis identified misclassified plants in Group 2 as would be expected from the inoculation results but no un-conforming plants were revealed in Group 1 and this group was considered to be confirmatory which agrees with results of breeding programmes at CRF. The results of recombinant F_2 plants located locus conferring the resistance between Sat 207 and AFLP-36 which covers a genetic distance of 26.3 cM. (Ansaldi, 2003).

The results of this study support the action of a major gene in one locus. There was no evidence of bias against heterozygotes in resistant plants though the phenotypic distribution indicates the possibility of other genetic action or gene x environment interaction. Van der Vossen and Walyaro (1980) described CBD resistance in Hibrido de Timor to be controlled by a gene of intermediate action in one locus (T), whose expression may be affected by presence of modifying genes. Such line of thought is possible considering that lines of cv Catimor used in breeding of the CBD resistant cv Ruiru 11 exhibit differences in specific and general combining abilities with male parents in regard to CBD resistance (Omondi, 1994). Resistance by plants to infection by *Colletotrichum* is quite complex (Esquerré-Tugayé et al., 1992) including Coffee-*C. kahawae* interaction in particular (Gichuru, 1997; Gichuru et al., 1999). However, it is concluded that the *C. canephora* chromosomal fragment T2

introgressed into *C. arabica* via Hibrido de Timor carries a major locus conferring resistance to *C. kahawae*. The designation *Ck*-1 is hereby suggested, as the first mapped locus of resistance to *C. kahawae*. This locus is most likely synonymous to the T locus described by van der Vossen and Walyaro (1980). The results will be of great use in selecting for CBD resistant plants resulting into rapid development of cultivars while excluding genomic fragments which are not linked to the resistance and reducing linkage drag. Future studies will focus on searching for more markers especially microsatelittes, analysis of more resistant plants in attempt to refine the location of the gene, develop SCAR markers from sequences of the AFLP markers and establishment of the relationship between this fragment and previously identified RAPD markers of CBD resistance (Agwanda et al., 1997). It is also of great importance to determine other possible functions of this fragment as well as functions of other fragments in Hibrido de Timor derivatives whether desirable or undesirable.

AKNOWLEDGEMENTS

This study was supported financially by IRD, France, EU (CBDRESIST Project) and CRF, Kenya. Technical assistance from IRD, CRF and CIRAD staff is gratefully appreciated. This paper is published with the authorization of the Director of Research, CRF.

REFERENCES

Agwanda, C. O., Lashermes, P., Trouslot, P., Combes, M C and Carrier, A. (1997). Identification of RAPD markers for resistance to Coffee Berry Disease, *Colletotrichum kahawae* in arabica coffee. Euphytica 97: 241-248

Ansaldi, C. (2003). Analyses moléculaires de l'introgression génétique chez le caféiér (Coffea arabica L.). DESS Bioingénierie, Université Paul, Toulose, France. 31p

Bettencourt, A. (1973). Consideraçoes gerais sobre o "Hibrido de Timor". Instituto Agronomico de Campinas, Circular No. 31, Campinas, Brazil.

Combes, M. C., Andrzejewski, S., Anthony, F., Bertrand, B., Rowelli, P., Graziosi, G. and Lashermes, P. (2000). Characterization of microsatellite loci in *Coffea arabica* and coffee species. Mol Ecol 9: 1178-1180.

Dancer, J. (1986). The evaluation of arabica coffee cultivars for resistance to coffee berry disease: some comments. Euphytica **35**: 125-126.

Diniz, L. E. C., Sakiyama, N. S., Lashermes, P., Caixeta, E. T., Oliveiera, Zambolin, E. M., Loureiro, M. E., Pereira, A. A. and Zambolin, L. (2005). Analysis of AFLP markers associated to the *Mex*-1 locus in Icatu progenies. Crop breed applied biotech 5: 387-393.

Esquerré-Tugayé, M. T, Mazau, D., Barthe, J. P., Lafitte, C. and Touzé, A. (1992). Mechanisms of resistance to *Colletotrichum* species. *In*: Bailey, J. A. and Jerger, M. J. (Eds), *Colletotrichum*; Biology, pathology and control. CABI Wallingford. UK. pp 121-133.

Gichuru, E. K. (1997). Resistance mechanisms in arabica coffee to coffee berry disease (*Colletitrichum kahawae* Sp. Nov.)- A review. Kenya Coffee 62: 2441-2444.

Gichuru, E. K., King'ori, P. N. and Masaba, D. M. (1999). Histochemical differences during infection on *Coffea arabica* by Colletotrichum kahawae isolates. ASIC, 2-6 August, Helsinki, Finland. Paper No. P 717.

Griffiths E., Gibbs, J. N. and Waller, J. M. (1971). Control of coffee berry disease. Ann Appl Biol 67: 45-74.

Lashermes, P., Combes, M. C., Robert, J., Trouslot, P., Hont, A. D., F Anthony, F. and Charrier, A. (1999). Molecular characterization and origin of the *Coffea arabica* L. genome. Mol Gen Gene 261: 259-266.

Lashermes, P., Andrzejewski, S., Bertrand, B., Combes, M. C., Dusert, S., Graziosi, G., Trouslot, P. and Anthony, F. (2000). Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.) Theor Appl Genet 100: 139-146.

Masaba, D. M. and Waller, J. M. (1992). Coffee Berry Disease: The current status. *In*: Bailey, J. A. and Jerger, M. J. (Eds), *Colletotrichum*; Biology, pathology and control. CABI Wallingford. UK. pp 237-249.

Omondi, C. O. (1994). Resistance to Coffee Berry Disease in Arabica Coffee Variety 'Ruiru 11'. Plant Breeding 112: 256-259.

Owour, J. B. O. and Agwanda, C. O. (1990). Comparison of the sensitivity of the mean and the mode in screening for resistance to coffee berry disease Colletotrichum coffeanum Noack sensu Hindorf (A short communication). Euphytica **45**: 247- 250.

Van der Vossen, H. A. M., Cook, R. T. A. and Murakaru, G. N. W. (1976). Breeding for resistance to coffee berry disease caused by *Colletotrichum coffeanum* Noack Sensu Hindorf in *Coffea arabica* L. I. Methods of preselection for resistance. Euphytica 25: 733-756.

Van der Graaff, N. A. (1978). Selection for resistance to coffee berry disease in arabica coffee in Ethiopia. Evaluation of selection methods. Neth J Pl Path 84: 205-215.

Van der Vossen, H. A. M. and Walyaro, D. J. (1980). Breeding for resistance to coffee berry disease in *Coffea arabica* L. Inheritance of the resistance. Euphytica 29: 777-791.

State-of-the-Art of Developing Arabica Coffee Cultivars with Durable Resistance to Coffee Berry Disease (*Colletotrichum kahawae*)

H. A. M. VAN DER VOSSEN

Plant Breeding & Seed Consultant, 1606 CA 18 Venhuizen, the Netherlands
E-mail: herbert.vandervossen@quicknet.nl

SUMMARY

In response to heavy crop losses caused by recurrent CBD epidemics in arabica coffee in Africa, breeding programmes aimed at developing cultivars with host resistance to this destructive fungal disease were initiated some 35-40 years ago in Kenya, Ethiopia and Tanzania. The release of CBD resistant cultivars to the coffee growers has been in progress in the first two countries since 1985 and more recently also in Tanzania. The resistance of cultivars like Ruiru II (Kenya) and Ababuna (and other cvs in Ethiopia) appears to be of a durable nature, since confirmed cases of a breakdown of host resistance under field conditions have not been reported over the past 20 years. Isolates of the CBD pathogen from divergent origins may vary considerably in aggressiveness when tested under controlled conditions by inoculation of berries and seedling hypocotyls, but statistically insignificant pathogen-plant interaction effects indicate the absence of physiologic races. Host resistance to the hemibiotrophic fungus *C. kahawae* appears to be of a quantitative nature but is nevertheless practically complete in some genotypes of arabica coffee. There is still no consensus among coffee breeders on the type of genetic system, some claiming convincing evidence for oligogenes (1-3 major genes) and others for polygenes determining CBD resistance. In this review, further evidence for major-gene resistance will be presented from earlier genetic studies based on arabica germplasm of Ethiopian origin. The discovery of closely linked molecular markers and very recently even the genetic mapping of one major gene for CBD resistance present in certain Hibrido de Timor genotypes provides additional support for oligogenic inheritance of CBD resistance. In the case of CBD, observed plant-pathogen interactions include an active defence mechanism of cork barrier formation preventing the pathogen from invading more host tissue at an early stage, which can be interpreted as race non-specific resistance. However, much more needs to be known of plant-pathogen interactions at the molecular level, in particular the cascade of defence responses through downstream signal transduction triggered by the initial invasion of host cells by the CBD pathogen, to understand and possibly improve the durability of CBD resistance. The development of cultivars combining yield and quality with durable host resistance to CBD is a most effective way of increasing economic sustainability of arabica coffee production in Africa. It has also considerable relevance to Latin America and Asia, where CBD is still a quarantine disease but with the constant threat of one day becoming endemic, just as has happened earlier with the coffee leaf rust disease. Adequate funding and close networking among all major coffee research centres in the world, in conventional and molecular breeding for durable CBD resistance, are prerequisites to achieving such cultivars.

INTRODUCTION

Coffee berry disease (CBD), caused by the fungus *Colletotrichum kahawae*, is still restricted to arabica coffee in Africa, although climatic conditions in certain high-altitude coffee areas

of Latin America and Asia appear to be very favourable to the fungus. CBD epidemics can quickly destroy 50-80% of the developing berries (6-16 weeks after anthesis) on susceptible arabica cultivars during prolonged wet and cool weather conditions. Preventive control by frequent fungicide sprays may account for 30-40% of total production costs. Annual economic damage to arabica coffee production in Africa, due to crop loss by CBD and cost of chemical control, is estimated at US$ 300-500 million. In response to the heavy crop losses caused by recurrent CBD epidemics in arabica coffee in Africa, breeding programmes aimed at developing cultivars with host resistance to this destructive fungal disease were initiated some 35-40 years ago in Kenya, Ethiopia and Tanzania (Van der Vossen, 2001). The release of CBD resistant cultivars to the coffee growers has been in progress in the first two countries since 1985 and more recently also in Tanzania (C.O. Omondi, personal communication, 2003; Bellachew et al., 2000; Teri et al., 2004). The resistance of these cultivars – like Ruiru II in Kenya (planted on an estimated 20,000 ha), Dessu, Ababuna, Melko CH2 and other released true-breeding selections in Ethiopia (planted on some 60,000 ha) – appears to be of a durable nature, since confirmed cases of a breakdown of host resistance under field conditions have not been reported over the past 20 years. This review presents an update of current information available from classical and molecular studies on CBD, in regard to the inheritance of host resistance and plant-fungus interactions.

INHERITANCE OF CBD RESISTANCE

Host resistance to CBD appears to be of a quantitative nature but is nevertheless practically complete in some genotypes of arabica coffee (Van der Vossen et al., 1976). There is still no consensus among coffee breeders on the type of genetic system, some claiming convincing evidence for oligogenic (Van der Vossen and Walyaro, 1980) and others for polygenic (Van der Graaff, 1981; Bellachew, 2001) inheritance of CBD resistance. Nevertheless, the results from genetic studies carried out in Kenya on arabica germplasm of Ethiopian origin (Coffee Res. Found. Kenya, Annual Report 1979) also indicated CBD resistance controlled by one or two major genes in certain accessions.

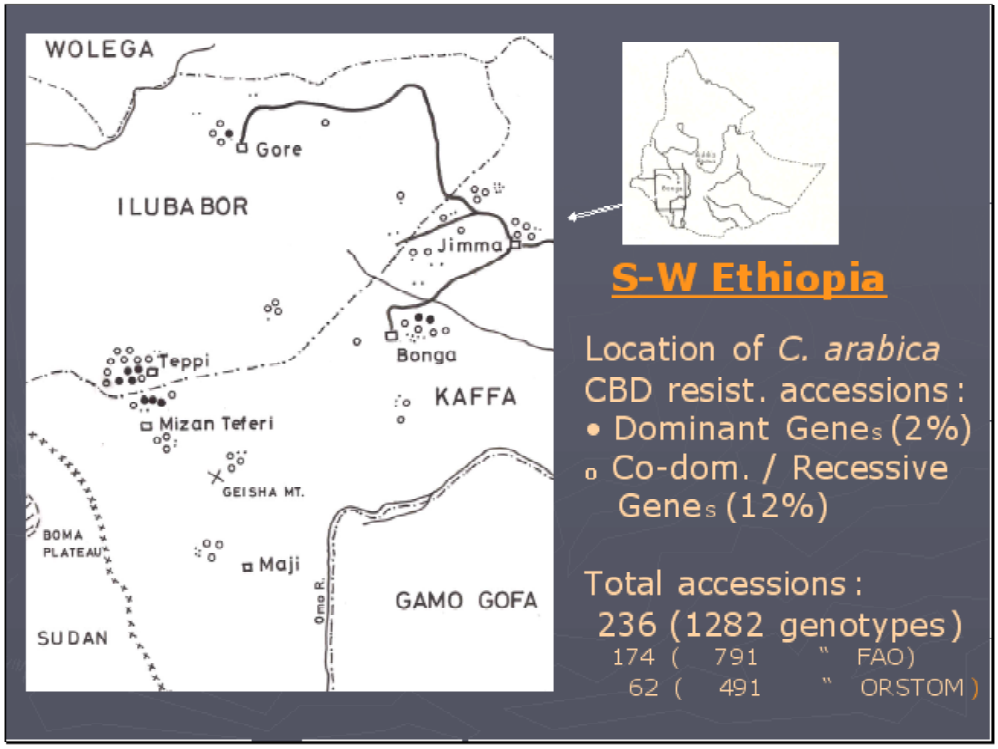


Figure 1.

The *Coffea arabica* germplasm of Ethiopian origin present at the Coffee Research Station near Ruiru, Kenya, consisted of 174 accessions (791 genotypes) from the 1964 FAO Mission (Meyer et al., 1968) and another 62 accessions (491 genotypes) from the 1966 ORSTOM Mission (Guillaumet and Hallé, 1967), seed samples collected from individual trees or groups of trees in the south-western highlands of Ethiopia. The altogether 1282 selfed progenies (equivalent to F2 offspring) of all genotypes were screened for CBD resistance by the inoculation test on 6-week old seedlings as developed by Cook (1973) and described in Van der Vossen *et a*l. (1976). A summary of conclusions based on the results of these inoculation tests is presented in Figure 1. In about 2% of the 1282 seedling progenies the frequency distribution of infection grades suggested the presence of 1-2 dominant resistance genes in the parental genotype in either homozygous or heterozygous condition. A few examples of genotypes with CBD resistance obviously conditioned by dominant gene(s) are given in Figure 2. In another 12%, inheritance of CBD resistance appeared to be conditioned by a few co-dominant or recessive genes. When mapping the locations of the accessions with CBD resistant genotypes in the Ethiopian centre of genetic diversity for *C. arabica*, "hot spots" for dominant genes for CBD resistance become apparent in the area from Teppi to Mizan Teferi, respectively in the Ilubabor and Kaffa zones in the Oromia Region of Ethiopia. It is also worth noting that this is relatively near to the Boma Plateau across the border in Sudan, from where the variety Rume Sudan was collected (Thomas, 1942). A selection from Rume Sudan was the progenitor of the dominant R gene used in the Kenyan breeding programme, which confers almost complete field resistance to CBD infection.

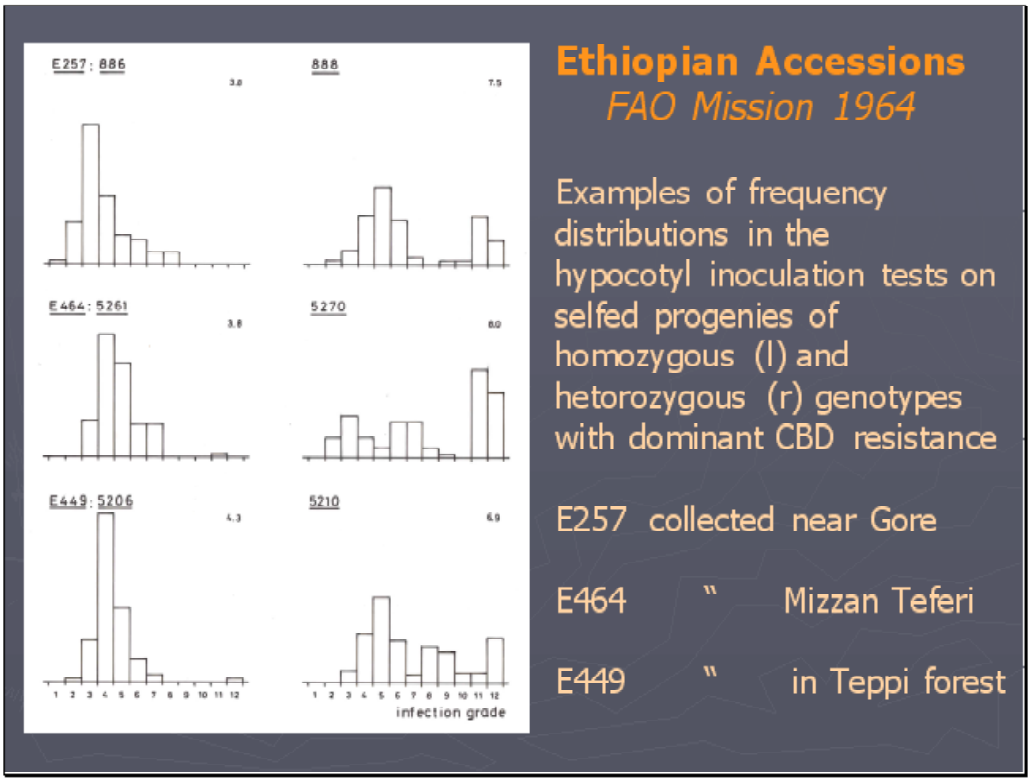


Figure 2.

This could indicate that the wild coffees at the Boma Plateau may have had their origin in the montane forests of south-west Ethiopia. A few accessions with dominant gene(s) for CBD resistance were also found in an area north of Bonga in the Kaffa zone and near Gore in the Ilubabor zone.

The inheritance of CBD resistance was studied further by applying the hypocotyl inoculation test to F2 progenies of a half diallel cross between five genotypes from Ethiopian accessions (E87, E98, E126, E71 and E257) with apparently homozygous resistance to CBD and two susceptible cultivars, viz. SL28 and Caturra. The mean grade of infection was used as data for an analysis of variance to obtain estimates of various genetic parameters, such as heritability, number of genes (effective factors) conditioning resistance and the dominance relations among the parent genotypes. For a detailed description of quantitative principles and methods applied in the analysis of the diallel cross, reference is made to an analogous study by Van der Vossen & Walyaro (1980). The results basically confirm that CBD resistance in Ethiopian germplasm is generally inherited through major genes with dominance, co-dominant or recessive expression. The heritability was high ($h^2 > 0.90$) and number of effective factors (genes) about one. A graph showing the dominance relations among parents according to the so-called Wr/Vr (covariances/variances) regression analysis is presented in Figure 3. Genotypes E87 and E 257 carry dominant, while E71, E126 and E98 appear to have co-dominant or recessive major gene or genes for CBD resistance.

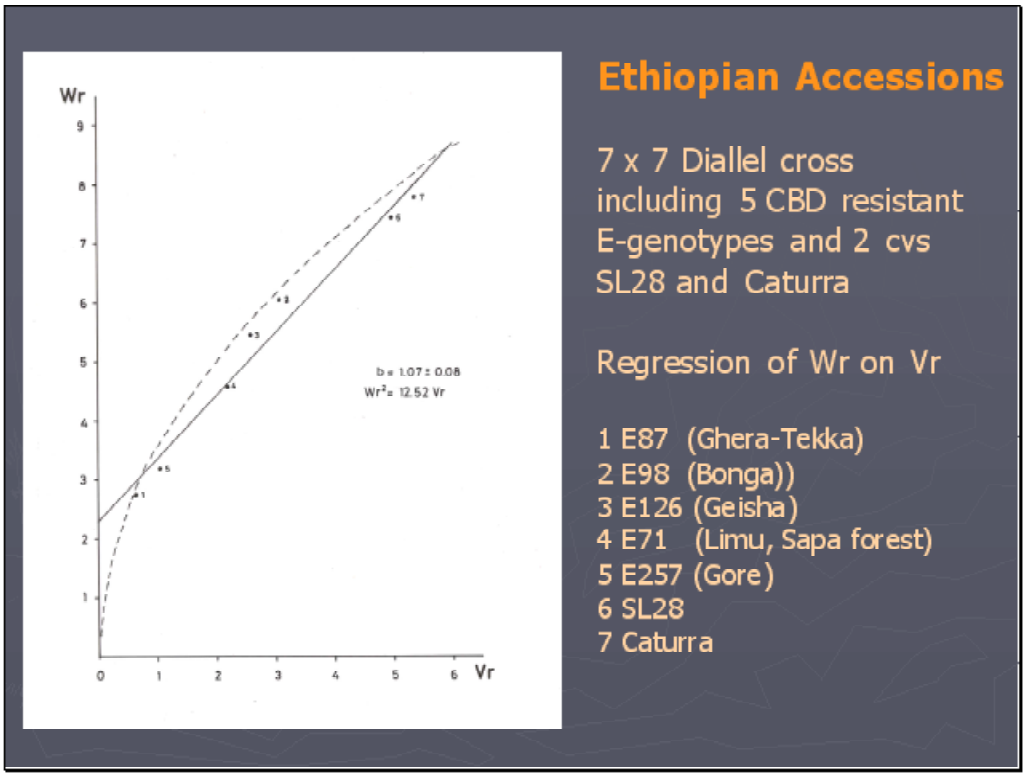


Figure 3.

These results are very similar to the Wr/Vr regression analysis of an 11 x 11 diallel cross between CBD resistant and susceptible varieties published by Van der Vossen and Walyaro (1980). The conclusion then was also major gene inheritance, with the variety Rume Sudan carrying a dominant and recessive, variety Hibrido de Timor one co-dominant and cultivar K7 a recessive major gene conditioning host resistance to CBD. Unfortunately, a follow-up programme of crosses, planned to investigate whether the gene(s) present in Rume Sudan were identical (same locus on a chromosome) or different from the resistance gene(s) of Ethiopian germplasm, was never implemented or completed.

The discovery of closely linked molecular markers (Agwanda et al., 1997) and very recently even the genetic mapping of the major gene (Gichuru et al., 2006) for CBD resistance present in certain Hibrido de Timor genotypes, provide additional evidence for oligogenic inheritance

of CBD resistance. However, this gene was almost certainly introgressed into *C. arabica* var. *typica* from *C. canephora* by spontaneous interspecific hybridization and backcrossing in the island of Timor (Bettencourt and Rodrigues, 1988). It will be very important to provide similar molecular and genomic evidence for the major genes in regular arabica varieties and germplasm from the Ethiopian centre of genetic diversity for *C. arabica*. The recently initiated research project (Omondi and Pinard, 2006, this conference) to develop molecular markers linked to genes for CBD resistance in the variety Rume Sudan is a significant step into the right direction in that respect.

PLANT – PATHOGEN INTERACTIONS

Like most *Colletotrichum species, C. kahawae* is a hemibiotrophic pathogen with a two-phase infection process, one short biotrophic (ref.: Figure 3 in Chen et al., 2004) followed by a destructive necrotrophic phase in susceptible host plants (Bailey et al., 1992). Early response to infection in resistant hosts was observed to include rapid cell death (Anon., 2005), but it is not necessarily similar to the hypersensitive response, which is so characteristic of biotrophic pathogens such as coffee leaf rust (*Hemileia vastatrix*). Instead, host resistance appears to be largely based on the rapid formation of a cork barrier in the pericarp of the fruit or cortex of hypocotyl stems distal from the initial infection site, which effectively prevents the pathogen from further invading healthy tissue. Such barriers are usually absent or incomplete in susceptible host plants (Masaba and Van der Vossen, 1982). Detached berries of resistant plants also do not develop cork barriers in response to CBD infection (Gichuru et al., 1999), which indicates that only physiologically active and undisturbed host tissues are capable of developing the resistance mechanism. However, the effectiveness of this resistance mechanism is strongly temperature dependent. At temperatures below 17 ºC over prolonged periods even the most resistant genotypes may develop some active lesions, while above 24 ºC susceptible genotypes show little or no CBD infection after inoculation (Van der Vossen & Waweru, 1977). The severity of CBD epidemics in Africa increases with altitude as the climate becomes cooler and wetter (Waller, 1971). High altitude coffee growing areas require, therefore, cultivars with strong CBD resistance (Bellachew, 2000). With similar cool temperatures, sites with a wetter climate during the developing crop will also require stronger host resistance to CBD (Omondi et al., 2004).

Isolates of the CBD pathogen from divergent geographic or ecological origins were found to vary considerably in aggressiveness when tested under controlled conditions by inoculation of berries and hypocotyl stems. However, pathogen-variety interaction effects remained statistically insignificant in all cases, thus indicating the absence of physiologic races (Bella Manga, 1999; Omondi et al., 2001; Anon, 2005). This would indicate that the available host resistance is not threatened as yet by race specification in the CBD pathogen.

So far, more research progress has been made in gaining insight into the biochemical and molecular basis of host resistance in arabica coffee to the biotrophic *Hemileia vastatrix* than to the hemibiotrophic *Colletotrichum kahawae*. Early resistance responses to CBD at infection sites were observed to include hypersensitive death of invaded host cells, deposition of callose around intracellular hyphae and accumulation of phenolic compounds (Silva et al., 2006). However, very little is still known about host-pathogen recognition events at the molecular level, in particular those activating layers of thin-walled cells near to the initial infection site to transform into a phellogen that leads to the cork barriers and apparently broad-spectrum host resistance.

CONCLUSIONS AND RECOMMENDATIONS

- General acceptance of the overwhelming evidence for oligogenic inheritance of host resistance to CBD would greatly facilitate the formulation of common research strategies (e.g. molecular marker-assisted selection and gene pyramiding) for efficient exploitation of available coffee germplasm for durable resistance to CBD in arabica coffee.
- The preselection test for CBD resistance by inoculation of hypocotyl stems of 6-week old seedlings should be rigolously standardized to obtain more consistent results between locations and in time. This applies in particular to: (1) temperature, (2) humidity, (3) incoculum preparation and spore concentration, (4) application of inoculum, (5) undisturbed growth of seedlings during infection and incubation for optimum physiological activity and consequently maximum expression of host resistance, and (6) scoring of infection by the scale with 12 classes developed by Cook (1973). The 0-4 scale adopted subsequently in many coffee research centres outside Kenya may be adequate for testing of the pathogenicity of CBD isolates, but it is definitely less precise and sensitive to establish segregation patterns in genetic studies. Excessive infection during screening tests can easily lead to frequency distributions characteristic of polygenic and recessive inheritance.
- Much more needs to be known of plant-pathogen interactions at the molecular level, in particular the early host-pathogen recognition events and subsequent cascade of defence responses through downstream signal transduction, to understand and possibly improve the durability of CBD resistance.
- The development of cultivars combining yield and quality with durable host resistance to CBD is a most effective way of increasing economic sustainability of arabica coffee production in Africa. It has also considerable relevance to Latin America and Asia, where CBD is still a quarantine disease but with the constant threat of one day becoming endemic, just as has happened earlier with the coffee leaf rust disease.
- Adequate funding and close networking among all major coffee research centres in the world, in conventional and molecular breeding for durable CBD resistance, are prerequisites to achieving such cultivars. The CIFC (Coffee Rust Research Centre, Oeiras, Portugal) with its recently acquired expertise in screen breeding lines for CBD resistance is ideally positioned to play a central role in this network.

ACKNOWLEDGEMENT.

Thanks are due to Dr.D.J.Walyaro, formerly senior coffee breeder and head CBU at the CRF in Kenya, for statistical analysis of the 7 x 7 diallel cross.

REFERENCES

Anon., 2005. Progress report 2004-2005. EU/INCO/ICA4-CT-2001-10008/CBDRESIST Project.

Agwanda, C.O., Lashermes,Ph., Trouslot, P., Combes, M.C. and Charrier, A., 1998. Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee. Euphytica, 97, 241-248.

Bailey, J.A., O'Connel, R.J., Pring, R.J. and Nash, C., 1992. Infection strategies of *Colletotrichum* species. In: Bailey, J.A. and Jeger, M.J. (eds). *Colletotrichum*: biology, pathology and control. British Society of Plant Pathology, CABInternational, Wallingford, UK. pp. 88-119.

Bellachew, B., Atero, B. and Tefera, F., 2000. Breeding for resistance to coffee berry disease in arabica coffee: progress since 1973. Proceedings of the workshop on control of coffee

berry disease (CBD) in Ethiopia, Addis Ababa, 13-15 August 1999. Jimma Agricultural Research Center (JARC), Ethiopia. pp. 85-98.

Bellachew, B., 2001. Arabica coffee breeding for yield and resistance to coffee berry disease (*Colletotrichum kahawae* sp. nov.). PhD Thesis, Department of Agriculture, Imperial College at Wye, University of London. UK. 272 pp.

Bella Manga, 1999. Etude de la diversité de *Colletotrichum kahawae* responsable de l'anthracnose des baies et caractérisation de la résistance du caféier Arabica à cet agent pathogène. Thèse Docteur en Sciences, Université Montpellier II, Sciences et Technique du Langedoc, France. 145 pp.

Bettencourt, A.J. and Rodrigues, C.J., 1988. Principles and practice of coffee breeding for resistance to rust and other diseases. In: Clarke, R.J & Macrae, R (eds). Coffee, vol. 4 Agronomy. Elsevier Applied Science, London & New York. pp. 199-234.

Chen, Z., Nunes, M.A., Silva, M.C. and Rodrigues Jr, C.J., 2004. Appressorium turgor pressure of *Colletotrichum kahawae* might have a role in coffee cuticle penetration. Mycologia 96: 1199-1208.

Cook, R.T.A., 1973. Screening coffee plants for CBD resistance. Annual report Coffee Research Foundation, Kenya 1972/73: 66-68.

Gichuru, E.K., Kingiori, P.N. and Masaba, D.M., 1999. Histological differences during infection of *Coffea arabica* varieties by *Colletotrichum kahawae* isolates. Proceedings 18th International Scientific Colloquium on Coffee, Helsinki, Finland. ASIC, Paris, France. pp. 477-479.

Gichuru, E.K., Combes, M-C., Mutitu, E.W., Ngugi, E.C.K., Bertrand, B. and Lashermes, Ph., 2006. Characterization and genetic mapping of a gene conferring resistance to coffee berry disease (*Colletotrichum kahawae*) in arabica coffee (*Coffea arabica* L.). Proceedings 21st International Scientific Colloquium on Coffee, Montpellier, France. ASIC, Paris, France, in the press.

Guillaumet, J.L. & Hallé, F., 1967. Etude de la variabilité de *Coffea arabica* dans son aire d'origine. ORSTOM Mimeo. Report.

Masaba, D.M. and Van der Vossen, H.A.M., 1982. Evidence of cork barrier formation as a resistance mechanism to berry disease (*Colletotrichum coffeanum*) in arabica coffee. Netherlands Journal of Plant Pathology 88: 19-32.

Meyer, F.G., Fernie, L.M., Narasimhaswamy, R.L., Monaco, L.C. and Greathead, D.J., 1968. FAO Coffee Mission to Ethiopia 1964-65. FAO Rome. 200 pp.

Omondi, C.O., Ayiecho, P.O., Mwang'ombe, A.W. & Hindorf, H., 2001. Resistance of *Coffea arabica* cv. Ruiru II tested with different isolates of *Colletotrichum kahawae*, the causal agent of coffee berry disease. Euphytica 121: 19-24.

Omondi, C.O., Agwanda, C.O. and Gichuru, E.K., 2004. Field expression of resistance to coffee berry disease (CBD) as affected by environment and host-pathogen factors. Proceedings 20th International Scientific Colloquium on Coffee, Bangalore, India. ASIC, Paris, France. pp. 1216-1221.

Omondi, C.O. and Pinard, F., 2006. Screening populations of arabica coffee for molecular markers linked to coffee berry disease resistance. Proceedings 21st International Scientific Colloquium on Coffee, Montpellier, France. ASIC, Paris, France, in the press.

Silva, M.C., Várzea, V., Guerra-Guimarães, L., Gil Azinheira, H., Fernandez, D., Petitot, A-S., Bertrand, B., Lashermes, Ph. and Nicole, M., 2006. Coffee resistance to the main

diseases: leaf rust and coffee berry disease. Brazilian Journal of Plant Physiology 18: 119-147.

Teri, J.M., Kilambo, D.L., Mtenga, D.J., Nyange, N.E., Nzallawahe, T.S., Chipungahelo, G.S., Kipokola, T.P. and Kullaya, I.K., 2004. Improved arabica varieties for the benefit of Tanzanian coffee growers. Proceedings 20th International Scientific Colloquium on Coffee, Bangalore, India. ASIC, Paris, France. pp. 1187-1191.

Thomas, A.S., 1942. The wild arabica coffee on the Boma Plateau of Anglo-Egyptian Sudan. Empire Journal of Experimental Agriculture 10: 207-212.

Van der Graaff, N.A., 1981. Selection of arabica coffee types resistant to coffee berry disease in Ethiopia. PhD Thesis (Communications no.8-11), Agricultural University of Wageningen, the Netherlands. 111 pp.

Van der Vossen, H.A.M., 2001. Coffee breeding practices. In: Clarke, R.J. & Vitzthum, O.G. (eds). Coffee – Recent Developments. Oxford: Blackwell Science, pp. 184-201.

Van der Vossen, H.A.M. and Waweru, J.M., 1977. A temperature controlled inoculation room to increase efficiency of preselction for resistance to coffee berry disease. Kenya Coffee 41: 164-167.

Van der Vossen, H.A.M. and Walyaro, D.J., 1980. Breeding for resistance to coffee berry disease in *Coffea arabica* L. II. Inheritance of the resistance. Euphytica 29: 777-791.

Van der Vossen, H.A.M., Cook, R.T.A. and Murakaru, G.N.W., 1976. Breeding for resistance to coffee berry disease caused by *Colletotrichum coffeanum* Noack (sensu Hindorf) in *Coffea arabica* L. I. Methods of preselection for resistance. Euphytica 25: 733-745.

Waller, J.M., 1971. The incidence of climatic conditions favourable to coffee berry disease in Kenya. Experimental Agriculture 7: 303-314.

Genetic Diversity of Wild *Coffea arabica* in Ethiopia: Analyses Based on Plastid, ISSR and Microsatellite Markers

G. K.TESFAYE[1,2], K. GOVERS[2], T. OLJIRA[1], E. BEKELE[1] AND T. BORSCH[2]

[1]Department of Biology, Addis Ababa University, Ethiopia,
[2]Nees-Institute for Biodiversity of Plants, University of Bonn, Germany

SUMMARY

Based on a sampling of about 300 individuals from well documented forest populations, genetic diversity of *Coffea arabica* in Ethiopia was assessed. Chloroplast (cp) and ISSR (Inter Simple Sequence Repeat) fingerprint data provide strong evidence that *C. arabica*, the only tetraploid *Coffea* species, evolved through a single allopolyploidization event. Phylogenetic analyses of chloroplast sequence data depict *C. eugenioides* as sister to *C. arabica* with high statistical support. Both species have their characteristic autapomorphies, suggesting that an ancestor of *C. eugenioides* was the maternal parent of *C. arabica*, but not extant *C. eugenioides*. Although > 7.5 kb of rapidly evolving cp spacers and introns were sequenced, and seven microsatellites were characterized, no deviating cp haplotypes were encountered among individuals of *C. arabica*. This points to a recent origin of the species followed by rapid spreading or to severe bottlenecks in the evolutionary history of the species. Populations of *C. arabica* were compared throughout forests of Ethiopia using ISSRs. This interregional analysis reveals complex geographical patterns of genetic diversity, with most regions possessing their own genotypes. Adding Ethiopian landraces (farmer's varieties) showed that wild populations of *C. arabica* are genetically different and can be distinguished from semi-domesticated plants. In previous studies, different Ethiopian individuals of *C. arabica* were subsumed under "subspontaneous material". Interregional patterns of genetic diversity in wild *C. arabica* indicate that a hierarchical-geographical structure is obscured by naturally occurring gene flow. Dense sampling of *C. arabica* with ISSRs in forests of Berhane Kontir and Yayu (Geba Dogi) shows a fine-scale spatial patterning of genotypes in wild populations. Work on nuclear microsatellites with the aim to establish a co-dominant marker system that allows to assess heterozygosity and gene flow in wild *Coffea* is in progress. Results of the first phase of the CoCE project provide a first assessment of genetic diversity throughout wild populations of *C. arabica* in Ethiopia. Molecular data support the existence of truly wild populations that differ from semi-domesticated plants, and underscore the need of conserving forest coffee. Effective strategies that well represent *C. arabica* genotypes require multiple sites for *in situ* conservation complemented with *ex situ* measures.

INTRODUCTION

The genus *Coffea* comprises approximately 100 species. However, only *C. arabica* and *C. canephora* are economically important species. *Coffea arabica* is the only species occurring in Ethiopia, geographically isolated from the remaining species of the genus. It is also the only allotetraploid (2n = 4x = 44), whereas all other *Coffea* species are diploids (2n = 2x = 22; Bridson and Verdcourt, 1988; Stoffelen, 1998). The reconstruction of phylogenetic relationships within *Coffea* appeared difficult because of low amounts of variability in the genomic regions sequenced such as ITS (Lashermes et al., 1997) and *trn*L-F (Cros et al., 1998). Resulting phylogenetic trees suffered from a lack in resolution and statistical support of nodes.

So far published analyses of genetic diversity of *C. arabica* (Lashermes et al., 1996; Anthony et al., 2002) largely sampled *ex situ* material outside Ethiopia, with individuals not exactly traceable in terms of geography and status of their respective populations. More recently, Aga et al. (2005) described polymorphism within and among forest populations in Ethiopia using RAPDs but their sampling was not exhaustive in terms of representing wild *C. arabica*. Despite the considerable importance of the wild gene pool of *C. arabica* in Ethiopia for improving coffee quality and productivity, research on wild populations has been very limited. This is even more surprising, considering that the wild Coffee populations are under great threat because of deforestation, caused by settlements and expansion of farm land (Gole, 2003; Senbeta, 2006).

In the framework of the CoCE project (Conservation and Use of Wild Populations of *Coffea arabica* in the Montane Rainforests of Ethiopia; www.coffee.uni-bonn.de) research is being undertaken in order to develop strategies for conservation and sustainable use of wild *C. arabica*. The purpose of this study was to analyse the origin of *C. arabica* and to assess patterns of genetic diversity of wild populations in Ethiopia.

MATERIALS AND METHODS

Sampling strategy

Sampling was carried out to cover all wild coffee areas and coffee producing regions of Ethiopia: Bale (= Harrena; I), Bonga (II), Berhane Kontir (III) and Yayu (= Geba Dogi; IV) are considered as main CoCE sites for multidisciplinary studies. In addition, forest coffee plots from Boginda (V), Bench Maji (VII), Anfilo (VIII), Daphe (IX), and Mankira (X) were also included to have a better representation of overall genetic diversity. Twenty five individual coffee trees were randomly sampled from 50 x 50 m plots. A representative selection of landraces was sampled to compare genotypes with forest populations. Moreover, two well known commercial cultivars of *C. arabica* (Blue Mountain and Caturra Yellow) were included. *Psilanthus leroyi* and *Ixora coccinea* (Rubiaceae) were used as outgroup. For phylogenetic analyses, samples of seven diploid species of *Coffea* were added. Genomic DNA was isolated from dried leaf tissue as described in Tesfaye et al. (submitted a).

Chloroplast genome sequencing and characterisation of cp microsatellites

Based on existing empirical data for angiosperms, a number of rapidly evolving chloroplast regions with known (Weising and Gardner, 1999) or probable occurrence of microsatellites were selected. These were the intergenic spacers *atp*B-*rbc*L, *trn*S-G, and *rpl*2-*rps*19, the group II introns in *atp*F, *trn*G, *trn*K, *rpl*16, and the *mat*K gene. Amplification and sequencing protocols are described in detail in Tesfaye et al. (submitted a). Sequence alignment followed Löhne and Borsch (2005). Inference of parsimony trees and ancestral character states was done with PAUP* (Swofford, 1998).

ISSR fingerprinting

Polymorphism in *C. arabica* and reproducibility were tested for 130 ISSR primers, available through the University of British Columbia (UBC 900 kit) and Raus et al. (2003). Eight di- (primers 810, 812, 813, 814, 818, 834, 844, 860), one tri- (866), and two tetra-nucleotide primers (CoIS001, CoIS002) were selected for routine screening. Interregional analysis considered ± 5 individuals from all plots throughout Ethiopia; intraregional analysis of all 25 individuals from five plots each in Berhane Kontir (III) and Yayu (IV). ISSR bands were scored as present '1' or absent '0'. The resulting binary matrix was employed to either

directly infer NJ trees, or to calculate NJ, PCO, and UPGMA diagrams based on Jaccard's similarity coefficient.

RESULTS AND DISCUSSION

Chloroplast microsatellites and origin of the *C. arabica* plastid genome

A sequencing approach served to characterize microsatellites, to screen for SNPs (Single Nucleotide Polymorphisms), and to generate a sequence dataset for phylogenetic. About 7500 nt were generated for seven different individuals of *C. arabica,* eight samples of diploid *Coffea* species, and outgroups. Seven microsatellite loci were characterized in the *Coffea* chloroplast genome. One is located in each of the spacers between *atp*B and *rbc*L*, trn*S and *trn*G, *rpl*2 and *rps*19, in the *rpl*16 and *trn*K introns. Two satellites are found in the *trn*G intron.

Satellites mostly consist of A/T mononucleotide strands, as also frequently observed in the chloroplast genome of other angiosperm species. In *Coffea*, only the microsatellite located in the *trn*S-*trn*G spacer differs in having dinucleotide repeats $(AT)_N$. All sequenced individuals of *C. arabica,* representing strongly divergent genotypes (based on ISSRs) from wild populations in different geographical regions of Ethiopia, and the Bourbon and Typica lines of commercial cultivars, share the same allele. Not a single SNP was found although a large sequence dataset was analysed (Tesfaye et al., submitted a). This is an unexpected finding since the investigated chloroplast regions are rapidly evolving and intraspecific variation has been encountered and utilized for population studies in a number of wild plants and crops (e.g., Petit et al., 1998). An explanation is that *C. arabica* is of very recent evolutionary origin, and after a single hybridization and allopolyploidization event populations of *C. arabica* have rapidly spread. Thus, there was not enough time to accumulate variation. It is also possible that there were severe bottlenecks in the evolutionary history of the species, for example caused by climatic changes.

Phylogenetic analyses of the plastid dataset show a sister group relationship of *C. arabica* and *C. eugenioides* (Figure 1) with maximum statistical support. Earlier indications (Cros et al., 1998; Raina et al., 1998; Lashermes et al., 1999) of possible maternal parentship of *C. eugenioides* are substantiated. However, the large sequence dataset now shows that cp genomes of both *C. arabica* and *C. eugenioides* possess apomorphies, indicating that not *C. eugenioides* is the maternal parent of *C. arabica,* but an ancestor of *C. eugenioides* (for details see Tesfaye et al., submitted a).

Interregional analysis

The topology shown in Figure 2 resolves four different groups. Group 1 (top of the tree) is dominated by Ethiopian landraces and cultivars (including 'Blue Mountain'), landraces from Eritrea, and includes individuals from Hararge and Sidamo. The remaining three groups are dominated by wild individuals. Plants from Boginda and Daphe cluster together, indicating the presence of closely related genotypes. To the contrary, genotypes found in Bonga appear as extremely heterogeneous, and are spread over the tree. Whereas *C. arabica* individuals from a single plot usually belong to similar genotypes, the levels of genetic diversity within different regions are extremely different (Tesfaye et al., submitted b).

ISSR markers differentiate individuals collected in wild populations (self regenerating, not selected by human activity for any trait) from individuals collected in gardens and grown under human control of reproduction (semi-domesticated plants, farmer's varieties). In

contrast to this project (Tesfaye et al., submitted b), previous studies using molecular markers did not employ exactly documented individuals, and could therefore not identify truly wild *C. arabica*.

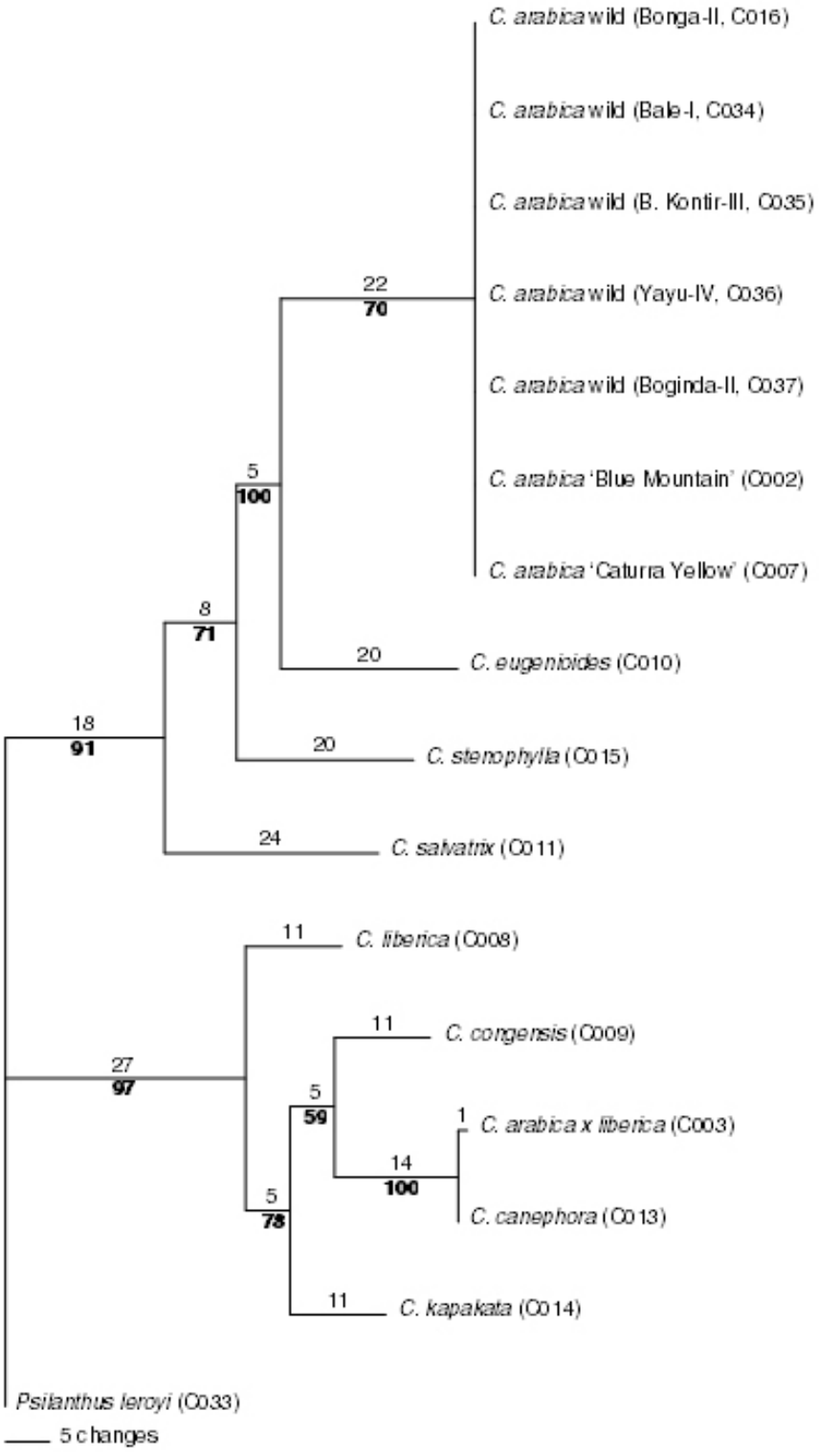


Figure 1. Single most-parsimonious tree from a combined analysis of sequences from all regions (7500 nt). Branch lengths are shown above and the Jackknife percentages below branches (modified from Tesfaye et al., submitted a).

Figure 2. Neighbour joining analysis of the interregional dataset based on eight di- and one tri-nucleotide primers, employing Jaccard's coefficient (Tesfaye et al., submitted b).

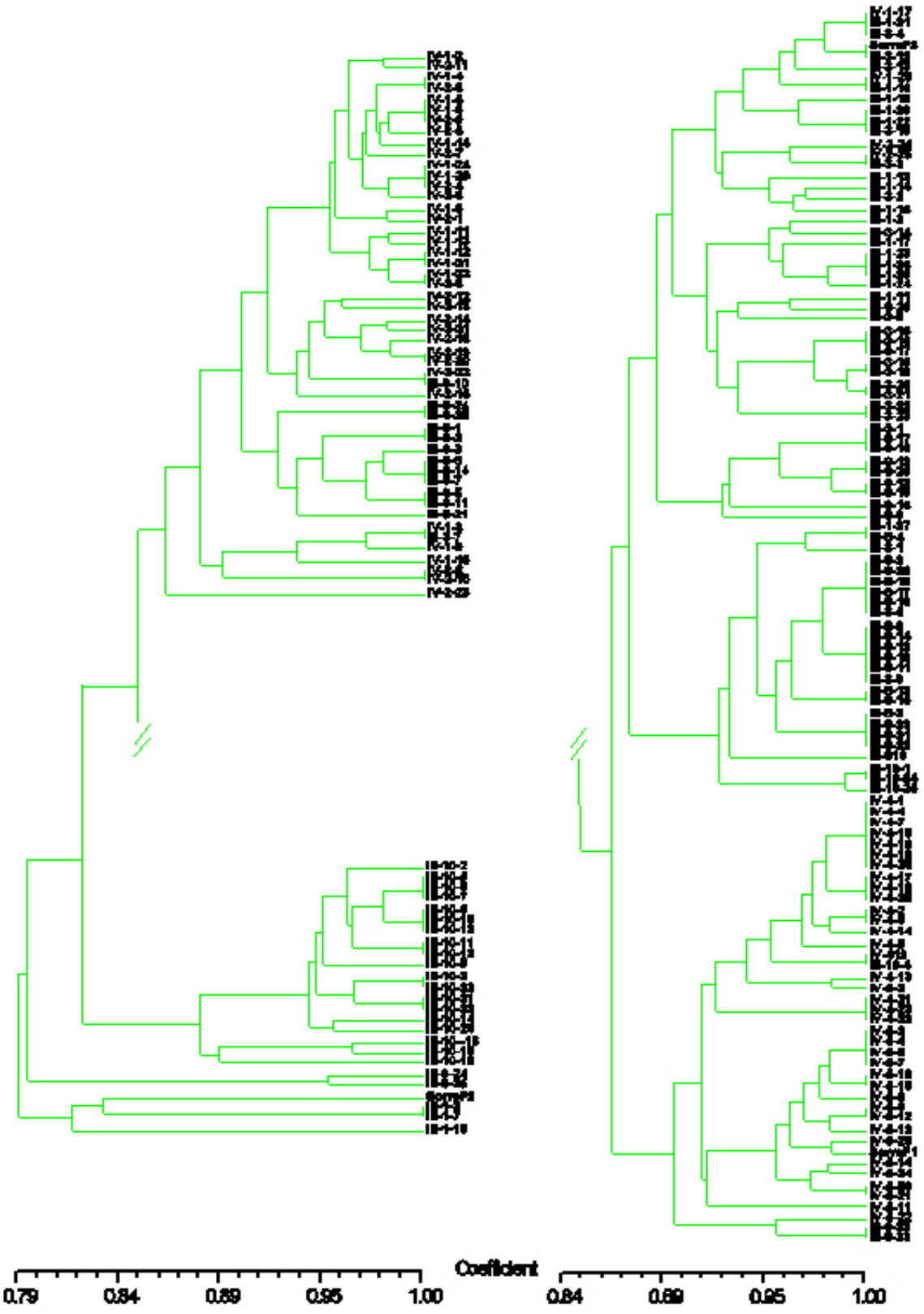


Figure 3. UPGMA diagram for 188 individuals from Berhane Kontir (III) and Yayu (IV) based on six dinucleotide primers, employing Jaccard's coefficients. The tree is split into two parts for graphical display (Tesfaye et al., submitted c).

Patterns of genetic diversity in *C. arabica* suggest considerable gene flow within and between regions. Such patterns are actually characteristic for outcrossing species, where the hierarchical patterning of populations is more or less obscured by gene flow. However, this is

contrast to existing reports of *C. arabica* as being autogamous (Bridson and Verdcourt, 1988; Free, 1993). Pollination data in coffee were obtained from cultivars in plantations, and these plants may in fact behave differently from wild *C. arabica*. Gene flow might be recent, and for example mediated by pollinators such as bees (Fichtl and Adi, 1994). Migration of plants in the species' evolutionary history due to changes in environmental conditions might also have played a role, and led to introgression of differing genotypes into geographically distant populations. In the case of Bonga the extraordinarily high diversity could be the result of historical human activity (Zewde, 2002).

Intraregional analysis

Dense sampling of individuals in Berhane Kontir and Yayu demonstrates considerable genetic diversity within both regions (Figure 3). Moreover, it shows a predominant fine scale spatial patterning of genotypes (Tesfaye et al., submitted c). Neighbouring individuals are more likely to be genetically similar than distant individuals. This could be caused by local pedigree structures as a result of limited gene dispersal (Chung et al., 2004). However, since the patterning of genotypes in the investigated plots is not totally hierarchical, gene flow may still play a role, which seems to be particularly evident in disturbed plots (III-9, IV-1 and IV-2). Genetic diversity of populations in disturbed forests may therefore be higher, but this seems a rather local effect, and does not necessarily mean that many unique genotypes exist in disturbed plots.

Characterization of nuclear microsatellites

Work on several nuclear microsatellite loci is currently underway for wild *C. arabica*. However, up to four fragments of different size are usually amplified in a single individual. In the tetraploid *C. arabica,* different fragments are potentially amplified from xenologous loci from the different parental genomes. Thus, in order to recognize true genetic diversity and heterozygosity, it is necessary to distinguish loci from the different parental genomes or to find loci with sufficient variability that show a diploid pattern of inheritance (nuclear microsatellites are co-dominant and inherited in a Mendelian manner). Characterisation of satellite loci through comparative sequencing of fragments amplified from *C. arabica*, *C. eugenioides*, and possible paternal parents is underway with the aim to develop a system for routine screening.

CONCLUSIONS AND FUTURE WORK

The fact that wild populations of *C. arabica*, as growing in forest coffee and semi-forest coffee systems, are genetically distinct from landraces (farmer's variety) underscores the need to conserve forest coffee. Moreover, since most regions possess their own unique genotypes, there is the need of a multi-site *in situ* conservation approach. Whereas the scientific data for a priority list of forest areas for *in situ* conservation of *Coffea* are available, more work is needed to evaluate and plan necessary conservation measures, e.g. minimum sizes of conserved forest areas, effects of forest management, and coffee cherry harvesting. Many of the remaining questions are connected to understanding levels of heterozygosity and gene flow. The establishment of a nuclear microsatellite marker system and their application in large scale screening has therefore high priority, and will be followed up in the CoCE project. Nevertheless, ISSR markers are already available and can be used for fingerprinting elite breeding materials and also certification of wild coffee.

ACKNOWLEDGEMENTS

The present research was carried out in the framework of the CoCE project (Conservation and use of wild populations of *Coffea arabica* L. in the montane rainforests of Ethiopia) in the BIOTEAM program, sponsored by the German Federal Ministry of Education and Research (BMBF). We acknowledge support by the Ethopian Institute of Agricultural Research (EIAR, formerly EARO), the Ethiopian Institute of Biodiversity Conservation (IBC), the Jimma Agricultural Research Center (EIAR), and the Sinana Agricultural Research Center (OARI). Without their help it would not have been possible to carry out this research.

REFERENCES

Aga E. 2005. Molecular genetic diversity study of forest coffee tree (*Coffee arabica* L.) populations in Ethiopia: Implications for conservation and breeding. Doctoral Thesis, Faculty of Landscape planning, Horticulture and Agricultural Science.

Anthony F., Combes M.C., Astorga C., Bertrand B., Graziosi G. and Lashermes P. 2002. The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. Theor. Appl. Genet. 104:894-900.

Bridson D.M. and Verdcourt B. 1988. Rubiaceae, Part 2. In: Polhill R.M., (ed.), Flora of Tropical East Africa. Balkema, Rotterdam The Netherlands, pp. 703-727.

Chung M.Y., Nason J.D. and Chung M.G. 2004. Spatial genetic structure in populations of the terrestrial orchid *Cephalanthera longibracteata* (Orchidaceae). Am. J. Bot. 91:52-57.

Cros J., Combes M.C., Trouslot P., Anthony F., Hamon S., Charrier A. and Lashermes P. 1998. Phylogenetic analysis of chloroplast DNA variation in *Coffea* L. Mol. Phylogenet. Evol. 9:109-117.

Free J.B. 1993. Insect pollination of crops. London: Academic Press.

Fichtl R. and Adi A. 1994. Honeybee flora of Ethiopia. DED, Margraf Verlag.

Gole T.W. 2003. Vegetation of the Yayu forest in SW Ethiopia: impacts of human use and implications for *in situ* conservation of wild *Coffea arabica* L. populations. Ecology and Development Series, No.10, ZEF, University of Bonn.

Lashermes P., Trouslot P., Anthony F., Combes M.C. and Charrier A. 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. Euphytica 87:59-64.

Lashermes P., Combes M.C., Trouslot P. and Charrier A. 1997. Phylogenetic relationships of coffee-tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. Theor. Appl. Genet. 94: 947-955.

Lashermes P., Combes M.C., Robert J., Trouslot P., D'Hont A., Anthony F., and Charrier A. 1999. Molecular characterization and origin of the *Coffea arabica* L. genome. Mol. Gen. Genet. 261: 259-266.

Löhne C. and Borsch T. 2005. Phylogenetic utility and molecular evolution of the petD group II intron in basal angiosperms. Mol. Biol. Evol. 22: 317-332.

Petit R.J., El Mousadik A. and Pons O. 1998 Identifying populations for conservation on the basis of genetic markers. Conserv. Biol. 12**:** 844-855.

Raina S.N., Mukai Y. and Yamanoto M. 1998. *In situ* hybridization identifies the diploid progenitor species of *Coffea arabica* (Rubiaceae). Theor. Appl. Genet. 97: 1204-1209.

Ruas P.M., Ruas C.F., Rampim L., Carvalho V.P., Ruas E.A. and Sera T., 2003. Genetic relationship in Coffea species and parentage determination of interspecific hybrids using ISSR (Inter- Simple Sequence Repeat) markers. Genet. Mol. Biol. 26: 319-327.

Senbeta W.F. 2006. Biodiversity and ecology of afromontane rainforests with wild *Coffea arabica* L. populations in Ethiopia. Ecology and Development Series No. 38, ZEF, University of Bonn.

Swofford D.L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony. Sinauer Associates, Sunderland (*and other methods).

Tesfaye G.K., Borsch T., Govers K. and Bekele E. (submitted a). Characterisation of *Coffea* chloroplast microsatellites and evidence for the recent divergence of *C. arabica* and *C. eugenioides* chloroplast genomes. Genome.

Tesfaye G.K., Govers K., Bekele E. and Borsch T. (Submitted b): ISSR fingerprinting of *Coffea arabica* throughout Ethiopia reveals high variability in wild populations and distinguishes them from farmer's varieties.

Tesfaye G., K., Govers, K., Oljira, T., Bekele, E. and Borsch, T. (submitted c) Genetic diversity of wild *Coffea arabica* in Ethiopia: Spacial patterning depends on scale. Biodiversity and Conservation.

Weising K. and Gardner R.C. 1999. A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyle-donous angiosperms. Genome 42: 9-19.

Zewde B. 2002. A history of modern Ethiopia, 1855-1991. 2nd edition, Addis Ababa University Press.

In situ Conservation and Use of the Wild Populations of *Coffea arabica* in the Montane Rainforests of Ethiopia

M. DENICH AND F. GATZWEILER

Center for Development Research (ZEF), University of Bonn, Walter-Flex-Str. 3, 53113 Bonn, Germany

SUMMARY

Coffea arabica has its center of origin in the highlands of southwest and southeast Ethiopia. Wild Arabica coffee grows there in the understory of montane rainforests and is traditionally used by local people for their own consumption and as a cash crop. Internationally, the wild coffee represents above all an important gene pool for breeding, but also supplies a small specialty niche market in industrial countries. The montane rainforests with the wild coffee populations and their gene pool, however, are highly threatened by deforestation due to settlement and agricultural land-use pressure. From August 2002 to July 2006, diversity and economic value of the Ethiopian coffee gene pool as well as its forest habitat were studied in a transdisciplinary German-Ethiopian research project which considers natural sciences, economics and social sciences. The respective field studies were carried out in five forest regions of southwest and southeast Ethiopia. Vegetation analyses reveal that the montane rainforests differ in their plant species composition from region to region. Molecular-genetic analyses confirm the high genetic diversity of wild *Coffea Arabica*. Physiological studies show that drought tolerance of wild coffee is site-specific. Furthermore, wild coffee populations contain germplasm which is tolerant of coffee leaf rust and coffee berry disease. By means of a cost-benefit analysis, the potential economic value of the Ethiopian wild coffee gene pool as a resource for coffee breeding was assessed. Its present value amounts to US$ 0.4-1.5 billion. Based on the project's results, concepts are to be developed for conservation and use of the genetic resources of wild *Coffea arabica* populations in Ethiopia. Wild *Coffea arabica* will only be conserved in the montane rainforests when the potential value of the coffee-genetic diversity can be transformed into real benefits for the people in the forest regions. For that, a financial incentive system is necessary. International fund-raising mechanisms for financing the incentives have to be developed. The coffee sector might play a prominent role in this context.

INTRODUCTION

Arabica coffee (*Coffea arabica*, Rubiaceae) has its center of origin in the highlands of southwest and southeast Ethiopia where wild coffee populations grow naturally in the undergrowth of the Afromontane rainforests at altitudes between 1,000 and 2,000 m. The wild coffee populations are highly endangered by land-use pressure on the montane rainforests (Tadesse et al., 2002; 2003; Feyera, 2005). Poverty and conflicting property rights make farmers convert forests into agricultural or pastoral land, thereby threatening the entire biodiversity of the rainforests. As wild coffee is collected by local people, the biodiversity of the forest habitat is also threatened by management interventions to increase the productivity of the wild coffee stands. Intensive management of wild coffee leads to the conversion of coffee forests into semi-forest coffee systems.

Ethiopia is currently the seventh largest coffee producer worldwide. Its annual production amounts to about 260,000 t (2005). Coffee is by far Ethiopia's most important export crop and, with 41% (FAO 2006), contributes decisively to the country's foreign currency income. Furthermore, for 700.000 households coffee is a source of income (EEA 2001). The most important cultivation areas are in southwest and south Ethiopia. Ninety-six percent (96%) of the Ethiopian coffee is produced in traditional coffee production systems. These are, forest coffee systems in which wild coffee is only collect by local people and management activities are minimal (5-6% of the Ethiopian coffee production) and semi-forest coffee systems (about 20% of the coffee production) where undergrowth which competes against coffee is removed and replaced with coffee plants traditionally gathered from wild populations. Seventy percent (70%) of the Ethiopian coffee is produced as so-called "garden coffee" in traditional small coffee plantations in pure culture or in mixed cultivation systems and only 4% comes from large-scale plantations (Demel, 1999).

International coffee breeding aims particularly at disease tolerance, production stability and quality improvement. Breeding and selection for disease tolerance focused on coffee leaf rust, coffee berry disease and have to focus on coffee wilt disease in the near future. Production stability can be achieved amongst others by adapting coffee cultivars to adverse ecological conditions, such as drought stress. In many parts of the coffee-growing world coffee cultivation is increasingly being displaced from the more favorable sites and moved to marginal land. With regard to quality, cup quality always is an issue as well as coffee beans with low caffeine content would reduce processing costs and improve the taste of decaffeinated coffee. Furthermore, coffee diseases develop local races and also the consumers' demands on coffee quality vary from region to region and in time. Therefore, a diverse gene pool is of paramount importance for breeding. Particularly, cross breeding of cultivars and wild genetic material leads to results above average due to heterosis effects. The Ethiopian wild coffee populations provide highly diverse genetic material for future coffee breeding and selection.

The gene pool of *Coffea arabica* is highly endangered by increasing land-use pressure on the montane rainforests. The importance of rainforest protection can be viewed against the background of man-made destruction or conversion in about 40% of the southwest Ethiopian forests during the last thirty years. Currently, only about 2,000 km^2 forests are left undisturbed (Tadesse et al., 2002), whereas the remaining are extremely fragmented. This development leads to an irreversible reduction of the gene pool of *Coffea arabica* resulting in huge benefits forgone in coffee breeding and production (Hein and Gatzweiler, in press). Therefore, the possibilities of in-situ conservation with the establishment of conservation areas – in addition to ex-situ conservation in field gene banks – must be exploited. The importance of in-situ conservation at the species' natural site is that natural selection and adaptation mechanisms with regard to changing site and environmental conditions are maintained.

The importance of the Ethiopian montane rainforests has been internationally acknowledged as they are part of the "Eastern Afromontane Biodiversity Hotspot" since January 2005 (http://www.biodiversityhotspots.org/xp/Hotspots/afromontane/).

RESULTS OF THE RESEARCH PROJECT ON WILD ARABICA COFFEE

From August 2002 to July 2006 (herein after referred to as CoCE I which stands for "Conservation and use of the wild populations of *Coffea arabica* in the montane rainforests of Ethiopia"), the diversity and the economic value of the Ethiopian coffee gene pool and its forest habitat have been assessed. Based on the findings, concepts of model character are to be

developed for conservation and use of the genetic resources of wild *Coffea arabica* populations in Ethiopia. The in-situ conservation of wild coffee offers a promising approach as the conservation of coffee genetic diversity is connected with the conservation of forest species diversity, i.e. the conservation of coffee gene pool becomes rainforest conservation and vice versa.

The investigation of wild coffee populations in their comprehensive biodiversity context called for a multidisciplinary approach which considers natural sciences, economics and social sciences. The respective field studies were carried out mainly in Bonga, Boginda, Berhane-Kontir, Maji and Yayu forest located to the West and in Harenna forest in the Bale Mountains East of the Great Rift Valley.

CoCE I was subdivided into six subprojects:

1. In forest areas with wild coffee occurrence and different use intensities, studies on vegetation structure and floristic composition as well as the structure of the coffee populations was performed along (altitudinal) transects.
2. Applying DNA fingerprinting techniques, the genetic diversity of wild coffee within and in between the different studies is being investigated. Additionally, genetic differences between wild coffee and land races as well as cultivars were assessed.
3. Investigations were carried out on the site-specific drought tolerance of the wild coffee populations along a precipitation gradient and, for comparison, of the respective accessions under controlled water conditions in an experimental station. The variability in drought tolerance can be seen as an example for wild coffee diversity with regard to an abiotic factor.
4. Important for profit-yielding coffee production is the availability of plant material tolerant of diseases and pests. In wild coffee populations along the above-mentioned rainfall gradient, the tolerance regarding the fungal diseases coffee berry disease and coffee leaf rust was studied in the field and in greenhouse experiments. The variability in fungal disease tolerance is an example for wild coffee diversity with regard to a biotic factor.
5. The economic value of the coffee-genetic resource for international breeding programs as well as of the forest with wild coffee occurrence was assessed. The assessment of the latter was carried out from farmer's and society's perspective considering conservation and use aspects.
6. The institutional framework (formal and informal) relevant for conservation and use of the wild coffee and the forest resources has been studied at local, regional and national level.

Summarized, the following results were achieved in CoCE I:

In the vegetation surveys more than 700 plant species were found. That amounts to 10% of the Ethiopian flora. The vegetation analyses reveal that the montane forests of the five study regions are floristically significantly different. The floristic compositions of forest areas with wild coffee occurrence, however, are cross-regionally very similar, thus, allowing the definition of "coffee forests". The occurrence of wild coffee very much depends on altitude (1,300-1,600 m) and management intensity by local coffee collectors. Management intensity, i.e. the extent to which competing vegetation is removed from the undergrowth, on its part determines species composition and structure of the coffee forests (Feyera, 2005; 2006; Schmitt, 2006).

Molecular-genetic analyses based on Inter-Simple Sequence Repeat (ISSR) markers confirm the high genetic diversity of wild *Coffea arabica* and show that wild coffee clearly differs genetically from landraces and cultivars. In particular, it can be shown that some regions have

genetically similar (Boginda, Berhane Kontir), other regions very different wild coffee populations (Bonga). In general, high levels of diversity within regions can be observed. Forest management by coffee collectors often increases the abundance of wild coffee plants; harvesting of the coffee berries, however, does not seem to have negative impacts on the genetic diversity given that coffee trees are self-regenerating (see Kassahun Tesfaye Geletu et al., this volume).

From a strict utilitarian point of view, genetic diversity is not a priori a value. In combination with the variability of ecological and physiological properties of the coffee plants, however, genetic diversity gains in importance. Therefore, the drought tolerance of wild coffee populations along a rainfall gradient has been analyzed. Ecophysiological studies reveal that drought tolerance is site-specific and the water use efficiency of wild coffee plants increases with decreasing mean annual rainfall of the study sites. In addition, drought-stressed coffee plants recover faster at the end of dry spells when they originate from dry environments such as Harenna or Bonga (see Taye Kufa et al. and Burkhardt et al., both this volume, Beining 2006).

To support the practical importance of the genetic diversity of wild coffee, the variability of coffee plants and populations regarding their tolerance towards coffee leaf rust (CLR) and coffee berry disease (CBD) was assessed. In wild coffee populations, 30-70% of the plants are infected by CLR, but major damages could never be observed, let alone the total eradication of a coffee stand as it happens in plantations in other parts of the world. Infection experiments in the field with the fungus *Colletotrichum kahawae* which is causing CBD demonstrate that 53-100% of the coffee berries are tolerant to CBD. It can be concluded that the disease situation in the coffee forests is relaxed and germplasm exists for breeding for CLR and CBD tolerance (see Hindorf et al., this volume). Furthermore, field studies revealed that coffee wilt disease, a fungal disease formerly known only from *Coffea canephora* (Robusta coffee), now also attacks *Coffea arabica* in Ethiopia, a fact that deserves closer attention.

By means of a cost-benefit analysis, the global economic value of the wild coffee gene pool as a resource for coffee breeding was assessed (Hein and Gatzweiler, in press). For this purpose, the following benefits of a breeding program based on wild coffee populations, were assumed: (1) reduction of yield losses due to diseases and pest attacks (CBD, CLR, nematodes) amounting to 2.1 billion US$ per year, (2) reduction of costs for chemical decaffeination through provision of low-caffeine plants (191 million US$ per year) and (3) 20% yield increase. The costs comprise a breeding program over 15 years and the introduction of the newly bred cultivars within another 15 years. Based on these assumptions, the net present value of the Ethiopian coffee genetic resource amounts to US$ 0.42 and US$ 1.46 billion at discount rates of 10% and 5%, respectively.

The economic valuation of the coffee forests has been carried out from the farmer’s and the society’s perspective (Rojahn, 2006). An income analysis reveals that from farmers’ perspective the conversion of the forest into arable land (selling of timber and maize production) is more profitable in the short run than sustainable forest management. The income from the latter would only amount to 65-75% of the former. Private and social economic analyses differ with regards to the type and amount of values taken into account. Looking at the value of coffee forests from the society's perspective means to take all values of the forest into account. These include, amongst others, sustainable timber production, collection of wild coffee and its sale for a premium price and ecosystem services the forest cover provides. Under those conditions sustainable forest management achieves higher net benefits as compared to exclusionary conservation or conversion into arable land which only achieve 50 and 70-85%, respectively, of the benefits from sustainable forest management.

Management concepts aiming at the conservation and use of natural resources might create conflicts. In Ethiopia, all forests are nationalized. Most coffee forest areas are located in so-called National Forest Priority Areas (NFPA), where local forest users have limited access and use rights, which conflict with traditional property rights. In combination with lacking incentives and absent monitoring and enforcement activities, this has led to a de facto open access situation. Institutional research found that the traditional use rights in the forests are still practiced, i.e. the forests are subdivided into clearly defined plots each of which is the property of an individual family (Stellmacher, 2006). This fact is very often unknown or ignored by new settlers and predetermines conflicts between traditional forest users and new settlers. Although there are as many functioning traditional and informal community-based institutions (e.g. norms, regulations, networks) as formal institutions (e.g. authorities, NGOs, official use rules for NFPA) operational at the local level, there is hardly any cross-institutional link which would be necessary for the pursuit of conservation and natural resource management concepts (Teklu 2006).

As has been shown, CoCE I produced important information and helped to advance our knowledge about wild *Coffea arabica* and its natural habitats, the montane rainforests of Ethiopia. In particular, it could be proven that

- there is a high degree of species diversity in the montane rainforests both at local and cross-regional level,
- there is also a high degree of genetic diversity in wild *Coffea arabica* populations differing from region to region (the differences in its disease and drought tolerance exemplify the genetic diversity),
- wild coffee and coffee forests have a considerable potential economic value at global and local scale, respectively, and
- a multitude of local, regional and national stakeholders with conflicting interests, mandate discontinuities, changing responsibilities as well as diverging property rights are involved in the use and management of forest resources.

In general, research results support the assumption that the wild coffee has contributed to maintain at least a minimum of forest resources as they have been used traditionally for time periods memorable although there were demands for arable land at all times. Nevertheless, high settlement and agricultural land-use pressure still exist and continually reduce the remaining forest fragments together with the wild coffee populations. Even though the potential economic value of the coffee-genetic resource and the forest could be demonstrated, a financial analysis from farmers' perspective verified that the conversion of forest into agricultural land is an economically sound decision. Influencing in the behavior of farmers is difficult due to lack of financial resources for conservation incentives and income alternatives or insecurity of land tenure which are just some of the factors which keep the pressure on the forest resources.

CoCE I concludes that basically four problem areas have to be tackled to realize the conservation and use of wild coffee populations in the montane rainforests of Ethiopia:

1. As natural forest areas are shrinking and coffee production is increasingly based on modern coffee cultivars instead of landraces and wild coffee, practical measures have to be developed to preserve the wild coffee gene pool *in situ* but even detached from strict forest conservation.
2. The potential economic value of the wild coffee-genetic resource has to be transformed into real economic benefits for the rural population through adequate incentive and financing mechanisms.

3. Implementation strategies have to be developed which include communication and public awareness building, education as well as strengthening institutions for conservation and sustainable use of forest resources.
4. In the course of CoCE I, new research questions have evolved which require further attention. These include genetic diversity, coffee diseases, coffee quality as well as the relationship between rules and regulations of forest management and the condition of the forests.

WHAT HAS TO BE DONE IN THE NEAR FUTURE?

The project "Conservation and use of the wild populations of *Coffea arabica* in the montane rainforests in Ethiopia" will contribute to the in-situ conservation of the genetic resources of wild *Coffea arabica* in its natural habitat, the montane rainforests in southwestern Ethiopia. As wild coffee is a component of the forests, its conservation can only be guaranteed if the montane rainforests themselves are protected. Thus, the overall objective of the project is to combine the conservation of the genetic diversity of the wild coffee populations with the conservation of the species and ecosystem diversity of the montane rainforests. Rainforest conservation thereby becomes conservation of the coffee gene pool and vice versa.

To further approach the overall objective, the research and development activities of the second project phase (2006-2009; herein after referred to as CoCE II) will focus on

- guidelines for specific sets of rules and regulations as incentive for conservation and sustainable use in different zones of the forest conservation area (biosphere reserve) and the generation of criteria for the development of a simple forest management and monitoring system,
- the development of financing mechanisms for the conservation activities,
- the assessment of costs and benefits of a prospective coffee-forest protected area,
- the development of a certification procedure for wild coffee, in particular concerning a geographical indication, to achieve premium prices on the market,
- the establishment and consolidation of a non-governmental organization which was recently founded (end of 2005) as Ethiopian Coffee Forest Forum (ECFF). This NGO is intended to guarantee the sustainability of the research results and their implementation by communication, public awareness building, education as well as strengthening the institutions involved in the conservation and use of forest resources, in particular wild coffee. ECFF itself is a potential future conservation NGO with responsibilities related to the establishment and management of a future protected area.

CoCE II will focus strongly on implementation-oriented research and activities as well as on the linkages between science, people and policy making. In a nutshell, our approach involves viewing the research objective from a perspective that goes beyond specific disciplines and is based on participation, characterised by systematic cooperation with the stakeholders concerned. Although research will continue on selected topics in the social and natural sciences, the outputs will be incorporated into implementation strategies or translated into practical activities. This approach strengthens the implementation orientation of the entire project. Part of the innovative strategy of disseminating and translating scientific results into action through the activities is the newly founded Ethiopian Coffee Forest Forum (ECFF), which is the first of its kind in Ethiopia to address coffee forest conservation issues.

REFERENCES

Beining, A. 2006. Contrasting adaptation to drought stress in wild populations of *Coffea arabica* in Southwest Ethiopia. Dissertation, University of Bonn, Germany.

Demel T., 1999. History, botany and ecological requirements of coffee. Walia 20: 28-50.

EEA – Ethiopian Economic Association 2001. Annual Report on the Ethiopian Economy. 1999/2000

FAO 2006 Special Report FAO/WFP Crop and Food Supply Assessment Mission to Ethiopia. http://www.fao.org/docrep/008/j7071e/j7071e00.HTM

Feyera S. W., 2006. Biodiversity and ecology of Afromontane rainforests with wild *Coffea* arabica L. populations. Ecology and Development Series 38. ZEF Bonn, Germany.

Feyera S., Schmitt, C., Denich, M., Sebsebe D., Vlek, P. L. G., Preisinger, H., Tadesse W. and Demel T., 2005. The diversity and distribution of lianas in the Afromontane rain forests of Ethiopia. Diversity and Distributions 11, 5: 443-452.

Hein, L., Gatzweiler, F.W. (in press). The Economic Value of Coffee *(Coffea arabica)* genetic resources. Ecological Economics.

Rojahn, A. 2006. Incentive mechanisms for a sustainable use system of the montane rain forest in Ethiopia. Dissertation, University of Kiel, Germany.

Schmitt, C. 2006. Montane rainforest with wild Coffea arabica in the Bonga region (SW Ethiopia): plant diversity, wild coffee management and implications for conservation. Dissertation, University of Bonn, Germany,

Stellmacher, T. 2006. Analysis of institutional factors influencing use and conservation of wild coffee in Ethiopia. A comparative study in Bonga Forest/Kaffa Zone and Harenna Forest/Bale mountains. Dissertation, University of Bonn, Germany.

Tadesse W. G., M. Denich, Demel T. and P.L.G. Vlek, 2002. Human impacts on *Coffea* arabica genetic pools in Ethiopia and the need for its in situ conservation. In: Managing plant genetic diversity. R. Rao, A. Brown, M. Jackson (eds), CAB International and IPGRI, 237-247.

Tadesse, W. 2003. Vegetation of the Yayu forest in SW Ethiopia: impacts of human use and implications for in situ conservation of wild *Coffea* arabica L. populations. Ecology and Development Series 10. ZEF Bonn, Germany.

Teklu Tesfaye Toli 2006. Coffee forest conservation: Local-level institutions influencing the conservation and use of coffee forests in southwest Ethiopia. Sozialwissenschaftliche Schriften zur Landnutzung und ländlichen Entwicklung 72. Margraf Publishers, Weikersheim, Germany.

Arabica Coffee (*Coffea arabica* L.) Local Landrace Development Strategy in its Center of Origin and Diversity

B. BELLACHEW[1] AND J-P. LABOUISSE[2]

[1]Jimma Agricultural Research Center, Jimma, Ethiopia, P.O. Box 192.
E-mail: bbellachew@yahoo.com
[2]CIRAD, Montpellier, France. E-mail:jean-pierre.labouisse @cirad.fr

SUMMARY

Arabica coffee (*Coffea arabica* L.) breeding and selection methods applied every where to improve production, productivity and quality are generally the same. However, the application of these methods may vary from country to country depending on the amount of genetic variability available, ecological conditions and research focus or prevailing production constraints of the country. Ethiopia is the center of origin and diversity of arabica coffee. The country is ecologically very diverse and coffees grown under these environments are different in quality, disease resistance, yield potential and many other traits. Development of breeding strategy that fits to these conditions is of paramount importance to exploit all the available advantages. In the past, the interest was to develop varieties that have wider adaptation and distribute to all coffee growing areas. It was, however, realized that distribution of such limited varieties to all coffee growing areas adulterates the typical quality of each specific locality or region, manifested poor adaptation and less preference by the local farmers compared to their respective local cultivars. A new breeding strategy, known as '*Local Coffee Landrace Development Program*', was designed to alleviate these problems. The new approach is aimed at development of varieties for each specific agro-ecology using the respective local landraces and this is elaborated in the text. The Collection of local landraces and establishments of seven research centers (Jimma, Agaro, Gera, Tepi, Awada, Haru and Mechara) that represent different agro-ecological zones of the major coffee growing areas have greatly facilitated the implementation and effectiveness of the new program. Currently, landraces roughly amounting to 1900 accessions from Harerge, 350 from Sidamo, 590 from West Wollega, and 200 from Limmu have been collected and established at the research centers available in the respective areas. Systematic evaluation of some part of the accessions in a crash program resulted in the identification of over 26 promising selections for three agro-ecologies in quite short time (five years). The selections were advanced to verification trial to confirm their performance before recommending for release as pure-line varieties for each agro-ecology. The implication of the new breeding strategy in promoting market oriented research and specialty coffee, the significance of hybrid development for increased yield and productivity and the focus of future breeding are discussed.

INTRODUCTION

Arabica coffee (*Coffea arabica* L.) of the family Rubiacae originates from the South Western montane rainforests of Ethiopia where it has its center of genetic diversity and grows as an understorey (Sylvain, 1958; Tadesse et al., 2001; FAO, 1968). In Ethiopia, Aarabica coffee grows under very diverse agro-ecologies covering ranges of altitudes (500m in the Gambella plain to 2600 m in Wollo, Northern Ethiopia) (Bayetta, 1987; Mesfin et al., 1987), temperature (Min. 8-15 ^{o}C, and Max. 24-31 ^{o}C), rainfall (800 to 2000 mm) (Zef and Earo, 2002), humidity (60-80%), and soil types. The coffees grown under these diverse

environments showed wide genetic variations within and between populations of different regions or environments for yield, quality disease resistance and other traits.

The availability of such genetic variations provides immense possibilities for improvement of the crop for any desirable traits of interest. On the other hand, the presence of such high environmental diversity, distinct variation in coffee quality within and between regions or localities (CTA, 1999) and location specificity of our improved varieties (Mesfin and bayetta, 1987) make the breeding program more complex. The coffees from Harerge, Limu, Yirgachefe, and Gimbi, fore example, have distinctly different quality attributes and fetches premium price in the world market. The CBD resistant varieties originated from south western Ethiopia did not adapt to Harerge and many other areas and farmers were reluctant to grow them except in south western areas. There fore, it would be difficult to easily obtain varieties that have wider adaptation and at the same time maintain the typical quality of each particular area. This challenge was an impetus for the development of a new breeding strategy that alleviates these problems, best fits to the Ethiopian conditions and enables to exploit all the available advantages of ecological and genetic diversities. In effect, a new improvement strategy known as '*local landrace development program*' has been initiated and the objective of this report is to provide a brief account of this approach in relation to the conventional method.

ARABICA COFFEE BREEDING METHODS IN ETHIOPIA

Arabica coffee breeding principles and methods applied in different coffee growing countries and the over all objectives, improved productivity and quality, are generally similar. Van der Vossen (1985; 2001) distinguished four basic methods – pure-line selection, pedigree selection, hybridization (intraspecific F1 hybrids), and interspecific hybridization followed by backcrossing and pedigree selection. However, the application of these methods may vary from country to country depending on the amount of genetic variability available, ecological conditions and prevailing production problems. In Ethiopia, pure-line selection and intra-specific hybridization are commonly used.

Pure-line selection

The coffee breeding program in Ethiopia was initiated in 1969 with the local coffee collections made available by the French coffee collection mission to Ethiopia in 1966. Since then, a lot of experience had been gained and considerable improvement had been made in developing proper breeding methods and procedures that best suit to Ethiopian conditions. Specifically, the pure-line variety development approach has been greatly improved.

The conventional approach (1969-1993)

In this approach, there were national and international collection programs. All the materials made available through local collections from different areas and introductions from abroad are first screened at one location, Jimma Agricultural Research Center (JARC) for yield, diseases, quality and other desirable agronomic traits. Materials that exhibited superior performance for yield and other characters are selected for advanced replicated multi-location trials in different parts of the country to test their adaptability and repeatability. The best selections are further armers verified in their respective areas of adaptation on a larger plot size under farmers condition. Selections that pass the final verification test are released as a new variety upon approval by Standing Committee on Coffee Research (SCCR). This process is so long particularly for perennial crops like coffee that it is known as 'long-term program'.

On the other hand, the outbreak of CBD in 1971 in Ethiopia had forced us to also develop short-term program and release CBD resistant cultivars in the shortest time possible. This program is sometimes known as 'crash' and was developed by Robinson (1974). It was called 'crash' because the normal procedure was ruled out and multiple step-wise activities were simultaneously undertaken to shorten the period of selection. This crash program involves mother tree selection, testing of the mother trees *in situ* and the progenies in the nursery and field. Selections that exhibited high level of resistance during mother tree and seedling progeny testing are immediately established in a large progeny block in order to distribute seeds as soon as a selection passes the final test.

The conventional method of screening at one location reduces initial cost of selection and facilitates close supervision of the experimental field and effective data collection. On the other hand, this method had several drawbacks: (1) difficulty to develop a number of varieties that adapt to all environments, (2) low preference of the released varieties by the local farmers when released to all areas, (3) adulteration of the typical quality of specific and known areas by introducing improved varieties originated in other areas.

The New (Modified) Approach (since 1994)

Cognizant of the aforementioned drawbacks noted with the conventional approach and the environmental diversity of the major coffee growing areas, a new breeding strategy has been designed by Bayetta Bellachew, senior coffee breeder, for the first time while preparing ten-years development plan during the 'Derg' regime, earlier to 1991. This new selection and breeding strategy is known as **'***local landrace development program***'.** In this program, varieties are developed for each major agro-ecology or coffee growing area independently based on local landrace collections of the respective areas. The basic assumptions are that (1) local landraces have better adaptation in their areas of origin than cultivars introduced from other geographical and ecological origins, (2) farmers show more preference for local cultivars than those improved varieties introduced from other areas, (3) it is possible to maintain the typical quality of each locality and (4) it is market oriented in a sense that consumers preference for a specific locality can be maintained and origin based diversity of specialty coffee can be produced.

Strategies for the Implementation of Local Landrace Development Program

Since the previous conventional approach largely focused on the development of widely adapted varieties, improved local varieties for each specific agro-ecologies are lacking in most of the coffee growing areas. This necessitated accomplishment of two major tasks : (a) designing of quick germplasm screening and selection methods in order to develop improved varieties using local landraces in the shortest time possible for the respective localities and (b) land acquisition and establishment of new sub-centers and renovation of existing ones in the major coffee growing areas, namely Sidamo, Wollega, Harerge and South Western (Kaffa, , Jimma and Illubabor).

a) Germplasm screening and selection methods – A crash program (short-term) and long-term selection programs were developed in order to provide improved varieties in the shortest time possible and in the long-term, respectively.

The crash program **–**In this crash program, mother trees are selected from the forest or garden coffee for CBD resistance and other desirable traits and marked, the marked mother trees are evaluated *in situ* for one or two seasons for yield and CBD resistance. Simultaneously, seeds are collected for progeny testing for two seasons in glass house (seedling test) and in

replicated field trials (Table 1). The plan was to collect enough and reliable data within five years in order to identify CBD resistant materials with good yield, quality and growth performance that can be recommended for release. The varieties released on the fifth year can be multiplied through tissue culture for immediate distribution to growers, but establishment of seed orchard should commence simultaneously for sustainable and cheaper distribution of seeds.

Table 1. Strategy for the crash program designed to release CBD resistant selections within five years.

Year	Activities	
	Mother tree	Progeny
1	Mother trees selection, evaluation for CBD(visual,ABT,ST) and yield estimation	Seed sowing
2	Evaluation: ▸ CBD – visual$_2$, ABT$_2$, ST$_2$ ▸ Yield est.$_2$	Transplanting and field management
3	–	Field management
4	–	Evaluation - Yield (1st crop), CBD
5	–	• Evaluation - Yield (2nd crop), CBD, growth, quality • Data summarization and variety release

ABT=attached berry test, ST=seedling test, CBD=coffee berry disease.

Table 2. Strategy designed to implement the modified long-term program in eight years.

Year	Activities	
	Original plot	Verification plot
1	Germplasm collection and Planting	-
2	Maintenance	-
3	Recording (year 1)	-
4	Recording (year 2)	-
5	Recording and selection (year 3)	Multi-location planting in large replicated plots
6	Recording (year 4)	Maintenance
7	Recording (year 5)	Recording (year 1)
8	Recording (year 6)	Recording (year 2)

Note: Recording refers to collection of data for yield, major diseases, growth characters, quality and related observations.

The long term program – under normal process, the long term plan in coffee takes a minimum of 15 years if variety trial and verification trials are merged. However, if the screening (9 years), variety trial (6 years) and verification trial (5-6 years) are separately carried out in that order, the duration will prolong up to 20 years to release a pure-line variety. Considering the present situation where improved local varieties for different coffee growing areas are lacking, a **modified long term program** has been designed to shorten the duration and release local varieties for each locality in a relatively shorter period. In this program, it is designed to make the first screening in five years and advance the selections to verification trial but still continuing data collection on the original plot as well (Table 2). In doing so, six years yield data from the original plot and two years yield data from the verification plot shall

be obtained in eight years. This will provide sufficient and reliable data on yield, disease resistance, quality and other parameters to select the best variety for release.

The other interesting advantage of the modified long-term program is that three important step-wise operations are simultaneously conducted – verification, local adaptation or variety trial and seed orchard establishment. In effect, 10-15 best and promising selections are planted in three replications over two or three locations in large progeny plots of 150 trees per plot within each agro-ecology to accommodate the three activities in one trial.

b) Establishment of Research Centers in Different Agro-ecologies – The availability of research centers in each of the major agro-ecologies is the most important factor to implement the local landrace development strategy. Therefore, it was deemed necessary to renovate the existing sub-stations and establish new sub-centers in order to represent the major coffee growing areas or agro-ecologies. Different externally funded projects have been initiated at different times to materialize the proposed plan. The fund raised through Ethio-Swiss Coffee Research Project (ESCORP) financed by Switzerland government and third and fourth coffee improvement projects (CIP III, CIP IV) both financed by European commission (EC) has enabled to Establish new sub-centers namely Awada in the South (sidamo) in 1997, Haru in west Wollega in 1998 and Mechara in Harerge in 2005, respectively. Today there are five coffee research sub-centers and four testing sites established across the major coffee growing areas all representing different agro-ecology (Table 3).

Table 3. Coffee Research Sub-centers and testing sites established in the major coffee producing areas.

Research Center	Year established	Altitude (m.a.s.l)	Land holding (hectare)	Location (zone, region)
Main center - Jimma	1967	1753	183	Jimma, Oromia
Sub-centers				
• Gera	1974	1900	166	Illubabor, Oromia
• Tepi	1976	1200	40	Bench-Maji, Southern
• Awada	1997	1740	31	Sidama, Southern
• Haru	1998	1750	69	West Wollega, Oromia
• Mechara	2005	1800	50	West Harerge, Oromia
Testing sites				
• Agaro	1973	1630	15	Illubabor, Oromia
• Mettu	1974	1550	32	Mettu, Oromia
• Mugi	1973	1553	27	West Wollega, Oromia
Wonago	1974	1850	10	Gedeo, Southern

Progress in local landrace development program

i) Germplasm collection and maintenance – The long-term national and international coffee germplasm collection program was launched in 1970. Collections were made using random and with major emphasis targeted or pointed collection methods in order to capture as many genetic variability as possible and also to capture desirable genotypes *per se* for immediate breeding work, respectively. So far about 5127 accessions have been collected from different coffee growing areas of the country and maintained at Jimma and its sub-centers, but some (approximately 200-300) of them have died from poor establishment, overbearing die-back and diseases (Table 4). In the international collection program about 190 material which includes 28 rust differentials, 6 diploid species and 156 known international Arabica varieties have been introduced. The introduction from abroad ad been discontinued since 1984 because

of poor performance of the varieties compared to the locals and death of most of them from poor adaptation and disease problems except that variety Geisha and Catimore lines did well at lower altitudes of Bebeka and Tepi.

Table 4. Germplasm collection and maintenance.

Maintenance and testing site	No of accessions	Duration of collection	Remark
Jimma	802	1970-1990	Collections from various areas
	190	1969-1984	Introduced arabica varieties, some diploid species and rust differentials from abroad
	172	2004	Bale landrace collections
Jimma, Mechara	1863	1998-2002	Harerge coffee landrace collections
Gera	973	1973-1982	CBD resistant mother trees from south western ethiopia
Awada	499	1994-2005	Sidamo coffee landrace collections
Haru	591	1998-2001	Wellega coffee landrace collections
Agaro	187	2001-2005	Limmu coffee landrace collections
Total	5317		

ii) Germplasm Evaluation and Variety Release – Once the genetic variability (germplam) is at hand, the next step is to evaluate the germplasm for yield, quality, disease resistance and other desirable traits in a series of trials (screening, variety trials and final verification test) over years to come up with a new variety. At present, there are 22 pure-line varieties that have been released through the conventional approach (Table 5). The release of varieties through the new approach, local landrace development program, is at early stage. However, because of the envisaged 'crash' and 'modified long-term' programs, one variety has been released for Sidamo area (Gedeo and Sidama zones) and a number of promising selections are under verification for Sidamo, Wollega, Limmu and Harerge coffee growing areas.

Table 5. Varieties released and those in pipe-line for different agro-ecology.

Variety	Target areas and year released	No of Varieties	Yield (qt/ha)	
			Res. plot	Farmers field
Puer-lines released	All coffee growing areas,1978-81	13	12-20	8-10
	Low and mid-alt., SW Ethiopia, 1997-2002	5	18-21	9-15
	High altitude CBD prone areas of SW Ethiopia, 2005	4	15-23	15-17
	Gedeo and Sidama zones, Southern Ethiopia	1	20	16.0
Pure-lines in pipe-line	Sidamo, S. Ethioipia	12	Under simultaneous variety and verification trials plus seed garden establishment	
	West Wollega, W. ETH	14		
	Harerge, E. Ethiopia	14		
	Harerge (Crash)	82	Under simultaneous mother tree and progeny tests	
	Limu area, SW Ethiopia (crash)	197		
2. Hybrids	SW Ethiopia, 1997-2002	3	24-26	15-24
Total		26		

Hybridization: Intraspecific F_1 Hybridization

Hybridization is a means of aggregating two or more desirable traits in to a single plant. However, while making crosses, it is necessary to know that the desirable traits from different parent sources are dominant over the accompanying contrasting undesirable traits in order to exploit the advantage of F_1 heterosis. In Ethiopia, sets of crosses have been made between different elite local cultivars to study heterosis for yield, components of yield and resistance to CBD, the most important traits of breeding interest.

From the hybridization program, it has been learnt that there is considerable and consistent degree of heterosis in crosses among elite local cultivars that can be exploited in commercial production as noted in different sets of crosses and under different locations (Table 6). These results were the base to determine a proper breeding method that follow development of pure-line varieties followed by further improvement of the productivity and growth of the elite cultivars through intra-specific hybridization for each agro-ecology. So far three heterotic hybrids with good yield, quality and moderate resistance to CBD have been released.

Table 6. The expression of heterosis in different sets of crosses among elite indigenous cultivars.

Set	Breeding objective & traits observed	Better parent heterosis (%)		F_1s & parents (No)
		Range	Mean of F_1s	
I (1978)*	Test for hybrid vigor			10 (5)+
	• Yield	-8 – 60	17.4	
	• Stem girth	-2 – 12	5.0	
	• Length of 1st pr.br.	-2 – 10	2.4	
II (1989)	Heterosis & parental diversity			15 (6)
	• Fresh yield	9.0 – 70.8	53.0	
	• Clean yield	9.6 – 90.5	57.5	
	• Girth	-2.4 – 16	10.2	
	• Length of 1st pr.br.	-3.2 – 15.3	4.0	
	• Plant height	-4.9 – 13.9	2.3	
III (1995)	Test Sidamo x SW crosses			10 (5)
	• Yield	-7.9 – 79.1	35.1	
IV (1996)	Yield & quality improvement			15 (6)
	Yield – Jimma	0.1 – 42.7	18.3	
	– Mettu	14.1 – 57.3	32.7	
	– Tepi	38.2 – 103.8	74.3	

**, + Figures in parenthesis indicate the year the experiment was initiated and number of parents employed, respectively.*

In the national coffee breeding program, one of the major challenges was the outbreak of CBD in 1971. A selection program for resistance was developed and it was so successful because of the proper strategy developed and availability of high genetic diversity in the population. In the case of breeding for resistance to the disease, as in any other breeding program for disease resistance, the primary step was to study the mode of inheritance of resistance to the disease and determine the best method of breeding. In effect, crosses were made between resistant, intermediate and susceptible parents using six-parent diallel cross in the F_1 (Mesfin and Bayetta, 1984) and F_2 (Bayetta, 2001) generations.

In both generations, mean grade susceptibilities of the crosses resistant x susceptible and moderately resistant x susceptible showed significantly higher levels of susceptibility over the

mid-parent values and negative, but non significant differences compared to their susceptible parents. The result indicated that there was partial to complete dominance of the susceptible alleles over the resistance alleles in the population studied and that the favorable character, resistance, was controlled by recessive allele(s) of the relevant gene(s). Based on this information a breeding strategy that follow selection for resistant and high or medium yielding cultivars and intra-specific crosses among the cultivars for further improvement of yield and growth charcters was designed and implemented.

In vitro Breeding

The main interest in the breeding program here was to assist the conventional breeding program in reducing the long cycles in coffee breeding and to multiply true-to-type F_1 hybrids for distribution to growers. However, this program was at its very early stage and not well developed in trained human power and facilities. Currently, only tissue culture protocol optimization is under way even though screening for disease resistance at calus level, germplasm characterization (fingerprinting), and identification of markers for important traits are in the plan.

REFERENCES

Bayetta Bellachew. 2001. Arabica coffee breeding for yield and resistance to coffee berry disease (*Colletotrichum kahawae* sp.nov.). Ph.D thesis, Imperial College at Wye University of London. 272pp.

CTA. 1999. Uniqueness of Ethiopian coffee. P28-30. In: Ethiopia: the cradle of the wonder bean Coffea arabica (abissinica). Coffee and Tea Authority (CTA), Addis ababa.

Charrier, A. and Berthaud, J. 1988. Principles and methods of coffee plant breeding: Coffea canephora Pierre. In: Coffee Vol. 4 Agronomy (eds R.J. Clarke and R. Macrae), PP. 167-97. Elsevier Applied Sciences, London and New York.

Mesfin Ameha and Bayetta Bellachew. 1982. Heterosis in crosses of indigenous coffee (*C.arabica* L.) selected for yield and resistance to coffee berry disease. I. At first bearing stage. Eth. J. Agr. Sci., IV: 33-43.

Mesfin Ameha and Bayetta Bellachew. 1983. Heterosis in crosses of indigenous coffee selected for yield and resistance to coffee berry disease. II. First three years. Eth.J.Agr. Sci.,V:13-21.

Mesfin Ameha and Bayetta Bellachew. 1984. Resistance of the F1 to coffee berry disease in six parent diallel crosses in coffee. P.107-117. In: Proc.1st Reg. workshop "coffee berry disease", 19-23 July 1982, Addis ababa.

Mefin ameha and Bayetta bellachew. 1987. Genotype x environment interaction in coffee (*Coffea Arabica* L.). P476-482. In: Fourth international colloquium on coffee (ASIC '87). 29 June-3 July, 1987. Montreux.

Robinson, R.A. 1974. Terminal report of the FAO coffee pathologist to the government of Ethiopia. FAO, Rome, AGO/74/443. 15pp.

Tadesse Woldemariam, M.Denich, Demel Teketay, P.L.G. Vlek. 2001. Human impacts on coffea arabica genetic pools in Ethiopia and the need for its *in situ* conservation. In: Managing plant genetic diversity. R.Rao, A.Brown, M. Jackson (eds), CABI International and IPGRI, 237-247.

Van der Graaff. 1981. Selection of Arabica coffee types resistant to coffee berry disease in Ethiopia. Doctoral thesisMededelingen Land bouwhogeschool, Wageningen, the Netherlands.

Van der Vossen, H.A.M. 1985. Coffee selection and breeding. In: Botany, Biochemistry and production of beans and beverages (eds M.N. Clifford and K.C. Willson), pp. 48-96. Croom Helm, London, New York and Sydney.

ZEF and EARO. 2002. Conservation and use of the wild populations of Coffea arabica in the montane rainforests of Ethiopia, Project proposal. 29pp.

Coffea spp. and *Coffea canephora* Diversity Evaluated with Microsatellites and SNPs. Lessons from Comparative Analysis

T. LEROY[1], P. CUBRY[1], N. DURAND[1], M. DUFOUR[1], F. de BELLIS[1], I. JOURDAN[1], L.G. VIEIRA[2], P. MUSOLI[3], P. ALUKA[3], H. LEGNATE[4], P. MARRACCINI[5], D. POT[2]

[1]CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) TA 80/03 Avenue d'Agropolis, 34398 Montpellier Cedex 5 France
[2]IAPAR (Instituto Agronômico do Paraná)
LBI-AMG CP 481 86001-970 Londrina (PR) Brazil
[3]NARO-CORI P.O. BOX 185 Mukono Uganda
[4]CNRA, BP 808, DIVO Ivory Coast
[5]EMBRAPA Recursos Genéticos e Biotecnologia CP 02372 70770-900 Brasilia (DF) Brazil

SUMMARY

Coffea genus includes more than 80 taxa, representing a high genotypic and phenotypic diversity. Our study leads to an evaluation of diversity, based on two types of markers, microsatellites (SSR) and Single Nucleotide Polymorphisms. *Coffea* diversity was evaluated at two levels: 20 species of the genus were analysed whereas a wider sampling was performed for *Coffea canephora*. 60 SSR markers were used; they provided new information concerning *Coffea* genus structuration and phylogenetic relationships between species. At the interspecific level, results are consistent with the geographical distribution of species. Within *C. canephora,* the knowledge of the genetic diversity of wild and cultivated populations is important as preliminary information for genetic improvement. Results obtained are consistent with previous studies. Furthermore, a new group including the Ugandan genotypes was identified, constituting the most eastern located independent genetic group, with a level of diversity comparable to Congolese genotypes. Analyses on SNPs diversity have also been performed for some specific genes implicated in sucrose and diterpen metabolisms. Results give complementary data on diversity, and indicate if these genes were subjected to natural of artificial selection, indicating the best putative candidate genes. Consequences for the identification of tools for Marker Assisted Selection are discussed.

INTRODUCTION

Coffee trees belong to the genus Coffea from the Rubiaceae family, consisting in approximately 80 species, all originated from inter-tropical forest of Africa and Madagascar. Across this genus, two species are economically important, *Coffea Arabica* and *Coffea canephora*.

Coffee diversity has been studied with molecular markers, including isozymes, RAPD, RFLP, ITS, AFLP and SSR (microsatellites). Previous molecular diversity studies using ITS (Internal Transcribed Spacers) have shown that the genus is organized in four groups from different geographical origins (Lashermes et al., 1997), i.e. Central and West Africa (WC clade), East Africa (E clade), Central Africa (C clade) and Madagascar (M clade). Recent studies also established that *C. canephora* and *C. eugenioides* are the putative parents of the species *C. arabica* (Lashermes et al., 1999; Cros et al., 1998).

Microsatellites (SSR) are widely used to analyze genetic diversity structure. Microsatellites are considered as a good tool for population genetics and diversity analysis due to their high variability and co-dominant nature. Within coffee genus, recent studies have confirmed the structure of the genus and moreover good cross-amplification and conservation of microsatellites sequences were pointed out for a subset of the genus (Poncet et al., 2004). At the intraspecific level, diversity groups were identified within the *Coffea canephora* species using molecular markers (Montagnon et al., 1992; Dussert *et al*, 2002; Cubry et al., 2005).

SNP (Single Nucleotide Polymorphisms) have been analyzed recently for some genes for two biosynthesis pathways (Bouchet et al., 2005; Durand et al., 2006, Pot et al., 2006). These analyses confirmed the structure of the diversity observed with other marker types, gave valuable information concerning the origin of *C. arabica*, and precised relationships between diversity groups within *C. canephora*.

This paper presents a comparative analysis of coffee diversity analyzed by both markers. The results have been obtained by our team in collaboration with teams from Uganda, Ivory Coast and Brazil in the frame of common projects.

MATERIAL AND METHODS

Material

SSR diversity

For interspecific studies, a total of 42 individuals representing 15 species of the genus *Coffea* were used. The material includes four species of particular interest represented by more than four individuals. Part of this material, especially several individuals of *Coffea canephora* came from the CNRA (Centre National de Recherche Agronomique) of Ivory Coast Republic where they were in collection at Divo Research station. Wild *Coffea canephora*, Erect and Nganda types from Uganda are a convenience of NARO-CORI. *Coffea arabica*, *Coffea congensis*, *Coffea liberica var liberica* and *var dewevrei*, and *Coffea sessiliflora* came from a collection in French Guyana except one individual from each species which came, like the other species, from the IRD (Institut de Recherche pour le Développement) collection in Montpellier, France. The species analysed were classified by previous studies in the different geographic groups.

Within *C. canephora* species, 232 individuals were genotyped from all diversity groups already identified within this species: Guinean group, SG1, SG2, B and C sub-groups from the Congolese group, Ugandan genotypes.

SNP diversity

For sucrose metabolism, analyses were performed on one individual from 14 different species, and on about 150 genotypes from the six diversity groups within *C. canephora*.

For lipid metabolism, analyses were performed on 48 genotypes from Ugandan origin. These genotypes came from field collections in the Kawanda Research Station (genotypes identified as Nganda and Erect phenotypes), or have been collected in Ugandan coffee farms. They have been analysed together with two genotypes of *C. eugenioides* species and five genotypes of *C. canephora* species representing 5 of the 6 groups of diversity within the species.

Methods

DNA was extracted and purified from fresh leaves using standard protocol used in CIRAD laboratories.

PCR and data acquisition for SSR

Markers used in this study were microsatellites or simple sequence repeats (SSR). SSR from three sources were used in our study: SSR obtained from enriched libraries; SSR obtained by sequencing ends of a genomic library obtained in our laboratory (BAC library); SSR defined within genes from sucrose metabolism.

At least, 60 markers were screened in this study. SSR analyses were performed with 59 different polymorphic markers for interspecific studies. For intraspecific studies, 24 markers were analyzed.

PCR (Polymerase Chain Reaction) was performed using 5 µl of DNA template (e.g. 2.5 ng) per reaction. PCR amplifications were performed in a thermocycler, 30 cycles at 60 °C. Fluorescently labelled PCR products were analysed by electrophoresis migration on a LiCor® 4300 automated sequencer with a 6% acrylamide gel. Gels images were retrieved and annotated with the manufacturer program SAGA® Generation Two. Alleles sizes were attributed manually to each individual in the basis of the automated analyse of SAGA, previously studied individuals of *Coffea canephora* (Cubry et al., 2005) were used as controls. Data matrix was exported as text file and formatted into Excel® software for the different programs used for the analysis.

SNP diversity

SNPs were analyzed in a few genes of two different biosynthetic pathways.

The first analysis was performed on 26 fragments from fives genes of the sucrose metabolism in coffee fruits: two Sucrose Synthase genes (SUSY 1 and SUSY 2), a cell wall Invertase gene (CWI), a vacuolar Invertase gene (INV), and a Sucrose Phosphate Synthase gene (SPS). These fragments cover an important part of genes concerned, covering promoters, non coding and coding regions.13, 000 bp were explored for SNP diversity in these sucrose biosynthesis genes.

The second analysis was performed on genes from lipid metabolism. These genes are implicated in diterpens metabolism, more precisely in Ent-Kauren metabolism, the main precursor of three diterpens present in coffee. These diterpens (Kawheol, Cafestol and 16- O Methyl Cafestol) are specific of coffee. Three genes of the Ent-Kauren metabolism were studied: Kauren Oxydase (KO), Kauren Synthase (KS) and Copalyl Diphosphate Synthase (CPS). For these genes, 7 fragments covering 3200 bp were explored for their polymorphism. The fragments were covering 3'UTR regions, introns and exons regions of the genes analysed.

Primers design for SNP study of all genes was performed from the Brazilian Genomacafe project. The sequences were then amplified by PCR, and directly sequenced without a cloning step. Sequences were then analyzed with Codon Code Aligner software. All the sequences obtained were aligned, and all the polymorphisms were identified. SNP were then pointed out and analyzed for all fragments.

Data analysis

SSR data were used to calculate a dissimilarity matrix between genotypes using allele frequencies for all the loci analyzed. Then, a diversity tree was constructed from the dissimilarity matrix sing neighbor joining method of the DARWIN software.

A gene tree has been constructed using SNP diversity, using neighbor joining method with Mega3 software. Gene flows between genetic groups within *C. canephora* were estimated from common haplotypes of the different groups.

RESULTS

Coffea genus diversity

Regarding diversity of the genus *Coffea*, SNP and SSR reveal a significant differentiation between populations analyzed, this structure being in accordance with geographical origin of the plants. On the trees resulting from dissimilarity analyses, species were classified in relation to their geographical origin for both markers (Figures 1 and 2). For both markers, western and Eastern origins are clearly separated.

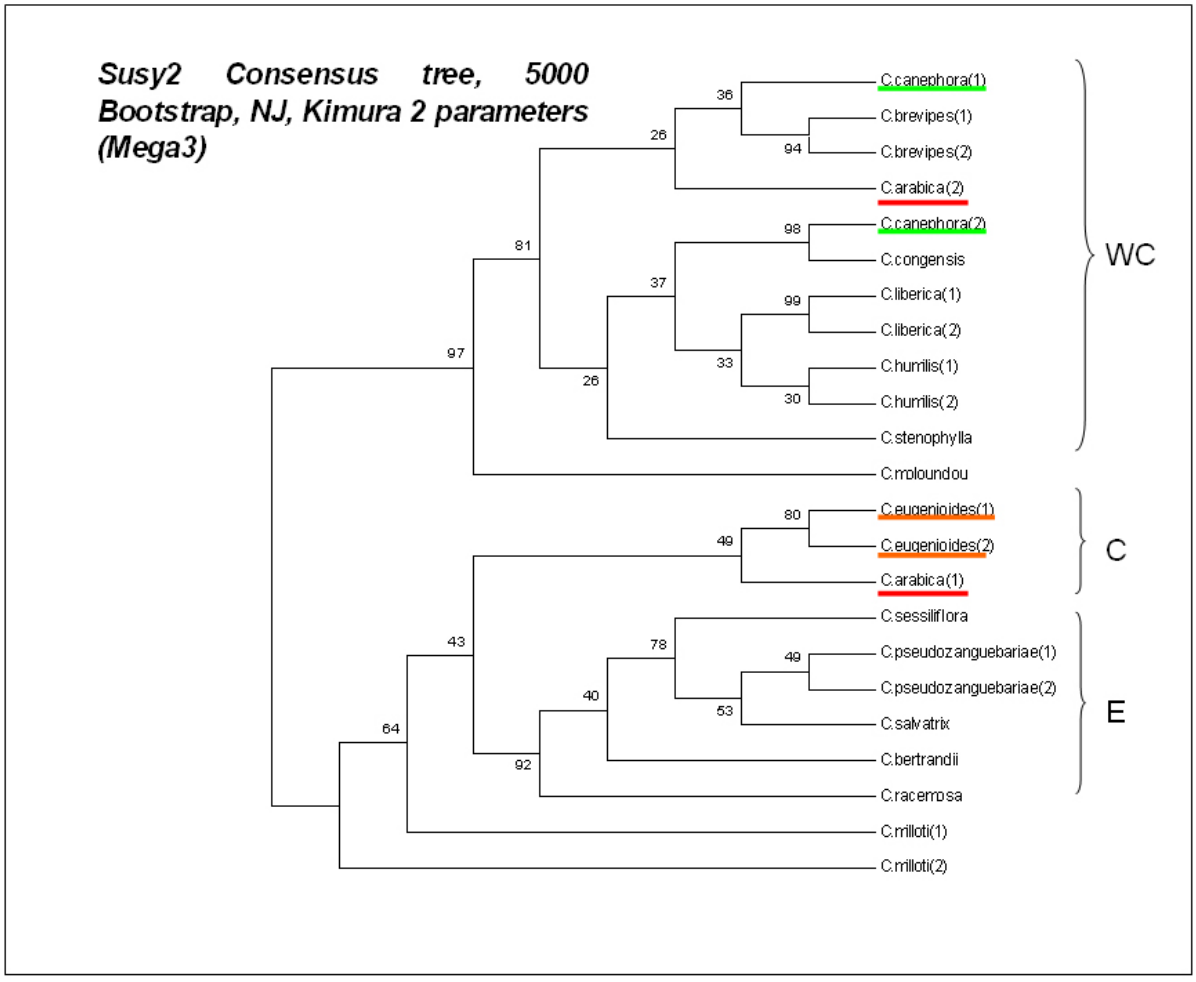


Figure 1.

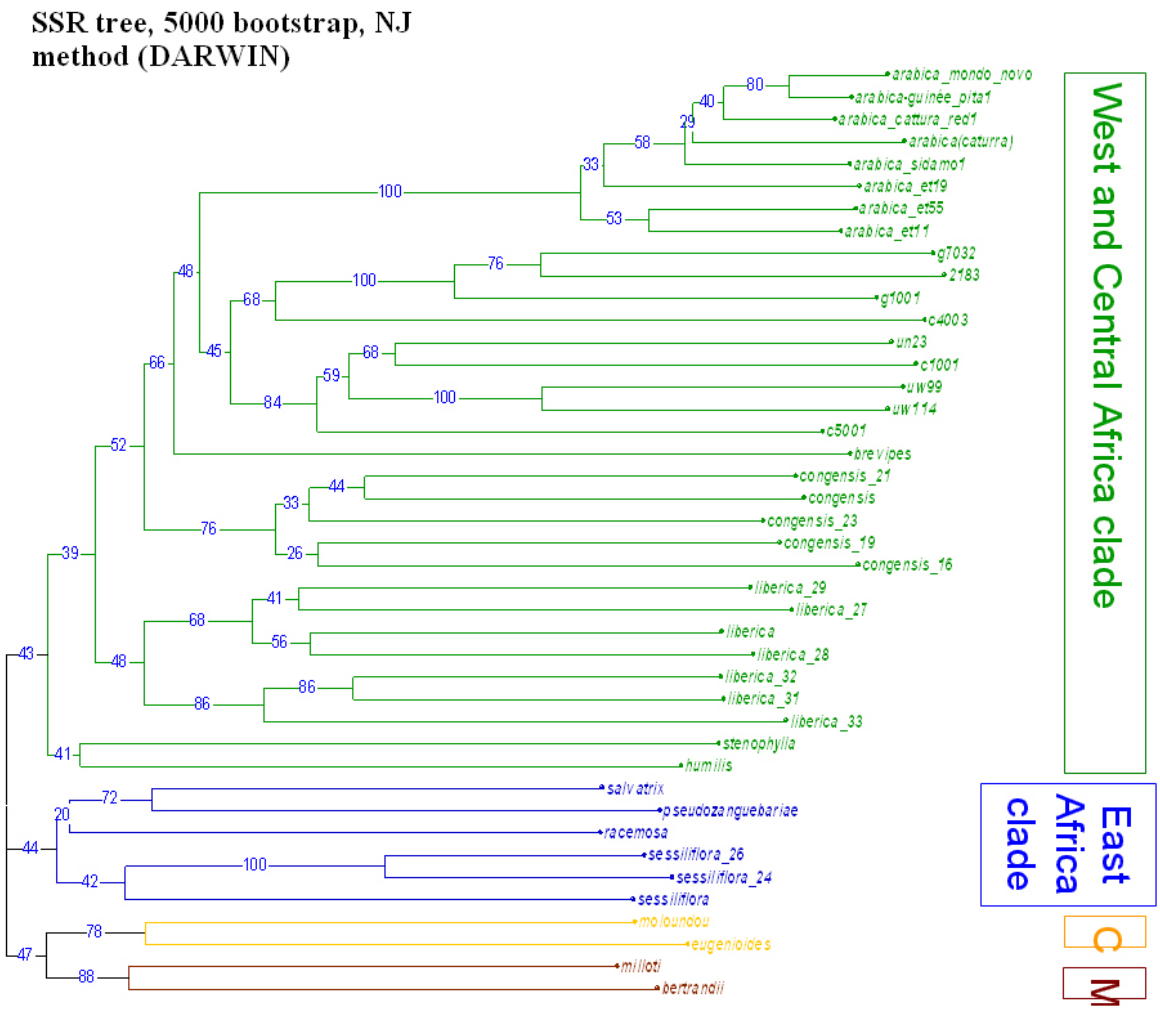


Figure 2.

We could analyze the history of *C arabica* species regarding these dissimilarity trees. This species is located in the same clade as *C. canephora* species for SSR polymorphism. On the tree presented for SNP analyses on SUSY 2 gene, both genotypes are located one close to *C.eugenioides*, one close to *C. canephora,* both being putative parents of *C. arabica* species.
A more specific analysis of comparative data between arabica and its putative parents (*C.canephora* and *C.eugenioides*) gave the following results:

- For SNP on sucrose metabolism, 92% of heterozygous sites observed in *C. arabica* correspond to sites for which the two alleles were found in *C. canephora* and *C eugenioides* (in the heterozygous or homozygous states). In 73% of heterozygous sites in Arabica, the two parental species present the alternative alleles in the homozygous state
- For SSR diversity, 65 % of the heterozygous sites observed in *C. arabica* are present in both parental species, a few of them (17%, 4) being fixed in both species.

The putative history of *C.arabica*, resulting from a cross between *C. canephora* and *C. eugenioides* is then confirmed by these results. For SSR markers that are neutral markers, the less proportion of alleles fixed in both parents could be explained by their high level of mutation.

For SSR, most of the homozygous loci present in *C.arabica* are *C.canephora* alleles. One hypothesis is that some backcrosses of the original interspecific hybrid to a *C.canephora*

genotype occurred, or the fact that our studies didn't consider the global allele diversity of the three species.

Nevertheless, the results confirm that *C.arabica* is a hybrid between a canephoroide species and a species of *Coffea* from Eastern Africa (*C. eugenioides*). The differences observed between both markers could be attributed to differences in the rapidity of evolution. SSR are known to have a very rapid evolution, compared to SNP located within genes of interest that could be submitted to selection pressure.

***Coffea canephora* diversity**

Within *C. canephora*, nucleotide diversity is important in both metabolisms, as presented in Table 1.

Table 1. Polymorphisms observed for sucrose and lipid metabolism.

polymorphisms	Sucrose metabolism	Lipid metabolism
Synonymous SNP in Exons	33 (1/393)	11 (1/289)
Non Synon. SNP in Exons	16 (1/813)	19 (1/167)
SNP in non coding regions	108 (1/120)	61 (1/52)
Total	157 (1/82)	91 (1/35)

The number of polymorphisms is indicated, frequency on the studied fragments is indicated between brackets.

Observed variability is high for sucrose metabolism, regarding to autogamous species like wheat (1/540 bp) and a tree like pine, but much lower than in maize. For diterpen genes, the values obtained are close to those obtained for maize (1/28bp). We observed three times more of non synonymous SNP in lipid metabolism than in sucrose metabolism.

These results indicate that sucrose metabolism genes are probably submitted to a higher conservative selection pressure (background selection) than some diterpen metabolism genes, particularly Kauren Synthase gene that presents a high percentage of non synonymous diversity (1/53bp). Two hypotheses could be discussed: this gene is not submitted to evolution pressures, meaning that modifications in the protein are of less importance, or this gene is submitted to a high adaptative selection pressure implicating a high proportion of mutations.

SSRs analyses performed in our work confirmed the diversity previously observed within *C. canephora*, the Guinean group and the four Congolese sub-populations are clearly identified and separated. The originality of genotypes from Uganda is also pointed out. Results on this new group will be presented in the next lecture.

Regarding the known structure of the species the results are indicating that SNP and SSR both discriminated the diversity groups within *Coffea canephora*. The global differentiation coefficient, estimated by the *Fst* value, is high with both markers:

- 0.464 for SNPs in Susy 1 gene, 0.548 for SNPs in SPS and Susy2 genes
- 0.380 for SSR markers.

All the identified groups are clearly separated with these markers

- Guinean genotypes from Western Africa
- Congolese genotypes from Central Africa

- Ugandan genotypes.

Working on haplotypes defined from SNP studies, or on SSR diversity, genetic relations between genotypes were evaluated: gene flows from haplotype data for SNPs, *Fst* values for SSR markers. Results were in good accordance for both markers.

A high differentiation of Guinean genotypes was observed, with few gene flows and high *Fst* values (> 0.4) with other groups. SG2 group is highly related to C group for both markers (*Fst* = 0.2), and some gene flows are present between Ugandan, B and SG2 regions (with *Fst* values < 0.3). In all cases, the SG2 region, mostly constituted by cultivated genotypes appears to be related to other Congolese groups.

Apart for Guinean genotypes, clearly separated for both markers, all the other groups, including Ugandan group, have the same genetic background from which they discriminated since the past glaciations period, from SG1 and SG2 regions. Differentiation between groups is then mainly based on differences in allelic frequencies.

Except for the relations between SG1 and SG2 that seems to be higher for SNP than for SSRs, both markers are indicating the same level of relations and differentiation between groups.

Globally, results obtained with both markers are quite similar, at the interspecific and intraspecific level. The diversity of *C. canephora* is now clearer: the different diversity groups, Guinean, Congolese and Uganda are identified, and their genetic relationships have been pointed out.

DISCUSSION - CONCLUSION

SSR and SNP markers allow to clearly defining the diversity within the *Coffea* genus and *Coffea canephora* species.

SNPs are quite interesting, since they are located within genes. The higher rate of SNPs observed for lipid metabolism indicate that these genes could be submitted to a stronger selection than those implicated in the sucrose metabolisms. Then, the genes implicated in sucrose metabolism could be considered as of great importance for the plants, and then should be highly conserved within the species.

All the groups confirmed by both markers within *Coffea* genus and *C. canephora* species indicate high similarity between geographical structure and genetic structure. Our results also confirm both species considered as the putative parents of *C. arabica*.

These molecular tools will allow us to build breeding programs for quality and pest resistance, taking into account candidate genes, SNP within them and SSR markers. Studying on sucrose and diterpen genes, some candidate genes will be identified, and SNP within these genes, if related to phenotypic variation, will constitute a valuable tool to Marker Assisted Selection programs. This type of work, associating neutral markers like SSR and SNPs that could be submitted to selection are of primordial importance, since they will allow us to identify precisely molecular markers of interest.

These markers could be used for genotyping populations, genetic mapping and definition of QTLs. Associated with phenotypical evaluation, they will lead us to define valuable markers for selection.

Finally, it has to be noted that this type of collaborative work is very efficient, since exchanges and common work between teams are important.

REFERENCES

Bouchet S, Marraccini P, Jourdan I, Leroy T, Vieira LGE, Ferreira LP, Musoli P, Pot D, 2005. Nucleotide diversity and molecular evolution of 5 genes involved in the sucrose biosynthesis pathway of Coffea canephora. in *Proceedings of the 4th Plant genomics European meeting*, *Amsterdam, September 20th-23th*.

Cros J, Combes MC, Trouslot P, Anthony F, Hamon S, Charrier A, Lashermes P, 1998. Phylogenetic analysis of chloroplast DNA variation in Coffea L. Mol. Phylogenet. Evol. 9, 109-117

Cubry P, De Bellis F, Pot D, Musoli P, Legnaté H *et al.*, 2005 Genetic diversity analyses and linkage disequilibrium evaluation in some natural and cultivated populations of *Coffea canephora,* in *Proceedings of the 4th Plant genomics European meeting*, *Amsterdam, September 20th-23th*.

Durand N, De Bellis F, Jourdan I, Rivalan R, Manez JC, Aluka P, Vieira LG, Ogwang J, Billot C, Pot D, Marraccini P, Leroy T, Guyot B, 2006. Biochemical and nucleotide variability in the diterpene metabolism for coffea canephora genotypes from Uganda. Poster, ASIC meeting, September 11-15 2006, Montpellier, France.

Dussert, S, Lashermes P, Anthony F, Montagnon C, Trouslot P et al., 2003 Coffee (*Coffea canephora)*, pp.239-258 in *Genetic Diversity of Cultivated Tropical Plants,* edited by P. Hamon, M. Seguin, X. Perrier and J. C. Glaszmann. Science Publishers. Inc. Enfield (NH), Plymouth. Lashermes P, Combes MC, Robert J., Trouslot P, D'Hont A., Anthony F and Charrier A, 1999. Molecular Characterisation and origin of the *Coffea arabica* L. genome. Mol Gen Genet 261: 259-266.

Lashermes P, Combes MC, Trouslot P, Charrier A, 1997. Phylogenetic relationships of coffee-tree species (Coffea L.) as inferred from ITS sequences of nuclear ribosomial DNA; Theor Appl Genet , 97: 947-955.

Montagnon, C, Leroy T, Yapo AB, 1992 Diversité génotypique et phénotypique de quelques groupes de caféiers (*Coffea canephora* Pierre) en collection. Conséquences sur leur utilisation en sélection. Café Cacao Thé **36:** 187-198.

Poncet, V, Hamon P, Minier J, Carasco C, Hamon S et al., 2004 SSR cross-amplification and variation within coffee trees. Genome **47:** 1071-1081.

Pot D, Bouchet S, Marraccini P, De Bellis F, Cubry P, Jourdan I, Vieira LGE., Ferreira LP., Musoli Pl, Leroy T., 2006. Nucleotide diversity of genes involved in sucrose metabolism. Towards the identification of candidates genes controlling sucrose variability in *coffea* spp. Oral communication, ASIC meeting, September 11-15 2006, Montpellier, France.

Fighting against Coffee Wilt Disease: Uganda wild *C. canephora* Genetic Diversity and its Usefulness

P.C. MUSOLI[1], P. ALUKA[1], P. CUBRY[2], M. DUFOUR[2], F. DE BELLIS[2], S. NAKENDO[1], A. NABAGGALA[1], J. OGWANG[1], D. KYETERE[1], T. LEROY[2], D. BIEYSSE[2], A. CHARRIER[3]

[1]Coffee Research Institute, P.O. Box 185 Mukono UGANDA
[2]CIRAD TA 80/03 Avenue d'Agropolis, 34398 Montpellier Cedex 5 France
[3]ENSAM, 2 Pl. P. Viala 34060 Montpellier Cedex 1, France

SUMMARY

Coffee wilt disease (CWD) caused by *Fusarium xylarioides* appeared in 1993 in Uganda and has become a serious problem of coffee production. From the 1940's to 1960's the disease caused considerable destruction to C. canephora in Central and West Africa. This disease was effectively controlled by uprooting and planting resistant varieties. For developing wilt resistant varieties in Uganda, wild *Coffea canephora* trees from Kibale and Itwara primary forests were studied together with cultivated genotypes of nganda and erecta phenotypes and other cultivated genotypes from Kalangala Islands in Lake Victoria. The genotypes were analyzed for genetic diversity using 24 SSR markers covering many parts of *C. canephora* genome. These studies found significant genetic differences between Kibale, Itwara, Kalangala and a group constituted of nganda and erecta genotypes. A comparison of Ugandan genotypes with known *C. canephora* diversity groups using 18 of the SSR markers revealed that Ugandan genotypes constitute a new diversity group in the species. Therefore it was interesting to test this Ugandan material for resistance to coffee wilt disease. Resistances tests were performed using a field isolates of *Fusarium xylarioides* on open pollinated progenies of some of Kibale, Itwara, Kalangala and Nganda and erecta individuals studied for genetic diversity. Results of these tests revealed presence of resistance among genotypes from all sources and significant genetic differences between sources. High variability between progenies within each source was also detected. In this paper importance of this diversity in developing improved coffee varieties is discussed

INTRODUCTION

Both arabica (*Coffea arabica*) and robusta (*Coffea canephora*) are cultivated in Uganda. Robusta constitutes about 90% of coffee production and about 80% of earnings from coffee exports. However coffee wilt disease, which emerged in Uganda in 1993 and has since then spread widely, has seriously devastated the crop. A survey carried out in 2002 found this disease in all Robusta coffee growing zones, affecting over 90% of robusta coffee farms and it had destroyed 44.5% of the crop nationwide (Oduor, 2005). The disease is also a high threat to gene pools available at research stations, farms and in the wild, not only in Uganda but also in other Robusta coffee growing countries.

Phytosanitory measures, such as elimination of diseased coffee trees on noticing symptoms, discriminate use and sterilizing farm tools when used in infected fields, and not replanting infected fields until after at least two years are being emphasized for short term management in cultivated coffee but there has been insignificant success (Wetala et al., 2000) although phytosanitory practices are reported to have been effective in Cameroon where it was

implemented in tandem with re-location of coffee growing to unaffected regions (Muller, 1997). Prospects for managing the disease effectively rely on host resistance (Delassus, 1954). Replanting with resistant varieties eliminated this disease from Ivory Coast in the 1950's

There is on-going research in Uganda to develop wilt resistant robusta coffee varieties. In this paper, results of a study on genetic variability of Ugandan *Coffea canephora* and prospects for developing wilt resistant varieties are presented. The study addressed 4 questions: 1) Are Ugandan *C. canephora* genotypes different from known global *C. canephora* diversity? 2) Is there significant genetic diversity among Ugandan *C. canephora*? 3) Is there resistance to coffee wilt disease among Ugandan *Canephora*? 4) What are the implications of the findings to the development of wilt resistant varieties? The Uganda genotypes studied include wild individuals from primary forests and cultivated individuals of nganda and erecta types.

MATERIALS AND METHODS

Genetic diversity

Plant materials

For purpose of studying genetic diversity of Ugandan populations, five sources were sampled, two of them (Kibale & Itwara) considered wild (Thomas, 1944), and one (Kalangala, once cultivated and now feral) involved sampling on islands in Lake Victoria and the other two, nganda and erecta, being cultivated (Table 1). Itwara sites separated by distances ranging from 0.6 to 5 km provided 7 to 18 individuals per site. Kibale sites separated by 7 to 45 km distances provided 4 to 30 individuals per site. Kalangala sites, located on three different islands separated by at least a 10km distance, provided 3 to 12 individuals per site. The cultivated sources include genotypes of erect phenotype with predominantly strong erect stems and Nganda phenotype with predominantly spreading stems. To compare Ugandan material with already known genetic diversity, previous sampling and analyses of Guinean genotypes and Congolese groups SG1, SG2, B and C were considered (Cubry et al., 2005).

Table 1. *Coffea canephora* materials studied for genetic diversity.

	Source	Type of material	Sampled sites	Studied individuals
Ugandan sources	Itwara	Wild	5	55
	Kibale	Wild	4	54
	Kalangala	Semi-wild/cultivated	5	35
	Nganda	Cultivated	1	31
	Erect	Cultivated	1	21
Core sources	Guinean	Wild	3	106
	SG2	Cultivated	4	25
	SG1	Wild	1	9
	Congolese B	Wild	1	39
	Congolese C	Wild	1	10

DNA extraction

Genomic DNA was extracted from grinded leaves following a MATAB buffer based extraction method adapted from the procedure used at CIRAD.

SSR genotypes

24 microsatellite markers (Simple Sequence Repeats, SSR) from different manufacturers and regions of the *Coffea canephora* genome were used to genotype the 196 individuals from Uganda. Eighteen of these markers were used for analyses including individuals from other diversity groups (Cubry et al., 2005).

PCR amplification and visualization of the microsatellites

PCR reactions were performed in 10µl volumes, containing 2.5 ng of DNA, 1mM Tri-HCl, 5 mM KCl, 2 mM MgCl2, 200 µM dNTP, 0.10 µM of reverse primer, 0.08 µM of forward primer tagged with M13 sequence, 0.10 µM of infrared fluorescently labelled M13 primer and 0.1 U of Taq DNA polymerase. PCR amplifications were run in an Eppendorf Ep384 thermocycler. Fluorescently labelled PCR products were analyzed by electrophoresis migration on a LiCor® 4300 automated sequencer with a 6% acryl amide gel. Gels images were retrieved and annotated with the manufacturer program SAGA® GT Generation Two. Allele sizes were evaluated on the basis of allelic controls previously defined by Cubry et al. (2005). Data matrix was used for further analyses. The genotyping was performed on the Genotyping Platform of the Genopole Montpellier Languedoc Roussillon, France.

Diversity analyses

Diversity statistics were calculated for all groups of genotypes using PowerMarker software. These analyses were performed on Ugandan genotypes in comparison with previous values obtained for other genetic groups (Cubry et al., 2005). Dissimilarity matrix between individuals based on simple matching index was computed with DARwin 5. A representation of the matrix was given in a tree built with weighted neighbour joining method (Saitou and Nei, 1987).

Genetic differentiation

Genetic differentiations between sites or sources were estimated with F_{ST} using Fstat software package. All the *F*-statistics were calculated using estimations proposed by WEIR and Cockerham (1984). Significance of all statistics was performed with 1000 permutations In order to test whether genetic differentiation follows a pattern of isolation by distance, correlation between genetic distance matrixes and geographic distances were tested with Mantel test using GenAlEx software. F_{ST} values obtained from fixation indices analysis calculated between pair sites were used to perform the Mantel test. We applied data transformation (Rousset, 1997) to perform regression analysis between logarithm of geographical distance and a genetic distance coefficient calculated as F_{ST} / (1-F_{ST}).

Resistance to coffee wilt disease

Plant materials

For purposes of relating coffee wilt disease resistance to genetic diversity among Ugandan *C. canephora*, open pollinated progenies of some of the individuals studied for genetic diversity were tested for resistance (Table 2). In Itwara forest four sites provided 1 to 4 progenies per site. In Kibale three sites also provided 1 to 4 progenies per site. In Kalangala five sites provided 2 to 9 progenies per site. Erecta provided 10 progenies and Nganda provided 16 progenies. Each progeny provided between 6 to 20 seedlings used in the studies.

Table 2. *Coffea canephora* materials studied for coffee wilt disease resistance.

Source	Type of material	Sampled sites	No. of progenies	No. of individuals
Itwara	Wild	4	10	110
Kibale	Wild	3	7	67
Kalangala	Feral	4	23	395
Nganda	Cultivated	1	16	311
Erect	Cultivated	1	10	177

Inoculation

Seedlings were inoculated by dipping their entire root section into the inoculum for at least 30 minutes with a water suspension inoculum of $1.0x10^6$ spores per ml of *Fusarium xylarioides* field isolate. After inoculation, seedlings were re-planted in polythene pots filled with fresh growth medium (soil) and kept in a screen house for incubation and assessment.

Data collection

Seedlings were assessed at 10 weeks after inoculation on a symptom progression scale of 1 to 5, where 1 = no disease, 2 = curling leaves and stunted growth, 3 = leaf drooping, weary and yellowing, 4 = leaf necrosis, leaf wilting, and abscission and 5 = plants are dead. A high value implies more disease and less resistance.

Data analysis

Analysis of variance was performed on assessment data using SAS soft ware and Student-Newman-Keuls mean separation test were performed to classify disease on progenies within sources and between sources. Analysis was not performed to classify disease by sites because most sites provided small numbers of progenies. Percent seedling mortality was computed for all progenies at this date.

RESULTS

Genetic diversity

Diversity statistics

There are differences between observed heterozygosity and gene diversity across all sources, indicating our sources have sub-structures (Table 3). Within Ugandan populations, wild Kibale and Itwara had lower observed heterozygosity and gene diversity than cultivated populations, including Kalangala, indicating crossing occurred for cultivated genotypes. Mean number of alleles is high in Ugandan sources, compared to other sources. Among Ugandan sources, wild sources and Kalangala have the highest number of private alleles. There is significant difference between wild Ugandan sources concerning number of private alleles. If we consider all sources, wild Guinean, Itwara and Kalangala regions have the highest number of private alleles. However, not a lot of loci have specific alleles in most sources, indicating they have strong genetic similarity.

Diversity tree of all 232 individuals separated individuals of Guinean and Congolese groups from those of Ugandan origin (Figure 1). Wild genotypes from Kibale and Itwara are separated from cultivated and semi-cultivated. The results indicate that Ugandan materials

are outside the previously known *C. canephora* genetic diversity. A tree (not shown) constructed with only 196 Ugandan genotypes identifies four groups of genotypes, Kibale, Itwara, Kalangala and a group constituted of Erecta and Nganda individuals.

Table 3. Diversity statistics.

Source	Observed heterozygosity & (p value)	Gene diversity & (p value)	mean No. of alleles	Private alleles (Ugandan sources)	Private alleles (all sources)
Itwara	0.396 (0.044)	0.586 (0.041)	193 (8.04)	37	14
Kibale	0.288 (0.043)	0.531 (0.051)	177 (7.38)	19	4
Kalangala	0.405 (0.045)	0.628 (0.049)	206 (8.58)	34	17
Nganda	0.407 (0.043)	0.623 (0.048)	194 (8.08)	14	7
Erect	0.397 (0.045)	0.625 (0.048)	172 (7.17)	12	6
Guinean	0.35	0.50	179 (5.29)		24
SG2	0.41	0.69	229 (6.74)		12
SG1	0.27	0.37	242(7.12)		11
Congolese B	0.37	0.50	173 (5.09)		7
Congolese C	0.37	0.45	101 (2.97)		14

Gene diversity for Guinean and Congolese regions was adopted from CUBRY et al., 2005. Number of private alleles for Ugandan regions was detected with 24 markers among 196 individuals. Number of private alleles for all regions was detected with 18 markers on 232 individuals.

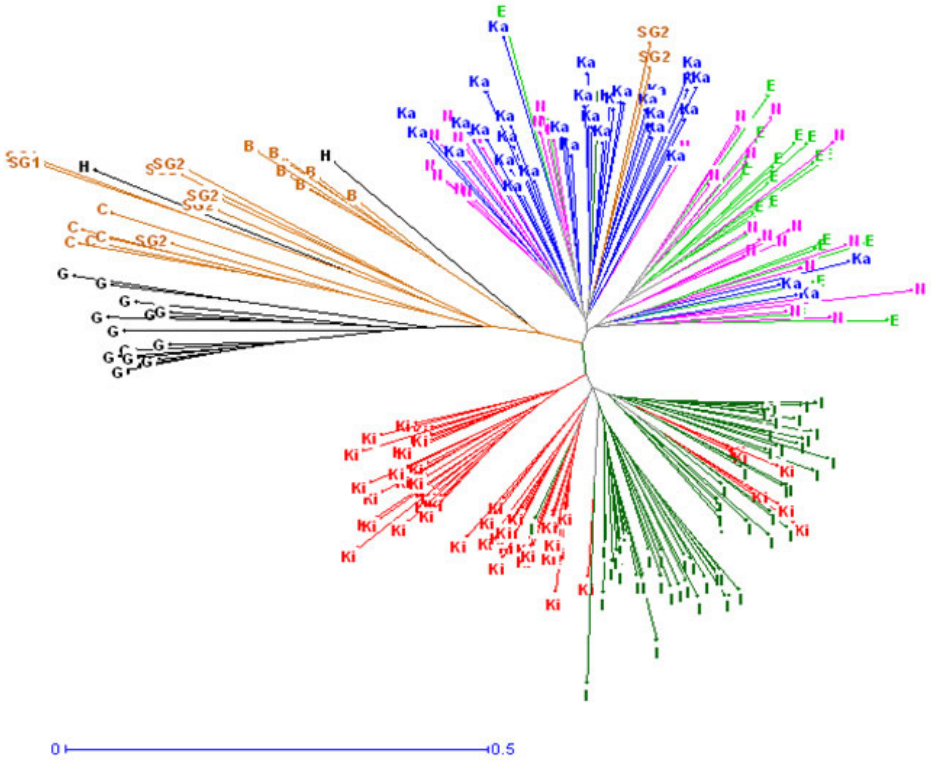


Figure 1. Phylogenic tree of 232 individuals using weighted neighbor joining method among 18 microsatellite loci. G (black) indicates Guinean; I (dark green) indicate Itwara forest; Ki (red) indicates Kibale forest; N (pink) indicates Nganda; E (green) is erect and Ka (blue) is Kalangala islands.

Genetic differentiation

F_{ST} coefficients revealed high (> 0.150) differentiation, whether you consider all the ten sources or only the five Ugandan sources. Considering separately the three Ugandan sources constituted by different sites, Kibale has a highly significant F_{ST} coefficient, meaning that sites in this source are highly differentiated into populations. On the other hand, F_{ST} coefficients are not significant within Itwara and Kalangala, meaning that the populations belonging to these sources are not differentiated.

Results of the Mantel tests for Ugandan populations pointed out a significant correlation between genetic and geographic distances, indicating geographical distance was responsible for independent evolution of these populations. Previous studies (Berthaud, 1986) indicated that pollen dispersal (mainly by insects) could be possible up to 2 or 3 km. Dispersal of seeds, due to animals especially mammals, was impossible between wild sources, but it is possible within each of the sources, especially in Itwara forest. Correlation of geographical distances and genetic distances was less evident ($p = 0.132$) when considering all the diversity regions than correlations within Ugandan country ($p = 0.015$). Given that the global geographic distances between the sources are quite long, more than 1,000 kms in most cases, it is quite normal. It is not possible that pollen can flow such distances.

Resistance to coffee wilt disease

Analysis of variance performed on 1-5 symptom progression data at 10 weeks after inoculation found significant genetic differences for disease levels between sources ($p = 0.0001$) and between progenies across sources ($p = 0.0001$)). Analyses for variances within sources found highly significant ($p < 0.0001$) genetic difference between progenies within Kibale, Kalangala, Erecta and nganda sources. Differences between Itwara progenies were not significant ($p = 0.08$). Mean separation tests placed sources into 3 classes: A consisting of nganda, B consisting of erecta and Kibale and C consisting of Itwara and Kalangala; in the decreasing order of disease development. These results indicate Itwara and Kalangala have high levels of resistance and nganda harbor the least level of resistance. Mean separation tests for progeny means within sources found large class overlaps in Kibale, Kalangala, nganda and erecta, implying resistance to coffee wilt is controlled by many genes that are not equally available among the progenies. There were no classes for Itwara, since difference between progenies means from this source are not significant. Percent seedling mortality varied widely between progenies (Table 4).

DISCUSSION

Results of diversity analyses and population structure in our study established that Ugandan *C. canephora* are different from known diversity groups, with high variability. This high diversity in Uganda, for both wild and cultivated populations, points out importance of Uganda in *C. canephora* diversity. The analyses pointed out specificity of Kalangala, which has a high mean number of alleles and private alleles. Phylogenic trees confirmed specificity of Ugandan genotypes; as a new diversity group within the species. F_{ST} values observed between sites and sources confirmed a high genetic differentiation within the species. A common genetic background exists between SG2 and cultivated Ugandan genotypes, since their F_{ST} values are much lower than values for Uganda relating to other regions.

Table 4. Percent mortality among *C. canephora* open progenies at 10 weeks after artificial infection with coffee wilt disease.

Kalangala		Nganda		Itwara		Erecta		Kibale	
Progeny	**Mortality**	**Progeny**	**Mortality**	**Progeny**	**Mortality**	**Progeny**	**Mortality**	**Progeny**	**Mortality**
UW206	0.0	UN015	35.0	UW136	0.0	UE012	5.0	UW008	0.0
UW182	0.0	UN004	35.0	UW091	0.0	UE010	20.0	UW012	15.0
UW194	0.0	UN018	47.4	UW098	6.7	UE011	40.0	UW022	22.2
UW198	0.0	UN016	52.6	UW106	14.3	UE020	40.0	UW005	28.6
UW217	5.0	UN020	55.0	UW090	14.3	UE024	65.0	UW004	50.0
UW203	5.0	UN010	60.0	UW123	20.0	UE016	75.0	UW009	70.0
UW209	5.0	UN005	60.0	UW146	28.6	UE005	80.0	UW010	90.0
UW201	5.3	UN006	65.0	UW155	33.3	UE031	80.0		
UW181	10.0	UN007	65.0	CPT11 TR2	37.5	UE006	85.0		
UW185	10.0	UN013	70.0	UW154	38.5				
UW215	10.0	UN024	70.0						
UW211	10.0	UN011	80.0						
UW219	15.0	UN003	90.0						
UW180	16.7	UN017	90.0						
UW210	20.0	UN001	91.0						
UW191	21.4	UN008	95.0						
UW199	25.0								
UW189	25.0								
UW205	25.0								
UW212	25.0								
UW183	25.0								
UW204	40.0								
UW218	45.0								
Mean	14.9		66.3		19.3		54.4		39.4

Phylogenic trees also established genetic separation of Kibale, Itwara and Kalangala sources. Nganda and Erect appeared as a mixed group on the trees, meaning that their phenotypic differences were not based on genetic differentiation. Pair wise F_{ST} values among Ugandan cultivated regions are very low, indicating clearly a low genetic differentiation. Nevertheless, Kalangala, pointed out as specific for global diversity characteristics was identified to be slightly different from Nganda and Erect. As confirmed by population structure, the islands have some specificity since they have been completely isolated from the mainland for at least three generations. Its genetic background, is clearly similar to the cultivated regions (Maitland, 1926), but its isolation allowed some specificity regarding allele diversity. Within forests, as expected, Kibale, which is bigger than Itwara, is more structured among the sites. Itwara sites are not differentiated enough and they could be considered as a single population. The short distances between sites in Itwara could partly count for this observation.

The correlations between geographical and genetic distances allowed us to establish that wild Ugandan genotypes have a genetic isolation related to geographical distance. Since dissemination of coffee pollen is limited to short distances (less than 5 km) and distances for seed movement are even shorter, this genetic differentiation is normal for the period of four generations. Correlations were not significant for the overall analysis, since geographical distances are very large and exchanges between populations are impossible. Further more, we did not have sufficient number of genotypes per site and sufficient number of sites per source to clearly study the possible genetic exchanges between sites. Since the sampling did not cover all countries and districts where coffee is found, it is still difficult to have a complete image of the global diversity. Countries like the Democratic Republic of Congo, Gabon and Angola should also be surveyed for more information about the genetic diversity and precise relationships between the regions. A systematic survey of all forests considered to be natural homes of wild coffee, sampling coffee trees at every 10 km distance shall be sufficient for understanding this discrimination.
In view of the observed global *C. canephora* genetic diversity, an international germplasm collection and conservation program would be important for preserving and availing the genetic variability to coffee breeding programs. Gathering representative genotypes of different genetic diversity groups in some specific germplam conservation fields should be initiated.

Our results establish presence of resistance against coffee wilt disease among Ugandan *C. canephora* population, with very high variability within and between sources. Results of other screen house tests performed on cultivated materials from farms and on-stations and results of field evaluation (not shown) also revealed high variability for resistance to coffee wilt disease among Ugandan *C. canephora* genotypes. However the high level of resistance among wild Itwara populations and genotypes cultivated on isolated islands in Lake Victoria is peculiar and validates usefulness of these populations as complementary sources of genes for genetic improvement of various agronomic traits of coffee; resistance against coffee wilt disease being the most immediate. Large scale screening of progenies and clones of individuals from Itwara and Kalangala will enhance our knowledge about the resistance and also hasten development of varieties resistant to coffee wilt disease.

REFERENCES

Berthaud, J., 1986 Les ressources génétiques pour l'amélioration des caféiers africains diploïdes: évaluation de la richesse génétique des populations sylvestres et de ses mécanismes organisateurs, conséquences pour l'application. ORSTOM, Paris.

Cubry P., F. De Bellis, D. Pot, P. Musoli, H. Legnaté 2005 Genetic diversity analyses and linkage disequilibrium evaluation in some natural and cultivated populations of *Coffea*

canephora, in *Proceedings of the 4th Plant genomics European meeting*, *Amsterdam, September 20th-23th.*.

Delassus E., 1954. La trachéomycose du caféier. Bulletin Scientifique du Ministère des Colonies, Section Agronomie, Tropicale, 5:345-348

Maitland, T. D., 1926. Coffea Robusta in Uganda, pp. 3-11 in *Annual Conference of the Uganda planters' Association,* edited by Uganda Protectorate, Department of Agriculture. Government Press, Uganda

Muller RA, 1997. Some aspects of past studies conducted in Western and Central Francophone Africa on Tracheomycosis (Cote d'Ivoire, Cameroon and Central African Republic. In: Hakiza JG, Birikunzira B, Musoli P, eds. Proceedings of the first regional workshop on Coffee Wilt Disease (Tracheomycosis). International Conference Centre, Kampala, Uganda, 15-26

Oduor G , Phiri N, Hakiza Gj, Abebe M, Asiimwe T, Kilambo Dl, Kalonji Mbuyi A, Pinard F, Simons S, Nyasse S and Kebe I, 2005. Surveys to establish the spread of coffee wilt disease, Fusarium (Gibberella) xylarioides, in Africa. ASIC 2004. Proceedings of 2Oth International Conference on Coffee Science, Bangalore, India, 1252-1255

Rousset, F., 1997 Genetic differentiation and estimation of gene flow from F-Statistics under isolation by distance. Genetics **147:** 1219-1228

Saitou N. and M. Nei 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution **4**: 406-425.

Thomas, A. S., 1944. The wild coffees of Uganda. The Empire Journal of Experimental Agriculture **12:** 1-12.

Weir, B. S., and C. C. Cockerham, 1984. Estimating F-statistics for analysis of population structure. Evolution **38:** 1358-1370

Wetala Mpe, Birikunzira Jb, Hakiza Gj And. Kabole C, 2000. Effect of uprooting and burning on affected plants on further spread of Coffee Wilt Disease. National Agricultural Research Organisation, Progress Report of Coffee Wilt Research and Development

Breeding for Durable Rust Resistance in Coffee (*Coffea arabica*) – Field Performance of Some Tetraploid Inter-specific Hybrid Derivatives in India

N. SURYA PRAKASH[1], V. V. K. M. LAXMI[1], P. SAILAJA[1], A. G. S. M. REDDY[3], M. S. SREENIVASAN[3], K. BHASKAR REDDY[1], P. K. VINODKUMAR[2], C. S. SRINIVASAN[3], A. SANTARAM[2], N. RAMAMURTHY[1], JAYARAMA[2]

[1]Regional Coffee Research Station, Coffee Board, R.V. Nagar – 531 133, A.P, India
[2]Central Coffee Research Institute, Coffee Board, Coffee Research Station 577 117, Karnataka, India
[3]Former Scientists, Central Coffee Research Institute, Coffee Board, India

SUMMARY

Coffee leaf rust caused by the obligate parasitic fungus *Hemileia vatatrix* Berk & Br is an important disease of concern for arabica coffee (*Coffea arabica* L) cultivation round the globe. In India, leaf rust disease is much more devastating on arabica coffee as the climatic factors are favourable for high disease build up, leading to severe crop losses. In coffee, so far nine resistance genes S_H1 to S_H9 have been identified to be conditioning the resistance to leaf rust, either singly or in combination. In Indian coffee breeding programme, all the nine resistance factors (S_H1 to S_H9) have been exploited and arabica strains that manifests varying levels susceptibility/resistance to rust have been developed. The durability of resistance incorporated into these commercial strains is often constrained by the appearance of new virulent races of rust fungus. Therefore, development of coffee cultivars with long lasting durable resistance by accumulating the resistance genes is a crucial priority. In this direction, a wide array of tetraploid interspecific hybrids have been developed by interspecific and introgressive breeding strategies and these materials are under field evaluation. During the last 10 years, as part of multi-location evaluation, 17 potentially elite hybrid progenies were selected and established at Regional Coffee Research Station, R.V. Nagar. These progenies have been evaluated for growth parameters, field tolerance to rust, clean coffee yield and bean characteristics. Analysis of variance of five morphological characters revealed significant differences between the genotypes in respect of four characters establishing that the genotypes are moderately heterogeneous for plant architecture. Among the reproductive/yield contributing characters, the differences were significant only with respect to yield. Heritability was high (60%) for yield suggesting low genotype X environment interaction. Among different genotypes, S.4643 recorded highest yield of 1055 kg/ha followed by S. 4637 (855 kg/ha) and S.4632 (827 kg/ha). The genotypes differ significantly in their field reaction to leaf rust as studied for 3 years. The genotype S.4643 shows the highest resistance followed by genotypes S.4422 and S.4595. The heritability of resistance was found to be 64% indicating low genotype X environment interaction for this trait. Based on the total index score for each genotype across all characters, S.4643 showed the highest total score of 12.20 followed by S.4622 (11.91), S.4637 (11.68), S.4595 (11.59) and S.4632 (11.44) as against 10.38 in Sln.4 (Agaro) used as control. Some of these promising lines have been advanced and field established for further monitoring and evaluation. The scope and potential of the selected progenies in deriving commercial lines for the zone are discussed.

INTRODUCTION

Globally, coffee is one of the most economically important crops and over 50 countries are involved in its production and trade. The earnings from coffee exports plays vital role in contributing significantly to the economic matrix of producing countries. This world coffee industry generates a total revenue of over $ US 40 billion annually to the producing countries besides providing employment to an estimated 20 million people in the areas of cultivation, processing and trade. In India, coffee has a place of pride among the plantation crops sector. This beverage crop is traditionally cultivated in an area of 0.35 million ha, mainly in the Southern states of Karnataka (57.1%), Kerala (24.4%) and Tamil Nadu (8.8%). Coffee is also grown to a small extent of about 30,000 ha in few other states like Andhra Pradesh, Orissa and North Eastern states which are considered non-traditional areas. India produces around 5 million bags (3 lakh tones) annually, approximately 4.5% of world's total production and 80% the bulk produce is exported while remaining 20% is consumed domestically. Based on the climatic suitability and topography, the arabica (*Coffea arabica*) and robusta (*C. canephora*) types are cultivated commercially in Indian coffee tracts. The area as well as quantum of production is distributed almost equally between these two species. Ofcourse for the past few years some of the farmers have been showing a tendency to shift for robusta because of the difficulties in arabica cultivation especially the disease & pest management.

Coffee cultivation in India is unique as it is grown under multi-tier shade in simulated micro-climate. This eco-friendly manner of coffee cultivation is instrumental for maintaining the forest cover and also in preserving rich bio diversity of flora and fauna. Among the major diseases and pests of coffee, leaf rust disease and white stem borer (*Xylotrechus quadripes* Chevrolat) are the major limiting factors for arabica coffee cultivation. Coffee leaf rust caused by the obligate parasitic fungus *Hemileia vatatrix* Berk & Br is a major disease of concern as the climatic factors of alternate wet and dry conditions that prevail in Indian coffee tracts are favourable for high disease build up, leading to crop losses up to 70% in susceptible cultivars, if proper control measures are not adopted (Anonymous, 2003). Although it is rather difficult to estimate precisely the global impact of rust disease, the economic damage to world arabica production has been estimated to be between US $ 1 billion and 2 billion per year (Van der Vossen, 2001) due to crop losses of 20-25%. The coffee farmers are mainly relying on use of prophylactic and systemic fungicides for disease management and also on cultivation of tolerant cultivars. Hence, development of coffee varieties with durable resistance to coffee leaf rust has been a breeding objective of the highest priority in many coffee producing countries. India is probably the first country to have initiated rust resistance breeding and constantly pursuing research on coffee leaf rust since 1920s.

Interestingly, the efforts to obtain durable resistance to coffee leaf rust have had a long history of initial successes followed by disappointments because of appearance of new virulent races of rust fungus (Van der Vossen, 2001). To date as many as 45 physiological races are known to be infecting different coffee genotypes (Rodrigues Jr et al., 1993; Varzea and Marques, 2005) in various coffee growing countries of which more than 37 rust races have been characterized from India (Prakash et al., 2005). Hence, in the Indian context, breeding for durable rust resistance has remained to be the crucial priority and current thrust in arabica coffee improvement programmes.

In rust resistance breeding programmes of India, all the nine resistance factors (S_H1 to S_H9) have been exploited and arabica strains that manifests varying levels of susceptibility/ resistance to rust have been developed. The durability of resistance incorporated into these commercial strains is often constrained by the appearance of new virulent races of rust fungus. From the past experience, resistance genes originated from diploid coffee species

seem to be more effective than those of tetraploid *C. arabica*. Hence, the combined use of resistance genes of diploid species in *C. arabica* varieties is expected to provide durable resistance. In this direction, a wide array of tetraploid interspecific hybrids have been developed by interspecific and introgressive breeding strategies and some of these new hybrid progenies were introduced in different agro-climatic zones for field evaluation. In the present paper, we report the field performance of 17 new hybrid progenies that were selected and established at Regional Coffee Research Station, R.V.Nagar as part of multi-location evaluation. The comparative performance of these hybrid progenies and the likely possibilities of deriving commercial lines for Andhra Pradesh and Orissa zones from these hybrid genotypes are detailed and discussed.

MATERIALS AND METHODS

The plant material included in the present field evaluation study comprised of 17 hybrid progenies that were selected and established at Regional Coffee Research Station, R.V. Nagar, Andhra Pradesh, during 1995. The parentage of the materials is presented in Table. 1. All the genotypes were planted in a compact block at spacing of 1.8 x 1.8 m, under a mixed canopy of shade. While planting, randomised progeny row design was followed in three replications and in each replication, 16 plants per genotype were planted. The plants were trained on topped single stem system and standard agronomic practices were adopted. The progenies have been evaluated for growth parameters, field tolerance to rust, yield and bean characteristics. A total of five morphological characters viz., stem girth, bush diameter, number of primary branches, length of longest primary, number of nodes on longest primary and five reproductive/yield contributing characters namely number of fruiting nodes on primary, number of fruits per node, progeny yield, Percentage of 'A' grade beans and weight of 100 'A' grade beans were recorded during 2000-01 i.e after five years of planting. The methodology detailed by Amaravenmathy and Srinivasan (2003) was basically followed for recording the data on morphological and reproductive parameters. Progeny yields were recorded for six years from 2000-2001 to 2005-2006 from all the 16 plants of each replication and thus pooled data of 48 plants per progeny was generated. However, considering the alternate cropping pattern in coffee, two-year blocks i.e average of 'off' and 'on' year's yields was considered for statistical analysis. Observations on incidence of leaf rust disease were carried out during Oct-Nov, the peak months of disease build up. Individual plants with even a single sporulating pustule were treated as susceptible for scoring the percentage of resistant/susceptible types. The level of disease build up and reaction type in the susceptible plants was also observed following the scale of Eskes and Toma-Braghini, 1981 cf Eskes 1989). Further, vigour of the plants and retention of infected leaves was also taken into consideration for assessing the resistance manifestation and average of three years data (Arc sin transformed values) was taken into account for analysis. Washed clean coffee samples of each genotype were prepared from 6 kg of uniformly ripened fruits by following the standard method and the same was used for analysing the bean parameters like percentage of 'A' grade beans (retained on 17 no sieve – 6.65 mm) and weight of 100 'A' grade beans. Average of three years data was considered for analysis of bean quality traits.

Analysis of variance was carried out for each character and if the progeny mean square was significant, critical difference was calculated. With the idea of developing an index that reflect the overall performance of a genotype across the characters studied, the mean value of each genotype in respect of each character was expressed as proportion of the grand mean and summed across all characters to arrive at a final score as followed by Amaravenmathy and Srinivasan (2003). The genotype that scored highest was considered the best performer and all the genotypes were ranked in that order.

RESULTS AND DISCUSSION

In general, all the genotypes evaluated in the present study exhibited vigorous growth. Basically these genotypes were the descendents of the crosses either between semi-dwarf x tall arabica types (Cauvery/Catimor crosses) or tall x tall types (crosses involving Sln.11 – Amphiploid of *C. liberica* x *C. euginioides* and other arabicas) as detailed in Table 1. Hence, two grades of phenotypes i.e vigorous semi-dwarfs and tall types were seen with good variation for phenotypic traits among different progenies. The character means, coefficient of variation and heritability for different morphological and reproductive characters is presented in Table 2. Among different progenies, S. 4599 recorded maximum values for stem girth (19.9 cm), bush diameter (119.8 cm) and length of longest primary (127.7 cm) while S.4633 recorded maximum number of primaries (12.3).

Table 1. Parentage of the genotypes included in the study

Sl. No	Acc. No.	Parentage
Cauvery (Catimor) crosses		
1	S.4622	Cauvery (Catimor) x Sln.9 (HDT x Tafarikela)
2	S.4632	(HDT x Geisha) x Cauvery
3	S.4633	Sib mated Cauvery
4	S.4634	Cauvery x Sarchimor
5	S.4635	Ethiopian line - Kaffa province x Cauvery
6	S.4636	Cauvery x Ethiopian line - Kaffa province
7	S.4637	Ethiopian line - wild Sidamo x Cauvery
8	S.4638	Cauvery x Ethiopian line - wild Sidamo
9	S.4643	Brazilian Catimor line (Green tip)
10	S.4644	Brazilian Catimor line (Bronze tip)
Sln.11 crosses and other hybrid progenies		
11	S.4595	Sln.11 (Amphiploid of *C. liberica* x *C. euginioides*) x HDT
12	S.4596	Sln.11 x (Arabica – Wallamo x HdeT)
13	S.4597	Tafarikela x Sln11
14	S.4599	HDT x Sln.11
15	S.4600	(Arabica – Wallamo x HDT) x Sln.11
16	S.4628	(KP423 x HDT) x (Geisha x HDT)
17	S.4422	Devamachy x S. 333
18	Sln.4 (Agaro)	Ethiopian Arabica line

Analysis of variance of five morphological characters studied revealed significant differences between the genotypes in respect of four characters namely stem girth, bush diameter, length of longest primary and number of primaries per plant while the differences were not significant for number of nodes per primary (Table 2). This shows that the genotypes are moderately heterogeneous for plant architecture. Among the four morphological characters that showed significant variation, no. of primaries showed highest phenotypic coefficient of variation (PCV) of 17.5% followed by bush diameter with 12.7% PCV. The Genotypic coefficient of variation (GCV) was highest in the case of bush diameter (10.8%). Heritability coefficient was relatively high for three characters viz., stem girth, bush diameter, length of longest primary that ranged between 67 to 72% and low for number of primaries (30%).

Table 2. Character means, coefficient of variation, heritability for different morphological and reproductive characters among different progenies.

Sl. No	Acc. No.	Stem girth (cm)	Bush diameter (cm)	Length of longest primary (cm)	No. of primaries	No. of nodes per longest primary	No. of fruits per node	No. of fruiting nodes/primary	% of rust resistant plants (Arc sin - transformed values)
1	S.4622	16.1	100.0	109.0	12.1	24.7	10.2	5.5	56.77
2	S.4632	15.6	93.3	102.9	10.7	20.9	9.3	5.0	56.82
3	S.4633	15.4	89.9	96.9	12.3	20.6	10.3	6.4	27.27
4	S.4634	14.0	81.1	90.7	11.4	19.3	9.3	5.3	49.38
5	S.4635	16.5	102.9	105.5	10.9	22.0	9.8	5.4	10.74
6	S.4636	16.3	97.6	106.0	11.4	24.4	8.8	5.4	8.79
7	S.4637	16.2	93.0	99.5	11.0	19.1	9.3	6.2	54.00
8	S.4638	16.2	95.5	102.6	11.6	22.3	9.7	5.1	41.82
9	S.4643	16.0	96.2	107.3	11.7	22.0	8.3	5.8	82.48
10	S.4644	14.5	84.6	89.4	9.1	20.8	6.7	5.7	68.04
11	S.4595	18.6	117.4	122.1	9.9	21.3	7.6	5.0	73.02
12	S.4596	17.9	110.5	122.4	11.2	19.5	9.2	4.8	48.76
13	S.4597	18.0	103.8	113.9	10.5	19.5	9.6	3.9	32.37
14	S.4599	19.9	119.8	127.7	9.7	21.3	6.5	3.8	45.67
15	S.4600	18.7	119.6	124.3	10,3	20,2	9.5	4.8	44.86
16	S.4628	15.7	88.1	94.9	12.0	19.1	8.7	6.1	59.53
17	S.4422	17.2	104.3	107.0	7.4	18.1	8.2	3.1	76.59
18	Sln.4 (Agaro)	17.2	111.0	119.9	8.0	21.8	7.0	4.8	24.39
Grand Mean		16.66	100.7	107.9	10.6	20.9	8.8	5.1	47.85
'F' test		**	**	**	*	NS	NS	*	**
CD – 5%		1.6	18.0	11.5	2.6	--	--	1.8	17.47
– 1 %		2.2	24.2	15.4	--	--	--	--	23.41
P.C.V %		10.2	12.7	12.0	17.5	13.9	19.2	24.1	36.6
G.C.V %		8.4	10.8	10.2	9.6	13.4	9.0	7.2	29.2
h^2 %		67	72	72	30	7	22	24	64

In case of the two reproductive characters, the differences among genotypes for number of fruits per node were not significant while differences in number of fruiting nodes per primary were less significant, indicating relative homogeneity for these traits. PCV was moderately high (19.2 and 24.1%) for both the characters where as GCV was low (9 and 7.2%), (Table 2). Heritability was also low (22 and 24%) indicating high genotype X environment interaction.

Among the three yield component characters studied, the differences among the genotypes were significant with respect to clean coffee yield and percentage of `A` grade beans while the differences for weight of 100 `A` grade beans were less significant (Table 3). The PCV and GCV were also moderately high, 22.8 to 27.6% and 15.5 to 21.5%, respectively for percentage of `A` grade beans and yield. Heritability was high (60%) for yield suggesting low genotype X environment interaction (Table 3). Among different genotypes, S.4643 (Brazilian Catimor line) recorded highest average yield of 1055 kg/ha followed by S. 4637 (855 kg/ha) and S.4632 (827 kg/ha). On the other extreme S.4644 recorded poor performance with average yield of only 423 kg/ha.

Table 3. Character means, coefficient of variation, heritability for yield and yield component characters among different progenies.

Sl. No	Acc. No.	Percentage of 'A' grade beans	Weight of 100 'A' grade beans (gm)	Mean yield – clean coffee (kg/ha)
1	S.4622	56.0	16.0	742
2	S.4632	56.0	15.6	827
3	S.4633	57.6	15.4	704
4	S.4634	58.4	16.7	615
5	S.4635	55.4	16.3	575
6	S.4636	56.6	16.3	525
7	S.4637	58.7	17.1	855
8	S.4638	64.2	16.9	703
9	S.4643	35.6	16.5	1055
10	S.4644	35.4	16.2	423
11	S.4595	46.5	16.8	585
12	S.4596	38.4	16.1	578
13	S.4597	38.6	15.2	517
14	S.4599	41.9	16.1	485
15	S.4600	46.6	16.3	719
16	S.4628	62.2	16.6	584
17	S.4422	53.4	16.5	545
18	Sln.4 (Agaro)	53.7	17.3	633
Grand Mean		50.66	16.33	648
'F' test		**	*	**
CD – 5%		14.06	1.02	374.5
– 1 %		18.84	--	501.0
P.C.V %		22.8	4.6	27.6
G.C.V %		15.5	2.6	21.5
h^2 %		46	32	60

Table 4. Growth and yield indices of the evaluated genotypes in the order of performance.

Sl. No	Acc. No.	Stem girth (cm)	Bush diameter (cm)	Length of longest primary (cm)	No. of primaries	No. of nodes per longest primary	No. of fruits per node	No. of fruiting nodes per primary	Percentage of rust resistant plant (Arc sin -transformed values)	Weight of 100 'A' grade beans (gm)	Percentage of 'A' grade beans	Mean yield	Total score
1	S.4643	0.96	0.96	0.99	1.10	1.05	0.95	1.13	1.72	1.01	0.70	1.63	12.20
2	S.4622	0.96	0.99	1.01	1.14	1.18	1.16	1.07	1.19	0.98	1.11	1.14	11.91
3	S.4637	0.97	0.92	0.92	1.03	0.91	1.06	1.21	1.13	1.05	1.16	1.32	11.68
4	S.4595	1.11	1.17	1.13	0.93	1.02	0.87	0.98	1.53	1.03	0.92	0.90	11.59
5	S.4632	0.93	0.97	0.95	1.01	1.00	1.06	0.98	1.19	0.96	1.11	1.28	11.44
6	S.4600	1.12	1.19	1.15	0.97	0.97	1.08	0.94	0.94	1.02	0.92	1.11	11.41
7	S.4638	0.97	0.95	0.95	1.09	1.07	1.11	1.00	0.87	1.03	1.27	1.08	11.39
8	S.4628	0.94	0.87	0.88	1.13	0.91	0.99	1.19	1.24	1.02	1.23	0.90	11.30
9	S.4633	0.92	0.89	0.90	1.16	0.99	1.17	1.25	0.57	0.94	1.13	1.09	11.01
10	S.4596	1.07	1.10	1.13	1.05	0.93	1.05	0.94	1.02	0.99	0.76	0.89	10.93
11	S.4634	0.84	0.81	0.84	1.07	0.92	1.06	1.04	1.03	1.02	1.15	0.95	10.73
12	S.4422	1.03	1.04	0.99	0.70	0.87	0.94	0.61	1.60	1.01	1.05	0.84	10.68
13	S.4599	1.19	1.19	1.18	0.91	1.02	0.74	0.74	0.95	0.99	0.83	0.75	10.49
14	S.4635	0.99	1.02	0.98	1.03	1.05	1.12	1.05	0.22	1.00	1.09	0.89	10.44
15	Sln.4 (Agaro)	1.03	1.10	1.11	0.75	1.04	0.80	0.94	0.51	1.06	1.06	0.98	10.38
16	S.4636	0.98	0.97	0.98	1.07	1.17	1.00	1.05	0.18	1.00	1.12	0.81	10.33
17	S.4597	1.08	1.03	1.06	0.99	0.93	1.09	0.76	0.68	0.93	0.76	0.80	10.11
18	S.4644	0.87	0.84	0.83	0.86	1.00	0.76	1.11	1.42	0.99	0.70	0.65	10.03

The genotypes differed significantly in their field reaction to leaf rust as studied for 3 years. The genotype S.4643 (Brazilian Catimor) showed the highest resistance followed by genotypes S.4422 and S.4595. Year to year stability for field resistance to rust is also high in these three genotypes and never reduced below 70%. Rest of the genotypes showed more variation for resistance ranging from 10.75% in S.4635 to 68.04% in S.4644. The heritability of resistance was found to be 64% (Table 2) indicating low genotype X environment interaction for this trait. PCV and GCV were moderate. Observations on disease build up and extent of defoliation revealed very low disease build up (3 to 4 on 0-9 scale) in majority of the genotypes with no defoliation of infected leaves. However, the disease build was observed to be high (6 to 7 on 0-9 scale) in S.4635, S.4636, S.4633, S.4638 and S.4644 leading to defoliation.

In order to identify the better performers across the genotypes, the total index score for each genotype is summed over morphological, reproductive and yield-contributing characters studied and the data is presented in the order of performance (Table 4). Among different genotypes, S.4643 showed the highest total score of 12.20 followed by S.4622 (11.91), S.4637 (11.68), S.4595 (11.59) and S.4632 (11.44) as against 10.38 in Sln.4 (Agaro) used as control. Srinivasan (1982) developed a selection index for arabica coffee based on five morphological characters and their correlation with initial fruit yield. It was reported that selection index based on morphological characters was found to be 16 to 19% more efficient compared to straight selection based on yield. Subsequently, Amaravenmathy and Srinivasan (2003) evaluated some HDT crosses at Regional Coffee Research Station, Chettalli, India and a total score method for differentiating genotypes for target characters including yield was reported. Based on this total score method, superior performance of S.4538 (as a composite line) was established.

The data of the present study clearly indicated the vegetative vigour and reproductive potential of the new genotypes and the findings are of immense use in deriving commercial lines for the zone. The top performers like S.4622, S.4637 and S.4632 are the derivatives of Catimor and its crosses involving Ethiopian lines like Geisha, Tafarikela and Wild Sidamo. As these lines are well stabilized, it is worth trying the above selected materials as a composite/multi line to achieve durable rust resistance. In this direction, selected plants from these lines were marked and advanced for further monitoring and evaluation. In Colombia, the multi line strategy adopted in releasing the composite variety 'Colombia' proved to be good in providing durable resistance. Although there has been a gradual increase in the susceptibility of these populations to rust over years, the multi-line strategy proved effective and perfect in checking the development and buildup of new virulent rust races in Colombia (Alvarado 2005). Further, with the experience in Brazil, Fazuoli (2005) opined that the strategies for use of resistant genetic plant material to increase the durability of resistance could be i) use of multi-lines; ii) composed variety (similar to multi lines) by mechanical mixture of seeds from various lines, not necessarily isogenic and iii) planting lines or cultivars with diverse resistance genes (with similar or not similar agronomic traits) in isolated strands with in the same estate.

Basically the Andhra Pradesh and Orissa tracts are the non-traditional areas of coffee cultivation in India and the climatic factors prevailing in these tracts are relatively harsh compared to the traditional areas. Further, the coffee cultivation in these areas is being taken up by socially and economically backward tribal farmers by adopting very low management practices. Hence, the performance of Cauvery/Catimor lines was observed to be poor as these lines require intensive management practices. In this context, even though S.4643 (Brazilian Catimor line – Green tip) topped the list in performance, exploitation of this line, in the tribal sector, has little scope. However, there exists a greater scope for its exploitation by

progressive private entrepreneurs who are cultivating coffee as in Orissa State. It is also worth studying the performance of S.4643 (Brazilian Catimor) under low management practices similar to that of tribal sector. Furthermore, some of the interspecific hybrid derivatives like Sln.5A (Devamachy x S.881- Rumesudan collection), Sln.6 (Robusa x arabica hybrid) and Sln.11 (Amphiploid of *C. liberica* x *C. euginioides*) are reported to be hardy and recorded promising performance even under low management practices, in this zone. However, inspite of the its high field tolerance to leaf rust, adoptability to low rainfall areas due to drought hardy nature and consistent production, Sln.11 could not be exploited due to small bean size (Dharmaraj, 1985; Gopal, 1985, Reddy et al., 1984; Reddy et al., 1985). In this context, the better performance of S.4595, a derivative of Sln.11 x HDT recorded in the present study, provides scope for its exploitation. Ganesh et al. (2002) evaluated the performance of S.4595 and its back cross progenies with HDT at Central Coffee Research Institute, Balehonnur and established the promising potential of S.4595 with respect to yield, field tolerance to leaf rust, bean and liquor quality traits. The S.4595, as a line developed to be a combination of vertical and horizontal resistance appears to be very promising even with respect to durable rust resistance. According to Alvarado (2005), incomplete resistance of post-infective action is manifested in the Timor hybrid derivatives (Colombia variety) when the complete resistance breaks down confirming that combined use of both type of resistances under known genetic back ground, guarantees durable protection against leaf rust in coffee. Considering these factors, elite plants in the progeny of S.4595 were marked and selfed progenies were advanced for further monitoring and exploitation.

In conclusion the present study confirmed the efficacy of the selection index based on total score method as suggested by Amaravenmathy and Srinivasan (2003) for identifying the superior lines. Furthermore the evaluation study enabled to identify the potential lines for commercial exploitation.

ACKNOWLEDGEMENTS

The help extended by Dr. C. S. Srinivasan, Joint Director of Research (Rtd), Indian Coffee Board, in analysing the data and for critically going through the manuscript is gratefully acknowledged. Special thanks are due to Dr. Jayarama, Director of Research, Coffee Board, for the constant support & encouragement

REFERENCES

Alvarado, A.G. 2005. Evolution of *Hemileia vastatrix* virulence in Colombia. *In* **Durable Resistance to Coffee leaf rust** (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 99-115. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Amaravenmathy, V.S. and Srinivasan, C.S. 2003. Phenotypic and genotypic variations for yield and plant architecture in some hybrid progenies of arabica coffee. J. Coffee Res. 31(2): 99-105.

Anonymous. 2003. Coffee Guide, Coffee Board, Bangalore, India, 200p.

Dharmaraj, P.S. 1985. Adaptation of of coffee selections in Andhra Pradesh. J Coffee Res. 15: 32.

Eskes, A.B. 1989. Resistance. In: Kushallapa, A.C and Eskes, A.B. (eds), *Coffee rust: Eidemiology, Resistance and Management*: 171-291. CRC Press, Boca Raton, Florida.

Fazuoli, L.C., Baiao de Oliveira, A.C., Toma-Braghini, M., Silvarolla, M.B. 2005. Identification and use of sources of durable resistance to coffee leaf rust at the IAC. *In* **Durable Resistance to Coffee leaf rust** (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 137-185. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Ganesh D., Ram A.S., Prakash, N.S., Mishra, M.K., Ahmed, J., Jagadeesan, M., Reddy, A.G.S. and Srinivasan, C.S. 2002. Evaluation of *Coffea liberica* x *Coffea eugenioides* and its progenies for yield, leaf rust tolerance and quality. Proc of Placrosym XV (Plantation Crops Symposium, Mysore, India, 2002), pp 72-77.

Gopal, N.H. 1985. Performance of coffee cultivars of Andhra Pradesh region with reference to drought. J Coffee Res. 15: 58.

Prakash, N.S., Ganesh, D. and Bhat, S.S. 2005. Population dynamics of coffee leaf rust (*Hemileia vastatrix* Berk & Br) and recent advances in rust research in India. *In* **Durable Resistance to Coffee leaf rust** (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 411-442. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Reddy, A.G.S., Raju, K.V.V.S.N. and Dharmaraj, P.S. 1984. Allopolyploidization in a spontaneously doubled hybrid of two diploid species of *Coffea*. Proc. VI Symposium on Plantation Crops, Kottayam, India, pp 31-39.

Reddy, A.G.S., Raju, K.V.V.S.N. and Dharmaraj, P.S. 1985. Breeding behavior of Ligenioides – A spontaneous amphidiploid between *Coffea liberica* and *C. eugenioides.* J Coffee Res. 15 (1 & 2): 33-37.

Rodrigues Jr, C.J., Varzea, V.M., Godinho, I.L., Palma, S. and Rato, R.C. 1993. New physiological races of *Hemileia vastatrix.* In: Proceedings of the 15th ASIC colloquium (Montpellier), ASIC, Paris, France, pp 318-321.

Srinivasan, C.S. 1982. Pre-selection for yield in coffee. India J. Genet. 42: 15-19.

Vander vossen, H.A.M. 2001. Coffee breeding practices. In: Clarke R.J and Vitzthum O.G (eds) Coffee Recent Developments – Agronomy 1 (eds), Blackwell Science Ltd, London, pp 184-201.

Varzea, V.M.P. and Marques, D. V. 2005. Population variability of ***Hemileia vastatrix*** vs. Coffee durable resistance. *In* **Durable Resistance to Coffee leaf rust** (Eds) Laercio Zambolim, Eunize Maciel Zambolim, Vitor Manuel Pinto Varzea, pp 53-74. (Proceedings of the 1st International Workshop on Durable Resistance to coffee leaf rust held at Vicosa, Brazil from 25-28 September 2005).

Investigation of Natural Interspecific Hybrids from New Caledonia: a Very Promising Source of Genetic Diversity for *C. arabica* Breeding

L. MAHÉ[1], D. LE PIERRÈS[1], M-C. COMBES[1], V. VARZEA[2] AND P. LASHERMES[1]

[1]Institut de Recherche pour le Développement, Montpellier, France
[2]Centro de Investigação das Ferrugens do Cafeeiro, Oieras, Portugal

SUMMARY

The development of cultivars that are resistant to coffee leaf rust caused by the fungal pathogen *Hemileia vastatrix* is a priority in coffee breeding. So far only a few descendants of interspecific hybrids between *C. arabica* and related diploid species have been used as sources of resistance. Identification of new sources of resistance is thus particularly worthwhile. As a consequence, we investigated the genetic diversity of spontaneous interspecific hybrids between *C. arabica* and *C. canephora* (HNC) from an exceptional sympatric zone in New Caledonia. AFLP and microsatellites amplification were used to evaluate the genesis and genetic diversity of 42 HNC plants, while rust resistance was evaluated by inoculation with a representative panel of rust races from a worldwide collection. Occurrence of a large assortment of hybridization events between the two coffee species is clearly established. An important genetic diversity was characterized in HNC plants originating from introgressions into *C. arabica* from various *C. canephora* progenitors. Out of the 14 plants tested for leaf rust resistance, half appeared to be resistant to all known races. Such high diversity in interspecific hybrids between *C. arabica* and *C. canephora* is exceptional and represents a highly valuable source for *C. arabica* breeding against coffee leaf rust.

INTRODUCTION

Commercial coffee production is mainly based on two closely-related species: *Coffea arabica* and *Coffea canephora* which account for, respectively, around 70% and 30% of world coffee production (International Coffee Organization, http://www.ico.org). *Coffea arabica* (2n = 4x = 44), the only polyploid species in the *Coffea* genus, is characterized by low genetic diversity (Anthony et al., 2002); whereas considerable variability has been reported among diploid coffee species (Berthaud and Charrier, 1988). The transfer of desirable characters, in particular disease resistance, from related diploid species into cultivars of *C. arabica* is thus an ongoing priority in coffee breeding (Van der Vossen, 2001).

Leaf rust caused by the fungal pathogen *Hemileia vastatrix* is considered to be one of the most serious diseases of the coffee tree. Inheritance studies of rust resistance demonstrated that the gene-for-gene theory is applicable to coffee rust reactions (Silva et al., 2006). The plant response to several races involves either complete resistance or susceptibility (low and high infection type) and enables classification of coffee genotypes in physiologic groups. Plants that are characterized by resistance to all the known rust races are classified in group A. They have been found in genotypes derived from crosses between *C. arabica* x *C. canephora*, either spontaneously (Timor hybrid) or man-made (Icatú) (Marques and Bettencourt, 1979; Kushalapa and Eskes, 1988).

The search for naturally occurring resistant coffee plants for the improvement of resistance to attacks by leaf rust pathogens dates back to 1875. Few spontaneous resistant plants derived from interspecific hybridization between *C. arabica* and diploid species such as *C. canephora* or *C. liberica* have been identified. In particular, a tetraploid genotype known as the 'Timor Hybrid' was found in a *C. arabica* field on the island of Timor in 1927 (Bettencourt, 1973). Molecular analysis established that the Timor hybrid originated from one spontaneous interspecific cross between *C. canephora* and *C. arabica* (Lashermes et al., 2000). Progenies of the Timor hybrid (HdT) have been widely distributed and intensively used in coffee breeding programmes as the main source of resistance to coffee leaf rust. Exploitation of Timor Hybrid populations has so far relied on conventional procedures in which a hybrid is produced with an outstanding arabica genotype, and the progeny is selfed and selected over 3-4 generations. Based on this strategy -which takes more than 30 years- improved cultivars have already been released in most coffee producing countries. However, in the last few years, some improved commercial varieties derived from HdT have been reported to be gradually loosing their resistance to leaf rust in some countries, due to the appearance of new virulent races (Várzea and Marques, 2005). The identification and use of new sources of resistance to leaf rust would therefore be particularly valuable.

Recently, prospecting missions in New caledonia (*Pacific island*) for genetically original material have revealed several areas of sympatric occurrence of *C. arabica, C. canephora* and plants showing intermediate phenotypes (Charmetant and Le Pierrès, 1991; Le Pierrès, 1999). Thus areas corresponded to forsaken fields since the 1940s and could be considered as neo-natural hybrid zone. Our objectives were to ascertain the presence of hybrid plants in those exceptional areas, to investigate the processes governing hybrid formation, to evaluate the diversity of this genetic material and the potential of fourteen of these plants to improve resistance of *C. arabica* to leaf rust.

HYBRID FORMATION

At all of the five prospected sites around le Col d'Amieu, hybrid plants were identified. The molecular characterization of hybrid plants indicated the occurrence of numerous and different hybridizations between *C. arabica* and *C. canephora* in the environmental conditions of the New caledonian central mountains. The repeated hybrid formation suggests a significant overlap between the flowering periods of both species, although differences in flowering phenology between the two species have been reported (Baranski, 196).

A noteworthy result emerged from the identification of various types of F_1 plants (Table 1). Triploid as well as tetraploid F_1 plants were detected. Triploid F_1 plants are usually obtained when crossing *C. arabica* and *C. canephora* (Carvalho and Monaco, 1978; Le Pierrès, 1995). On the other hand, the identified tetraploid F_1 plants are likely to result from the involvement of unreduced gametes of *C. canephora*. Such gametes, which contain the full somatic chromosome number, are commonly considered as the consequence of "gametic non-reduction" or "meiotic nuclear restitution" during micro- and megasporogenesis. These unreduced gametes resulted either from an incomplete first meiotic (= first division restitution or FDR) division, or from an incomplete second meiotic division (= second division restitution or SDR). The FDR 2n-gametes comprise non-sister chromatids of each homologous pair of chromosomes whereas in SDR 2n-gametes the sister chromatids are included (Hermsen, 1984). In the present study, both processes seem to be concerned since of the 8 identified tetraploid F_1 plants, 3 seemed to involve FDR gametes (i.e. presence of loci exhibiting two canephora alleles) while 5 appeared to result from SDR gametes. Several researchers have found than 2n pollen production is stimulated by environmental factors (Hermsen, 1984; Ramsey and Schemske, 1998). Temperature and especially variation in

temperature have particularly large (Lewis, 1943; McHale1983). In Coffea, Lanaud (1979) reported the production of such unreduced gametes in *C. canephora* and *C. liberica,* two diploid species, under cold treatment. The New caledonian mid-mountain conditions could therefore favour the production of 2n gametes and tetraploid hybrid plants. Furthermore, both micro- and megasporogenesis appeared to be affected. Based on the characterization of hybrid plant cpDNA, of the 8 identified tetraploid F_1 plants, 6 result from crosses with *C. arabica* as female parent (i.e. Arabica type cpDNA) while 2 plants derive from crosses with *C. canephora* as female parent (i.e. Canephora type cpDNA).

Table 1. List and characterization of identified first-generation hybrids (Mahé et al., in press).

Plant codes	Origin (site)	Ploidy level	CpDNA type	Presence of locus exhibiting two *C. canephora* alleles
	Leonard			
L 1		4 X	*C. arabica*	
L 2		4 X	*C. arabica*	+
	Couli			
C 23		4 X	*C. canephora*	
C 28		3 X	*C. arabica*	
C 30		4 X	*C. arabica*	
C 34		4 X	*C. arabica*	+
C 38		4 X	*C. arabica*	+
C 39		4 X	*C. canephora*	
C 40		3 X	*C. canephora*	
	Friquet			
F 5		3 X	*C. canephora*	
F 6		3 X	*C. arabica*	
	Dogny			
D 92		4 X	*C. arabica*	

Post-F_1 individuals were detected in high proportion (i.e. 71%) and at the 5 collection sites. All the 30 identified post-F_1 hybrid plants invariably showed the *C. arabica* DNA markers and diverged only for the number and type of canephora SSR-alleles or AFLP-bands suggesting an asymmetrical gene flow (Ostberg et al., 2004). Furthermore, all post-F_1 individuals exhibited DNA content close to that of *C. arabica*. Although allowing only a crude estimation, this observation indicated that most post-F_1 plants were nearly tetraploid, with about 44 chromosomes. Thus, the collected post-F_1s in the present study are likely to result from either backcross events to *C. arabica* or from intercrosses between hybrid plants. The presence in *C. canephora* of a gametophytic self-incompatibility system (Berthaud, 1986; Lashermes et al., 1996) could restrain the possibility of backcross to *C. canephora*. Furthermore, as observed in artificial hybrids (Herrera et al., 2002; 2004, an evolution toward the tetraploid condition also seemed strongly favoured by a preferential transmission from hybrid plants of gametes carrying a diploid number (i.e. 22) of chromosomes. This process is most likely the consequence of both gametic selection and post-zygotic barriers, as reported elsewhere (Herrera et al., 2002; Ehlenfeldt and Ortiz, 1995; Carputo et al., 1999).

Table 2. List of identified *C. canephora* alleles for all analyzed SSR loci. For each allele, the presence in the different populations (Dogny, D; Couli, C; Leonard, L; Szemmelveisz, S; Friquet, F) and mean frequencies are reported (Mahé et al., in press).

Locus	Alleles	Populations					Mean frequencies	Locus	Alleles	Populations					Mean frequencies	Locus	Alleles	Populations					Mean frequencies
		D	C	L	S	F				D	C	L	S	F				D	C	L	S	F	
SAT32	Ca	+	+	+	+	+	0,31	SAT171	Ca		+				0,05	SAT235	Ca	+	+				0,04
	Cb		+			+	0,09		Cb	+	+				0,05		Cb	+	+		+	+	0,36
	Cc	+					0,03		Cc	+	+		+	+	0,33		Cc					+	0,04
	Cd	+	+			+	0,15		Cd	+					0,03		Cd	+	+	+	+	+	0,22
	Ce	+		+	+		0,18		Ce				+	+	0,08		Ce	+	+			+	0,22
	Cf		+				0,03		Cf		+	+		+	0,29		Cf	+					0,03
SAT41	Ca	+	+				0,07		Cg			+			0,02	SAT251	Ca		+				0,03
	Cb	+	+				0,08	SAT177	Ca	+	+		+		0,14		Cb		+		+		0,07
	Cc	+	+				0,06		Cb	+	+				0,04		Cc	+	+			+	0,12
	Cd	+	+		+		0,08		Cc	+	+				0,10		Cd				+	+	0,12
	Ce	+	+				0,06		Cd	+	+	+		+	0,31		Ce	+	+	+		+	0,31
	Cf	+			+		0,15		Ce		+				0,08	SAT252	Ca		+				0,02
	Cg		+	+		+	0,23	SAT189	Ca					+	0,04		Cb	+					0,06
SAT47	Ca	+	+	+			0,20		Cb		+				0,03		Cc	+	+		+	+	0,33
	Cb	+	+		+	+	0,30	SAT193	Ca	+	+	+	+	+	0,81		Cd		+	+		+	0,29
	Cc	+	+	+		+	0,29	SAT225	Ca		+		+		0,13		Ce	+	+				0,07
	Cd	+	+				0,06		Cb		+				0,06	SAT253	Ca		+	+			0,09
	Ce	+	+				0,04		Cc	+	+	+		+	0,36		Cb		+				0,01
	Cf		+				0,01		Cd	+	+			+	0,22		Cc	+	+	+	+		0,20
SAT65	Ca	+	+	+	+	+	0,93		Ce	+					0,03		Cd	+	+		+	+	0,28
SAT109	Ca	+	+	+		+	0,51	SAT227	Ca					+	0,04		Ce	+	+	+		+	0,30
	Cb	+	+		+	+	0,21		Cb	+					0,03	SAT254	Ca		+	+			0,08
	Cc	+	+				0,05		Cc					+	0,04		Cb		+				0,04
	Cd		+				0,01		Cd					+	0,04		Cc		+	+			0,04
SAT157	Ca	+	+	+		+	0,36		Ce	+	+			+	0,13		Cd	+	+			+	0,22
	Cb	+	+	+	+	+	0,67		Cf		+				0,01		Ce	+			+		0,16
SAT160	Ca	+	+		+	+	0,43		Cg		+			+	0,05		Cf	+					0,03
	Cb	+	+	+			0,07		Ch		+		+		0,09		Cg					+	0,04
	Cc	+	+	+			0,16		Ci	+	+	+			0,16		Ch					+	0.01
	Cd	+					0,03		Cj		+		+		0,19		Ci		+				0,01
SAT166	Ca	+	+	+		+	0,24		Ck	+	+				0,06	SAT262	Ca					+	0,04
SAT170	Ca	+	+	+	+	+	0,77	SAT228	Ca	+					0,03		Cb	+	+			+	0,09
	Cb		+				0,02		Cb	+	+	+	+	+	0,50		Cc	+	+	+		+	0,17
																SAT280	Ca					+	0,04
																	Cb	+		+	+		0,18
																	Cc	+	+				0,06
																	Cd		+			+	0,09
																	Ce	+	+			+	0,19

HYBRID DIVERSITY

The genetic variability of hybrid plants from the 5 collected sites was evaluated. The arabica alleles were invariably present among the hybrid plants at all loci except the locus M177, which showed a polymorphism similar to the variation observed among the *C. arabica* accessions. In contrast, a considerable variation for the canephora alleles was observed. The different canephora alleles identified for each of the 24 SSR loci analyzed are listed in Table 2. The total number of canephora alleles per locus varied from 1 to 11, with a mean of 4.6. Most alleles were shared by plants originating from different collecting sites. However, few alleles appeared restricted to one single population. Differences between sites were observed. While the plants collected at the sites Dogny (D), Couli (C) and Friquet (F) showed marked polymorphism, the plants originating from the Leonard (L) and Szemmelveisz (S) sites appeared more homogeneous. Moreover, for several loci, more than 3 canephora alleles per loci were observed among the plants collected at a given site (i.e. D, C and F), suggesting the involvement of several canephora genotypes in the hybridization process. Furthermore, of the 42 hybrid plants, we identified 10 plants exhibiting at least one SSR locus presenting two *C. canephora* alleles; three pertain to the F_1 group of hybrids and the others to the post-F_1.

RUST INOCULATION TEST

An inoculation test was carried out with the most common race (II) and the most virulent races (isolate 5a, 2191, 1321) on fourteen HNC. This coffee population showed similar characteristics to other interspecific tetraploid hybrids e.g. instability of intermediate reaction types. This phenomenon was observed in an Icatú population studied by Marques & Bettencourt 1979 and is frequently observed in some derivatives of HDT (Várzea, personal communication). From the results obtained (Table 2) it was possible to identify coffee plants like L2, L7, L8, L9, L10, C23 and C34 that showed resistance to the most virulent rust isolates at CIFC, and we predict that they very likely belong to physiologic group A. The L4 coffee plants showed susceptibility to rust isolate 5a and resistance to isolate 2191. Coffee plants C28, D95 and S2 showed resistance to isolate 5a and susceptibility to isolate 2191. The coffee plant was susceptible to rust isolates 2191, 5a and 1321. The S1 coffee plant Cou30 was the only one characterized as group E, susceptible to isolate 1427 (race II-v5). It should be noted that Cou30 is a F1 hybrid, and its sensitivity to race II was unexpected. It is possible that the alleles brought by *C. canephora* are similar to those presents in arabica.

In conclusion, both AFLP and SSR were very effective at detecting genetic variation. The molecular data points towards high levels of genetic diversity of *C. canephora* progenitors at the origin of HNCs (Mahé et al., in press). Comparison with Timor Hybrid-derived genotypes illustrated the considerable new diversity present in HNC plants. Finally rust inoculation tests clearly established the resistance potential of these HNCs. Although only a representative set of HNCs was studied, HNC plants appear to be a very promising new source of resistance against leaf rust which would justify extended collection and preservation measures. Individuals with a low level of introgression classified in the physiologic group A could be used directly in breeding programmes and could contribute significantly to the development of improved Arabica cultivars exhibiting durable resistance to rust.

Table 1. Reaction type of HNC plants inoculated with the most virulent rust isolates (Mahé et al., in press).

Plants	Rust isolates and virulence genotype		
	2191	5a	1321
	v2,5,6,7,9, ?	v2,5,7,8,9,?	v5,6,7,8,9
L 1	nd	nd	**R**
L 2	**R**	**R**	**R**
L 4	**R**	S	**R**
L 7	**R**	**R**	**R**
L 8	**R**	**R**	**R**
L 9	**R**	**R**	**R**
L 10	**R**	**R**	**R**
C 23	**R**	**R**	**R**
C 28	S	**R**	**R**
C 30	S	S	S
C 34	**R**	**R**	**R**
Dog 95	S	**R**	**R**
SZE01	S	S	S
SZE02	S	**R**	**R**

nd: not determined

ACKNOWLEDGEMENTS

We are grateful to the Province Sud of New Caledonia for financial support (Convention 531-PVF/DDR).

REFERENCES

Anthony F, Combes M-C, Astorga C, Bertrand B, Graziosi G, Lashermes P (2002). The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. Theoretical Applied Genetic 104: 894-900.

Baranski O (1996). Etude des caractères morphologiques, phénologiques et de la fertilité de 29 taxons de caféiers sauvages africains. Rapport VSN, ORSTOM. Paris. pp. 38.

Berthaud J (1986). Les ressources génétiques pour l'amélioration des caféiers africains diploïdes. Collection ''Travaux et Documents'' ed ORSTOM, Paris, pp.188.

Berthaud J and Charrier A (1988) Genetic resources of Coffea. In "Coffee vol 4: Agronomy". In R. J. Clarke and Macrae R. (Eds), 1-40, London.

Bettencourt AJ (1973). Cosideradoçoes gerais sobre o "Hybrido de Timor", Instituto Agronomico de Campinas, Campinas.

Carputo D, Monti L, Werner JE, Frusciante L (1999). Uses and usefulness of endosperm balance number. Theoretical Applied Genetics 98: 478-484.

Carvalho A, Monaco LC (1968). Relaciones geneticas de especies selecionadas de Coffea. Cafe, Lima, 9: 1-19.

Charmetant P, Le Pierrès D (1991). Rapport de mission en Nouvelle-Calédonie: prospection et collecte d'hybrides naturels de caféiers cultivés. IRCC-CIRAD/ORSTOM, Paris, pp. 21

Ehlenfeldt MK, Ortiz R (1995). Evidence on the nature and origins of endosperm dosage requirements in *Solanum* and other angiosperm genera. Sexual Plant Reproduction, 8: 189-196.

Hermsen JGTh (1984). Some fundamental considerations on interspecific hybridization. Iowa State J. research. 58: 461-474.

Herrera J-C, Combes M-C, Cortina H, Alvarado G, Lashermes P (2002). Gene introgression into *Coffea arabica* by way of triploid hybrids. Heredity, 89: 488-494.

Herrera J-C, Combes M-C, Cortina H, Lashermes P. (2004). Factors influencing gene introgression into the allotetraploid *Coffea arabica* L. from its diploid relatives. Genome, 47: 1053–1060.

Kushalapa AC, Eskes AB (1988). Coffee Rust: epidemiology, Resistance and Management. CRC Press, Inc. Boca Raton, Florida.

Lanaud C (1979). Etude de problèmes génétiques posés chez le caféier par l'introgression de caractères d'une espèce sauvage *C. kianjavatensis, Mascarocoffea*. dans l'espèce cultivée *C. canephora Eucoffea*. Café Cacao Thé, 23: 3-28.

Lashermes P, Couturon E, Moreau N, Paillard M, Louarn J (1996). Inheritance and genetic mapping of self-incompatibility in *Coffea canephora* Pierre. Theor. Appl. Genet. 93: 458-462.

Lashermes P, Paczek V, Trouslot P, Combes M.-C, Couturon E, Charrier A (2000). Single-locus inheritance in the allotetraploid *Coffea arabica* L. and interspecific hybrid *C. arabica x C. canephora*. Journal of Heredity 91, 81-85.

Le Pierrès D (1995). Etude des hybrides interspécifiques tétraploïdes de première génération entre *Coffea arabica* L. et les caféiers diploïdes. PhD thesis, University of Paris-XI.

Le Pierrès D (1999). Etudes des hybrides naturels entre *Coffea arabica* et *Coffea canephora* de Nouvelle-Calédonie. IRD, Centre de Nouméa. pp. 44

Lewis D (1943). The Incompatibility sieve for producing polyploids. Journal Genet. 45: 264-264.

Mahé L, Le Pierrès D, Combes M-C, Lashermes P (in press) Introgressive hybridization between the allotetraploid *Coffea arabica* and one of its diploid ancestors *C. canephora* in an exceptional sympatric zone in New Caledonia. Genome.

Marques DV and Bettencourt AJ (1979). Resistência à *H. vastatrix* numa população de Icatú. Garcia de Orta (Série de Estudos Agronómicos) 6: 19-24.

McHale NA (1983). Environmental induction of high frequency 2n pollen formation in diploid *Solanum*. Canadian. J. of genetics and cytology, 25: 609-615.

Ostberg CO, Slatton SL, Rodriguez RJ (2004). Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout *Oncorhynchus mykiss*., coastal cutthroat trout *O. clarki clarki*. and interspecific hybrids. Mol. Ecol. 13: 2773-2788.

Ramsey J, Schemske DW (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. Ann. Rev. Eco. and Syst. 29: 467-501.

Silva MDS, Várzea V, Guerra-Guimarães L, Azinheira HG, Fernandez D, Petitot A-S, Bertrand B, Lashermes P, Nicole M (2006). Coffee resistance to the main diseases: leaf rust and coffee berry disease. Braz. J. Plant. Physiol. 18: 119-147.

Van der Vossen HAM (2001) Coffee breeding practices. In: R.J. Clarke and Vitzthum, O.G. (eds) *Coffee – Recent Developments*. Oxford: Blackwell Science, 184-201.

Várzea VMP, Marques DV (2005). Population variability of *Hemileia vastatrix* vs coffee durable resistance. In: L. Zambolim, Zambolim E. and Várzea V.M.P (eds), Durable resistance to coffee leaf rust. 53-74. Universidade Federal de Viçosa, Brasil.

New Coffee (*Coffea* L.) Species from Cameroon Bring Original Characters for Breeding

F. ANTHONY[1], M. NOIROT[2], E. COUTURON[2], F. RICHARD[1], P. STOFFELEN[3]

[1]Institut de Recherche pour le Développement, BP 64501, 34394 Montpellier cedex 5, France
[2]Institut de Recherche pour le Développement, Université de la Réunion,
Campus du Chaudron, BP 172, 97492 Sainte Clotilde cedex, La Réunion
[3]National Botanic Garden of Belgium, Domein van Bouchout, 1860 Meise, Belgium

SUMMARY

Documentation on wild coffee (*Coffea* L.) native to Cameroon has considerably increased during the last ten years. Only five species were known until the early 1990s. Up to 15 species growing in the Cameroonian forests have been reported so far, which makes this region as rich in coffee species as the Kenya-Tanzania region. Several unknown coffee were collected in Cameroon by an ORSTOM (now IRD) team and identified using provisional names. *Coffea* sp. "Bakossi" and *Coffea* sp. "Moloundou" are presented here as new species and described as *C. charrieriana* and *C. anthonyi*, respectively. These species bring original characters such as the absence of caffeine in *C. charrieriana* beans and the self-compatibility of *C. anthonyi*. Moreover the genome of *C. anthonyi* is closely related to the maternal ancestor genome of the allotetraploid species *C. arabica*.

RÉSUMÉ

Les informations sur les caféiers sauvages endémiques du Cameroun ont considérablement augmenté au cours des dix dernières années. Seulement cinq espèces étaient connues jusqu'au début des années 90. Quinze espèces poussant des les forêts camerounaises ont été reportées maintenant, ce qui fait cette région aussi riche en espèces de caféier que la région Kenya-Tanzanie. Plusieurs caféiers non connus furent collectés au Cameroun par une équipe de l'ORSTOM (maintenant IRD) et identifiés en utilisant des noms provisoires. *Coffea* sp. "Bakossi" et *Coffea* sp. "Moloundou" sont présentés ici comme de nouvelles espèces et décrits respectivement comme *C. charrieriana* et *C. anthonyi.* Ces espèces possèdent des caractères originaux tels que l'absence de caféine dans les grains de *C. charrieriana* et l'auto-compatibilité de *C. anthonyi*. De plus, le génome de *C. anthonyi* est très proche du génome de l'ancêtre maternel de l'espèce allotétraploïde *C. arabica*.

INTRODUCTION

The number of coffee species has considerably increased since the creation of the genus *Coffea* L. in the 18th century with the only species known at that time, *C. arabica* L. Fifteen species were described at the end of the 19th century, but they were 64 new species added in the following century. A total of 103 *Coffea* species is now accepted (Davis and Stoffelen, 2006). The recent increase in the number of coffee species can be related to intensive explorations of African and Madagascan forests, which constitute the natural habitat of wild coffee, and to major revisions of herbarium samples from East Africa (Bridson and Verdcourt, 1988), Central Africa (Stoffelen, 1998), Madagascar (Davis and Rakotonasolo, 2000; 2001; 2001b; 2003) and the Mascarenes (Dulloo et al.).

Awareness of the lack of diversity conserved in the existing coffee genebanks made the FAO and French organizations (IRD ex-ORSTOM, CIRAD, MNHN) joint their efforts to collect coffee genetic resources in the last 40 years. IRD has organized and conducted collection missions in Ethiopia (Guillaumet and Hallé, 1978), Kenya (Berthaud et al., 1980), Tanzania (Anthony et al., 1987), Central Africa Republic (Berthaud and Guillaumet, 1978), Cameroon (Anthony et al., 1985), Congo (de Namur et al., 1987), Ivory Coast (Berthaud, 1986) and Guinea (Le Pierrès et al., 1989). A total of 7800 wild coffee genotypes belonging to 20 species were collected and introduced in a field genebank in Ivory Coast (Anthony, 1992). New species were collected in East Africa, then described as *C. costatifructa* Bridson, *C. fadenii* Bridson, *C. mufindiensis* Bridson, *C. pocsii* Bridson, *C. pseudozanguebariae* Bridson and *C. sessiliflora* Bridson. In Central Africa, some coffee trees could not be identified using the botanical classifications and flora mentioned above. Here we present two new species from Cameroon, previously named *Coffea sp*. "Bakossi" and *Coffea sp*. "Moloundou", of which description has been submitted under the names *C. charrieriana* (Stoffelen et al., 2005) and *C. anthonyi* (Stoffelen et al., 2006).

DISCOVERY OF THE NEW SPECIES

The collecting mission of Cameroon took place from February 4th to March 3rd 1983 (Anthony et al., 1985). The collector team was composed of two Geneticists and a Botanist. About 5500 km were covered in the forest area of the country. *C. charrieriana* and *C. anthonyi* were discovered in the South West and East provinces, respectively (Figure 1). *C. charrieriana* was found in only one population of 17 individuals while *C. anthonyi* was collected in three populations. This species was also collected in Congo (de Namur et al., 1987), which has indicated that *C. anthonyi* is distributed in the boarder region of Cameroon and Congo. The new species were growing in primary rain forest at 160 m and 360 m a.s.l. for *C. charrieriana* and *C. anthonyi*, respectively.

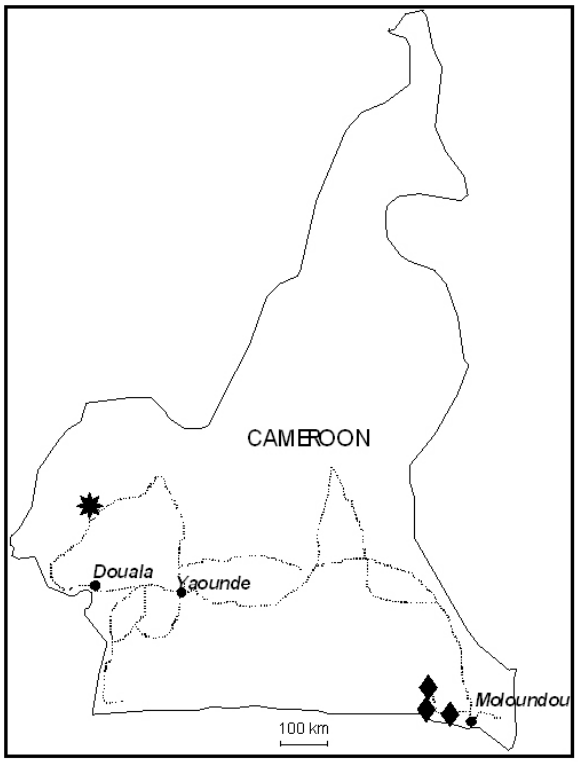


Figure 1. Travel in the forest area of Cameroon and collecting sites of *C. charrieriana* (✸) and *C. anthonyi* (◆).

Cuttings and seedlings were introduced in the field genebanks of Cameroon and Côte d'Ivoire. Herbarium samples were then prepared in Côte d'Ivoire and sent to the National Botanic Garden of Belgium for identification. After morphological study of those herbarium samples and re-examination of *Coffea* samples from Meise, Kew, Paris and Waganingen, it was confirmed that the collections were new species.

RECOGNITION OF THE NEW SPECIES

The new species display morphological characters which are typical for the genus *Coffea*: the corolla tube is short, the style is long and the anthers are exserted. The seeds are also typical for coffee, showing an asymmetrical invagination. But these coffee have no resemblance to species already described.

C. charrieriana is a small shrub of 1-1.5 m high. The most discriminating character is found in the seeds (Stoffelen et al., 2005): sclereids are lacking in the parenchymatic seed coat of *C. charrieriana* while they are present in all other *Coffea* species. *C. charrieriana* differs from all other Central African species by its small (5-7 x 2.2-3.5 cm) and thin (100-130 µm) leaves, with a rounded tip acumen (Figure 2a). Other discriminating characters are the length of its corolla tube and anthers, and the size of the corolla lobes and fruits. Only one small leaved species has been reported in Central Africa, *C. kapakata* (A.Chev.) Bridson, which has typical costae fruits and lobed calyces. Moreover *C. kapakata* is native to a savannah region from Angola.


2a


2b

Figure 2. Photographs of leaves of *C. charrieriana* (Figure 2a) and *C. anthonyi* (Figure 2b).

C. anthonyi is another small shrub up to 2 m high, with small leaves (5-9 x 2.2-4 cm) and an acumen with a rounded tip (Figure 2b). It can be easily distinguished from the other small leaved species from Central Africa (i.e. *C. kapakata* and *C. charrieriana*) because of its leaf shape, which is obovate or seldom elliptic (Stoffelen et al., 2006). Other discriminating characters can be found in flower and fruit morphology.

EVALUATION & CHARACTERIZATION OF THE NEW SPECIES

C. charrieriana is a caffeine-free species (Campa et al., 2005) with low contents in chlorogenic acids Campa et al., 2005), sucrose and trigonelline (Campa et al., 2004).This is the first caffeine-free species discovered in Central Africa. Previously, caffeine-free species were only known from several Malagasy species (Rakotomalala, 1992; Anthony et al., 1993) and one species from East Africa, *C. pseudozanguebariae* Bridson (Anthony et al., 1993;

Hamon et al., 1984). Another original biochemical attribute of *C. charrieriana* is its sucrose content (3.8% dmb) which is the lowest content reported in the genus *Coffea* (Campa et al., 2004).

C. anthonyi is a self-compatible species, closely related to the allotetraploid species *C. arabica*. Self-compatibility was suspected during the collecting mission because an individual was bearing a fruit containing two seeds, which has indicated a successful hybridization of both ovules. Two young seedlings were collected under the tree, but no other adult tree could be found, suggesting self-pollination. Self-compatibility was then confirmed in the field genebank of Côte d'Ivoire, making controlled self-pollination (Anthony, 1992). The fruit set (29.7%) was similar to the fruit set of open-pollination in self-incompatible species. Except the allotetraploid species *C. arabica*, only one self-compatible species has been reported in the genus *Coffea*, *C. heterocalyx* Stoff., also native to Cameroon (Louarn, 1992).

Phylogenetic analyses grouped *C. anthonyi* with the East African species *C. eugenioides* S.Moore (Lashermes et al., 1996; Lashermes et al., 1997; Cros et al., 1998). No difference was detected in the chloroplastic sequences of both species (Cros et al., 1998). Moreover the two species were classified with the allotetraploid species *C. arabica*. Since the chloroplastic genome has a maternal inheritance in coffee (Lashermes et al., 1996), *C. anthonyi* appears closely related to the maternal ancestor of *C. arabica*. Therefore *C. anthonyi* could represent a key species for understanding the origin of the cultivated species *C. arabica*.

DISCUSSION & CONCLUSION

The discovery of new species in Cameroon confirms that this region is a centre of diversity of importance for coffee. Only five species (i.e. *C. brevipes* Hiern, *C. canephora* Pierre ex A.Froehner, *C. congensis* A.Froehner, *C. liberica* Bull. ex Hiern, *C. mayombensis* A.Chev.) were known until the early 1990s. Up to 15 species growing in the Cameroonian forests have been reported so far, which makes this region as rich in coffee species as the Kenya-Tanzania region.

The discovery of new coffee species indicates that the inventory of coffee genetic resources is not complete. Nineteen new species have been described since 2000, principally from Cameroon and Madagascar (Davis and Rakotonasolo, 2000; 2001; 2001b; 2003; Davis, 2001; Cheek et al., 2002; Davis and Mvungi, 2004; Sonké and Stoffelen, 2004). Global climatic changes increase the need of setting up new collecting missions of living material. This also points out the need of implementing conservation programs for long-term preservation of coffee genetic resources.

REFERENCES

Anthony F (1992). Les ressources génétiques des caféiers: collecte, gestion d'un conservatoire et évaluation de la diversité génétique. Collection Travaux & Documents Microfichés n° 81, ORSTOM (now IRD), Paris.

Anthony F, Berthaud J, Guillaumet J-L, Lourd M (1987). Collecting wild *Coffea* species in Kenya and Tanzania. Plant Genetic Resources Newsletter 69: 23-29.

Anthony F, Clifford MN, Noirot M (1993). Biochemical diversity in the genus *Coffea*: chlorogenic acids, caffeine and mozambioside contents. Genet. Res. Crop Evol. 40: 61-70.

Anthony F, Couturon E, Namur (de) C (1985). Résultats d'une mission de prospection effectuée par l'ORSTOM en 1983. Proc. 11th international scientific colloquium on coffee, Lomé, Togo. ASIC, pp. 495-505.

Berthaud J (1986). Les ressources génétiques pour l'amélioration génétique des caféiers africains diploïdes. Collection Travaux & Documents n° 188, ORSTOM (now IRD), Paris.

Berthaud J, Guillaumet J-L (1978). Les caféiers sauvages en Centrafrique : résultats d'une mission de prospection (janvier-février 1975). Café, Cacao, Thé 3: 171-186.

Berthaud J, Guillaumet J-L, Le Pierrès D, Lourd M (1980). Les caféiers sauvages du Kenya : prospection et mise en culture. Café, Cacao, Thé 24: 101-112.

Bridson D, Verdcourt B (1988). *Coffea*. In: Polhill R. M. (ed.) Flora of Tropical East Africa. Rubiaceae (Part 2). A. A. Balkema: Rotterdam, pp. 703-727.

Campa C, Ballester J-F, Doulbeau S, Dussert S, Hamon S, Noirot M (2004). Trigonelline and sucrose diversity in wild *Coffea* species. Food Chemistry 88: 39-43.

Campa C, Doulbeau S, Dussert S, Hamon S, Noirot M (2005). Diversity in bean caffeine content among wild *Coffea* species: evidence for a discontinuous distribution. Food Chem. 91: 633-637.

Campa C, Doulbeau S, Dussert S, Hamon S, Noirot M (2005). Qualitative relationship between caffeine and chlorogenic acid contents among wild *Coffea* species. Food Chem. 93: 135-139.

Cheek M, Csiba L, Bridson D (2002). A new species of *Coffea* (Rubiaceae) from western Cameroon. Kew Bull. 57: 675-680.

Cros J, Combes M-C, Trouslot P, Anthony F, Hamon S, Charrier A, Lashermes P (1998). Phylogenetic relationships of *Coffea* species: new evidence based on the chloroplast DNA variation analysis. Mol. Phylogenet. Evol. 9: 109-117.

Davis A, Stoffelen P (2006). A new taxonomy conspectus of the genus *Coffea* L. Proc. 21st international conference on coffee science, Montpellier, France. ASIC, in press.

Davis AP (2001). Two new species of *Coffea* L. (Rubiaceae) from eastern Madagascar. Kew Bull. 56: 479-489

Davis AP, Mvungi EF (2004). Two new endangered species of *Coffea* (Rubiaceae) from the Eastern Arc Mountains (Tanzania) and notes on associated conservation issues. Bot. J. Linn. Soc. 146: 237-245.

Davis AP, Rakotonasolo F (2000). Three new species of *Coffea* L. (Rubiaceae) from Madagascar. Kew Bull. 55: 405-416.

Davis AP, Rakotonasolo F (2001). Two new species of *Coffea* L. (Rubiaceae) from northern Madagascar. Adansonia 23: 337-345.

Davis AP, Rakotonasolo F (2001b). Three new species of *Coffea* L. (Rubiaceae) from NE Madagascar. Adansonia 23: 137-146.

Davis AP, Rakotonasolo F (2003). New species of *Coffea* L. (Rubiaceae) from Madagascar. Bot. J. Linn Soc. 142: 111-118.

Dulloo et al

Guillaumet J-L, Hallé F (1978). Echantillonnage du matériel récolté en Ethiopie. Bull. IFCC 14: 13-18.

Hamon S, Anthony F, Le Pierrès D (1984). La variabilité génétique des caféiers spontanés de la section *Mozambicoffea* A. Chev. 1). Précisions sur deux espèces affines : *Coffea pseudozanguebariae* Bridson et *C. sp. A* Bridson. Adansonia 2: 207-223.

Lashermes P, Combes M-C, Trouslot P, Charrier A (1997). Phylogenetic relationships of coffee tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. Theor. Appl. Genet. 94: 947-955.

Lashermes P, Cros J, Combes M-C, Trouslot P, Anthony F, Hamon S, Charrier A (1996). Inheritance and restriction fragment length polymorphism of chloroplast DNA in the genus *Coffea* L. Theor. Appl. Genet. 93: 626-632.

Le Pierrès D, Charmetant P, Yapo A, Leroy T, Couturon E, Bontems S, Tehe H (1989). Les caféiers sauvages de Côte d'Ivoire et de Guinée : bilan des missions de prospection effectuées de 1984 à 1987. Proc. 13th international scientific colloquium on coffee, Paipa, Colombia. ASIC, pp. 420-428.

Louarn J (1992). La fertilité des hybrides interspécifiques et les relations génomiques entre caféiers diploïdes d'origine africaine (genre *Coffea* sous-genre *Coffea*). Doctoral thesis, Université de Paris-Sud, Orsay, France.

Namur (de) C, Couturon E, Sita P, Anthony F (1987). Résultats d'une mission de prospection des caféiers sauvages du Congo. Proc. 12th international scientific colloquium on coffee, Montreux, Switzerland. ASIC, pp. 397-404.

Rakotomalala JJR (1992). Diversité biochimique des caféiers: analyse des acides hydroxycinnamiques, bases puriques et diterpènes glycosidiques. Particularités des caféiers sauvages de la region malgache (*Mascarocoffea* Chev.). Doctoral thesis, Université de Montpellier II, Montpellier, France.

Sonké B, Stoffelen P (2004). Une nouvelle espèce de *Coffea* L. (Rubiaceae) du Cameroon, avec quelques notes sur ses affinités avec les espèces voisines. Adansonia 26: 153-160.

Stoffelen P (1998). *Coffea* and *Psilanthus* (Rubiaceae) in tropical Africa: a systematic and palynological study, including a revision of the West and Central African species. Doctoral thesis, Katholieke Universiteit Leuven, Leuven, Belgium.

Stoffelen P, Noirot M, Couturon E, Anthony F (2005). A new caffeine-free coffee species in the deep rain forest of Cameroon. Bot. J. Linn. Soc., submitted.

Stoffelen P, Noirot M, Couturon E, Anthony F (2006). A new self-compatible coffee species from Cameroon and Congo, closely related to the cultivated species *C. arabica*. Bot. J. Linn. Soc., submitted.

Phylogenetic Relationships in *Coffea* (Rubiaceae) Inferred from Molecular Sequence Data

A. P. DAVIS[1], O. MAURIN[1], M. CHESTER[1], E. F. MVUNGI[2] AND M. F. FAY[1]

[1]Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK
[2]Department of Botany, University of Dar es Salaam, P.O. Box 35060. Dar es Salaam, Tanzania

SUMMARY

The phylogenetic relationships of *Coffea* and the closely related genus *Psilanthus* are inferred using plastid sequence data from four plastid regions (*trnL-F* intron, *trnL-F* IGS, *rpl16* intron and *accD-psa1* IGS), and the ITS region of nuclear rDNA (ITS 1/5.8S/ITS 2). Our results show that *Coffea* plus *Psilanthus* forms a well-supported and easily characterized clade but that the monophyly of these genera are unresolved. In terms if the present classification of *Coffea*, *Coffea* subgen. *Baracoffea* is monophyletic but *Coffea* subgen. *Coffea* is paraphyletic. Well supported clades corresponding to phytogeographical regions, or having geographical coherence, are recovered in *Coffea* and *Psilanthus*. Low levels of sequence divergence do not allow complete resolution of relationships, most notably for the species of *Coffea* occurring in Madagascar. The origin of *C. arabica*, by recent hybridization between *C. canephora* and *C. eugenioides,* is supported.

INTRODUCTION

The genus *Coffea* occurs naturally in tropical Africa, Madagascar (including the Comoros) and the Mascarenes (Mauritius and Reunion) and comprises 103 species (Davis et al., in press). Most species are restricted to humid evergreen forests, although some are found in seasonally dry deciduous forest and/or bush-land. The most recent classifications of *Coffea* (Bridson, 1988a; 1988b) divide the genus into two subgenera: *Coffea* subgen. *Coffea* (95 spp.) and *Coffea* subgen. *Baracoffea* (eight spp.). *Coffea* subgen. *Coffea* occurs throughout the distribution range of the genus, whereas *Coffea* subgen. *Baracoffea* is only found in the seasonally dry forest and scrubland of western Madagascar (Davis et al., 2005).

It is now well established that *Psilanthus* is the closest relative of *Coffea* (Davis et al., 2005; in press). *Psilanthus* comprises 22 species and occurs in tropical Africa, southern and SE Asia, and tropical northern Australia (Davis, 2003).

Coffea has been the focus of several recent molecular systematic studies, using data from various sources, including random amplified polymorphic DNA (RAPD) (Lashermes, 1993), sequences from plastid DNA (Cros, 1994; Cros et al., 1998; Lashermes et al., 1996), and ITS sequences of nuclear ribosomal DNA (Lashermes et al., 1997). At the species level and above, the studies of Lashermes et al. (1997) and Cros et al. (1998) provide the most useful data so far.

According to Lashermes et al. (1997) (see 953, Figure 4), on the basis of ITS2 data, *Coffea* falls into four main geographical groups: Madagascar (BP 53), East Africa (BP 22), Central Africa (BP 67), and West and Central Africa (an unresolved group). Using sequence data from the *trnL-trnF* intergenic spacer region, Cros et al. (1998) separated *Coffea* into the same

four groups but with better support values, i.e. Madagascar (BP 82), East Africa (BP 64), Central Africa (BP 100) and West and Central Africa (BP 41). Cros et al. (1998) noted conspicuous incongruence for the systematic position of *C. arabica*, suggesting a hybrid origin involving two separate lineages within *Coffea*. This incongruity was further resolved by two further studies. Raina et al. (1998) used genomic in situ hybridization (GISH) and fluorescent in situ hybridization (FISH) to study the genome organization and evolution of *C. arabica*, and they concluded that *C. congensis* and *C. eugenioides* are the likely diploid progenitors. Using restriction fragment length polymorphism (RFLP) markers in combination with GISH data, Lashermes et al. (1999) suggested that *C. arabica* is an amphidiploid formed by hybridisation between *C. eugenioides* and *C. canephora*, or ecotypes related to these diploid species. The results of Raina et al. (1998) are not incompatible with the study by Lashermes et al. (1999) as *C. congensis* and *C. canephora* are genetically very similar (Lashermes et al., 1997; Prakash et al., 2005). One other incongruity identified by Cros et al. (1998) was the placement of the West Africa species *C. stenophylla*: for *trnL-trnF* it is sister to *C. humilis* and with the Central African and East African and Madagascan species (BP 35) and for ITS2 (Lashermes et al., 1997) with species from West and Central Africa (BP 4). According to Cros et al. (1998) this discrepancy was the result of interspecies transfer of plastid DNA mediated by hybridization.

A further important finding of Lashermes et al. (1997) and Cros et al. (1998) was the limited sequence divergence between *Coffea* and *Psilanthus*, inferring that these two genera are very closely related and that they should be considered as representing a single genus (i.e. *Coffea*).

Despite recent advances in the molecular systematics of *Coffea* and closely related genera (Davis et al., in press), the phylogenetic relationships within the genus and between *Coffea* and *Psilanthus* are still imperfectly known. In this contribution we present an overview of our study on *Coffea* and *Psilanthus* (Maurin et al., sibmitted) which uses plastid sequence data from the *trnL-F* intron, *trnL-F* IGS, *rpl16* intron and *accD-psa1* IGS, and ITS1/5.8S/ITS2 sequences of nuclear ribosomal DNA. Better sampling of *Coffea* has been made possible due to recent collecting activities in Cameroon, Tanzania, Madagascar and the Mascarenes. In the study reported on here we analysed 76 samples of *Coffea*, representing 73 species, and seven species samples of *Psilanthus*. This represents by far the most complete and comprehensive molecular phylogenetic study of *Coffea* and *Psilanthus* to date.

MATERIALS AND METHODS

The samples used in this study, together with voucher information and GenBank accession details are available from the authors on request, and will be publicly available at a later date (Maurin et al., sibmitted). The methodology for DNA extraction, amplification, sequence alignment and cladistic analysis using parsimony are given in Davis et al. (in press) and Maurin et al. (submitted). Support for trees is given using Bootstrap percentages (BP) and are described as strongly/well-supported (85-100%), moderate (75-84%), or low (50-74%).

RESULTS

The single plastid analyses, combined plastid and ITS analysis will be reported in detail elsewhere by us (Maurin et al., sibmitted). Analyses from these data sources are largely congruent, except for the placement of *C. arabica* and a clade of three species (*C. humilis*, *C. stenophylla*, *C. togoensis*) from the Upper Guinea region. The position of *C. arabica* was the only strongly supported point of incongruence, and this species was removed from the combined (plastid and ITS) analysis, owing to its hybrid origin. The incongruent (plastid vs. ITS) position of the Upper Guinea species is not strongly supported and was retained in the

combined analysis. The results and discussion presented here mainly concern *Coffea*; further discussion of *Psilanthus* will be elsewhere (Davis et al., in press; Maurin et al., sibmitted).

Coffea and *Psilanthus* form a well-supported group (BP 100; Figure 1), but the relationship between *Coffea* and *Psilanthus* is unresolved. Several well-supported groupings within *Coffea* are recovered, including an Upper Guinea (UG) clade (BP 100), a Lower Guinea Congolian (LG/C) clade (BP 86), an East-Central African (E-CA) clade (BP 100), a Mascarene (MAS) clade (BP 98), and *Coffea* subgen. *Baracoffea* (BP 100). An east African-Indian Ocean (EA-IO) clade (BP 52) and an East African (EA) clade (BP 52) are also recovered but are only weakly supported. There are a number of strongly supported groupings of East African species, several well-supported species pairs. Asian *Psilanthus* (BP 100), African *Psilanthus* (BP 99) and *Psilanthus* subgen. *Psilanthus* (BP 96) are also well-supported. Apart from *Coffea* subgen. *Baracoffea* and some small groupings of species, the relationships between most Madagascan species are unresolved.

DISCUSSION

The well-supported monophyly of *Coffea* plus *Psilanthus* is in agreement with other molecular studies (Davis et al., in press; Andreasen and Bremer, 2000) and is consistent with the presence of a of a unique carpel morphology for these genera (Robbrecht and Puff, 1986), i.e. the presence of 'coffee beans' (Robbrecht and Puff, 1986). The robust morphological and molecular support for *Coffea* plus *Psilanthus*, versus low sequence diversity between these genera (Maurin et al., sibmitted), may be taken as evidence for merging these genera (Davis et al., in press; Cros et al., 1998; Lashermes et al., 1997) into a single genus. These data concur with the morphological study by Davis et al. (2005), which shows that there are few morphological characters separating *Coffea* and *Psilanthus*. It should be said, however, that the systematic relationships between *Coffea* and *Psilanthus* are still largely unresolved and any decision regarding the taxonomy of these genera must wait until further data is available.

Our study clearly shows that *Coffea* subgen. *Baracoffea* is nested within *Coffea* subgen. *Coffea* (Figure 1), and forms a well-supported group restricted to the drylands of western Madagascar (Davis et al., 2005). *Coffea rhamnifolia*, which occurs in NE Kenya and SE Somalia, can no longer be considered a member of *Coffea* subgen. *Baracoffea* (Leroy, 1982). *Coffea rhamnifolia* is a morphologically unique species of uncertain taxonomic position (Davis et al., 2005). Our combined molecular analysis places *C. rhamnifolia* with two Indian species of *Psilanthus* (BP 57; Figure 1), although further data are still required for this species.

The systematic position of the well-supported Upper Guinea (UG) clade, which includes *C. humilis*, *C. stenophylla* and *C. togoensis*, is different in the plastid and ITS analyses. The ITS analysis puts the UG clade sister to a lineage containing species from the Lower Guinea/Congo region, and the combined plastid analysis sister to a group of species from East and East-Central Africa (see Cros et al., 1998; based on *C. stenophylla*). The combined molecular analysis places the UG clade in approximately the same position as the combined plastid analysis (Fiure. 1), although this relationship is not well supported. It seems likely that the incongruence between nuclear and plastid data sets is due to (maternal) plastid genome transfer and lineage sorting, which may have predated speciation in the UG clade. The Upper Guinea region is separated from the Lower Guinea/Congo region by the Dahomey Interval (White, 1979), which is an extension of the woodland savannah of the Sahel to the Gulf of Guinea (Poorter et al., 2004) presently some 250 km wide (White, 1979), and this may have played a role in the isolation of the Upper Guinea and Lower Guinea/Congolian species groups.

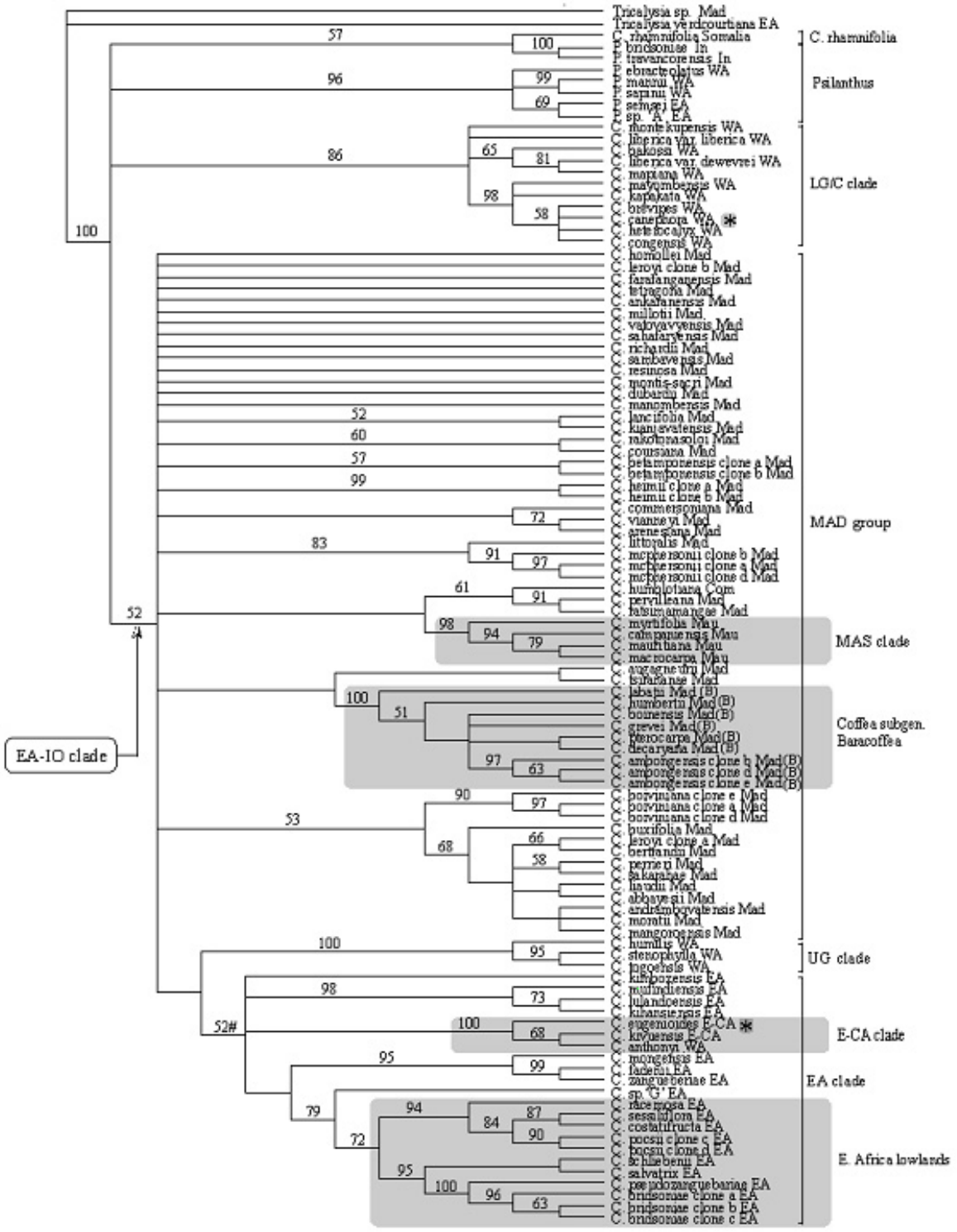


Figure 1. Strict consensus tree (of 12, 680 most parsimonious trees) generated from combined molecular (plastid and ITS) data. Boostrap values of > 50% are placed above the branches. Abbreviations (clades and groups): LG/C clade = Lower Guinea/Congolian clade; EA-IO clade = East Africa-Indian Ocean clade; UG clade = Upper Guinea clade; EA clade = East Africa clade; E-CA clade = East-Central Africa clade; MAS clade = Mascarene clade; MAD group = Madagascar group. Abbreviations (regions): EA = East Africa; E-CA = East Central Africa; Com = Comoros; In = India; Mad = Madagascar; Mau = Mauritius; WA = West Africa. Abbreviations (other): (B) = species belonging to *Coffea* subgen. *Baracoffea*. # = Indicates BP following removal of UG clade. * Denotes progenitor species of *C. arabica.*

The Lower Guinea-Congolian (LG/C) clade is a cohesive group of predominately lowland rainforest *Coffea* species, which is largely restricted to the Lower Guinea/Congolian region. It does, however, include the widespread *C. canephora* and *C. liberica*, which also occur in Upper Guinea region, and *C. kapakata* from Angola. In our combined analysis the LG/C clade is unresolved in relation to *Psilanthus* and *C. rhamnifolia*.

An East African-Indian Ocean (EA-IO) clade is consistently retrieved in our combined analysis but the support for this group is weak. The West African UG clade is placed within the EA-IO clade but this position may be due to (maternal) plastid genome transfer (see above). The separation of West/Central African *Coffea* species (UG and LG/C clades) and East African and the Indian Ocean species (EA-IO clade) would be expected given the geological history of Africa.

All species from East Africa and East-Central Africa are placed within the East Africa (EA) clade (Figure 1). There is < 50% bootstrap support for this group, although deleting the UG clade from our combined molecular-morphological analysis gives the EA clade a higher support value (BP 52). Within the EA clade there are several well-supported groups that have either geographical/ecological or morphological correspondence. The clade formed by *C. mufindiensis*, *C. lulandoensis* and *C. kihansiensis* represents a group of high altitude (800-2300 m) species from the Udzungwa Mountains, for example.

The East-Central Africa (E-CA) clade (*C. eugenioides*, *C. kivuensis* and *C. anthonyi* ined./*C.* sp. 'Mouloundou'), is placed in an unresolved position at the base of the EA clade. The distribution of *C. eugenioides* and *C. kivuensis* falls almost entirely within Lake Victoria Regional Mosaic (White, 1983), at altitudes normally well above 1000 m, whereas *C. anthonyi* ined. occurs in a few isolated locations at low to mid-altitude (350-650(-900) m) in SE Cameroon and NW Congo.

All Madagascan *Coffea* species (MAD group) are placed within the EA-IO clade, with species and species groups unresolved in relation to the African groups within this clade, due to low levels of sequence divergence. *Coffea* subgen. *Baracoffea* is the only well-supported Madagascan clade, apart from *C. pervilleana* and *C. ratsimamangae*, two closely allied species from northern Madagascar (Davis and Rakotonasolo, 2001a). It is noteworthy that the species of *Coffea* subgen. *Baracoffea*, which are restricted to the seasonally dry forests (including, spiny/xerophytic deciduous forest) of western Madagascar, are on a long branch (14) and are by far the most morphologically distinct group within *Coffea* (Davis et al., 2005), having evolved as a response to a distinctly seasonally dry environment.

All four *Coffea* species from the Mascarenes are placed within the EA-IO clade (Figure 1), and form a well-supported Mascarene (MAS) clade. The position of the MAS clade within the combined analysis, coupled with the relative proximity of the Mascarenes to Madagascar, infers an 'out of Madagascar' origin for the Mascarene species.

Our data support a recent hybrid origin for *C. arabica* (Cros et al., 1998; Lashermes et al., 1997; Raina et al., 1998; Lashermes et al., 1999). Our ITS analysis (Maurin et al., submitted) places *C. arabica* sister to *C. canephora* (BP 65), with only two base pair differences between these species. In our combined plastid analysis (Maurin et al., submitted) *C. arabica* is placed within the E-CA clade (see above). The relationship of *C. arabica* to other species in this clade (*C. eugenioides*, *C. kivuensis*, *C. anthonyi* ined.) is unresolved, although the sequences of *C. arabica* and *C. eugenioides* are identical. Our results concur with those of Lashermes et al. (1999), which infer a relatively recent origin for *C. arabica*.

Our results are not in accordance with the present subgeneric classification of *Coffea* and *Psilanthus* (see Introduction), owing to the paraphyly of *Coffea* subgen. *Coffea* and *Psilanthus* subgen. *Afrocoffea*. Disregarding the need to resolve the relationship between *Coffea* and *Psilanthus*, as discussed above, taxonomic changes are needed if the classification of these genera is to be constructed within a phylogenetic framework.

CONCLUSIONS

Despite relatively low levels of sequence variation we were able to recover a number of well-supported clades and consistently retrieved species groups (i.e. those in strict consensus trees) with geographical correspondence. Our data support an African origin for *Coffea* (Charrier, 1978; Leroy, 1980a; 1980b), although an origin in East Africa is unlikely (Leroy, 1982). The origin of the Madagascan species seems to follow the same pattern as *Begonia* (Plana et al., 2004): long distance dispersal (a single dispersal or a limited number of dispersal events) from mainland Africa followed by insular speciation and subsequent dispersal to offshore islands in the Indian Ocean (Comoros and the Mascarenes). A recent hybrid origin for *C. arabica*, with *C. canephora* and *C. eugenioides* as the progenitor species, is supported (Lashermes et al., 1999; Maurin et al., submitted).

ACKNOWLEDGMENTS

We would to thanks the following organizations and individuals for providing us with plant material for this study, including P. De Block, IRD-Montpellier, Y. Jaufeerally-Fakim, Q. Luke, F. Mbago, F. Rakotonasolo, B. Sonké and P. Stoffelen. In Madagascar fieldwork was undertaken under collaborative agreements between the Parc Botanique et Zoologique de Tsimbazaza (PBZT), the University of Antananarivo, the Association Nationale de Gestion des Aires Protegées (ANGAP), and the Royal Botanic Gardens, Kew. We would also like to acknowledge the Tanzania Commission for Science and Technology (COSTECH), the staff and Directors at the Université de Yaoundé, National Herbarium of Cameroon and Limbe Botanical and Zoological Gardens. E.F. Mvungi gratefully acknowledges the DANIDA-ENRECA biodiversity project, for their financial support This work would not have been possible without the generous support of Kraft Foods (UK).

REFERENCES

Andreasen, K., Bremer, B. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology, nuclear and chloroplast DNA data. Am. J. Bot. 87: 1731-1748.

Bridson, D.M. 1988a. *Coffea*. In: Polhill, R.M., Bridson, D.M., Verdcourt, B. (Eds), Flora of Tropical East Africa, Rubiaceae, 2. Balkema, Rotterdam/Brookfield, pp. 703-723.

Bridson, D.M. 1988b. Classification. In: Wrigley, G., Coffee. Longmans, New York, pp. 61-75.

Charrier, A. 1978. La structure génétique des caféiers spontanés de la région Malgache (*Mascarocoffea*). ORSTOM, Paris.

Cros, J. 1994. Implications phylogénétiques des variations de l'ADN chloroplastique chez les caféiers (genres *Coffea* L. et *Psilanthus* Hook.f.). PhD dissertation, Université Montpellier II, France.

Cros, J., Combes, M.C., Trouslot, P., Anthony, F., Hamon, S., Charrier, A. Lashermes, P., 1998. Phylogenetic analysis of chloroplast DNA variation in *Coffea* L. Mol. Phyl. Evol. 9: 109–117.

Davis, A.P. 2003. A new combination in *Psilanthus* (Rubiaceae) for Australia, and nomenclatural notes on *Paracoffea*. Novon 13: 182-184.

Davis, A.P., Bridson, D.M., Rakotonasolo, F. 2005. A reexamination of *Coffea* subgenus *Baracoffea* and comments on the morphology and classification of *Coffea* and *Psilanthus* (Rubiaceae-Coffeeae). In: Keating, R.C., Hollowell, V.C., Croat, T. (Eds), Festschrift for William G. D'Arcy: the Legacy of a Taxonomist (Monograph in Systematic Botany 104), MBG Press, Missouri, pp. 398-420.

Davis, A.P., Chester, M., Maurin, O., Fay, M. In Press. Searching for the relatives of *Coffea* (Rubiaceae, Ixoroideae): the circumscription and phylogeny of Coffeeae based on plastid sequence data and morphology. Am. J. Bot.

Davis, A.P., Govaerts, R., Bridson, D.M., Stoffelen, P. In press. An annotated checklist of the genus *Coffea* L. (Rubiaceae). Bot. J. Linn. Soc.

Davis, A.P., Rakotonasolo, F. 2001a. Three new species of *Coffea* L. (Rubiaceae) from NE Madagascar. Adansonia*,* sér. 3, 23: 137-146.

Lashermes, P., Combes, M.-C., Robert, J., Trouslot, P., D'Hont, A., Anthony, F., Charrier, A. 1999. Molecular characterisation and origin of the *Coffea arabica* L. genome. Mol. General Genet. 261: 259-266.

Lashermes, P., Combes, M.-C., Trouslot, P., Charrier, A. 1997. Phylogenetic relationships of coffee-tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. Theor. Appl. Genet. 94: 947-955.

Lashermes, P., Cros, J., Combes, M.-C., Trouslot, P., Anthony, F., Hamon, S., Charrier, A. 1996. Inheritance and restriction fragment length polymorphism of chloroplast DNA in the genus *Coffea* L. Theor. Appl. Genet. 93: 626-632.

Lashermes, P., Cros, J., Marmey, P., Charrier, A. 1993. Use of random amplified DNA markers to analyse genetic variability and relationships of *Coffea* species. Genet. Res. Crop Evol. 40: 91-99.

Leroy, J.-F. 1980a. Les grandes lignées de caféiers. Association Scientifique Internationale du Café (ASIC), 9th Colloque, 473-477.

Leroy, J.-F. 1980b. Evolution et taxogenèse chez les caféiers (*Coffea* L., *Psilanthus* Hook.f. et *Nostolachma* Durand). Hypothèse sur leur origine. C. R Acad. Sci. 291: 593-596.

Leroy, J.-F. 1982. L'origine kenyane du genre *Coffea* L. et la radiation des espèces à Madagascar. Association Scientifique Internationale du Café, 10th Colloque, 413-420.

Maurin, O., Davis, A.P., Chester, M.., Mvungi, E.F., Fay, M.F. [Submitted]. Phylogenetic relationships in *Coffea* (Rubiaceae) inferred from sequence data and morphology. Mol. Phyl. & Evol.

Plana, V., Gascoigne, A., Forrest, L.L., Harris, D., Pennington, R.T. 2004. Pleistocene and pre-Pleistocene *Begonia* speciation in Africa. Mol. Phyl. Evol. 31: 449-461.

Poorter, L., Bongers, F., 'Kouamé, F.N., Hawthorne, W.D. 2004. Biodiversity of West African Forests: and Ecologhical Atlas of Woody Plant Species. CABI Publishing, Oxon (UK)/Cambridge(USA).

Prakash, N.S., Combes, M.-C., Dussert, S., Naveen, S., Lashermes, P. 2005. Analysis of genetic diversity in Indian robusta coffee genepool (*Coffea canephora*) in comparison with a representative core collection using SSRs and AFLPs. Gen. Res. Crop Evol. 52: 333-343.

Raina, S.N., Mukai, Y. Yamamoto, M. 1998. In situ hybridization identifies the diploid progenitor species of *Coffea arabica* (Rubiaceae). Theor. Appl. Genet. 97: 1204-1209.

Robbrecht, E., Puff, C. 1986. A survey of the Gardenieae and related tribes (Rubiaceae). Bot. Jahrb, Syst. 108: 63-137.

White, F. 1979. The Guineo-Congolian Region and its relationships to other phytochoria. Bull. Jard. Bot. Nat. Belg. 49: 11-55.

White, F. 1983. The vegetation of Africa. A descriptive memoir to accompany the Unesco/AETFAT/UNSO vegetation map of Africa. Unesco, Paris.

Antioxidant Metabolism of Coffee Cell Suspension Cultures in Response to Cadmium

R. A. GOMES JR[1], C. MOLDES[1], F. S. DELITE[1], G. B. POMPEU[1], P. GRATÃO[1], P. MAZZAFERA[2], P. J. LEA[3], R. A. AZEVEDO[2]

[1]ESALQ-USP, Piracicaba, SP, Brazil
[2]UNICAMP, Campinas, SP, Brazil
[3]Univ. Lancaster, UK

SUMMARY

The antioxidant responses of *Coffea arabica* cell suspension cultures to Cd were investigated. Cd accumulated very rapidly in the cells and this accumulation was directly correlated $CdCl_2$ concentration in the medium. At 0.05 mM $CdCl_2$, growth was stimulated, but it was reduced at 0.5 mM $CdCl_2$. Cells became brown with Cd and an increase in lipid peroxidation was observed at 0.5 mM $CdCl_2$. Catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) activities increased with Cd. Ascorbate peroxidase (APX) in the cells was higher at 0.05 mM $CdCl_2$, but could not be detected at 0.5 mM $CdCl_2$ after 24h of growth, whilst guaiacol peroxidase (GOPX) did not show a clear response to Cd treatment. Activity staining in non-denaturing PAGE one CAT isoenzyme, nine SOD isoenzymes and four GR isoenzymes. The SOD isoenzymes were differently affected by Cd treatment and one GR isoenzyme specifically responded to Cd. The results suggest that the higher concentrations of $CdCl_2$ may lead to oxidative stress. The main response appears to be via the induction of SOD and CAT activities for the removal of active oxygen species (AOS), and by the induction of GR to ensure the availability of reduced glutathione for the synthesis of Cd-binding peptides, which may also be related to the inhibition of APX activity probably due to glutathione and ascorbate depletion.

INTRODUCTION

Naturally occurring amounts of Cd are normally low, however the concentration can be significantly increased by anthropogenic activities (Gratão et al., 2005). In general, Cd in plants reduces growth and may lead to cell death depending on the metal dose and time-length of exposure (Benavides et al., 2005).

Active oxygen species (AOS) such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and singlet oxygen (O_2^1), are normally produced by the cell but under adverse environmental factors the production of AOS may be enhanced. The major AOS scavenging mechanisms of plants include the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (GOPX) among others (Gratão et al., 2005). These enzymes have been shown to be affected by exposure to Cd in several plant species studied (Gratão et al., 2005). SODs dismutate $O_2^{\bullet -}$ to form H_2O_2 and O_2, whereas CAT, APX, GOPX are enzymes that catalyse the conversion of H_2O_2 to water and O_2. APX and GR are important components of the ascorbate-glutathione cycle responsible for the removal of H_2O_2 in different cellular compartments.

Sewage sludge has been tested as a fertilizer in coffee. Therefore, the aim of this work was to study the effect of $CdCl_2$ on the metabolism of coffee cells as a model.

MATERIAL AND METHODS

Cell suspensions and treatments

Friable callus were obtained from leaves of *C. arabica* variety Catuaí Vermelho (Neuenschwander and Baumann, 1992) and used to produce suspension cultures (100 rpm, dark, 25 °C) being transferred to new medium every week. Seven-day-old cells were transferred to media containing $NiCl_2$ at 0.05 and 0.5 mM and the cells were grown for 288 h being harvested at distinct periods during the growth cycle.

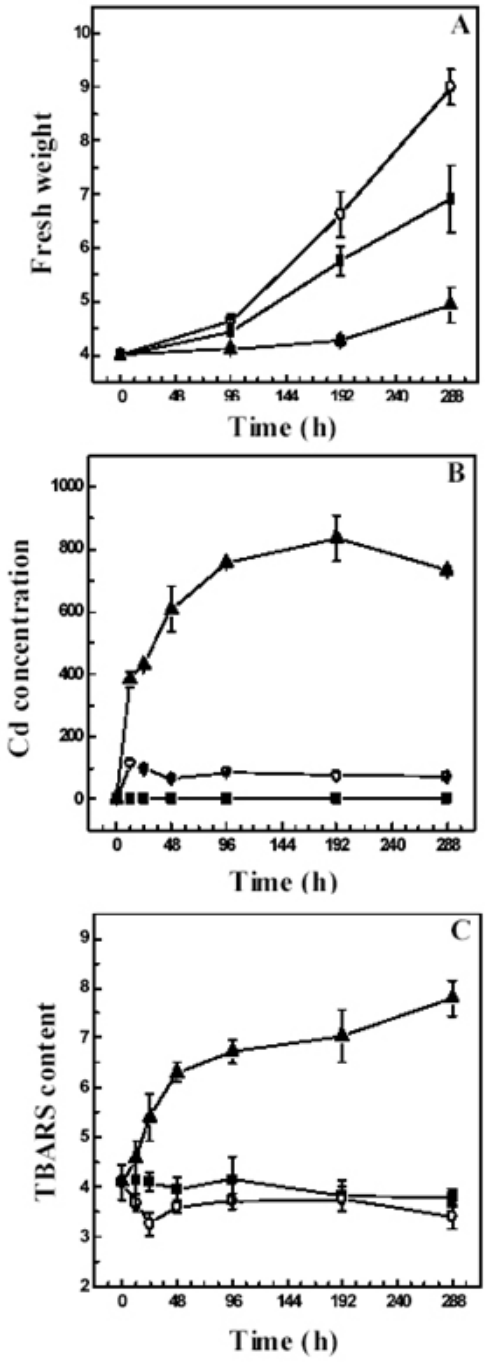


Figure 1. Cell growth (g fr. Wt) (A); Cd accumulation (µg g^{-1} dry wt) (B) and TBARS content (nmol g^{-1} fr. Wt) (C) in coffee cells grown for a 288 h period in three concentrations of $CdCl_2$. Control zero $CdCl_2$ (■), 0.05 mM $CdCl_2$ (○) and 0.5 mM CdCl2 (▲).

Analyses

Ni was analysed by energy disperse X-Ray fluorescence. Lipid peroxidation was determined by TBARS method. Enzymes were extracted with 100 mM KP buffer, pH 7.5, 1 mM EDTA, 3 mM DTT and 5% PVPP. After centrifugation the supernatant was used for CAT, GR, APX, GOPX and SOD analyses. CAT, GR, APX (Azevedo et al., 1998) and GOPX (Medici et al., 2004) were assayed by spectrophotometric methods. Additionally CAT, GR and SOD were assayed by activity staining on non denaturing PAGE. SOD (Azevedo et al., 01998), CAT (Woodbury et al., 1971) and GR (Nakano and Asada, 1981) were stained with already established protocols.

RESULTS

Cell growth, accumulation and induction of oxidative stress

A considerable stimulation of growth was observed at 0.05 mM $CdCl_2$, whereas an inhibition of growth was observed at 0.5 mM (Figure 1A). Cd entered the cells very rapidly (Figure 1B). Cells showed a brown coloration at the higher dosage of $CdCl_2$ and lipid peroxidation was shown by TBARS (Figure 1C). The lower Cd concentration did not affect the TBARS.

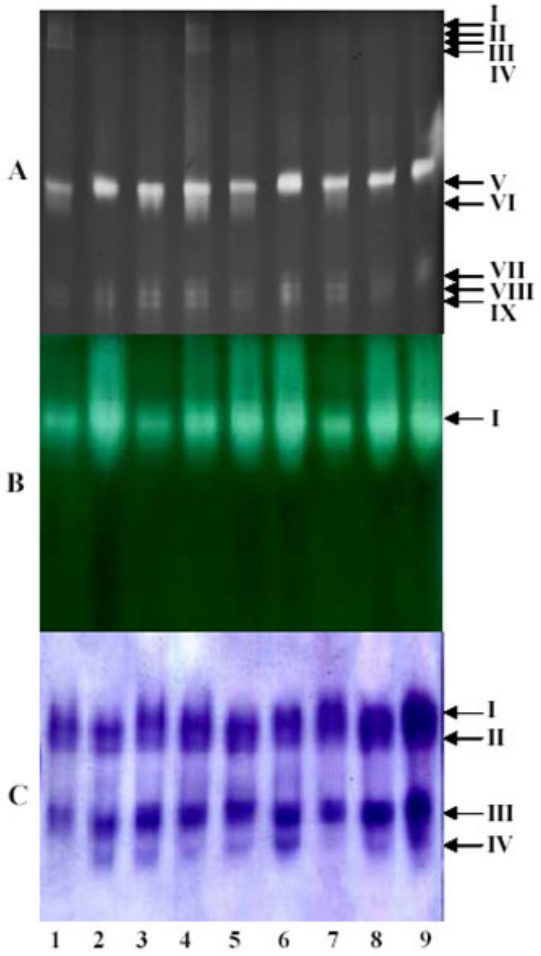


Figure 2. Activity staining for SOD (A), CAT (B) and GR (C) isolated from coffee cells. Lane 1, control (zero $CdCl_2$) after 96 h; lane 2, 12 h, lane 3, 24 h; lane 4, 192 h and lane 5, 288 h of growth in 0.05 mM $CDCl_2$; Lane 6, 12 h lane 7, 24 h, lane 8, 192 h and lane 9, 288 h of growth in 0.5 mM $CdCl_2$.

Enzyme activities

SOD activity staining revealed several isoenzymes in coffee cells (Figure 2A), including 6 Mn-SODs (bands I, II, III, IV, V and VI) and 3 Fe-SODs (bands VII, VIII and IX), but no Cu/Zn-SOD isoenzymes were detected (Figure 3). A more general increase in activity in the $CdCl_2$ treated coffee cells was observed, particularly for band V. The four minor Mn-SOD isoenzymes (bands I, II, III and IV) were inactivated by $CdCl_2$ at t he lower concentration. Fe-SODs isoenzymes VII, VIII and IX were not affected by Cd.

CAT activity staining (Figure 2B) revealed the presence of only one CAT isoe nzyme, which increased in both Cd treatments, exhibiting an almost identical pattern of activity variation to that observed with the spectrophotom eter assay (Figure 4).*GR activity* staining revealed at least four isoenzymes (bands I, II, III and IV) (Figure 2C) and all exhibited increases in intensity in response to Cd treatment, but one (band IV) appeared to responds more specifically to Cd. The total activity of GR increased with Cd treatment and time of exposure (Figure 4B). After an initial fall, *APX activity* increased in coffee cells subjected to 0.05 mM $CdCl_2$ and reached a 3-fold increase after 288 h of treatment (Figure 4C). On the other hand, APX activity in the cells at 0.5 mM Cd was not detectable throughout the experiment (Figure 4C). *GOPX* exhibited erratic variations during the first 96 h of $CdCl_2$ exposure, but after that the lower Cd concentration induced a steady increase in enzyme activity (Figure 4D). In cells subjected to the higher Cd concentration, the GOPX activity followed the trend of the control cells.

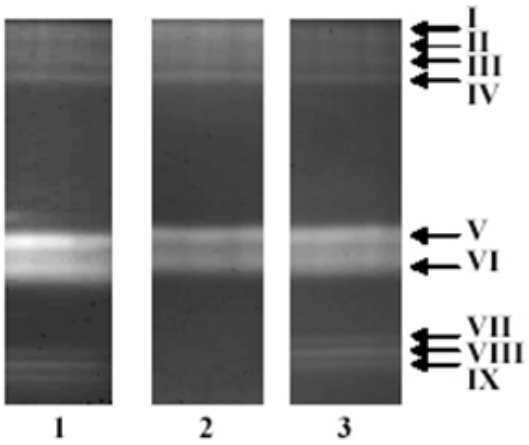


Figure 3. Coffee cell extract was loaded onto the gel and stained for individual SOD isoenzymes. Lane 1, control SOD activity; lane 2, plus 5 mM hydrogen peroxide and lane 3, plus 2 mM potassium cyanide.

DISCUSSION

Cd can enter coffee cells very quickly and accumulate to high concentrations and although final destination of the metal has not been determined, it is known that Cd can be complexed by phytochelatins and sequestered in the vacuole (Wójcik and Tukiendorf, 2005).

In plants, Cd can cause inhibition of shoot and root growth as observed for several species tested (Vitória et al., 2001) but a number of reports have demonstrated that Cd can either stimulate or inhibit the growth of *in vitro* cell cultures (Fornazier, 2002). Cells treated with Cd developed a brown colour and showed an enhancement in TBARS at the higher Cd concentration tested indicating the occurrence of lipid peroxidation and therefore, of oxidative stress, as shown by other plants (Sandalio, 2001).

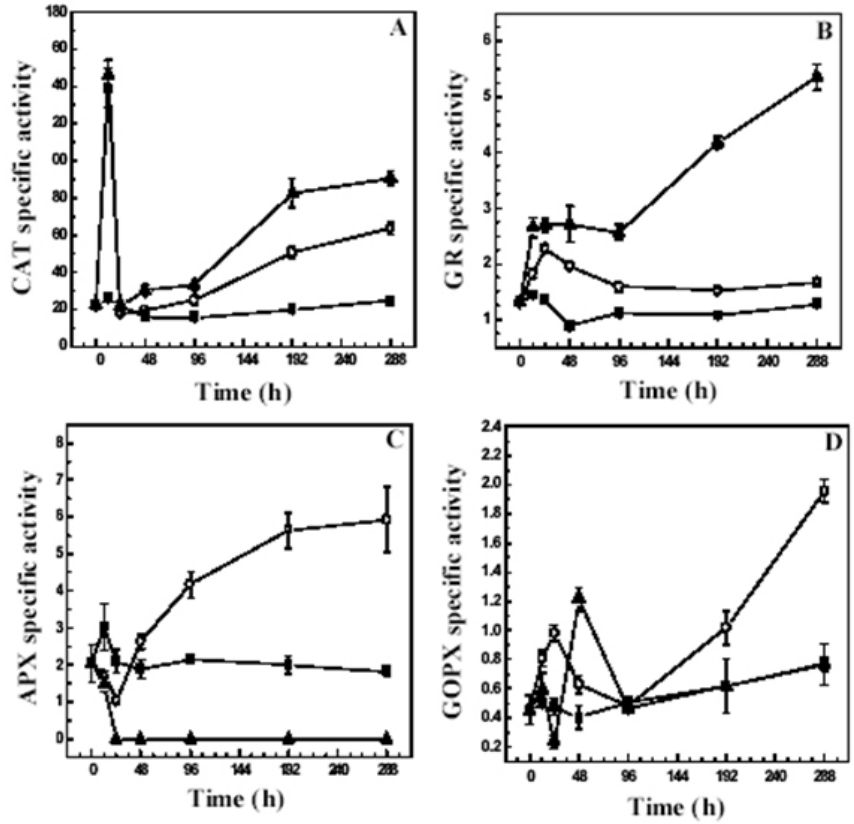


Figure 4. Specific activity of CAT (mmol min^{-1} mg^{-1} prot (A); GR (mmol min^{-1} mg^{-1} prot) (B); APX (mmol min^{-1} mg^{-1} prot) (C) and GOPX (u mg^{-1} prot) (D) in coffee cells grown for a 288 h period in three concentration of $CdCl_2$. Control zero $CdCl_2$ (■), 0.05 mM $CdCl_2$ (○) and 0.5 mM $CdCl_2$ (▲).

Cd is not a redox metal, a nd therefore cannot catalyse Fenton-type reactions yielding AOS. As the coffee cells were grown in dark in this experiment, it is probable that the Cd induced oxidative stress was due to mitochondrial activity, which produces more AOS if the electron transport is affected.

Mn-SOD is located in the mitochondria and peroxisomes, Fe-SOD is associated with the chloroplasts and the abundant Cu/Zn-SODs are located in the cytosol, chloroplasts and peroxisomes (Gratão et al., 2005). Two major Mn-SOD bands (V and VI) were detectable in PAGE gels. The band V was responsible for the majority of the total increase in the Cd treated coffee cells. Fe-SOD isoenzymes did not vary and are likely to be associated with plastids.

In coffee cells, $CdCl_2$ induced a considerable increase in CAT activity at both concentrations tested. An elevation of CAT activity has been associated with Cd toxicity in some plant species (Vitória et al., 2001; Ferreira et al., 2002). Only one CAT isoenzyme appears to accounted for all CAT activity detected in the suspension culture even though plants have often shown to contain a varying number of CAT isoenzymes.

GR activity was also shown to increase considerably with $CdCl_2$ treatment. The majority of the studies determining the response of GR to Cd exposure have shown that GR activity increases (Vitória et al., 2001; Ferreira et al., 2002). Band IV showed the greatest increase induced by Cd. The coffee GR activity detected is more likely to be due to a cytosolic GR isoenzyme since the cell cultures were maintained in the dark, since most of the GR activity detected in plants is localized in the chloroplasts.

In coffee cells, the activity of APX increased at the lower $CdCl_2$ concentration, but was severely inhibited by the higher $CdCl_2$ concentration tested. Although the finding appears strange, it has been observed previously, when low-concentration Cd treatments led to an increase in APX, but the activity was reduced at higher concentrations of Cd (Balestrasse et al., 2001; Zhang et al., 2003). The reduction in APX activity at the higher $CdCl_2$ concentration may be due to GSH depletion and a subsequent reduction in the ascorbate-glutathione cycle.

Cd treatment has been shown to be able to increased or decrease GOPX activity in plants (Balestrasse et al., 2001). In coffee cells GOPX did not show a clear response to $CdCl_2$, when concentration and time of the experiment were considered, suggesting that in coffee cells, GOPX is not directly involved in the response mechanism to Cd, in a similar way to that reported for *A. thaliana* (Cho and Seo, 2005).

In conclusion, the lower Cd concentration tested did not induce oxidative stress according to cell growth and TBARS content, even though some alterations in enzyme activities were observed. The Cd oxidative stress tolerance may be due to the rapid and significant increase of the activities of some of the major antioxidant enzymes APX, CAT, SOD and GR. The higher Cd concentration appeared to have induced AOS production and established some level of oxidative stress, since the activities of CAT, SOD and GR were increased.

REFERENCES

Azevedo RA et al. (1998) Physiol. Plant 104, 280-292.

Balestrasse KB et al. (2001) Aust. J. Plant Physiol. 28, 497–504.

Benavides MP (2005) Braz. J. Plant Physiol. 17, 21-34.

Cho U, Seo N (2005) Plant Sci. 168, 113-120.

Ferreira RR et al. (2002) J. Plant Nutr. 25, 327-342.

Fornazier RF (2002) Plant Cell Tiss. Org. Cult. 71, 125-131.

Gratão PL et al. (2005) Braz. J. Plant Physiol. 17, 53-64.

Medici LO et al. (2004) Funct. Plant Biol. 31, 1-9.

Nakano Y, Asada K (1981) Plant Cell Physiol. 22, 867-880.

Neuenschwander B, Baumann TW (1992) Plant Cell Rep. 10, 608-612.

Sandalio LM (2001) J. Exp. Bot. 52, 2115-2126.

Vitória et al. (2001) Phytochemistry 57, 701-710.

Wójcik M, Tukiendorf A (2005) Biol. Plant. 49, 237-245.

Woodbury W et al. (1971) Anal. Biochem. 44, 301.

Zhang FQ et al. (2003) J. Plant Nutr. 26, 1779-1788.

Synchronization of Coffee Somatic Embryos using Abscisic Acid (ABA)

J. W. KAHIA[1] , S. H MANTELL[2] AND E. W. KAHANGI[3]

[1]Coffee Research Foundation, P.O.BOX 4, Ruiru Kenya. E-mail:jakahi2002@yahoo.co.uk
[2]Abrahamsang 37045 Fagelmara, Sweden. E-Mail address sinclair.mantell@nakhlatec.se
[3]Jomo Kenyatta University Of Science And Technology - P.O.BOX 6200, Nairobi, Kenya
E-mail: esterkahangi@yahoo.com

SUMMARY

Breeding work at the Coffee Research Foundation Ruiru, Kenya has developed a new coffee Cultivar that is resistant to the two major diseases Coffee Berry Diseases (CBD) caused by *Collectotrichum kahawae* sp. Nov., and Coffee Leaf Rust (CLR) caused by *Hemilea vastatrix* B. et. Br. The propagation of the new cultivar is by hand cross-pollination, which is time consuming. Recently the Coffee Research station has developed a tissue culture protocol (somatic embryogenesis) for propagating the new variety. In somatic embryo system asynchrony development of embryos is evident. Of the various growth regulators tested for controlling precocious development of somatic embryos *in vitro*, ABA has been found to be the best. During the study, when globular embryos were subjected to ABA 1-10 µM most of the embryos were found to be more mature (cotyledonary Stage).

INTRODUCTION

Somatic embryogenesis has the potential for rapid and efficient clonal propagation of plants. The yield and quality of somatic embryos depend on the culture media. Somatic embryos regenerated from Ruiru 11 leaf explants showed asynchronous development with globular-stage, heart-stage, torpedo-stage and cotyledonary-stage embryos being present in the same culture (Kahia, 1999). Cotyledonary-stage are the most desirable and therefore this experiment was conducted to improve the coffee regeneration system such that high numbers of high quality cotyledonary-stage somatic embryos could be produced in preference to mixtures of embryos. Asynchronous development is often the key constraint to commercialization of somatic embryogenesis and its application to production of synthetic seeds on a large scale. Interestingly, such embryo abnormalities are usually absent or only occur at extremely low frequencies in natural zygotic embryogenesis. Synchronisation of somatic embryos has been successful using ABA in caraway (Ammirato, 1977), alfalfa (Senaratna et al., 1989), celery (Nadel et al., 1990) and oilseed rape (Senaratna et al., 1991). Although ABA has been reported to inhibit the initiation of somatic embryos (Tisserat and Murashige, 1977), it has been found to be essential for the normal growth of somatic embryos and only in its presence do they resemble zygotic embryos in their development and structure (George, 1993).

Manipulation of endogenous and/or exogenous ABA levels increases frequencies of embryos that reached maturity and thereby facilitate the handling of large numbers of somatic embryos, which are required for mass propagation (Ammirato, 1987).

MATERIALS AND METHODS

Leaf explants from a six-month old seedling growing in Wye College, UK greenhouses were sterilized using 25% commercial bleach for 20 min. and rinsed four times in sterile distilled water. They were cultured in Murashige and Skoog (MS) media supplemented with Thidiazuron (TDZ) 0.5 µM, 100mg/l Meso inositol, 3% sucrose (Induction media) for 14 days. After 14 days the leaf explants were transferred to a plain MS media for another one month when globular embryos were regenerated. The globular embryos were transferred to 150 ml Erlenmeyer flasks containing 50 ml of plain MS liquid media. These were incubated in darkness at 25 °C on a gyratory shaker at 100rpm. After one month they were mechanically sorted by sieving through a metal sieve of pore size 250 µm. They were cultured in Petri dishes each containing 50 mg of embryos on 10 ml medium semi solid Modified Linsmaeir and Skoog media supplemented with different concentrations of ABA. The ABA was tested at concentrations of 0, 1, 5, 10.20, 30 and 40 µM. Each treatment had five Petri dishes and was replicated four times. Records were taken after one month of the different stages of somatic embryo development.

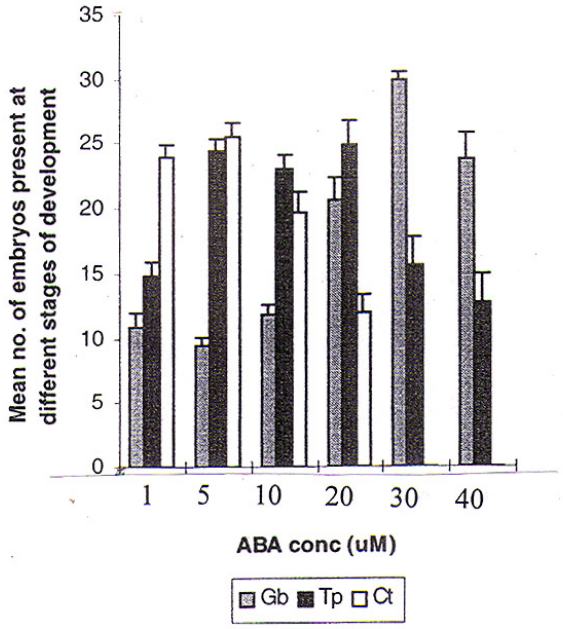


Figure 1. The effect of different concentrations of ABA on developmental stages of Ruiru 11 somatic embryos. Gb = Globular; Tp = Torpedo and Ct = Cotyledonary stage embryos.

RESULTS

Plate 1-4 shows the different stages of development of somatic embryos observed in a single culture. Increasing the concentration of ABA from 1-10 µM increased the number of embryos in the cotyledonary stage although the increase was not significantly different. ABA at 10 µM gave the highest mean number (25) of cotyledonary stage embryos (Figure 1) although it was not significantly different at p = 0.005 from ABA at 1 µM. Embryos cultured on media devoid of ABA did not develop further and eventually turned brown and died. The globular embryos followed the normal pattern of somatic embryo development and gave raise to both torpedo and early cotyledonary-stage in an apparently highly synchronized manner. The higher concentrations of ABA tested (30-40 µM) did not support development beyond the torpedo-stage. It was also observed that the higher concentrations of ABA led to browning of the embryos.

DISCUSSION

When globular embryos were cultured on medium supplemented with ABA (1-10 μM) most of them were found to be more mature. When higher levels of ABA (30-40μM) were used the globular did not develop past the torpedo stage. The results of this work are in agreement with that of Nadel et al. (1990) working on celery and Senaratna et al. (1991) working on oilseed rape. These workers observed that low levels of ABA allow the development in a more synchronized manner and higher concentrations inhibit further growth. It can be concluded from the results of the current study that synchronization of Ruiru 11 coffee somatic embryos can be achieved by using ABA (1-10 μM). This is the first report on the use of ABA on synchronization of coffee somatic embryos.

REFERENCES

Ammirato, P. V. (1977). Hormonal control of somatic embryo development from cultured cells of caraway: Interaction of abscicic acid , zeating, and gibberellic acid . *Plant Physiology*. 59: 579-586.

Ammirato, P. V. (1987). Organization events during somatic embryogenesis. In C.E. Green, D. A. Somers, W. P. Hackectt and D. D. Biersboer (eds.). Plant Tissue and Cell Culture. Alan R. Liss Inc, New York. pp 57-81.

George, E. F. (1993). Plant propagation by tissue culture. Part 1. The technology Exegetics Limited, UK.

Kahia, W. J. (1999). *In Vitro* Propagation of the New Disease Resistant *Coffea Arabica* L. Cultivar Hydrid –Ruiru 11. Ph D thesis University of London.

Nadel, B. L., Altman, A. and Ziv, M. (1990). Regulation of somatic embryogenesis in celery suspension. 11. Early detection of embryogenic potential and the induction of synchronized cell cultures. *Plant cell Tissue Organ Culture*. 20: 119-124.

Senaratna, T., Mckersie, B. D. and Bowley, S. R. (1989). Desiccation tolerance of alfalfa (*Medicago sativa* L.). somatic embryos. Influence of abscisic acid, stress pretreatment and drying rates. *Plant Science*. 65: 253-259.

Senaratna, T., Kott, L., Beverdof ,W. D. and Mckersie, B. D (1991). Desiccation of microspore-derived embryos of oilseed rape (*Brassica napus* L.). *Plant cell Report*. 10: 342-344.

Tisserat, B. and Murashige, T. (1977). Effects of ethephon, ethylene, and 2,4 Dichlorophenoxyacetic acid on asexual embryogenesis *in vitro. Plant Physiology.* 60: 437-439.

Somatic Embryogenesis in Genotypes of *Coffea arabica* with Reduced Caffeine Content

J. A. S. ALMEIDA, M. B. SILVAROLLA, M. T. BRAGHINI, L. C. FAZUOLI

Centro de Café 'Alcides Carvalho', Instituto Agronômico de Campinas, CP 28, 13.001-970. Campinas, SP, Brazil. E-mail: julietasa@iac.sp.gov.br

SUMMARY

Somatic embryogenesis is an excellent method of micropropagation, which can occur indirectly or directly. In this study, three single accessions with reduced caffeine seed content from the Instituto Agronômico de Campinas (IAC) Coffee Germplasm Collection were evaluated for somatic embryogenesis capacity, aiming at the establishment of clones of the same. It were used leaf explants of the AC1, AC2 and AC3 genotypes of *C. arabica* just as the cultivar Mundo Novo IAC 376-4 (MN), inoculated in the following culture media: **1.** Callus induction medium, MS salts (2,4-D and kinetin). The calluses formed were transferred to a 1/2 MS medium (NAA and kinetin) (Sondahl and Sharp, 1977); **2.** Modified medium of Yasuda et al. (1985) containing 1/2 MS, plus 6-BA. In the firsty medium, after 90 days, the induction of callus was verified for all the genotypes. These genotypes differed with regard to the embryos formation so, the AC1 and AC3 formed more number of embryos than AC2 and MN. In second medium, at 5 μM 6-BA, the AC3 and MN presented a reduced formation of embryos. These embryos were formed on the border of the explants without calluses, which suggested the occurrence of direct somatic embryogenesis. In the doses of 10, 15 and 20 μM 6-BA all genotypes formed calluses, but they were small (about 4 mm). At 150 days, the AC1 and AC3 explants showed more number of embryos than AC2 and MN. Regardless of being trees from a same progeny, the results suggested genetic differences in the capacity of somatic embryogenesis among ACs.

INTRODUCTION

The three *Coffea arabica* plants with reduced caffeine seed content (Silvarolla et al., 2004) designated AC1, AC2 and AC3, are single accessions in the coffee germplasm collection of the Instituto Agronômico de Campinas (IAC). The propagation of these plants through seeds would not be the most appropriate form since the progenies formed could present segregation for different traits and impair an evaluation of the mother plants. For an agronomical characterization in field trials the plants have to be cloned.

Micropropagation is an excellent method of establishing uniform populations in the short term. Among the methods of micropropagation is somatic embryogenesis which leads to the formation of a bipolar structure, similar to an embryo, that develops from a non-zygotic cell without vascular connection with the original tissue (von Arnold et al., 2002; Gaj, 2004).

Somatic embryogenesis can occur indirectly or directly. In the former case, the differentiated cells are first redetermined, followed by cell proliferation, which leads to callus formation and posterior differentiation of embryos on the surface (Sondahl et al., 1984; Williams and Maheswaran, 1986; Yeung, 1995). In the direct form, embryos are formed directly on the edge of the explant (Dublin, 1982; Ramos et al., 1993). Sondahl and Sharp (1977) established the use of 2,4-D and kinetin for the indirect somatic embryogenesis of *C. arabica* to induce

calluses and another medium with NAA and kinetin for the embryo formation from these calluses. On the other hand, cytokinins are associated to embryo formation through embryogenic calluses in *C. arabica*, though under low or zero auxin concentrations as well (Chée and Cantliffe, 1988; Raghavan, 1997). The effect of 6-BA in embryogenic callus induction for immediate embryo formation was verified in leaf explants of *C. arabica* var. Típica at 5 µM (Yasuda et al., 1985), cv IAPAR 59 at 5 µM (Ayub & Gebieluca, 2003) and at 5 and 25 µM for the cv Rubi and Catuaí Vermelho IAC 81 (Cid and Cruz, 2004).

In this study, the somatic embryogenesis of leaf explants of the AC1, AC2 and AC3 genotypes of *C. arabica* with reduced caffeine seed content were evaluated in two culture media: Sondahl and Sharp (1977) and Yasuda et al. (1985), aiming at the establishment of clones of the same.

MATERIAL AND METHODS

It were collected leaves from AC1, AC2, AC3 and the Mundo Novo IAC 376-4 (MN) *C. arabica*, grown under field and greenhouse conditions at the IAC. Square-shaped explants (2 cm^2) from these leaves were inoculated in the following media: **1**. Callus induction, MS salts plus 2,4-D and kinetin (Sondahl and Sharp, 1977). The calluses formed were transferred to a 1/2 MS medium with NAA and kinetin. **2**. Yasuda modified medium (Yasuda et al. 1985) containing 1/2 MS, with 6-BA, at concentrations of 5, 10, 15 and 20 µM/L.

The treatments cultured in Sondahl medium comprised (up to) 15 replications and the ones using Yasuda medium eight replications, which were maintained in presence or absence of light, at 25 °C. Each replication of a treatment consisted of one explant per flask. The following parameters were evaluated in the treatments: explant with callus formation, number of explant sides with callus formation, estimated callus size and the number of embryos formed.

RESULTS AND DISCUSSION

All AC genotypes initially evidenced capacity of indirect somatic embryogenesis (Figures 1A, 1B) once their explants had formed calluses and embryos. The callus formation however only occurred in explants maintained in the dark (Figure 1C), which attained about 15 mm while those in the light developed smaller calluses (about 3 mm). The response of the ACs was similar to that of most *C. arabica* genotypes, which formed calluses in the dark only (Menéndez-Yuffi and Garcia of Garcia, 1977; Almeida et al., 2001; Santana et al., 2004).

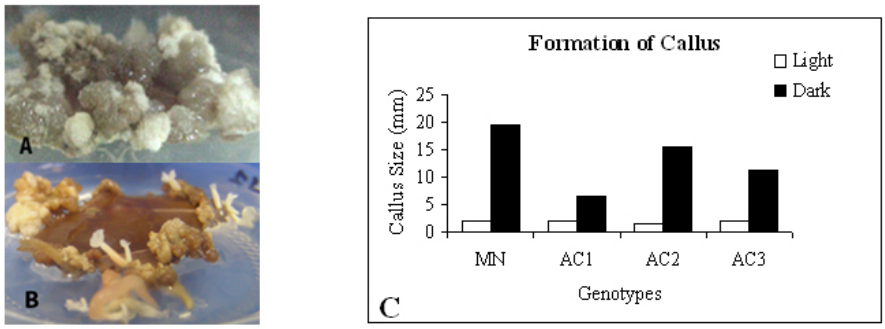


Figure 1. The somatic embryogenesis from the leaf explant of AC genotype of *C. arabica*, in callus induction (A) and embryogenic (B) medium (Sondahl and Sharp, 1977). Effect of the illumination on callus formation (C).

It was verified that the ACs presented different responses regarding to the capacity of indirect somatic embryogenesis (Figure 2). The AC3 and MN genotypes attained a higher percentage of explants with callus formation (Figure 2A), number of explant sides with callus formation (Figure 2B) and callus size (Figure 2C) than AC1 and AC2. But, the number of embryos formed was higher for the AC1 and AC3 genotypes than AC2 and MN (Figure 2D). Carman (1990) coined the term "embryogenic competence" to describe the easiness of embryo induction *in vitro* and indicate an association with embryogenesis-controlling genes.

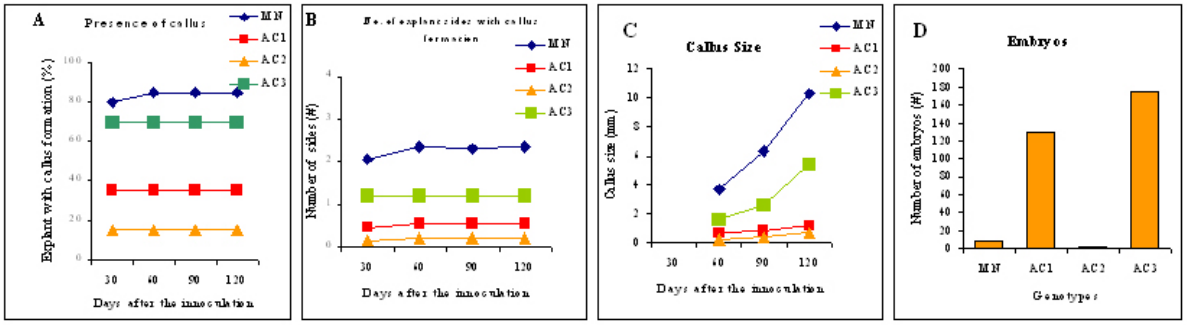


Figure 2. Indirect somatic embryogenesis in leaf explants of decaffeinated *C. arabica* genotypes.

In order to evaluate if explants of the ACs and MN could form calluses of the embryogenic type, explants of the same were inoculated in medium with 5 µM 6-BA. It was observed that all genotypes initiated callus formation, though of reduced size (4 mm). However, after about 150 days some AC3 and MN explants formed a reduced number of embryos along the edges (Figure 3). But these embryos were formed without a visible presence of calluses, suggesting an occurrence of direct somatic embryogenesis.

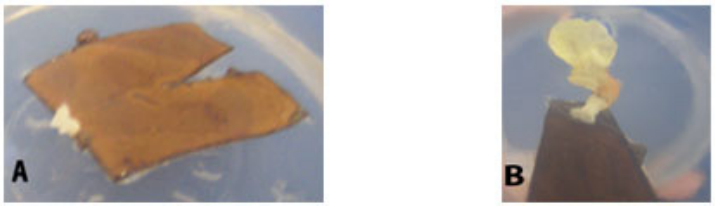


Figure 3. Somatic embryo (A, B) of the AC1 genotype of *C. arabica* in medium with 5 µM 6-BA (Yasuda et al., 1985).

In another experiment the AC and MN were submitted to doses of 10, 15 and 20 µM 6-BA (Figure 4). All genotypes formed calluses, in all tested 6-BA doses, but they were small (4 mm) (Figures 4A, B, C). At 150 days, the genotypes AC1 and AC3 explants showed more number of embryos along their edges than AC2 and MN (Figure 5D). Besides this, the genotypes AC1 and AC3 also formed a light-colored callus mass, mainly at concentrations of 15 and 20 µM 6-BA (Figure 5). These facts show that the tested 6-BA doses did not induce embryogenic callus formation, in agreement with observations of Yasuda et al. (1985), Ayub and Gebieluca (2003) and Cid & Cruz (2004) in *C. arabica* genotypes.

Regardless of being trees of a same progeny, the results suggested genetic differences in the capacity of somatic embryogenesis among ACs.

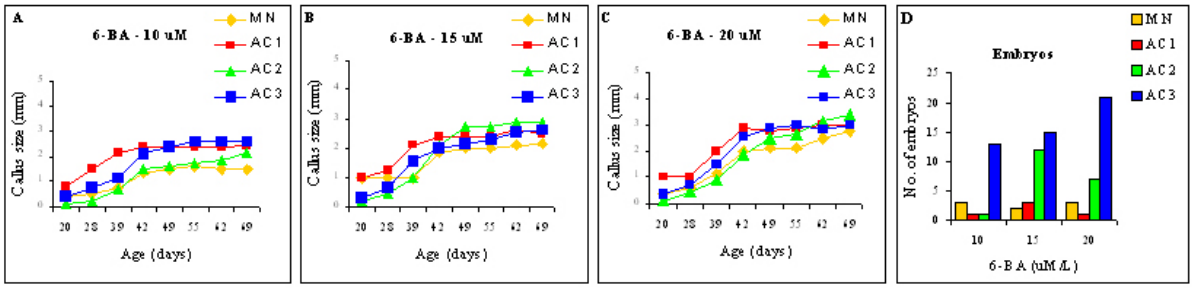


Figure 4. Somatic embryogenesis from leaf explants of AC and Mundo Novo IAC 376-4 genotypes of the *C. arabica* in medium with 6-BA (Yasuda et al., 1985).

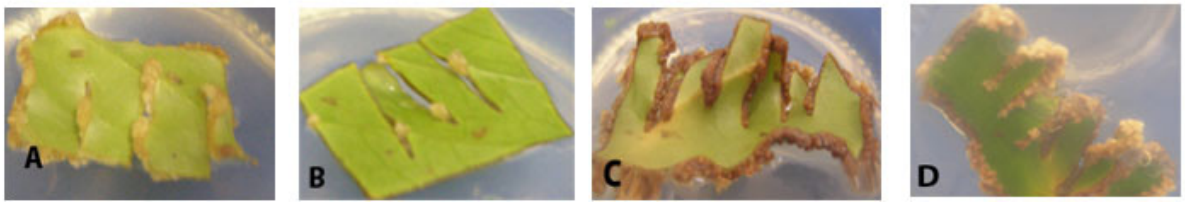


Figure 5. Leaf explants of AC genotypes in medium with 20 µM 6-BA. Light-colored callus mass in A. AC1; B. AC2; C. AC3; D. AC3.

REFERENCES

Almeida, J.A.S.; Simioni, K.C.M.; Fazuoli, L.C.; Ramos, L.C.S. 2001. Obtenção de embriões a partir de calos de genótipos de *Coffea.* In II Simpósio de Pesquisa dos Cafés do Brasil. Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café, Vitória. pp. 1: 34-35.

Ayub, R.A; Gebieluca, A.N. 2003. Embriogênese somática em genótipos de café (*Coffea arabica*) é citocinina dependente. Publ.UEPG Ci. Exatas Terra. Ci.Agr.Eng.Ponta Grossa. 9(2):25-30.

Carman, J.G. 1990. Embryogenic cells in plant tissue cultures occurrence and behaviour. In Vitro Cell Development Biology. 26:746-753.

Chée, R.P.; Cantliffe, D.J. 1988. Selective enhancement of *Ipomea batatas* Poir. Embryogenic and non-embryogenic callus growth and production of embryos in liquid culture. Plant Cell, Tissue and Organ Culture. 15:149-159.

Cid, L.P.B.; Cruz, A.R.R. 2004. Somatic embryogenesis from three coffee cultivars: ´Rubi`, Catuaí Vermelho 81 and IAPAR 59. HortScience. 39(1):130-131.

Dublin, P. 1982. Culture de tissus et amélioration génétique des caféiers cultivés. ASIC, 10^{o} Colloque. pp 433-459.

Gaj, M.D. 2004. Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.) Heynh. Plant Growth Regulation. 43:27-47.

Menéndez-Yuffi, A.; Garcia de Garcia, E. 1977. Morphogenic events during indirect somatic embryogenesis in coffee "Catimor". Protoplasma. 199:208-214.

Raghavan, V. 1997. Molecular embryology of flowering plants. Cambridge University Press. pp.690.

Ramos, L.C.S.; Yokoo, E.Y.; Gonçalves, W. 1993. Direct somatic embryogenesis is genotype specific in coffee. ASIC, 15^{o} Colloque, pp. 763-766.

Santana, N.; González, M.E.; Valcárcel, M.; Canto-Flick, A.; Hernández, M.M.; Fuentes-Cerda, C.F.J.; Barahona, F.; Mijangos-Cortês, J.; Loyola-Vargas, V.M. 2004. Somatic embryogenesis: a valuable alternative for propagating selected robusta Coffee (*Coffea canephora*). In Vitro Cell Dev Biol - Plant. 40:95-101.

Silvarolla, M.B.; Mazzafera, P.; Fazuoli, L.C. 2004. A naturally decaffeinated arabica coffee. Nature. 429(24):826.

Sondahl, M.R.; Nakamura, T.; Medina-Filho, H.P.; Carvalho, A.; Fazuoli, L.C.; Costa, W.M. 1984. Coffee. In: Handbook of plant cell culture. Eds. Ammirato, P.V., Evans, D.A., Sharp, W.R., Yamada, Y. Macmillan Publishing Company, New York. pp.564-590.

Söndahl, M.R.; Sharp, W.R. 1977. High frequency induction of somatic embryos in cultured leaf explants of C*offea arabica* L. Z.Pflanzenphysiol. 81:395-408.

von Arnold, S.; Sabala, I.; Bozhkov, P.; Dyachok, J.; Filonova, L. 2002. Developmental pathways of somatic embryogenesis. Plant Cell Tissue Organ Culture. 69:233-249.

Yasuda,T.; Fujii,Y.;Yamaguchi,T. 1985. Embryogenic callus induction from *Coffea arabica* leaf explants by benzyladenine. Plant Cell Physiol**.** 26(3):595-597.

Yeung, E.C. 1995. Structural and development patterns in somatic embryogenesis. In: In vitro embryogenesis in plants. Thorpe, T.A. (ed.). Kluwer Acdemic Publishers, Netherlands. pp.205-248.

Williams, E.G.; Maheswaran, G. 1986. Somatic embryogenesis: factors influencing coordinated behaviour of cells as an embryogenic group. Ann. Bot. 57:443-462.

Transformation of *Coffea arabica* for Root-knot Nematode Resistance Using Cysteine and Serine Proteinase Inhibitors

R. CABOS[1], B. SIPES[1], D. SCHMITT[1], H. ATKINSON[2], C. NAGAI[3]

[1]University of Hawaii at Manoa, Honolulu, HI, USA
[2]University of Leeds, Leeds, UK
[3]Hawaii Agriculture Research Center, Aiea, HI, USA

SUMMARY

Biotechnology procedures were applied for the control of *Meloidogyne konaensis* on coffee trees. This nematode species is a parasite of *Coffea arabica* cv. Typica grown in the Kona region of Hawaii. Cystatin, a modified cysteine proteinase inhibitor from rice, alone or in combination with a cowpea trypsin inhibitor was inserted into arabica somatic embryos using *Agrobacterium tumefaciens* or by particle bombardment. Over 2500 viable lines remained after a 7 month selection period on antibiotic media. Plants were regenerated from 71 lines of selected somatic embryos. A nematode biological assay was conducted in a secured laboratory. Ninety-one of the transformed plants, representing 41 lines, were challenged with *M. konaensis*. Roots were harvested 347 days after inoculation and the nematode eggs and juveniles were compared among transgenic and non-transgenic lines. Twelve lines demonstrated resistance to *M. konaensis* and supported fewer than 70% of the nematodes found on wild-type coffee plants. This technology is a promising control method for root-knot nematodes in *C. arabica*.

INTRODUCTION

Root-knot nematodes cause significant economic loss in coffee plantations throughout the world. In Kona, Hawaii, *Meloidogyne konaensis*, the Kona Coffee Root-knot Nematode, parasitizes *Coffea arabica* cv. Typica 'Guatemala' (Eisenback et al, 1994). An infection by *M. konaensis* is characterized by less feeder roots, as well as, swelling and corkiness of the main tap root (Serracin et al., 1999).

Cysteine and serine proteinase inhibitors have significantly reduced plant-parasitic nematode populations when engineered into crops such as banana, potato, and rice (Atkinson, 2006). The inhibitors work by binding proteinases in the nematode's digestive system, reducing nematode growth and fecundity (Atkinson et al., 1994). In this experiment, cystatin, a modified cysteine proteinase inhibitor from rice, was used alone or in combination with a cowpea trypsin inhibitor (Urwin et al., 1998). Tubulin, a preferential root-expressing promoter, or the constitutive CaMV35S promoter was used to drive the effector gene.

MATERIALS AND METHODS

Tissue Culture and Transformation

Young leaves were collected from field-grown *C. arabica* cv. Typica 'Guatemala'. Leaf discs were placed into tissue culture using a modified protocol developed by Sondahl et al. (1977). Somatic embryos developed 8 months later.

The nematode resistance genes were inserted into *C. arabica* somatic embryos via *A. tumefaciens* strain EHA 105 or by particle bombardment (Nagai et al., 1992). The gene constructs used were CaMV35S-OcI-ΔD86/GO/CpTI (Urwin et al., 1998), CaMV35S-OcI-ΔD86 (Atkinson et al., 1998), and Tubulin-OcI-ΔD86 (Atkinson et al., 2001) (Figure 1).

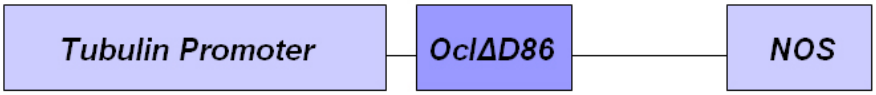


Figure 1. The Tubulin-OcI-ΔD86 gene construct

Somatic embryos were multiplied on media supplemented with 30 mg/L of geneticin sulfate for 7 months to select for transformants. Screening was also conducted using the Polymerase Chain Reaction (PCR). Genomic DNA was extracted from leaves of selected plantlets using BioSystems 101 FastDNA kit. RNA was extracted from root tips with the Qiagen RNeasy Plant Mini Kit and used for Reverse Transcriptase PCR (RT-PCR) and Quantitative RT-PCR reactions.

Nematode Resistance Assay

An assay was conducted in the laboratory in 15.5 cm diameter clay pots containing a sterilized 2:1 soil and sand mixture. Ninety-one of the transformed plants were inoculated with eggs of *M. konaensis*. Depending upon their size, the plants received 4,000, 2,000, or 1,000 root-knot nematode eggs. Wild-type *C. arabica* from tissue culture and *C. arabica* transformed with the GUS gene were used as controls. After 347 days, plant growth and root weight were recorded. The root system was rated for galls and overall health. The roots were blended in a 0.6% NaOCl solution and poured through nested 149-µm and 20 µm pore sieves. The nematode eggs and juveniles (J2s) were collected and counted. The number of eggs and J2s were combined to get the final nematode population and transformed $\log_{10}(x+1)$ for analysis. The reproduction factor (Rf) was derived by dividing the final population by the inoculation number.

RESULTS AND DISCUSSION

Tissue Culture and Transformation

After the initial selection period, 1,251 lines transformed by *A. tumefaciens* remained in culture (Table 1) as well as 1,262 lines from particle bombardment (Table 2). Plants were regenerated from 71 lines: 18 by *Agrobacterium*-mediated transformation with the dual construct, 11 by *Agrobacterium*-mediated transformation with the tubulin-driven construct, 35 lines through bombardment with 35S-OcI-ΔD86/GO/CpTi and 7 lines through bombardment with Tubulin-OcI-ΔD86.

Table 1. Transformation of Somatic Embryos with *Agrobacterium tumefaciens.*

Construct	# Plates Transformed	# Lines After Selection	# Lines Regenerated	# Lines Tested	# PCR + Lines
ΔD86/GO/CpTI	103	584	18	10	5
Tubulin-ΔD86	62	657	11	5	1

Table 2. Transformation of Somatic Embryos with Particle Bombardment.

Construct	# Plates Transformed	# Lines After Selection	# Lines Regenerated	# Lines Tested	# PCR + Lines
ΔD86/GO/CpTI	20	786	35	14	9
Tubulin-ΔD86	10	163	7	2	2
35S-ΔD86	10	313	0	0	0

Nematode Resistance Assay

Nematode reproduction among the individual coffee plants was highly varied. The Rf values were compared between the transgenic and control plants of similar size. Each plant was categorized as resistant, average, or very susceptible. If the plant had less than 70% of the nematodes found in the susceptible control, it was considered resistant. If the plant had greater than 150% more nematodes than the control plants, it was considered very susceptible. Of the plants containing the construct 35S-OcI-ΔD86/GO/CpTi, 21 were scored as resistant, 41 susceptible, and 16 very susceptible (Table 3). In plants containing the construct Tubulin-OcI-ΔD86, 3 were resistant, 8 susceptible, and 3 very susceptible. High levels of resistance occurred in 26% of the modified plants tested.

Table 3. Nematode Resistance Levels Among Transformed Plants.

Construct	Resistant	Susceptible	Very Susceptible
35S-ΔD86/GO/CpTI	21	41	16
Tubulin-ΔD86	3	8	3

Twelve lines containing the cystatin gene had a > 70% reduction in the *M. konaensis* population as compared to the wild-type. These lines also had at least a 70% smaller Rf value, 70% less eggs per gram of shoot, and 70% less eggs per gram of fresh root than the controls. Plant growth was not correlated to susceptibility. The resistant plants showed less galling and had an overall healthier root system than the wild-type controls.

CONCLUSIONS

Out of 41 lines transformed with the nematode resistance genes, 12 lines, 29%, demonstrated high levels of resistance to *M. konaensis*. Properly expressed, cysteine and serine proteinase inhibitors are an effective control method against root-knot nematodes, suppressing their growth and reproductive ability.

REFERENCES

Atkinson, H.J.et al., 1994. Advances in Molecular Plant Nematology 197-210.

Atkinson, H.J. et al., 1998. CAB International. The Physiology and Biochemistry of Free-Living and Plant-Parasitic Nematodes: 381-413.

Atkinson, H.J. 2006. http://www.biology.leeds.ac.uk/nem/home.htm

Eisenback, J.D. et al., 1994. Journal of Nematology 26(4):363-374.

Nagai, C. et al., 1992. Hortscience 27(6): 661.

Serracin, M. et al., 1999. College of Tropical Agriculture and Human Resources Plant Disease PD-16.

Sondahl, M.R., et al., 1977. Z. Pflanzenphysiol. Bd. 81. S. 395-408.
Urwin, P.E. et al., 1998. Planta 204:472-479.

Coffee Seed Cryopreservation: Current Research Progress

M. T. S. EIRA, V. S. RIBEIRO[1], L.C. FAZUOLI[2], O. GUERREIRO FILHO[2], C. WALTERS[3]

[1]Embrapa, Brasília, DF, Brasil. E-mail: mirian.eira@embrapa.br
[2]IAC, Campinas, SP, Brasil
[3]NCGRP/USDA, Fort Collins, CO, USA

SUMMARY

Viability of seeds stored in genebanks must be maintained for several years or even centuries. Because of the difficulties in storing the seeds, coffee germplasm is maintained in field collections, presenting significant problems, such as land and labor costs and susceptibility to environmental hazards and pathogens. Storage of *Coffea* species in *ex situ* genebanks may help to preserve the threatened diversity of this important genus. Since 1976 the International Plant Genetic Resources Institute has considered coffee as a high priority for genetic conservation. For non-orthodox seed species, cryopreservation is the only technique available for long-term germplasm conservation. The genebank of Embrapa Genetic Resources and Biotechnology, in Brazil has now established a program to cryopreserve genetic resources of *Coffea*. The protocol was first determined for *C. arabica* and *C. racemosa*. Seeds were first dried to 0.20 g/g (in equilibrium with 78-80% RH). Sufficiently rapid cooling and warming was achieved in hermetically-sealed foil-laminate bags containing 10-11 g (or 50 seeds) by plunging bags directly into liquid nitrogen – LN containers and placing bags removed from LN directly into a 40 °C bath. Successfully cryopreserved *C. arabica* and *C. racemosa* seeds showed minimal viability loss after two-year storage in liquid nitrogen. The same protocol is being adapted to other species of *Coffea.*

INTRODUCTION

Coffee is extensively cultivated as a cash crop in many countries, including Brazil, which is the main producer of coffee beans in the world. There is a great variability between and within species, which is of importance for breeding. A living collection of *Coffea*, comprehending most species of the genus, is maintained at the Instituto Agronômico de Campinas, IAC, Brazil. Significant problems appeared with the maintenance in field genebanks: i) genetic erosion in some species due to their poor adaptation to the local environment and to attacks by pests and pathogens; and, ii) significant labor costs and large space requirements. Thus, research for conservation of coffee genetic resources, in genebanks, as seeds, became a priority.

Seeds of *Coffea* species do not behave as the majority of seeds with respect to storage, according to the definitions of Roberts (1973). They are not recalcitrant, since they survive desiccation to water contents less than 0.20 g/g, and some seeds survive exposure to subzero temperatures, and they are not orthodox either, as the seeds do not survive complete desiccation and the combined effects of desiccation and low temperatures. Ellis et al. (1990; 1991) introduced the “intermediate” category of desiccation tolerance to describe seeds such as coffee, which can tolerate some drying but do not survive complete desiccation or the combined effects of desiccation and low temperature.

For non-orthodox seed species, cryopreservation is the only technique available for long-term germplasm conservation. In the case of intermediate seed-propagated species, seeds are partially desiccation tolerant and, therefore, the option which has to be always tested first is whole seed cryopreservation.

A certain amount of work has been carried out on the cryostorage of coffee seeds. Most of the papers reported coffee seeds as desiccation tolerant and liquid nitrogen sensitive. Nevertheless, Normah and Vengadasalam (1992) showed that seeds of *C. liberica* can survive storage in liquid nitrogen if the water content is around 17% (fwb). Eira et al. (1999) reported survival of *C. arabica* and *C. racemosa* seeds with 0.20g H2O/g dry mass after liquid nitrogen exposure. Dussert et al. (2001) reported that seed survival was strictly depended on avoidance of intracellular ice formation, and that 0.20 g/g corresponded to the seed unfrozen water content .According to those results, coffee seeds are not liquid nitrogen sensitive at appropriate water content, around 17% (fwb) or 20% (dwb).

OBJECTIVE

Our objective was to establish a program to cryopreserve *Coffea* seeds as a complementary strategy of long term germplasm conservation of that important genus.

The protocol was first determined for *C. arabica* and is now being adapted to other species, such as *C. racemosa*.

RESULTS

Successfully cryopreserved *C. arabica* and *C. racemosa* seeds showed minimal viability loss after two-years storage in liquid nitrogen.

The best protocol were obtained with seeds are extracted from ripe fruits at 0.20 g/g dw (in equilibrium with 78-80% RH), as reported by Eira et al. (1999) and Dussert et al. (2001). Sufficiently rapid cooling was achieved in by plunging the bags directly into LN containers and thawing was carried out by placing bags removed from LN directly into a 40 °C bath. Viability was evaluated by seed germination tests carried out at 30 °C, in the presence of light.

PERSPECTIVES

The results are promising to the definition of the long term conservation protocol for *Coffea* genetic resources. A core collection of *C. arabica* germplasm is being organized and will be preserved in liquid nitrogen. The protocol is now being adapted to other species of *Coffea*.

ACKNOWLEDGEMENTS

This research was supported by the Brazilian Consortium for Research of Development of Coffee.

REFERENCES

Dussert S, Chabrillange N, Rocquelin G, Engelmann F, Lopez M, Hamon S (2001) Tolerance of coffee (*Coffea* spp.) seeds to ultralow temperature exposure in relation to calorimetric properties of tissue water, lipid composition and cooling procedure. Physiol Plant 112:495-504.

Eira MTS, Walters C, Caldas LS, Fazuoli LC, Sampaio JB, Dias MCC (1999) Tolerance of *Coffea* spp. seeds to desiccation and low temperature. Braz J Plant Physiol 11:9-105.

Ellis RH, Hong TD, Roberts EH (1990) An intermediate category of seed storage behaviour? I. Coffee. J Exp Bot 41:1167-1174.

Ellis RH, Hong TD, Roberts EH (1991) An intermediate category of seed storage behaviour? II. Effects of provenance, immaturity and imbibition on desiccatio-tolerance in coffee. J Exp Bot 42:653-657.

Normah MN, Vengadasalan M (1992) Effects of moisture content on crypreservation of *Coffea* and *Vigna* seeds and embryos. CryoLetters 13:199-208.

Roberts EH (1973) Predicting the storage life of seeds. Seed Sci Technol 1:499-514.

Mechanisms of Seed Ageing in Coffee

S. DUSSERT[1], A. LAFFARGUE[1], M. DAVEY[2], S. DOULBEAU[1], H. ETIENNE[1,3]

[1] IRD, 911 Av. Agropolis, BP 64501, 34394 Montpellier Cedex 5, France
[2] K.U.Leuven, Kasteelpark Arenberg 13, B-3001 Leuven, Belgium
[3] CIRAD, Av. Agropolis, 34398 Montpellier Cedex 5, France

SUMMARY

The biochemical and physiological basis of the intermediate seed storage behaviour was examined by investigating the effects of equilibrium drying under relative humidities (RH) of 9-81% and of storage at 20 or 5 °C on coffee seed viability and antioxidant, lipid and sugar status. Slow drying induced a significant decrease in the concentrations of the pools of two major antioxidants, glutathione and ascorbate, and an increase in the free fatty acid (FFA) content of seeds, independent of the RH employed. Seeds stored at 81% RH and 20 °C lost their viability very rapidly and showed an extensive loss and oxidation of antioxidants, an accumulation of FFA, and a selective loss of phospholipids, in particular phosphatidylethanolamine (PE). Interestingly the changes in PE content were not due to fatty acid de-esterification and the increase in FFA levels resulted from neutral lipid hydrolysis. Decreasing the storage temperature to 5 °C considerably slowed both the loss of seed viability, the level of oxidative stress as well as the rates of lipid hydrolysis. After 1 year under 45% RH/5 °C, the loss of seed viability (40%) was found to be due to imbibitional damage and could be circumvented by pre-humidifying or pre-heating seeds before sowing.

INTRODUCTION

Three categories of seed storage behaviour are generally recognized among plant species: orthodox, intermediate and recalcitrant (Roberts, 1973; Ellis et al., 1990). Using coffee (*Coffea arabica*) as a model, Ellis et al. (1990) defined the "intermediate" category as being for seeds which display the following two main characteristics regarding their level of desiccation tolerance and storage behaviour: i) seeds that are able to withstand considerable drying (down to a relative humidity (RH) of 30-40%) in comparison with recalcitrant seeds, but which cannot tolerate extreme water loss as is the case in orthodox seeds, ii) in contrast with orthodox seeds, lowering the storage temperature decreases intermediate seed longevity at low water contents (RH < 50%). Therefore, intermediate seeds cannot be stored under conventional genebank conditions (FAO/IPGRI, 1994), and for intermediate seed species, cryopreservation is the only technique available for long-term germplasm conservation (Dussert et al., 2001).

Despite the importance of this category of storage behaviour (Hong et al., 1996), the physiology of intermediate seeds during drying and storage is still largely unknown. However, significant progress in the understanding of the intermediate storage behaviour has been brought by recent work from Sacandé et al. (2000) using neem seeds. By comparing seeds desiccated and stored under two RHs (32 and 75%), these authors have shown that the rapid loss of seed viability at 75% RH in comparison with 32% RH was associated with an increase in the free fatty acid (FFA) content and decreases in the phospholipid (PL) and glutathione contents. Therefore the first objective of the present study was to confirm that oxidative stress, PL loss and lipid de-esterification are key mechanisms of loss of viability in

intermediate seeds, using coffee seeds as the reference system for this storage category, and to further understand the origin of these damages.

In addition, for the first time, we investigated the effects of lowering the storage temperature on these three deteriorative processes to understand one of the two main features of intermediate seeds, *i.e.* the apparent negative effect of low temperature on their longevity at low water contents.

Besides, Walters et al. (2001) showed that in pea and tea embryonic axes, desiccation tolerance is positively correlated to the drying rate and negatively correlated to O_2 consumption. These authors assumed that the oxidative stresses induced by slow drying are due to the longer time spent in a partially dehydrated state, where cells continue to respire but cannot scavenge reactive oxygen species and other toxic by-products (Leprince et al., 2000). The second objective of this study was thus to verify whether physiological changes could be detected during the course of drying in coffee seeds and whether they could be correlated with oxidative damage.

MATERIALS AND METHODS

Fresh mature seeds of *C. arabica* L. (variety Caturra) were provided from CICAFE, San Jose, Costa Rica. Three seed-lots were employed corresponding to three consecutive years of production at CICAFE (2001, 2002 and 2003). Seeds were desiccated by equilibration over various saturated salt solutions for 20 days at 25 °C in the dark, as previously described by Dussert et al. (2001; 2003). Storage behaviour at 5 °C and 20 °C was investigated using seeds desiccated over K_2CO_3 and $(NH_4)_2SO_4$ saturated solutions. Batches of 100 seeds were sealed hermetically in aluminium foil-polyethylene bags and stored for 0, 3 and 12 months in the dark. Seed pre-humidification, osmoconditioning, pre-heating and germination procedures were performed as described in Dussert et al. (2003). Simultaneous analysis of ascorbate and glutathione contents was carried out by ion-suppression RP-HPLC essentially as described in Davey et al. (2003). Total lipids were extracted using a modified Folch method (1957) with methylene chloride replacing chloroform. PL and FFA were purified by Solid Phase Extraction as described in Dussert et al. (2006). The PL fraction was analyzed by HPLC (Beckman Coulter) equipped with a Lichrospher 100 Diol column from Merck and an evaporative light-scattering detector (Chromachem, Eurosep). The FFA fraction containing heptadecanoic acid (17:0) as internal standard was methylated with 5 ml of 14% BF_3 methanolic solution at 90 °C for 1 min. Fatty acid methyl esters were analyzed by Gas Chromatography using an HP 5890 system with flame ionisation detection and a Famewax capillary column (RESTEK, France).

RESULTS

Slow drying induced a significant decrease in the concentrations of the pools of two major antioxidants, glutathione and ascorbate, and an increase in the FFA content of seeds (Figure 1). Seeds stored at 81% RH and 20 °C lost their viability very rapidly and showed an extensive loss and oxidation of antioxidants, an accumulation of FFA, and a selective loss of phospholipids, in particular phosphatidylethanolamine (PE) (Figure 2). Interestingly the changes in PE content were not due to fatty acid de-esterification and the increase in FFA levels resulted from neutral lipid hydrolysis. Decreasing the storage temperature to 5 °C considerably slowed both the loss of seed viability, the level of oxidative stress as well as the rates of lipid hydrolysis (Figure 2). No decline in seed viability was observed under storage conditions of 45% RH/20 °C (Table 1). After 1 year under 45%RH/5 °C, the loss of seed

viability was found to be due to imbibitional damage and could be circumvented by pre-humidifying or pre-heating seeds before sowing.

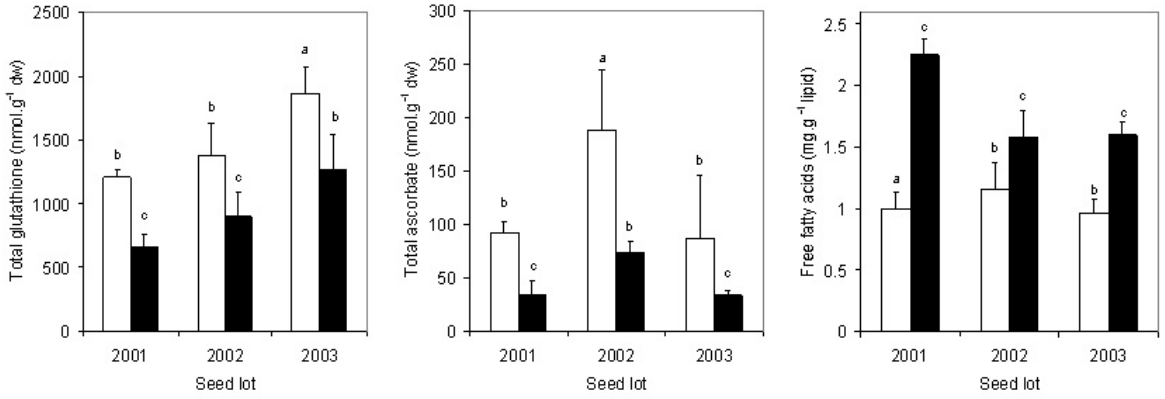


Figure 1. Seed glutathione, L-ascorbate, and FFA content in three seed lots of *C. arabica* before (white bars) and after (black bars) drying under 81% RH at 25 °C.

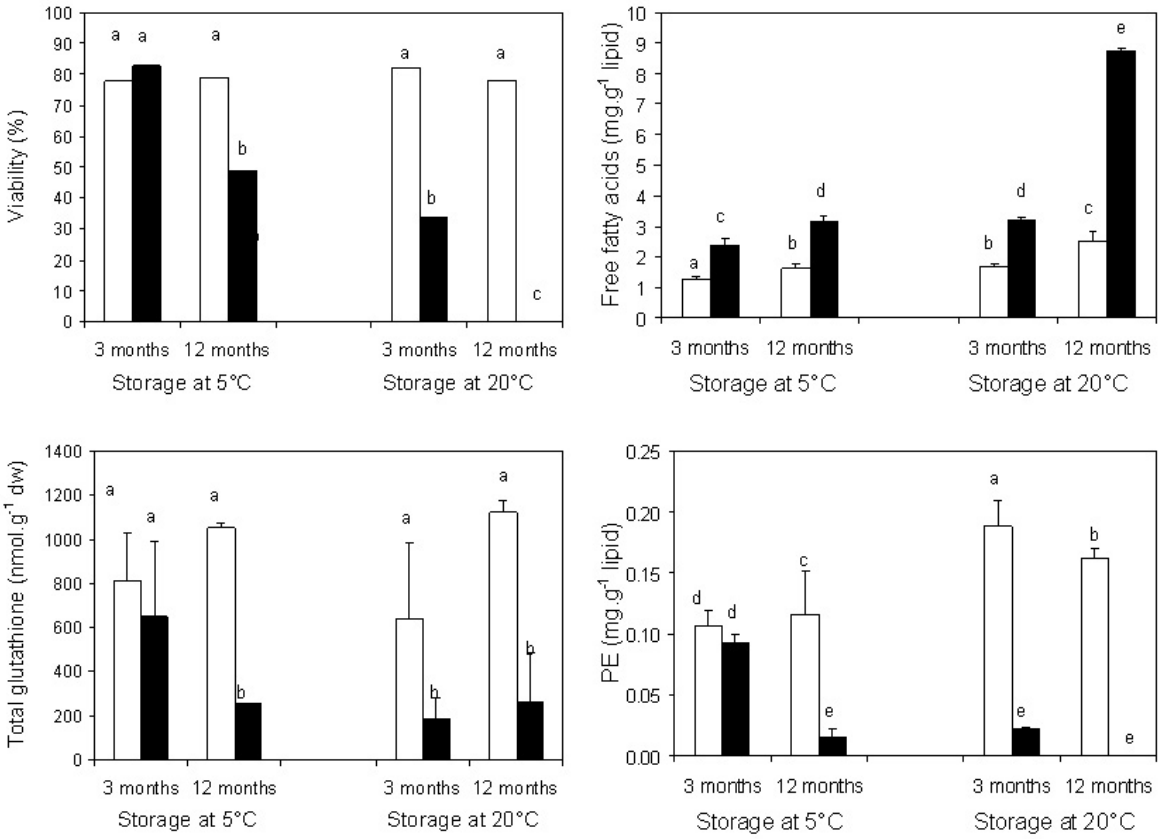


Figure 2. Effect of the time of storage at 5 °C and 20 °C on viability (under optimal rehydration conditions), free fatty acid content, glutathione and phosphatidylethanolamine (PE) contents in *C. arabica* seeds (seed-lot 2001) after drying under 45% RH (white bars) and 81% RH (black bars).

Table 1. Effect of the rehydration procedure on the percentage of normal seedlings recovered from *C. arabica* seeds stored for 1 year at 5 °C and 20 °C after equilibrium drying under 45% and 81% RH. Means followed by the same letter were not significantly different at *P* = 0.05 according to the Newman and Keuls test.

	Storage at 5 °C			Storage at 20 °C	
	45% RH	81% RH		45% RH	81% RH
Rapid rehydration	39 a	49 a		72 b	0
Osmoconditioning	35 a	32 a		65 b	0
Pre-humidification	81 b	35 a		82 b	0
Pre-heating	79 b	38 a		78 b	0

The present study clearly demonstrates that oxidative stress, lipid hydrolysis and PL loss are involved in the ageing of coffee seeds stored at high temperature (20 °C) and at intermediary RHs (81%). It also offers an important new perspective on the effect of low temperature on the storage behaviour of intermediate seeds. In the initial studies that led to the definition of the intermediate category, Ellis et al. (1990) observed that decreasing the storage temperature led to a decreased longevity of dry coffee seeds, and this characteristic still represents one of the main criteria to distinguish orthodox from intermediate seed storage behaviour. According to our work, the apparent decrease in longevity of seeds stored at low RH and temperature is due to imbibitional damage. We have not only shown the importance of the rehydration procedure after storage at 5 °C, but also that decreasing the storage temperature slows down all the deteriorative processes observed at 20 °C, i.e. lipid hydrolysis, oxidative stress and PL loss. This important result was relevant at both hydration levels tested and may offer important applied perspectives for germplasm conservation. It also questions one of the two criteria retained by Ellis et al. (1990) in their definition of the intermediate category, i.e. the negative effect of lowering the storage temperature on seed longevity.

REFERENCES

Davey MW, Dekempeneer E, Keulemans J (2003) Anal Biochem 316: 74-81

Dussert S, Chabrillange N, Montillet JL, Agnel JP, Engelmann F, Noirot M (2003) Physiol Plant 119: 534-543

Dussert S, Chabrillange N, Rocquelin G, Engelmann F, Lopez M, Hamon S (2001) Physiol Plant 112: 495-504

Dussert S, Davey M, Laffargue A, Doulbeau S, Swennen R, Etienne H (2006) Physiol Plant, 127: 92-204

Ellis RH, Hong TD, Roberts EH (1990) J Exp Bot 41: 1167-1174

FAO/IPGRI (1994) Genebank standards. Rome: Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute.

Folch J, Lees M, Sloane Stanley GH (1957) J Biol Chem 226: 497-509

Hong TD, Linington S, Ellis RH (1996) *Compendium of information on seed storage behaviour*. International Plant Genetic Resources Institute, Rome.

Leprince O, Harren FJM, Buitink J, Alberda M, Hoekstra FA (2000) Plant Physiol 112: 597-608

Roberts EH (1973) Seed Sci Technol 1: 499-514

Sacandé M, Hoekstra FA, van Aelst AC, De Vos CHR (2000) Seed Sci Res 10: 381-392

Walters C, Pammenter NW, Berjak P, Crane J (2001) Seed Sci Res 11: 135-148

Effects of the *Laurina* Mutation on the Shoot Apex in *C. arabica* cv. Laurina

A. LECOLIER[1], J. L.VERDEIL[2], H. CHRESTIN[3], M. NOIROT[1]

[1]IRD, UMR 1097 – DGPC (AGRO-M, CIRAD, INRA, IRD), «Diversité et Génome des Plantes Cultivées», Pôle de Protection des Plantes, 97410, Saint Pierre, La Réunion
[2]CIRAD PHIV, UMR 1098, IFR, 34 000 Montpellier, France
[3]IRD, UMR 1097 – DGPC (AGRO-M, CIRAD, INRA, IRD), Centre IRD de Montpellier, 34 000, Montpellier, France

SUMMARY

Coffea arabica var. Laurina, also called Bourbon Pointu, appeared on the island of La Réunion following a spontaneous mutation in the Bourbon variety. One of the major visible effects of a mutation is the modification of the plant architecture towards a dwarf pyramidal shape. In order to understand this effect, the lateral organs initiation pattern was performed through shoot apex comparison of the two Bourbon varieties. The observations were performed through two ways: 1/ macroscopic description of an internode emergence cycle (phyllochron); 2/ microscopic descriptions of leaf primordium emergence cycle in an apical shoot apex and Shoot Apical Meristem (SAM) (plastochron). The last procedure allowed a fine histological description of the *Coffea* apex, between two leaf primordial emissions. Whatever the environmental conditions (field or greenhouse), within one month, the original cv. Bourbon grew six-fold higher than the cv. Bourbon pointu. Plastochron could not explain this difference. Whatever the environmental conditions (field or greenhouse), within one month, the original cv Bourbon grew six-fold higher than the cv Bourbon pointu. Plastochron could not explain this difference. In fact, the *Laurina* mutation acts very early on the periphery of the shoot apex concerning the future leaf and internode. By contrast, the meristem sensu stricto was not modified by the mutation.

INTRODUCTION

Coffea arabica var. Laurina, also called Bourbon pointu (BP), appeared in La Réunion island, following a spontaneous mutation in the Bourbon variety (B). Monolocus and recessive (Krug and Carvalho, 1951), the main scientific interest in this mutation concerns its pleiotropic effects such as drought resistance, lower caffeine content and seed shape which accounts for its vernacular name. Among all the effects, BP has a characteristic Christmas tree shape with short internodes (Chevalier, 1947). However the mutation does not affect the phyllotaxy and the leaves are still opposed and positioned in a decussate pattern.

Our first objective was thus to describe on the variety B: i/ the cycle of internode emergence at the macroscopic level, estimating the plastochron; ii/ the internal structure of the shoot apex; and iii/ the microscopic evolution of the SAM during the plastochron. Then our aim was to analyse the effects or not of the *Laurina* mutation on BP for all of these parameters (i.e phyllochron, plastochron, structure and size of the shoot apex).

MATERIAL AND METHODS

The plant material involved the variety C. arabica var. Bourbon (B) and its mutant, the variety *C. arabica* var. Laurina (BP). Fine histological description of the shoot apex was performed through widthwise resin-embedded sections.

RESULTS

Shoot apex functioning in *C. Arabica* L. var Bourbon

Macroscopic description of an internode emergence cycle

The time origin (T0) of the cycle was arbitrarily set as the moment when leaves Lf1 are fully apart with visible interpetiolar stipules S1 at the top of the shoot. Four main macroscopic phases were observed: ϕ1, the latency phase: no morphological change occurred; ϕ2, young conjugate leaves Lf2 appeared between stipules S1, but their petioles were still not visible); ϕ3, the petioles of growing Lf2 had fully emerged. During this step, the new internode and stipules appeared; and ϕ4, leaves Lf2 were growing apart with from each other. Lf2 and internode growth continued during this phase (Figure 1).

Internal description of the shoot apex

An absence of external changes during the latency phase does not mean that there is an absence of development processes between stipules S1. Figure 2A shows a cross-section of the shoot apex constituted by stipules S1, leaves Lf2 and meristem. Stipules S1 form its external envelope that protects leaves Lf2. In Figure 2B, the shoot apex is in the _2 phase: the young leaves Lf2 emerged between stipules S1, while stipules S2 were fully visible. At this moment, primordia (P) were emerging (Figure 2C and D). They are developing into leaves during phases _3 and _4, thus looping the loop. Consequently, the apical meristem regularly and invariably emitted organs without dormancy.

Microscopic description of the shoot apex

Form and volume variations of the shoot apex were also microscopically observed during the plastochron.

Four microscopic phases were well-defined: 1) “dome-shaped” meristem (Figure 3.A) without initium nor primordia, 2) “trapezoidal” apex (Figure 3.B), divisions of L1 tunica layer lead to a pair of diametrically opposed primordia , 3) “small foliar anlage” with larger primordia (Figure 3.C), and, 4) “large foliar anlage” (Figure 3.D).

Effects of the *Laurina* mutation on Shoot apex and Shoot Apical Meristem size

The impact of the *Laurina* mutation on the phyllochron depended on the environment ($F_{1, 36} = 5.24$; $p = 0.028$). At Montpellier (France), in greenhouse, the leaf emergence rate was 39% higher in B than in BP, whereas it was similar at St Pierre (Reunion island, France). However, whatever the environmental conditions, Bourbon growth six-fold higher than BP. Plant size difference is mainly due to internodes length.

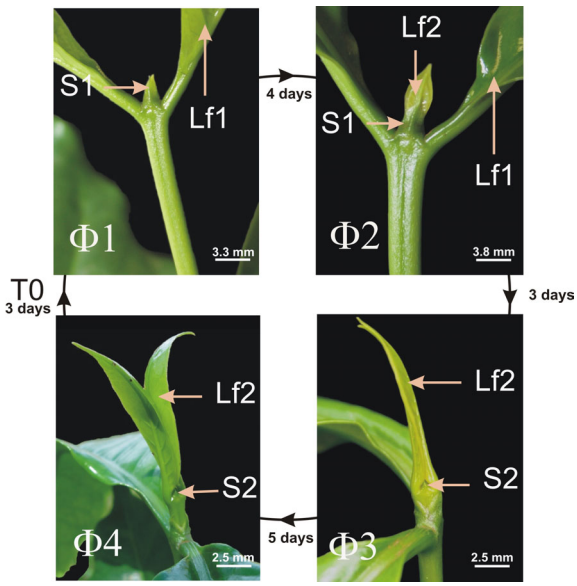


Figure 1. Macroscopic phases (f1 to f4) of leaf emergence in *C. arabica* var. Bourbon. Lf1, Lf2: first and second leaf pair, respectivement, numbered by appearance. S1 and S2: Stipule pairs, respectivement of Lf 1 and Lf2.

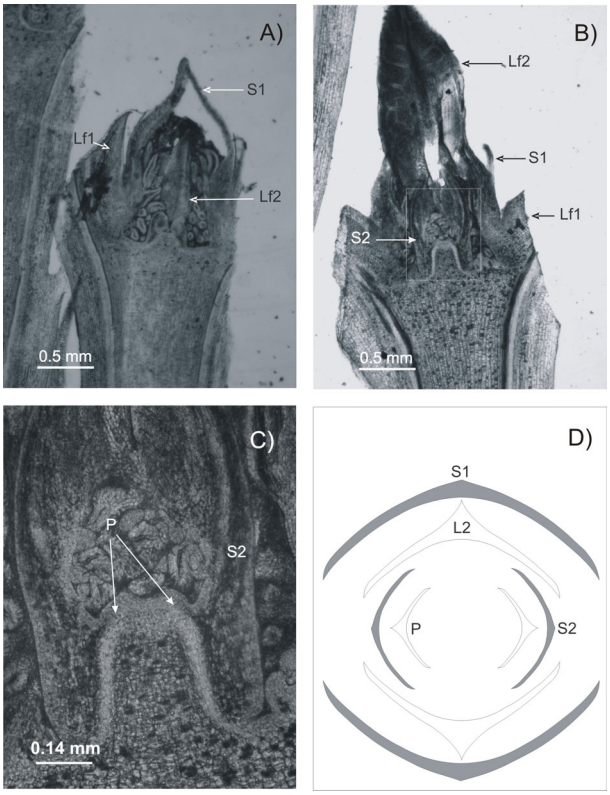


Figure 2. Internal description of the shoot apex. A) apex at T0 with stipules S1 protecting leaves Lf2. B) apex in the second phase. Leaves Lf2 are emerging between stipules S1. C) zoom on the meristem corresponding to B showing leaf primordium. D) schematic representation of the apex at T0 in transversal section.

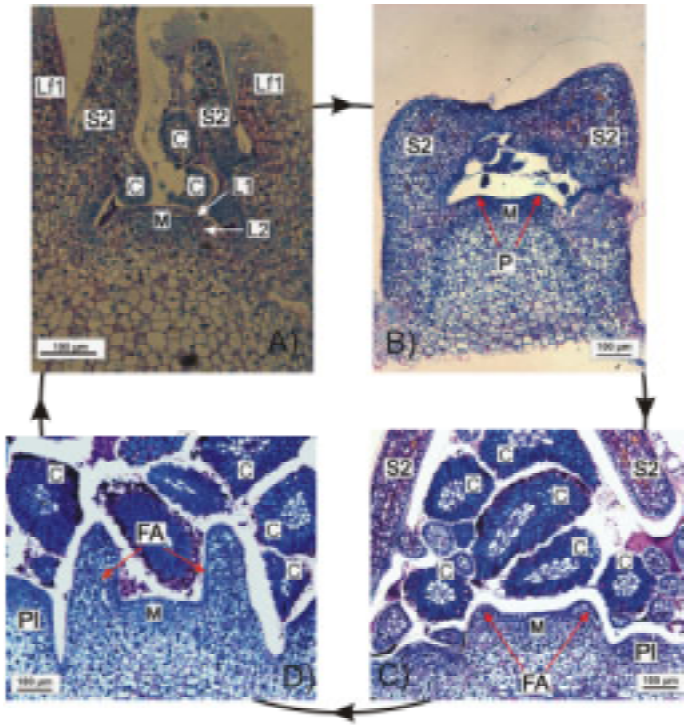


Figure 3. Microscopic description of a shoot apex at the meristem level. A) Dome-shaped apex; B) Primordium emergence; C) Small leaf anlage; and D) Large leaf anlage. Lf1: leaf #1; S2: stipule #2; M: meristem; P: primordium; L1: layer L1; L2: Layer L2; Pl: future plagiotropic axis; FA: foliar anlage; and C: colleter, called also trichomes.

At the microscopically level, form and volume variations of the shoot apex diverged between BP and B: although apex zonation and functionning are not modified by the mutation, BP show a lower apex diameter during leaf initiation (Figure 3 B, C and D). The impact of the *Laurina* mutation was similar whatever the apex stage (trapezoidal, small foliar anlage, large foliar anlage).

CONCLUSION

Our objective was to analyse the effects of the *Laurina* mutation on the shoot apex functioning in order to understand tree shape variations between B and BP. The first step was to describe, for the first time, the structure and the functioning of the shoot apex and the SAM in B, this knowledge being unknown up to date.

Change of apex diameter in BP could play a role in the observed differences in the leaves or internodes size. The *Laurina* mutation seems to act very early on two types of meristems: the foliar anlage meristem and the young internode meristem, resulting in smaller primordia, smaller and foliar anlages, and furtherly thereafter smaller leaves and smaller internodes than in the wild type. By contrast, the meristem *sensu stricto* was not affected by the mutation.

Two independent perspectives can be proposed. The first one would be to monitor the consequences of the *Laurina* mutation on the internode growth and size, while the second one would be to test candidate-genes. Theses one could play a role in founder cell maintenance and number patterns (Wuschel and Clavata for example (Weigel and Jurgens, 2002)) or in future leaf cell division (Antegumenata transcription factor for example (Mizukami and Fischer, 2000)).

REFERENCES

Chevalier A. 1947. Les caféiers du globe: III. Sistématique des caféirs et faux-caféiers maladies et insectes nuisibles. In: Le Chevalier P, ed. *Encyclopedie Biologique*. Paris.

Krug CA, Carvalho A. 1951. The genetics of coffea. *Adv Genet* 4: 127-158

Mizukami Y, Fischer RL. 2000. Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. *Proc Natl Acad Sci U S A* 97: 942-947

Weigel D, Jurgens G. 2002. Stem cells that make stems. *Nature* 415: 751-754

Analysis of Dwarfism at the Microscopic Level in *C. arabica* cv. Laurina

A. LECOLIER[1], J. L. VERDEIL[2], H. CHRESTIN[3], M. NOIROT[1]

[1]IRD, UMR 1097 – DGPC (AGRO-M, CIRAD, INRA, IRD), «Diversité et Génome des Plantes Cultivées», Pôle de Protection des Plantes, 97410, Saint Pierre, La Réunion
[2]CIRAD PHIV, UMR 1098, IFR, 34 000 Montpellier, France
[3]IRD, UMR 1097 – DGPC (AGRO-M, CIRAD, INRA, IRD), Centre IRD de Montpellier, 34 000, Montpellier, France

SUMMARY

Most Arabica varieties cultivated worldwide belong to the Typica or the Bourbon groups. In the island of Réunion, a dwarf Bourbon tree appeared, which is known as the Bourbon pointu (or the Laurina variety), due to the so-called *Laurina* mutation. The internode length – more than internode number variations – mainly account for plant size difference between the two varieties. The aim of this study was to explain internode length differences due to the *Laurina* mutation in terms cell size and cell number. Histological analysis results showed that the two varieties (Bourbon vs Bourbon pointu) differed in two main aspects: 1/ smaller and fewer cells in Bourbon pointu; 2/ In both varieties, due to their lignification, the internodes reached their final size, after two new internodes had been emitted from the apex. In Bourbon, stem growth occurred via cell division (meresis) in the last emitted internode, then to cell elongation (auxesis) in the previous one (classical kinetic of growth), whereas in Bourbon pointu, growth was due to meresis only (almost no auxesis) in the two last internodes. Understanding the physiological mechanism controlling dwarfism in the Bourbon pointu variety will be our next challenge. Internode growth is generally under the control of plant hormones. We hypothesize that the *Laurina* mutation could modify internodes growth towards a modified plant growth regulation.

INTRODUCTION

Most *Arabica* varieties cultivated worldwide belong to the Typica or Bourbon groups. On the Réunion island, a dwarf Bourbon (B) tree appeared, which is known as "Bourbon pointu" (BP) (or the Laurina variety), due to the so-called *Laurina* mutation. The main scientific advantage of the *Laurina* mutation is its pleiotropic effect. Various traits, including resistance to dryness, seed shape, aroma and caffeine content are clearly concerned, including the presence of short internodes (Chevalier, 1947). The internode length – more than internode number variations – mainly accounts for plant size differences between the two varieties. The aim of this paper was to understand the effects of the *Laurina* mutation on internode growth in terms of cell division and elongation

MATERIALS AND METHODS

The plant material included B and BP varieties of the *Coffea arabica* L species. Widthwise sections were cut from fresh material using a vibratome. Varieties were compared at the macroscopic level on the basis of the internode number in 5-month-old and 18-month-old plants, under two environmental conditions. Observations consisted of measurements (cell width, cell height and pith diameter) and pith cell counts. Four locations were taken into

account: L1 at the first internode 5 mm below the apex (internode #K); L2, L3 and L4 sampled at the top, the middle and the base of the third internode from the top (internode #K-2), respectively.

RESULTS

In both varieties, due to lignification, internodes reached their final size after two new internodes had been emitted from the apex, so after three plastochrons (cycles of leaf primordium emergence in the shoot apex).

Among the cell dimensions, height appears to be the most important trait to explain internode length differences. In fact, differences between varieties change according to the cell location (L1 to L4), as shown by the presence of a "variety-location" interaction (Table 1). Whereas cell height did not change from L1 to L4 in BP, it doubled in the control variety. In the latter case, it could explain the lengthwise internode growth. Lastly, the BP/B ratio decreased from L1 to L4 (Table 2), i.e. when the distance from the shoot apical meristem increased. It could be around 64% at #K-3 internodes and lower, but this was not observed.

Table 1. Two-way ANOVA results for cell height (Cell_Ht), cell width (Cell_Wd) and cell shape (Cell_Shp) in Bourbon and Bourbon pointu. Significant effects are in bold.

	Between-variety		Between-location		Interaction	
	$F_{1, 88}$	p	$F_{3, 88}$	p	$F_{3, 88}$	P
Cell_Ht	5.76	**0.018**	34.5	**< 0.00001**	17.1	**< 0.00001**
Cell_Wd	293	**< 0.00001**	4.28	**0.007**	1.61	0.19
Cell_Shp	83.7	**< 0.00001**	11.4	**< 0.00001**	4.60	**0.005**

Table 2. Cell characteristics in Bourbon (B) and Bourbon pointu (BP) at four locations on the orthotropous axis. Letters in exposant indicate Tukey's test results.

	Bourbon pointu	Bourbon	Ratio (%)
	Cell_larg (µm)		
L1	36.1^{a}	53.4^{c}	67.6
L2	39.1^{b}	62.2^{d}	62.9
L3	38.2^{b}	60.4^{d}	63.2
L4	39.0^{b}	61.8^{d}	63.1
	Cell_Ht (µm)		
L1	36.9^{ab}	28.7^{a}	128.6
L2	38.6^{b}	34.9^{ab}	110.6
L3	41.8^{bc}	48.9^{c}	85.5
L4	41.0^{b}	57.6^{d}	71.2
	Cell_Form		
L1	0.99^{bc}	0.54^{a}	183.3
L2	1.01^{bc}	0.56^{a}	180.4
L3	1.07^{c}	0.82^{b}	130.5
L4	1.11^{c}	0.95^{bc}	116.8

Cell_Wd was always smaller at BP internodes (Table 2). Especially, the between-variety ratio was relatively constant whatever the cell location (64% on average).

The cell shape index confirmed the above observations (Tables 1 and 2). In BP, it was constant from L1 to L4, with 1.05 on average (cells were roughly square-shaped), whereas control cells were roughly rectangular at L1 and L2 and became square at L4 (Figures 1 and 2).

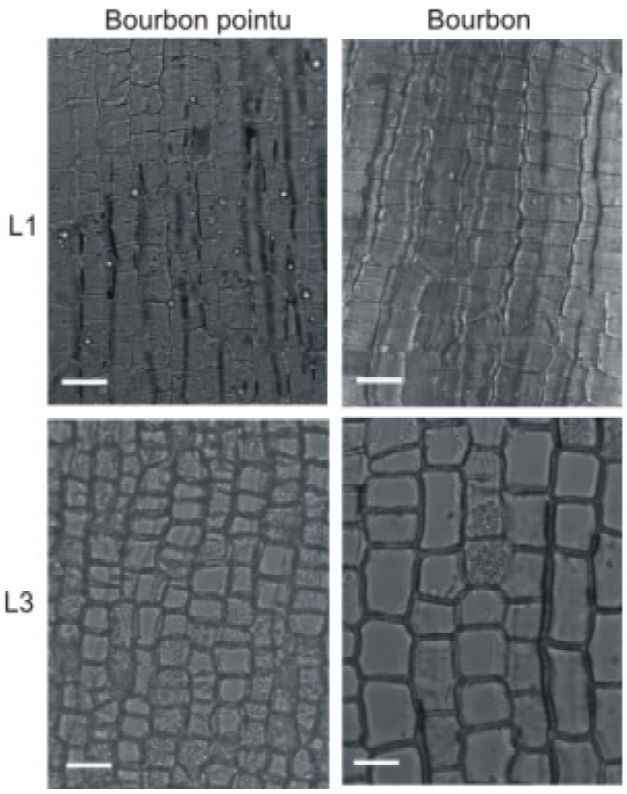


Figure 1. Cell shape in pith at locations L1 and L3 on the orthotropous axis in varieties Bourbon pointu and Bourbon. Bars represent 100 µm.

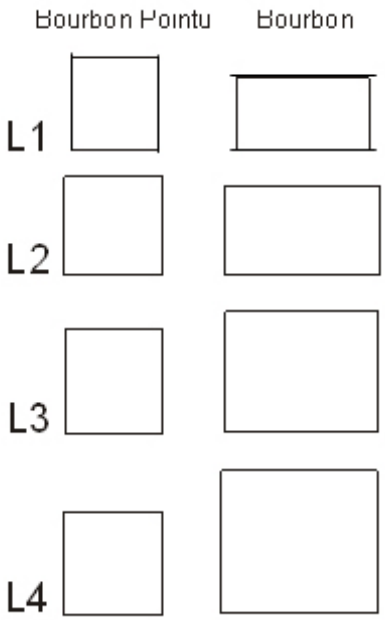


Figure 2. Variations in size and shape in pith cells at four localistions (L1 to L4) in varieties Bourbon pointu and Bourbon.

To sum up, in BP the cell shape is fixed as of L1, even though the cell size (height and width) increased from internodes #K to #K-2. On the contrary, cells in B were rectangular at L1, grew in size without shape modification from L1 to L2, and became increasingly square, without any width changes, from L2 to L4 (Figure 2). Consequently, the cell shape became similar in both varieties at end of growth.

Lengthwise cell number is the second trait that could explain differences in internode length. For example, in the last internode emitted, this parameter was six-fold lower in BP than in the control. Especially, the lengthwise cell number doubled from internode #K to internode #K-2 in BP, whereas it was fixed very early (as of #K) in the control.

CONCLUSIONS AND PERSPECTIVES

The varieties differed according to two main features:. In B, stem growth could be due to cell division (meresis) at the last emitted internode (L1), then to cell elongation (auxesis) at the previous one (L2 to L4) (classical growth kinetics), whereas in BP, growth was only due to meresis (almost no auxesis) at the last two internodes. Overall BP cells were smaller and fewer (Figure 1 and 2).

The basic internode elongation and histogenesis pattern in B is qualitatively similar in *Aesculus sylvatica, Quercus rubra, Liquidambar styraciflua, Salix nigra* and *Pinus taeda*, but with different development patterns along their orthotropic and plagiotropic axes (Brown and Sommer, 1992). In contrast, observations on the mutant BP did not entirely follow the traditional maturation pattern.

In L1 position, pith cells are uniformly small throughout and highly active mitotically as for a classic, newly formed internodes (Brown et al., 1992; Garrison, 1973). But during internode maturation (L2, L3 and L4), the *Laurina* mutation seemed to stop cell elongation. Consequently, the cell length gradient disappeared and cells kept the same length of about 40 µm, i.e. –33% relative to the cell length of the control in L4. It could be argued that cell elongation would happen latter. Nevertheless, as the lignification process is already quite advanced in L4, the ultimate internode length could be considered to be reached. In addition, when cell division was still present, its rate was slower than in B, leading to fewer cells at the end of growth.

In BP, cell number and cell height contributed to the final internode length. In sub-mature internode locations (L4), cell height and number were 71% and 40% of control, respectively. If only the cell height were affected, the final internode length would be 34 mm in BP *vs* 39 mm in control. In the opposite situation (only cell number decreases), the final length would be 19 mm. Therefore, cell number is the predominant histogenetic factor contributing to final internode length differences, as already recorded over a century ago (Harting, 1845; Moll, 1876).

The physiological origin of dwarfism in BP constitutes a future challenge. Internode growth is often regulated by plant hormones (Kende and Zeevaart, 1997; Reid, 1993). The *Laurina* mutation could regulate internode growth through regulation of plant hormones.

REFERENCES

Brown CL, Sommer HE. 1992. Shoot Growth and Histogenesis of Trees Possessing Diverse Patterns of Shoot Development. *American Journal of Botany* 79: 335-46.

Brown CL, Sommer HE, Wetzstein HY. 1994. Morphological and Histological Differences in the Development of Dwarf Mutants of Sexual and Somatic Origin in Diverse Woody Taxa. *Trees* 9: 61-66.

Chevalier A. 1947. Les caféiers du globe: III. Systématique des caféiers et faux-caféiers - Maladies et insectes nuisibles. In: Lechevalier P, ed. *Encyclopédie Biologique*. Paris.

Garrison R. 1973. The Growth and Development of Internodes in Helianthus. *Botanical Gazette* 134: 246-55.

Harting M. 1845. Recherches micrométriques sur le développement des parties élémentaires de la tige annuelle des plantes dicotylédonéees. *Ann Sci Nat Bot, Sér. 3*. 4: 210-79.

Kende H, Zeevaart AD. 1997. The Five "Classical" Plant Hormones. *The Plant Cell* 9: 1197-210.

Moll J. 1876. *The Influence of Cell Division and Cell Elongation on Growth (in Dutch)*. Utrech: L.E Bosh en zoom.

Reid JB. 1993. Plant Hormone Mutants. *J.Plant Growth Regul.* 12: 207-26.

Genetic Diversity within *Coffea canephora* Germplasm Maintained in RCI Using SSR Markers, Results and Future Prospects

P. CUBRY[1], P. MUSOLI[3], H. LEGNATE[4], P. ALUKA[3], M. DUFOUR[1], D. POT[2], F. DE BELLIS[1], T. LEROY[1]

[1]CIRAD, Perennial Crops Department, UMR 1096 PIA TA 80/03 Avenue Agropolis, 34398 Montpellier Cedex 5, France
[2]IAPAR (Instituto Agronômico do Paranà) LBI-AMG CP 481 86001-970 Londrina (PR) Brazil
[3]NARO-CORI P.O. Box 185 Mukono, Uganda
[4]CNRA, BP 808, DIVO, République de Côte d'Ivoire

SUMMARY

Robusta coffee, produced by *Coffea canephora*, represents about 35% of the world coffee production. As part of studies aiming at the improvement of coffee cup quality, the knowledge of the genetic diversity of the wild and cultivated populations is an important task. Up to 210 individuals from different geographic origin were screened for diversity with a set of microsatellites markers take from the whole genome. The results of this analysis are consistent with the previous studies made with other kind of markers (RFLP and isozymes). Several genetic parameters were computed and indicate a very high diversity and structuration of the species. Original wild material from Uganda (1) was also studied in order to replace it within the previously known groups. Several genetic parameters (e.g. PIC, F statistics) were computed in order to better know the dynamic of these populations. These results will be discussed in the context of *Coffea canephora* genetic improvement.

INTRODUCTION

Robusta coffee, produced by *Coffea canephora*, represents about 35% of the world coffee production. In the frame of studies aiming to the improvement of coffee quality and productivity, a study of genetic diversity of some wild and cultivated populations from the Divo reference collection (Ivory-Coast Republic) and from a new geographic origin, i.e. some Ugandan forests, has been performed. These analyses on the global diversity are of great importance to perform efficient association mapping studies.

MATERIALS AND METHODS

210 individuals from different geographic origin (see Figure 1), from wild and cultivated populations were used in this study, the exact composition of the panel was:

- 2 wild populations from the Guinean group (Western Africa)
- 1 population for each of the 4 subgroups of the Congolese group (Central Africa) i.e. SG1, SG2, B and C
- A new geographic origin from the forests of Uganda, named UW.

All those individuals were genotyped using 34 microsatellites markers (SSR). Those markers were covering the whole genome, since they were spread on a genetic intraspecific map of *Coffea canephora* in development at CIRAD.

A set of descriptive statistics including Gene Diversity (also referred to as Expected Heterozygosity), Observed Heterozygosity or Allele Number were calculated using PowerMarker software (Liu et al., 2005). A dissimilarity matrix was computed from the microsatellite data using DARwin 4.0 (Perrier et al., 2003). Moreover a factorial analysis on the dissimilarity matrix (AFTD) was performed using DARwin 4.0, in order to have an image of the diversity structure within the species. In order to estimate population differentiation, the F_{ST} coefficient was computed for all defined groups (Arlequin 3.0, Excoffier et al., 2005).

RESULTS

As expected, summary statistics show an important amount of diversity for the global sample (see Table 1).

Table 1. Mean values upon 34 microsatellites loci for summary statistics. SG1, SG2, B and C are sub-groups of the Congolese group (see Dussert et al., 2003)

	Allele number	Gene diversity	Observed Heterozygosity
SG1	2.38	0.37	0.27
SG2	6.74	0.69	0.41
B	5.09	0.50	0.37
C	2.97	0.45	0.37
Congolese	9.71	0.72	0.37
Guineans	5.29	0.50	0.35
Uganda	3.94	0.39	0.27
Global	12.50	0.73	0.35

Congolese group is the most diverse with a mean number of 9.71 alleles and a Gene Diversity coefficient of 0.72. Ugandan individuals show the lowest diversity in this sample.

Analyses performed with microsatellite markers confirm previous results obtained with other markers such as isozymes (Montagnon et al., 1992) or RFLP (Dussert et al., 1999), leading to a genetic structure in accordance with geographic distribution of the genotypes (see Figure 1). Ugandan wild individuals could be considered as a new sub group of the Congolese group, since they are clearly separated from the other groups.

Matrix of F_{ST} (Table 2) pointed out the high genetic differentiation between all the genetic groups. Guinean group seems to be the most particular since it presented only pairwise F_{ST} values higher than 0.4. Structure within the Congolese group is also important with some high values of F_{ST} between subgroups. UW seems to be related with SG2 and B Congolese groups which correspond to the closest geographic origins.

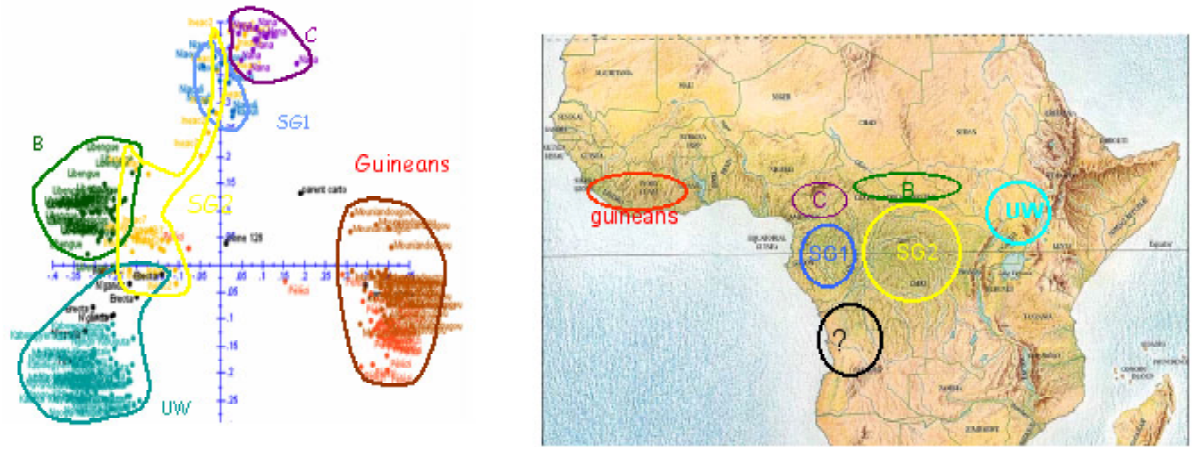


Figure 1. Factorial analyses based on dissimilarity for genotyping data and relation to geographical structuration

Table 2. Matrix of pairwise Fst by genetic groups. All Fst values are significative according to a permutation test.

	B	SG2	SG1	C	Guineans	UW
B	0					
SG2	0.2054	0				
SG1	0.4639	0.3277	0			
C	0.4106	0.2473	0.4976	0		
Guineans	0.4353	0.3270	0.4884	0.4354	0	
UW	0.2879	0.2789	0.5386	0.5406	0.4617	0

CONCLUSION

The high diversity among the *Coffea canephora* species and its structure in geographic groups was confirmed by new type or molecular markers. Moreover, a new genetic sub-group from Central Africa have been identified and possibly provide some interesting properties. This genetic diversity will be of great interest in association mapping studies to discover interesting traits for quality improvement.

ACKNOWLEDGEMENT

We are grateful to CNRA, République de Côte d'Ivoire for providing vegetal material. All the experiments were made on the genotyping platform of the Genopole Montpellier Languedoc Roussillon.

REFERENCES

Berline, M. **2002** Définition de marqueurs microsatellites polymorphes et cartographie génétique préliminaire d'une descendance intraspécifique de Coffea canephora. *In UFR SVT, DESS Bioingénierie, 34, Université Paul Sabatier, Toulouse III*

Cubry P. et al., **2005** Genetic diversity analyses and linkage disequilibrium evaluation in some natural and cultivated populations of *Coffea canephora,* in *Proceedings of the 4th Plant genomics European meeting*, *Amsterdam, September 20th-23th*.

Dussert, S. et al., **2003** Coffee (*Coffea canephora)*, pp.239-258 in *Genetic Diversity of Cultivated Tropical Plants, edited by P. Hamon, M. Seguin, X. Perrier and J. C. Glaszmann. Science Publishers. Inc. Enfield (NH), Plymouth*.

Montagnon, **2000** Optimisation des gains génétiques dans le schéma de selection recurrente réciproque de *Coffea canephora* Pierre, *Phd Thesis, ENSAM – CIRAD, Montpellier, France*

Perrier et al., **2003** Data analysis methods. *In Genetic diversity of cultivated tropical plants (Hamon, P., Seguin, M., Perrier, X.,Glaszmann, J. C., ed), 43 - 76, Enfield, Science Publishers*

Genetic Diversity in *Coffea* Genus Using Microsatellites Loci

P. CUBRY[1], D. POT[2], F. DE BELLIS[1], P. MUSOLI[3], P. ALUKA[3], H. LEGNATE[4], M. DUFOUR[1], T. LEROY[1]

[1]CIRAD – Tree Crops Department – UMR 1096 PIA (Polymorphismes d'Intérêt Agronomique) TA 80/03 Avenue Agropolis, 34398 Montpellier Cedex 5, France
[2]IAPAR (Instituto Agronômico do Paranà) LBI-AMG CP 481 86001-970 Londrina (PR) Brazil
[3]NARO-CORI P.O. Box 185 Mukono, Uganda
[4]CNRA, BP 808, DIVO, République de Côte d'Ivoire

SUMMARY

The objective of this study was to precise the known diversity in this genus with a whole genome coverage microsatellite set. Microsatellites markers derived from three different origins (SSR enriched library, gene sequences and BAC library?) were applied to a set of 45 individuals representing 20 species. The aim of this work was to validate with a large set of microsatellites previous diversity studies made with other markers (RFLP, isozymes) and to relocate the genetic diversity of *C. canephora* in a global context. Our results clearly validate the previously described geographic groups of the *Coffea* genus. Considering the rapid evolution of microsatellites, the genome organisation and the origin of the *C. Arabica* genome is discussed. Moreover, the interspecific transferability of microsatellites defined on a specific species was very good. The focus made on several species allowed us to make some comparisons on several genetic parameters e.g. PIC and mean number of alleles. The results and the future work on the basis of this set of markers and individuals are discussed.

INTRODUCTION

A better knowledge of *Coffea* genus diversity is a prerequisite for the genetical improvement of the two main cultivated species: *Coffea arabica* and *Coffea canephora*. This diversity is a potential reservoir for genes of agronomical interest, mainly those linked to quality, transferable to cultivars through marker-assisted selection. Previous studies have been performed using several markers-types such as RFLP, AFLP and isozymes. A set of microsatellites markers from different origins was built on their easiness of use, in order to enlarge knowledge on genetic structure and genetic relationships among the genus. Furthermore, stressing on the *Coffea canephora* data is of importance for our future works considering genetic diversity studies and linkage disequilibrium.

MATERIALS AND METHODS:

- 42 individuals representing 15 species were genotyped using 60 microsatellites loci from four different origins:
  - microsatellites enriched library from *Coffea arabica* var. Cattura
  - microsatellites enriched library from *Coffea canephora* clone 126
  - a BAC library from *Coffea canephora* clone 126
  - gene sequences of *Coffea canephora*

  The 60 microsatellites loci have been chosen to cover the entire genome

Table 1. List of vegetal material and providers.

Species	Work Name	Variety or diversity group	Collection
arabica	Arabica_caturra	caturra	IRD, France
arabica	Arabica_catuai_red1	catuai red 1	Cirad, French Guyana
arabica	Arabica_guinee_pita1	guinee pita 1	Cirad, French Guyana
arabica	Arabica_sidamo1	sidamo 1	Cirad, French Guyana
arabica	Arabica_mondo_novo	mondo novo	Cirad, French Guyana
arabica	Arabica_et19	wild ethiopian arabica	Cirad, French Guyana
arabica	Arabica_et11	wild ethiopian arabica	Cirad, French Guyana
arabica	Arabica_et55	wild ethiopian arabica	Cirad, French Guyana
canephora	C1001	congolese group B	CNRA, République de Côte d'Ivoire
canephora	C4003	congolese group C	CNRA, République de Côte d'Ivoire
canephora	C5001	congolese group SG2	CNRA, République de Côte d'Ivoire
canephora	G1001	guinean	CNRA, République de Côte d'Ivoire
canephora	G7032	guinean	CNRA, République de Côte d'Ivoire
canephora	UN23	nganda	CORI, Uganda
canephora	UW114	uganda wild	CORI, Uganda
canephora	UW99	uganda wild	CORI, Uganda
canephora	02183	guinean	CNRA, République de Côte d'Ivoire
congensis	Congensis		IRD, France
congensis	Congensis_16		Cirad, French Guyana
congensis	Congensis_19		Cirad, French Guyana
congensis	Congensis_21		Cirad, French Guyana
congensis	Congensis_23		Cirad, French Guyana
liberica	Liberica		IRD, France
liberica	Liberica_27	liberica	Cirad, French Guyana
liberica	Liberica_28	liberica	Cirad, French Guyana
liberica	Liberica_29	liberica	Cirad, French Guyana
liberica	Liberica_31	dewevrei	Cirad, French Guyana
liberica	Liberica_32	dewevrei	Cirad, French Guyana
liberica	Liberica_33	dewevrei	Cirad, French Guyana

bertrandii	Bertrandii		IRD, France
brevipes	Brevipes		IRD, France
eugenioides	Eugenioides		IRD, France
humilis	Humilis		IRD, France
milloti	Milloti		IRD, France
anthoniy	anthonyi		IRD, France
pseudozanguebariae	Pseudozanguebariae		IRD, France
racemosa	Racemosa		IRD, France
salvatrix	Salvatrix		IRD, France
sessiliflora	Sessiliflora		IRD, France
sessiliflora	Sessiliflora_24		Cirad, French Guyana
sessiliflora	Sessiliflora_26		Cirad, French Guyana
stenophylla	Stenophylla		IRD, France

- 4 species of interest have a higher number of individuals, the two cultivated ones *Coffea arabica* and *Coffea canephora* and two species used for breeding purposes *Coffea liberica* and *Coffea congensis*.
- A dissimilarity matrix was computed from the data file using a simple matching distance index to serve as the basis of a Neighbor-Joining tree construction (DARWin 5.0, Perrier et al., 2003).

Descriptive statistics (for results of amplifications) and summary genetic parameters were calculated. We estimate amount of amplifications by dividing markers presenting at least one scorable band upon the total number of markers for each individual. Gene diversity, an analogue of expected heterozygosity, observed heterozygosity and allele number were computed using PowerMarker software (Liu et al., 2005) and confidence intervals were calculated for each parameter using 5000 bootstrap iterations.

RESULTS

Amount of amplifications, ranging from 51.67% to 98.33% with a mean of 82.60%, demonstrate a good transferability of microsatellites markers through the genus.

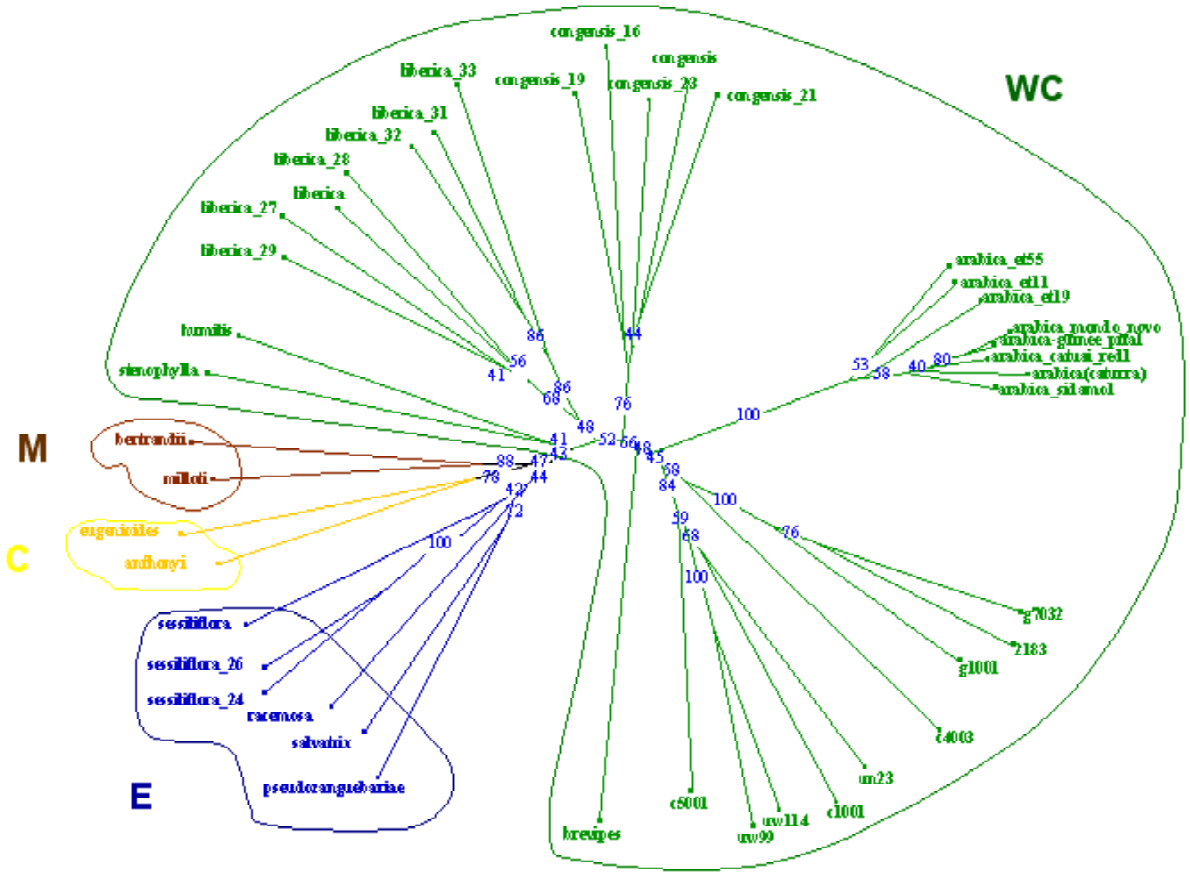


Figure 1. NJ-tree for the 42 individuals analyzed based on the dissimilarity matrix calculated by simple matching. Bootstrap values are calculated with 5000 reps, only values more or equal to 40 are indicated

Genetic groups shown on Figure 1 (i.e. Central Africa clade (C), West and Central Africa clade (WC), East Africa clade (E) and Madagascar clade (M) as described by Lashermes et al., 1997), are congruent with previous studies and well cover the geographic distribution of the species.

Table 2. Summary statistics on microsatellites observed alleles. Mean values and Standard Deviation (SD) are represented for Number of Alleles (Allele No), Gene Diversity, analogue to Expected Heterozygosity (Gene Div.) and Observed Heterozygosity (Obs. Het.). Data show values for the overall set of 15 species and for the 4 species of breeding interest.

	Overall 15 species			*Coffea arabica*			*Coffea canephora*		
	Allele No	Gene Div.	Obs. Het.	Allele No	Gene Div.	Obs. Het.	Allele No	Gene Div.	Obs. Het.
Mean	10.82	0.72	0.32	2.10	0.30	0.48	4.81	0.55	0.30
SD	0.57	0.03	0.02	0.14	0.03	0.06	0.30	0.03	0.03

	Coffea congensis			*Coffea iberica*		
	Allele No	Gene Div.	Obs. Het.	Allele No	Gene Div.	Obs. Het.
Mean	2.86	0.35	0.27	3.62	0.45	0.35
SD	0.21	0.03	0.04	0.24	0.03	0.04

Global diversity is high (see Table 2) with a mean number of allele of 11. The four species of interest show some important differences. Indeed, *Coffea arabica* is the less diverse and the only one to show gene diversity smaller than observed heterozygosity, likely due to its particular system of reproduction. The other three species reveal the same properties, even if *Coffea canephora* shows higher gene diversity.

CONCLUSION

This study have shown the interest of SSR markers to make genetic studies within the *Coffea* genus, even if their evolution is faster than other usable markers like RFLP or AFLP. Moreover, their properties (neutrality, codominance) make those markers particularly interesting in population genetics. The high genus transferability of SSR markers might be an important basis for comparative genomic studies, breeding assistance and knowledge transfer.

As expected, the four species on which we focused our attention are all grouped in the Central Africa clade. The amount of diversity in those species is very large. Those results clearly validate *Coffea liberica* and *Coffea congensis* as potential usable diversity resources for the improvement of *Coffea arabica* and *Coffea canephora*. The knowledge on diversity and genetic structuration within and between those species is of interest for future Arabica breeding purposes.

ACKNOWLEDGEMENT

We are grateful to IRD, CNRA and CORI for providing vegetal material used in this study. All the experiments were made on the genotyping platform of the Genopole Montpellier Languedoc Roussillon.

REFERENCES

Cubry, P et al.; Diversity in *Coffea* genus: a comparative approach using SSR markers from different origins. *in prep*

Dufour et al., **2002** Potential use of SSR markers for *Coffea* spp. genetic mapping. [CD-ROM]. In : *19th international colloquium on coffee science ASIC, 2001-05-14/2001-05-18 ; Trieste, Italy*. ASIC, Paris, France.

Leroy et al., **2005** Construction and characterization of a *Coffea canephora* BAC library to study the organization of sucrose biosynthesis genes. Theor Appl Genet **111:** 1032-1041.

Poncet, **2004** SSR cross-amplification and variation within coffee trees (*Coffea* spp.). Genome 47:1071-1081.

Biochemical and Nucleotide Variability in the Diterpene Metabolism for *Coffea canephora* Genotypes from Uganda

N. DURAND[1], F. de BELLIS[2], I. JOURDAN[2], J. C. MANEZ[1], P. ALUKA[3], L. G. VIEIRA[4], J. OGWANG[4], D. POT[4], P. MARRACCINI[5], T. LEROY[2], B. GUYOT[1]

[1]CIRAD-CP UPR Quality of perennial products TA 80/16 Rue JF Breton, 34398 Montpellier Cedex 5, France
[2]CIRAD-CP UMR T52 PIA TA 80/03 Avenue Agropolis, 34398 Montpellier Cedex 5, France
[3]NARO-CORI P.O. Box 185 Mukono, Uganda
[4]IAPAR (Instituto Agronômico do Paraná) LBI-AMG CP 481 86001-970 Londrina (PR), Brazil
[5]EMBRAPA-CENARGEN, parquet estacao biologica, CEP 70770-900, Brasilia DF, Brasil

SUMMARY

In the current context of overproduction and low prices, coffee producers give a particular attention to coffee quality, impact on human health being one of its components. In this context, diterpens content constitutes a relevant target trait (Cavin et al., 1998). Coffee diterpens (Cafestol, Kahweol and 16-O-Methylcafestol) are specific of the coffee genus(Finnegan et Djerassi, 1960; Speer and Mischnick, 1989). Little information is available on coffee diterpens metabolism. Today only the enzymes implied in the way of biosynthesis of Ent-Kaurene are known. The ultimate stages between Ent-Kaurene and Cafestol, Kahweol, 16-O-MC are not identified yet.

ENT-KAURENE PATHWAY

Studies are currently in hand to identify these enzymes and the genes (Figure 1).

The three genes analysed are: *ko (Kaurene Oxydase), ks (Kaurene Synthase) and cps (Ent-copalyl diphosphate synthase)*. For each of these genes, using ESTs (Expressed Sequenced Tag) developed by the "Genoma café" project (http://www.lge.ibi.unicamp.br/cafe) two to three regions located either in the protein coding domain or in the 3'UTR have been selected.

The three genes analysed are: *ko (Kaurene Oxydase), ks (Kaurene Synthase) and cps (Ent-copalyl diphosphate synthase)*. For each of these genes, using ESTs (Expressed Sequenced Tag) developed by the "Genoma café" project (http://www.lge.ibi.unicamp.br/cafe) two to three regions located either in the protein coding domain or in the 3'UTR have been selected.

Aims of the study were to know:

- The variations of diterpens and lipids content in the genotypes of coffee-trees originating from Uganda,
- The nucleotidic variability of genes implied in the biosynthesis of diterpens.

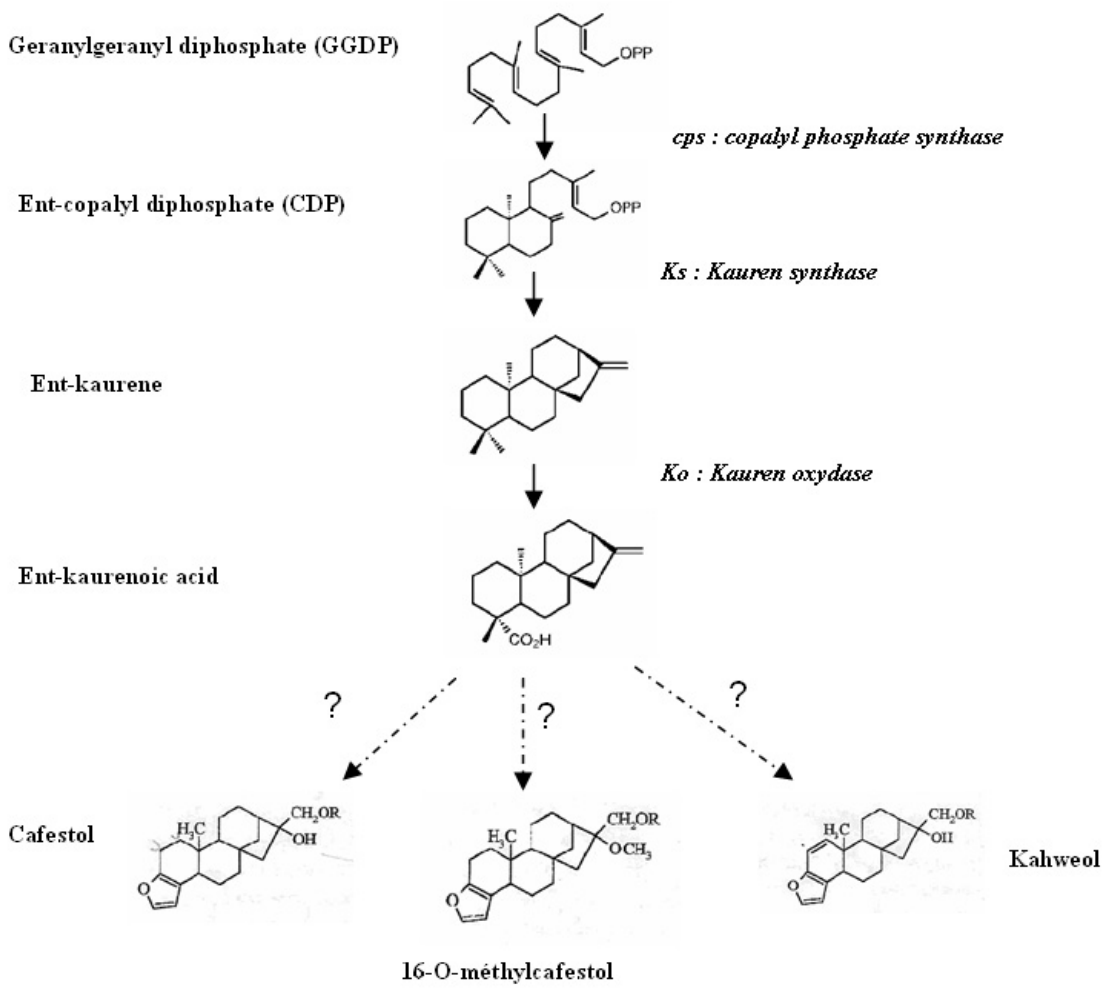


Figure 1. Ent-Kaurene pathway.

MATERIAL AND METHODS

- 48 genotypes from Uganda have been analysed for their biochemical content in diterpens and nucleotide diversity:
  - Erect "UE" forms imported,
  - N'Ganda "UN" presumedly native of Uganda,
  - Hybrid "UH" between UE and UN,
  - Farm "UF" prospected in farms located in various Ugandan areas.

- The quantification of the diterpens was carried out according to the method described by Eloy Dias (communication personal, 2006).
- The quantification of the lipids was carried out according to the method described by Folstar et al. (1976).
- The sequencing was carried out on 3 genes (*ko, ks, cps*) involved in diterpens metabolism.
- The alignment of the sequences was carried out using the software Codon Code Aligner v 1.5.2 .

RESULTS

- **Lipids and diterpens content (Figure 2 et Figure 3)**

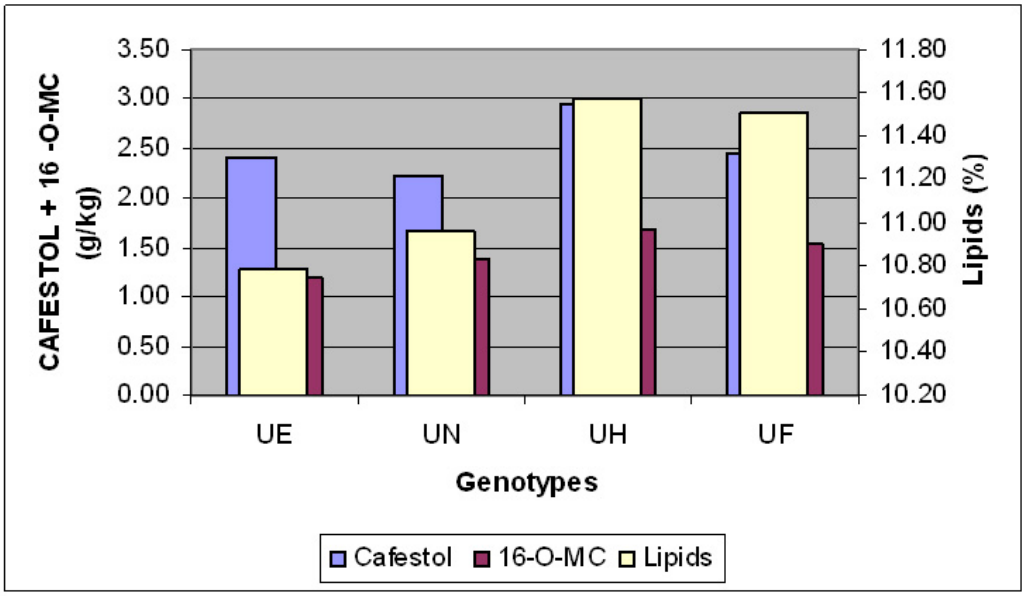


Figure 2. Lipids and diterpens content.

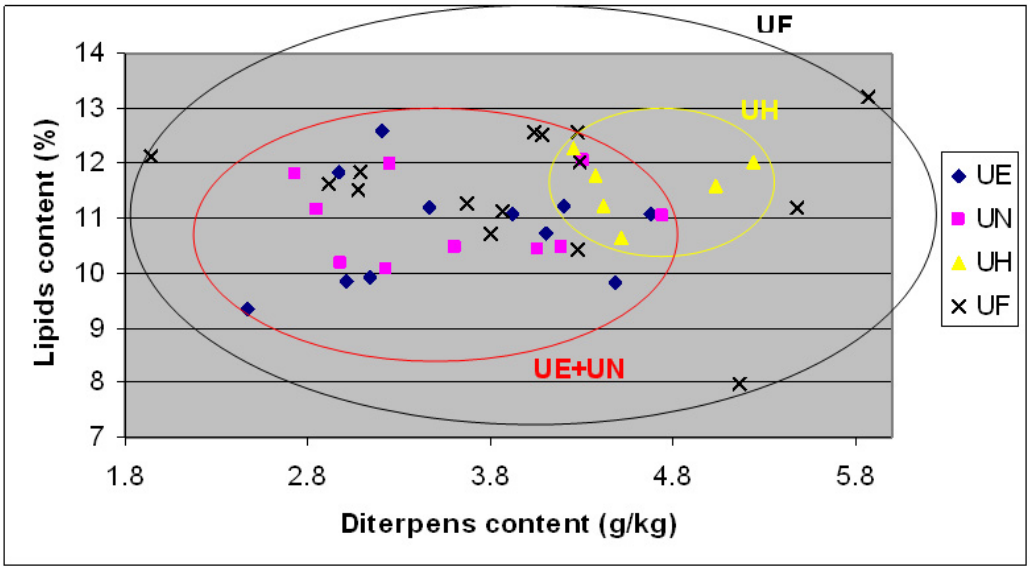


Figure 3. Individual variability for diterpens and lipids content.

- Lipids content *C. canephora* Uganda (11.2%) > *C. canephora* (9%).
- Kahweol content < 0.1 g/kg on all samples.
- Diterpens : Cafestol + 16-O-MC (g/kg):
  - UE (3.61) and UN (3.59) are similar,
  - UH higher content (4.64),
  - UF higher variability (1.94 to 5.87).

- **Nucleotide diversity (Figure 4)**

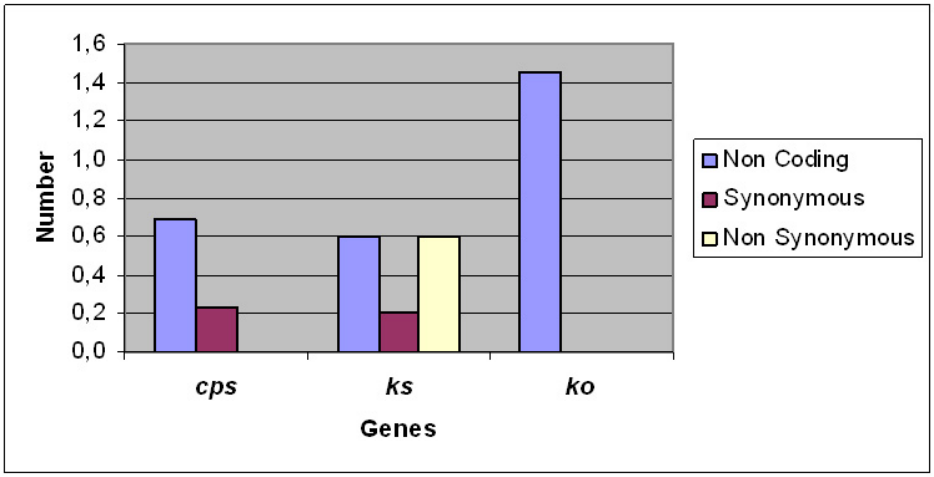


Figure 4. Number of polymorphisms for 100 pb.

- *cps* : Nucleotidic variability relatively not significant.
- *ks* : Presence of non synonymous mutations.
- *ko :* Very polymorphic on the non coding areas.
- Polymorphisms have been identified: allowing to separate UE versus others genotypes.

CONCLUSION

This study confirms the interest of the genotypes of Uganda's *C. canephora* for their quality. A high content of lipid is a major element for the quality of the coffees. In addition one observes an important variability of the terpenic compounds in the analyzed genetic groups. The important variability observed on the genotypes of farm are explained probably mainly by environmental effects.

Polymorphisms have been identified within analysed sequences in the Ugandan population. And particularly, polymorphisms allowing to separate the genotypes UE from the others Ugandan genotypes.

REFERENCES

Cavin C, Holzhaüser D, Constable A, Hugget AC, Schilter B (1998) The coffee-specific diterpenes cafestol and kahweol against aflatoxin B1-induced genetoxicity through a dual mechanism. *Carcinogenesis 19*, 1369-1375

Finnegan, R.A, et Djerassi, C (1960) Terpenoids XLV. Further studies on the structure and absolute configuration of cafestol. *J. Am. Chem. Soc*., 82, 4342-4

Folstar, P., Pilnick, W., de Heus, J.G. et van der Plas, H.C (1976) The composition of fatty acids in coffee oil and wax. *Lebensmittelwiss. technol*., 8, 286-8

Speer,K. and Mischnick, P.(1989) 16-O-Methylcafestol-ein neues Diterpen im Kaffee. *Z. Lebensm. Unters.-Forsch*., 189, 219-22.

This research project was supported by the USDA and the ICRAF. N° 58-4001-3-F157 project USDA-ICRAF

Collection and *ex situ* Conservation of Coffee Landraces in Ethiopia – The Example of Harerge

J.-P. LABOUISSE[1], B. BELLACHEW[2], C. HAMELIN[3], S. KOTECHA[4], B. BERTRAND[1]

[1]CIRAD, UMR DGPC, Montpellier, F-34398 France; E-mail: labouisse@cirad.fr
[2]JARC-EIAR P.O. Box 192, Jimma, Ethiopia
[3]CIRAD, UMR PIA, Montpellier, F-34398 France
[4]Consultant ICO-EU-WB, London W9-1BQ, England

SUMMARY

The south-western highlands of Ethiopia are recognized as the primary centre of diversity of *Coffea arabica* L. However, relatively little is known about the situation of coffee genetic resources collected and conserved *ex situ* within Ethiopia. In 2005, we started compiling an electronic database of coffee genetic resources conserved at the Jimma Agricultural Research Centre (JARC). Since 1967, 48 campaigns of collections have been undertaken by the Ethiopian research organization, with the main objectives to capture the genetic diversity and search for genotypes tolerant to coffee berry disease. To date (July 2006), 4731 distinct accessions are presently conserved and evaluated by JARC in field gene-banks at Jimma-Melko centre and at 9 sub-centres located in the main coffee growing areas. In Harerge, a coffee zone in the East of the country, coffee is produced in highly diversified farming systems adapted to different ecological niches. Harerge fetches premium prices in the world market but this resource is under threat of erosion. Out of 30 Harerge *woredas* (districts) with significant coffee production, JARC has collected coffee landraces in 20 woredas. In all, a total number of 1952 Harerge accessions are conserved in JARC field gene-banks at the Jimma-Melko centre and Mechara sub-centre. They are assessed regularly for yield, disease tolerance, and quality.

INTRODUCTION

Ethiopia holds a unique position in the world as *Coffea arabica* L. has its primary centre of diversity in the south-western highlands of that country. This fact is strongly substantiated by observations of travellers and scientists (Sylvain, 1958; Meyer, 1965) and, more recently, by studies using DNA-based genetic markers (Lashermes et al., 1996; Anthony et al., 2001).

On a world scale, commercial exploitation of Arabica coffee is based on a small number of cultivars, Typica and Bourbon varieties, along with their mutants and hybrids, which makes this crop particularly vulnerable to biological and climatic risks. Consequently, collection and conservation of the wild coffee populations and landraces existing in Ethiopia have long been acknowledged as being of major interest in capturing genetic diversity for future crop improvement (Meyer et al., 1968; Guillaumet and Hallé, 1967). However, relatively little is known about the situation of coffee genetic resources collected and conserved *ex situ* within Ethiopia, despite a brief overview drawn up about ten years ago (FAO, 1996; Bellachew, 1997).

In 2005, we started building up an electronic database of coffee genetic resources conserved at the Jimma Agricultural Research Centre (JARC), which is in charge of coffee research

within the Ethiopian Institute of Agricultural Research (EIAR). A summary of the information generated by that database is presented here. We have chosen Harerge, a coffee zone in the East of the country, to illustrate this collection and conservation work on Ethiopian coffee landraces.

MATERIAL AND METHODS

We used Microsoft® Access 2002 for constructing a relational database and DIVA-GIS Version 5.2.0.3 software (Hijmans et al., 2005) for mapping of the collection and conservation sites.

RESULTS

Current status of coffee germplasm conserved ex situ by JARC

The collection made by ORSTOM (Guillaumet and Hallé, 1967) was the first one successfully established at Jimma research center in 1967 under the name of French collection. From this date, 47 other collections have been undertaken by Ethiopian researchers. The main objectives were the capture of the genetic diversity and the search of genotypes tolerant of coffee berry disease (CBD). This disease, caused by *Colletotrichum kahawae***,** a parasite unknown until 1970 in Ethiopia, provokes a significant drop in the production. A research programme to select naturally CBD-tolerant coffee landraces has been under way at JARC since 1973 (Robinson, 1973; Van der Graaff, 1981).

To date (July 2006), 5109 genotypes were collected in 101 *woredas* (or districts) and 63 % of the *woredas* with more than 500 hectares planted with coffee are represented in the collections. In total, 4731 distinct accessions are conserved and evaluated by JARC in field genebanks at Jimma-Melko center and at 9 sub-centres or testing stations located in the main coffee growing areas (Figure 1).

Collection of Harerge coffee landraces

In the eastern part of Ethiopia, coffee is found in the East Harerge, West Harerge, and Arsi zones at an elevation of between 1,600 and 2,000 metres.

Local coffee landraces are well adapted to these drought-prone areas where they are grown in association with other crops in open sunlight or under a few shade trees. Farmers have developed many names – 17 in number were recorded by Bayetta (1987) – to distinguish between coffee landraces according to their morphological characteristics. By segregating these landraces and using different ecological niches and farming practices, farmers have maximized the use of the genetic diversity within the species.

Harerge coffee fetches premium prices in the world market. This sun dried coffee has an overall cup taste profile displaying a typical mocha flavour, with chocolate notes, in a medium-dense body and a mild, soft acidity with light fruitiness. Usually coffees from the East Harerge zone achieve an additional premium especially for the more distinctive golden yellow coloured long-berry beans which are also differentiated by softer tones of characteristics described above and yet still balanced in aroma and flavour. Arsi zone coffee was mixed with Harerge coffee earlier and is now further differentiated and marketed separately.

However, this coffee germplasm is under threat of genetic erosion due to its high susceptibility to coffee berry disease and coffee leaf rust. Die-back frequently occurs due to poor management standards and inadequate shade. Coffee cultivation also faces competition from food crops and **chat** (*Catha edulis*), the tender leaves and shoots of which are chewed as a mild drug in the Horn of Africa.

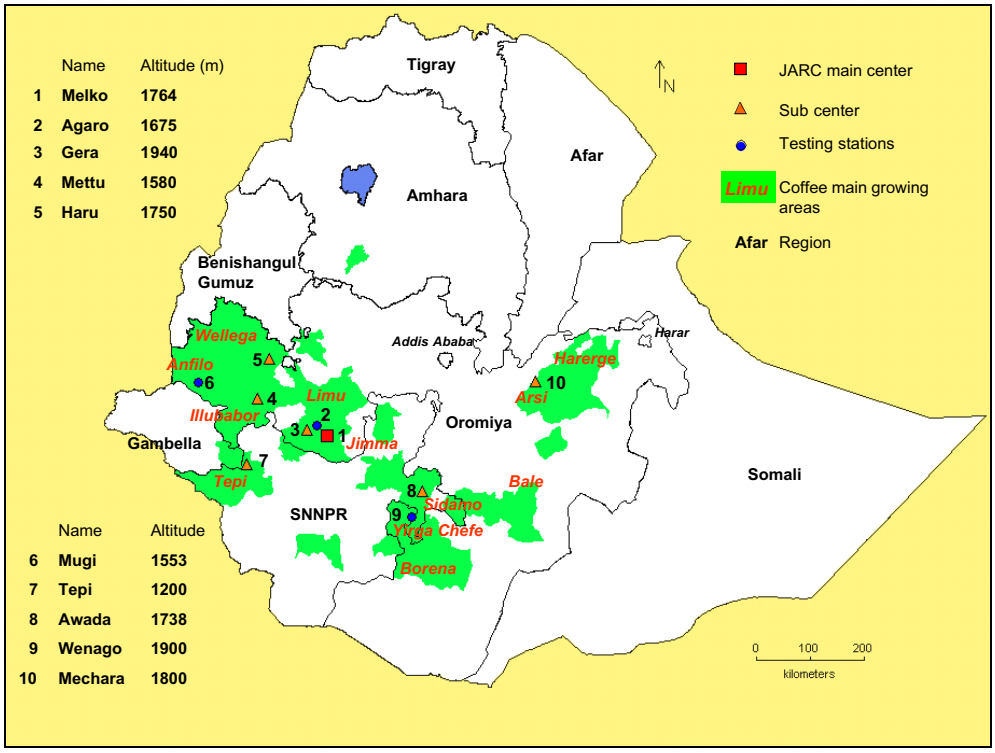


Figure 1. Sites for *ex situ* conservation and evaluation of coffee landraces in Ethiopia.

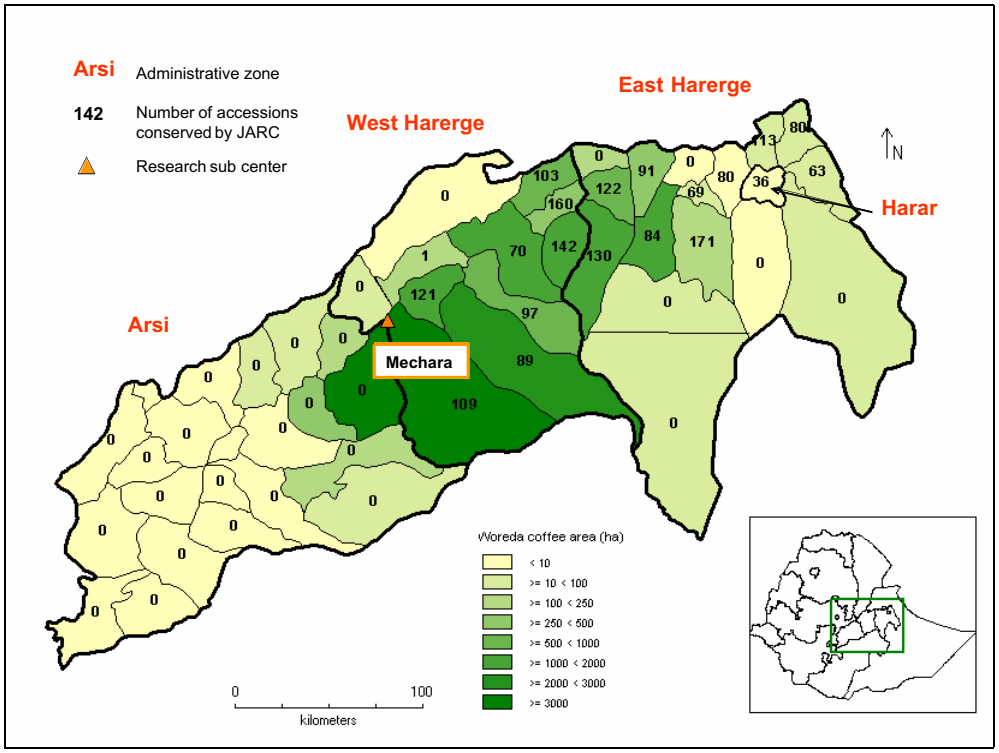


Figure 2. Number of accessions originated from different *woredas* in Harerge and Arsi zones conserved by JARC.

Out of 30 *woredas* with significant coffee production, 20 have been surveyed so far (Figure 2). In all, a total of 1952 Harerge accessions is conserved in the JARC field genebanks at the Jimma-Melko centre and Mechara sub-centre, and assessed regularly for yield, disease tolerance, and quality.

The medium-term objective of the JARC breeding programme is to supply Harerge farmers with CBD-tolerant, high-yielding landraces, suitably adapted to their ecological niches, and with the unique Harerge coffee flavour profiles.

CONCLUSION

By using passport data and GIS technology, collection gaps are quickly recognized. This analysis will be further refined by overlaying thematic base maps (climate, soils) combined with agricultural censuses, satellite imagery, and field work data to identify areas under imminent threat of genetic erosion. Maps can also be adapted for marketing, with clearer identification of origins and taste profiles to increase buyer awareness.

ACKNOWLEDGEMENTS

We thank Coffee Improvement Project IV in Ethiopia and European Development Fund for the financial support.

REFERENCES

Anthony F, Bertrand B, Quios O et al. (2001). Genetic diversity of wild coffee (Coffea arabica L.) using molecular markers. Euphytica, 118: 53-65.

Bellachew B (1997). Arabica coffee breeding in Ethiopia: a review. In: 17ème Colloque Scientifique International sur le Café, Nairobi, Kenya, 20-25 juillet 1997., Paris: ASIC, 406-414.

Bayetta B (1987). Coffee (Coffee arabica L) genetic and germplasm collection in Harerge region. PGRC/E - ILCA germplasm Newsletter, 15: 8-13.

Bellachew B, Atero B, Tefera F (2000). Breeding for resistance to coffee berry disease in arabica coffee: Progress since 1973. In: Proceedings of the workshop on control of Coffee Berry Disease (CBD) in Ethiopia held in Addis Abeba. 13-15 August 1999, Addis Abeba: Ethiopian Agricultural Research Institute, 85-98.

FAO (1996). The state of the world's plant genetic resources for food and agriculture. Background documentation prepared for the International Technical Conference on Plant Genetic Resources. Leipzig, Germany, 17-23 June, 1996. FAO, Rome.

Guillaumet JL, Hallé F (1967). Etude de la variabilité du Coffea arabica dans son aire d'origine. Rapport sur la mission ORSTOM dans le Sud-Ouest de l'Ethiopie. 12 novembre-18 décembre 1966. ORSTOM, Adiopodoumé, Côte d'Ivoire.

Hijmans R, Guarino L, Jarvis A, O'Brien R, Mathur P (2005). DIVA-GIS Version 5.2.0.3, available at http://www.diva-gis.org/.

Lashermes P, Trouslot P, Anthony F, Combes M, Charrier A (1996). Genetic diversity for RAPD markers between cultivated and wild accessions of Coffea arabica. Euphytica, 87: 59-64.

Meyer FG (1965). Notes on wild Coffea arabica from southwestern Ethiopia, with some historical considerations. Econ.Bot., 19, 2: 136-151.

Meyer FG, Fernie LM, Narasimhaswamy RL, Monaco LC, Greathead DJ (1968). FAO coffee mission to Ethiopia 1964-1965. FAO, Rome.

Robinson RA (1973). Annual report to the national Coffee Board of Ethiopia. IAR, Addis Abeba.

Van der Graaff NA (1981). Selection of arabica coffee types resistant to coffee berry disease in Ethiopia.Thesis. Wagenigen.

Sylvain PG (1958). Ethiopian coffee. Its significance to world coffee problems. Econ.Bot., 12: 111-139.

Chemical Characterization of *Coffea arabica* Accessions from Instituto Agronômico Coffee Germplasm Collection

T. J. G. SALVA, V. B. DE LIMA, G. MARTIN, A. SANVIDO, M. B. SILVAROLLA, L. C. FAZUOLI, O. GUERREIRO-FILHO

Instituto Agronômico (IAC), Centro de Café 'Alcides Carvalho', Campinas, Brazil
E-mail: tsalva@iac.sp.gov.br

SUMMARY

Instituto Agronômico (IAC) Coffee Germplasm collection is composed of 16 species of *Coffea* and 2 of *Psilanthus* genera available for breeding programs. Highly productive varieties currently cultivated in Brazil and in other coffee producer countries have been originated from this germplasm collection. In order to explore the potential of utilization of the available material in future genetic improvement programs, forty four *C. arabica* accessions were analyzed for soluble solids, reducing sugars, total reducing sugars, trigonelline, total chlorogenic acids and caffeine. Chemical diversity was confirmed in all these grain components. Ethiopian and Yemen introductions enriched the possibilities of breeding focusing cup quality and nutraceutical characteristics. Some of these gene types are very different of genetic improved cultivars and varieties currently available. Although typical total reducing sugar concentration in Arabica is around 7% according the methodology employed, an Ethiopian accession exhibited concentration as high as 9.43%. Also, trigonelline, total chlorogenic acids and caffeine were characteristically high in accessions from Ethiopia.

INTRODUCTION

Traditionally, Instituto Agronômico (IAC) coffee breeding program have focused mainly productivity, plant height and resistance to several biotic and abiotic stress. The development of cultivars such as Mundo Novo, Catuaí Vermelho and Icatu Vermelho are examples of progressive genetically improved cultivars with economic returns to coffee growers. The main characteristic of Mundo Novo cultivar is the plant vigour and yield stability and Catuaí Vermelho combines the high productivity of Mundo Novo and compact architecture of Caturra. Both, Mundo Novo and Catuaí Vermelho are responsible for about 90% of the total Arabica coffee plants growing in Brazil. Other successful strategy of coffee breeding employed interspecific hybridization between a tetraploid plant of *C. canephora* and *C. arabica* cultivar Bourbon Vermelho. The technique resulted in Icatu germplasm, resistant to nematodes and *Colletotrichun kahawae.* However, nowadays this program is focusing also nutraceutical properties and cup quality. It is known that some chemical compounds of coffee beans like sugars (De Maria et al., 1996), protein, polysaccharides (Nunes et al., 1997), chlorogenic acids (De Menezes, 1994), trigonelline (Viani and Horman, 1974), caffeine (Webster et al., 2000) and lipids (Kurzrock et al., 2004) determine these characteristics. The aim of this research was to explore the genetic diversity of coffee plants belonging to the IAC Coffee Germplasm Collection. In this paper results regarding to soluble solids, reducing sugars, total reducing sugars, trigonelline, total chlorogenic acids and caffeine concentration in representative varieties and cultivars of *C. arabica* germplasm are presented.

MATERIAL AND METHODS

Plant material

22 cultivars and botanical varieties (codes 1 to 22), 11 Ethiopian introductions (codes 23 to 33) from the Kaffa region (FAO, 1968)and 11 introductions (codes 34 to 44) from Yemen (Table 1) belonging to the IAC Coffee Germplasm Collection were evaluated.

Table 1. *C. arabica* germplasm chemically analyzed.

Genetic Material	Code	Genetic Material	Code	Genetic Material	Code
Bourbon Vermelho* IAC	1	Mundo Novo	16	IAC 3856-3	31
Agaro	2	Catuaí	17	IAC 3996-4	32
Geisha	3	Catuaí	18	IAC 3996-5	33
Vila Sarchi	4	Sudan Rume	19	IAC 4128 PDRY	34
Bourbon Amarelo*	5	Gláucia	20	IAC 4120 PDRY	35
Nacional	6	Amphylo	21	IAC 4123 PDRY	36
Laurina	7	IAC 4309	22	IAC 4118 PDRY	37
Moka Grande	8	IAC 3856-1Ethiopia	23	IAC 4116 PDRY	38
Caturra Vermelho* IAC	9	IAC 3971-1 Ethiopia	24	IAC 4114 PDRY	39
Caturra Amarelo* IAC 476	10	IAC 3971-2 Ethiopia	25	IAC 4117 PDRY	40
Semperflorens	11	IAC 3996-1 Ethiopia	26	IAC 4135 PDRY	41
Mundo Novo Vermelho*	12	IAC 3971-3 Ethiopia	27	IAC 4122 PDRY	42
Mundo Novo Vermelho*	13	IAC 3996-2 Ethiopia	28	IAC 4129 PDRY	43
Ibaaré	14	IAC 3996-3 Ethiopia	29	IAC 4124 PDRY	44
Mundo Novo Amarelo* 2	15	IAC 3856-2 Ethiopia	30		

*Code numbers were used instead of cultivar names in Results and Discussion. *Vermelho = Red Fruits and Amarelo = Yellow Fruits.*

Sample Preparation

Fully ripe fruits were harvested in 2004, processed by wet method and sun dried to around 11% moisture**.** Only hulled non-defective grains were employed in the analyses. Duplicates of whole grains were used to moisture determination and duplicates of ground grains (≤ 0.5 mm) were used in chemical analyses.

Free carbohydrates

Soluble sugars and carbohydrates were extracted in hot water as described in Rogers et al. (1999). Reducing sugars concentration was determined by Somogyi-Nelson, using glucose in standard curve. Free low molecular carbohydrates were previously transformed to reducing sugars by treatment with HCl (37%) at 70 °C, followed by neutralization, and then quantified by that same methodology. Therefore, they were nominated total reducing sugars.

Soluble Solids

Concentration of soluble solids was determined according to method 30.1.21 of AOAC (1997).

Trigonelline, Total Chlorogenic Acids and Caffeine

Samples of 100 mg of coffee were kept 70% aqueous methanol at 60 °C during 1 hour. After centrifugation and filtration, the components were quantified using C_{18} Shim-pack CLC-ODS(M) column and UV detector at 272 nm. Elution was performed with methanol/water/AcOH (50/49.5/0.5 v/v/v) at 1ml/min.

RESULTS AND DISCUSSION

Soluble solid content ranged from 27 to 32.8% with the distribution showed in Figure 1 (A). Varieties with concentration between 29.3 and 30.3% were in greatest proportion (36.4%). The second major group (31.8%) included varieties plus Yemen and Ethiopian accessions 4, 7, 11, 15, 18, 24, 28, 30, 32, 35, 36, 40, 42 and 43. In this group soluble solid concentrations were between 28.4 and 29.3%. The highest soluble solid concentration was measured in the accession 41, and the lowest was measured in accessions 27 and 33. Reducing sugars content varied from 0.169 to 0.269% with the greatest number of varieties accumulating from 0.170 to 0.210% (Figure 1B).

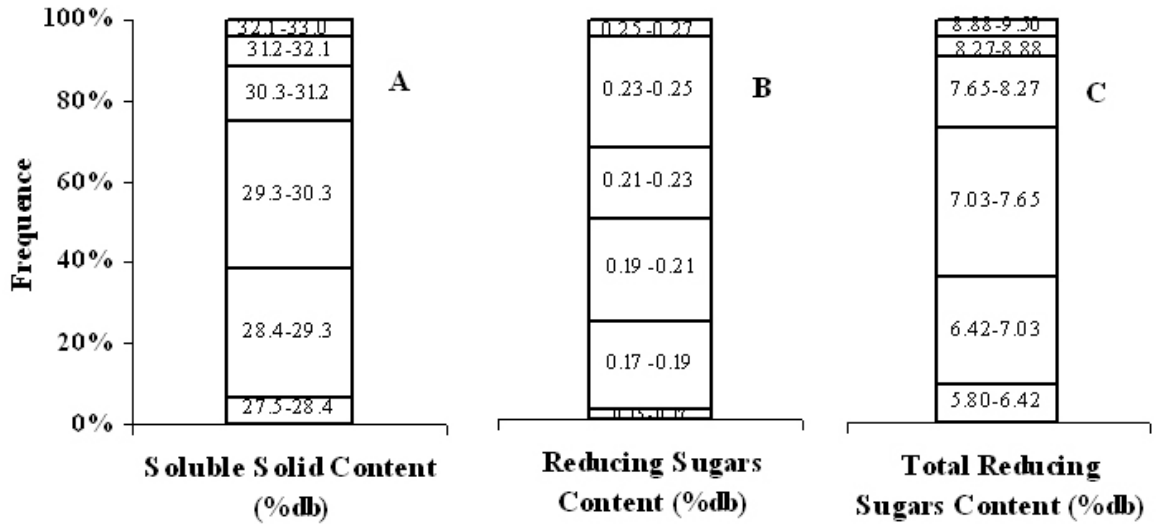


Figure 1. Genetic diversity in *C. arabica* regarding soluble solid (A) reducing sugars (B) and total reducing sugars (C) contents.

The lowest concentration was measured in accession 30. Although the majority of varieties (63.7%) exhibited total reducing sugar concentration between 6.42 and 7.65% (Figure 1C), an Ethiopian accession (25) showed 9.43%. Since total reducing sugars extracted in the conditions carried out in the experiments is mainly sucrose this accession could be of interest for cup quality improvements.

Most of cultivars (29.5%) presented total chlorogenic acid concentration between 4.7 and 5.2% (Figure 2A). Chlorogenic acid concentration varied from 3.7%, in variety 12, to 6.7%, in variety 33. Concerning trigonelline concentration, the major group (34.1%) included varieties with 1.40 to 1.50% (Figura 2B). This component that is also related to coffee nutraceutical properties, was found in concentration ranging from 1.00, in plant 21, up to 1.58%, in plant 27. Seventy percent of the analyzed Arabica accessions showed trigonelline concentration between 1.20 and 1.50%. Figure 2C shows that 41% of varieties have caffeine concentration between 1.12 and 1.34%. All commercial varieties, except Caturra Amarelo, were included in this group. Other 41% of accessions presented caffeine from 1.34 to 1.55%.

Around 70% of Ethiopian and Yemen introductions were in this group. The lowest caffeine concentration occurred in variety 7 (0.76%) and the highest (1.77%) was in plant 33.

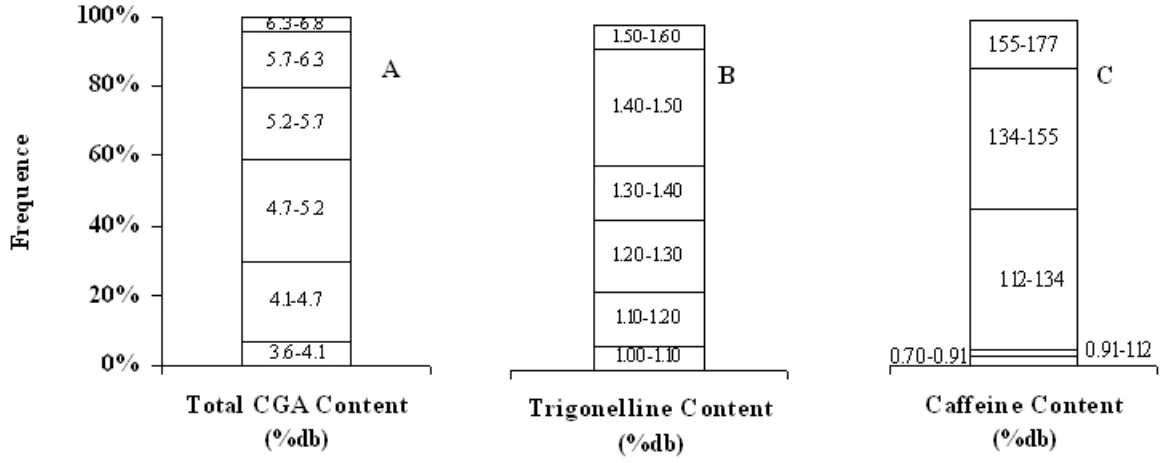


Figure 2. Genetic diversity in *C. arabica* respect to total chlorogenic acids (A) trigonelline (B) and caffeine (C) contents.

CONCLUSIONS

C. arabica accessions from IAC Coffee Germplasm Collection showed important chemical diversity. Ethiopian and Yemen accessions were highlighted for potential exploration in coffee breeding purposes focusing nutraceutical properties and cup quality. Ethiopian exhibited the highest concentrations of all components, except soluble solid, which was greatest in a Yemen introduction.

ACKNOWLEDGEMENTS

This research was supported by FAPESP, SP, BR – Project N. 04/04609-0

REFERENCES

De Menezes, H. C. *Food Chem*. 50:293-296, 1994.

De Maria, C.A.B.; Trugo, L.C.; Aquino Neto, R.R.; Moreira, R.F.A.; Alviano, C.S. *Food Chem*. 55:203-207, 1996.

FAO Report: *Coffee mission to Ethiopia 1964-65* (Food and Agriculture Organization, Rome, 1968).

Kurzrock, T., Kölling-Speer, I., Speer, K. 20th ASIC Colloquiun (Bangalore): 161-168, 2004.

Nunes, F. M., Coimbra, M. A., Duarte, A. C., Delgadillo, I. J. of Agric. *Food Chem*. 45:3238-3243, 1997.

Rogers, W. J.; Michaux, S., Bastin, M.; Bucheli, P. *Plant Science* 149: 115-123, 1999.

Viani, R. and Horman, I.. *Journal of Food Science*. 39:1216-1217, 1974.

Webster Ross, G., Aboot, R. D., Petrovitch, H., Morens, D. M., Grandinetti, A., Tung, K. *JAMA*, (S. I.), 283: 2674-2679, 2000.

Tree Shape Modification Induced by the *Laurina* Mutation in *Coffea arabica* L.

A. LECOLIER[1], J. L. VERDEIL[2], H. CHRESTIN[3], M. NOIROT[1]

[1]IRD, UMR 1097 – DGPC (AGRO-M, CIRAD, INRA, IRD), «Diversité et Génome des Plantes Cultivées», Pôle de Protection des Plantes, 97410, Saint Pierre, La Réunion
[2]CIRAD PHIV, UMR 1098, IFR, 34 000 Montpellier, France
[3]IRD, UMR 1097 – DGPC (AGRO-M, CIRAD, INRA, IRD), Centre IRD de Montpellier, 34 000, Montpellier, France

SUMMARY

Coffea arabica var. Laurina is a dwarf mutant of *Coffea arabica* var. Bourbon, with a characteristic pyramidal shape. The aim of this study was to understand the origin of shape changes between these two varieties. Tree-shape variation could theoretically depend on the angle formed between plagiotropic and orthotropic axes. Nethertheless the two Bourbon varieties exhibit a same (about 50°) angle between these axes, with a within-variety variation of 3° only. As the angle does not explain the main shape differences between the two varieties, a growth ratio (Ri) was defined between the length of plagiotropic and orthotropic axis. This parameter allowed differentiating the Bourbon and the Bourbon pointu. The elongation patterns of the last internodes on the orthotropic axis in regards to the plagiotropic branches could explain mostly the pyramidal shape of Bourbon pointu. In fact, the *Laurina* mutation affected the growth of last internodes of the orthotropic axes without affecting the plagiotropic ones.

INTRODUCTION

Coffee trees belongs to the Rubiaceae De Jussieu family. They are defined by their coffean placentation and belong to genus *Coffea* L. and *Paracoffea* Leroy. The genus *Coffea*, which includes coffee trees *sensus stricto,* is characterised exclusively by their axillary inflorescences, monopodial axis, and presence of epicalyx.

The *Laurina* mutation appeared spontaneously at the end of the 18th century on the Bourbon variety (B) on the island of Réunion. Recessive and monolocus, its main scientific interest concerns its pleiotropic effect. For example, the mutation modifies dryness resistance, caffeine content and seed shape, the latter being at the origin of the vernacular name Bourbon pointu (BP) (Krug and Carvalho, 1951). Nonetheless, mutation does not affect phyllotaxy and leaves are still opposed and positioned in a decussate pattern. If internode size decreases proportionally on orthotropic and plagiotropic axes, the mutation should only lead to a dwarf tree in regards to the Bourbon variety, without impact on tree shape. This is the case of the *caturra* mutation.

However, BP shows a characteristic Christmas tree shape (Chevalier, 1947). Changes in tree shape should imply branching angle modification between orthotropic and plagiotropic axes, and/or, differential growth between orthotropic and plagiotropic internodes.

Our aim was to understand the origin of shape modifications between B and BP.

MATERIAL AND METHODS

Plant material concerned varieties B and BP of the *Coffea arabica* L. species. Experiments and observations were carried out in fields at Makes (897 m above sea level) (Réunion, France). Five 2.5-year-old plants of each variety were sampled for macroscopic and microscopic observations.

Two types of macroscopic measures could explain shape variation:

- The first one is the angle between orthotropic and plagiotropic axes. Four angles were measured - in degrees - per plant, on different nodes, but always on the third upper part of the tree.
- The second one is the ratio between orthotropic and plagiotropic lengths observed at different node level (fig. 1). Internodes were numbered from the tree top. Let Lo_i the length of the orthotropic axis between the tree top to the node "i". Let Lp_i, the length of the plagiotropic axis emerging from the node "i" (as they are two plagiotropic branches at each orthotropic node, in pratice Lp_i was the average of these two plagiotropic lengths). Lengths Lo_i and Lp_i allowed to compute the four growth ratio $Ri = Lp_i/Lo_i$ ».

Lastly, microscopic sections were carried out at the mid of the last internode on the orthotropic axis, but also on plagiotropic P_1 and P_3. This allowed to measure pith cell height.

RESULTS AND DISCUSSION

Tree-shape variation would theoretically depend on, among others, the angle between plagiotropic and orthotropic axis. Nonetheless, this trait did not differ between B and BP ($F_{1, 8} = 4.43$; $p = 0.069$), exhibiting a same (about 50°) angle between these axes, with a within-variety variation of 3° only.

Tree-shape would also theoretically depend on the relative length of the plagiotropic axis in regards to the orthotropic one. Indeed, in purely conical shape, the ratio R_i should not vary according to "i" (see fig. 1). In fact, R_i differed between B and BP ($F_{1, 32} = 59.2$; $p = 0.00024$), but also decreased from R_1 to R_4 ($F_{3, 32} = 8.61$; $p < 1.10^{-6}$). Nevertheless, decreasing was not linear (Figure 2).

For both varieties, the relation between the ratio R_i and the variable « i » was fitted to the function $y = a.e^{bx} + c$ with $b < 0$. The parameter c represents the asymptote, i.e. the value of R_i when i tends toward infinite. It reached 0.5 for BP and 0.81 for B. Thus, as far as the mutant concerned, mean length of mature internodes was 4.1 and 2.4 cm for orthotropic and plagiotropic axis, respectively. These lengths were 9.3 and 7.5 cm, respectively, for Bourbon.

Internode growth end both depended of variety and axis type (Figure 3). For Bourbon, internode stopped its growth after three plastochrons, i.e. when internode reached the second position from the top. This growth pattern was observable on the plagiotropic and the orthotropic axis. For the mutant, orthotropic internode reached its definite length (4.1 cm) in one plastochron, but plagiotropic one (2.4 cm) reached still in three plastochrons.

Lastly, cell height didn't explain internode length variation for the youngest internode of the orthotropic axes, the first plagiotropic branch and the third one. Consequently, as cell height cannot explain internode length variation between them, only cell number could be implied.

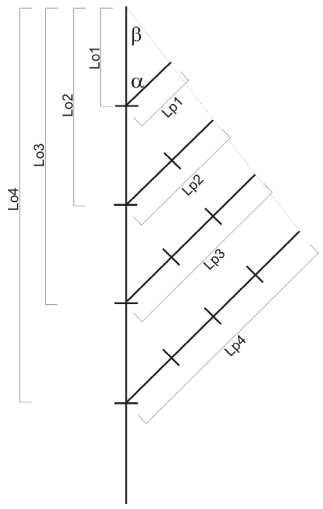


Figure 1. Diagram with different measures done on the top of each tree.

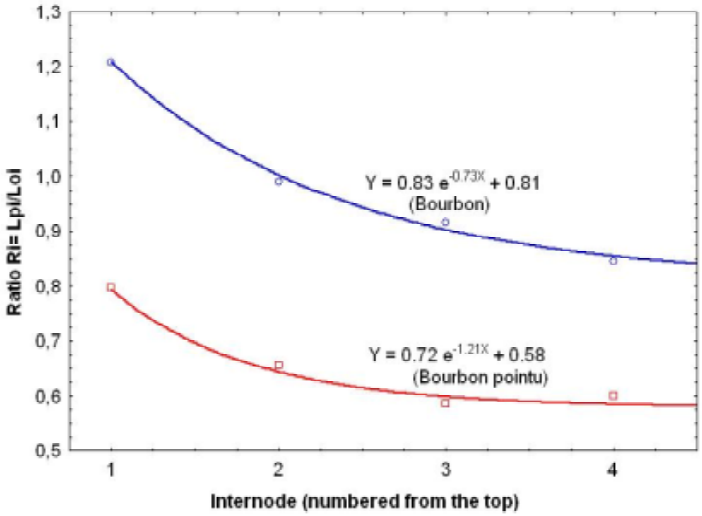


Figure 2. Relation between the ratio R_i and the variable « i ». Internodes were numbered from the top of the tree.

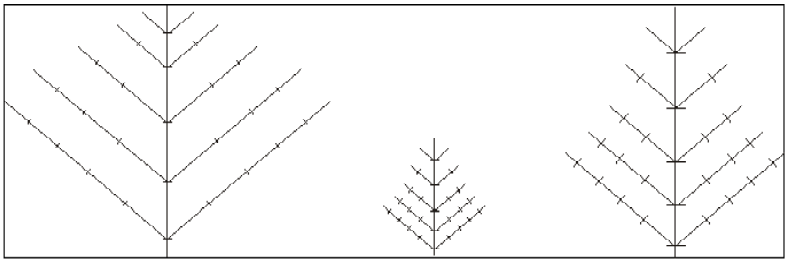


Figure 3. Representation (on the same scale) and pictures of the five last internodes of B and BP. On the left: "Bourbon" upper part (five last internodes), with above corresponding picture. In the middle: "Bourbon pointu" upper part (five last internodes), with above corresponding picture. On the right: "Bourbon pointu" (corrected scale)

CONCLUSIONS AND PERSPECTIVES

Elongation patterns of the last internodes on the orthotropic axis in regards to the plagiotropic branches could mostly explain the BP pyramidal shape. Both axis types had reduced size, but

the *Laurina* mutation affected the last internodes growth pattern of the orthotropic axis without affecting the plagiotropic ones.

In orthotropic axis, BP has fewer and smaller cells compared to Bourbon (Lecolier et al., submitted). A perspective could be to analyse cell elongation and division in plagiotropic branches.

Plant growth internal regulators are in majority in relation with the difference of geotropism between orthotropic and plagiotropic axes. Observations led to the hypothesis of a desequilibrum in one or many of them. Investigations are now required in order to understand a potential hormone roles in the *Laurina* mutation.

REFERENCES

Chevalier A. 1947. Les caféiers du globe: III. Sistématique des caféirs et faux-caféiers maladies et insectes nuisibles. In: Lechevalier P, ed. *Encyclopedie Biologique*. Paris.

Krug CA, Carvalho A. 1951. The genetics of coffea. *Adv Genet* 4: 127-158

Lécolier, A., Verdeil, J-L., Escoutes, J., Chrestin, H., Noirot, M. Submitted. Effects of the *Laurina* mutation on the shoot apex in *Coffea arabica* L., Annals of botany.

Variability and Association of Agronomic Characters in the Hararge Coffee Germplasm Accessions

M. KEBEDE AND B. BELLACHEW

Jima Agricultural Research Center, P O Box 192, Jima, Ethiopia

SUMMARY

A field experiment was conducted at Awada Agricultural Research Sub-Center using one hundred Hararge coffee germplasm accessions for morphological characterization at pre-bearing stage. These coffee germplasm accessions representing 16 weredas of Hararge coffee growing areas and 4 standard checks from southwest Ethiopia were evaluated for 14 quantitative characters. A wide range of phenotypic variations was observed among accessions in respect of majority of the characters studied. Among the 14 characters, total number of internodes per plant, canopy diameter, leaf area and number of secondary branches are the most important ones. The differences between the minimum and maximum mean values for other characters were also high. The estimates of PCV and GCV for the 14 quantitative characters considered ranged from 5.9 to 54.8% and 3.2 to 37.5%, respectively. The phenotypic and genotypic correlation analyses for the 14 quantitative traits showed positive and significant associations in the majority of characters. Among the characters, plant height, length of the longest primary branch, average length of primary branches, number of internodes on the longest primary branch, total number of internodes per plant in particular showed positive and highly significant correlations with majority of the characters both at phenotypic and genotypic levels. High h_b^2 values were also obtained for internode length of branches (90%), canopy diameter (51.9%) and plant height (50.8%). For the rest of the characters, moderate h_b^2 values were obtained. The estimates of genetic advance for the 14 characters were also calculated. Relatively higher estimates were obtained for number of secondary branches, plant height and canopy diameter. In view of this, it may be surmised that the above-mentioned characters could be of potential importance to the improvement of Hararge coffee population through selection and hybridization

INTRODUCTION

The identification and manipulation of plant characters contributing towards the increment of yield level is important since they were found to increase the breeding efficiency (Tyagi and Sharma, 1985). A due emphasis on the characters having useful relationship with grain yield has proved to be important in upgrading yield level through visual selection of desirable lines (Hayes et al., 1955). Correlation studies furnished information on the nature and degree of associations among characters contributing to yield and between yield components and seed yield.

In relation to coffee, it was reported that morphological characters such as stem girth, width of canopy, number of primary branches and number of secondary branches influenced yield in coffee (Dancer, 1964; Srinivasan, 1982). Based on 4 years observations on yield, stem diameter, height, and number of primaries on arabica coffee, Bouharmont et al. (1998) found significant genetic correlation between the said morphological traits and yield. Charier (1978) obtained high and positive correlation between height and stem diameter of arabica coffee. According to Berthaud et al. (1978), among the seven vegetative characters studied in thirty

four *C. arabica* populations in Ivory Coast, number of nodes of the side branches and of the main stem and their basal diameter were found to have positive correlation.

Several workers estimated the heritability values of quantitative characters in coffee. For instance, Walyaro and Van der Vossen (1979) obtained high heritability values for internode length (90%) and number of primaries (85%) whereas moderate values for stem diameter (43%) and nodes on the longest primaries (30%). Srinivasan (1982) also obtained high heritability for length of longest primaries (90%). Bayetta (2001) reported high broad sense heritability for 15 of the 18 morphological characters studied on six elite parental lines and their 15 F_1 crosses. Previous work on Ethiopian coffee revealed that the expected genetic advance was higher for number of primary branches (67 percent), internodes length on primary (26 percent) and total nodes per plant (87 percent). The above characters had also exhibited higher heritability values, thereby indicating their amenability for selection (Srinivasan and Vishveshwara, 1978).

In the same manner, the present study was conducted to investigate the genetic variability, heritability, expected genetic advance, and association of the 14 quantitative characters for the 104 sampled accessions of Hararge coffee germplasm.

MATERIALS AND METHODS

The experiment was carried out at Awada Agricultural Research Sub-Center (AARSC) located near Yirgalem town, 45 km south of Awassa at an altitude of 1750 meters above sea level. The sources of test materials were 902 Hararge coffee accessions that were collected from 16 Woredas of eastern and western Hararge zones and maintained in the field at AARSC. These accessions were planted in July 2000. For the present study, 100 accessions were taken at random. Four CBD (coffee berry disease) resistant cultivars well adapted to Sidamo environment were included as standard checks. Data were collected on 14 quantitative agronomic characters from 4 plants per accessions for each of the 104 Hararge coffee germplasm accessions.

DATA ANALYSIS

Analysis of variance using nested ANOVA was computed for each of the 14 quantitative characters in order to identify the variability among accessions. Phenotypic correlation coefficient analysis between yield components such as stem girth, number of primary branches, internodes length, plant height and canopy diameter were computed using the procedure suggested by Miller et al. (1958).

Test of significance for the results of phenotypic correlation coefficient analysis was made by comparing 'r' values from the table of simple linear correlation at n-2 degrees of freedom. Where, n=number of treatments (germplasm accessions in this case).

Estimation of heritability (h^2) and genetic parameters

Heritability in broad sense (h_b^2) for the quantitative traits was computed as suggested by Allard (1960). By considering all the accessions (observations) tested here were genetically uniform, the mean sum of squares for error (MSe) for each character was assumed to be purely a random environmental variance (V_E). The genotypic and phenotypic variances were calculated from the ANOVA table for each character using the formula given by Singh and Chaudhary (1987).

Phenotypic Coefficient of variation (PCV)

$$PCV=(\sqrt{Vp}/X)*100$$

Genotypic Coefficient of variation (PCV)

$$GCV=(\sqrt{Vg}/X)*100$$

X =Grand mean for each of the 14 characters of 104 germplasm accessions
Genetic advance (GA)
$GA=k.\sigma_p.H^2$
K=constant (selection differential where k=2.056 at 5% selection intensity)
σ_p=phenotypic standard deviation on mean basis
H^2=heritability in broad sense

RESULTS AND DISCUSSIONS

Morphological variation for quantitative characters

Summary of the grand mean, range, heritability values, expected genetic advance and percent coefficients of genetic and phenotypic variations (PCV&GCV) for all the fourteen quantitative characters are presented in Table 1. The mean range of characters for the 104 accessions showed the existence of high morphological variation among accessions for each character considered. For instance, number of secondary branches, total number of internodes per plant, plant height and canopy diameter showed higher mean ranges along with higher coefficient of variation indicating the presence of relatively higher morphological variation among germplasm accessions for these characters as compared to the others. On the contrary, the mean ranges for the characters such as angle of primary branches and internode length on the stem showed relatively smaller mean ranges accompanied by smaller coefficient of variation implying that their contribution to the total variation is minimum (Table 1). Except for the four characters i.e. number of internodes on the longest primary branch, stem diameter, angle of primary branches and number of secondary branches, the mean minimum values for the rest of the characters were obtained from accessions of east Hararge areas specifically, Girawa, Bedeno and Kombolcha. Nine of the mean maximum values in respect of characters as referred above belonged to accessions from Mesela and Kuni Woredas out of which seven were scored by accessions of Kuni itself. Germplasm accessions from Bedeno, Girawa and Kombolcha woredas scored maximum mean values in respect of the remaining five characters, viz., canopy diameter, stem diameter, leaf area, number of secondary branches and angle of primary branches.

Estimates of phenotypic (PCV) and genotypic (GCV) variations

The estimate of PCV ranged from 5.9% for angle of primary branches to 54.8% for number of secondary branches. Except for characters total number of internodes per plant (24.5%) and internode length of branches (39.6%), estimate of PCV for the rest of 10 characters is within the range of 11-17%. In the same manner estimate of GCV ranged from 3.2% for angle of primary branches to 37.5% for internode length of branches. Total number of internodes per plant (14%) and number of secondary branches (34.2%) showed higher percentage values of GCV (Table 1).

It was observed that PCV for all characters was higher in magnitude than GCV values, which might explain the influence of environment on the expression of the characters. Those characters such as number of secondary branches, total number of internodes per plant and internode length of branches that showed higher percentage values for both GCV and PCV, had also exhibited higher magnitudes of both genotypic and phenotypic variances which intern indicates availability of immense genetic variation for improvement of Hararge coffee.

Table 1. Mean, range, standard deviation, heritability, and phenotypic and genotypic coefficients of variations and expected genetic advance of the 14 quantitative characters of Hararge coffee germplasm accessions.

Characters	Mean*	Range	Standard deviation	Heritability (%)	PCV (%)	GCV (%)	GA (%)
PLH	130.58	103.25-169.00	15.030	50.8	14.57	10.38	15.77
ILS	6.00	4.00-6.22	0.495	36.9	11.43	6.932	0.376
ILB	5.17	3.65-5.95	1.960	90.0	39.56	37.53	3.638
NIS	20.67	14.00-26.25	2.630	40.3	15.14	9.608	1.938
NILPB	20.64	16.50-27.25	2.140	24.6	15.77	7.821	1.086
TNIP	430.46	267.25-653.75	73.870	32.8	24.53	14.04	50.05
CD	107.51	74.38-143.13	13.100	51.9	15.36	11.06	14.03
SD	3.38	2.63-4.80	0.341	25.1	15.24	7.629	0.176
LA	50.32	35.40-62.39	6.620	41.5	17.66	11.38	5.673
NPB	37.46	27.25-47.50	4.660	40.0	16.84	10.65	3.848
APB	64.44	58.75-69.75	2.580	29.3	5.91	3.2	1.563
NSB	113.39	30.25-236.75	45.660	39.0	54.8	34.21	36.79
LLPB	80.24	61.75-100.50	8.610	43.5	14.21	9.37	7.741
ALPB	55.48	39.67-67.25	5.300	36.2	13.2	7.936	115.4

PLH=plant height, ILS=inernode length of stem, ILB=internode length of branch, NIS=number of internodes of stem, NILPB=number of internodes on the longest primary branch, TNIP=total number of internodes per plant, CD=canopy diameter, SD=stem diameter, LA=leaf area, NPB=number of primary branches, APB=angle of primary branches from the main stem, NSB=number of secondary branches, LLPB=length of the longest primary branch, ALPB=average length of primary branches

Correlation among quantitative characters

The phenotypic and genotypic correlation analyses were computed for the 14 quantitative characters using the 104-germplasm accessions (data not shown). The result of the analysis showed positive and significant associations (both at $P < 0.05$ and $P < 0.01$) among the characters in majority of the cases. However, negative and significant associations were also observed in some cases.

The phenotypic correlation coefficients analysis in general indicated higher degree of associations among the characters. The characters that correlated positively and significantly with majority of the characters included canopy diameter, stem diameter, length of longest primary branch and average length of primary branches where each had positive and significant associations with ten of the quantitative characters. Further, the characters such as plant height, number of internodes on the stem, number of internodes on the longest primary branch, total number of internodes per plant, number of primary branches and number of secondary branches correlated positively and significantly with seven characters each. On the contrary, angle of primary branches and average internode length of primary branches did not

show significant associations with any of the characters considered. Leaf area correlated positively and significantly only with internode length of the stem and plant height while it associated negatively and significantly with average internode length of branches and number of primary branches. Srinivasan and Vishveshwara (1978) also reported positive and significant associations of leaf area with plant height and internode length of the stem. In view of this, it may be reasonable to surmise that stem diameter, total number of internodes per plant and length of the longest primary branch are the most important characters with respect to selection and improvement of Hararge coffee population. On the same analogy, it could be stated that angle of primary branches and average internode length of branches are less relevant.

Heritability and estimates of genetic advance

Broad sense heritability values obtained for the 14 quantitative characters of the 104 coffee germplasm accessions ranged from 24.6 per cent (number of internodes on the longest primary branch) to 90 per cent (average internode length of branches). High heritability values were obtained for average internode length of branches (90%), canopy diameter (51.9%) and plant height (50.8%). This result is in agreement with the previous works of Mesfin and Bayetta (1988) who obtained higher estimates of broad sense heritability for stem diameter, length of first single primary branch and plant height. This is suggestive that selection for these characters is likely to result in positive response to selection, which is supported by the observations of Falconer and Mackey (1989). Moderate values were found, on the other hand, for the length of longest primary branch (43.5%), leaf area (41.5%), number of internodes of the stem (40.3%), number of primary branches (40%), number of secondary branches (39%), internode length of the stem (36.9%), average length of primary branches (36.2%), total number of internodes per plant (32.8%), angle of primary branches from the main stem (29.3%), stem diameter (25.1%) and number of internodes on the longest primary branch (24.6%). This indicated that majority of the characters considered have moderate heritability values hence, selection for any of these characters is likely to produce intermediate responses (Falconer and Mackay, 1989). Similar results were reported by Walyaro and Van der Vossen (1979) for majority of these traits. However, they also reported low and high heritability value for plant height (18%) and stem diameter (65%), respectively. The results of present study in respect of plant height and stem diameter are at variance with the results of Walyaro and Van der Vossen (1979) that may be partially attributed to the difference in the test material and environment (Srinivasan et al., 1979).

Estimate of genetic advance as percentage of the grand mean obtained for the 14 quantitative characters ranged from 0.18 (for stem diameter) to 50% (for total number of internodes per plant). Relatively higher estimates of genetic advance were exhibited for number of secondary branches (36.8%), plant height (15.8%) and canopy diameter (14%). This result is in agreement with Srinivassan (1988). However, internode length of branches, number of primary branches and number of internodes of the stem showed lower estimates of genetic advance, despite their higher heritability values; which might be attributed to their low value of standard deviation due to the mean.

The information obtained on heritability and genetic advance could be used for planning effective breeding programs in the genetic improvement of the coffee population (Bayetta, 2001) since it provides a useful indication of the relative value of selection in the material at hand especially when selection of genotypes is based on phenotypic performance of quantitative characters (Allard, 1960 and Hayes et al., 1955). This is corroborated by the findings of Kumar et al. (1985) that estimation of broad sense heritability is one of the prerequisites in any hybridization program. In light of this observation, heritability values and

estimates of genetic advance obtained could be baseline information in the improvement of Hararge coffee through selection and crossing.

REFERENCES

Allard, R.W. 1960. *Principles of Plant Breeding*. John Wiley & Sons, Inc. New York, London.pp.75-99.

Bayetta Bellachew. 2001. Arabica coffee breeding for yield and resistance to coffee berry disease (*Colletotrichum kahawae sp. nov*.) *Ph.D. Dissertation*, University of London, UK.

Bouharmont, C.C., P. Boccara, M. Eskes, and A.B. Baradat. 1998. Prediction of genetic value for coffee production in *Coffea arabica* from a half-diallel with lines and hybrids. *Euphytica.*104: 49-59.

Charrier, A. 1978. Analysis of the phenotypic variability of *C.arabica L.* collection in Madagascar. *Pl. Breed. Abst*., 49:243

Dancer, J. 1964. The measurement of growth in coffee. II. The relationship between components of shoot and stem diameter at the base of the shoot. *East Afri. J. of Agri. Sci*., 33: 21-25.

Falconer, D.S. and T.F.C. Mackey. 1996. *Introduction to Quantitative Genetics*, 4th ed. Longman, Essex, UK.pp.115-119.

Hayes, H.K., F.R. Immer and D.C. Smith. 1955. *Methods of Plant Breeding*.2nd ed. McGraw-Hill Inc. New York, London, Toronto.pp.446-451.

Kumar, A., S. C. Misra, Y. P. Singh, and B. P. S. Chauhan. 1985. Variability and correlation studies in Triticale. *J. Maharashtra Agric. Univ*., 10:273-275.

Mesfin Ameha and Bayetta Bellachew. 1988. Genotype-environment interactions in coffee (*C. arabica* L.). *In: Proc. Twelfth International Scientific Colloquium on Coffee. Montreux, 29 June-3 July*, Switzerland. pp.476-482.

Miller, P. A., J. C. Williams, Jr., H. F. Robinson, and R. E. Comstock. 1958. Estimates of genotypic and environmental variances and covariances in upland cotton and their implication in selection. *Agron J*., 50:126-131.

Singh, R.K. and B.D. Chaudhary. 1987. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi. p. 318.

Srinivasan, C.S. and S. Vishveshwara. 1978. Heterosis and stability for yield in arabica coffee. *Indian J. Genet. and Pl. Breed.,* 38:13-21.

Srinivasan, C.S., S. Vishveshwara and H. Subramanaya. 1979. Genotype-environment interaction and heritability of yield in *Coffea arabica* L. *Indian J. Coffee Res*., 9: 69-73.

Srinivasan, C.S. 1982. Pre-selection for yield in coffee. *Indian J. of Genet. and Pl. Breed.*, 42:15-19.

Tyagi, M.C. and B. Sharma. 1985. Association among economic traits in lentil. *Lens.* 12: 10-12.

Walyaro, D.J. and H.A.M. Van der vossen. 1979. Early determination of yield potential in arabica coffee by applying index selection. *Euphytica*. 28: 465-472.

Welsh, J.R. 1981. Fundamentals of Plant Genetics and Breeding. Wiley: New York. p.272

Diversity in the South Ethiopian Coffee (*Coffea arabica* L.)

M. KEBEDE, B. BELLACHEW AND S. SEIFU

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

SUMMARY

A field experiment on evaluation of 41 south Ethiopian coffee accessions with 2 standard checks of the southwest Ethiopian origin was conducted using Randomized Complete Block Design at Wonago Research Sub-Station during 1999-2003 cropping seasons. Data on 7 morphological agronomic characters, average of three years data on severity of CBD and CLR infestations and clean coffee yield was obtained for the 43 genotypes. The germplasm accessions differed significantly for all the 7 morphological agronomic characters and yield in the univariate analyses of variances indicating the prevalence of variability among south Ethiopian coffee germplasm accessions. Further, the first four principal components explained 82.63 percent of the total variation prevalent within the germplasm accessions out of which 32.52 percent was explained by the first principal component. Average linkage cluster analysis using Mahalanobis (D^2) distance for the 10 characters grouped the 43 accessions in to 9 clusters. The number of accessions per cluster ranged from 1 in cluster IX to 13 in cluster II. The clustering pattern of the accessions revealed the prevalence of genetic diversity in the south Ethiopian coffee for the characters considered. The maximum inter-cluster distance was observed between clusters V and VIII while the minimum was observed between clusters VI and VII. The study highlighted the possibility of using accessions of the distant clusters as potential candidates for the genetic improvement of south Ethiopian coffee through crossing and selection.

INTRODUCTION

Coffee is the most important export crop of the south Ethiopian region with more than 46 percent share of the national market. It covers more than 185 000 ha of land in 50 Woredas (districts) with 11 are high, 7 medium and 32 are low coffee producers. Garden coffee comprises 130 000 ha, semi forest 45 000 ha and forest coffee 10 000 ha where the semi forest and forest coffee production systems are pertinent to the western part of the region. In 2005 cropping season, the annual coffee production of the region was 131 000 tons out of which 100 302 tons was exported as 60 percent washed and 40 percent dry processed (SNNPR BOA and NRD, unpublished). The average yield of coffee in the region is 500 kg/ha (for local or landrace cultivars) while 800 kg/ha for the released coffee berry disease resistant cultivars. Though the region is highly endowed with suitable environments and immense genetic diversity for coffee production, the productivity of coffee per unit area remains very low as compared to world average. This is attributed mainly due to the lack of improved cultivars for central and eastern coffee growing areas of the region, shortage of improved agronomic technologies and prevalence of diseases mainly Coffee berry disease and coffee wilt disease. Moreover, physiological problems such as die back partly caused by absence of shade trees coupled with minimum use or absence of agricultural inputs in the smallholder coffee orchards of Central and Eastern Zones of the Region is very common (SNNPR BOA and NRD, 2004).

The exploitation of genetic diversity for crop improvement should be the ultimate objective of genetic resources exploration and conservation. The vital stages of evaluation and

incorporation of valuable characters such as disease resistance and/or tolerance to environmental stress factors into new varieties appeared to be justifications of genetic resources conservation, characterization, and evaluation (Ford-Lloyd and Jackson, 1986). In cognizant of this fact, renewed efforts of coffee germplasm collection were undertaken consecutively for 3 years (1995-1997) from different coffee growing areas of Central and Southeastern part of the South Ethiopian Region by Jima Agricultural Research Center (JARC) and as a result more than 350 accessions were collected and maintained at Awada Agricultural Research Center.

Several workers have estimated the extent of genetic diversity present from the different sources of arabica coffee germplasm collections. For instance, a study by Catter (1992) on second progeny arabica coffee collections of Ethiopian origin indicated the prevalence of high level of variability in morphological, agronomic and biochemical characteristics. The genetic diversity analysis conducted by (Lashermes et al., 1996) using RAPD markers on cultivated and sub-spontaneous accessions of arabica coffee confirmed the narrow genetic base of commercial cultivars (3 typica and 3 bourbon types). On the other hand, they reported the existence of large genetic diversity within the sub-spontaneous material, which consisted of 11 samples representing the different coffee growing areas in Ethiopia. Further, they have suggested the prevalence of an east-west differentiation in the Ethiopian coffee germplasm. Similarly, Montagnon and Bouharmont (1996) characterized 148 arabica coffee accessions for phenotype diversity under field condition. They have evaluated the accessions using eighteen different morphological and agronomic traits by employing multivariate analysis and identified two main groups in the coffee accessions. According to them, accessions of group I have a more erect branching habit, narrower leaves, and were more resistant to coffee leaf rust and coffee berry disease than accessions of group II. They further opined that group I mostly contained Ethiopian arabica coffee accessions collected from west of Great Rift Valley, whereas group II contained commonly cultivated varieties throughout the world and Ethiopian accessions collected from east of Great Rift Valley in Ethiopia. On the same basis, the present study was conducted in order to estimate the genetic diversity among South Ethiopian coffee germplasm collections and to facilitate for use in the ongoing breeding program.

MATERIALS AND METHODS

The experiment was carried out at Wonago Agricultural Research Sub-Station (WARSS) located near Wonago town, 99 km south of Awassa town at an altitude of 1850 meters above sea level. The sources of test materials were 41 South Ethiopian coffee accessions that were collected from 6 Woredas of Gedeo, Sidama and Wolayta zones and maintained in the field at WARSS (Table 1). The 41 accessions and 2 released coffee berry disease (CBD) resistant cultivars were planted in July 1999 using Randomized Complete Block Design in 4 replications. Data on 7 morphological agronomic characters vis-à-vis stem girth, plant height, number of primary branches, number of stem nodes, length of longest primary branches, canopy diameter and internode length of the main stem; percent disease infestation levels on CBD and coffee leaf rust (CLF) and average of 3 years clean coffee yield was obtained on the 43 genotypes.

DATA ANALYSIS

A two-way analysis of variance (using MSTATC statistical software package) was computed for each quantitative character in order to identify the variability among the genotypes. Further, the data were standardized to a mean of zero and a variance of unity, to avoid differences in scales used for analyses before undertaking principal component and divergence analyses. Genetic divergence between clusters was determined using the

generalized Mahalanobis's D^2 statistics. Clustering of the accessions was performed using the proc cluster procedure of SAS version 8.2 software package (SAS Institute, 2001) by employing the method of average linkage cluster analysis of observations. The D^2 values obtained between and within clusters (inter and intra-cluster distances) were tested for their significance at the required level of probability against the tabulated values of X^2 for p degrees of freedom where p is number of characters (Singh and Chaudhary, 1987).

Table 1. Details of germplasm accessions used in the study.

Place of collection (Woreda)	Collection number (genotype identity)	Total number of genotypes per Woreda	Remark
Wonago	85190, 85181, 85188, 85196,85195, 85193, 85200, 85180, 3170, 3270, 1377, 2077, 2777, 3677, 3977, 2181	16	Gedeo Zone
Yirgachefe	85245, 85238, 85257, 85237, 85241, 85252, 85260, 85246, 85259, 2970, 3070	11	Gedeo Zone
Aleta Wondo	85264, 85296, 85265, 85263, 85288, 85269, 85294	7	Sidama Zone
Dale	3470, 3670	2	Sidama Zone
Bolososore	1681, 2081	2	Wolayta Zone
Sodozuria	1870	1	Wolayta Zone
Southwest Ethiopia	75227, 744	2	Released CBD Resistant cultivars
Unknown	85213, 85232	2	Gedeo Zone

RESULTS AND DISCUSSIONS

Analyses of variances

Univariate analyses of variance were computed using MSTATC version 2.10 statistical software program for the seven quantitative morphological characters and the three years combined yield data. The ANOVA showed a highly significant difference among the genotypes for all the characters considered. Southeast Ethiopian coffee population was stated to be of narrow genetic base (Lashermes et al., 1996; Anthony et al., 2001), however, the findings of this study indicates the presence of wide variations among Southeast Ethiopian (Sidama, Gedeo and Wolayta) landrace coffee populations located east of the Great Rift Valley. This might be attributed to the differences in the type of collections used i.e. forest coffee versus landraces. Since landraces are traditional varieties that have evolved over generations of selections by farmers (Harlan, 1992; Frankel et al., 1995), they are characterized by high genetic heterogeneity, good adaptation to local environmental conditions and low productivity. In view of this it may be reasonable to state that there is a good chance to improve Sidama, Gedeo (Yirgachefe) and Wolayta coffees through selection and breeding. Such a view was endorsed by the work of earlier researchers (Leroy et al., 1993; Catter, 1992).

PRINCIPAL COMPONENT ANALYSIS (PCA)

The first four principal components with eigen values greater than unity explained 82.63 percent of the total variation among the 43 genotypes for the 10 quantitative characters measured. The first principal component accounted nearly one third (32.52%) of the total variation (Table 2). Accordingly, canopy diameter, number of nodes on the main stem, number of primary branches and plant height in that order are the most important characters that contributed for the variation in the first PC. On the same basis, internode length, number of nodes on the main stem, number of primary branches and plant height had significant contributions for the variation in the second PC. In the 3rd PC yield, severity of coffee berry disease, plant height and internode length are the most important characters that contributed for the variation obtained.

In light of the results obtained from the PCA, it may be possible to deduce that more than half (53%) of the variation obtained was primarily due to such characters as; number of nodes, primary branches, and plant height of the main stem. This perhaps emphasized the significance of these characters to the appraisal of genetic diversity in the south Ethiopian landrace coffee populations. More over, these characters could be used as a selection criterion for improving the productivity of the crop since they represent the lion's share in the variability of the coffee population in the specified area.

Table 2 Eigenvalues, total variance, cumulative variance, and eigenvectors for the 10 characters.

Characters	PC 1	PC 2	PC 3	PC 4
Stem girth	0.3202	0.2181	0.0278	–0.4966
Plant height	0.3657	0.2544	0.3688	0.2707
Number of primary branches	0.3934	-0.4099	–0.0944	0.0404
Number of nodes on the stem	0.4007	-0.4265	–00025	0.0620
Length of longest primary	0.3473	0.2676	–0.0210	–0.3109
Canopy diameter	0.4949	0.1001	0.0418	–0.0852
Internode length of the stem	0.0856	0.5465	0.3488	0.2857
% CBD infestation	0.0039	–0.2906	0.5251	–0.3920
% CLR infestation	0.1878	–0.2377	0.1982	0.5299
Average yield	0.2007	0.1286	–0.6097	0.2340
Eigenvalues	3.2519	2.0916	1.7386	1.1810
%Total variance	32.52	20.92	17.39	11.81
%Cumulative variance	32.52	53.43	70.82	82.63

Note: PC1, PC2, PC3, and PC4 are the first four principal components with eigenvalues greater than unity.

CLUSTER ANALYSIS

The 41 southeast Ethiopian coffee selections including 2 southwest Ethiopian CBD resistant cultivars were grouped in to 9 clusters suggesting the prevalence of wide phenotypic variations in the coffee populations. The number of genotypes per cluster varied from 1 in cluster IX to 13 in cluster II. Cluster III contained selections only from Gedeo Zone (Yirgachefe & Wonago Woredas). On the same manner, in cluster V except 1 from Wonago, was composed of selections from Sidama Zone (Dale and Aleta Wondo Woredas). The 2 CBD resistant cultivars (75227 and 744) used as checks were grouped in clusters VI and VII where each cluster had 3 selections.

The selections from Wonago Woreda distributed in to 6 clusters where 7 out of 16 were grouped in cluster II. Similarly the selections from Yirgachefe distributed in to 5 clusters where 4 out of 11 were grouped in cluster III. Relatively low mean yield and higher scores of both CBD and CLR infestations characterized cluster IX that contains only 1 selection from Yirgachefe Woreda.

The cluster analysis failed to clearly show relatedness of the selections due to geographical origin. Rather it is evident that there is overlapping of clustering patterns in respect of all Woredas, which could be explained as lack of differentiation among Woredas arising partly due to gene flow (Amsalu Ayana and Endashaw Bekele, 1999)

Table 3. Distribution of the 43 genotypes over nine clusters based on the 10 characters considered in the study.

Woreda	Clusters									Total genotypes per Woreda
	I	II	III	IV	V	VI	VII	VIII	IX	
Yirgachefe	-	4	4	1	-	1	-	-	1	11
Wonago	3	7	2	1	1	1	1	-	-	16
Dale	-	-	-	-	2	-	-	-	-	2
Aleta Wondo	1	2	-	-	2	-	1	1	-	7
Sodozuria	-	-	-	1	-	-	-	-	-	1
Bolososore	-	-	-	-	-	-	1	1	-	2
Southwest	-	-	-	-	-	1	-	1	-	2
Unknown	1	-	1	-	-	-	-	-	-	2
Total	5	13	7	3	5	3	3	3	1	43

Table 4. Inter (Bottom) and intra (bold and diagonal) cluster distances among the 43 genotypes.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	**4.3**								
II	26.4**	**2.4**							
III	34.1***	30.6***	**3.6**						
IV	42.6***	18.7*	26.5**	**5.3**					
V	60.6***	55.2***	37.3***	33.2***	**4.3**				
VI	56.4***	28.3**	30.0***	22.9*	18.8*	**5.3**			
VII	68.4***	41.5***	27.9**	49.4***	33.2***	18.6*	**5.3**		
VIII	85.3***	31.1***	81.2***	43.9***	134.7***	75.8***	110.6***	**5.3**	
IX	50.0***	42.6***	40.0***	33.0***	58.0***	56.8***	62.5***	74.6***	**0.0**

** = Significant at P< 0.05 (X^2 = 18.307)*
*** = Significant at P< 0.01 (X^2 = 23.209)*
**** = Significant at P< 0.001 (X^2 = 29.588)*

INTER AND INTRA-CLUSTER DISTANCE (D^2) ANALYSIS

Almost all clusters showed a highly significant (P < 0.01) difference among each other. The smallest inter-cluster distance (18.6) was observed between clusters VI and VII while the highest (134.7) was between clusters V and VIII. In most of the cases, the genotypes among

the clusters are significantly ($P < 0.001$) divergent from each other. Considering the intra-cluster (within cluster) distance, no significant genetic dissimilarity was detected.

Since the magnitude of heterosis largely depends upon the degree of genetic divergence in the parental lines, the germplasm selections belonging to the pairs of distant clusters such as V & VIII, VII & VIII and I & VIII could be very useful in hybridization program to obtain a wide variation among the segregates and to maximize heterosis in the F1. Similar view was held by earlier researchers (Souza and Sorrels, 1991).

REFERENCES

Amsalu Ayana and Endashaw Bekele. 1999. Multivariate analysis of morphological variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea. *Genetic Resources and Crop Evolution.* **46**: 273-284.

Anthony, F., M.C. Combes, J.C. Herrena, N.C.Prakash, B.Bertrand and P.Lashermes. 2001. Genetic Diversity and Introgression Analysis in Coffee (*Coffea arabica L.*) using Molecular Markers.

Catter, R. 1992. Study and structure of the phenotypic variation of *Coffea arabica* from Ethiopia. TROPAG Data Base. pp.51.

Ford-Lloyd, B.V. and M. Jackson. 1986. *Plant Genetic Resources*: *An Introduction to Their Conservation and Use.* Edward Arnold Publishers, London. p.168.

Frankel, O.H., Brown, A.H.D. and J.J. Burdon. 1995. *The Conservation of Plant Biodiversity*. Cambridge univ. press, Cambridge.

Harlan, J.R. 1992. *Crops and Man*. 2nd ed. American Society of Agronomy and Crop Science Society of America. Madison, WI.

Lashermes, P., P. Trouslot, F. Anthony, M.C. Combs and A. Charier. 1996. Genetic diversity for RAPD markers between cultivated wild accessions of *Coffea arabica*. *Euphytica.***87**: 59-64.

Leroy, T., C. Montango, A. Charrier, and A.B. Eskes. 1993. Reciprocal recurrent selection applied to *Coffea canephora* Pierre. I. Characterization and evaluation of breeding populations and value of inter-group hybrids. *Euphytica.* **67**:113-125.

Montagnon, C. and P. Bouharmont. 1996. Multivariate analysis of phenotype diversity of *C. arabica* L. *Genetic Resources and Crop Evolution.***43** : 221-227.

SAS Institute. 2001. SAS user's guide: Statistics. 5th ed. SAS Inst., Cary, NC.

Singh, R.K. and B.D. Chaudhary. 1987. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi. p. 318.

Southern Nations Nationalities People's Region Biro of Agriculture and Natural Resources department (SNNPR BoA and NRD). 2004. Agricultural Development Efforts, Extension Service, and Technology Transfer. In: Proceedings of the workshop on national planning of Ethiopia. June 2004, Addis Ababa, Ethiopia.

Souza, E. and M.E. Sorrels. 1991**.** Relationships among 70 North American oat germplasms: I. Cluster analysis using quantitative characters. *Crop Sci.,* **31**: 599-605.

Variability and Interrelationships between Coffee (*Coffea arabica* L.) Seedling Characters and their Implication in Selection

S. SEIFU[1] AND B. BELLACHEW[2]

[1]Jimma Agricultural Research Center, Awada Agricultural Research Sub-Center, P.O.Box 205, Yirgalem, Ethiopia. E-mail: seyoum03@ yahoo.com
[2]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, P.O. Box 192, Jimma, Ethiopia

SMMARY

Eighty one accessions of the Ethiopian coffee (*Coffea arabica* L.) germplasm were evaluated for fifteen characters at the seedling stage at Jimma Agricultural Research Center during 2002/2003 cropping season with the objectives of estimating the magnitude of phenotypic and genotypic variability, correlation coefficient, heritability and expected genetic advance. In effect, seedlings from the 81 accessions were raised and grown up in the nursery after arranging them in simple lattice with sixteen seedlings per plot. Eight months old seedlings were evaluated for different characters. The accessions differed significantly for all the traits except shoot (SDW) and root (RDW) dry weight, tap root length (TRL) and number of lateral roots (NLR) indicating that there is sufficient variability to have an effective selection for traits of interest. Stem diameter showed the minimum phenotypic (PCV) (10.1%) as well as genotypic coefficient (6.4%) of variation (GCV) whereas both PCV (33.5%) and GCV (21.7%) were highest for shoot fresh weight. Estimates of broad sense heritability ranged from 37.1% for root fresh weight (RFW) to 65.7% for leaf width (LW). Values of expected genetic advance (GA) expected from selection of the top 5% of the accessions as expressed relative to the means ranged from 9.4% for stem diameter (SD) to 31.9% for leaf area (LA). High heritability estimates for internode length, total number of stem nodes, leaf length, leaf width and leaf area coupled with high genetic advance indicated that these traits could be improved through mass selection. Of all the traits considered in the present investigation, seedling height with internode length; number of true leaves with total number of stem nodes; leaf length with leaf width, leaf area and shoot fresh weight; leaf width with leaf area and shoot fresh weight; leaf area with shoot fresh weight; and root fresh weight with lateral root length exhibited significant phenotypic and genotypic correlations suggesting that selection directed towards one character may also directly affect the others. However, care should be taken in controlling environmental effects due to significant positive phenotypic correlations. Overall, the study confirmed the presence of trait variability and higher estimates of genetic parameters for most of the characters in the Ethiopian coffee germplasm evaluated indicating the presence of immense opportunity for genetic improvement of the crop.

INTRODUCTION

Similar to other crops, yield in coffee (*Coffea arabica* L.) is a complex trait as it is polygenically controlled. Thus, knowledge of the association of various plant characters with yield and among themselves is essential. Such knowledge aids to identify morphological characters that are best indicators of yield potential and may be used for selecting for yield at the prebearing stage. Moreover, information on the nature and magnitude of variation in a population and the extent of environmental variation on the expression of these characters are necessary for selection to be efficient.

Establishment of the association between seedling and matured plant characters could help in advancing early selection and saving considerable amount of time particularly in a perennial crop like coffee where a single of generation selection takes ten or more years. Studying plants at seedling stage could help in advancing selection and early evaluation of morphological traits in plants may potentially select better plants at maturity (Ramos and Carvallho, 1997). Moreover, it was indicated that nursery pot experiments are preferred due to reduction of variability resulting from environmental factors. Ramos (1980) and Ramos et al. (1982), evaluating some coffee genotypes, showed that the study of young plants germinating in homogeneous substrates is feasible, allowing the simultaneous study of a large number of plants.

Various characteristics related to root systems of seedling and young plants have been studied with the purpose of characterizing varieties and species within the genus coffea (Monaco *et al*. 1973); determining the root fresh matter (Leon and Umana, 1961); the main (apical) root length (Ramos, 1980); the relative root surface (Ramos et al., 1978) and the shoot diameter, the root volume and the main root length (Ramos and Lima, 1980). Franco and Inforzato (1946) showed that the developmental pattern of coffee roots depends on the plant genotype. Shoots and seedling vigor also varied in different clones of Coffea canephora (Naidu et al., 1992).

Though nursery evaluation of seedlings in arabica coffee is not a common practice, there are some evidences that warrant the effectiveness of seedling evaluation. Root and shoot characteristics in coffee were associated with yield and environmental tolerances by Dublin (1968). Walyaro (1983) made nursery evaluation of certain traits in arabica coffee and reported high and positive correlation between heights measured in the nursery and field ($r = 0.91^{**}$), angles of laterals measured in the nursery and field ($r = 0.77^{**}$). He also noted positive correlation between mean over three years yield and earlier records of seedling girth, number of laterals, length of longest lateral, number of leaves and leaf area in the nursery. Mesfin (1982), working with Ethiopian arabica coffee, reported good association between growth of 21 months old seedling and girth ($r = 0.79^{*}$), number of flowers and fruits ($r = 0.48^{*}$) measured on three years old F_1 plants. Yehasab (1988) has also reported good correlation between three years average yield of F_1's and seven F_2 seedling characters ($r = 0.31 – 0.80^{*}$).

Yehasab (1988) reported heritability estimate of seedling characters such as stem diameter, number of leaves, height, shoot fresh weight, root dry weight, number of nodes and root volume, which ranged from 70 to 88.77 percent. Recently, Bayetta (2001) reported high broad sense heritability estimates for all the characters measured (stem diameter, number of leaves, height, shoot fresh weight, root dry weight and number of nodes). This ranged from 71.43 to 97.32 percent. Therefore, estimation of genetic parameters for growth characters, for which earlier studies confirmed strong correlation with coffee yield, is worth considering.

In view of these facts, the present study was conducted with the objective of estimating the magnitude of phenotypic and genotypic variability, correlation coefficient, heritability and expected genetic advance of certain traits at the seedling stage.

MATERIALS AND METHODS

Eighty-one coffee accessions were evaluated based on seedling characters at Jimma Agricultural Research Center (JARC) of the Ethiopian Institute of Agricultural Research. The research center receives an average annual rainfall of 1500 mm and has mean maximum and minimum temperatures of 25.0 °C and 11.2 °C, respectively. The experiment was laid in a 9 x

9 Simple Lattice Design. Sixteen (16) coffee seeds were sown per accession following the conventional method used for raising coffee seedlings (Taye, 1998). All management practices such as watering, mulching, shading; weeding and other operations were applied timely and uniformly as per the recommendations (Tesfaye, 1995) throughout the trial period.

Five eight months old seedlings were recorded per plot for root and shoot characters. Both destructive and non-destructive methods were used to record the required characters. The characters considered were, Seedling height, Number of true leaves, Mean leaf length, Mean leaf width, Total number of main stem nodes, Internode length, Stem diameter, Shoot fresh weight, Shoot dry weight, Root fresh weight, Root dry weight, Leaf area, Tap root length, Number of lateral Roots and Lateral root length.

All statistical analyses and data processing were performed using SAS (SAS Institute, 2001) version 8.2 Software. Analysis of variance for the traits considered was done using the following model (Singh and Ceccarelli, 1995):

$$Y_{ij} = \mu + g_i + r_j + \varepsilon_{ij}$$

Where: Y_{ij} = the response of Y trait from the i^{th} accession, j^{th} replication.

μ = Population mean.
g_i = the effect of the i^{th} genotype.
r_j = the effect of the j^{th} replication.
ε_{ij}= experimental error/error variance.

Estimates of genetic parameters were computed as shown below:

1) **Estimation of variance components:** The genotypic and phenotypic variance components and coefficients of phenotypic and genotypic variability were estimated based on the method suggested by Burton and De Vane (1953), Al-Jibouri *et al.* (1958) and Miller et al. (1958).

Genotypic Variance (σ^2_g):

$$\sigma^2_g = \frac{MSg - MSe}{r}$$

Environmental Variance (σ^2_e):

σ^2e= error mean square (MSe)

Phenotypic Variance (σ^2_p):

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where; MSg= mean square due to genotypes
MSe= environmental variance (error mean square)
r= number of replications

Phenotypic Coefficient of variation (PCV):

$$PCV= \frac{\sqrt{\sigma^2 p}}{\overline{X}} X100$$

Genotypic Coefficient of variation (GCV):

$$GCV=\frac{\sqrt{\sigma^2 g}}{\overline{X}} X100$$

2) **Heritability (H)**: Heritability in broad sense for all characters was computed using the formula suggested by Falconer (1989).

$$\text{Heritability (H)} = \frac{\sigma_g^2}{\sigma^2_p} \times 100$$

Where; H= heritability (in broad sense)
σ^2_g= genotypic variance
σ^2_p= phenotypic variance

3) **Genetic advance (GA) under selection**: Expected Genetic advance for each character (at 5% selection intensity) was computed using the methodology described by Johansson et al. (1955).

$$GA= k. \sigma_p. H$$

Where,
GA= expected genetic advance
K= constant (selection differential where k=2.056 at 5% selection intensity)
σ_P= phenotypic standard deviation on mean basis
H= Heritability in broad sense

4) **Genetic advance as percent of mean (GAM):** was also calculated to compare the extent of predicted genetic advance of different traits under selection using the following formula:

$$GAM=\frac{GA}{\overline{X}}$$

Where, GAM= genetic advance as percent of mean, $\overline{X}$ = mean of the population in which selection was employed.

RESULTS AND DISCUSSION

Analysis of Variance

The results of the present study revealed that all the seedling parameters except shoot dry weight (SDW), root dry weight (RDW), tap root length (TRL) and number of lateral roots (NLR) exhibited highly significant ($P < 0.001$) mean squares due to coffee accessions

(Table 1) indicating the presence of considerable variation among the coffee accessions evaluated. Significant variations for the characteristics considered in the study indicate that they were genotype dependent, or under genetic control. Previous works have also confirmed the genotypic effect in coffee seedling measurements (Vishveshwara and Raju, 1974; Naidu et al., 1992). The present finding is partly in agreement with previous findings of Bayetta (1991) and Yehassab (1988) who reported the presence of highly significant variation for traits studied in coffee seedlings.

Table 1. Summary of mean squares.

Characters	Mean Squares		
	Replication (1) [a]	Error (80) [a]	Genotypes (80) [a]
Seedling height	3.67	4.92	12.15***
Internode length	0.0001	0.096	0.349***
Total number of main stem nodes	0.48	0.16	0.555***
Leaf length	0.72	0.37	1.687***
Leaf width	0.35	0.07	0.361***
Stem diameter	0.001	0.001	0.0014***
Number of true leaves	0.75	0.91	2.279***
Leaf area	13.40	7.33	25.25***
Shoot fresh weight	0.38	1.16	2.836***
Shoot dry weight	0.97	0.66	0.85[ns]
Root fresh weight	3.16	0.35	0.76***
Root dry weight	0.07	0.07	0.017 [ns]
Number of lateral roots	3.49	178.11	205.27 [ns]
Lateral root length	34.83	2.27	5.27***

*Note: ***= highly significant (P <0.001); a = degrees of freedom for the respective source of variation; ns = non-significant.*

Estimates of Variability

The results of the analysis for the mean range between the accessions (Table 2) revealed a considerably wide deviation for all the seedling characters studied. The highest value was almost twice of the minimum value for seedling height, total number of stem nodes, leaf length, number of true leaves and leaf width; three fold for lateral root length; four fold for leaf area; five fold for root fresh weight and seven fold for shoot fresh weight. This result clearly indicated the presence of significant variation between the accessions for these characters and the possibility to bring considerable improvement of the traits through selection alone.

The estimate of phenotypic coefficient of variation (PCV) (Table 2) ranged from 10.1% for stem diameter to 33.5% for shoot fresh weight. Among the characters measured, stem diameter, root fresh weight, leaf area, lateral root length, internode length, seedling height and leaf width had a PCV higher than 15%. Similarly, a range of 6.4 % for stem diameter to 21.7 % for shoot fresh weight was observed for the genotypic coefficient of variation (GCV). The GCV was also higher than 15% for shoot fresh weight, leaf area, root fresh weight, lateral root length and internode length indicating the availability of sufficient variation for the improvement of these traits through selection and confirming the conclusions made earlier based on mean range value analysis.

Table 2. Summary of Estimates of Variability.

Characters	Range		Mean	S.D	σ^2_p	PCV%	σ^2_g	GCV%	H%	GA	GAM%
	Min.	Max.									
Seedling height	9.95	22.58	17.29	2.47	8.54	16.9	3.62	11.0	42.4	2.55	14.75
Internode length	1.20	3.20	2.35	0.42	0.23	20.1	0.13	15.1	56.9	0.55	23.40
Total number of stem nodes	4.80	8.00	5.84	0.53	0.35	10.3	0.19	7.60	54.5	0.67	11.47
Leaf length	4.61	9.01	7.15	0.92	1.03	14.2	0.66	11.3	63.9	1.34	18.74
Leaf width	1.90	3.79	2.98	0.42	0.22	15.7	0.15	12.7	65.7	0.63	21.14
Stem diameter	0.26	0.38	0.32	0.03	0.22	10.1	0.002	6.40	40.1	0.03	9.38
Number of true leaves	6.00	13.30	9.33	1.07	1.59	13.5	0.68	8.90	43.1	1.12	12.00
Leaf area	6.09	23.22	14.32	3.55	16.29	28.2	8.96	20.9	55.0	4.57	31.91
Shoot fresh weight	1.29	7.06	4.22	1.19	2.00	33.5	0.84	21.7	42.1	1.22	28.91
Root fresh weight	0.86	3.93	2.28	0.62	0.56	32.8	0.21	20.0	37.1	0.57	25.00
Lateral root length	4.99	6.49	7.90	1.63	3.77	24.6	1.50	15.6	40.0	1.60	20.25

SD = Standard deviation, σ^2_p = Phenotypic variance, PCV=phenotypic coefficient of variation, σ^2_g = Genotypic variance, GCV=Genotypic. Coefficient of variation, H = Broad sense heritability, GA = Genetic Advance, GAM =Genetic Advance expressed as percent of the mean.

Table 3. Phenotypic (above diagonal) and Genotypic (below diagonal) correlation coefficient among fifteen traits considered in the study.

Trait	SH	IL	TNMSN	LL	LW	SD	NTL	LA	SFW	RFW	LRL
SH	—	0.892**	0.539**	0.776**	0.786**	0.737**	0.598**	0.809**	0.903**	0.691**	0.535**
IL	0.882**	—	0.145	0.758**	0.807**	0.675**	0.288*	0.798**	0.772**	0.551**	0.438**
TNMSN	0.284	-0.159	—	0.300**	0.271*	0.338**	0.890**	0.310**	0.580**	0.510**	0.380**
LL	0.714	0.671	0.092	—	0.896**	0.708**	0.394**	0.939**	0.855**	0.704**	0.618**
LW	0.749	0.758	0.055	0.890**	—	0.719**	0.350**	0.929**	0.860**	0.711**	0.561**
SD	0.666	0.647	0.003	0.718	0.782	—	0.355**	0.746**	0.785**	0.741**	0.568**
NTL	0.355	-0.062	1.000**	0.183	0.148	-0.068	—	0.391**	0.628**	0.499**	0.394**
LA	0.776	0.747	0.098	0.974**	0.974**	0.773	0.184	—	0.901**	0.742**	0.612**
SFW	0.851	0.693	0.388	0.866*	0.892**	0.717	0.458	0.936**	—	0.797**	0.625**
RFW	0.573	0.439	0.321	0.782	0.787	0.709	0.332	0.809	0.779	—	0.713**
LRL	0.458	0.324	0.244	0.724	0.600	0.635	0.203	0.749	0.690	1.098**	—

SH =Seedling height, IL = Internode length, TNSN = Total number of main stem nodes, LL = Leaf length, LW = Leaf width, SD = Stem diameter, NTL = Number of True Leaves, LA = Leaf area, SFW = Shoot Fresh Weight, RFW = Root fresh weight, LRL = Lateral root length.

Estimates of Heritability and Genetic Advance

In the present investigation, estimates of heritability in the broad sense ranged from 37.1% for root fresh weight to 65.7% for leaf width. This finding is in contrast to the higher heritability estimates obtained for seedling parameters by Yehasab (1988) and Bayetta (2001). Heritability estimates of greater than 50% were observed for internode length, total number of main stem nodes, leaf length, leaf width and leaf area reflecting that these characters are highly heritable and thus improvement of these traits through selection could be efficient. Previous investigations by various authors have also indicated the existence of genotypic effect for Coffee seedling measurements (Vishveshwara and Raju, 1974; Naidu et al., 1992). The expected genetic advance as percent of the grand mean was highest for leaf area (31.91%); followed by shoot fresh weight (28.91%), root fresh weight (25.00%), internode length (23.40%), leaf width (21.14%) and lateral root length (20.25). In addition, internode length, total number of stem nodes, leaf length, and leaf width and leaf area exhibited high estimates of heritability and genetic advance indicating their greater amenability for selection.

All the characters investigated in the present study (Table 3), except internode length with total number of stem nodes, exhibited positive and significant phenotypic correlation among themselves. However, the genotypic correlation coefficient was significant only for seedling height with internode length; number of true leaves with total number of stem nodes; leaf length with leaf width, leaf area and shoot fresh weight; leaf width with leaf area and shoot fresh weight; leaf area with shoot fresh weight; and root fresh weight with lateral root length. The strong genotypic correlation observed among these characters may indicate that these characters are most probably controlled by the same genetic system that might be linked. This implies that selection directed for one trait will directly affect the other associated trait. Some of the characters, such as leaf length with leaf area and shoot fresh weight, have also showed high correlation because of their obvious close association.

Moreover, the high positive association of yield with stem diameter and shoot fresh weight; positive and highly significant association of bearing primaries with number of leaves, number of nodes and root fresh weight; and positive and significant association of number of fruits with number of leaves, number of nodes and shoot fresh weight observed earlier in eight months old seedlings by Yehasab (1988) indicated the importance of these characters in selecting for yield potential at seedling stage.

CONCLUSION

The results of the study revealed the presence of considerable variation among the coffee accessions evaluated and this can be utilized for further improvement of the crop. Certain traits (Shoot fresh weight, Leaf area, Root fresh weight, Lateral root length and Internode length) exhibited higher GCV indicating that there is sufficient variation for improving these traits. Thus they should further be evaluated to verify their possible association with field performance and be considered in selection.

Traits that had higher genotypic coefficient of variation, heritability and genetic advance (SFW, RFW and TNMSN) and for which earlier reports confirmed strong and positive association with yield and yield components in coffee may be utilized in selecting high yielding genotypes at the seedling stage. Moreover, traits that had significant and positive genotypic association with each other (seedling height with internode length; number of true leaves with total number of stem nodes; leaf length with leaf width, leaf area and shoot fresh weight; leaf width with leaf area and shoot fresh weight; leaf area with shoot fresh weight; and root fresh weight with lateral root length) may indicate that these characters are most

probably controlled by the same genetic system that might be linked. This implies that selection directed for one trait will directly affect the other associated trait. Conversely, low or non-significant genotypic correlations exhibited by most of the traits suggest independence of association, an indication that it could be possible to characteristics to diverse directions. However, care should be taken in controlling the environmental effects because of significant and positive phenotypic coefficient of correlations and the study should continue to verify possible association of measurements at the seedling stage with known features of the respective genotypes at adult plant stage.

ACKNOWLEDGEMENT

We acknowledge the Agricultural Research and Training Project (ARTP) of Ethiopian Institute of Agricultural Research (EIAR) for funding the research. We are indebted to Jimma Agricultural research center for provision of germplasm as well as research facilities for the field and laboratory evaluation and hosting the study. We sincerely appreciate the assistance rendered by the staff of Coffee Breeding and Genetics Division of Jimma Agricultural Research Center.

REFERENCES

Al-jibouri, H.A., P.A., Miller and H.P. Robinson. 1958. Genetic and environmental variance in upland cotton crosses of interspecific origin. *Agron. J*.**50**: 633-636

Bayetta, B. 1991. Nursery Evaluation of Hetrosis and Combining Ability in Reference to Origin and Morphology of Parents in Coffee (Coffea arabica L.). M.Sc thesis, Alemaya University of Agriculture, Alemaya.

Bayetta, B.2001. Arabica Coffee Breeding for Yield and Resistance to Coffee Berry Disease (Colletotrichum Kahawae sp.nov.), Doctoral Thesis, Imperial College at Wye University of London.

Burton, G.W. and E.H. Devane. 1953. Estimation of heritability in tall Festuca (Festuca arundinacea) from replicated clonal material. *Agron. J*. **45**:478-481.

Dublin, P.1968. Le rapport longueur pivot/longueur hypocotyle des plantes de coffea racemosa Lour.et de quelques autres especes du genre.*Cafe Cacao the,* **12**(2):127-134.

Falconer, D.S.1989. Introduction to Quantitative Genetics. 3rd edition Longman Scientific and Technical publishing, London. 438pp.

Franco, C.M. and R.O. Inforzato. 1946. Sistema radicular do cafeeiro nos principais tipos de solo do Estado de Sao Paulo. *Bragantia, Campinas*, **6**:443-478.

Johansson, H.W., H.F. Robinson, and R.F. Comstock. 1955. Genotypic and phenotypic correlation in soybean and their implication in selection. *Agron. J*. **47**:477-483.

Leon, J and R. Umana.1961. Diferencias varietales en el sistema radical del café IICA.*Turrialba*, Costa Rica, **3**(11): 130-133.

Mesfin, A. 1982. Hetrosis in crosses of indigenous coffee (Coffea arabica L) selected for yield and resistance to CBD. I. At first bearing stage. *Eth. J.Agric. Sci*. **4**: 33-43.

Meyer, F.G. 1965. Notes on wild coffea arabiaca L. from southwestern Ethiopia with some historical consideration. *Econ. Bot*. **19**(2): 136-151.

Miller, P.A., J.C. Williams, H.F. Robinson and R.E. Comstock.1958. Estimates of Genotypic and Environmental Variances in upland cotton and their implications in selection. *Agron. J*. **50**:126-131.

Monaco, L.C., M.H., Scali, A. Carvalho and L.C. Fazuoli. 1973. Variabilidade no sistema radicular de genotipos de café, Ciencia e Cultura, Sao Paulo. **25**:247 (Resumos)

Naidu, M.M., Muniswamy and H.L. Sreenath. 1992. Comparison of seedling vigor in three clones of *Coffea canephora* (Robusta). *Journal of Coffee Research*, Karnataka, **22**(2): 131-134

Ramos, L.C.S. 1980. Desenvolvimento de plantulas de quarto cultivares de café. *Braganita, Campinas*, **39**: 215-218.

Ramos, L.C.S. and A., Carvalho.1997. Shoot and Root Evaluations on Seedlings from *Coffea* Genootypes.Bragantia. Vol.56 n. Campinas.Brasil.

Ramos, L.C.S., M.M.A. Lima and A., Carvalho. 1982. Crescimento do sistema radicular e da parte aerea em plantas jovens de cafeeiros. *Braganita, Campinas*, **41**: 93-99.

SAS Institute. 2001. SAS User's Guide. Version 8.2 ed. SAS Institute, Cary, NC, USA.

Singh, M. P and S. Ceccarelli. 1995. Estimation of heritability using variety trials data from incomplete blocks. *Theor. Appl.Genet*. **90:**142-145.

Taye, K. 1998. Response of Arabica Coffee (Coffea arabica L.) to Various Soil Fertility Management. M.Sc. Thesis, AUA, Ethiopia.

Tesfaye, S. 1995. Influence of Nursery Watering Frequency, Moisture Status of Rooting Media and Media Moisture Conservation on Growth of Coffee (coffea arabica L.) Seedlings. M.Sc. thesis, Alemaya University of Agriculture, Alemaya.

Vishveshwara, S. and S.K. Raju. 1974. Root systems of dwarf and tall plants in San Ramon hybrids of C*. arabica*. *Journal of Coffee Research*, Karnataka, **4**(4): 94-104.

Walyaro D.J. and H.A.M. Van der Vossen. 1979. Early determination of yield potential in arabica coffee by applying index selection. *Euphytica*. **28**:465-72.

Yehasab A. 1988. Segregation and Correlation Studies of Agronomic Characters in the F_2 Progenies of Coffee (Coffea arabica L). M.Sc. Thesis, Alemaya University of Agriculture, Alemaya, Ethiopia.

Genetic Erosion – A Threat to the Conservation and Sustainable Utilization of Ethiopian Coffee (*Coffea arabica* L.) Germplam

S. SEIFU[1] AND B. BELLACHEW[2]

[1]Jimma Agricultural Reszearch Center,Awada Agricultural Research Sub-center, P.O.Box 205,Yirgalem,Ethiopia. E-mail: seyoum03@yahoo.com
[2]Ethiopian Institute of Agricultural Research, Jimma Agricultural Reszearch Center, P.O. Box 192, Jimma, Ethiopia

SUMMARY

A number of reports are being published which indicated that the arabica coffee genepool of Ethiopia, which represents an important heritage of mankind and the most important resource for breeding of varieties and biological research, is under a severe threat of genetic erosion. The effort made so far to conserve the vast coffee genetic resource that the country is endowed with and to assess and monitor genetic erosion in this crop of high economic significance and world heritage has also been reported to be very little. Consequently, there has been a rising concern about possible genetic erosion of landrace coffee types and wild relatives due to replacement by few Coffee Berry Disease (CBD) resistant coffee varieties, shift in land use from Coffee growing to chat (Catha edulis Forks.ex Endl), deforestation of and incidental fire on the forest coffee ecosystem which harbour the arabica coffee genepool, and settlement of immigrants in portions of the forest coffee. In this article, the major factors contributing to the dewindling of the arabica coffee gene pool are discussed, the weak link between private public partnerships and international collaboration is highlighted and measures to be taken to save the threatened arabica coffee genepool are suggested.
Key words: Genetic erosion, Coffea arabica, Germplasm

INTRODUCTION

It has long been recognized that Ethiopia is the center of origin and genetic diversity of arabica Coffee (*Coffea arabica* L.) which fetches premium prices in the world market, provides a livelihood for over 25% of the Ethiopian population, covers 4-5% of GDP and 60% of the country's foreign exchange earnings and contributes for employment of rural and urban communities involved in the production and sale of the crop, source of income and food security for the producing countries. No other product or service in Ethiopia offers these opportunities.

Ethiopian Coffee (*Coffea arabica* L.) represents an important heritage of mankind and the most important resource for breeding of varieties and biological research. Various researchers have reported that the types of production systems (Melaku, 1982b), the occurrence of wild coffee types with distinct phenotypic differences, distinct variation observed in quality and bean size (Dawit, 1986), and the existence of distinct agrotypes adapted to the system of production (Watkins, 1987) could be considered as indicators of the vast diversities existing in the Ethiopian coffee. Moreover, Paulos and Demil (2000) reported that almost the entire south-west Ethiopia and more than half of South Ethiopia could have been covered by coffee plantations due to suitability of the land and agro ecology had it not been for growing other food crops. Despite its importance to biological research and cultural and socio-economic values to all those involved in its production and trade, however, it is one of the most

neglected crops in the world with respect to genetic conservation (Tewoldebirhan, 1990), the effort made so far to conserve the vast coffee genetic resource that the country is endowed with is very little. Consequently, there has been a rising concern about possible genetic erosion of landrace coffee types and wild relatives due to man-made and other natural calamities and the effort made so far to assess and monitor genetic erosion in this crop of high economic significance and world heritage has also been very little. Therefore, this article is prepared with the following objectives:

1. to highlight the threat of genetic erosion to Ethiopian Arabica coffee germplasm and
2. suggest/recommend possible solutions

Genetic erosion

Genetic erosion (the extinction of land races by natural and man made calamities) of agricultural crops is a threat to global food security as a consequence of enhanced risks to pests, diseases, and extreme environmental conditions. Continued existence of genetic diversity of crops ensure against these problems but is threatened by increasing dependence on modern, high yielding crop varieties, changes in historic land use patterns, changes in local and regional economies, and shortages of agricultural labor. Scarascia-Mugnozza and Perrino (2002) reported that the spread of new and more productive crop varieties, which were generally less heterogeneous than primitive populations, paradoxically started the well-known process of genetic erosion. Moreover, they have reported that outbreak of Southern Corn leaf blight in the 1970s' and a catastrophic outbreak of Coffee leaf rust in Brazil provoked publicity of genetic erosion on a global scale. Engles and Hawkes (1971) reported that the diversity in the Ethiopian coffee gene pool is highly threatened by erosion due to extensive deforestation and replacement of primitive coffee populations by maize, chat and other crops, and changing patterns in land use.

Apart from preliminary observations and speculations, systematic study of genetic erosion (causes, extent and consequences) in the arabica coffee gene pool of Ethiopia is limited and the effort made so far to assess and monitor genetic erosion within this crop on-farm is very little and the crop seems particularly threatened due to the following factors:

CHANGES IN LAND USE

The genetic resources of coffee and the associated flora and fauna are disappearing rapidly as a result of deforestation of the ecosystems due to increased demand for more cultivable land for crop production (as a result of population growth, decline in soil fertility and productivity of the currently cultivated land), fuel wood and timber. A shift in the land use pattern from coffee cultivation to that of khat (Catha edulis Forsk.ex Endl.) has also been observed with the decline in the market price of coffee and other socio-economic factors Zenebe et al. (2002). Watkins (1986) and the IAR team concluded that 80 percent of the Coffee from Habro Awraja, which produces 40 percent of the Harer coffee, is rapidly declining in production and suggested a prompt action to save the germplasm of this coffee which has a tremendous worldwide reputation for quality. A recent study conducted by Teklu and Thomas (2004) in Yayu and Sheko woredas indicated continuous price reduction as a number one threat (Table 1) to population of wild coffee in the forest. With the decline in the market price of coffee, framers start to look for other alternatives like growing cereals and selling them in the market. This in turn meant that they engage themselves in clearing forests in search of more agricultural lands (Teklu and Thomas, 2004). This has also been reported by other researchers (Demil, 1999; Zerihun, 1999; Tadesse et al., 2002).

An unpublished report of a survey carried out by Bayetta et al.(1996) in the southern part of Ethiopia have also revealed that farmers were forced to uproot a large number of coffee farms covered by landraces and replace it with khat (Catha edulis Forsk.ex Endl.) with the decline in the market price of coffee. Similar trends have also been observed with the current decline in the market price of coffee. In a recent (2005) coffee collection mission in the Dale and Aletawondo Woredas of southern Ethiopia, the team have witnessed replacement of coffee landraces by the very few improved selections and shift in land use from Coffee growing to Khat (Catha edulis Forsk.ex Endl) pineapple and maize in drought prone areas where these crops have a comparative advantage over coffee particularly when coffee prices are very low. If the prevailing conditions continue without appropriate intervention by the state and the local community, we may end up with a narrow genetic base of our coffee genetic resource for which no immediate remedy can be sought.

Table 1. Farmers' perception of threat (%) to wild coffee population in Yayu and Sheko forest.

Threats to the wild coffee Population	Yayu	Sheko	Total
Indiscriminate deforestation	22.5	18.60	20.60
Population increase	15.80	24.40	20.10
Agricultural expansion	10.50	12.50	11.50
Price reduction	30.0	25.00	27.50
Disease	21.20	19.50	20.30
Total	100.0	100.0	100.0

Source: Teklu and Thomas (2004).

REPLACEMENT OF LAND RACES BY IMPROVED CULTIVARS

With the advent of scientific plant breeding, the rapid spread of high yielding varieties, characterized by a narrow genetic base caused the displacement of traditional unimproved cultivars, which had larger genetic bases.

The Coffee Improvement Project (CIP) initiated by the former government of Ethiopia to combat the deadly Coffee Berry Disease (CBD) has resulted in the development and release of thirteen CBD resistant selections that have originated from southwestern parts of the country. The development and release of these lines all over the country and the subsequent replacement of landraces by these improved but narrow spectrum of varieties in the last three decades witnesses the prevalence of genetic erosion in our coffee genetic resources unless necessary precautions are taken.

Mesfin Ameha (1971) reported that with the advent of Coffee Berry Disease (CBD) in 1971 and the subsequent identification CBD resistant selections, the distribution of resistant cultivars resulted in the retention of relatively invariable individuals in some typical coffee forests where they were replanted after forest clearing. He further noted that this has caused significant losses in genetic diversity and indicated that between 25,000 and 35,000 hectares of semi-forest coffee has so far been replaced with CBD resistant cultivars leading to at least 10 percent loss. Moreover, it was estimated that about 120,000 hectares of land will be replanted with the advanced cultivars and about 80 percent of the remaining 230,000 hectares will be lost through other factors by the year 2000 (Mesfin Ameha, 1971) if agricultural development, forest utilization and population growth continue with the pace that prevailed during that time.

An unpublished report of Dale Woreda Pilot Learning Site Diagnosis and Program Design (2004) indicated that the proportion of improved selections versus landraces varies between regions depending on the presence of road infrastruture. The report further noted that farmers' interviewed in one peasant association near the road reported up to 90% of their coffee being from improved varieties. Hence, it is generally assumed that there is a rapidly diminishing diversity within the arabica coffee genepool on farmers' fields. However, Jimma Agricultural Research Center has taken the initiative to minimize, if not to stop, the loss of coffee genetic resources by establishing sub-centers, sub-stations and testing sites in the major coffee growing regions, where landraces are collected, characterized, utilized for various coffee research activities and thereby conserved.

GEO-POLITICAL PROBLEMS

The permanent settlement of immigrants who come in search of job opportunities during the peak coffee harvesting season and remaining behind has also been reported to contribute to the loss of our coffee genetic resource. These immigrants were reported to demarcate portions of the forest coffee area, claim as their possession illegally and start inflicting damage on the resources. Political unrest may also result in mass destruction of forest ecosystems, field gene banks and infrastructures installed for conservation.

DEFORESTATION AND FOREST FIRE

The coffee forest and wild coffee are disappearing rapidly mainly because of deforestation (Teklu and Thomas, 2004). Quite recently, Ethiopia experienced a catastrophic event of incidental fire in which over 70,000ha of forest in the south-eastern part of the county was burnt down in February-March 2000 (Tadesse, 2002). Deforestation of and incidental fire on the forest coffee ecosystem that harbours the arabica coffee genepool contributed to the loss of forests housing much of the arabica coffee genepool (Tadesse, et al., 2002). Paulos and Demil (2000) reported that erosion of the vast coffee genetic resource base of Ethiopia is being caused by destruction of habitats by deforestation. In a recent study conducted by Teklu and Thomas (2004) in Yayu and Sheko Woredas, deforestation was mentioned by farmers as one of the factors to which decrease in the population of wild coffee is attributed (Table 2).

Table 2. Farmers' perception (%) of factors that have led to the change in population of the wild coffee in the forest in Yayu and Sheko.

Factor	Yayu	Sheko	Total
Ageing	16	22	23
Deforestation	47	34	40.5
Agri.Expansion	17	36	26.5
Disease	10	8	10

Source: Teklu and Thomas (2004).

PREVALENCE OF COFFEE PESTS

Prevalence of severe diseases-particularly of Coffee berry and wilt diseases has been reported to be one of the major factor threatening arabica coffee population in the forest. Adugna and Hindorf (2000) and Girma and Hindorf (2001) reported diseases and pests (Coffee Berry Disease (CBD), Coffee Leaf Rust (CLR), and other pathogens as well as pests) as factors threatening wild coffee population in the forest

ISSUES THAT NEED TO BE ADDRESSED

Improved Partnerships in conservation

It is well known that the genetic base on which the coffee industries around the world depend is narrow and thus endanger the coffee based industries and the people depending on them, if production fails due to such unforeseen calamities as diseases and pests as that occurred due to the outbreak of Coffee Leaf Rust (CLR) in 1869 and 1870 Srilanaka, Java and Sumatra respectively where coffee plantations were completely wiped out because of a narrow genetic base. Moreover, it is widely accepted that the future coffee breeding efforts will depend on a continuing and expanding supply of germplasm. Thus, an urgent task for the future is to strengthen collaborative efforts to conserve our coffee genetic resources that play a major role in the socio-economic development of the country.

The role of Local communities in decision making

Previous attempts to conserve the forests in general and the coffee forests in particular were precarious and did not have significant impact. One of the many possible factors to past failures is failure to understand farmers' perception of the need to conservation and hence lack of participation of the local communities in the planning, decision making and implementation processes of conservation activities (Kumelachew, 2001; Yonas, 2001). Therefore, local communities at various levels should be involved in decision making and formulation of policies for the use and management of coffee genetic resources., failure to recognize this dimension may result in developing superficial policy measures and designing and implementing less effective and perhaps redundant conservation and use concepts that fail to address the structural differences between resource users on the one hand and resource users and policy makers on the other (Teklu and Thomas, 2004).

Institutional linkage and international partnership

A number of Ethiopian institutions are involved in the improvement, conservation and trade of coffee. Among these Bureaus' of agriculture of the Ministry of Agriculture and Rural Development (MoARD), Institute of Biodiversity conservation (IBC), Environmental Protection Authority (EPA) and Research Centers play greater role in the collection, improvement and conservation of coffee genetic resources. Nonetheless, it largely appears that the institutional linkage is very weak and this might have contributed to the loss of variability in the arabica coffee genepool. Therefore, it is urgently required to strengthen institutional arrangements between various stakeholders if we are to save remnants of the dwindling arabica coffee genepool.

Considering the risk that might occur if conservation of this most important beverage is left only for Ethiopia, Paulos and Demil (2000), suggested a coordinated effort to help Ethiopia conserve the coffee germplasm on behalf of the interest of all arabica coffee consumers. Moreover, Tewoldebirhan (1990) reported that collection and preservation of this resource of higher economic importance cannot be left only to Ethiopia because of its poor economic strength. Nonetheless, apart from some collaborative efforts with the Food and Agriculture Organization (FAO) of the United Nations (UN) and the former ORSTOM now IRD, for the collection of Ethiopian arabica coffee germplasm in the 1960's; with the European Union (EU) for landrace development of Ethiopian coffee and the government of Switzerland for the Ethio-Swiss Coffee Research Project (ESCORP), the effort made so far to strengthen international collaboration is very little. Hence, concerned institutions should focus their

efforts in this regard if the Ethiopian Coffee industry is to undergo desired improvement from its present condition.

The role Private public partnership

Though a number of stakeholders (private investors, NGOs, etc.) are involved in the coffee system, the role they played so far in the improvement and conservation of coffee germplasm has been very little and fund for research and conservation efforts is allocated only by the central government contrary to the case in some developing African countries (eg.Kenya) and developed nations.

CONCLUSION AND RECOMMENDATION

There has been a global effort over the past decade to collect and preserve the germplasm of many crop species in response to the fear that much of their genetic diversity could disappear due to pressures exerted by mankind. Despite the significant contribution of arabica coffee to the Ethiopian economy in particular and the world at large, however, there has been little effort by concerned institutions to conserve the vast genetic resources that the country is endowed with. The loss of heterogeneous coffee populations, which represent the genepool for hundreds of agronomic traits, will be catastrophic. The well known coffee of Limu, Nekemte, Gimbi, Harer, Sidama and Yirgacheffe, which fetch high premium, will no longer exist unless immediate ways are found to preserve it.

Apart from preliminary observations and speculations, systematic study of genetic erosion (causes, extent and consequences) in the arabica coffee gene pool of Ethiopia is limited and the crop seems to be threatened due to increasing dependence on modern, high yielding varieties, changes in historic land use patterns, deforestation and forest fire, geo-political problems and prevalence of diseases and insect pests. Therefore, studying the underlined causes of genetic erosion, determining the extent, defining key indicators for the same and identifying areas threatened by genetic erosion in various coffee growing regions is indispensable for the conservation and sustainable use of coffee genetic resources and this will ultimately provide baseline information to develop an early warning system at the national level. Moreover, there is a need to involve local communities at various relevant decision making levels, engage in international collaboration, and strengthen private public partnerships to save remnants of the Ethiopian arabica coffee genepool.

In general, upgrading the awareness of the local communities and improving their participation in the planning and implementation of conservation efforts, urging the concerned institutions to fully address the collection, characterization and conservation of this crop of high economic importance is an indispensable task to the state if conservation of our renowned coffee genetic resource and the associated development of our coffee industry is to be realized. In order to alleviate the fear of extinction of the wide coffee genetic resources that our country possesses and maintain the genetic variability of wild and cultivated coffee populations, conservation of the natural coffee farms around the country, especially the natural forest ecosystems is urgently required. This will consequently assist in the maintenance or collection of germplasm that could be used to establish living collections and improve the quality, quantity, and resistance to pests and abiotic stresses.

ACKNOWLEDGEMENT

We acknowledge with thanks the travel grant extended by the International Foundation for Science (IFS) to the first author to present this paper at the 21st ASIC Conference. Further

thanks are due to the ASIC' 06 Organizing Committee for the invitation that they sent us to present this paper at the 21st ASIC Conference in France, Montpellier. We also thank EIAR/JARC management for granting permission to the author to participate in the conference.

REFERENCES

Adugna, G. and H. Hindorf. 2000. Recent outbreaks and investigations on Gibberella xylarioides (Fusarium xylarioides) on coffee in East and Central Africa. 6 Eur. Fusarium Sem., Berlin.Mitt.BBA 377, 66.

Bayetta B., Zebene, M., Tesfaye, S. and Woldemichael, W. (Unpublished). Survey of coffee production potential in Sidama Zone, Southern Region. Ethio-Swiss Coffee Research Project-ESCORP report. Institute of Agricultural Research, Jimma agricultural Research Center.

Bayetta B., Zebene, M., Tesfaye, S. and Woldemichael, W. (Unpublished). Survey of coffee production potential in Sidama Zone, Southern Region. Ethio-Swiss Coffee Research Project-ESCORP report. Institute of Agricultural Research, Jimma agricultural Research Center.

Dale Woreda Pilot Learning Site Diagnosis and Program Design. 2004. Report on Priority commodity description, analysis and potential interventions.ILRI,Ethiopia.

Dawit, T. 1986. Coffee genetic variability study among three major coffee growing regions based on phenotypic characters

Demel, .1999. History, Botany and Ecological requirements of Coffee.Walia, **20:**28-50.

Engles, J.M.M. and Hawkes, J.G. 1971. The Ethiopian gene center and its genetic diversity. In: Plant Genetic Resources of Ethiopia. J.M.M. Engles, J.G., Hawkes and Melaku Worede (Eds.). Cambridge University Press, Cambridge.

Girma, A., H.Hindorf.2001. Tracheomycosis (Gibberella xylarioides) on Coffee (Coffea arabica L.). Deutscher Tropentag, Bonn.Book of abstracts.p.143 and CD ROM.

Kumilachew Yeshitela. 2001. Loss of forest biodiversity associated with changes in land use: the case of Chewaka-Utto tea plantation. In: Biological Society of Ethiopia: Imperative problems associated with forestry in Ethiopia. Proceedings of a workshop. Addis Ababa University, Addis Ababa.Pp:115-122.

Melaku, W. 1982b. Coffee Genetic Resources in Ethiopia, Conservation and Utilization with Reference to CBD Resistance. Proceedings of the First Regional Workshop on Coffee Berry Disease.pp:203-211.

Mesfin Ameha. 1971. Significance of Ethiopian coffee genetic resources to coffee improvement. In: Plant Genetic Resources of Ethiopia. J.M.M. Engles, J.G., Hawkes and Melaku Worede (Eds.). Cambridge University Press, Cambridge.

Paulos, D. and Demil, T. 2000. The Need for Forest Coffee Germplasm Conservation in Ethiopia and its Significance on the Control of Coffee Berry Disease(CBD). In: Proceedings of the Workshop on Control of Coffee Berry Disease(CBD) in Ethiopia.

Scarascia-Mugnozza, G.T. and Perrino, P.2002. The history of ex-situ conservation and use of Plant genetic resources. In:Engles,J.M.M., Ramanatha Rao,V., Brown, A.H.D., Jackson, M.T.(eds.).2002. Managing Plant Genetic Diversity. IPGRI. Rome, Italy.

Tadesse, W.Gole, M.Denich, Demel, T, P.L.G. Vlek.2002. Human impacts on Coffea arabica genepools in Ethiopia and the need for its in situ conservation. In: Managing Plant

Genetic Diversity. Engles, J.M.M, R. Rao, A.H.D. Brown, J.M.M, Jackson (eds.) CAB International and IPGRI, 237-247.

Teklu T. and Thomas B. 2004. Wild Arabica Coffee Populations under Severe Threat: Farmers' Perception of Existence, Access to and Conservation needs in the Montane Rainforests of Ethiopia. Conference on International Agricultural Research for Development. Deutscher Tropentag 2004-Berlin, 5-7 October 2004.

Tewoldebirhan, G. 1990. The importance of Ethiopian forests in the conservation of arabica coffee genepool. In: H.D. Inlenfeldt(Ed.). Proceedings of the Twelfth Plenary Meeting of AETFAT.Mitt.inst.Allg.Bot.Hamburg 23a:65-72.

Watkins, R.1986. Proposed actions for maintenance and production of Harer coffee. Coffee Improvement Project (CIP).A.A., Ethiopia.

Watkins, R.1987. Selecting, Breeding, Testing and Releasing New Ethiopian Cultivars and Multi-cultivars Which Combine High Quality, Good Yield, CBD and other Resistance and Which are Adapted to the Relevant Farm Systems of Ethiopia. Wye College/ Coffee Improvement Project.

Yonas Yemshaw. 2001. Status and prospects of forest policy in Ethiopia. In: Biological Society of Ethiopia: Imperative problems associated with forestry in Ethiopia. Proceedings of a workshop. Addis Ababa University, Addis Ababa.Pp:9-30.

Zenebe, W., Million, A., Tesema, C., Etana, E., and Bayu, T.2002. Present situation of Harrar Coffee and Khat production and marketing. Biodiversity Newsletter.Vol.1, No.2.

Zerihun Woldu.1999 Forests in the vegetation types of Ethiopia and their status in the geographical context. In: Forest Genetic Resources Conservation: Principles, Strategies and Actions. Edwards, S., Abebe Demissie, Taye Bekele, G.Haase (eds.), IBCR/GTZ.Addis Ababa, 1-36.

Physiochemical Evaluation of Green Bean of Some Coffee *Canephora* Varieties in Nigeria

S. O. OGUNWOLU AND S. S. OMOLAJA

Cocoa Research Institute of Nigeria, P. M. B. 5244, Ibadan, Oyo state, Nigeria

SUMMARY

Three Coffee *canephora* varieties (Quillou, Ex-java, and Niaoulli) in Nigeria were wet processed by depulping, fermentation, washing and dried in the sun. Screen test was carried out on the samples of these coffee varieties, using screen of 7.5 mm, 6.5 mm, and 5.5 mm apertures for size classification. Chemical compositions of the coffee beans were determined. In terms of physical characteristics, Quillou could be graded as small bean as it was retained by 5.5 mm screen, with 2.4% (w/w) passed through and its 100 bean weight was 13.0 g. Ex-java could be graded as medium bean, it was retained by 6.5 mm screen with 2.3% (w/w), and its 100 bean weight was 16.0 g. Niaoulli could be graded as large bean, it was retained by 7.5 mm screen with 2% (w/w) passing through the screen, and its 100 bean weight was 17.0 g. Caffeine content of Quillou (2.2%) was found to be the highest among others, while Ex-java has the highest ash content of 4.4% and highest protein content of 2.9%. Niaoulli has the least Caffeine content (1.0%)

INTRODUCTION

Coffee comes from all around the world and is therefore a truly international trade. It is the world's second largest traded commodity next to oil. Around two-thirds of it comes from the Americas but there are many large and small producers in countries such as Arabia, India, Africa, West Indies, Java and Sumatra. The coffee from each different area of the world has its own unique taste (Clarke and Macrae, 1989).

Besides location, other factors affect the quality and flavour of coffee. These include the variety of the plant, the chemistry of the soil in which it is grown, the weather, and the precise altitude at which the coffee grows. Such variables combined with the way the cherries are processed after being picked contributes to the distinctions between coffees from countries, growing regions and plantations worldwide. The combination of factors is so complex, that even from a single plantation one finds variation in quality and taste (Wrigley, 1988).

There are two main species of commercial coffee – *Coffea arabica* and *C. canephora* (robusta). Arabica is a higher quality and higher value coffee normally grown in cooler, elevated areas of the tropics and sub-tropics at 1000 m or more above sea level. Arabica is used in the roast and ground coffee market and is added to blends of Robusta to improve the quality of instant coffee.

Robusta is a lower quality coffee and is used mainly in instant coffee and for blending with Arabicas to add body and crema. Robusta is normally grown in warmer areas at lower elevations unsuited to Arabica. Compared with Arabica, Robusta is generally more vigorous, more productive and less vulnerable to rust. On the west coast of Africa, the Ivory Coast is one of the world's largest producers of robusta coffee. In the mid 1990 s it was the largest

African coffee producer, fifth in the world overall and second for the production of Robusta (Rene, 1992).

Coffee is a long-term crop with a lifespan of more than 10 years, and considerably longer under good management, thus the choice of variety (cultivar) is very important. As quality of the coffee bean is crucial for production of high-grade coffee, choose only varieties that are recommended for your area. These will be the best yielding, best quality varieties that will grow productively in the local soils and climate (Njoroge, 1998).

All food products possess characteristics which are related to their state, aspect or appearance such as weight, volume, size, shape, colour, solubility, moisture content, texture, etc. Coffee is no exception. From the tree to the cup, the various physical characteristics of coffee in its different forms play an important part in the way it is treated and in the design of equipment to process it (Bucheli, 1998).

MATERIALS AND METHODS

Fresh berries of Quillou, Exjava and Nialloui varieties were harvested from CRIN Headquarters plantation, Ibadan Nigeria.

Processing

The Coffee berries were weighed, and wet processed separately by depulping, fermentation, washing and drying in the sun until the moisture content was about 12%. The dried parchment coffee was then dehulled manually, and the films removed to obtain clean green beans.

Physical analysis

100 green beans of each coffee variety (Quillou, Exjava, and Nialloui) were selected and weighed to obtain 100 green bean weights.

Screen Test

This was carried out using screen of 7.5 mm, 6.5 mm, and 5.5 mm aperture for size classification.

Chemical analysis

The chemical compositions of the 3 coffee varieties (Quillou, Exjava and Nialloui) were determined, using the method of Clifford and Wilson (1985).

RESULTS AND DISCUSION

Table 1. Physical and Chemical properties of Three Coffee canephora Varieties.

CHEMICAL COMPOSITIONS	QUILLOU	EX-JAVA	NIAOULLI
100 Green bean weight (gm)	13.0	16.0	17.0
Moisture Content (%)	12.0	11.4	11.8
Caffeine Content (%)	2.2	1.2	1.0
Fat Content (%)	11.0	13.8	12.3
Ash Content (%)	3.8	4.4	3.9
Protein (%)	1.5	2.9	1.8

SCREEN TEST RESULTS

NIAOULLI: Retained by 7.5 mm screen with 2% (w/w) passing through.

EX-JAVA: Retained by 6.5 mm screen with 2.3% (w/w) passing through.

QUILLOU: Retained by 5.5 mm screen with 2.4% (w/w) passing through.

From the Screen test results above, Quillou was retained by 5.5 mm screen, with 2.4% (w/w) passed through this screen and could be graded as small bean and its 100 bean weight was 13.0 g. Ex-java was retained by 6.5mm screen with 2.3% (w/w) passed through this screen and could be graded as medium bean, and its 100 bean weight was 16.0 g. Niaoulli was retained by 7.5 mm screen with 2% (w/w) passing through this screen, and its 100 bean weight was 17.0 g.

According to Table 1, Caffeine content of Quillou (2.2%) was found to be higher than that of the Ex-java, which was higher than that of Niaoulli. Also, from the same Table 1, the Ash content which is an indication of minerals content was found to be highest in Ex-java (4.4%) and lowest in Quillou (3.8%). Ex-java has highest protein content (2.9%), followed by Niaoulli (1.8%) and Quillou (1.5%).

REFERENCES

Bucheli P, et al, (1998). Industrial Storage of Green Coffee under Tropical Conditions and its impact on raw material Quality and Ochratoxin A content. J Agric. Food Chem. 1998, 46, 4507-4511.

Clarke R. J. and Macrae R. (1989) – Coffee Vol. 2 – Technology. London, Elsevier Applied Science Publishers, 1989.

Clarke R. J. and Macrae R. (1988) – Coffee Vol. 3: Physiology. London, Elsevier Applied Science Publishers, 1988.

Clifford, M.N. and Willson, K.C. (1985) – Coffee; botany, biochemistry and production of beans and beverage. London, Croom Helm, 1985.

Njoroge, J.M, (1998). Agronomic and processing factors affecting coffee quality. Outlook on Agriculture Vol 27, No. 3, 1998, pp 163-166.

Rene Coste, (1994) Coffee: The Plant and the Product, Macmillan Press Ltd, London 1992. International Coffee Agreement 1994.

Wrigley, G. (1988): Coffee. London, Longman, 1988.

Seedling Emergence and Growth Response of Wild Arabica Coffee Accessions in Southwest Ethiopia

T. KUFA[1] AND J. BURKHARDT[2]

[1]Ethiopian Institute of Agricultural Research, Jimma Research Center, P. O. Box 192, Jimma, Ethiopia
[2]Institute of Plant Nutrition, University of Bonn, Karlrobert-Kreiten-Str.13, D-53115 Bonn, Germany. E-mail: kufataye@yahoo.com

SUMMARY

The study was conducted with the primary objective to compare the variability in seed germination and seedling growth of wild Arabica coffee accessions under controlled optimal nursery conditions at Jimma Agricultural Research Center, southwest Ethiopia. The experiment was laid dawn in a randomised complete block design with six replicates. Seedling emergence and subsequent growth stages were recorded to calculate mean days of emergence, percentage and rate of emergence and seedling vigor indices. The analysis of variance for percentage of emerged seedlings depicted highly significant differences among coffee progenies between 55 and 97-days after sowing. Consequently, the overall mean germination percents were highest and lowest for PIS3 (83.5%) and PIIIS2 (48.3%) accessions, respectively. There were also highly significant variations in seed germination rate with superior and lowest results obtained from Harenna and Berhane-Kontir accessions. In addition, the accessions exhibited highly significant differences in speed of growth stages (emergence, soldier, butterfly and first true leaf) and seedling vigor. In addition, seedlings of wild Arabica coffee populations showed highly significant variations for all the morphological growth characters considered. Consequently, most of the progenies from Harenna and Yayu had maximum mean values as compared to others, particularly Berhane-Kontir accessions. In general, the rapidity of growth responses followed the descending order: Yayu > Harenna > Bonga > Berhane-Kontir accessions and their latter stage productivity could also vary accordingly. The findings would, therefore, provide baseline information on the extent of variability in early growth stages of wild coffee accessions and thus, the need to target specific management options on the use and conservation of Arabica gene pools in the montane rainforests of Ethiopia.

INTRODUCTION

Seed physiologist describe four germination stages: 1) hydration or imbibitions, during which water penetrates into the embryo and hydrates proteins and other colloids, 2) the formation or activation of enzymes, leading to increased metabolic activity, 3) elongation of radicle cells, followed by emergence of the radicle from the seed coat (germination proper) and 4) subsequent growth of the seedlings. The seed covering layers can interfere with the penetration of water and oxygen or both and they can prevent emergence of he radicle by acting as a mechanical barrier (Salisbury and Ross, 1992).

Germination process is complete when nutrition no longer depends upon reserve materials, but is autotrophic. By this time, the root has secured a hold in the soil the cotyledons or the primary leaves are unfolded and the seedlings attained independence growth and ensure plant establishment. According to Larcher (2003), the duration of germination is the time elapsing

between hydration of the seed and the appearance of the radicle and its rate is the percent increase in germinating seeds per unit time.

Coffee seed consists mainly of endosperm, which is coved by endocarp (parchment) and seminal tegument-silver skin. It contains starch, fat, reducing sugars, saccharides, tannins, caffeine and water. There is an embryo a radicle and cotyledons (Coste, 1992; Wrigley, 1988). The water content of coffee seed should be gradually reduced under shade and it should not be dropped below 18% during storage, most probably indicating an intermediary behaviour of coffee seeds between an orthodox and recalcitrant seeds.

In Ethiopia, the natural ecology of wild Arabica coffee is disturbed largely due to the escalating deforestation rates. There are still diverse coffee types, which have established from the self-sown seeds in the prevailing heterogeneous forest conditions. However, information is scanty on seed germination and early growth performances. In perennial crops like coffee, basically the ultimate measure of early screening of coffee seedling is the growth potential which influences the chance of survival of any seedling (Walyaro and Vander Vossen, 1979). The work done by Yacob (1993) also showed the possibility of early screening of Arabica coffee cultivars grown under specific management conditions. The primary objective of this study was, therefore, to compare the variability in patterns of seed germination and seedling growth response of wild Arabica coffee accessions under a controlled nursery condition at Jimma Agricultural Research Center, southwest Ethiopia.

MATERIAL AND METHODS

The study was conducted in southwest Ethiopia at Jimma Agricultural Research Centre (7º46' N and 36º0' E, 1753 m above sea level). Ripe red cherries were collected from selected mother coffee trees at three forest fragments within four forests in southeast and southwest where wild coffee populations grow naturally in the undergrowth of montane rainforest. These include Harenna, Bonga, Berhane-Kontir and Yayu. Except Harenna of southeast, the others are located in southwest where Arabica coffee has its specific origin. The accessions were designed as I-1, I-2, I-3, II-1, II-2, II-3, III-1, III-2, III-3, IV-1, IV-2 and IV-3. The seeds were harvested from the selected mother trees between October and December 2003. The seeds were prepared and stored between 3 and 5 months in a well-ventilated conventional cold room at Jimma Research Centre. To ensure maximum germination, two coffee seeds were sown on March 29 and 30, 2004 in a black plastic plant pot (volume = 5.8 cm^3). The pots were properly filled with the same media type prepared from topsoil and decomposed coffee compost at respective ratio of 3:1 (Taye et al., 2002).

A randomised complete block design with six replicates was used to arrange coffee accessions with a plot consisted of 25 seedlings. In this case, a total of 3600 coffee seedlings were raised. All post-sowing nursery operations (mulching, watering, shading, weeding, disease and insect control) were uniformly and timely applied. Starting from the appearance of the seedlings above the soil surface, the number of emerged seedlings and subsequent growth stages (butterfly, soldier and first true leaf pairs) were counted at a week intervals between May 24, 2004 and July 12, 2004 (55-112 days after sowing). From this, germination percent, germination rate and mean days to each stage were calculated following the procedures outlined by Steiner (1990). After most seedlings produced the first true leaf pairs, thinning to one seedling per pot was accomplished on July 12, 2004. Subsequently, five uniform seedlings were selected and arranged in the central row of each plot and morphological growth parameters were recorded on a month basis between 10 and 13 November 2004, 224-227 days after sowing (DAS). These included seedling height, diameter at base, number of main stem nodes, main stem internode length, leaf number, maximum leaf

length and width. Intact leaf area was estimated using leaf dimensions (maximum length and width) and a constant (K = 0.66) developed by Yacob et al. (1993) for Arabica coffee seedlings. Further, seedling vigour indices (SVI) were determined for two growth stages (55 and 112-DAS) using the measurements on percent germination, girth and height of coffee seedlings as described by Steiner (1990). Finally, analysis of variance (ANOVA) was computed in a randomised complete and means values were compared according to Tukey's at 5 % probability level.

RESULTS AND DISCUSSION

Seed germination

Coffee seeds started to emerge above the potting soil after 8 weeks (55-days after sowing, DAS), though it varied among coffee accessions. This was longer than the normal nursery calendars, due primarily to the reduced temperatures and hard seed cover. Accordingly, the results of analysis of variance depicted highly significant variations among wild Arabica coffee accessions between 55 and 97-DAS. Furthermore, the growth response of coffee seedlings on 55 and 112-DAS indicated highly significant differences in the rates of emergence, germination, growth stages and vigour index. Although there were no significant variations during the initial stage (55-DAS), the seedlings exhibited highly significant differences in the rapidity of growth stages. There were remarkable differences within accessions of each forest coffee population, particularly Bonga and Yayu (Table 1a). Coffee seeds from Berhane-Kontir population, II-2 and IV-3 had significantly low percent mean values for the early growth parameters. This is in line with early harvesting and prolonged storage time, indicating in part the decline in seed viability with increased time of storage. This, however, depends on the moisture content of seeds (Wondifraw, 1994) and storage conditions (Yacob et al., 1996). In addition, the reduced germination percent for Berhane-Kontir and Yayu accessions could be attributable to seed damage by Antestia bug (*Antestiopsis intricatata*). Consequently, the over all percent emergence values ranged between 48.3 and 83.5% for III-2 and I-3, respectively. However, the slightly lower mean emergence for 97 than 90-DAS shows that the seedlings attained subsequent growth stage. As a result, except Berhane-Kontir accessions, the seedlings produced the first true leaf pair on 112-DAS (Table 1b). Above all, the significantly prolonged mean days to the germination and subsequent early growth of Berhane-Kontir accession may demonstrate their specific microclimatic requirements similar to the area of origin, hot humid climate.

During the initial stage, the least and highest values of emergence, germination rates, soldier stage and seedlings vigor were obtained from III-3 and IV-2 accessions. The same growth responses were observed with time and on 112-DAS Yayu accessions had significantly the highest first true leaf stage, Harenna and Bonga populations followed this. During this time, significantly highest germination rates (0.79) and seedling vigour (487.62) were calculated from Harenna accessions, followed by Yayu. In contrast, most Berhane-Kontir accessions were at a soldier stage and the appearance of true leaf was accordingly inhibited as compared to others. As a result, the most inferior seedling vigour was determined from Berhane-Kontir accessions (Tables 1 and 2).

Table 1. Variability in growth stages (means±SD) of coffee seedlings of wild Arabica coffee accessions during different days after sowing (DAS).

a) 55 DAS

Accession	ER	SR	GR	MDE	MDS	SVI
Pr>F	***	**	***	Ns	*	***
I-1	0.07±0.08abc	0.15±0.20ab	0.22±0.24abc	16.00±12.15	39.00±12.15	70.96±85.88ab
I-2	0.09±0.04abc	0.07±0.07b	0.15±0.10bc	35.14±12.67	19.86±12.67	42.13±30.07b
I-3	0.13±0.06a	0.16±0.11ab	0.29±0.13ab	27.92±11.61	27.08±11.61	72.18±41.83ab
II-1	0.05±0.03bc	0.19±0.18ab	0.24±0.20abc	15.29±13.84	39.71±13.84	66.18±64.60ab
II-2	0.03±0.03bc	0.04±0.03b	0.07±0.06bc	25.48±18.08	20.35±16.45	15.99±14.14b
II-3	0.09±0.07abc	0.12±0.11ab	0.21±0.17abc	24.14±9.79	30.87±9.79	49.86±43.10b
III-1	0.04±0.03bc	0.06±0.04b	0.09±0.06bc	19.72±14.22	26.12±16.51	19.29±14.26b
III-2	0.04±0.03bc	0.04±0.04b	0.07±0.07bc	25.72±19.29	20.12±17.62	15.28±16.21b
III-3	0.01±0.02c	0.02±0.04b	0.03±0.06c	12.22±22.20	15.28±24.37	6.06±11.30b
IV-1	0.09±0.04ab	0.19±0.08ab	0.28±0.08abc	19.42±6.77	35.58±6.77	77.87±29.16ab
IV-2	0.13±0.03a	0.29±0.25a	0.42±0.28a	20.55±9.13	34.45±9.13	126.08±77.82a
IV-3	0.06±0.04abc	0.08±0.04ab	0.14±0.07bc	19.43±12.04	35.57±12.04	30.81±19.98b
Mean	0.07	0.12	0.18	21.75	28.66	49.39
CV (%)	57.85	95.93	69.50	66.40	49.50	76.42

b) 112 DA

Accession	SR	BR	FTLR	GR	MDS	MDB	MDFTL	SVI
Pr>F	***	***	***	***	***	***	***	***
I-1	0.12±0.06bc	0.60±0.02ab	0.07±0.07bc	0.78±0.04a	16.23±8.76cd	85.75±3.80ab	10.02±9.98bc	487.62±88.47a
I-2	0.09±0.03bcd	0.66±0.04a	0.04±0.05bc	0.79±0.02a	12.88±4.66cde	92.95±5.30a	6.17±7.36bc	420.60±81.43ab
I-3	0.10±0.07bc	0.65±0.08a	0.04±0.05bc	0.79±0.04a	14.30±9.60cd	91.69±7.25ab	6.02±6.78bc	378.22±80.89bc
II-1	0.11±0.03bc	0.51±0.03bcd	0.07±0.07bc	0.69±0.03b	17.13±5.35cd	83.68±6.36ab	11.19±10.41bc	360.20±66.73bc
II-2	0.07±0.03cde	0.45±0.05c-f	0.03±0.03bc	0.54±0.05cde	13.52±6.37cd	92.51±7.11a	5.97±6.73bc	232.54±51.87d
II-3	0.05±0.02cde	0.58±0.08ab	0.04±0.06bc	0.67±0.03b	9.42±2.18def	96.40±10.18a	6.18±10.30bc	316.55±56.29c
III-1	0.11±0.04bc	0.44±0.04c-f	0.01±0.01c	0.55±0.02cd	22.33±7.40bc	87.28±5.27ab	2.39±3.31c	223.78±45.00d
III-2	0.14±0.03ab	0.37±0.07f	0.00±0.00c	0.50±0.05de	30.91±8.78ab	81.10±8.78ab	0.00±0.00c	196.99±63.05d
III-3	0.19±0.07a	0.38±0.08ef	0.00±0.01c	0.58±0.04c	37.08±13.19a	74.35±13.05b	0.57±0.88c	220.03±62.58d
IV-1	0.02±0.02e	0.55±0.07abc	0.11±0.09ab	0.68±0.02b	2.76±3.21ef	90.81±12.97ab	18.43±14.66ab	378.74±62.21bc
IV-2	0.01±0.01e	0.51±0.09b-e	0.17±0.10a	0.68±0.03b	1.70±1.42f	82.52±15.93ab	27.79±16.63a	432.28±90.44ab
IV-3	0.04±0.02de	0.42±0.03def	0.02±0.02c	0.48±0.02e	8.43±4.40def	98.79±4.59a	4.78±4.18c	202.87±46.56d
Mean	0.09	0.51	0.05	0.64	15.56	88.15	8.29	320.87
CV (%)	37.23	12.33	81.90	5.30	34.90	10.45	80.44	13.25

*Ns = Not significant, ** and *** = significant at P≤0.01and P≤0.001 significance levels, respectively. Means with the same letter within each column are not significantly different according to Tukey test. Abbreviations: ER = emergence rate, SR = soldier rate, GR = germination rate, MDE = mean days of emergence, MDS = mean days of soldier, BR = butterfly rate, MDB = mean days of butterfly, FTLR = first true leaf rate, MDFTL= mean days of first true leaf and SVI = seedling vigor index.*

Table 2. Morphological characters of 7-month-old seedlings of wild Arabica coffee accessions at nursery site, Jimma Research Center, Ethiopia.

Accession	Height (cm)	Girth (cm)	Leaf						Node number	Internode length (cm)
			Number	Length (cm)	Width (cm)	Total area (cm^2)	Mean area (cm^2)	LAI		
I-1	16.39a	0.33a	8.52a	7.09abc	3.05a	122.47ab	14.35ab	0.35ab	4.33a	3.77ab
I-2	15.05bc	0.30ab	8.50ab	6.97bcd	3.07a	121.09ab	14.18ab	0.35ab	4.23ab	3.55bc
I-3	13.23de	0.31ab	8.37abc	6.53def	2.90abc	106.81a-d	12.67bcd	0.31a-d	4.20ab	3.14d
II-1	14.17dc	0.31ab	8.02dc	6.79cde	2.85abc	103.73a-d	12.88bc	0.30bcd	4.02bcd	3.52bc
II-2	13.42de	0.29bcd	7.62e	6.33def	2.72cd	87.62de	11.48cd	0.25de	3.82d	3.52bc
II-3	13.89dc	0.29bc	8.10bcd	6.75cdef	2.79bcd	101.64b-e	12.50cd	0.29b-e	4.03bcd	3.45c
III-1	13.19e	0.27cd	7.08f	6.30f	2.63d	78.07e	10.97d	0.23e	3.47e	3.81a
III-2	12.37e	0.27cd	7.07f	6.80cde	2.71cd	87.09de	12.25cd	0.25de	3.52e	3.52bc
III-3	12.18e	0.26d	6.85f	6.53def	2.81bcd	84.57de	12.22cd	0.25de	3.51e	3.48c
IV-1	15.71ab	0.30ab	8.07dc	7.32ab	2.94ab	115.49abc	14.29ab	0.34abc	4.05bc	3.87a
IV-2	16.78a	0.33a	8.37abc	7.53a	3.06a	128.16a	15.30a	0.37a	4.18ab	4.01a
IV-3	13.46de	0.29bcd	7.73de	6.59def	2.74bcd	93.42cde	12.01cd	0.27cde	3.95cd	3.41c
F-test	**	**	**	**	**	***	**	***	**	**
Mean	14.15	0.30	7.86	6.79	2.86	102.51	12.93	0.30	3.94	3.59
CV (%)	10.24	10.47	5.78	7.69	8.36	12.23	14.75	12.15	6.33	8.16

***, *** = Significant at P≤0.01 and P≤0.001 levels, respectively. Means with the same letter(s) within a column are not significantly different at P = 0.05 significance level (Tukey test).*

Most accessions attained peak emergence on 62-DAS and sharply declined thereafter (Figure 1) with the highest and least values obtained for coffee accession IV-1 and III-3, respectively. Accordingly, coffee accessions from Yayu sites were fast to reach a soldier growth stage, followed by Harenna and least being Berhane-Kontir coffees. Most seedlings attained a butterfly growth stage on 69-DAS (Figure 1) and the results increased with increased DAS. Thus, significantly highest mean percent butterfly was obtained for coffee types from Yayu forest (IV-1 and IV-2), though coffee seeds from accession IV-3 were significantly delayed to reach this stage. This was also least and took long mean days for Berhane-Kontir coffee types (Figure 2). The findings are in agreement with the characteristics of the seeds especially with seed density of the wild coffee populations (Taye et al., 2004). This reflects the influence of genetic and environmental factors on seed characteristics of wild coffee populations in the montane rainforests of Ethiopia, though determination on the magnitude of the effects, singly or in interactions, calls for further investigations.

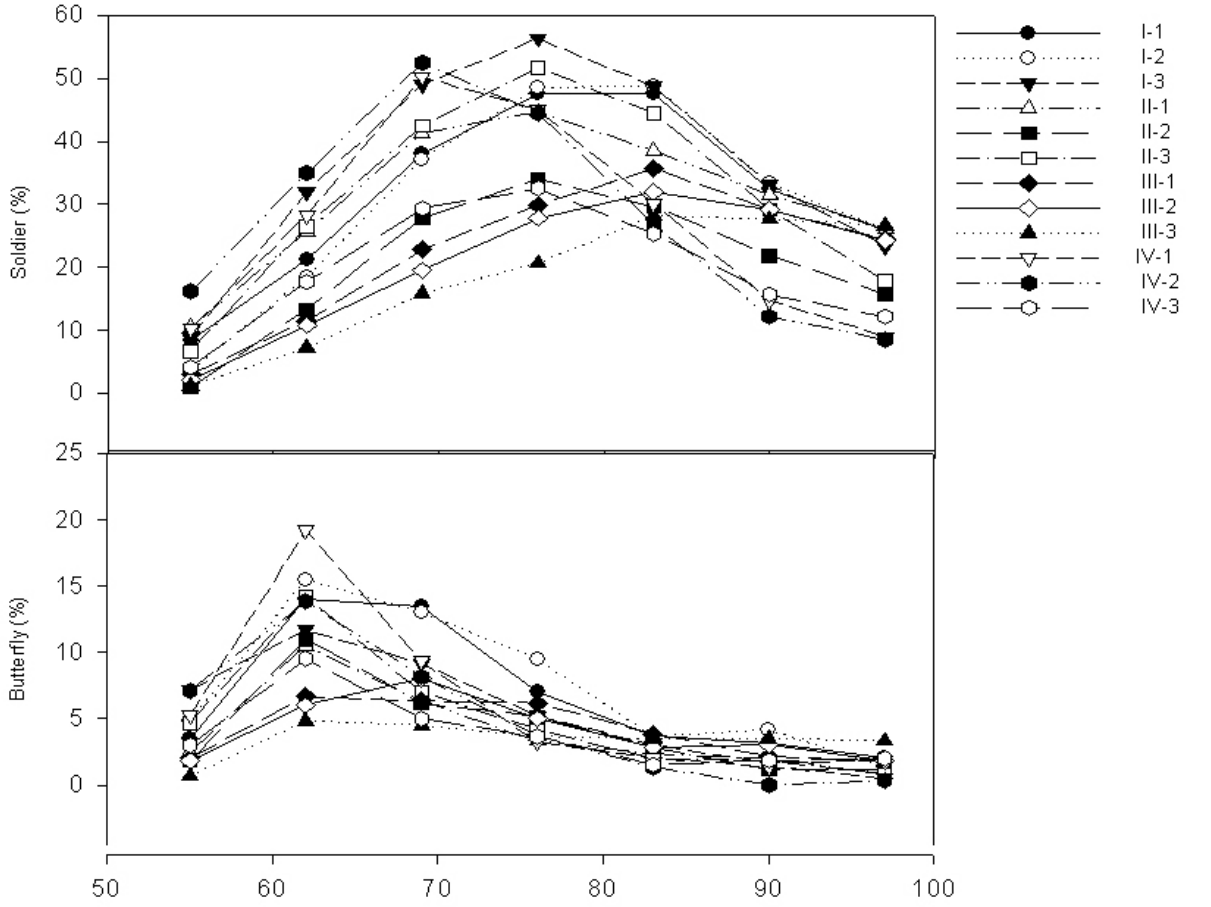


Figure 1. Mean percent of soldier and butterfly growth stages in seedlings of wild coffee accessions for different days after sowing (DAS).

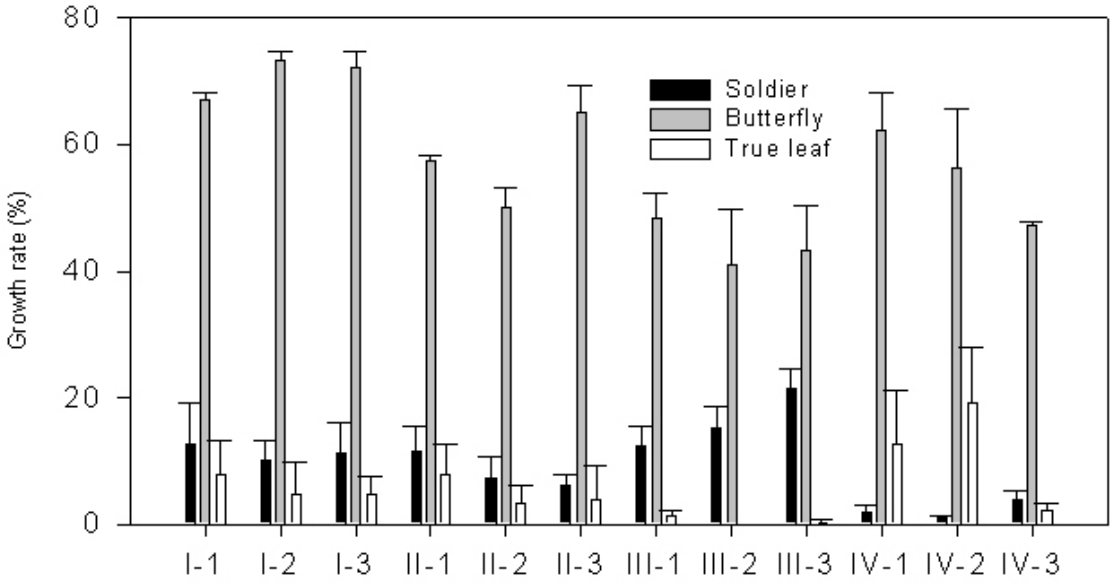


Figure 2. Mean proportion of soldier, butterfly and true leaf pair in seedlings of wild coffee accessions at the appearance of first true leaf pair (112 DAS).

Seedling growth

Seedlings of wild Arabica coffee accessions showed highly significant variations for all the extension growth parameters considered (Table 2). Accordingly, most of the progenies collected from Harenna and Yayu forests had maximum values as opposed to the least values recorded from Bernahe-Kontir population. The tallest seedlings with the highest leaf dimensions were found for IV-2, followed by seedling from I-1 with significantly more numbers of leaves and nodes. Moreover, significant variations in growth parameters were noticed among seedlings raised from coffee seeds collected from the three sites within each forest coffee unit. This is more prominent for the least seedling height and length of main stem internode length recorded for the I-3, III-3 and IV-3. Similarly, leaf number and leaf dimensions were lowest for accessions from II-2 and IV-3. The last data recorded on October 20, 2004 (204 days after sowing, 6.8-months-old) also revealed that most coffee seedlings produced 3 to 4 true leaf pairs (Figure 3), whereas accessions from Bernahe-Kontir had 2 to 3 pairs of true leaves. This is quite in agreement with the speed of seed germination of the accessions shown above, most probably suggesting the differences in seed reserves to detect germination and seedling growth.

In general, the present results corroborated with our findings (Taye et al., 2004) on the morphological variability of the mother wild coffee trees in the natural forest conditions. This shows the stability of the agronomic traits that can be used in the selection and evaluation of coffee accessions. This supports the works done by Walyaro and Vander Vossen (1979) and Yacob (1993) who showed the possibility to screen and predict that latter performances of coffee cultivars using early growth responses. However, further studies are still required to examine the consistency of the findings and identify promising coffee cultivars in an endeavour to conserve and exploit the immense wild Arabica gene pools in the montane rainforests of Ethiopia.

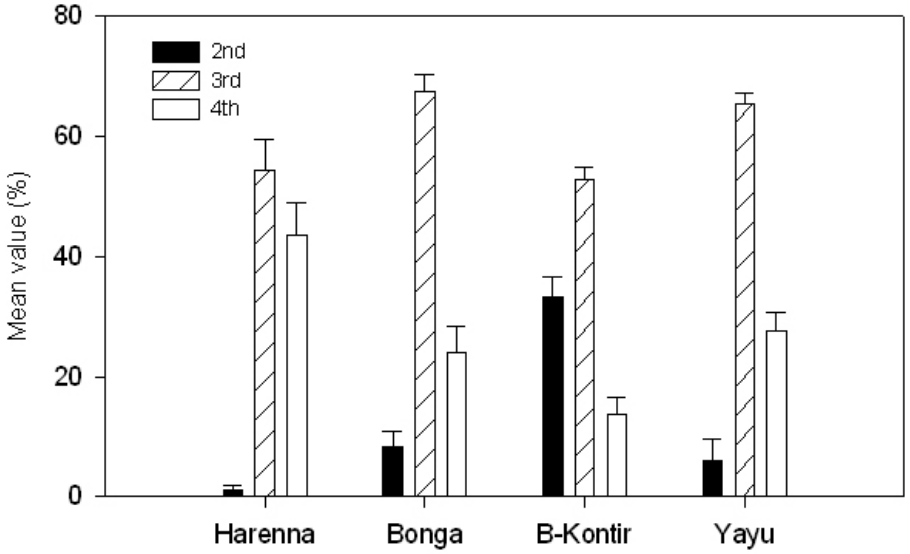


Figure 3. Mean values of true leaf growth stages (3rd-4th) in seedlings of coffee accessions of the four wild coffee populations (204 days after sowing).

CONCLUSIONS

The present findings showed inter- and intra-variability in seed germination and seedling growth under controlled optimal nursery environments, largely indicating the immense genetic diversity in seed characteristics among wild Arabica coffee populations in Ethiopia. However, repeated investigations are required, among others, on the influence of environmental and plant factors on the patterns of flowering, fruit phenology, seed growth and storage conditions (pre-and post-harvesting operations) on seed viability, seed germination and subsequent growth of coffee seedlings. This is crucial for *in-situ* and *ex-situ* conservation and utilization of the wild Arabica coffee populations in the monatne rainforest in the country.

REFERENCES

Coste R. 1992. Coffee: The plant and the product. Macmillan, London.

Larcher W. 2003. Physiological Plant Ecology: Ecophysiology and stress physiology of functional groups (4th ed.). Springerr-Verlag Berlin Heidelberg, NewYork.

Salisbury F. B and Ross C.W. 1992. Plant Physiology (4th ed). Wardsworth Learning, Inc., Thomson Asia Pte Ltd., Singapore.

Steiner J. J. 1990. Seedling rate of development index: Indication of vigor and seedling growth response. *Crop Sci*. 30: 1264-1271.

Taye K., Tesfaye S., and Alemseged Y. 2002. Influence of media mixture and watering frequency on seed germination and seedling growth of Arabica coffee in Ethiopia. Proceedings of the 19th International Conference on Coffee Science (ASIC), May 14th-18th, 2001, Trieste, Italy.

Taye K., Burkhardt J. and Goldbach H. 2004. Ecophysiological variability of forest Arabica coffee populations in hydraulic characteristics along a climatic gradient in Ethiopia: Morphological and physiological variability. Proceedings of 20th International Conference on Coffee Science (ASIC), 11-15 October 2004, Bangalore, India.

Walyero D.J. and Van der Vossen H.A.M. 1979. Early determination of yield potential in Arabica coffee by applying index selection. *Euphytica*, 28: 465- 472.

Wrigley G. 1988. Coffee. Tropical Agriculture Series, London, John Wiley and Sons, Inc., N.Y.

Wondifraw T. 1994. The influence of initial moisture content and type of container on the viability of coffee (*Coffea arabica* L.) seeds during storage. Alemaya University of Agriculture, School of Graduate Studies, Pp 116.

Yacob E. 1993. Relative performance of three CBD cultivars under varying light regimes. Proceedings of the 15th International Scientific Colloquium on Coffee, Montpellier, France.

Yacob E., Mohammednur A. and Taye K. 1993. Leaf area estimation in CBD resistant Arabica coffee to design a prototype area meter (the third awarded paper of the year). Bulletin of the Crop Science Society of Ethiopia (CSSE), *SEBIL,* 5: 29-30.

Yacob E., Tesfaye S., Taye K., Alemseged Y., Takele N., Anteneh N. and Bekele B.1996. Advances in coffee agronomy research in Ethiopia. Pp 40-45. *In:* Tenywa J.S., Adipala Ekwamu M.W. Ogengalatogo (eds.). Proceedings of Inter-Africa Coffee Organization (IACO) Workshop, 4-6 Sept 1995, Kampala, Uganda.

Early Bearing Stage Evaluation of *Coffea arabica* L. Germplasm for Yield and Morphological Characters

E. HABTE, B. BELLACHEW AND H. SINGH

Jimma University, Ambo College, P.O box 19, Ambo, Ethiopia
Jimma Agriculture Research Center, P.O.box 192, Jimma, Ethiopia

SUMMARY

The study conducted on coffee trees of Western Ethiopia with first two productions reveals that the morphological characters such as fruit length, canopy diameter, girth of the main stem, average length of the primary branch and average internode length of the main stem showed a higher estimate values of genetic component, heritability in broad sense as well as in genetic advance as percent of the mean at early stage of production. Hence these characters are worth to be considered as indirect selection criteria to conduct early selection, moreover, these traits have revealed a positive and significant association with average yield of the first two year production, which recorded lower heritability estimate in broad sense, at genotypic and phenotypic level. The path coefficient analysis at genotypic level further revealed the importance of main stem node and plant height for use as indirect selection for higher yield as reflected by the highest and positive direct effect values.

INTRODUCTION

Ethiopia is the primary center of origin and center of genetic diversity of *Coffea arabica* L. (Krug, 1958). Due to this, there is immense genetic variability that offers great potential for improvement of this crop. Nevertheless, the duration of breeding program in *C. arabica* L. to produce new cultivars largely depends upon the efficiency of the methods of selection for yield. Under such circumstances, the time spent in variety development could be shortened through early selection for production potential. This could be a fundamental improvement strategy to satisfy the urgent needs of the growers. In this line, significant phenotypic correlation was found between plant vigor and yield (Walyaro van der Vossen, 1979) and early selection for yield potential was possible based on plant vigor and or 2-3 years yield data (Van der Vossen, 1976).

Study was conducted in Jimma -Haru agricultural research Sub center in 2004 to evaluate some Western Ethiopia coffee germplasm collections at early bearing stage, with the objectives of investigating early performance of growth characters and for early yield potential, for the supply the local farmers with improved local cultivars in the shortest time possible.

MATERIAL AND METHODS

The experiment was carried out at Haru Agricultural Research Sub-Center which is located Western part of Ethiopia. The experimental materials consisted seventy-five accessions and six CBD resistant selections included as check. These materials were collected from different areas of Haru area in 1999. The experiment was laid down in a 9x9 simple lattice design. Each incomplete block consisted of nine plots with seven trees per plot. The plant-to-plant spacing used was two meters by two meters. Immediately after field transplanting coffee

seedlings, sesbania trees were planted as temporary shade to provide a relatively durable shade.

The data collected were, fruit length in mm, canopy diameter in cm, girth of the main stem in mm, average length of the primary branch and average internode length in cm of the main stem Number of primaries Number of main stem nodes Plant height in cm. Phenotypic and genotypic correlations among yield, yield related traits were estimated using the method described by Miller et al. (1958).

The direct and indirect effect of yield related traits on yield per plot were worked out through path coefficient analysis Dewey and Lu (1955).

RESULT AND DISCUSSION

Most of the accessions were superior to the check cultivars for most of the growth characters considered, particularly for plant height average length of primaries and canopy diameter. Therefore, improvement of coffee landraces may be possible by indirect selection for these three characters.

Table 1. Estimates of components of variance, broad sense heritability (H^2), and expected genetic advance (GA) in coffee.

Characters	PCV (%)	GCV (%)	Estimates of components of variance			H^2 (%)	GA	GA as the % of the mean
			Vg	Ve/r	Vph			
ALP	9.82	7.51	21.42	15.21	36.63	58.48	7.29	11.83
AVY	42.53	21.36	33479	99269.5	132749	25.22	189.29	22.1
FL	7.21	5.44	0.007	0.0053	0.0123	56.91	0.13	8.46
H	9.90	5.87	100.74	186.09	286.83	35.12	12.25	7.16
NMN	8.48	4.36	1.55	4.32	5.87	26.41	1.32	4.61
AILM	9.14	7.23	0.15	0.09	0.24	62.5	0.63	11.77
CANOPY	9.17	6.51	72.71	71.33	144.04	50.48	12.48	9.53
G	8.35	6.59	0.083	0.05	0.133	62.41	0.47	10.73
NPB	9.93	5.13	6.47	17.76	24.23	26.7	2.71	5.46

Vph,Vg and Ve/r are phenotypic, genetic and error variances of genotype means ,respectively ALP =Average length of primary, AVY =Average fresh cherry yield, FL= Fruit length, H =Plant height, NMN =Number of main tem node,AILM= Average main stem internode length ,CANOPY= Canopy diameter ,G= Girth ,NPB Number of primary branch.

In the present study, it has been shown that, accessions with lower mean values for average main stem internode length had also lower mean values for plant height and canopy diameter. As indicated by earlier investigators coffee yields per unit area can be increased considerably by close spaced planting systems (Van der Vossen, 2001). Therefore, these accessions could give an opportunity for high density planting and ease of harvesting.

Canopy diameter, length of primary and plant height showed a relatively intermediate to high estimates for GCV, H and GA (as % of the mean) which indicated direct selection may be useful to improve these characters (Table 1). Similarly, Walyaro and van der Vossen (1979) Srinivasan (1982) and Sera (1987) reported that coffee yield showed low broad sense

heritability. Yield in the present study exhibited higher GCV and GA estimates, but with low broad sense heritability.

Table 2. Genotypic (upper diagonal) and Phenotypic (lower diagonal) correlation coefficients.

	H	G	NPB	NMN	AILM	CANOPY	ALP	FL	AVY
H		0.7**	-0.03	-0.16	0.84**	0.77**	0.82**	0.24*	0.31**
G	0.57**		0.04	-0.03	0.54**	0.78**	0.78**	0.42**	0.41**
NPB	0.55**	0.25*		0.94*	-0.46**	-0.07	-0.31**	0.14	0.17
NMN	0.5**	0.16	0.89**		-0.6**	0.01	-0.28*	0.1	0.31**
AILM	0.69**	0.5**	-0.07	-0.23*		0.62**	-0.73**	0.31**	0.13
CANOPY	0.57**	0.63**	0.18	0.04	0.6**		0.89**	0.07	0.53**
ALP	0.6**	0.68**	0.06	0.01	0.45**	0.78**		0.34**	0.67**
FL	0.17	0.22	0.06	0.11	0.18	0.01	0.16		0.58**
AVY	0.23*	0.33**	0.23*	0.18	0.12	0.39**	0.31**	0.13	

**, ** significant at p= 0.05 and at p= 0.01 respectively.*

Table 3. Genotypic Path coefficient analysis

	H	G	NPB	NMN	AILM	Canopy	ALP	FL	GC
H	1.342	0.041	0.12	-0.651	0.341	-0.291	0.023	0.041	0.31
G	0.610	0.08	-0.16	-0.122	0.004	-0.041	0.041	0.074	0.41
NPB	-0.041	0.003	-3.63	3.826	-0.003	0.004	-0.016	0.025	0.17
NMN	-0.217	-0.002	-3.55	4.071	-0.005	-0.001	-0.015	0.018	0.31
AILM	1.129	0.043	1.84	-2.442	0.008	-0.033	-0.468	0.055	0.13
Canopy	1.044	0.063	0.28	0.041	0.005	-0535	0.046	0.012	0.53
ALP	1.112	0.063	1.24	-1.14	-0.569	-0.168	0.052	0.06	0.67
FL	0.325	0.034	-0.56	0.407	0.157	-0.004	0.018	0.177	0.58

R =0.21, GC Genotypic correlation coefficient average yield with the characters. The underlined values are direct effects of the respective characters on average yield. ALP = Average length of primary, AVY = Average yield, FL = Fruit length, H = Plant height, NMN = Number of main tem node, AILM = Average main stem internode length, CANOPY = Canopy diameter, G = Girth, NPB Number of primary branch.

Positive and highly significant genotypic and phenotypic correlations were observed between the average yield and most growth characters, particularly with average length of primary, fruit length and canopy diameter and girth (Table 2). This implies that indirect selection through these characters may be applied for screening high yielding genotypes. Plant height and number of main stem node had high direct effect on yield. However, the other two characters average length of primaries and canopy diameter had lower direct effects (Table 3). Taking in to account practical situations in coffee improvement, canopy diameter, average length of primary, and height of the tree are worth considering as indirect criteria for selection (Sera 2000; Srinivasan- 1982; Walyaro and Van der Vossen, 1979).

In addition (Table 3) the traits such as average length of primaries and canopy diameter displayed intermediate to high estimates of GCV, H and GA. Nevertheless, their indirect effects via plant height were high. Moreover, these three traits showed strong association among each other. There fore, simultaneous improvement of these characters may be possible by selecting only for one of these traits.

REFERENCES

Dewey, D.R. and K.H. Lu, 1959. Acorrelation and path coefficient analysis of components of crested wheat grass seed production. *Agronomy Journal* ,51:515-518.

Krug,C.A.1958. The genetics of Coffea.*advan. in Genet*. 4:127-158.

Miller, P.A., J.C. Williams, H.F.Robinson and R.F. Comstock,1958. Estimation of genotypic and environmental variances and covariances in upland cotton and their implications in selection. *Agronomy Journal* 50:126-131.

Sera, T., 2000, Development of coffee cultivars in reduced time by using biotechnology in the iapar model for high density planting. Coffee biotechnology and quality. Proceeding of the third international seminar on biotechnology in the coffee agro industry, Dondria, Brasil.47-70.

Srinivasan, C.S, 1982 Preselection for yield in coffee .*Indian Journal of Genetics and plant breeding* 42 (1):15-19.

Van der Vossen H.A.M., 2001. Coffee breeding and selection review achievements and challenges: 18th international colloquium on coffee (volI).2001, Italy. 401-408.

Van der Vossen, H.A.M., R.T.A. Cook, and G.N.W. Murakaru, 1976. Breeding for resistance to coffee berry disease caused by *Collletotricum coffeanum* noak in *Coffea arabica* L..I. Methods of preselection for resistance. *Euphytica*,25:733-745.

Walyaro, D.J.and H.A.M Van der Vossen, 1979. Early determination of yield potential in arabica coffee by applying index selection. *Euphytica*.28: 465-472.

Inheritance of Caffeine in Interspecific Hybrids of *Coffea arabica* x *Coffea canephora*

R. H. G. PRIOLLI[1], M. MÖLLER[1], M. I. ZUCCHI[1], L.C.S. RAMOS[1], P. MAZZAFERA[2], W. J. SIQUEIRA[1], C. A. COLOMBO[1]

[1]IAC, Campinas, SP, Brasil
[2]UNICAMP, Campinas, SP, Brasil

SUMMARY

Caffeine inheritance was studied in plants derived from a cross between an artificially doubled di-haploid *C. arabica* var. Bourbon Vermelho (2n = 22 → 2n = 44) and a normal doubled *Coffea canephora* (2n = 22 → 4n = 44). Caffeine in seeds was determined in the parent lines, F_1 and F_2 hybrids, as well as in backcrosses with *C. arabica* (BC_1) during 2004 and 2005. The caffeine in seeds of the F_2 plants showed a normal distribution and a mean value intermediate to those of the parents, whereas BC_1 plants tended to have values closer to the *C. arabica* parent line. By pooling the data of two years it was estimated that a broad-sense heritability coefficient was 67.3% and that five additive genes might be controlling the trait in the seeds. The results confirm the existence of distinct mechanisms controlling the caffeine content in seeds and leaves of coffee and indicate favorable conditions to breed for caffeine content in this coffee population.

INTRODUCTION

Some studies have suggested that about 94% of the caffeine variation observed in coffee species is genetically determined (Barre et al., 1998; Montagnon et al., 1998). Campa et al. (2005) proposed that discontinuity of caffeine distribution in 21 species and taxa of coffee was due to major genes and that variation within groups might be due to accumulation of mutations with minor effects. In several interspecific coffee populations the mean caffeine content is usually an intermediate value to that of the parents (Charrier and Berthaud, 1975; Le Pierres, 1987). Moreover, intra- and inter-specific hybrids have indicated that the variability in the progeny depends on the heterozygosis of the parents, which is more evident in out-breeding species. Montagnon et al. (1998) studied intraspecific hybrids of *C. canephora* and observed that the narrow-sense heritability was high (0.80) for caffeine content. From studies with diploid hybrids of *C. liberica* and *C. pseudozanguebariae* Barre et al. (1998) concluded that the great variation observed was under polygenic control and that the absence of caffeine in the latter species was due to a pair of recessive alleles.

Here we studied the genetic variability and the inheritance of the caffeine content in seeds and leaves of interspecific hybrids of *C. arabica* x *C. canephora* 4n.

MATERIALS AND METHODS

Plant material

An original cross was carried out between *C. arabica* var. Bourbon Vermelho (P_1) and *C. canephora* var. Robusta 4n (P_2) and the only tetraploid hybrid (F_1) from this cross was named H2460-10-1. The P_1 parent is an artificially doubled di-haploid *C. arabica* var. Bourbon

Vermelho (2n = 22 → 2n = 44), whereas P_2 was originally diploid (2n = 2x = 22) and had the chromosome number duplicated with colchicine (2n = 22 → 4n = 44). Due to their low fertility, P_2 parent and hybrid F_1 were used as pollen donors in controlled crossings. F_2 individuals were obtained by selfing the hybrid F_1. Back cross individuals (BC_1) were obtained from controlled crosses between P_1 and the hybrid F_1. Altogether, 150 F_2 and 88 BC_1 individuals were obtained and among them, 71 F_2 and 24 BC_1 plants produced seeds in 2004 and 2005. The other plants consistently did not produce seeds and they might be sterile. All plants have been maintained in a field experiment since 1999.

Caffeine extraction

Seeds (from red cherries) were dried (70 °C), ground, extracted with 80% metOH (70 °C) and analysed by HPLC.

Statistical analysis

Seed caffeine contents were distributed as frequency histograms for both evaluation years with a class interval (i) according to the formula i = A/k, where A = amplitude of the variation between the maximum and minimum value observed in the dataset and k = square root of the number of observations per dataset. The hypothesis of the number of loci controlling caffeine in the seeds was tested according to Wright gene estimates. The null hypothesis $H_0 = 0$ was tested by the chi-square test, according to a segregation model represented by the equation $(a + b)^m$, where m represents the number of segregant alleles, and a and b represent non effective and effective alleles for caffeine, respectively. The interactions between treatments and years were evaluated by considering the generations as treatments and each year of data collection (2004 and 2005) as an experiment in a completely randomized design. Comparisons between means were carried out using the Tukey test at 5% of probability.

RESULTS

Caffeine in the seeds of F_2 and BC_1: During 2004 and 2005 evaluations of the caffeine content in the 71 F_2 plants ranged from 1.33% to 3.28%, with a mean content of 2.23% (Figure 1A). The values were normally distributed, which is a characteristic of additive inheritance, and transgressive segregation was not observed. Variation in the caffeine content was also observed for the 24 individuals of the BC_1 population (Figure 1B).

However, the mean value (2.05%) was lower and the amplitude of variation (ranging from 1.57% to 2.55%) was narrower than the values observed for the F_2 population, tending towards the *C. arabica* parent (P_1) with lower caffeine content, as would be expected considering the backcross to this species as the recurrent parent. Analysis of variance of the caffeine content in the seeds showed that data from two years evaluation were not significantly different. On the other hand, significant difference was observed between generations (Table 1). Tukey test (5%) comparisons showed phenotypic divergence between the parents, and segregating generations as intermediaries.

Since experiments (years) were not statistically different, the pooled data from 2004 and 2005 were used to calculate means and variances. The estimate of the broad-sense heritability coefficient was 67.28%. Additionally, the number of loci estimated to control the trait in seeds was five, according to the additive segregation tested for F_2 generation (Table 2). The $(a + b)^5$ distribution in F_2 (observed frequency) did not differ significantly ($\chi^2 = 16.336$, $P < 0.05$) from the expected frequency of five genes controlling the trait.

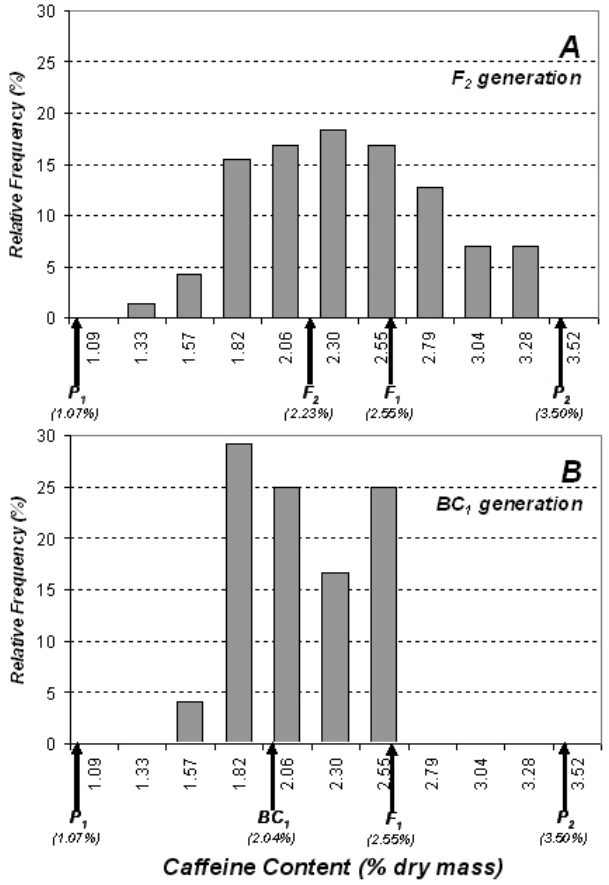


Figure 1. Distribution of the caffeine content (% dry mass) in the seeds of the F_2 (71 plants) and BC_1 (24 plants) hybrids in the years 2004 and 2005. Arrows and values in parenthesis indicate the caffeine content of the parents P_1 (*C. arabica* var. Bourbon Vermelho) and P_2 (*C. canephora* 4n), and of the F_1, F_2 and BC_1 generations.

Table 1. Comparison of the caffeine content in seeds of *Coffea* plants two parent lines (P_1 = *C. arabica* var. Bourbon Vermelho, and P_2 = *C. canephora* 4n) and their respective hybrids F_1, F_2 and RC_1 during the years of 2004 and 2005. ANOVA results for the effect of years and generations: *F* and probability (*P*) test.

	Year 204[a]		Year 2005	
	n	**Caffeine (%)**	**n**	**Caffeine (%)**
P_2	1	3.20 a	1	3.80 a
F_1	3	2.70 a	3	2.40 ab
F_2	71	2.13 ab	71	2.31 ab
BC_1	24	2.11 ab	24	1.98 b
P_1	3	1.10 b	3	1.30 b
***F* test (years)**	0.4718			
P	0.5332			
***F* test (generations)**	0.0070*			
P	23.365			
CV%	10.6			

[a] *= Means followed by different letters are statistically different at 5% probability by the Turkey test; * indicates significance at 5% probability; n = nimber of plants studied in each generation; CV% = experimental 1 coefficient of variation.*

Table 2. Hypothesis test for the segregation of five genes controlling trait caffeine content in seeds of the F_2 population. ^{ns} = not statistically significant at 5% probability.

Phenotype frequency	Caffeine alleles		Phenotype (% caffeine)	Observed frequency	Expected frequency	$(OF-EF)^2$ EF
	Non effective	Effective				
1/1024	0	10	1,10	0	0,0693	0,0693
10/1024	1	9	1,34	1	0,6934	0,1356
45/1024	2	8	1,59	3	3,1201	0,0046
120/1024	3	7	1,83	11	8,3203	0,8630
210/1024	4	6	2,07	13	14,5605	0,1673
252/1024	5	5	2,31	15	17,4727	0,3499
210/1024	6	4	2,56	9	14,5605	2,1235
120/1024	7	3	2,80	9	8,3203	0,0555
45/1024	8	2	3,04	7	3,1201	4,8247
10/1024	9	1	3,28	3	0,6934	7,6736
1/1024	10	0	3,53	0	0,0693	0,0693
				N = 71	N = 71	$?^2 = 16{,}336^{ns}$

DISCUSSION

Distribution of caffeine content

The histograms of the distribution of the caffeine in seeds of the segregating populations showed that the P_1 and P_2 parent lines used to obtain the F_2 and BC_1 populations presented contrasting values for caffeine, thus generating phenotypic variation what is adequate to study the genetic control.

The extensive variation observed in seeds of the F_2 and BC_1 populations reflects mainly the diversity from the *C. canephora* parent, since this species out-breeds and is heterozygous. On the other hand, *C. arabica* is autogamous and it has allotetraploid origin (Lashermes et al., 1999). In spite of the large variation in the distribution of the frequencies in the F_2 generation, the mean caffeine content was intermediate to the parental content, and statistically not different from the mean content of BC_1. The same has been observed in previous studies (Charrier and Berthaud, 1975; Le Pierres, 1987; Mazzafera and Carvalho, 1992), although for hybrids from *C. liberica* x *C. pseudozanguebariae* it has been suggested that the absence of caffeine was a recessive trait, controlled by one major gene (Barre et al., 1998).

In the present study the means and the caffeine content distribution in seeds of the F_2 and BC_1 populations led us to propose that genetic determinism of caffeine fits a oligogenic (with a continuous distribution) and additive model. Barre et al. (1998) initially rejected the hypothesis of an additive model for caffeine inheritance due to the fact that all inter-specific hybrids between species with or without caffeine had the compound. However, the square root of the alkaloid content in the hybrids studied by these authors was additive, allowing them to propose that caffeine content might depend linearly on either squared variables XY, X and Y being additive.

Caffeine content analysis of variance

The experimental coefficient of variation for caffeine in seeds was approximately 10% (table 1), indicating high experimental precision. Moreover, the absence of a significant source of

variation for years allowed us to group these data in order to obtain the variances necessary to determine the number of genes or loci controlling the trait "caffeine content" in seeds. The elevated values of the broad sense heritability coefficient in seeds indicate favorable conditions for the selection of plants with both high and low caffeine contents in the studied coffee material. Using Wright´s model we estimated the presence of five additive genes controlling the caffeine content in seeds of a coffee population originated from *C. arabica* x *C. canephora* 4n. This model was the most adequate for such estimation, since the parents were contrasting for the trait, and the loci seemed to segregate independently and additively. It is noteworthy that gene number estimation based on Wright's model is conservative, expressing the minimum number of genes controlling a character. This might be valuable for a breeding program. For instance, considering the F_2 hybrids, it would be necessary to have at least 1,042 individuals in order to find at least one genotype identical to the parents. The comparative study of the expected and observed frequencies for the segregation of five genes using a chi-square test was not significant. However, due to the complexity of caffeine metabolic pathway we can not rule out that the number of genes estimated in this study may be under- or over-estimated. Campa et al. (2005) suggested that major genes control large variations of caffeine in coffee seeds while small variations would be due to several mutational events with minor effects.

In the genetic breeding context of the species studied here (*C. arabica* and *C. canephora* 4n), our results could provide new insights for further studies aiming to alter caffeine content. These are important findings considering the fact that the commercial variety Icatu, originated from a hybrid between the *C. arabica* and *C. canephora* and with several backcrosses to the former species, has high yield and is resistant to the fungus causing orange leaf rust, but its caffeine content is approximately 1.2%, similar to commercial varieties of *C. arabica*.

REFERENCES

Barre P. et al. (1998) Theor Appl Genet 96:306-311.

Campa C. et al. (2005) Food Chem 91:633-637.

Charrier A., Berthaud J. (1975) Café Cacao Thé 19:251-264

Lashermes P. et al. (1999) Mol Gen Genet 261:259-266.

Le Pierres D. (1987) XII ASIC, Montreaux, pp 468-475.

Mazzafera P., Carvalho A. (1992) Euphytica 59:55-60.

Montagnon C. et al. (1998) Plant Breed 117:576-578.

TUPI RN IAC 1669-13: A Coffee Cultivar Resistant to *Hemileia vastatrix* and *Meloidogyne exigua* Nematode

L. C. FAZUOLI, W. GONÇALVES, M.TOMA-BRAGHINI, M. B. SILVAROLLA

Centro de Café 'Alcides Carvalho', Instituto Agronômico (IAC/APTA) Campinas – P.O. Box 28, 13.001-970. Campinas, SP, Brazil. E-mail: fazuoli@iac.sp.gov.br
(Support: SAA /CBP&D-Café/CNPq)

SUMMARY

The objective of this work was to develop a coffee cultivar resistant to rust (*Hemileia vastatrix*) and to the *Meloidogyne exigua* nematode. A F_5 coffee progeny from the hybrid CIFC H361/4 (Villa Sarchi x Timor Hybrid 832/2) was characterized. Evaluation of rust resistance was performed regarding to the prevalent races in experimental fields. Tests for resistance to *M. exigua* were accomplished under greenhouse conditions, using the cv. Catuaí Vermelho IAC144 as control. Twenty young plants were inoculated with 4000 nematode eggs/plant, and 150 days after the inoculation the following parameters were determined: galls index (GI), number of eggs in the radicular system (NE), reproduction factor (RF) and reduction of the reproduction factor (RRF), in percent numbers. Evaluated progenie exhibited a gall index of 0.5 but without typical galls, reproduction factor of 0.025, reduction of this factor of 98.2% and number of eggs per radicular system of 100. In control plants GI was of 4.5 and NE was 5550. The new cultivar called TUPI RN IAC 1669-13 when cultivated under irrigation produced 91, 50 and 89 saks (60 kg bags) of green coffee per hectare in the first three years, respectively. It presents large beans with average screen size of 18.4 and 90.2%, 8.0% and 1.8% of flat (normal beans), peaberry and elephant beans, respectively. The cultivar has low height, exhibiting green young leaves and large red fruits with medium maturation. Due to the multiple resistance to rust and nematode *M. exigua* (*MeMe*) the cultivar TUPI RN IAC 1669-13 can be planted through seeds providing a new option for the coffee growers.

RESUMO

O objetivo deste trabalho foi desenvolver uma cultivar de café com resistência à ferrugem e ao nematóide *Meloidogyne exigua*. Utilizaram-se progênies de cafeeiros do híbrido CIFC H361/4 (Villa Sarchi X Híbrido de Timor 832/2). Os testes para ferrugem foram feitos em relação às raças prevalecentes nos locais dos experimentos e os de resistência a *M. exigua* foram realizados em casa de vegetação, usando-se como testemunha a cv. Catuaí Vermelho IAC144. Inocularam-se 20 plantas jovens com 4000 ovos do nematóide/planta, determinando-se aos 150 dias após a inoculação, o índice de galhas, o número de ovos por sistema radicular, o fator de reprodução e a redução do fator de reprodução, em números percentuais. O índice de galhas foi 0,5, sem galhas típicas sendo que, na testemunha foi 4,5. O fator de reprodução foi 0,025 e a redução deste fator foi 98,2%. O número de ovos por sistema radicular foi 100 e na testemunha 5550. A cultivar TUPI RN IAC 1669-13 em plantios irrigados, produziu 91, 50 e 89 sacas de café beneficiado/ha nos três primeiros anos. Apresenta grãos grandes com peneira média 18,4 e 90,2%, 8,0% e 1,8% de grãos tipos chato, moca e concha, respectivamente. O seu porte é baixo, as folhas novas são de coloração verde e os frutos grandes e vermelhos, com maturação média. Devido a sua resistência múltipla à ferrugem e ao nematóide *M. exigua* (*MeMe*) a cultivar TUPI RN IAC 1669-13 poderá ser plantada via sementes constituindo-se em mais uma opção para os cafeicultores.

INTRODUCTION

The use of coffee varieties or cultivars with multiple resistance to phytopathogenic agents is an ever-increasing need in modern coffee culture. The favorable effects of this technology on coffee culture are directly related to reducing production costs and, above all, environmental contamination caused by chemical compounds that may be hazardous to human health.

Among the diseases affecting the coffee plant, coffee leaf rust (caused by the fungus *Hemileia vastatrix*) e nematode damage (caused by *Meloidogyne exigua*) are biotic fatores responsible for significant yield losses.

The objective of this research study was to develop a coffee variety with multiple resistance to both coffee rust and the nematode *M. exigua*.

MATERIAL AND METHODS

In 1971, the Institute of Agronomy of Campinas (Instituto Agronômico de Campinas – IAC) received seeds of the hybrid CIFC H361/4 (Villa Sarchi X Timor Hybrid) of the F_2 generation, which constituted the basis and point of departure for the development of cultivar TUPI RN IAC1669-13 described in this paper. The materials used for the initial cross-breeding process of hybrid H361/4 - performed in 1968 at the Coffee Rust Research Center (Centro de Investigação das Ferrugens do Cafeeiro – CIFC) in Portugal-were Timor Hybrid 832/2 and Villa Sarchi. Timor Hybrid CIFC 832/2 has high resistance to rust since it carries the genes SH_6, SH_7, SH_8 and SH_9, in addition to carrying the dominant gene *Me* that confers resistance to the nematode *M. exigua*, identified for the first time by Fazuoli et al. (1974), and later confirmed by Fazuoli (1981) and in 2003 Noir et al. (2003), using data collected on progeny T 5296, derived from Timor Hybrid CIFC 832/2. The same authors also identified molecular markers linked to the dominant gene *Mex-1* that confers this resistance. The Villa Sarchi cultivar is native to Costa Rica and is thought to have been found in Typica, Bourbon and other varieties of *C. arabica* plantations. Experiments conducted at IAC showed that the Villa Sarchi cultivar carries the same *Ct* allele, which confers low height to plants of the "Caturra Vermelho" variety. (Carvalho et al., 1984; 1991).

The tests to evaluate rust resistance against prevailing races in the experimental plots and selection fields were conducted over a several-year-long period of time and using a 1 to 5 scale. The tests to assess resistance to *M. exigua* were conducted under greenhouse conditions, in the period from March 16th to August 16th 2005, and employed the Catuaí Vermelho IAC 144 variety as check cultivar for comparison and evaluation ("control"). The gall index, number of eggs per root system and per gram root, the reproduction factor and reduction in the reproduction factor were determined on coffee plants 150 days after inoculation of 20 young plants with 4000 nematode eggs/plant. The agronomic evaluations were conducted under field conditions, whereas the seeds were evaluated in the laboratory.

RESULTS

Second generation (F_2) H361/4 coffee plants received at the Institute of Agronomy the designation IAC 1669. The results of an evaluation experiment conducted in Campinas showed that plant IAC 1669-13 exhibited outstanding features in terms of production yield, seed size and rust resistance as compared to the other specimens investigated. For that reason, seeds from this progeny were sent to Eng.º Agro. Saulo Roque de Almeida, Varginha/MG and, at a later stage, to Eng.º Agro. José Carlos Grossi, in Patrocínio/MG, who enlarged the total area planted with this specimen. A series of new selections was made, one of which

resulted in the Tupi RN IAC 1669-13 cultivar. Irrigated areas planted with this cultivar yielded 91, 50 and 89 saks (60kg bags) of green coffee per hectare in the first three years, planted at a spacing of 3.68 x 0.5 m, thereby confirming its agronomic value. Analysis of the seeds collected in 2004 showed that cultivar TUPI RN IAC 1669-13 produces large beans with average screen size 18.4 and 90.2% flat beans, 8.0% peaberry and 1.8% elephant beans.

New leaves of TUPI RN IAC 1669-13 are green, different from cultivar Tupi IAC1669-33, which has bronze-colored shoots. It is a low growing plant that bears large, red fruits of medium maturation (more precocious than Catuaí). Total height and crown diameter dimensions are similar to those of cv. Tupi IAC1669-33 and a little smaller than those cv. Catuaí Vermelho IAC 144. Cultivar Tupi RN IAC 1669-13 has high nutritional demands and requires special care. Bourbon constitutes 50% of its genetic makeup, since the Villa Sarchi variety is a Bourbon mutant. As for organoleptic or sensory properties, cultivar Tupi RN 1669-13 has good drinking quality, a fact later confirmed by Eng.º Agro. José Carlos Grossi, who found that its beans have good market acceptance.

Coffee plants of the Tupi RN IAC 1669-13 cultivar tested for resistance to *M. exigua* had a gall index of 0.5 with small enlargements, however, without typical galls, whereas cv. Catuaí Vermelho IAC 144, used as "control" or check cultivar, exhibited a gall index of 4.5, with the presence of typical galls. The reproduction factor was 0.025 and the reduction in this factor was 98.2%. The number of eggs per root system of progeny IAC 1669-13 RN was 100 *versus* 5550 on the nematode susceptible check cultivar. These data indicate that cv. Tupi RN IAC 1669-13 is highly resistant to the nematode *M. exigua* and homozygous for the *Me* gene. As for reaction to rust, it was found that, under field conditions, up to the present time the material remains resistant to the prevailing races on both the test sites and seed production fields.

Resistant (without galls) ***Susceptible (with typical galls)***

Figure 1.

cv. Tupi RN IAC 1669-13

Figure 2.

Coffee fruits
cv. Tupi RN IAC 1669-13

Figure 3.

CONCLUSION

Due to its multiple resistance to both rust and the nematode *M. exigua* with homozygosis for the resistance gene (*MeMe*), cultivar TUPI RN IAC 1669-13 may be seed-planted and constitutes an excellent alternative and option for coffee growers

REFERENCES

Fazuoli, L.C. 1981. Resistance of coffee to the root-knot nematode species *Meloidogyne exigua* and *M. incognita*. Resumes. Colloque International sur la Protection de Cultures Tropicales. Lyon, France. p. 57.

Fazuoli, L.C.; Mônaco, L.C.; Carvalho, A.; Medina Filho, H.P. 1974. Herança da resistência ao nematóide Meloidogyne exigua. Ciência e Cultura 7:30 (Suplemento).

Carvalho, A.; Medina Filho, H.P.; Fazuoli, L.C. and Costa, W.M. 1984. Número de locos e ação gênica de fatores para porte pequeno em *Coffea arabica* L. Bragantia 43:425-442.

Carvalho, A.; Medina Filho, H.P.; Fazuoli, L.C.; Guerreiro Filho, O. and Lima, M.M.A. 1991. Aspectos genéticos do cafeeiro. Revista Brasileira Genética. 14:135-183.

Noir, S.; Antony, F.. Bertrand, B.; Combes, M.C. and Lashermes , P. 2003. Identification of major gene (Mex-1) from *Coffea canephora* conferring resistance to *Meloidogyne exigua* in *Coffea arabica*. Plant Pathology 52:97-103.

Genetic Basis of Species Differentiation between *Coffea liberica* Hiern and *C. canephora* Pierre: Analysis of an Interspecific Cross

A. N'DIAYE, M. NOIROT, S. HAMON, V. PONCET

UMR 1097 Diversité et Génome des Plantes Cultivées, Centre IRD, 911 avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France

SUMMARY

The phenotypic and genetic differentiation between the two related *Coffea* species (*C. liberica* Hiern and *C. canephora* Pierre) was examined. These species differed markedly in terms of leaf, inflorescence, fruit and seed characters. A genetic map of the interspecific cross *Coffea liberica* x *C. canephora* was constructed on the basis of 72 BC1 hybrids. Eighty-three AFLP markers, four inter simple sequence repeats (ISSR) and five microsatellites corresponding to *Coffea liberica* species-specific markers were mapped into 16 linkage groups. The total length of the map was 1502.5 cM, with an average of 16.3 cM between markers and an estimated genome coverage of 81%. The two species were evaluated relative to 19 quantitative traits and found to be significantly different for 15 of them. Eight QTLs were detected, associated with variations in petiole length, leaf area, number of flowers per inflorescence, fruit shape, fruit disc diameter, seed shape and seed length. Results on segregation distortion and the under-representation of particular markers were interpreted in terms of genome differentiation. The implications for the introgression of QTLs involved in advantageous morphological traits (number of flowers per inflorescence, fruit and seed shape) are discussed.
Keywords: *Coffea liberica*, *Coffea. canephora*, Species differentiation, Genetic map, QTL

RÉSUMÉ

La différenciation phenotypique et génétique entre *C. liberica* Hiern and *C. canephora* Pierre a été étudiée. Ces deux espèces ont montré de grandes différences au niveau des caractères foliaires, des inflorescences, des fruits et des graines. Une carte génétique du croisement interspécifique *Coffea liberica* x *C. canephora* a été construite à partir de 72 hybrides BC1. 83 marqueurs AFLP, 4 ISSR et 5 microsatellites correspondant à des marqueurs espèces spécifiques de *Coffea liberica* ont été cartographiés sur 16 groupes de liaison. La carte a une longueur totale de 1502,5 cM avec un espacement moyen entre marqueurs de 16,3 cM et couvrant 81% du génome. Les deux espèces évaluées sur la base de 19 caractères morphologiques ont montré des différences significatives pour 15 caractères. Huit QTL, impliqués dans la variation de la longueur du pétiole, la surface foliaire, le nombre de fleurs par inflorescence, la forme et le diamètre du disque du fruit, la forme et la longueur de la graine, ont été détectés. Les distorsions de ségrégation et la sous-représentation de certains marqueurs ont été interprétées en termes de différenciation du génome. L'introgression de QTL associés à des caractères morphologiques avantageux (nombre de fleurs par inflorescence, forme du fruit et de la graine) a été discutée.

INTRODUCTION

Two main coffee species, Coffea Arabica and C. canephora (Robusta) provide most of the world coffee production. Only *C. liberica* Hiern of the Pachycoffea subsection is still cultivated to a minor extent. Currently, *C. liberica* is of interest for *C. canephora* breeding programs for its clustered fruit maturation, high seed weight and low caffeine content. Despite the close phylogenetic relationship between *C. liberica* and *C. canephora*, these species differ substantially according to their morphological characters (N'Diaye et al., 2005). *C. liberica* could thus be of interest for interspecific breeding programs. Moreover, *C. liberica* and *C. canephora* have an overlapping geographical distribution and studying their divergences also provides an opportunity to investigate the genetic basis of species differentiation in the same environmental context.

Here we present the analysis of the phenotypic and genetic differentiation (some likely adaptive) between *C. liberica* and *C. canephora*. The features of an interspecific map based on *C. liberica*-specific markers were analysed.

MATERIALS AND METHODS

The plant material consisted of 26 *C. liberica* (LIB) and 26 *C. canephora* (CAN) samples representative of the variability within both species and the BC_1 mapping progeny. The BC_1 population derived from hand-pollination of *C. liberica* x *C. canephora* F_1 hybrids with bulk pollen from *C. canephora*. A total of 72 BC_1 progeny were evaluated. A total of 19 morphological characters, involving leaves, flowers, fruits and seeds, were analysed. Total genomic DNA from adult leaves was extracted according to Ky et al. (2000b). Genotyping was performed using AFLP, microsatellite (SSR) and Inter simple sequence repeat (ISSR) markers. PCR amplifications and analyses on an IR^2 Automated Sequencer (LI-COR, Lincoln, Neb, USA) were carried out according to Poncet et al. (2004). Before analyzing the segregating population, 15 LIB and 15 CAN genotypes were screened to identify LIB-specific bands. Since the BC_1 progeny involved different *C. liberica* and *C. canephora* parents, only LIB-specific AFLP or ISSR bands present in all LIB (representative of the species) and absent in all CAN genotypes were scored. Since microsatellite markers are co-dominant, we selected diagnostic markers with no shared alleles between the two parental species. Linkage analyses were performed using MAPMAKER/EXP 3.0b (Lincoln and Lander, 1992) and Mapdisto (version 1.37, available via http://www.mpl.ird.fr/mapdisto) software packages. The genome coverage was estimated assuming a random marker distribution (Lange and Boehnke, 1982): $c = 1 - e^{-2nd/Ge}$, where c is the percentage of the genome within d cM of a marker, Ge is the estimated genome length and n is the number of markers. QTL analysis was performed on 48 BC_1 hybrids having both quantitative trait notations and molecular marker data, by the composite interval mapping (CIM) procedure (Zeng, 1994).

RESULTS

C. liberica and *C. canephora* differed dramatically for all the phenotypic traits assayed, except for leaf shape index. In particular, LIB had larger leaves (leaf area index: 109.6 vs. 84.0 cm^2) and bigger fruits (fruit length: 17.5 vs. 12.0 mm) than CAN. Conversely, CAN had more flowers per inflorescence (NFI: 3.9 vs. 2.8) and more inflorescences per node (NIN: 7.5 vs. 5.3) than LIB. None of the evaluated traits exceeded the parental mean values.

A total of 138 LIB-specific polymorphic markers (121 AFLP, 9 ISSR and 8 microsatellites) were mapped into 16 linkage groups. The estimated map length (Ge) was 1795.6 cM, with a genome coverage (c) of 81%. Among all of the segregating markers, 42 (30%) deviated significantly ($\alpha = 5\%$) from the 1:1 Mendelian segregation expected in the BC_1 progeny.

A total of eight QTLs were detected, associated to variations in petiole length, leaf area, number of flowers per inflorescence, fruit shape, fruit disc diameter, seed shape and seed length.

DISCUSSION

A total of 121 LIB-specific AFLP markers (present in all LIB genotypes and missing in all CAN genotypes) were generated by 100 tested AFLP primer combinations. This polymorphism was lower than that of [*C. pseudozanguebariae* (PSE) x *C. liberica* var. *dewevrei* (DEW)] (Ky et al., 2000b) and [*C. canephora* (CAN) x *C. heterocalyx* (HET)] (Coulibaly et al. 2003b) interspecific crosses with 192 PSE- and 207 HET-specific. The transferability of SSR or EST-SSR markers across different *Coffea* species, even genetically distant, has been shown (Poncet et al. 2004). Only diagnostic loci – no shared alleles between the parental species – were informative for the mapping. Interestingly, EST-SSR primers, with allele sizes that differ more between species, were shown to reveal more diagnostic markers than SSR primers (Poncet et al. unpublished data). Because of the limited number of mapped markers, the 81% genome coverage should be taken cautiously. For instance, in *Pinus sylvestris*, a similar coverage rate was observed with more markers (188) but a lower average distance (8.9 cM) (Yin et al., 2003). In the LIB x CAN linkage map, despite good genome coverage, markers should be added to improve the marker density. Interestingly, among the microsatellite markers we analysed, the EST-SSRs tended to be more informative in revealing differences between species. This highlights the necessity of further development and analysis of this type of marker. Distorted segregation was noted in one third (36%) of the studied loci in the *C. liberica* x *C. canephora* cross. In the *Coffea* genus, the frequency of skewed loci did not seem to be associated with the parental phylogenetic relationship. Indeed, *C. liberica* and *C. canephora* are more closely related than *C. pseudozanguebariae* and *C. liberica* var. dewevrei but showed similar levels of distorted loci in their progeny. Besides commonly described distorting factors (see, for example, Fishman et al. 2001), the heterogeneity of our progeny might also have been partially responsible for the segregation distortions. The role of genome size differences was not relevant in LIB x CAN which have a similar sized genome (2C = 1.4 pg, Noirot et al. 2003). Based on the strategy used for BC_1 progeny generation, this interspecific map allowed us to identify QTLs involved in species differentiation and could provide insight into phenotypic divergence patterns. Eight QTLs associated with leaf, fruit and seed characters were detected. Three of them, i.e. *FS2*, *SL* and *SS,* associated with fruit shape, seed length and seed shape variation, respectively, were tightly linked, and the strong correlations suggest the presence of a major QTL with a pleiotropic effect. No QTL was detected for nine (56%) of the evaluated. However, the QTL number and effects (magnitude) should be interpreted cautiously given the reduced number of plants that were assessed. QTLs for fruit and grain features, in particular seed length (*SL*) could potentially be interesting for *C. canephora* breeding). In this study, only one QTL, i.e. *NFI,* involved in the expression of the number of flowers per inflorescence, was located within one of these negatively selected blocks. This suggests that the genomes of both species might be relatively permeable to

introgression for the other QTLs. Since these QTLs are related to species differentiation, they might also represent advantageous traits related to adaptive features.

CONCLUSION

This linkage map, the first obtained from the interspecific cross (*C. liberica* x *C. canephora*) x *C. canephora*, clearly showed that generating species-specific markers from an interspecific cross may be difficult when species are closely related. However, the map obtained could provide a reference for any potential crosses involving the parents of the two species considered. EST-SSR markers, which were informative for the LIB x CAN linkage map, are also potentially useful for any study based on diagnostic markers distinguishing *C. liberica* and *C. canephora* species. However, for population studies, it would be recommended to first check, with a larger sample, whether these diagnostic alleles are frequency dependent.

REFERENCES

Coulibaly I., Revol B., Noirot M., Poncet V., Lorieux M., Carasco-Lacombe C., Minier J., Dufour M., Hamon P. 2003b AFLP and SSR polymorphism in a Coffea interspecific backcross progeny [(*C. heterocalyx* x *C. canephora*) x *C. canephora*]. Theor Appl Genet 107:1148-1155

Fishman L., Kelly A.J., Morgan E. and Willis J.H. 2001. A genetic map in the *Mimulus guttatus* species complex reveals transmission ratio distortion due to heterospecific interactions. Genetics 159:1701-1716

Ky C.L., Doulbeau S., Guyot B., Akaffou S., Charrier A., Hamon S., Louarn J. and Noirot M. 2000b. Inheritance of coffee bean sucrose content in the interspecific cross *Coffea pseudozanguebariae* X *Coffea liberica* 'dewevrei'. Plant Breeding 119:165-168

Lange K. and Boehnke M. 1982. How many polymorphic genes will it take to span the human genome? Am. J. Hum. Genet. 34:842-845

Lincoln S.E. and Lander E.S. 1992. Systematic detection of errors in genetic linkage data. Genomics San Diego 14:604-610

N'Diaye A., Poncet V., Louarn J., Hamon S. and Noirot M. 2005. Genetic differentiation between *Coffea liberica* var. *liberica* and *C. liberica* var. *Dewevrei* and comparison with *C. canephora*. Pl. Syst. Evol. 253:95 - 104

Noirot M., Poncet V., Barre P., Hamon P., Hamon S. and De Kochko A. 2003. Genome size variations in diploid African *Coffea* species. Ann. Bot. Lond.. 92:709-714

Poncet V., Hamon P., Minier J., Carasco C., Hamon S. and Noirot M. 2004. SSR cross-amplification and variation within coffee trees *Coffea* spp... Genome 47:1071-1081

Yin T.M., Wang X.R., Andersson B. and Lerceteau Kohler E. 2003. Nearly complete genetic maps of *Pinus sylvestris* L. Scots pine constructed by AFLP marker analysis in a full-sib family. Theor. Appl. Genet. 106:1075-1083

Zeng Z.B. 1994. Precision Mapping of Quantitative Trait Loci. Genetics 136:1457-1468

Progress in Developing Coffee Berry Disease (*Colletotrichum kahawae*) Resistant Compact Hybrid Varieties (*Coffea arabica*) in Tanzania

D. J. MTENGA, D. L. KILAMBO, J. M. TERI, L. MASUMBUKO

Tanzania Coffee Research Institute (TaCRI) P.O. Box 3004 Moshi, Tanzania
E-mail: tacriced@kicheko.com – Fax no: +255 27 2756773

SUMMARY

A crossing programme to develop compact-dwarf varieties was initiated in 2003/04 whereby 32 Colombian (dwarf) and 61 tall hybrid lines were involved. Lines involved in the crossing programme are good sources of coffee berry disease (CBD) and coffee leaf rust (CLR) resistance. A total of 67 compact hybrid lines were developed. Out of 67, 15 lines considered as the best crosses were subjected to CBD Pre-selection test at hypocotyls stage to study inheritance of resistance. VC 298, VC 506 and HdT 1593 (CBD resistant candidate), and N 39 and KP 423 (CBD susceptible varieties) were included as check varieties. Results show that Disease Intensity Reaction (DIR) of hybrid compact lines ranged from 0.0 to 3.5. Line with DIR < 25 is considered to be CBD resistant candidate. Resistance inherited appears to be controlled by VC 298, VC 506, HdT 1593 from tall hybrid lines, and Colombian 086, 088, 089 and 090 lines used as female parents. The resistance of these compact lines will be qualified with CBD attached berry test at fruit bearing stage.

INTRODUCTION

Since 1880s tall coffee varieties have been the most popular planted by growers in Tanzania (Robinson, 1964). The varieties; N 39, KP 423 and H 66 produce fine coffees and are preferred by buyers and roasters, but are highly susceptible to anthracnose of green berries (CBD) caused by *Colletotrichum kahawae* Waller & Bridge and CLR incited by *Hemileia vastatrix* Berk et Br. In addition, for easy management tall varieties are trained as single or double stem capped. But the system results in excessive production of shoots, increasing turns of handling and pruning and therefore labour costs. Experience from other countries shows that compact varieties assist in easy management, and can accommodate 2.5 more plants per unit area than tall varieties (Njoroge, 1991; van der Vossen and Walyaro, 1981). Njoroge (1991) also revealed that compact-dwarf varieties have 3 times economic benefits compared to conventional varieties.

Accordingly, TaCRI initiated hybridization programme to breed new varieties of Arabica coffee that combined resistance to CBD and CLR with improved yield and quality, and more compact to suit close-spaced plantings. This report covers the progress made so far in getting compact varieties for the benefits of farmers in Tanzania.

MATERIALS AND METHODS

Parentage

Parents involved as tall varieties in the crossing programme represents a composite of hybrids already indicated resistance to CBD and CLR (Kilambo and Swai, 1998; 1999). The hybrids

involves combinations of N 39, KP 423, SL 28 and SL 34 to impart attributes of flavour and yield (Millot, 1969); and VC 298, VC 506 and HdT 1593 to impart genes for CBD resistance (van der Vossen and Walyaro, 1980). These were used as male parents. Summary of characteristics of Colombian lines accumulated from 2001 to 2003 before initiation of the crossing programme in 2004 are presented in Table 1. They were used as female parents to impart compactness; thick and strong laterals, internodes on both main stem and laterals very short.

Table 1. Summary of characteristics of Colombian lines; CBD and CLR, and liquor scores 2001-2003.

S/no	Line	Details of the line	Mean % CBD incidence (Semi Detached Berry)	Mean % CLR incidence (Field assessment)	Mean cupping taste results
1	PNI 086 10/5	Cattura x HdT 1343/219 F_{5-6}	0.0	0.0	6
2	PNI 088 10/2	Cattura x HdT 1343/219 F_{5-6}	0.0	0.0	6
3	PNI 089 208/16	Cattura x HdT 1343/219 F_{5-6}	2.3	0.0	5
4	PNI 090 2/10	Cattura x HdT 1343/219 F_{5-6}	0.0	0.0	5
5	HdT 1593		0.8	0.0	NA
6	VC 298		0.0	0.0	NA
7	VC 506		0.0	0.0	NA
8	KP 423		87.3	85.0	5
9	N 39		90.4	97.2	4
Mean SE ±			20.0 12.99	20.24 13.42	5 0.30

Key: Beverage quality: 2 = Good; 3 = Fair to Good; 4 = Fully Fair; 5 = Fair Average Quality (FAQ); 6 = About Fair; 7 = Poor to Fair; 8 = Poor. CBD incidence: < 5 % Resistance; > 75 % Susceptible. CLR incidence: < 5 % Resistance; > 75 % Susceptible.

Crossing

Technique used for emasculation was that developed by Krug (1935) of removing the petals. Branches of emasculated flowers (in this case Colombian lines) were pollinated by tall hybrid lines then enclosed in muslin sleeves supported on a frame of wire. Fruits were then harvested, seeds processed eventually sown.

CBD test

Hypocotyls (5-6 weeks) were sprayed inoculated twice at 48 hrs intervals with suspension of *C. kahawae* at 2.0 x 10^6 spores/ml using the method by van der Vossen et al. 1976. To allow infection a temperature of about 22-24 °C was required during the first four days, and R.H in the boxes maintained at 100 %, followed by an incubation period of three weeks at 19-20 °C. Coffee seedlings were individually scored for CBD symptoms developed on the hypocotyls stem using a scale with a range of 0-4 developed by van der Graff (1981).

Data analysis

For each genotype DIR was determined by counting number of hypocotyls in disease description multiplied by numerical value of disease description divide by number of hypocotyls in all descriptions multiplied by 4. Four is a factor of categories: Resistance (DIR 0-25), Moderately Resistance (DIR 26-50), Moderately Susceptible (DIR 51-75) and Susceptible (DIR 76-100).

Table 2. Disease Intensity Reaction (DIR) of the best compact lines.

S/ No.	Line	DIR
1	(HdT 1593 x N 39) x SL 28 x (N 39 x VC 298) x PNI 108/10	3.0
2	PNI 090 2/19 x (N 39 x HdT 1593) x (HdT 1593 x N 39) x VC 298	0.7
3	PNI 090 2/10 x (N 39 x HdT 1593) x (HdT 1593 x N 39) x VC 298	2.5
4	PNI 086 x (N 39 x VC 298)	3.5
5	PNI 089 101/13 x SL 34 x (HdT 1593 x N 39) x VC 506	3.5
6	PNI 089 101/13 x KP 423 x (HdT 1593 x N 39) x VC 298	0.0
7	(N 39 x HdT 1593) x (HdT 1593 x N 39) x VC 298 x PNI 089	0.0
8	PNI 087 15/11 x (SL 34 x (HdT 1593 x N 39) x VC 506	0.0
9	(N 39 x HdT 1593) x (HdT 1593 x N 39) x VC 298 x 089 203/3	0.0
10	(HdT 1593 x N 39) x SL 28 x VC 506 x PNI 089 107/13	0.0
11	(HdT 1593 x N 39) x SL 28 x VC 506 x PNI 089 10/5	0.0
12	(N 39 x HdT 1593) x (HdT 1593 x N 39) x VC 298 x PNI 089 108/10	1.0
13	Padang x (KP 423 x HdT 1593) x PNI 089 10/5	1.0
14	PNI 089 208/15 x Padang x (HdT 1593 x N 39) x VC 506	0.0
15	PNI 089 101/13 x KP 423 x (KP 423 x HdT 1593) x N 39 x VC 298	0.0
16	HdT 1593	2.6
17	VC 298	2.3
18	KP 423	72.4
19	N 39	75.6
Mean		8.8
SE ±		5.3

Key DIR: Resistance (DIR 0 – 25), Moderately Resistance (DIR 26 – 50), Moderately Susceptible (DIR 51 – 75) and Susceptible (DIR 76 – 100).

RESULTS AND DISCUSSION

Although the hybrids are still at seedling stage, leaves shows typical characteristics of compact type of varieties. They are broad and fairly large and darkish green, thick and leathery when mature; a typical characteristic for leaves of compact varieties (Njoroge, 1991). Their genetic heterogeneity is of considerable advantage as it is expected to confer stability of resistance with regard to protection against diseases but also imparts hybrid vigour and improves on the liquor quality. This is possible because tall lines selections involving multiple crosses of VC 298 and or VC 506 and or HdT 1593 were back-crossed to N 39 and or KP 423 and or SL 28 and or SL 34 (Ndondi and Nyange, 1990). The lines are expected to be highly resistant to CBD because the population contains genotypes carrying different combinations of disease resistance genes from VC 298, VC 506, HdT 1593 (Millot, 1969) and Colombian varieties (containing HdT 1343) (van der Vossen, 2005). A high level of rust resistance is derived from HdTs and Colombian varieties. CBD hypocotyls test revealed DIR scores between 0.0 and 3.5, indicating high level of resistance (Table, 2). These seedlings will

be established in clonal mother garden to produce true to type seedlings for on-station, multi-locational and on-farm trials establishment to confirm their resistance to CBD and CLR, compact characteristics, yield potential and liquor quality attributes.

REFERENCES

Kilambo, D. L. and Swai, F. B. (1998) Coffee Pathology Progress Report. Presented during the National Coffee Research Coordinating Committee Meeting, at Selian Agricultural Research Institute Arusha, February 1998. Lyamungu miscellaneous reports.

Kilambo, D. L. and Swai, F. B. (1999) Coffee Pathology Progress Report. Presented during the National Coffee Research Coordinating Committee Meeting, at Uhuru Hostel Moshi, February 1999. Lyamungu miscellaneous reports.

Krug, C. A. (1935) Hybridization of coffee. Journal of Hered. 26: 325-330.

Millot, F. (1969) An Inventory of the coffee varieties and selections imported into and growing within East Africa. Records of previous selections and breeding work. Kawanda Agricultural Research Station miscellaneous reports.

Ndondi, R. V. and Nyange, N. E. (1990) A review of coffee breeding programme; Lyamungu Research Institute. An occasional publication presented to the Coffee Coordinating Committee Meeting held in Moshi at Kahawa House.

Njoroge, J. M. (1991) Management of Hybrid Ruiru II Arabica coffee-A review. In Kenya Coffee Volume 56 no 652 February 1991.

Robinson, J. B. D. (1964) A handbook on Arabica coffee in Tanganyika. Tanganyika Coffee Board.

Van der Graaff (1981) Selection of Arabica coffee types resistant to coffee berry disease in Ethiopia. Mgen Landbouwhogeschool. Wageningen 110 pp.

Van der Vossen, H. A. M. (2005) Report on coffee advisory mission to TaCRI, Lyamungu, Tanzania. UM Project 33166 MTZ.

Van der Vossen, H. A. M and Walyaro, D. J. (1981) The coffee breeding programme in Kenya: A review of progress made since 1971 and Plan of action for the coming years. Kenya coffee, 46 (541):113-130.

Van der Vossen, H. A. M. and Walyaro, D. J. (1980) Breeding for resistance to coffee berry disease in coffee Arabica L. II Inheritance of the resistance. Euphytica 29: 777-791.

Van der Vossen, H. A. M. and Cook, R. T. A and Mukaku, G. N. W (1976) Breeding for the resistance to coffee berry disease caused by *Colletotrichum coffeanum* in coffee Arabica. Euphytica 25: 733-745.

Carbon Allocation between Growth and Production in Fruit Trees

M. GENARD[1], J. DAUZAT[2], N. FRANCK[3], F. LESCOURRET[1], N. MOITRIER[1], P. VAAST[2], G. VERCAMBRE[1]

[1]Institut National de la Recherche Agronomique, Avignon, France
[2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Montpellier, France
[3]Universidad de Chile, Santiago, Chile

SUMMARY

Carbon allocation within a plant depends on complex rules linking source organs (mainly shoots) and sink organs (mainly roots and fruits). The complexity of these rules comes from both regulations and interactions between various plant processes involving carbon. This paper presents these regulations and interactions, and analyses how agricultural management can influence them. Ecophysiological models of carbon production and allocation are good tools for such analyses. The fundamental bases of these models are first presented, focusing on their underlying processes and concepts. Different approaches are used for modelling carbon economy. They are classified as empirical, teleonomic, source-sink or based on transport and chemical/biochemical conversion concepts. These four approaches are presented with a particular emphasis on the regulations and interactions between organs and between processes. The role of plant architecture in carbon partitioning is also discussed and the interest of coupling plant architecture models with carbon allocation models is highlighted. The link between roots, shoots and reproductive compartments is analysed through a model developed for peach trees, describing carbon transfer within the plant, and based on source-sink and Munch transport theory. On this basis, the consequences of fruit load or plant pruning on fruit and vegetative growth can be evaluated.

INTRODUCTION

Plants invest in competing activities for carbon such as growth, reproduction or maintenance costs. In wild plants, allocation to reproduction and above and below ground vegetative parts is particularly important because it is closely related to plant dissemination and survival and hence fitness. Several theories have been proposed to explain the relative investment in the reproductive compartment, and between above and below ground vegetative parts. An important framework for explaining patterns of plant carbon allocation is the theory of allometry (West et al., 1997) wich predicts intraspecific and interspecific scaling relationships among leaves, roots and reproductive biomass (Enquist, 2002; Niklas and Enquist, 2003). This theory, validated for wild plant clearly, shows that allometric rules dictate how metabolic production and biomass are partitioned between different plant parts. In agricultural crops, plants have been manipulated by human for a long time through plant breeding and cultural practices. Although it may at first appear that the great increase in productivity since a century has been achieved through an improved efficiency of the photosynthesis apparatus, it does not seem to be the case (Ericsson et al., 1996). Indeed, the large gain in productivity among agricultural species is basically a result of a change in carbon allocation pattern within the plant. Increasing the number and size of fruits is one of the main objectives of plant breeding in fruit trees. As a consequence, modern cultivars have to invest an increased amount of

carbohydrates produced via photosynthesis into fruit production. Fruit competition for carbohydrates can subsequently lead to a reduced vegetative growth of shoots and roots. This is typically the case of coffee where branch development is strongly reduced on heavy fruit-bearing trees often leading to branch dieback and resulting in a strong alternate production pattern (Cannell, 1971; Vaast et al., 2005). Therefore, agronomic practices seek to balance fruit and vegetative growth, either by manual or chemical fruit thinning or by increasing the vegetative growth through nitrogen supply or pruning. Additionally, irrigation and fertilization may counterbalance the reduced growth of the root system. The plant architecture is also an important feature for management because it influences the heterogeneity of fruit size within the plant. Indeed, the architecture is a network which connects the organs to each others and provides the support for carbon transfers within the plant. Architecture determines the spatial position of organs and hence their activity (photosynthesis, respiration) mediated by energy exchanges.

Carbon allocation within a plant depends on complex rules linking source organs (mainly leaves) and sink organs (mainly roots and fruits) for assimilates. The complexity comes essentially from regulations due to feedback mechanisms and interactions between different functions. In order to analyse this complexity, carbon models of plant growth have been developed during the last thirty years (Le Roux et al., 2001). These models are powerful tools to analyse how source and sink number, size and position within the plant affect the carbon partitioning and as a consequence the vegetative and reproductive growth.

The first objective of this review is to address the conceptual framework of carbon allocation in plant based on the current theories. The second objective is to exemplify the control of carbon partitioning through regulatory mechanisms and the interactions between processes in the plant system. The third objective is to analyse the role of plant architecture on carbon allocation. Finally, to exemplify the interest of carbon partitioning modelling, plant models will be used to assess the effect of management practices such as fruit thinning and plant pruning on vegetative growth and fruit production.

THEORIES OF CARBON ALLOCATION

As stated by Lacointe (2000), Leroux et al. (2001) and Thornley and Johnson (1990), four main approaches have been used in models of carbon economy. These approaches are either empirical and allometric, teleonomic, source-sink, or based on transport and chemical/biochemical conversion concepts.

The models of carbon balance based on empirical allocation coefficients give usually reasonable predictions in the range of conditions for which these coefficients have been measured. These coefficients can be constant or variable during the season or be modulated by external conditions by means of empirical relationships. The biomass allocation between plant parts X and Y can be derived from the assumption that allometric relationships exist between differents parts of the plant ($Y= \alpha X^{\beta}$). West et al. (1997) proposed a general model for the origin of allometric laws in biology. The model proposes that evolution by natural selection resulted in optimal fractal-like vascular networks. These networks minimize the total hydrodynamic resistance and yet maximize the whole organism ressource use by maximizing the scaling of surfaces where ressources are exchanged with the environment. It has been showed using this theory that the mass and growth rate of one plant part can be expressed in terms of mass and growth rate of another part. One example is given for mango fruit for which there is strong allometric relationship between stone dry weight and fruit dry weight (Figure 1). Derivation of such an allometric equation has been used by Lescourret and Génard (2005) to partition carbon between flesh and stone in a model of virtual peach fruit. However,

such a model does not allow neither to explicit the regulations between processes nor to simulate easily the effect of practices such as fruit thinning or shoot pruning on carbon allocation coefficients.

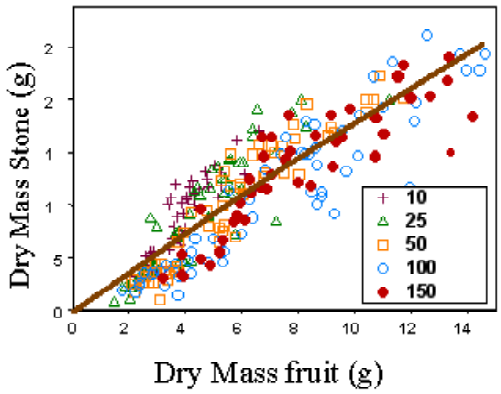


Figure 1. Allometric relationships between stone mango dry weight and fruit dry weight. Five different leaf to fruit ratio were used (from Léchaudel et al., 2002).

Many models of plant carbon allocation are based on teleonomic approaches where some goal is assumed, e.g. a functional balance between shoot and root (Davidson, 1969) or a relationship between foliage and conducting tissue as in the pipe-model (Shinozaki et al., 1964).

The pipe model theory states that the sapwood area at height x (A) and the foliage biomass above x (W) are related through a constant ratio (Shinozaki et al., 1964). This can be interpreted, in terms of functional balance, as a condition for an efficient supply of water to foliage biomass:

$$W = k\,A$$

The proportionality constant is often assigned different values at different plant heights (Lacointe, 2000). The pipe model is one of the most commonly used model to distribute ressources between foliage and woody structure in process based models (Sievänen et al., 2000), however it is probably inadequate to predict plant response to disturbances such as pruning or thinning (Le Roux et al., 2001).

Beside the hydraulic point of view as used in the pipe model, the stem also provides a mechanical support to the foliage. It has been suggested that the stem taper profile is in many case close to that just required for a safe and "harmonious" growth indicating a functional balance (Lacointe, 2000). Assuming very simplified tree shape, MacMahon and Kronauer (1976) predicted logitudinal profile of stem and branches diameter with a power law:

$$D = kL^{-}$$

with D is the diameter at distance L from the point where D=0.

A differential expression of that law was used by Ford et al. (1990 dans Lacointe) to partition assimilates between elongation and radial growth in coniferous branches.

Another important teleonomic approach concerns the root:shoot functional balance. Models of carbon allocation between shoots and roots have been based largely on carbon supplied by

the shoots and nitrogen supplied by the roots (Charles-Edwards, 1976; Reynolds and Thornley, 1982 dans Chen et Reynolds 1997). This approach has been extended to water supplied by the roots (Chen and Reynolds, 1997). According to the root:shoot functional balance, total nitrogen (or water) acquisition by the root system is proportional to total carbon assimilation by the shoots. This functional balance was described by Davidson (1969 voir Le Roux) as:

$$W_s/W_r = k\,(S_r/S_s)$$

where W_s and W_r are the shoot and root biomasses, respectively, S_s and S_r are the shoot and root specific activities, respectively, and k is a constant. The specific activities are usually allowed to vary with external N or water availability (for S_r) and with above-ground environment such as light intensity and atmospheric CO_2 concentration (for S_s). Grechi et al. (2006) found experimentally on grapevine strong allometric relationships between roots and shoots. These relationships were highly dependant on nitrogen and light supply, so that a strong relationship was found between the root:shoot ratio and the C:N ratio (Figure 2) which is in agreement with the root:shoot functional balance theory.

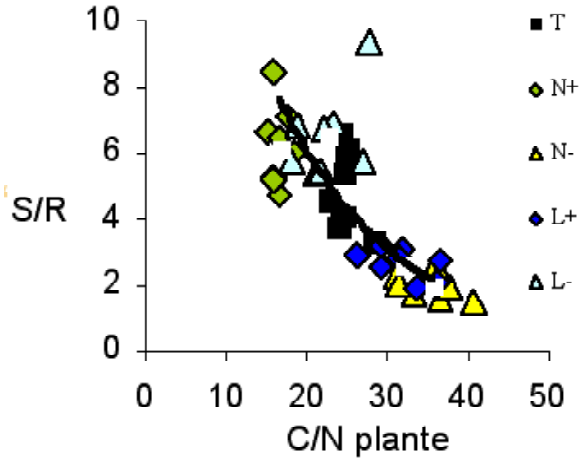


Figure 2. Relationship between the root:shoot ratio and the C:N ratio of *Vitis vinifera* for different levels of irradiance and nutrient supply (from Grechi et al., 2006).

Many models utilize optimization principles whereby the relative root:shoot allocation is considered in the context of maximizing the relative growth rate of the plant or to constrain the C/N ratio to a target value (Thornley and Johnson, 1990; Lacointe et al., 2000; Le Roux et al., 2001). However optimization must rely on major simplifications in order to facilitate analytical solutions to the optimal control strategy and assume that plants "anticipate" the environmental conditions (Reynolds and Chen, 1996). As an alternative to optimisation, Reynolds and Chen (1996 and 1997) proposed a "coordination theory" where the allocation coefficients are driven by the imbalance between root N or water supply and shoot carbon supply. Such a coordination has been also proposed for young peach trees by Génard et al. (1998). In this case, a variable RS reflecting the balance between the mass of roots younger than one year (*Wr*) and the leafy shoot mass (*Ws*) is defined as:

$$RS = \frac{1}{RSe}\frac{Wr}{Ws}$$

The parameter *RSe* is equal to the ratio of weight of young roots and weight of shoots when the tree is at equilibrium. When *RS* is greater than one, there is an imbalance in favour of

roots and assimilates are preferentially allocated to shoots, whereas when *RS* is less than one, assimilates are preferentially allocated to roots. The consequence is a fluctuation of the root:shoot ratio along the season. Equilibrium is reached when *RS* is equal to one.

In the source-sink relationships-based models, the carbon allocation is assumed to depend on the respective ability of the different sinks to import available assimilates from the sources (Lacointe, 2000). According to Grossman and DeJong (1994), this ability or "sink strength" or "sink demand" is based on the genetically determined potential growth respiration rate, maintenance respiration rate and net sink strength (g d^{-1}). The potential net sink strength is the maximum rate at which the organ can accumulate dry matter per unit of time. It is the product of sink size (g) and potential net sink activity (d^{-1}) expressed as the relative growth rate. The potential net sink strength of an organ can be decreased by suboptimal environmental conditions. That new sink strength is called by Grossman and DeJong (1994) the conditional net sink strength. The sink growth rate, also called apparent net sink strength, is calculated from the conditional net sink strength, taking into account the resource availability.

Experimentally determined seasonal patterns of organ growth potential are frequently used in the literature to represent potential net sink strengths. Regarding fruits, Marcelis (1996) argued that series of growth rate measured under conditions of non-limiting assimilate supply provide a correct estimation, based on the idea that potential growth only changes with time. However, this idea implies that constrained fruit growth is able to reach the potential growth rate after removal of competing sinks. This capability was observed for cucumber (Marcelis, 1993) or tomato, but not for other species such as peach (Grossman and DeJong, 1995). For such species, the conditional net sink strength at a given time depends on its state at this time, i.e. on the past growth. Moreover, the dynamic of fruit growth and particulary the time at which fruit growth slows down or stops depends on the accumulation of matter in the fruit (Havis, 1962; Proebsting and Mills, 1981; Johnson, 1995).

Lescourret et al. (1998) proposed the following equation for the conditional net sink strength (*CSS*). It emphasizes the role of fruit history by means of the accumulated growth (*W*), both in terms of sink size and sink activity. It also emphasizes the role of time by means of accumulated degree-days (Figure 3).

$$CSS = RGR_{ini} \times W \times \left(1 - \frac{W}{W_{max}}\right) \times f(dd) \qquad \text{with} \quad f(dd) = 1 \text{ if } dd < dd_{min}$$

$$f(dd) = \frac{dd_{max} - dd}{dd_{max} - dd_{min}} \quad \text{if } dd \text{ is between } dd_{min} \text{ and } dd_{max}$$

$$f(dd) = 0 \text{ if } dd > dd_{max}$$

where RGR_{ini} is the initial relative growth rate, W_{max} refers to the limiting final weight and dd_{min} and dd_{max} are parameters.

In some cases, sink strength strongly varies according to the equilibrium between sinks within the plant which makes it very difficult to define potential or conditional sink strengths. It is typically the case for shoots and roots which are growing according to the functional equilibrium (see above). The potential (conditional) sink strength of shoots will depend on the growth of roots and conversely. Génard et al. (1998) proposed an approach only based on the apparent sink strength. They assumed that the conditional sink strength at time *t* was

proportional to its apparent sink strength at *t-1*. The proportionality coefficient RS (see above), which can be above or below 1, varied according to the root:shoot ratio at *t-1*.

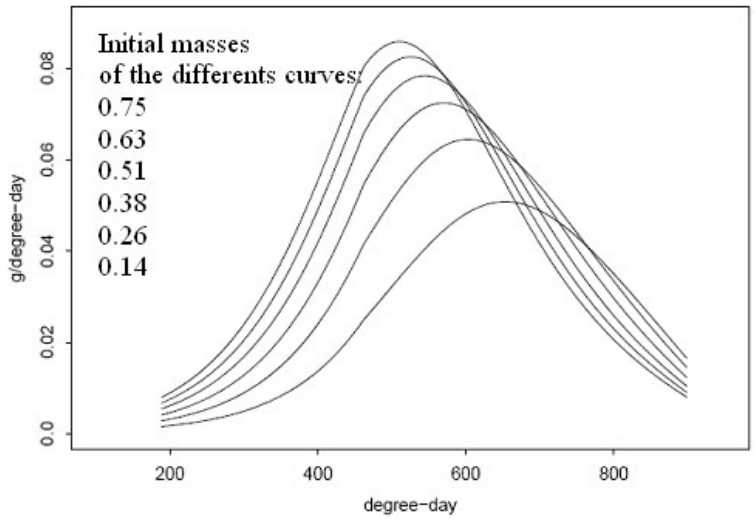


Figure 3. Conditional net sink strength (CSS) of peach fruit for different initial masses. The fruit history (here the initial fruit mass) has strong effect on CSS.

An important step after the quantification of organs demands is the carbon allocation from sources to sinks. Le Roux et al. (2001) summarized the allocation rules which are applied in most of the models. When the demand is less than the supply, each sink gets its own demand and the excess supply goes to reserves. On the other hand, there are two approaches to deal with the case of supply shortage. In the "proportional" approach, the carbon supply from sources is shared by the sink organs which get each the same proportion of their demand. Alternatively, in the "hierarchical-priority" approach, the sink with the highest priority is served first, then the component with the next priority level is considered, and so on. The maintenance respiration requirements are assigned the highest priority because they are vital for the organ survival. Most of the models use a mixture of these rules. It is the case of the model proposed by Vaast et al. (2002) for the coffee branch.

Models based on transport and chemical/biochemical conversion concepts opened the way for a more mechanistic description of the carbon partitioning. They made it possible to avoid the empirical allocation coefficients, functional balance rules, or fixed allometric relationships. Thornley (1972, 1998) first proposed a transport-resistance model for shoot:root partitioning in relation to the availability of C and N. In this approach, C and N enter the plant through uptake processes, plant compartment are connected via transport pathways, and substrates are used for growth. Transport rate is proportional to concentration differences. This approach can account for the effect of nitrogen on the shoot-root ratio. It is generally assumed that the mechanism governing transport of assimilates is the Münch pressure-driven flow (Dale and Sutcliffe, 1986). Using the simplified formulation of the Münch hypothesis presented by Thornley and Johnson (1990), Minchin et al. (1993) presented a simple mechanistic model of phloem transport that explains sink priority as a function of sugar gradient. In the same theoretical framework, Bassow and Ford (1990) proposed a space-time model of carbon translocation in forest trees. More recently, Bruchou and Génard (1999) proposed a space-time model of carbon translocation along a shoot bearing fruits (Figure 4). The novelty of this model comes from the aggregation of physiological processes taking into account spatial aspects. The stem is represented as a set of compartments connected to source (leafy shoots) and sink (fruits) compartments.

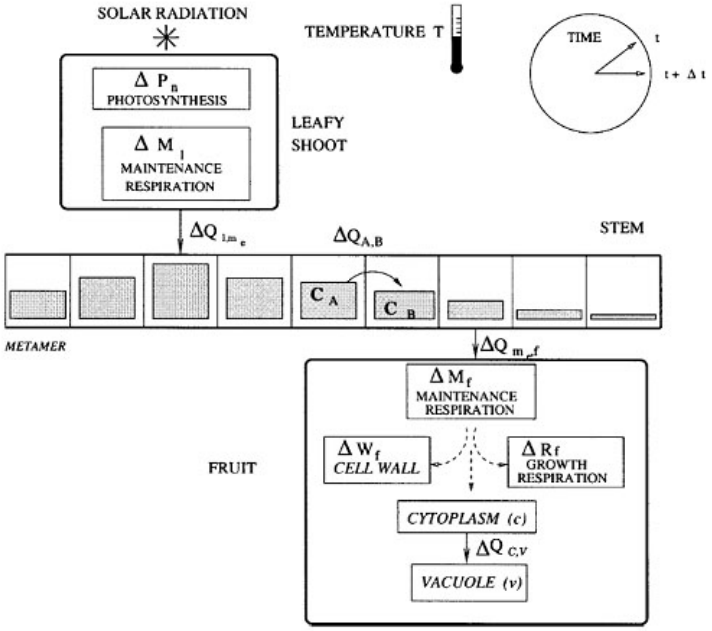


Figure 4. A model of carbohydrate translocation driven by concentration difference between compartments of a branch. The branch is divided in metamer which are compartments between which transferts occur. The sources are the leaves and the fruits are the sinks. (from Bruchou and Génard, 1999).

The considered physiological processes are photosynthesis, respiration of fruits and leaves, translocation of assimilates and fruit growth. Assimilate production is regulated by sink strength. Carbon translocation between two compartments depends on the gradient of assimilate concentration using the simplified formulation of the Münch hypothesis (Thornley and Johnson, 1990). A similar approach has been used by Bidel et al. (2000) to model the growth of the root. The authors used their model to analyze how differences in meristem sink strength and/or phloem conductivity can control the morphology of the root system. Recently, Allen et al. (2005) presented a carbon allocation model within the whole tree architecture based on electric analogy. Daudet et al. (2002) proposed a more complete theoretical model considering phloem and xylem flows in order to account for the carbon-water interactions.

An important step concerning fruit trees is the sugar unloading to the fruit and the biochemical transformations of sugars within the fruit. The sugars can be transported from the phloem to the fruit by active transport, mass flow or diffusion. Fishman and Génard (1998) proposed the following equation to represent the total uptake of carbohydrates (U) by the fruit:

$$U = Ua + (1-\sigma p)*((Cp+Cf)/2)*Up + A*ps*(Cp-Cf)$$

where Ua is the rate of uptake due to active transport obeying a Michaelis-Menten equation, Up is the phloem flow of liquid entering the fruit, Cp and Cf are the sugar concentrations in the phloem and fruit, respectively, σp is the reflexion coefficient which is a measure of impermeability of the cell membrane to the solute, A is the membrane area through which the solutes diffuse and ps is the solute permeability coefficient. If $\sigma p = 1$ the membranes are impermeable and there is no sugar uptake through mass flow. As ps is usually small, the diffusion component can often be neglected. The rate (Ua) obeys the Michaelis-Menten equation:

$$Ua = vm\ Cp / (KM + Cp)$$

where *vm* is the maximum uptake rate and *KM* is the Michaelis-Menten constant.

The transformation of phloem sugars (sucrose, sorbitol,..) into sink soluble carbohydrates (sucrose, glucose, fructose,…), starch or cell walls is one aspect of carbon partitioning that is usually ignored. It is a major process of growth because sink soluble carbohydrates drive the sink osmotic potential which in turn drives the sink water uptake. When a sink is harvested for its quality, as in the case of fruits, the transformation of phloem sugars into other sugars, starch and cell walls components determines their quality which is an important component of their market value. This is important not only for fruits produced for their flesh such as mango or peach but also for fruits produced for their seeds or beans such as coffee.

Génard and Souty (1996) and Génard et al. (2003) designed a mechanistic model, called SUGAR (Génard and Lescourret, 2004) to predict the changes in sugar composition during fruit development (Figure 5). The model was designed for peach, but the main principles can be used for other fruits. In this model, the unloaded sugars are either directly stored in the tissues or transformed in CO_2 through the respiration process or transformed into other sugars or used to synthesize other compounds (structural carbohydrates, etc.). The enzymatic reactions were described according to the "rate law" of chemical kinetics (Chang, 2000), which states that the rate of a reaction is proportional to the reactant concentration. Thus, the rates of change in the amounts of carbon in sugar compounds depend on sugars already accumulated in the fruit flesh. They are described through a set of differential equations, each equation being of the following form:

$$\frac{dC_j}{dt} = E_j + \sum_{i \neq j} k_{ij}(\theta, x) C_i - C_j \sum_{i \neq j} k_{ji}(\theta, x) - R_j$$

where C_j is the carbon amount in sugar *j*, E_j and R_j, which can be equal to zero, depending on the compartment, are respectively the carbon flow from the phloem and the carbon loss by respiration, k_{ij} is a function of parameter (θ) and variable (x) describing the relative rate of sugar transformation of sugar *i* into sugar *j*.

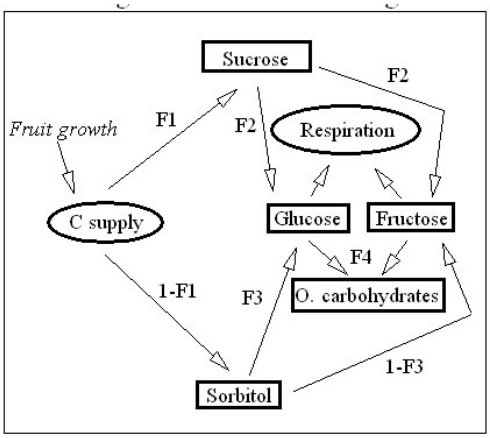


Figure 5. Diagram illustrating the carbon partitioning by the SUGAR model to predict the changes in sugar composition along the peach fruit development. Arrows, ellipses and boxes represent carbon flows, carbon supply and losses, and carbohydrate compartments, respectively. The Fi are allocation functions (from Génard and Lescourret, 2004).

REGULATIONS AND INTERACTIONS

The carbon allocation within a plant and between different biochemical compounds results from strong regulation of source and sink strength. These regulations are at the basis of teleonomic approaches such as the functional equilibrium in which the sink strength of shoot and root systems is regulated by the state of the equilibrium between them. These regulations are also considered in the source-sink models where the conditional net sink strength, and thus the carbon partitioning, is regulated by the history of the sink itself. Moreover, in some source-sink models, the source strength is regulated by the sink strength (Léchaudel et al., 2005) or by the amount of reserves in the leaves (Lescourret et al., 1998). It is interesting to notice that the high genotypic differences observed in peach photosynthesis by Quilot et al. (2002) is not related to the variation of the potential photosynthesis that is very similar between genotypes, but to differences in fruit sink strength. Indeed genotypes with low fruit sink strength accumulate reserves in the leaves, which depress the actual photosynthesis through a feedback mechanism. Such a feedback mechanism has also been documented on coffee plants with low fruit loads (Vaast et al., 2005; Franck et al., 2006).

In models based on transport and chemical/biochemical conversion concepts, the regulation are often emerging properties of the system. The model of Bruchou and Génard (1999) is used hereafter to exemplify this fact.

The model was run for leaves:fruit ratios equals to 5 and 30. Simulated photosynthesis was independent of the leaf:fruit ratio early in the morning, but after midday it decreased concomitantly with the ratio value (Figure 6). Similarly, a decrease in photosynthesis with low fruit loads has been observed for different tree species (Gucci et al., 1995; Chalmers et al., 1975; Ben Mimoun et al., 1996; Franck et al., 2006). The diurnal variation of leaf carbohydrate content (Figure 6) followed a classical pattern, increasing during the day and decreasing at night (Upmeyer and Koller, 1973; Sharkey and Pate, 1976; Franck et al., 2006). The leaf carbohydrates content increased with the leaf:fruit ratio (LF) whereas the export of carbohydrates from leaves decreased from LF = 5 to LF = 30 (Figure 6). The concentration of carbohydrates simulated in the woody shoot exhibited a diurnal periodicity and increased with the leaf:fruit ratio. The simulated unloading rate from phloem to fruit increased strongly with the leaf:fruit ratio. The diurnal variations closely followed the variations of loading rate (Figure 7) showing that the system quickly reacted to changes in assimilate supply.

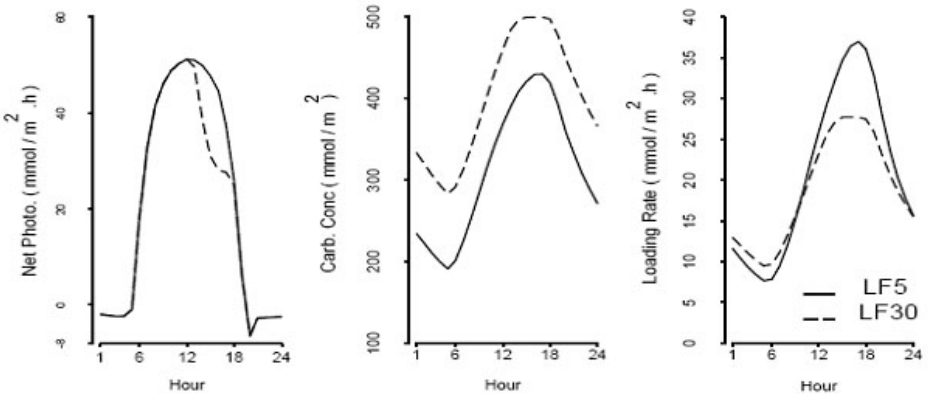


Figure 6. Simulated diurnal variation of peach leaf photosynthesis and carbohydrate content, and phloem loading rate for leaves:fruit ratio equals to 5 and 30 (from Bruchou and Génard, 1999).

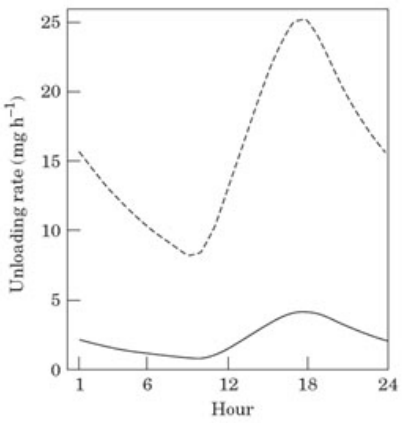


Figure 7. Simulated diurnal variation of unloading rate from phloem to fruit according to leaf:fruit ratio in peach (from Bruchou and Génard, 1999).

The consequence of these different loading rates is an increase of fruit mass with leaf:fruit ratio. These simulations showed that a change in source:sink ratio had a strong influence on (i) source carbon status and activity, (ii) the different steps of carbon translocation and (iii) sink activity. These effects are emergent properties of the system which result mainly from the sugar concentrations in the different organs. The sugars can be considered as a signal which operates as a regulator, i.e. an increase of leaf sugar content regulates the photosynthesis by feedback inhibition, or an increase in phloem sugar concentration induces an increase of sugar unloading in the fruit.

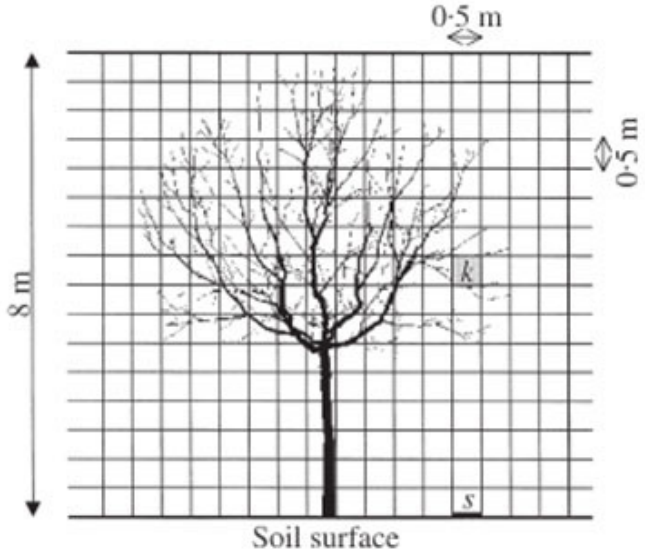
The partitioning of carbon also results from the interaction between different processes. The transport resistance model of Thornley (1972; 1998) considers the transport and chemical conversion of substrate C and N to structures. It is clear through this model that carbon allocation is highly dependant on the nitrogen supply and allocation within the plant. Another example is from the theoretical work of Daudet et al. (2002). These authors showed how the carbon allocation can be affected by the water status of the plant. They showed that the fruit growth rate in terms of dry matter can be decreased by plant water stress despite the fact that the sucrose source is unchanged. Interestingly, using the model of Fishman and Génard (1998), Lescourret et al. (2001) found that increasing the skin surface conductance for water increased the fruit dry weight and decreased the fruit fresh weight. When surface conductance increases, fruit transpiration increases strongly, and this can explain the decrease of fruit fresh weight. On the opposite, the mass flow of sugar is increased, because the water inflow from the plant to the fruit is higher, leading to an increase of the fruit dry weight.

From these examples about regulation and interaction between carbon, nitrogen and water, it can be seen that mechanistic models are important tools to analyse and understand the carbon allocation within the plant.

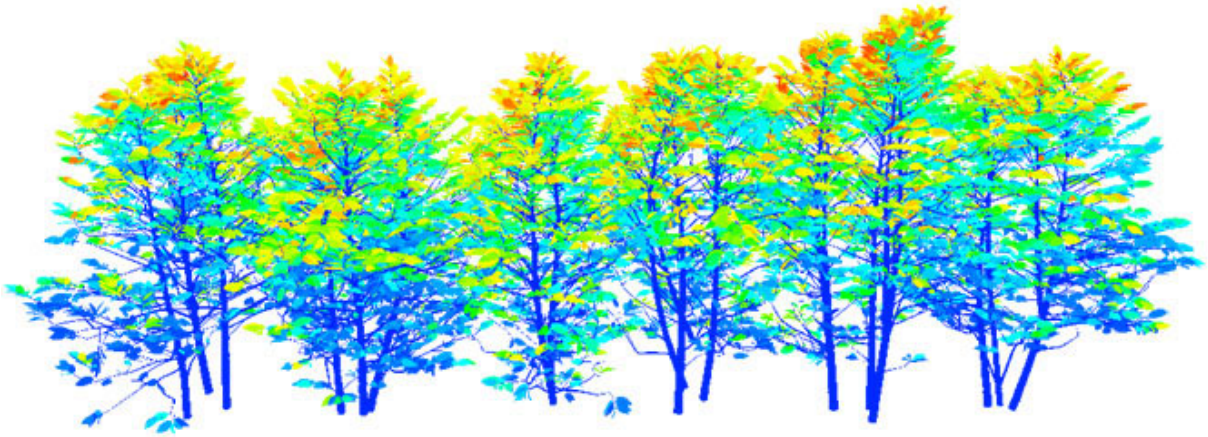
PARTITIONING AND ARCHITECTURE

During the last decade, many researchs were initiated in order to understand the complex interactions between plant architecture and the physical and biological processes that drive plant development. For this objective, functional-structural plant models were used (Ford, 1992; Bosc, 2000; King, 2005). Coste (2004) reviewed such models, and Sievänen et al. (2000) and Godin and Sinoquet (2005) discussed the different questions raised around functional-structural plant models. Two points are particularly important to analyze, (i) the impact of plant architecture on leaf photosynthesis variability within the canopy and (ii) the architecture impact on carbon partitioning within the plant.

It is well known that light interception is very variable within the plant, especially in the case of trees (Génard and Baret, 1994). The use of 3D plant models for calculating light capture and carbon assimilation throughout plant architecture was first proposed by Sinoquet et al. (RATP model, 2001) and then Dauzat et al. (Archimed model, ASIC 2006). Both models lay on the Farquhar leaf photosynthesis model (Farquhar et al., 1980) coupled with a stomatal regulation model but they diverge relatively to the spatial scale at which photosynthesis calculations are performed:



Spatial discretizing of a 20-year-old walnut tree in the RATP model



Simulation of *coffea arabica* photosynthesis at leaf scale by the Archimed model.

Figure 8. In the RATP model (A), the 3D plant model is used for spreading foliage into 3D cells. Light interception and photosynthesis are then calculated at cell scale. In Archimed model (B), light interception and photosynthesis are calculated at the scale of individual leaves.

In the RATP model, the 3D plant geometry is used to distribute the plant foliage into cells within a 3D space (Figure 8). The light interception is then calculated by assuming that foliage is randomly arranged within each cell (each cell being therefore considered as filled with a turbid medium). Then, the light intercepted by cells is shared into sunlit and shaded

foliage portions in order to calculate their photosynthesis. The carbon assimilated within a cell has subsequently to be shared into the shoots proportionally to their foliage area within the cell.

Alternatively, the Archimed model considers the light interception at the scale of individual leaves (Figure 8). The integration of assimilated carbon can therefore be directly calculated at any scale, from individual shoots to individual branches up to the tree scale. Because all plants organs are connected through the plant topology, sap flows resulting from transpiration can be calculated throughout the plant and, finally, water potential of stems and leaves can be assessed (Dauzat et al., 2001). This feature opens the opportunity to infer the fruit transpiration.

The model of Bruchou and Génard (1999) has been used to study the effect of fruit position on the branches on phloem sugar concentrations and specific mass transfer. Considering a branch with two fruits and a regular distribution of leaves, it was noticed that the sugar concentration along the branch was at its lowest at the fruit location, hence generating concentration gradients between leaves and fruits (Figure 9 A). It is interesting to notice that when fruits and leaves were regularly distributed along the branch, these concentration gradients generate phloem flux in different directions on the same branch. When the leaves and the fruits were on the opposite sides of a branch, the concentration gradient between source and sink was almost linear (Figure 9 B). The mean gradient was in the range of 0.05-0.2 g cm^{-3} m^{-1} which are values similar to the gradients (0.02-0.3 g cm^{-3} m^{-1}) measured in different tree species (Zimmermann, 1957; Grange and Peel, 1978; Hocking, 1980). Using this model, Bruchou and Génard (1999) analysed the competition between peach fruits. They studied a branch bearing two fruits and four leafy shoots, fruits and shoots being on the opposite extremities of the branch. The simulation predicted a decrease of 22% in dry matter for the fruit farthest from the leaves, which was consistent with experimental results. In order to analyse the distance effect by means of the model, the cases where only the proximal or distal fruit was kept on the stem were simulated. In both situations, fruit growth was the same, which shows that, according to our model, there is no distance effect but a relative position effect. This means that topology of the system was the most important.

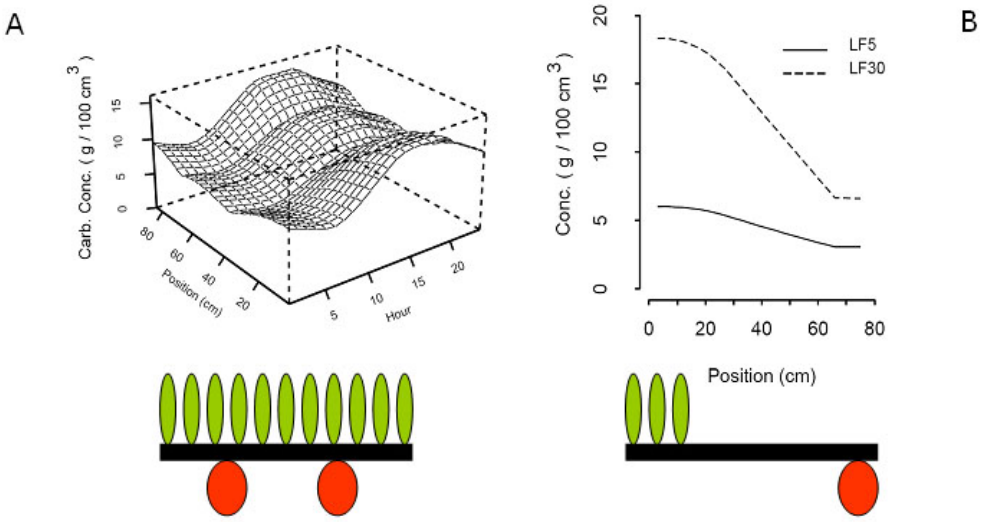


Figure 9. Effect of fruit position on the branches, on phloem sugar concentrations. (A) Diurnal variation of phloem concentration according to position on branch with two fruits and a regular distribution of leaves. (B) Phloem concentration according to position on branch when the leaves and the fruits were on the opposite sides of the branch (from Génard and Bruchou, 1999).

A modelling framework has been recently developed to analyse carbon transfer within a complex root architecture (Vercambre in prep). Movement of the phloem sap is caused by a gradient in hydrostatic pressure, the pressure in the phloem being osmotically induced accordingly to the Munch's theory. The sieve plate is assumed to be totally permeable to solutes, i.e. the reflection coefficient is null. Only one form of carbohydrate is considered in the present case and other solutes are neglected. Carbohydrate unloading is supposed to be limited by an enzymatic process. At the collar of the root system, the carbohydrate concentration is imposed. The model allows the simulation of the carbohydrates distribution in the root system (Figure 10) and assess the assimilate concentration throughout the root system according to its topology and the root properties.

Large concentration gradients appear along the axis, especially for the branched roots with limited phloem transfer capacities. The carbohydrate concentration gradient is relatively low for the main axis compared to the branched roots. Along the main axis, presence of lateral roots lead to a large decrease in carbohydrate availability, due to carbohydrate unloading in lateral roots. The carbohydrate concentration is highly variable in the secondary root tips, depending on the location of the root insertion on the main axis as well as on the length of the secondary root; the longer the root, the lower the carbohydrate concentration. Furthermore, a steep decrease in carbohydrate concentration is observed at the root tips. This decrease results from high sink activity of the root tips due to a high metabolic and growth activity.

These examples clearly showed that we are now able to take into consideration the architecture-function interactions in models which can help us to analyse how the plant is functionning. The challenge for the future is probably to be able to provide to these models experimental data on roots conductances, phloem concentrations, ... etc, in order to test their underlying hypotheses.

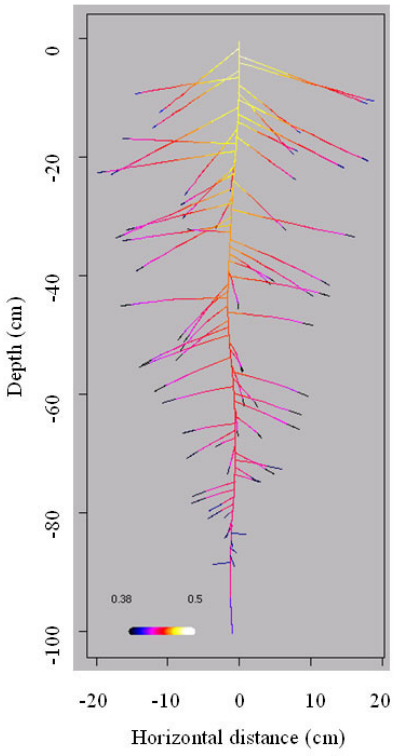


Figure 10. Simulated phloem carbohydrate concentration throughout a taprooted system 100 days old. Root collar carbohydrate concentration is fixed at 0.5 M, and the xylem water potential is assumed as uniform and null.

Effect of cultural practices

Most of the cultural practices are able to change the cabon partitioning within the plant. There are numerous papers showing that high levels of nutrient supply increase shoot growth relatively to root growth (Cannell, 1985). The root/shoot models predict that water stress would increase root growth relatively to shoot growth, which is actually what is observed in most cases (Cannell, 1985; Ericsson et al., 1996). Effect of pruning and fruit thinning are the two other important practices having a strong influence on carbon partitioning. The effect of both these practices will be analysed hereafter using structural-functional models.

Génard et al. (1998) studied the effect of pruning on carbon allocation, using a model simulating growth and development of peach trees during their first growing season. This model distinguishes four types of compartments: the rootstock, the main axis, the secondary axes and the new roots. Tree structure is described by the position of secondary axes on the main axis. The processes considered by the model are: (1) the plastochron activity of the main axis, which defines the time needed to produce a new metamer and its related ramification; (2) the mobilization of reserves and carbon acquisition for tree growth; and (3) the partitioning and use of carbon for maintenance and growth. The balance between root and shoot growth is managed using the principles of "coordination theory". If the shoot:root ratio is altered by pruning, it tends to be restored by compensatory growth through an alteration of the partitioning scheme. Growth correlations between the main and secondary axes are also considered in order to define the rules of assimilate partitioning.

The model, validated on experimental data, accounted for several previously reported pruning responses. For example, as classically observed the model predicts that pruning intensity and date of pruning have little effect on the seasonal carbon balance and consequently on the total dry weight at the end of the growing season. The model also predicts an increase in the growth of the axes remaining after pruning, tending to recreate a more functional root:shoot ratio which is well documented in the literature (Cannell, 1985). Pruning also increased the rhythmic growth of shoot and root in the model simulations as related by Atkinson (1980). More recently, a structural-functional model simulating walnut tree growth in response to pruning has been proposed by Balandier et al. (2000) for several year old trees.

These models concern trees without fruits and are not not usable to analyse the effect of management on trees bearing fruits. We are currently developing a "virtual tree" model describing carbon transfer within the plant between shoots bearing fruits, trunk and branches, and roots, which is usable for such an analysis. The model assumes that the plant is a set of shoot bearing fruits connected to each other by the branches. The carbon allocation between shoots bearing fruits and with the other parts of the plant is based both on source-sink concepts and a simplified version of the Munch transport theory. The leafy shoots have the first priority for growth. The balance between root and shoot growth is managed using the principles of "coordination theory". The physiological processes considered are the leaf and fruit photosynthesis, the respiration of all the plant organs, the carbon storage and remobilisation in leaves, branches-trunk and roots, the growth of the organs. The leaf photosynthesis is regulated by their reserve concentration.

Simulations were performed from 63 to 111 days after bloom, on a peach tree with two main branches, 20 shoots bearing fruits, 188 fruits and 119 shoots before pruning and thinning. The effect of summer pruning and fruit thinning was analysed considering an unpruned high loaded tree, an unpruned thinned tree (80% of fruits removed), a pruned (50% of leafy shoots removed) high loaded tree, and a pruned thinned tree (Figure 11).

The photosynthesis rate was the highest for high loaded trees. The pruned thinned tree exhibited also a high photosynthesis rate during the 10 first days of simulation, and a decreased rate when the tree recovered its foliage area. Pruning and thinning had both an effect on growth and reserve accumulation of the different organs. However, the effect was very different on sinks and source organs. The sink organs grew more and accumulated more reserves when the leaf:fruit ratio was greater. The shoots grew more when the tree was pruned and, for a given pruning intensity, they grew more when the tree was thinned. The effect of pruning on shoot growth can be interpreted according to the coordination theory. But why do shoots grow more after thinning when the model assumes they have a priority for carbon? The analysis of simulations shows that there is more carbon available for roots when the tree is thinned. The subsequent increase of root growth induces an increase of shoot sink strength due to the application of the "coordination" theory.

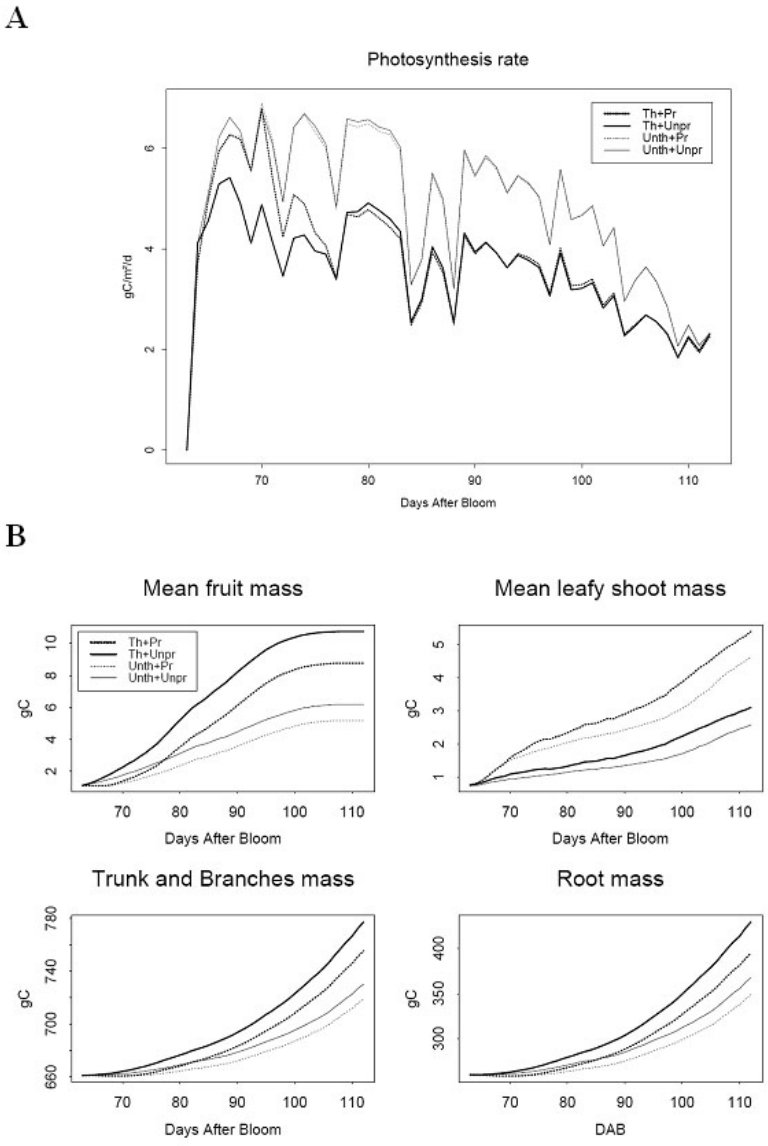


Figure 11. Peach leaf photosynthesis rate (A) and carbon partitioning between fruits, leaves, aerial wood and roots (B) as predicted by the virtual tree for different levels of fruit thinning and summer pruning. The treatments are unpruned unthinned tree (Unpr+Unth), unpruned thinned tree (80% of fruits were removed, Unpr+Th), a pruned (50% of leafy shoots were removed) unthinned tree (Pr+Unth), and a pruned thinned tree (Pr+Th).

In most of the published studies, the negative effect of high fruit load on shoot growth is interpreted as a direct competition between fruits and shoots (Hurd et al., 1979; Grossman and DeJong, 1995). Our simulation leads to an alternative interpretation of the fruit load effect on vegetative growth: the competition between fruits and roots for carbon decreases both the fruit and root growth, but the decrease of shoot growth only results from the decrease of root growth.

CONCLUSIONS

Carbon partitioning in plants is controled by a number of factors which include photosynthesis, the number and location of competing sinks, storage capacity and vascular tranport. Although there is considerable information on individual processes in plants such as photosynthesis, translocation and cell growth, it appears that the control actually regulating the carbon partitioning at the whole plant level are still poorly understood (Wardlaw, 1990; Le Roux et al., 2001). Indeed, many processes are closely interrelated and more integrative research work based on modelling approach is needed. One interesting way is to follow the teleonomic approach which assumes that the different processes are interrelated through a common goal. That approach can bring general macroscopic laws applicable to a large range of species (West et al., 1997 and 1999; Enquist, 2002) and is able to represent carbon partitioning strategies selected by evolutionary pressure. Another way is to integrate basic knowledge in source-sink and transport and chemical/biochemical models able to link the different processes. Such models are based on different theories corresponding to more or less accurate description of mechanisms. They allow to take into consideration the regulation of processes and their interactions. They are able to simulate complex allocation processes and could be useful tools to analyse the genetic variability (Quilot et al., 2005), and to interpret some genomic studies. With the increasing power of computers and informatic languages, it becomes possible to link plant architecture and function in functional-structural plant models which opens new avenues to link plant growth and development with environmental and management factors. It is clear that the body of knowledge on coffee tree is now important enough to undertake such approach. As far as multiple interactions link functions (carbon assimilation, transpiration, growth…) and plant organs, we need integrated simulation tools that interactively calculate:

1. the carbon assimilation throughout the plant canopy;
2. the transpiration throughout the plant canopy;
3. the sink demand for assimilates throughout the aerial and root systems;
4. the resulting assimilate flow throughout the phloem system;
5. the water flow and resulting water potential throughout the xylem system.

Points 1, 2 and 5 have been already addressed in coffee tree and will be presented in the following lectures.

REFERENCES

Allen M. T., Prusinkiewicz P., DeJong T. M. 2005. Using L-systems for modeling source-sink interactions, architecture and physiology of growing trees: the L-PEACH model. New Phytologist 166, 869-880.

Atkinson D. 1980. The distribution and effectiveness of the roots of tree crops. Hortic. Rev. 2, 424-490.

Balandier P., Lacointe A., Le Roux X., Sinoquet H., Cruiziat P., Le Dizes S. 2000. SIMWAL: A structural-functional model simulating single walnut tree growth in response to climate and pruning. Annals of Forest Science 57, 571-585.

Bassow S.L., E.D. F. 1990. A process based model of carbon translocation in trees: an exploration of the branch autonomy theory. Silva Carelica 15, 77-87.

BenMimoun M., Longuenesse J. J., Génard M. 1996. Pmax as related to leaf: Fruit ratio and fruit assimilate demand in peach. Journal of Horticultural Science 71, 767-775.

Bidel L. P. R., Pagès L., Riviere L. M., Pelloux G., Lorendeau J. Y. 2000. MassFlowDyn I: A carbon transport and partitioning model for root system architecture. Annals of Botany 85, 869-886.

Bosc A. 2000. EMILION, a tree functional-structural model: Presentation and first application to the analysis of branch carbon balance. Annals of Forest Science 57, 555-569.

Bruchou C., Génard M. 1999. A space-time model of carbon translocation along a shoot bearing fruits. Annals of Botany 84, 565-576.

Cannell M. G. R. 1971. Production and distribution of dry matter in trees of Coffea arabica L. in Kenya as affected by seasonal climatic differences and the presence of fruit. Ann. Appl. Biol. 67, 99–120.

Cannell M. G. R. 1985. Attributes of trees as crop plants. In Dry matter partitioning in tree crops. Eds Cannell, M.G.R. Jackson, J.E. National Environment Research Council, Penicuik, Great Britain. 160-193.

Chalmers D. J., Canterford R. L., Jerie P. H., Jones T. R., Ugalde T. D. 1975. Photosynthesis in relation to growth and distribution of fruit in peach trees. Australian Journal of Plant Physiology 2, 635-645.

Chang R. 2000. Physical chemistry for the chemical and biological sciences. University Science Books, Sausalito, CA. 1018 p.

Charles-Edwards D. A. 1976. Shoot and root activities during steady-state plant growth. Annals of Botany 40, 767-772.

Chen J., Reynolds J. F. 1997. A coordination model of whole-plant carbon allocation in relation to water stress. Annals of Botany 80, 45-55.

Chen J. L., Reynolds J. F. 1997. A coordination model of whole-plant carbon allocation in relation to water stress. Annals of Botany 80, 45-55.

Costes E. 2004. Integrating knowledge of tree biology and physiology into models of fruit tree development: a review. Acta Horticulturae 636, 575-589.

Dale J. E., Sutcliffe J. F. 1986. Water relations of plant cells. Plant Physiology, Vol 9, Water and Solutes in Plants 9, 1-48.

Daudet F. A., Lacointe A., Gaudillère J. P., Cruiziat P. 2002. Generalized Munch coupling between sugar and water fluxes for modelling carbon allocation as affected by water status. Journal of Theoretical Biology 214, 481-498.

Dauzat J., Rapidel B., Berger A., 2001. Simulation of leaf transpiration and sap flow in virtual plants: description of the model and application to a coffee plantation in Costa Rica. Agricultural and Forest Meteorology, 109: 143-160.

Davidson R. L. 1969. Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover. Annals of Botany 25, 59-104.

Enquist B. J. 2002. Universal scaling in tree and vascular plant allometry: Toward a general

quantitative theory linking plant form and function from cells to ecosystems. Tree Physiology 22, 1045-1064.

Ericsson T., Rytter L., Vapaavuori E. 1996. Physiology of carbon allocation in trees. Biomass & Bioenergy 11, 115-127.

Farquhar G.D., von Caemmerer S. & Berry J.A., 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta 149, 78-90.

Fishman S., Génard M. 1998. A biophysical model of fruit growth: simulation of seasonal and diurnal dynamics of mass. Plant, Cell and Environment 21, 739-752.

Ford E. D. 1992. The control of tree structure and productivity through the interaction of morphological development and physiological processes. International Journal of Plant Sciences 153, 147-162.

Ford R., Ford E. D. 1990. Structure and basic equations of a simulator for branch growth in the Pinaceae. J. Theor. Biol 146, 1-13.

Franck N., Vaast P., Génard M., Dauzat J. 2006. Soluble sugars mediate sink feedback down-regulation of leaf photosynthesis in field-grown Coffea arabica. Tree Physiology 26, 517-525.

Génard M., Baret F. 1994. Spatial and temporal variation of light inside peach trees. Journal of the American Society for Horticultural Science 119, 669-677.

Génard M., Lescourret F. 2004. Modelling fruit quality : ecophysiological, agronomical and ecological perspectives. Production practices and quality assessment of food crops, Vol. 1, "Preharvest practice". 47-82.

Génard M., Lescourret F., Gomez L., Habib R. 2003. Changes in fruit sugar concentrations in response to assimilate supply, metabolism and dilution: a modeling approach applied to peach fruit (Prunus persica). Tree Physiology 23, 373-385.

Génard M., Pagès L., Kervella J. 1998. A carbon balance model of peach tree growth and development for studying the pruning response. Tree Physiology 18, 351-362.

Génard M., Souty M. 1996. Modeling the peach sugar contents in relation to fruit growth. Journal of the American Society for Horticultural Science 121, 1122-1131.

Godin C., Sinoquet H. 2005. Functional-structural plant modelling. New Phytologist 166, 705-708.

Grange R. I., Peel A. J. 1978. Evidence for solution flow in the phloem of willow. Planta 138, 15-23.

Grechi I., Vivin P., Hilbert G., Milin S., Robert T., Gaudillère J.-P. 2006. Effect of light and nitrogen supply on internal C:N balance and control of root-to-shoot biomass allocation in grapevine. Environmental and Experimental Botany, in press.

Grossman Y. L., Dejong T. M. 1994. Peach - a Simulation-Model of Reproductive and Vegetative Growth in Peach-Trees. Tree Physiology 14, 329-345.

Grossman Y. L., Dejong T. M. 1995. Maximum Fruit-Growth Potential Following Resource Limitation During Peach Growth. Annals of Botany 75, 561-567.

Gucci R., Grappadelli L. C., Tustin S., Ravaglia G. 1995. The Effect of Defruiting at Different Stages of Fruit-Development on Leaf Photosynthesis of Golden-Delicious Apple. Tree Physiology 15, 35-40.

Havis A. L. 1962. Effects of time of fruit thinning of Redhaven peach. Proc. Am. Soc. Hort. Sci. 80, 172–176.

Hocking P. J. 1980. The composition of phloem exudate and xylem sap from tree tobacco (*Nicotiana glauca* Grah.). Annals of Botany 45, 633-643.

Hurd R. G., Gay A. P., Mountifield A. C. 1979. The effect of partial flower removal on the relation between root, shoot and fruit growth in the indeterminate tomato. Annals of Applied Biology 93,

Johnson D. S. 1995. Effect of flower and fruit thinning on the maturity of 'Cox's Orange Pippin' apples at harvest. J. Hort. Sci. 70, 541–548.

King D. A. 2005. Linking tree form, allocation and growth with an allometrically explicit model. Ecological Modelling 185, 77-91.

Lacointe A. 2000. Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. Annals of Forest Science 57, 521-533.

Léchaudel M., Génard M., Lescourret F., Urban L., Jannoyer M. 2002. Leaf-to-fruit ratio affects water and dry-matter content of mango fruit. Journal of Horticultural Science and Biotechnology 77, 773-777.

Léchaudel M., Génard M., Lescourret F., Urban L., Jannoyer M. 2005. Modelling effects of weather and source-sink relationships on mango fruit growth. Tree Physiology 25, 583-597.

LeRoux X. l., Lacointe A., Escobar-Gutierrez A., Dizes S. l. 2001. Carbon-based models of individual tree growth: a critical appraisal. Annals of Forest Science 58, 469-506.

Lescourret F., Ben-Mimoun M., Génard M. 1998. A simulation model of growth at the shoot-bearing fruit level. I. Description and parameterization for peach. European Journal of Agronomy 9, 173-188.

Lescourret F., Génard M. 2005. A virtual peach fruit model simulating changes in fruit quality during the final stage of fruit growth. Tree Physiology 25, 1303-1315.

Lescourret F., Génard M., Habib R., Fishman S. 2001. Variation in surface conductance to water vapor diffusion in peach fruit and its effects on fruit growth assessed by a simulation model. Tree Physiology 21, 735-741.

Marcelis L. F. M. 1993. Effects of assimilate supply on the growth of individual cucumber fruits. Physiol. Plant. 87, 313–320.

Marcelis L. F. M. 1996. Simulation of biomass allocation in greenhouse crops: a review. Acta Hort. 328, 49–67.

McMahon T. A., Kronauer R. E. 1976. Tree structures: deducting the principle of mechanical design. J. Theor. Biol. 59, 443-466.

Minchin P. E. H., Thorpe M. R., Farrar J. F. 1993. A Simple Mechanistic Model of Phloem Transport Which Explains Sink Priority. Journal of Experimental Botany 44, 947-955.

Niklas K. J., Enquist B. J. 2003. An allometric model for seed plant reproduction. Evolutionary Ecology Research 5, 79-88.

Proebsting E. L., Mills H. H. 1981. Effects of season and crop load on maturity characteristics of 'Bing' cherry. J. Am. Soc. Hort. Sci. 106, 144–146.

Quilot B., Génard M., Kervella J., Lescourret F. 2002. Ecophysiological analysis of genotypic variation in peach fruit growth. Journal of Experimental Botany 53, 1613-1625.

Quilot B., Génard M., Lescourret F., Kervella J. 2005. Simulating genotypic variation of fruit quality in an advanced peach x Prunus davidiana cross. Journal of Experimental Botany 56, 3071-3081.

Reynolds J. F., Chen J. L. 1996. Modelling whole-plant allocation in relation to carbon and nitrogen supply: Coordination versus optimization: Opinion. Plant and Soil 185, 65-74.

Reynolds J. F., Thornley J. H. M. 1982. A shoot:root partitioning model. Annals of Botany 49, 585-597.

Sharkey P. J., Pate J. S. 1976. Translocation from leaves to fruits of a legume, studied by a phloem bleeding technique: diurnal changes and effects of continuous darkness. Planta 128, 63-72.

Shinozaki K., Yoda K., Hozumi K., Kira T. 1964. A quantitative analysis of plant form. The pipe model theory. II. further evidence of the theory and its application in forest ecology. Japanese Journal of Ecology 14, 133-139.

Sievanen R., Nikinmaa E., Nygren P., Ozier-Lafontaine H., Perttunen J., Hakula H. 2000. Components of functional-structural tree models. Annals of Forest Science 57, 399-412.

Sinoquet H., Le Roux X., Adam B., Ameglio T. and Daudet A., 2001. RATP: a model for simulating the spatial distribution of radiation absorption, transpiration and photosynthesis within canopies: application to an isolated tree crown. Plant, Cell and Environment, 24, 395-406.

Thornley J. H. M. 1972. A model to describe the partitioning of photosynthate during vegetative plant growth. Annals of Botany 36, 419-430.

Thornley J. H. M. 1998. Modelling shoot:root relations: the only way forward? Annals of Botany 81, 165-171.

Thornley J. H. M., Johnson I. R. 1990. Plant and crop modelling. Oxford University Press, Oxford, 669 p.

Upmeyer D. J., Koller H. R. 1973. Diurnal trends in net photosynthetic rate and carbohydrate levels of soybean leaves. Plant Physiology 51, 871-874.

Vaast P., Angrand J., Franck N., Dauzat J., Génard M. 2005. Fruit load and branch ring-barking affect carbon allocation and photosynthesis of leaf and fruit of *Coffea arabica* in the field. Tree Physiology 25, 753-760.

Vaast P., Dauzat J., Génard M. 2002. Modeling the effects of fruit load, shade and plant water status on coffee berry growth and carbon partitioning at the branch level. Acta Horticulturae 584, 57-62

Wardlaw I. F. 1990. Tansley Review No. 27. The control of carbon partitioning in plants. New Phytologist 116, 341-381.

West G. B., Brown J. H., Enquist B. J. 1997. A general model for the origin of allometric scaling laws in biology. Science 276, 122-126.

West G. B., Brown J. H., Enquist B. J. 1999. A general model for the structure and allometry of plant vascular systems. Nature (London) 400, 664-667.

Zimmermann M. 1957. Translocation of organic substances in trees. II on the translocation mechanism in the phloem of white ash (*Fraxinus americana* L.). Plant Physiology 32, 399-404.

Coffee a Shade-Adapted Plant: Implications on its Carbon Balance and Consequences on Coffee Yield and Quality in Agroforestry Systems

N. FRANCK[1], P. VAAST[2], J. DAUZAT[3]

[1]Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Correo Central, Santiago, Chile
[2]Département des Cultures Pérennes, Centre de Coopération International en Recherche Agronomique pour le Développement (CIRAD), CATIE 7170, Apdo. 3, Turrialba, Costa Rica
[3]Département d'Amélioration des Méthodes pour l'Innovation Scientifique, CIRAD, TA40/PS2, bd. de la Lironde, 34398 Montpellier Cedex 5, France

SUMMARY

Coffee has been classified as a shade-adapted plant species and exhibits typical features of such species: (i) acclimation of leaves in order to photosynthesize in low light, (ii) high leaf area: woody structure ratio and (iii) absence of a self-thinning mechanism that regulates fruit load. These features are of key importance for understanding the acclimation of coffee plants to the shade environment imposed by agroforestry systems because they affect the plant carbon balance, the basis of coffee yield and quality. A trial was established in a commercial coffee plantation under the optimal coffee cultivation conditions of the Orosi valley in Costa Rica by applying four shading regimes and four fruit load levels within each shading regime. In parallel, high and low fruit load treatments were established on girdled coffee branches. Gas exchange and chlorophyll fluorescence measurements, biometrical and biochemical measurements were performed and used to (i) parameterize a leaf carbon assimilation (*A*) model and (ii) calculate the carbon balance at the branch level. Our results showed that coffee leaves acclimated to efficiently photosynthesize under very low light in shading treatments. It was demonstrated that the light-saturated *A* was reduced in case of low fruit demand and that this reduction was related to the accumulation of photosynthetic products in the leaves. Because the flower initiation was reduced by shade, this effect was more important in shaded treatments. On the other hand, full sun grown coffee had a high carbon demand by fruits which resulted in a carbon shortage leading to reduced bean size and vegetative growth, thereby hampering the flowering and production potentials for the next season.

INTRODUCTION

Coffee (*Coffea arabica* L.) cultivation in agroforestry systems is regaining popularity (Beer et al., 1997) and shade cast by associated trees strongly alters the plant carbon balance through its effects on vegetative growth, leaf morphology, flower induction and resulting fruit setting (Cannell, 1971; Da Matta, 2004; Vaast et al., 2005). The fact that coffee exhibits typical features of a shade plant (acclimation of leaves in order to photosynthesize in low light, high leaf area: woody structure ratio and the absence of a self-thinning mechanism that regulates fruit load, among other) should be taken into account in order to analyze its agronomic performance in agroforestry systems. Actually, these typical shade plant features have a direct effect on the carbon balance of the plant and may directly act on coffee growth, fruit yield and bean size under different shade levels. The carbon balance of a plant is the result of the activities of carbon source organs (i.e. carbon assimilation (*A*), mainly by leaves and, to a

lesser extent, by fruits), and carbon sink organs (i.e. carbon fixation in biomass, accumulation as reserves and consumption by respiration of plant organs). Concerning coffee *A*, it has been shown that when the carbon demand by sink organs, especially fruits, is low, *A* is down-regulated (Cannell, 1971; Vaast et al., 2005a). This feature is known as source:sink down-regulation of photosynthesis. Other factors which have been shown to restrict coffee *A* are stomatal conductance (g_s) (Nunes, 1988), especially under hot and dry conditions (Da Matta, 2004), and photoinhibition (PI) (Nunes et al., 1993). Regarding carbon allocation to different organs of coffee, a high fruit load implies high demand for carbon which induces lower vegetative growth and even branch mortality (Cannell, 1971). Under these conditions, the amount of new fructiferous nodes is reduced and the fruit production for the next season is hampered (Cannell, 1971).

In order to study how different shading rates and fruit loads affect coffee carbon assimilation and allocation, we established a trial on a commercial farm in the Orosi Valley of Costa Rica in which the coffee plants were submitted to four growth irradiance (GI) levels and four fruit load levels (FL). Gas exchange and chlorophyll fluorescence measurements were performed in order to parameterize an *A* model for coffee leaves. Additionally, growth dynamic and carbon content of the different plant organs were followed in order to estimate carbon demand and allocation. The effect of coffee shade acclimation on the different components of the carbon balance and their relationship to coffee performance in agroforestry systems are discussed.

MATERIALS AND METHODS

Experimental site and plant material

Measurements were performed on coffee plants (*Coffea arabica* L.) of the highly productive, dwarf cv. 'Caturra' in a homogenous commercial orchard in the Orosi valley of Costa Rica (9.79 N, 83.82 W; 1108 m above sea level) planted in 1999 on an Inceptisol. The coffee plants were in their second (2003) and third (2004) production cycles at a 1 x 2 m spacing with east-west oriented rows.

Growth irradiance and fruit load treatments

Four growth irradiance (GI) treatments were established in December 2002. Each treatment included eight coffee rows of 10 plants each. Treatments consisted in full solar irradiance (GI_{100}) and three shade treatments with 75% (GI_{75}), 50% (GI_{50}), and 25% (GI_{25}) of the full solar irradiance. GI treatments were achieved by constructing shade houses covered with black shade screens with the required light transmittances. Within each GI treatment, four fruit load treatments (full fruit load [FL_{100}], 50% [FL_{50}], 25% [FL_{25}] and 5% [FL_5]) were established in September 2002. Each FL treatment was applied on eight plants per GI treatment.

Carbon assimilation model

For modelling the intrinsic photosynthetic capacity of coffee leaves we used the model developed by (Farquhar et al., 1980) as modified by (Harley and Tenhunen, 1991). This model is based on biochemical limitations to carbon assimilation which relies on incident photon flux density (PFD) and mesophyll CO_2 concentration (C_i). The limitations to *A* imposed by g_s were modelled following the approach of (Ball et al., 1987) who presented an empirical relationship which incorporates the often observed correlation between *A* and g_s (Leuning, 1995) and includes the effects of air humidity (HR) and ambient CO_2 concentration.

We used this model as modified by (Leuning, 1995) and coupled it to the A model hereby allowing the simultaneous estimation of A and g_s. To include the limitation to A caused by PI we adjusted the model proposed by (Ögren, 1991) which can be derived from leaf fluorescence and gas exchange measurements and allows estimating the effect of PI on two parameters of the (Farquhar et al., 1980) model as a function of incident PFD integrated over a given period.

Determination of the parameters of the photosynthesis sub-model

A and g_s were measured with a CO_2/H_2O infrared gas analyser (LCPro, ADC BioScientific Ltd., Hoddesdon, U.K.) connected to a broadleaf chamber and with automatic control of leaf temperature, PFD and CO_2 and H_2O concentrations. For the parameterisation of the photosynthesis model, light response curves and C_i response curves were performed on fully developed exposed leaves (3^{rd} to 6^{th} pair of leaves from the branch tip) sampled on plagiotropic branches at mid-height in the crown of plants in the four GI treatments at a reference leaf temperature (T_l) of 25 °C.

Determination of the parameters of the stomatal conductance sub-model

For the g_s sub-model, spot measurements of A and g_s were performed with the infrared gas analyser in the four GI treatments during the months of June and July 2004 under current PFD, T_l, and CO_2 and H_2O concentrations. In each GI treatment, measurements were performed at three periods of the day (5:30-9:30; 11:30-14:00; and 15:00-17:30) on eight fully-exposed mature leaves. The following variables were simultaneously recorded for each measurement: PFD, T_l, HR, C_i, g_s and A.

Determination of the parameters of the photoinhibition sub-model

For the four GI treatments, chlorophyll fluorescence measurements were performed on horizontal leaves, fully exposed to solar irradiance during the whole day, on girdled branches with a minimal fruit load of 12 fruits per leaf in order to restrict the probability of sampling sink-limited leaves (Vaast et al., 2005a). The ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) was measured on leaves that were previously dark adapted for a period of ~25 minutes with a portable, pulse-modulated fluorometer (FMS2, Hansatech Instruments Ltd., Pentney, Norfolk, UK).

Source: sink feedback trial

High (10 Fruit/leaf) and low (1 Fr/l) treatments were established on girdled coffee branches. Three month later, maximal A (A_{max}) was measured at 1056 $\mu mol\ m^{-2}\ s^{-1}$ PFD on leaves which were then collected and lyophilised together with the twigs. Carbohydrate contents were measured in all sampled leaves and twigs: glucose, fructose and sucrose were determined using high performance liquid chromatography (HPLC) with ionic separation (DIONEX Co., Sunnyval, CA, USA) and starch was analysed on the residual pellet after sugar extraction by hydrolysis with amyloglucosidase (Robinson et al., 1988) and its concentration was determined enzymatically (Bergmeyer et al., 1984).

Biometrical and tissue chemical composition measurements

On each of the eight plants of the GI x FL treatments, three plagiotropic branches (A_1) were selected at different heights on the plant (high: 10^{th}-14^{th} node from the top [H]; middle:

22^{nd}-26^{th} node [*M*]; and low: 34^{th}-38^{th} node [*L*]). Biometrical measurements on these branches were performed at a monthly interval from May 2003 to January 2004.
Measurements included a full description of primary (A_2) and secondary (A_3) ramifications in terms of length, diameter and number of nodes, fruits and leaves. Length and width of all leaves were measured. Allometrical functions were adjusted in order to estimate leaf area from their length and width and twig dry weight from their number of internodes. Leaf mass to area ratio (M_A) was estimated by dividing the dry weight of each leaf sample by its corresponding estimated leaf area. Potential fruit growth was estimated by adjusting a logistic equation to fruit dry weight measurements performed at each measurement date. Carbon, nitrogen and ash content of the different organs of the branches were measured with automatic element analyser (Flash EA 1112, Thermo Finnigan Italia S.P.A., Rodano-Milano, Italy) and used to estimate growth respiration (RG) by means of the equation proposed by (Vertregt and Penning de Vries, 1987) as modified by (Penning de Vries et al., 1989). Maintenance respiration (RM) was estimated following (Génard et al., 1998).

RESULTS

Carbon assimilation model and it's relationship with leaf mass to area ratio

M_A linearly decreased with decreasing GI from 153.3 ± 5.7 at GI_{100} to 94.3 ± 2.5 at GI_{25}. The adjusted leaf carbon assimilation model revealed that all parameters could be directly or indirectly related to M_A on a 1:1 basis, at the exception of the parameters of the PI submodel which were related to M_A by logarithmic equations (Table 1). This allowed expressing the parameters of the model as a function of M_A hereby enabling the use a single set of parameter for all of the GI treatments. The performance of the model was tested for two contrasting days (cloudy and sunny) and showed a good agreement with observed *A* but with a tendency to overestimate *A* as the afternoon proceeded, which was more marked with decreasing GI (Figure 1). Taking PI into account increased the predictions of the model, especially for higher GI treatments (Figure 1).

Table 1. Equations for calculating the parameters of the carbon assimilation model as a function leaf mass to area ratio (M_A). M_A^{ref}: reference M_A.

Submodel	Parameter	Function used	R^2
A	α_c	$\alpha_c = 0.62 M_A^{ref}/M_A$	0.94
	P_{ml}	$P_{ml} = 31.22 M_A/M_A^{ref}$	0.67
	Vc_{max}	$Vc_{max} = 57.78 M_A/M_A^{ref}$	0.92
	R_d	$R_d = 0.844 M_A/M_A^{ref}$	0.60
g_s	a_1	$a_1 = 31.05 M_A^{ref}/M_A$	0.91
PI	*m*	$m = 0.0006 \mathrm{Ln}(M_A) - 0.0035$	0.83
	L_{fluxB}	$L_{fluxB} = 212.95 \mathrm{Ln}(M_A) - 820.06$	0.43

Parameters: photosynthesis (A): maximum quantum yield (α_c), CO_2- and light-saturated rate of A (P_{ml}), maximum carboxylation rate (Vc_{max}), day respiration (R_d); stomatal conductance (g_s): coefficient of g_s sensibility to A, intercellular CO_2 concentration and vapour pressure deficit (a_1); photoinhibition (PI): coefficient PI sensibility to photon flux density (m) and light PI threshold (L_{fluxB}).

Effect of treatments on the branch carbon balance

Shade had a strong significant effect on flowering intensity and consequent fruit load with fruit set levels of 4620 ± 821 (fruits per plant) for GI_{100}, 3052 ± 525 for GI_{75}, 1500 ± 183 for GI_{50} and 605 ± 139 for GI_{25}. The logistic growth functions adjusted to the seasonal evolution

of fruit dry weight (data not shown) were used to estimate potential carbon demand by fruits (ΨCD_{Fr}) by adding the carbon fixed by the fruits (i.e. fruit dry weight x fruit carbon content) and the estimations of carbon consumed by RG and RM (Figure 2). Then, an indicator of the source:sink relationship (Si/So) of each branch and measurement date was calculated by relating the estimated ΨCD_{Fr} multiplied by the total amount of fruits on each branch (N_{Fr}) to the total leaf area of each branch (LA_{Br}; $Si/So = [\Psi CD_{Fr} \times N_{Fr}]/LA_{Br}$). Different plant growth traits were related to Si/So showing that, independently of GI treatment, leaf abscission linearly increased with increasing Si/So for *H* and *M* branches whereas, for these same branches, leaf initiation rate linearly decreased with increasing Si/So (Figure 3ab). No relationship between these traits and Si/So was found for *L* branches and no relationship between Si/So and fruit drop was found for any branch height (Figure 3a-c). The analysis for the entire season showed that the mean initiation rate of leaves, nodes and ramifications decreased with increasing Si/So, whereas, the incidence of dieback increased with increasing Si/So (Figure 3d-g). On the other hand, yield increased whereas the proportion of big beans and the floral intensity of the following season decreased with increasing Si/So (Figure 3h-k). Concerning these traits, shade had a beneficial effect on yield and the proportion of big beans and a negative effect on flowering intensity of the following season (Figure 3h-k).

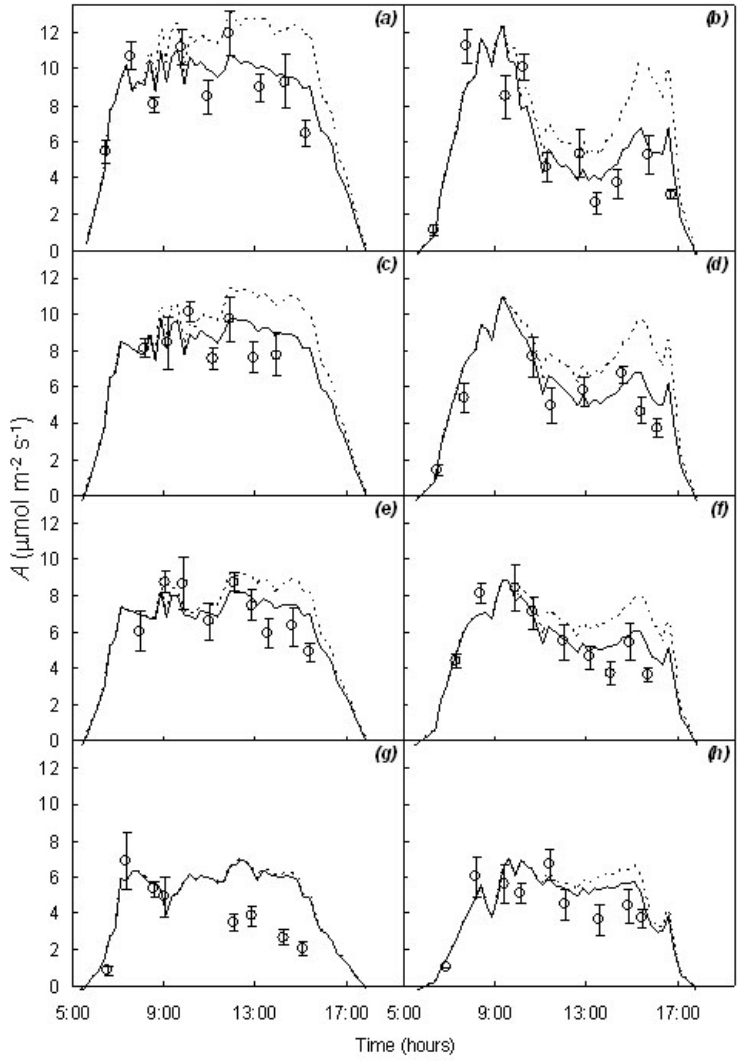


Figure 1. Measured and predicted daily evolution of net carbon assimilation rate (*A*) with (full lines) and without (broken lines) accounting for the effect of photoinhibition during two contrasting days (cloudy day: a, c, e, g; sunny day: b, d, f, h) for the different growth irradiance (GI) treatments (GI_{100}: ab; GI_{25}: cd; GI_{50}: ef; GI_{25}: gh).

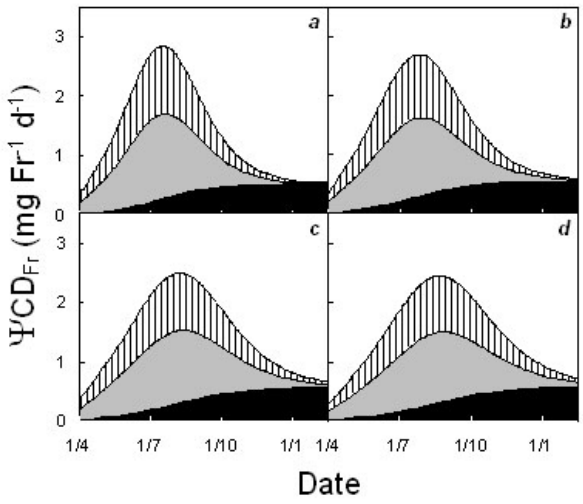


Figure 2. Estimated potential carbon demand by fruits (ΨCD_{Fr}) as a function of time for the different growth irradiance (GI) treatments: GI_{100} (a), GI_{75} (b), GI_{50} (c) and GI_{25} (d). Vertical lines: fixed carbon, grey: growth respiration and black: maintenance respiration.

Source:sink down-regulation of carbon assimilation

The results of the experiment on girdled coffee branches shows that low sink demand induced a significant increment of sucrose, soluble sugar (SS) and starch content in twigs and leaves and an increased fruit dry weight whereas A_{max} was reduced (Table 2). Furthermore, a negative relationship between A_{max} and leaf soluble sugar content could be established (Figure 4a) and this response was mirrored when the mean estimated seasonal branch carbon assimilation (A_{Br}) was expressed as a function of Si/So, showing an increment of A_{Br} with increasing Si/So (Figure 4b).

Table 2. Total non structural carbohydrates (TNC) in twigs and fruit dry weight (DW_{Fr}) at the onset (initial) and the end (final) of the source: sink feedback trial; starch, sucrose, soluble sugar (SS) and maximal photosyntheic rate (A_{max}) of leaves in the morning and at noon for high (H_{FL}) and low (L_{FL}) fruit load treatments of girdled coffee branches. Different letters on a same line indicate statistical significant differences between treatments (Bonferroni, $\alpha = 0.05$).

Organ	Trait	Initial		Final	
				H_{FL}	L_{FL}
Twig	TNC (mg g^{-1})	14.9 ± 1.2b		0.5 ± 0.1c	89.8 ± 7.1a
Fruit	DW_{Fr} (g $fruit^{-1}$)	0.23 ± 0.02c		0.32 ± 0.01b	0.40 ± 0.01a
		Morning		Noon	
		H_{FL}	L_{FL}	H_{FL}	L_{FL}
Leaves	Starch (mg g^{-1})	7.6 ± 6.5c	163.8 ± 57.9ab	22.2 ± 14.6c	189.1 ± 59.5a
	Sucrose (mg g^{-1})	37.1 ± 2.5ef	56.3 ± 4ab	46.6 ± 9cd	63.8 ± 3.6a
	SS (mg g^{-1})	43.8 ± 4.2de	62.8 ± 4.1a	54.1 ± 8.5bc	69.6 ± 4.5a
	A_{max} (μmol m^{-2} s^{-1})	9,54 ± 1,31a	5,55 ± 1,61c	7,24 ± 1,68b	2,89 ± 0,76d

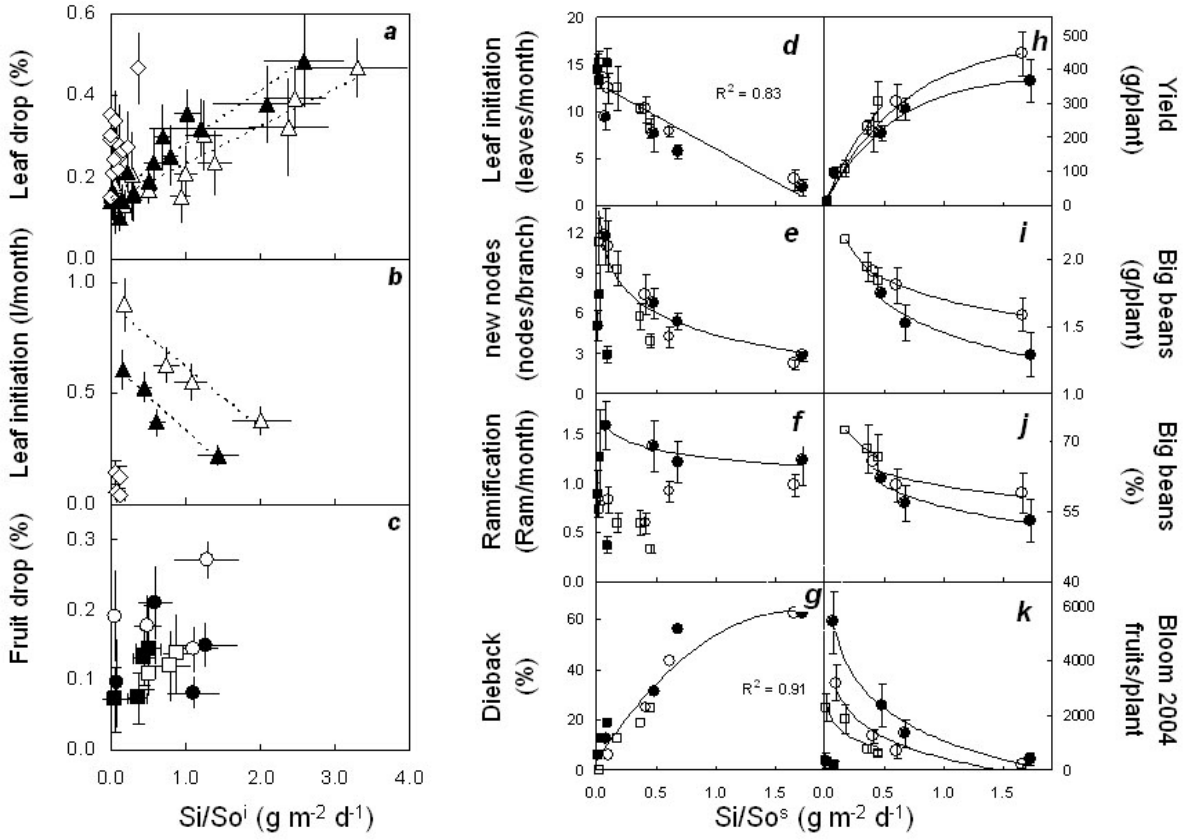


Figure 3. Some growth traits as a function of the indicator of the source:sink ratio estimated for each measurement date (Si/So^i; a-c) and seasonal mean estimations (Si/So^s; d-k). a-c: high (△), middle (▲) and low (◊) branches; d-k: GI_{100} (●), GI_{75} (○), GI_{50} (□) and GI_{25} (■).

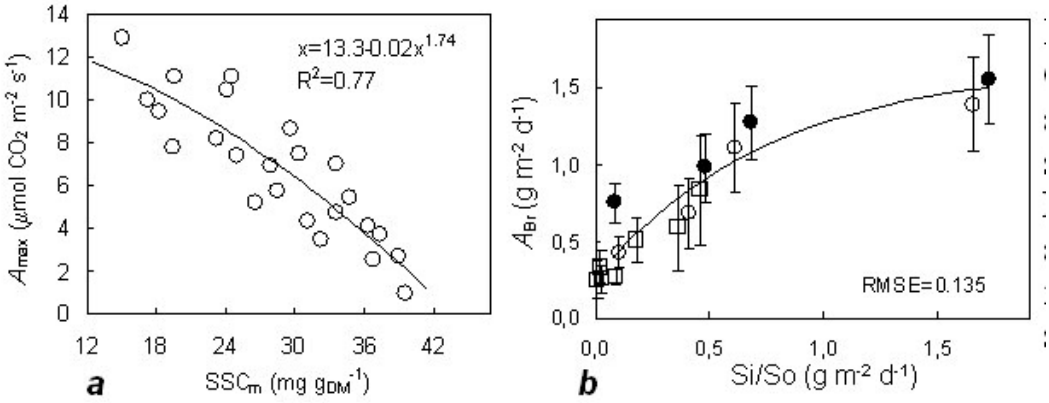


Figure 4. A: Maximum leaf CO_2 assimilation (A_{max}) rate as a function of leaf soluble sugar content (SSC_m). B: Estimated branch A (A_{Br}) as a function of the source:sink indicator (Si/So); same symbols as in Figure 3d-k.

DISCUSSION

The results obtained with the leaf A model confirm the acclimation of coffee leaf A to shade showing that, even at GI levels of 25%, the carbon assimilation of coffee leaves is only slightly reduced as compared to full sun owing to (i) an increased quantum use efficiency (ii) a more efficient investment of dry matter in leaf area (i.e. lower M_A), (iii) a lower limitation by g_s and PI due to lower leaf to air VPD (Nunes, 1988) and lower incident PFD (Nunes et al., 1993), respectively (Figure 1 and Table 1). Concerning the carbon balance, we confirm the

hypothesis enounced by (Cannell, 1971), indicating that coffee lacks a self-thinning mechanism in response to high fruit loads achieved in full sun (Figure 3c). This leads to very high carbon demand by fruits which results in a decreased vegetative growth, an increased leaf drop and an incremented incidence of branch dieback, which all negatively affects return to bloom (Figure 3). With regard to yield, high carbon demand by fruits reduces the proportion of big beans but increases yield. Shade appears to have a beneficial effect on bean size and yield whilst reducing potential yield owing to a negative effect on bloom intensity (Figure 3h-k).

Our results, both at the leaf level and the branch level, confirm a source:sink down-regulation of carbon assimilation which is mediated by an increasing accumulation of carbohydrates in the leaves when sink demand for carbon decreases (Figure 4). Even under high fruit load conditions, carbohydrates accumulate in coffee leaves inducing a reduction of *A* towards the afternoon (Table 2). This feature could explain why the *A* model overestimated *A* in the afternoon (Figure 1) as it does not include down-regulation by carbohydrate accumulation in leaves. Moreover, the observed decrease in fruit load by shade might be at the origin of the increasing overestimation of *A* with decreasing GI levels (Figure 1).

Taken together, our results indicate that shade plant characteristics severely alter coffee plants carbon balance in agroforestry systems. As the woody structure (i.e. carbon storage capacity) of the coffee plant is rather small, insufficient carbon can be stored during periods of low fruit carbon demand or when fruit load is low (Table 2). Consequently, in shading treatments where fruit load is low, carbon surpluses rapidly accumulate in leaves inducing a down-regulation of photosynthesis (Table 2, Figure 4). On the other hand, when plants are grown in full sun, the absence of a self-thinning mechanism leads to excessive fruit load which rapidly consumes the insufficient reserves and then hampers vegetative growth and bean size. This further leads to alternate bearing cycles. The fact that under high fruit loads, despite a reduction of fruit size, an accumulation of soluble sugars in the leaves is observed, indicates that sugars are not readily translocated from leaves to fruits. Based on our results we propose that future studies dealing with increasing the storage capacity and the translocation rate from source organs to sink organs, as well as increasing floral induction in agroforestry systems, may improve coffee yield and bean size. Regarding leaf photosynthetic capacity, the present *A* model reveals beneficial effects of shade by decreasing photoinhibitory and stomatal restrictions to photosynthesis, the latter showing a higher effect.

ACKNOWLEDGMENTS

The authors would like to thank: Mr. Ricardo Falla from 'Finca las Chúcaras' and the European Commission (ICA-4-CT-2001-10071) for financial support.

REFERENCES

Beer J, Muschler RG, Kass D et Somarriba E. 1997. Shade management in coffee and cacao plantations. *Agroforestry Systems* 38, 139-164.

Cannell MGR. 1971. Effects of fruiting, defoliation and ring-barking on the accumulation and distribution of dry matter in branches of *Coffea arabica* L. in Kenya. *Experimental Agriculture* 7, 63-74.

Da Matta FM. 2004. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. *Field Crops Research* 86, 99-114.

Vaast P, van Kanten R, Siles P, Angrand J et Aguilar A. 2005. Biophysical interactions between timber trees and Arabica coffee in suboptimal conditions of Central America. *Agroforestry Systems* In press.

Vaast P, Angrand J, Franck N, Dauzat J et Génard M. 2005a. Fruit load and branch ring-barking affect carbon allocation and photosynthesis of leaf and fruit of *Coffea arabica* in the field. *Tree Physiology* 25, 753-760.

Nunes MA. 1988. Environmental effects on the stomatal and mesophyll regulation of photosynthesis in coffee leaves. *Photosynthetica* 22, 547-553.

Nunes MA, Ramalho JC et Dias M. 1993. Effect of nitrogen supply on the photosynthetic performance of leaves from coffee plants exposed to bright light. *Journal of Experimental Botany* 44, 893-899.

Farquhar G, von Caemmerer S et Berry J. 1980. A biochemical model of photosynthetic CO_2 assimilation of C_3 species. *Planta* 149, 78-90.

Harley P et Tenhunen J. 1991. Modelling the photosynthetic response of C_3 leaves to environmental factors. In: *Modelling crop photosynthesis: from biochemistry to canopy* (eds K. Boote & R. Loomis), pp. 17-39. Special Publication of the Crop Science Society of America, Madison, Wisconsin.

Ball J, Woodrow I et Berry J. 1987 *A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions*. Paper presented at the VII international congress on photosynthesis, Dordrecht, 221-224. (Leuning 1995)

Leuning R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants. *Plant, Cell and Environment* 18, 339-355.

Ögren E. 1991. Prediction of photoinhibition of photosynthesis from measurements of fluorescence quenching components. *Planta* 184, 538-544.

Robinson N, Hewitt J et Bennett A. 1988. Sink metabolism in tomato fruit. I Developmental changes in carbohydrate metabolizing enzymes. *Plant Physiology* 87, 727-730.

Bergmeyer H, Bernt E, Schmidt F et Stork H. 1984. D-Glucose determination with hexokinase and glucose-6-phosphate dehydrogenase. In: *Methods of enzymatic analysis* (ed H. Bergmeyer), pp. 1196-1201. Academic Press, New York.

Vertregt N et Penning de Vries F. 1987. A rapid method for determining the efficiency of biosynthesis of plant biomass. *Journal of Theoretical Biology* 128, 109-119.

Penning de Vries F, Jansen D, ten Berge H et Bakema A. 1989. *Simulation of ecophysiological processes of growth in several annual crops*. Pudoc, Wageningen.

Génard M, Lescourret F, Ben-Mimoun M, Besset J et Bussi C. 1998. A simulation model of growth at the shoot-bearing fruit level. II. Test and effect of source and sink factors in the case of peach. *European Journal of Agronomy* 9, 189-202.

Different Drought Adaptation Strategies of *Coffea arabica* Populations Along a Rainfall Gradient in Ethiopia

J. BURKHARDT[1], T. KUFA[2], A. BEINING[2], H. E. GOLDBACH[1], M. FETENE[3]

[1]University of Bonn, INRES – Plant Nutrition, Karlrobert-Kreiten-Str. 13, D-53115 Bonn, Germany
[2]Centre of Development Research, University of Bonn, Germany
[3]Addis Abeba University, Biology Department, Ethiopia

SUMMARY

We compared the water use of four wild *Coffea arabica* populations along a rainfall gradient in Ethiopia. While the driest site, Harenna, lies east of the Rift valley, the three moister sites are situated in the southwestern part of Ethiopia. Measurements were carried out *in situ*, as well as in an extended *ex situ* experiment, where seeds of the four original sites were used to raise seedlings. Measurements tackled all relevant parts of water transport, i.e. soil conditions, the hydraulic conductivity of the root and the shoot system, stomatal control of gas exchange, and the atmospheric demand for water vapour. Water use efficiency *in situ*, measured by isotopes as well as by gas exchange, was found to be higher in the dry than in the wet season, and on dry sites compared to wet sites, thus reflecting the availability of water. Unexpectedly, no correlation with the rainfall gradient was observed neither when measuring the hydraulic system *in situ*, nor when looking at the reaction of seedlings to drought and radiation stress under *ex situ* conditions. Plants from the driest site, Harenna, showed highest transpiration and production. While the root system of Harenna trees was the most extensive one compared to the other sites, their hydraulic system also showed the highest efficiency for water transport, and stomatal behaviour was liberal. As a consequence these plants were the most vulnerable ones to drought stress, and eventually they were the first of all to be damaged and to die during an extended drying period. Plants from the wettest site were much more conservative in terms of water use and withstood much longer against drought stress, at the same time having lower productivity. The results showed that the precipitation gradient was not reflected in a simple way by drought stress tolerance of trees. It can be concluded that populations follow different strategies when conserving or spending water under drought stress conditions. Modern coffee varieties have a very narrow genetic basis, likely to based on genotypes from east of the Rift valley (Montagnon and Bouharmont, 1996). The present results confirm that genotypes west of the Rift Valley are highly valuable for enriching the genetic basis of cultivated *C. arabica* germplasm.

INTRODUCTION

Ethiopa is the center of genetic diversity of *Coffea arabica*. Wild coffee populations colonize most potential and marginal sites along the climatic gradients of the major coffee growing areas with immense ecophysiological diversity in terms of adaptation to biotic and abiotic stresses. However, information on the mechanisms underlying the adaptation of coffee plants to specific environmental stress, including drought stress, is lacking. In this study, four Afromontane rainforests with the occurrence of wild coffee populations spanning a broad climatic gradient were selected.

Ths study was part of the CoCE project (Conservation and use of wild populations of *Coffea arabica* in the montane rainforests of Ethiopia), assessing the diversity and the economic value of the Ethiopian coffee gene pool and to develop concepts of model character for conservation and use of the genetic resources. Thereby it focuses on traits inherent to the wild coffee populations and their usefulness for breeders. The aim was to characterize the water use of Arabica coffee by measuring gas exchange, hydraulic properties and water potentials to achieve an ecophysiological analysis as a basis for conservation and use of wild coffee populations in Ethiopia. The results could support the design of forest resource management concepts or transfer ecophysiologically desired plant traits through breeding programs. If coffee populations or accessions with outstanding drought adaptability in terms of growth architecture and hydraulic conditions could be identified, this would be an important argument to preserve and use the wild Arabica coffee populations by the protection of the respective montane rainforests of Ethiopia.

It was hypothesized that a rainfall gradient would promote regional differentiation in ecophysiological traits that would allow the identification of drought-tolerant coffee populations. In this regard, there are many indications of genetically based traits in the coffee plants for adaptation to drought stress. The hydraulic conductivity of the roots and shoots are among the key ecophysiological factors for identifying drought-tolerant coffee cultivars. This study was directed towards identification of some of the most important functional traits and the underlying mechanisms for coping with environmental stress in wild coffee populations.

METHODS

Measurements were carried out *in situ*, as well as in an extended *ex situ* experiment, where seeds of the four original sites were used to raise seedlings. *In-situ*-investigations were made within four rainforests along a rainfall gradient between 2,350 and 950 mm per year, following the order of Berhane-Kontir > Yayu > Bonga > Harenna, with the driest site, Harenna, situated in the southeast. Moreover, coffee accessions from these wild coffee populations were established at the Jimma Research Center. In the *ex-situ* experiment, the one-year-old coffee seedlings were evaluated under contrasting daylight and drought stress regimes over a period of 17 days.

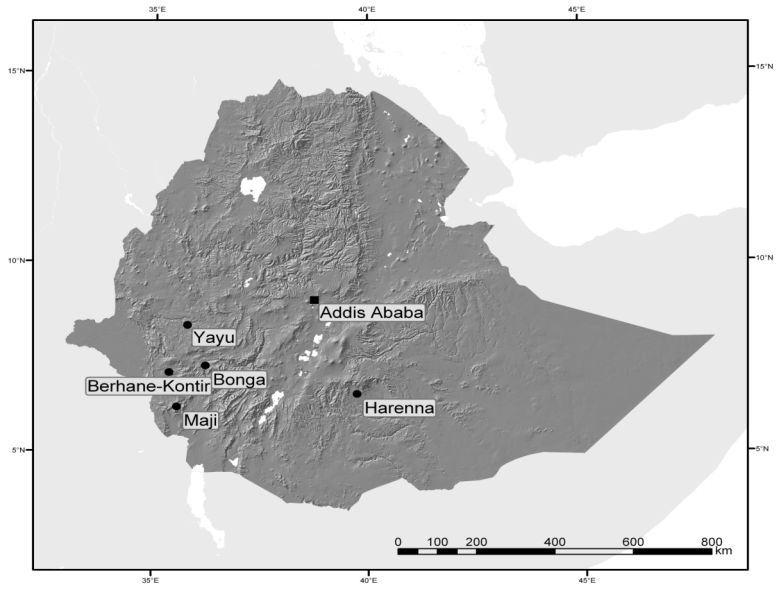


Figure 1. Map of Ethiopia indicating the four in situ study sites Berhane-Kontir, Bonga, Harenna and Yayu. The site of the ex-situ experiment, Jimma, is situated near Bonga.

Measurements tackled all relevant parts of water transport, i.e. soil conditions, the hydraulic conductivity of the root and the shoot system, stomatal control of gas exchange, and the atmospheric demand for water vapour. A porometer (Lcpro, ADC, UK) was used to measure gas exchange. A high-pressure flow meter (HPFM, Dynamax, USA) was employed to measure hydraulic flows in root and shoot segments of mature trees and coffee seedlings.

RESULTS

Water use efficiency *in situ*, measured by isotopes as well as by gas exchange, was found to be higher in the dry than in the wet season, and on dry sites compared to wet sites, thus reflecting the availability of water (Figure 2).

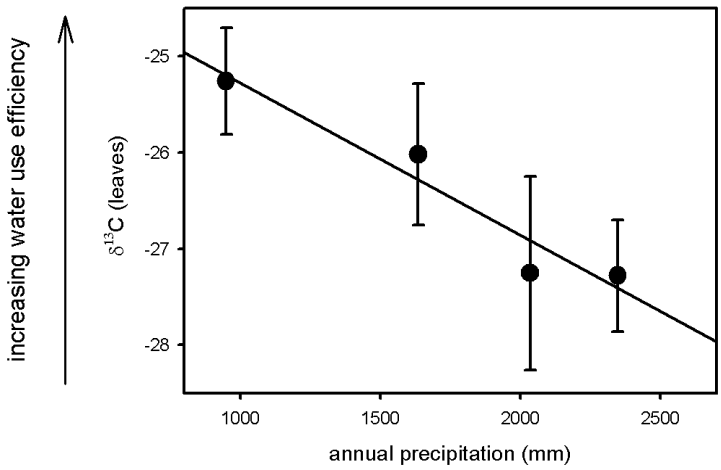


Figure 2. $\delta^{13}C$ values of leaf samples collected after the dry season at the four experimental sites.

No correlation with the rainfall gradient, however, was observed neither when measuring the hydraulic system *in situ*, nor under *ex situ* conditions (Figure 3) In both cases, Harenna trees showed the highest values, follwed by Berhane Kontir, while Bonga trees showed the lowest values.

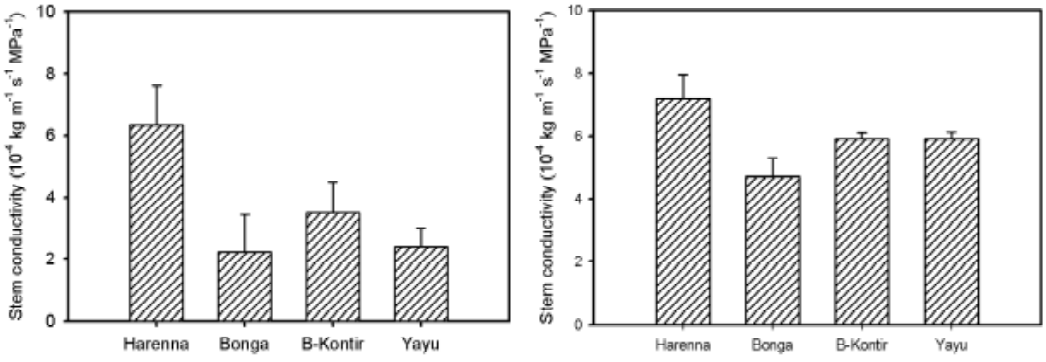


Figure 3. Stem hydraulic conductivity of trees in situ (left) and ex situ (right).

Looking at gas exchange measurements, plants from the driest site, Harenna, showed highest transpiration when under drought and light stress (Figure 4). Trees from Berhane Kontir showed the lowest values. Harenna trees also had the highest biomass, while Berhane Kontir trees showed the lowest values. The root/shoot ratio for Harenna trees was highest, followed by Bonga, Yayu and Berhane Kontir (Figure 5).

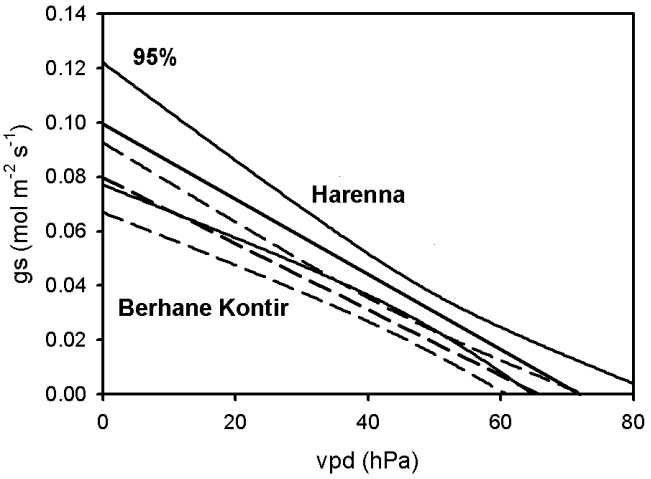


Figure 4. Stomatal conductance gs vs. vapour pressure deficit under drought and light stress during the ex-situ experiment.The 95% confidence intervals are indicated. Only results from Harenna and Berhane Kontir are shown, results from the other two sites were in between.

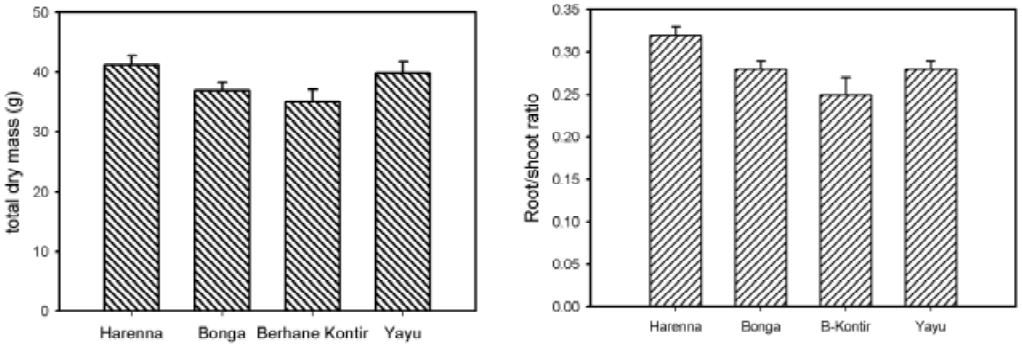


Figure 5. Total dry mass (left) and root/shoot ratio (right) of unstressed trees.

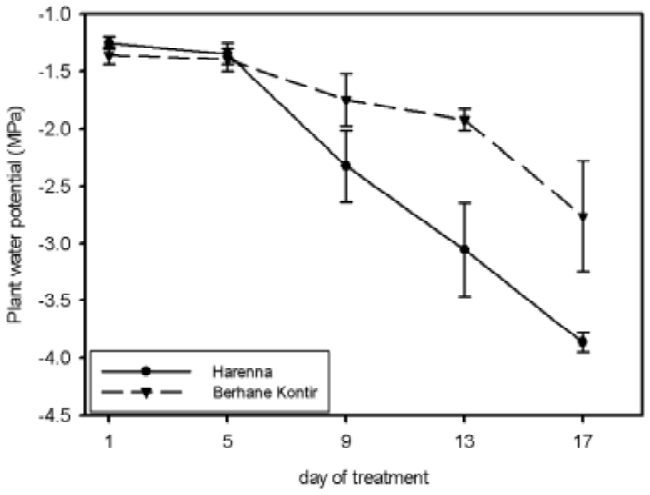


Figure 6. Predawn water potential of trees under drought and light stress. Only Harenna and Berhane Kontir trees are shown, the other two groups were in between.

When looking at plant water potentials during the drought and light stress experiment, the predawn values were decreasing fastest for Harenna indicating fastest dehydration. These

slowest decrease was measured for Berhane Kontir trees (Figure 6). According to this, wilting started first for Harenna trees, and first plants died after about 20 days.

DISCUSSION

The results showed that the precipitation gradient was not reflected in a simple way by drought stress tolerance of trees. While isotopic measurements confirmed a higher water use efficiency for trees with low water availability, measurements of hydraulic conductance were surprisingly indicating Harenna trees to have less limitations for water transport in the shoot. High hydraulic conductivity poses a threat under drought conditions due to the formation of cavitation, for which reason an order according to the rainfall gradient would have been expected. The trees from the three southwestern site in fact were confirming such an order, with Bonga showing lowest hydraulic conductivity both *in situ* and *ex situ*. Trees from Harenna, the driest site, also behaved diferently from the others when looking at transpiration measurements. Highest stomatal conductances were maintained under drought stress, leading to strongest dehydration. On the other hand, the biomass production was highest for Harenna trees.. The root/shoot ratio of the *ex situ* seedlings again reflected the expected pattern according to the precipitation patterns of the original sites, with Harenna and Bonga having the highest, and Berhane Kontir trees having the lowest values.

Summarising, the plants from Harenna showed a liberal water use under drought stress, at the same time achieving high productivity. Trees from Berhane Kontir, the wettest site, were conservative in terms of water use, with lower stomatal conductance, but less biomass production. It is speculated that different water use strategies might be expressed by these patterns, letting find Harenna populations their way out of serious droughts by putting their main effort into biomass and seed production (and thus conservation of the population as a whole), while trees from other populations might be more oriented to ensure individual survival.

Modern coffee varieties have a very narrow genetic basis, likely to based on genotypes from east of the Rift valley (Montagnon and Bouharmont, 1996). The present results confirmed ecophysiological differences between populations from east and west of the Rift Valley, respectively, indicating that especially populations from soutwestern Ethiopia are highly valuable for enriching the genetic basis of cultivated *C. arabica* germplasm.

REFERENCES

Montagnon C. Bouharmont P., 1996. Multivariate analysis of phenotypic diversity of *Coffea arabica*. Genetic Resources and Crop Evolution 43, 221-227.

Using Virtual Plants for Upscaling Carbon Assimilation from the Leaf to the Canopy Level. Application to Coffee Agroforestry Systems

J. DAUZAT[1], N. FRANCK[2], P. VAAST[1]

[1]Centre de Coopération International en Recherche Agronomique pour le Développement, Montpellier, France
[2]Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile

SUMMARY

Models of carbon assimilation are regularly developed at the leaf scale and must be afterwards integrated at the canopy scale. Analytical methods for this integration imply several simplifying assumptions and, in complex cases like coffee agro-forestry systems, face the difficulty of a proper assessment of the actual light interception by coffee plants. Alternatively, the ARCHIMED method of numerical integration proposed in this study allows simulating the irradiation of individual leaves within any 3D virtual stand. With this information, carbon assimilation models can be properly integrated from leaf to canopy scale. Moreover, the energy balance can be solved to output the temperature of leaves which can be subsequently accounted for the calculation of their assimilation. A comprehensive carbon assimilation model was developed for coffee leaves under different growth irradiances by Franck et al. (ASIC, 2006). In order to accurately upscale this model at canopy level, virtual 3D canopies were reconstructed from field digitizing of shaded and unshaded coffee plants. These virtual canopies were then used to simulate the carbon assimilation (*An*) of coffee in pure stand or within agroforestry systems. Results revealed assimilation potentialities of the shaded coffee stand comparable to that of the unshaded canopy because of its higher LAI. In contrast the unshaded canopy assimilation was strongly hampered by branch defoliation attributed to an excessive fruit load. Sensitivity analyses demonstrated that the photoinhibition effect is negligible during the rainy season and of secondary importance during the dry season prevented leaves are acclimated to high irradiances. On the contrary, excessive leaf temperature is the major reducing factor of *An* during the dry season and/or in lowlands. These results illustrate the particular interest of agroforestry systems in warm and dry conditions. Through these simulation experiments, the carbon assimilation potentialities of coffee orchards can be assessed *vs*. the climatic conditions and the shading rate of the above tree strata. Recommendations can then be driven regarding the optimal density of trees according to their crown development as well as the expected gain of *An* resulting from tree pruning.

INTRODUCTION

The simulation of canopy carbon assimilation is the basis for estimating the potential production that plants can sustain. Different modelling approaches were developed for this purpose: Big-Leaf models [Sellers et al., 1992; Sellers et al., 1996), Sun-Shade models (de Pury and Farquhar, 1997) or Multi-Layers Models (Raupach, 1991). All these models lay on analytical integration of a leaf photosynthesis model in the case of homogeneous pure stands. Despite Multi-Layers Models are more versatile and can also include the physical and biological processes influencing the canopy microclimate and atmospheric exchanges

(Raupach, 1991), they are one-dimensional and inappropriate for row crops such as coffee. A fortiori, multi-strata agroforestry systems are out of their scope.

Alternatively, the approach developed in this paper lay on light models enabling the calculation of individual leaves irradiance in any complex stand (Dauzat et al., 2001). On this basis, it becomes possible to numerically calculate the photosynthesis of individual leaves and to properly integrate it at the plant level and at the canopy level. This 3D model was applied to coffee canopies receiving full radiation or half of it. Using this model, we analyzed the effect of micrometeorological variables on coffee canopy photosynthesis and the effect of shading.

MATERIAL AND METHODS

Experimental site and measurements

Measurements were performed on dwarf cv. 'Caturra' of *Coffea arabica* in the Orosi valley of Costa Rica (9.79 N, 83.82 W, 1108 a.s.l). Shadowing treatments were applied using a shading net as described in Franck et al. (Franck et al., 2006) and leaves photosynthesis was measured in each treatment [ibid]. Two treatments were selected for integrating leaf photosynthesis to canopy level: GI_{100}, receiving 100% of solar radiation and GI_{50}, receiving 45% of it.

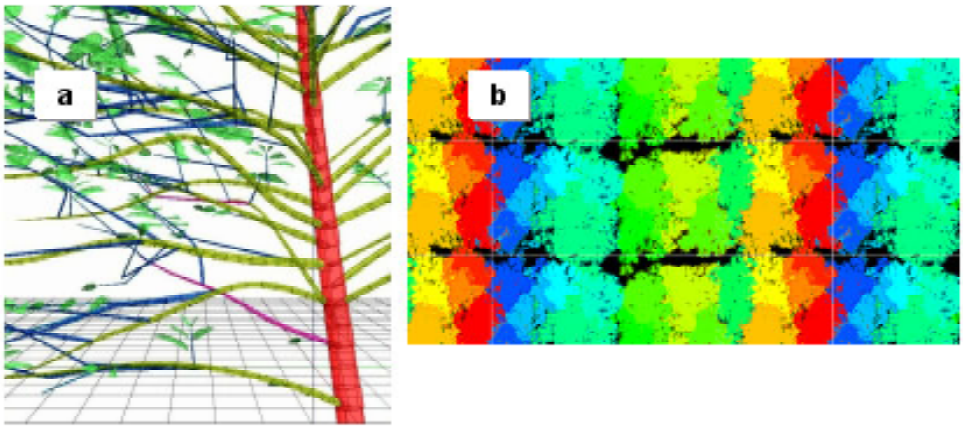


Figure 1. Reconstruction of 3D canopies. a: Detail of a virtual plant with internodes and leaves. b: Top view of reconstructed row (GI_{50}) which is virtually duplicated for simulating an infinite canopy.

For representing virtual canopies, 12 m long portions of rows were selected within each treatment. The woody structure geometry of each plant was measured with an electromagnetic 3D digitizer (Polhemus, Inc., Winooski; http://www.polhemus.com) in a similar way as presented by Sinoquet and Rivet (Sinoquet and Rivet, 1997) The plant sampling systematically included the 3D coordinates at the origin and the extremity of each axis. The shortest internodes, denoting the growth rest during the dry season, were also digitized. Additional points were sampled to render the geometry of curved or kneed axes. Concomitantly, the plant structure was described and coded in a "Multiscale Tree Graph" file (Godin and Caraglio, 1998) which indicates the relative position of internodes and leaves within the plant topology. Later on, the plant topology was used for interpolating the position of nodes that were not digitized to finally output 3D virtual plants at the detail level of individual organs (Figure 1a). Leaves were individually positioned on nodes and were affected a specific leaf area in function of their altitude within the canopy.

Micrometeorological calculations

The principle for simulating light interception and subsequent irradiance of leaves is based on image calculations from 46 points of view as described in Dauzat et al. (2001). For these simulations it was supposed that the reconstructed portions of rows described in each treatment (Figure 2) constituted an elementary pattern of the corresponding plot (Figure 1b). Leaves irradiance were calculated in the Photosynthetically Active and near-infrared ranges (PAR and NIR) at 15mn time steps. Given these irradiances, the temperature of each leaf was calculated by solving its energy balance as detailed in Dauzat et al. (2001).

Simulations were performed using micrometeorological data collected during the rainy season. In some runs, conditions of completely clear sky were simulated by fixing solar radiation (*Rs*) at its maximum, i.e. to 75% of extra-terrestrial radiation (*Ra*).

Calculation of photosynthesis

Photosynthesis was calculated with the Farquhar et al. model (1980). The effect of photoinhibition was accounted according to the Ögren and Sjöström's model (1990) and the stomatal conductance was modelled following Ball et al. (1987) and Leuning (1995). All models were parameterized for the different treatments by Franck et al. (2006).

RESULTS

Comparison of full sun and shaded canopies

Virtual reconstructed canopies were used to infer biometric data such as LAI. Compared to the shaded canopy, the full sun canopy exhibited much lower LAI at the end of the rainy season (2.07 against 5.71) despite their trunk and branch areas were similar (Figure 3a-b). The shaded treatment exhibited larger and thinner leaves distributed mainly in the upper and middle part of the canopy whereas the full sun canopy exhibited an obvious defoliation at mid height, i.e. on fruit bearing branches.

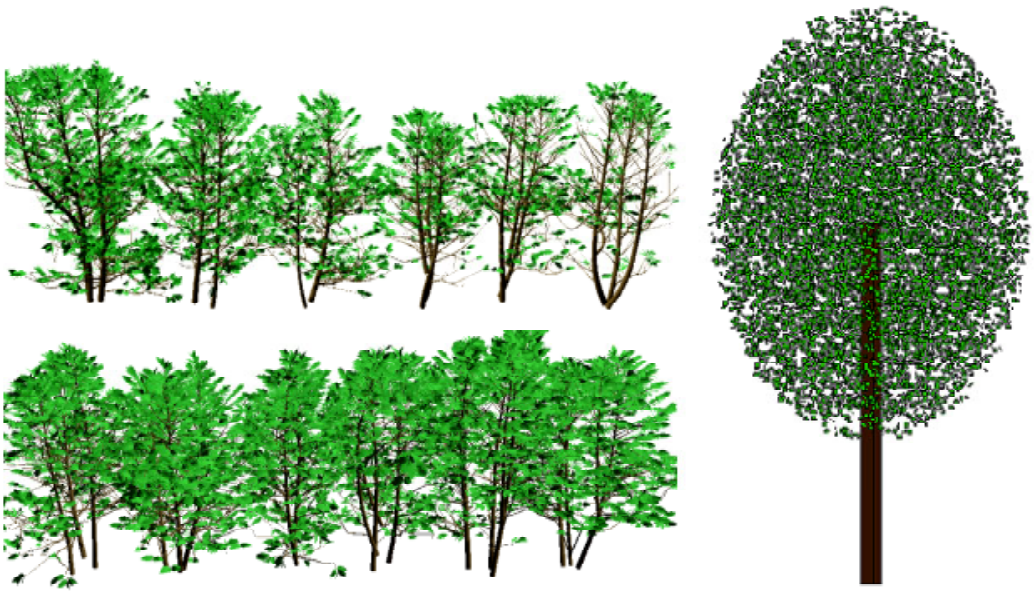


Figure 2. Virtual coffee rows reconstructed from digitizing (up: full sun GI_{100}; down: shaded GI_{50}) and simulated shade tree.

The average interception rate of incident radiation during the fruit growing season was 70.11 % for GI_{100} and 93.72 % for GI_{50}. Trunks and branches accounted for 20.63 % of total interception for GI_{100} and 5.12 % for GI_{50}. Most of the incident light was intercepted by the upper half of the GI_{50} canopy whereas the interception is more evenly distributed for the GI_{100} canopy (Figure 3c-d).

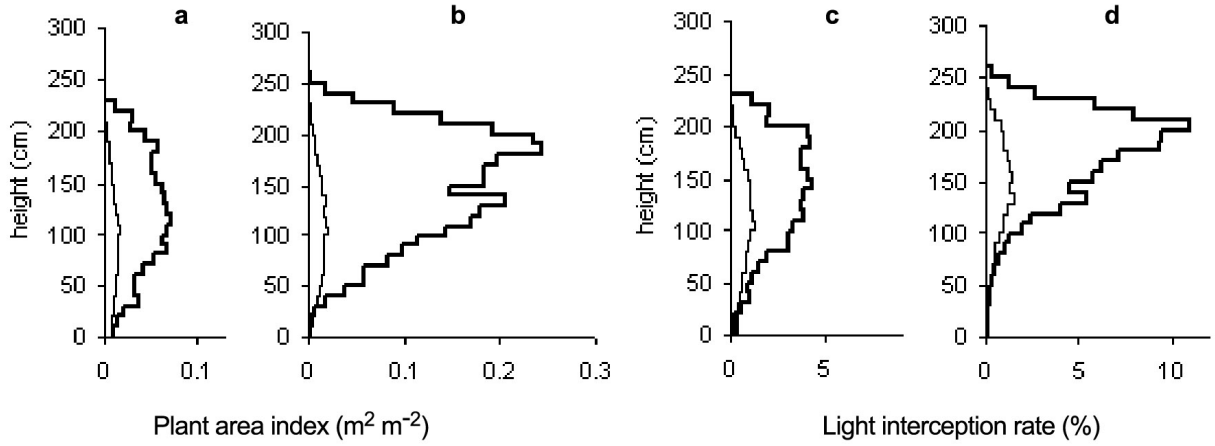


Figure 3. a-b: Profiles of Stem (–) and Leaf Area Indices (-) for GI_{100} and GI_{50} respectively and b-c: corresponding rates of light interception. Note: Stem area was divided by π in order to express SAI in terms of intercepting area.

Photosynthesis was calculated over the whole period of fruit growing at a time step of 15mn. Despite it received 45% of full radiation, the shaded canopy fixed about the same quantity of carbon (0.45 mol C m^{-2} d^{-1}) that the full sun canopy (0.47 mol C m^{-2} d^{-1}). The photoinhibition was responsible of a 3.5% reduction of assimilation for the full sun canopy and had negligible effect on the shaded canopy.

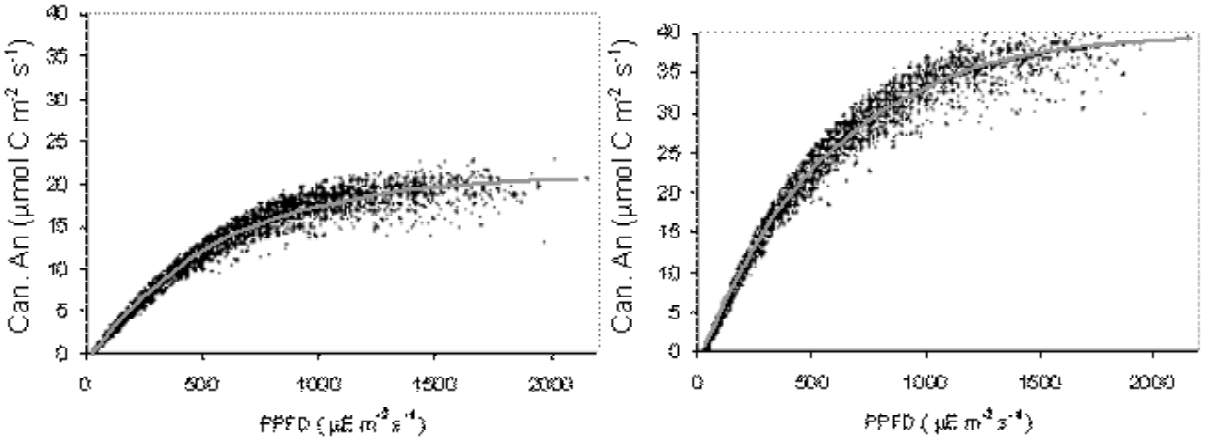


Figure 4. Canopy net assimilation per plot square meter vs. incident PPFD (PAR Photon Flux Density) during the rainy season. Left: GI100 in full irradiance, right: GI50 with 55% shading. Each point represents 15mn integration at one time of the day. Lines represent fittings with a monomolecular equation.

Analysis of canopy assimilation vs. micrometeorological conditions

The sub-hourly canopy assimilation depends mainly on incident PAR (Figure 4) and can be fitted satisfactorily with a monomolecular equation: $An = a + b\,(1\text{-}e^{\,c\,PAR})$. The daily canopy assimilation is roughly a linear function of incident solar radiation (Figure 5). The residuals from this linear relationship mainly result from effects of (i) the maximal air temperature and (ii) the temporal variability of incident radiation: the more even the radiation over the day, the better the light use efficiency.

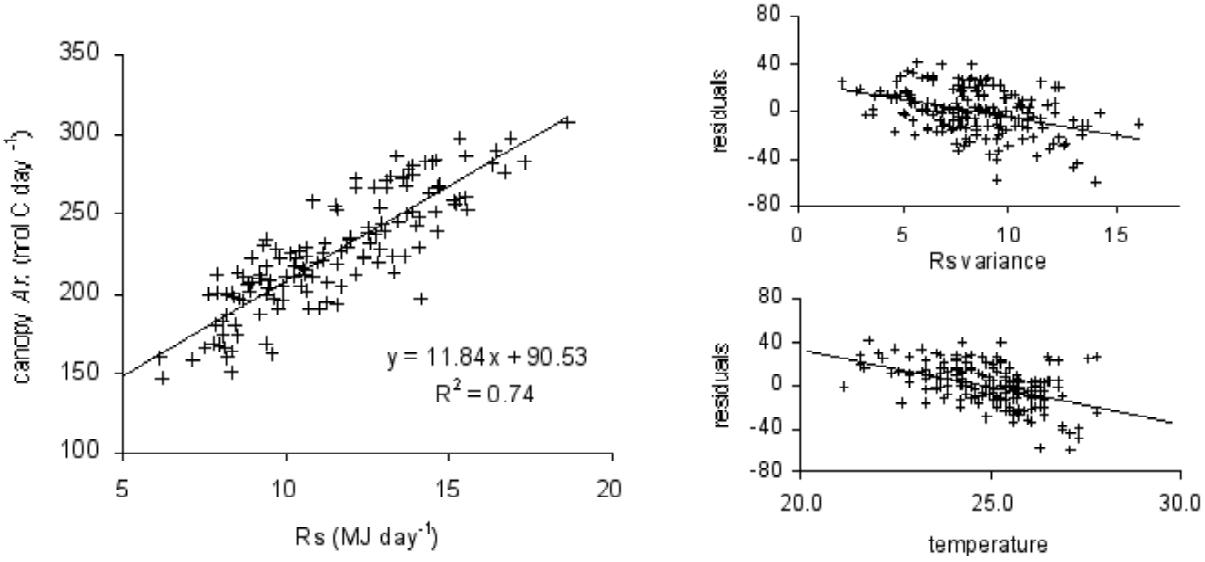


Figure 5. Linear model of daily canopy An vs. Rs for the full sun canopy and analysis of residuals vs. the Rs variance and the air temperature.

When submitted to maximal solar radiation (Rs/Ra = 0.75) and under average air temperature, the GI_{50} assimilation largely overpass the GI_{100} assimilation because of its much higher LAI. In such conditions, photoinhibition is responsible for a 10.2% *An* reduction for GI_{100} and a 4.2% reduction for GI_{50}. Decreasing the air temperature by 5°C increased notably canopy *An* whereas increasing it by 5°C drastically decreased *An* (Figure 6).

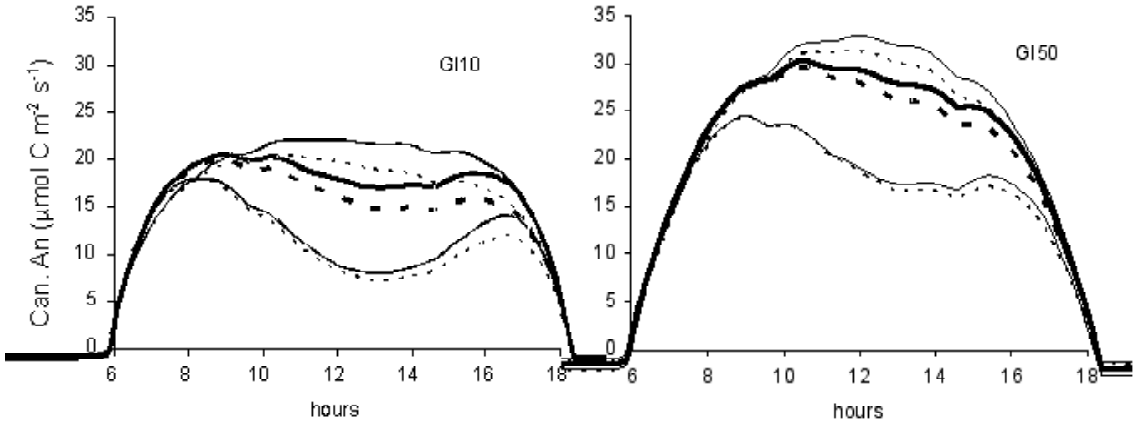


Figure 6. Canopy net assimilation when submitted to maximal solar radiation (Rs/Ra = 0.75) with the average air temperature measured at Orosi (thick lines), with air temperature decreased by 5 °C (upper curves) or increased by 5 °C (lower curves). Solid lines represent An_{can} without photoinhibition and dashed lines when accounting for photoinhibition.

Application to an agroforestry system

The effect of tree shading was simulated by adding in the GI_{50} plot ellipsoidal trees at a density of 208 trees ha^{-1}. The light transmission rate under trees ranged from 27% (in early morning and late afternoon) up to 67% (around noon), with a daily average transmission of 45%. Assuming leaves are acclimated to their light regime, tree shade would reduce An_{can} by 23.5% on average.

Compared to an artificial shading of the same intensity, the tree shading is characterized by its variability over time and its spatial heterogeneity. Because An_{can} is not a linear function of light, coffee photosynthesis is therefore 4.4% lower under tree shade than under artificial shade on average (Figure 7).

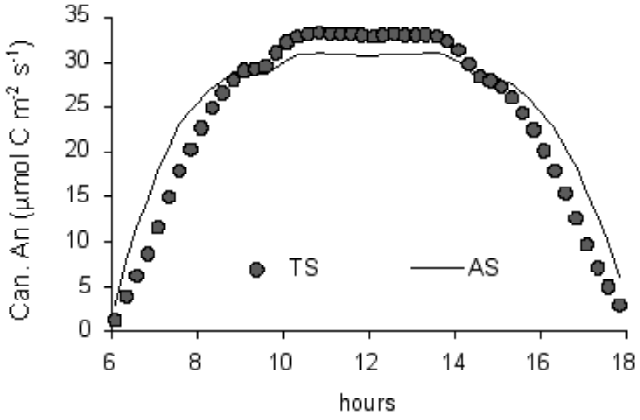


Figure 7. Daily course of canopy assimilation for canopy under artificial shade (AS) and under tree shade (TS) for a same daily shading rate of 45%.

DISCUSSIONS

The main feature of the present model is the use of 3D representations of canopies at the scale of individual organs which allows simulating photosynthesis of individual leaves with accounting of their own environment. Processes such as photoinhibition, stomatal conductance and thermal balance of leaves can then be simulated with minimal assumptions.

Not surprisingly, the coffee canopy photosynthesis is highly correlated to incident PAR. It was shown that the photoinhibition effect is of little impact on coffee photosynthesis during the rainy season because of low solar radiation on average: 3.5% reduction in full sun and 0.9% under shade. However, in conditions of maximal radiation as during the dry season, the photoinhibition can reduce the canopy assimilation by a factor of 9% in full sun and 4% under shade.

Because tree shading is variable over time and space, its reduction effect on *An* is stronger than the sole effect of reducing incident light. A strong negative effect of air temperature on canopy *An* was put in evidence. In this respect, the tree shading can be beneficial (i) by lowering the air temperature by 1 or 2 degrees and (ii) by limiting the leaves heating submitted to high irradiances. Such effects, that were not simulated here, would be particularly beneficial in hot lowland areas.

Another major result is that plant defoliation drastically reduces canopy photosynthesis: with a LAI value of 5.71, a canopy receiving 45% of full radiation may assimilate carbon as efficiently as a canopy that has a LAI of 2.07 in full sun. Excessive fruit loads are responsible

of defoliation and branch die-back (Vaast et al., 2002). This has two detrimental effects on the subsequent production because (i) lowered LAI means less potential *An* and (ii) reduced vegetative growth means less new internodes able to bear fruits the next year. High nitrogen fertilizations are generally applied to boost vegetative growth in order to counteract defoliation effects but such practice leads to alternate productions. Though, because canopy *An* is less than proportional to LAI, the guiding rule should be to maintain an average LAI able to sustain an average production.

Given its high LAI, the shaded canopy performs canopy photosynthesis comparable to the one of the full sun canopy and would therefore be able to sustain a much higher production than its actual production. This would necessitate to enhance floral initiation which is naturally decrease by shading. Traditionally, a drastic pruning of shade trees like Erythrina was effective for this purpose. In modern agroforestry systems using timber trees, pruning can not be so drastic and the choice may be oriented to species loosing their leaves during the dry season.

ACKNOWLEDGMENTS

this study received a support from the European Commission (ICA-4-CT-2001-10071) within the framework of the Central American Coffee Agroforestry Project (www.casca-project.com).

REFERENCES

Ball J, Woodrow I. and Berry J. 1987 A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. Paper presented at the VII international congress on photosynthesis, Dordrecht, 221-224.

Dauzat J., Rapidel B., Berger A., 2001. Simulation of leaf transpiration and sap flow in virtual plants: description of the model and application to a coffee plantation in Costa Rica. *Agricultural and Forest Meteorology*, 109: 143-160.

de Pury, D.G.G, Farquhar G.D., 1997. Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopy. *Plant, Cell and Envi*ronment 18, 1183-1200.

Farquhar G.D., von Caemmerer S. & Berry J. 1980. A biochemical model of photosynthetic CO2 assimilation of C3 species. *Planta* 149, 78-90.

Franck N., Vaast P., Dauzat J. 2006. Coffee: A shade-adapted plant – Implications on its carbon balance and consequences on coffee yield and quality in agroforestry systems. *In* 21st International Conference on Coffee Science.

Godin, C. and Y. Caraglio (1998). A multiscale model of plant topological structures. *Journal of Theoretical Biology* 191: 1-46.

Leuning R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants. *Plant, Cell and Environment* 18, 339-355.

Ögren E. et Sjöström M. 1990. Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy. *Planta* 181, 560-567.

Raupach 1991. Vegetation-atmosphere interaction in homogeneous and heterogeneous terrain: some implications of mixed-layer dynamics. *Journal Plant Ecology* 91, 105-120.

Sellers, P.J., Berry, J.A., Collatz, G.J., Field, C.B., Hall, F.G., 1992. Canopy reflectance, photosynthesis and transpiration. III: A reanalysis using improved leaf models and a new canopy integration scheme. *Remote Sensing of Environment* **42**, 187-216.

Sellers, P.J., Randall, D.A., Collatz, G.J., Berry, J.A., Field, C.B., Dazlich, D.A., Zhang, C., Collelo, G.D., Bounoua, L., 1996. A revised land surface parameterization (SiB2) for atmospheric GCMs Part I: Model formulation. *Journal of Climate* **9**, 676- 705.

Sinoquet, H. and P. Rivet (1997). Measurement and visualization of the architecture of an adult tree based on a three-dimensional digitising device. Trees **11**(5): 265-270.

Vaast P., Dauzat J. and Génard M. 2002. Modelling the effect of fruit load, shade and plant water status on coffee berry growth and carbon partitioning at the branch level. *Acta Horticulturae* **584**, 57-62.

Nitrogen Dynamics and Nitrate Leaching in *Coffea arabica* Systems in Costa Rica According to Site Conditions, Fertilization and Shade Management

JM. HARMAND[1], V. CHAVES[2], H. ÁVILA[3], P. CANNAVO[4], L. DIONISIO[2], G. CROUZET[1], B. ZELLER[4], P. VAAST[1,3], R. OLIVER[1], E. DAMBRINE[4]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), TA 10/D, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France
[2]Instituto de Café de Costa Rica (ICAFE), Heredia, Costa Rica
[3]Centro Agronómico Tropical de Investigación y Enseñensa (CATIE), Departamento de Agroforesteria, 7170 Turrialba, Costa Rica
[4]Institut National de la Recherche Agronomique (INRA), INRA-Nancy, Biogéochimie des Ecosystèmes Forestiers, 54280 Champenoux, France

SUMMARY

For the last 5 years, research has been undertaken on nitrogen (N) cycling in full sun coffee plantations and coffee agroforestry systems in Costa Rica, to improve N management (N fertilization and shade management) in order to optimize production of coffee and associated trees while reducing negative environmental impacts such as nitrate (NO_3^-) water contamination. These studies showed that Costa Rican soils where coffee is grown, exhibited (1) high permeability allowing large water drainage, but (2) different adsorption patterns resulting in different NO_3^- dynamics. Acrisols (Ultisols, USDA classification) from the southern zone of Costa Rica presented a dominance of positive charges in the subsoil which were responsible for strong NO_3^- retention hence mitigating NO_3^- leaching and water contamination. Andisols from the Central Valley did not show this mechanism at such a magnitude so that NO_3^- can potentially be leached in large amounts into the aquifers. Fertilization experiments in full sun coffee plantation showed that increasing the N fertilizer level from 150 to 350 kg N ha^{-1} yr^{-1} resulted in a low additional coffee production and a high potential for NO_3^- water contamination. In optimum growing conditions for coffee in full sun with high inputs, the inclusion of shade trees (*Eucalyptus deglupta* or *Inga densiflora*) increased N accumulation in litter and permanent biomass and slightly reduced water drainage, but also reduced coffee production by 25-33%. One experiment using ^{15}N labelled fertilizer in a highly fertilized coffee-*Inga densiflora* association (250 kg N ha^{-1} y^{-1}) showed a low efficiency of N fertilizer use by both coffee and trees. Only ≈ 24% of the annual fertilizer input contributed to the vegetative growth and the coffee production. These results highlight the need to adapt N fertilization to the current annual production and to reduce N fertilization input in shaded coffee systems intensively managed in order to better match plant needs and reduce NO_3^- leaching. In an organically managed system fertilized with coffee pulp (≈150 kg N ha^{-1} yr^{-1}), but without any chemical N fertilizer input, coffee production was acceptable under the shade legume tree *Erythrina poeppigiana*. Compared to the highly fertilized agroforestry system (250 kg N ha^{-1} y^{-1}) previously described, NO_3^- leaching in this organically managed agroforestry system was reduced by 3 times.

RÉSUMÉ

Durant les 5 dernières années, des recherches ont été menées sur le cycle de l'azote dans les monocultures et les systèmes agroforestiers caféiers au Costa Rica. Il s'agissait d'améliorer la

gestion de l'azote en particulier la fertilisation et la gestion de l'ombrage afin d'optimiser la production du caféier tout en réduisant les impacts environnementaux négatifs comme la pollution nitrique des eaux. Les conditions climatiques pluvieuses et la perméabilité élevée des sols au Costa Rica favorisent un drainage important s'accompagnant d'un fort potentiel de lixiviation des cations et des anions du sol. Nos études ont montré que les sols ont des comportements variables du point de vue de l'adsorption des anions en particulier des nitrates (NO_3^-) : les Acrisols (Ultisols, classification USDA) de la zone Sud du Costa Rica présentent dans le sous-sol des charges positives qui retiennent les NO_3^- et limitent fortement les pertes par lixiviation. Les Andisols de la Vallée Centrale, formés sur cendres volcaniques ne présentent pas un phénomène de rétention de NO_3^- avec une telle amplitude, et par conséquent, les nitrates présents dans ces sols peuvent être lixiviés rapidement. Les essais de fertilisation ont montré que l'augmentation de la dose annuelle d'engrais au-delà de 150 kg N ha^{-1} a peu d'effet sur la production du caféier à long terme mais présente un fort potentiel de pollution nitrique. En conditions favorables pour la production de café en plein soleil, l'introduction d'arbres d'ombrage (*Eucalyptus deglupta* ou *Inga densiflora*) dans la plantation de caféiers hautement fertilisée, a eu pour effets (1) d'augmenter l'accumulation d'azote dans la litière et la biomasse permanente, (2) de réduire légèrement le drainage, mais aussi (3) de réduire la production de café de 25 à 33%. Une expérimentation utilisant l'engrais marqué ^{15}N dans une plantation hautement fertilisée (250 kg N ha^{-1} y^{-1}) a montré une faible utilisation de l'engrais de l'année par le caféier et l'arbre d'ombrage (≈ 24% de l'apport), même en année de forte production de café. Ces résultats montrent globalement que dans les systèmes fertilisés, il est nécessaire d'adapter les apports d'engrais au phénomène d'alternance des récoltes du café et de réduire les apports sous ombrage pour limiter les pertes de nitrates par drainage profond. Dans les systèmes biologiques conduits sans apport d'azote minéral, mais avec apport de drupes de café à raison de 150 kg N ha^{-1} an^{-1}, l'ombrage d'*Erythrina poeppigiana* élagué deux fois par an a permis une production acceptable de café tout en divisant par trois les quantités de N lixivié par rapport au système agroforestier hautement fertilisé précédemment décrit.

INTRODUCTION

In the last decades, intensively managed coffee (*Coffea arabica*) systems have been developed in Central America, particularly in Costa Rica where old coffee varieties, grown under a variety of shade trees, were replaced by more productive varieties (e.g., Caturra, Catuai) planted under heavily pruned leguminous trees (e.g. *Erythrina poeppigiana)* or in unshaded monocultures (Babbar and Zak, 1995). These heavily fertilized coffee plantations (annual fertilizer input of about 250 kg N ha^{-1}) grown on permeable soils under high rainfall intensities have been presented as a suspected cause of nitrate (NO_3^-) contamination of groundwater (Reynolds-Vargas and Richter, 1995). N fertilization is a key factor for coffee growth and production but the efficiency of N fertilization is apparently low in these coffee systems. Using ^{15}N labeled fertilizer, Salas et al. (2002) estimated that only 30 to 40% of the applied N was absorbed by the coffee plants. Fertilizer N not used by the crop or soil microbes may be leached as NO_3^- into groundwater, therefore improving the fertilizer efficiency may reduce NO_3^- leaching.

In recent years, fast growing timber trees like *Eucalyptus deglupta* Blume have been planted in unshaded coffee in Costa Rica or have replaced the conventional leguminous shade trees in order to reduce labour cost (legume tree pruning) and to diversify income. In these systems, coffee is still being grown with a high fertilization, typically from 150 to 250 kg N ha^{-1} y^{-1}. Meanwhile, organic coffee production which began to extend in the mid-90's, is currently developed at a very low scale in Costa Rica (~1% of the coffee area under certified organic production in 2005). In this country, many of the alternative technologies for organic

production are based on the regular application of composted organic matter (mainly outside nutrients), "natural" methods of disease and pest control and the use of leguminous shade tree such as *Erythrina poeppigiana*. In these systems, farmers have also continued to look for economic diversification of the tree component with non leguminous and fruit trees.

The inclusion of fast growing timber trees in coffee agroforestry systems may limit nutrient leaching by interception (increased root length density in top soil), by taking up nutrients from deeper soil horizons and by accumulating nutrients in biomass, litter and topsoil. Nevertheless, the timber trees may compete significantly with coffee for light, water and soil nutrients and reduce coffee production (Beer et al., 1997), whereas legume shade trees can contribute to improve coffee N availability through N_2 fixation and N recycling.

This report presents some results from experiments undertaken during the last five years in *Coffea arabica* systems in Costa Rica on the effects of site conditions, fertilization and shade tree species (timber or legume tree) on N fluxes, especially N accumulation in permanent biomass, N export in coffee berries harvest and NO_3^- leaching.

MATERIAL AND METHODS

Over a four year period (2002-2005), in situ measurements of key components of the N cycle of different target coffee agroforestry systems were conducted at a patch scale. In each study, a full sun coffee plot with the same date of establishment and similar agricultural management as the shaded plot was used as a control. Studies focused on inputs (N Fertilizer), soil N mineralization, N accumulation in biomass (including N_2 fixation by the legume shade tree) and soil (NO_3^- retention), and N outputs (coffee berry harvest, NO_3^- leaching, mineral N loss in runoff and nitrous oxide emissions).

This report mainly presents results on N accumulation in permanent biomass, N export in coffee berries harvest and N loss through NO_3^- leaching.

The first agroforestry system studied was a heavily fertilized coffee plantation receiving 180 kg N ha^{-1} y^{-1} as ammonium-nitrate and shaded by *Eucalyptus deglupta* of 7 year old and planted at a density of 110 trees ha^{-1}. This system was located on a farm (Verde Vigor SA) in the low wet pacific southern zone of Costa Rica (altitude of 600 m, mean annual temperature of 23 °C and average annual rainfall of 2700 mm). The soil is a fine textured Acrisol (Ultisol) derived from sedimentary rocks rich in mafic materials.

Other studied agroforestry systems were located on the experimental station of ICAFE (Coffee Institute of Costa Rica) in Barva de Heredia in the Central Valley, a wet medium altitude zone of Costa Rica (altitude of 1180 m, mean annual temperature < 22 °C and average annual rainfall of 2500 mm). The soil is a fine textured Andisol derived from volcanic ash. One system was a highly fertilized coffee plantation (5000 plants ha^{-1}) receiving 250 kg N ha^{-1} y^{-1} (2 x 90 kg N as urea and 70 kg N as ammonium-nitrate) and shaded by *Inga densiflora* of 7 year old and planted at a density of 278 trees ha^{-1}. The other system was a coffee plantation shaded by a leguminous tree species, *Erythrina poeppigiana* (420 trees ha^{-1}). This 6 year old plantation received N inputs via coffee pulp at a rate of 150 kg N ha^{-1} yr^{-1} but did not receive any chemical N fertilizer.

In Barva de Heredia, two other independent studies were conducted in full sun coffee plantations: (1) N accumulation in coffee biomass was monitored over a two year period in a coffee plantation (6500 plants ha^{-1}) established in 1996, pollarded in 2001 and receiving 250 kg ha^{-1} yr^{-1} as N fertilizer. In 2003-2004, 12 pre-selected plants were excavated every two

months, and sorted into stem, branches, leaves and fruits for biomass and N content evaluation: (2) a fertilization experiment was established in 1999. The trial was a block design of 4 blocks, containing 4 rates of fertilizer application of NH_4-NO_3 in granular form (150, 250, 350 and 0 kg N ha^{-1} yr^{-1}). Annual N export in coffee berries was measured between 2001 and 2005 and NO_3^- leaching was measured in 2003.

RESULTS AND DISCUSSION

Influence of soil characteristics on NO_3^- leaching

The studies showed that Central American soils where coffee is grown, exhibited high permeability allowing large water drainage (1000 to 1500 mm) (Imbach et al., 1989; Harmand et al., submitted), but different adsorption patterns resulting in different NO_3^- dynamics. Acrisols from the southern zone of Costa Rica presented a dominance of positive charges in the subsoil, below 80 cm depth which were responsible for strong NO_3^- retention, and hence mitigating NO_3^- leaching and water contamination (Avila et al., 2004). Under full sun coffee and coffee shaded with *Eucalyptus deglupta*, concentration in leaching water strongly decreased with increasing depth, coinciding with soil NO_3^- accumulation. Lysimeters located at 0.6, 1.2 and 2 m below the soil surface, detected mean NO_3^--N concentrations of 13-16, 6-9 and 1-2 mg l^{-1}, respectively. NO_3^--N concentrations in soil solution at 2 m soil depth and in spring water (1.8 mg l^{-1}) at catchment level were similar and low. In 2002, nitrate lixiviation estimated at 2m depth was about 27 and 15 kg N ha^{-1} in full sun and shaded coffee, respectively. In the 2 m soil profile, the NO_3^- accumulation was about 600 kg NO_3-N ha^{-1} (Avila et al., 2004). Nevertheless the duration of the anion adsorption process in the subsoil, which may be widespread in Central America Acrisols, is not presently known.

In the Central Valley of Costa Rica, Andisols derived from the weathering of volcanic ashes did not show this mechanism at such a magnitude so that NO_3^- can potentially be leached in large amounts. For example, in highly fertilized full sun coffee (*Coffea arabica*) system and coffee shaded with *Inga densiflora* (≈250 kg N ha^{-1} yr^{-1}), lysimeters located at 0.3, 0.6 and 2 m below the soil surface, detected mean annual NO_3^--N concentrations of 18, 17 and 12-16 mg l^{-1}, respectively despite high NO_3^- leaching estimated at 35 to 65% of the annual N fertilizer input.

N accumulation and fertilization experiments in full sun coffee plantations

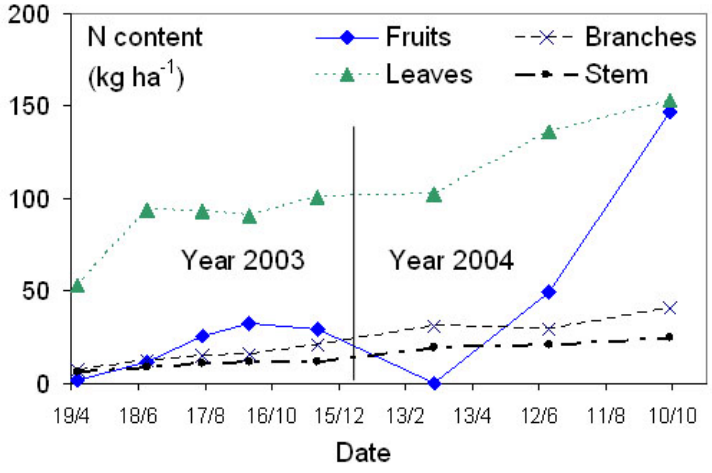


Figure 1. N dynamics in different components of a full sun coffee plantation, Barva de Heredia, Costa Rica.

On Figure 1 showing N dynamics in the different components of the coffee plants, the alternate production pattern of coffee trees appears with a year (2003) of low coffee production (1.6 t dry matter berries) followed by a year (2004) with high coffee production (8 t dry matter berries). From these data, N exportation in coffee berries was estimated in 2003 and 2004 at 28 and 140 kg ha^{-1} yr^{-1}, respectively, while annual N accumulation in the total aerial biomass was estimated in 2003 and 2004 at 95 and 230 kg ha^{-1} yr^{-1}, respectively. N allocation to the coffee berries accounted for 29 and 63% of total N accumulation in year of low and high production, respectively, showing that coffee fruits were the most important sink for N, especially for years of high production.

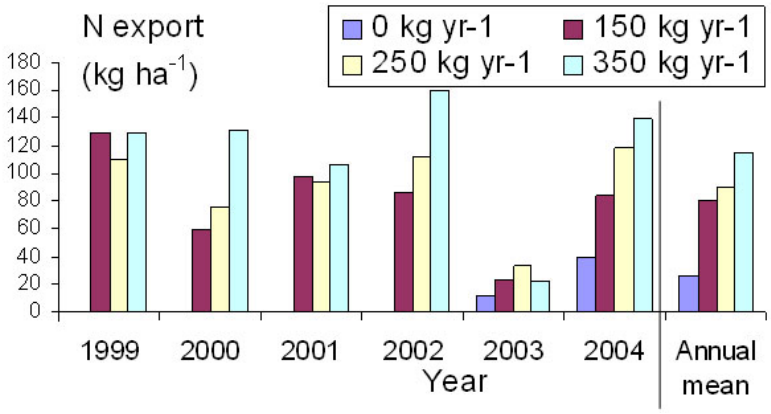


Figure 2. N export in harvest of coffee berries for different levels of N fertilizer input (0, 150, 250, 350 kg N ha^{-1}), Barva de Heredia, Costa Rica.

Results from the fertilization experiment showed that there was a linear relationship between N applied and coffee production every two years (Figure 2). Despite a high level of N mineralization and nitrification (more than 200 kg ha^{-1} yr^{-1} at this site, data not shown), coffee production in full sun conditions without any fertilizer input was low and there is a clear response of coffee production to fertilizer input. Nevertheless, over a 6 year period, increasing the N fertilizer level from 150 to 350 kg N ha^{-1} yr^{-1} resulted in a low additional coffee production (+0.9 t dry matter ha^{-1} yr^{-1}), and hence a low additional N exportation by coffee berries (+35 kg N ha^{-1} yr^{-1}) and a high potential of NO_3^- water contamination.

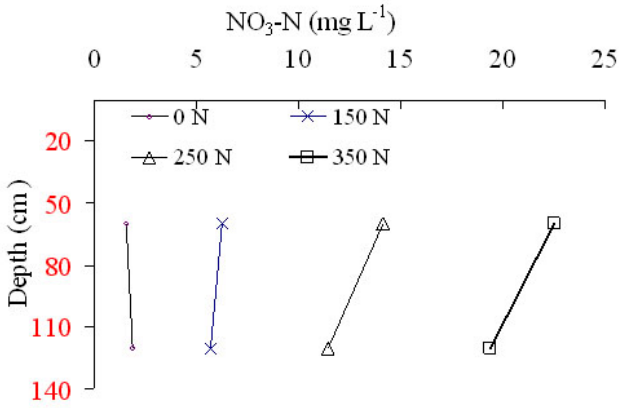


Figure 3. Mean annual NO_3^--N concentrations in leaching water at 60 cm and 120 cm depth under full sun coffee for different levels of N fertilizer input (0, 150, 250, 350 kg N ha^{-1}), Barva de Heredia, Costa Rica.

As shown on Figure 3, there was in 2003 a positive relationship between N applied and the mean NO_3^- concentration in leaching water. For N fertilizer levels larger than 150 kg N ha^{-1}

yr^{-1}, these NO_3^- concentrations were higher than 10 mg NO_3^--N L^{-1} considered as a health hazard according the World Health Organisation. By increasing N fertilization from 150 to 350 kg N ha^{-1} yr^{-1}, NO_3^- leaching increased from 60 to more than 160 kg N ha^{-1} yr^{-1}. These results highlight the need to adapt N fertilization to the current annual production taking into account the alternate bearing pattern in order to better match plant needs and reduce NO_3^- leaching. Increasing the N fertilizer level above 150 kg N ha^{-1} yr^{-1} does not seem to provide significant additional coffee production in the long term but has high potential for NO_3^- water contamination.

N cycling in a highly fertilized coffee -*Inga densiflora* system in Costa Rica

In 2004, one experiment using ^{15}N labelled fertilizer was carried out in the coffee-*Inga densiflora* plot receiving 250 kg N ha^{-1} y^{-1} in order to evaluate the fate of N fertilizer (Cannavo et al., submitted). Results showed that only ≈ 24% of the annual fertilizer input contributed to vegetative growth and coffee production. In these site conditions (intense rainfall combined with highly permeable soil and low anion exchange capacity), the main flux is the loss of NO_3^- in leaching water (Table 1). The inclusion of shade trees increased N accumulation in litter and permanent biomass and slightly reduced water drainage, but also reduced coffee production. For years of low coffee production (e.g. 2003), the larger N accumulation in biomass in shaded coffee was associated with lower NO_3^- leaching under shade than in full sun coffee. Nevertheless, for years of high coffee production (e.g. 2004) and hence high N exportation by coffee berries in full sun, the lower coffee production under shade was associated with a greater amount of NO_3^- leaching (Table 1). These experiments showed a low efficiency of N fertilizer use by both coffee and trees, highlighting the need to reduce N fertilization input in shaded coffee systems intensively managed in order to reduce NO_3^- leaching.

Table 1. Annual N fluxes (kg N ha^{-1}) in full sun and shaded coffee (*Inga densiflora*) systems in Costa Rica.

N Flux	Full sun coffee	Coffee - *Inga densiflora*
Fertilizer input	250	250
N accumulation in biomass and litter	46	115
N losses (Year 2003)		
- N export in coffee beans harvest	38	43
- Nitrate N leaching at 2m depth	120	95
N losses (Year 2004)		
- N export in coffee beans harvest	143	95
- Nitrate N leaching at 2m depth	74	90-130

N cycling in an "organically" managed *Coffea arabica – Erythrina poeppigiana* system in Costa Rica

Without any chemical N fertilizer input, coffee production was very low in full sun conditions (0.75 t berries ha^{-1} yr^{-1} during 3 years) but acceptable and sustainable under the shade legume tree (more than 3 t berries ha^{-1} yr^{-1} for the same period). This was the result of (1) the beneficial effect of shade and (2) higher N availability in the system resulting from N_2 fixation by the associate legume tree species and high rates of N recycling. The rates of N recycling through tree pruning residues, roots and litter were much larger in the shaded system than in full sun: 350 and 40 kg N ha^{-1} yr^{-1}, respectively. To conclude, incorporating a legume tree stratum into an organic coffee system strongly increased the speed and volume of the N cycle, resulting in a greater coffee yield response and a slight increase of NO_3^- leaching

(Table 2). Compared to the highly fertilized agroforestry system previously described, NO_3^- leaching in this organically managed agroforestry system was reduced by 3 times.

Table 2. Annual N fluxes (kg N ha^{-1}) in full sun and *Erythrina poeppigiana* coffee systems in Costa Rica (Year 2004).

N flux	Full sun coffee	*Coffee - Erythrina poeppigiana*
Organic fertilizer input	150	150
N accumulation in biomass and litter	23	80
N losses		
- N export in coffee beans harvest	15	62
- Nitrate-N leaching at 1.2 m depth	31	46

CONCLUSION

These results highlight the need to adapt N fertilization to the current annual production taking into account the alternate bearing pattern of coffee plants in order to better match plant needs and reduce NO_3^- leaching. Increasing the N fertilizer level above 150 kg N ha^{-1} yr^{-1} does not seem to provide significant additional coffee production in the long term but has high potential for NO_3^- water contamination.

The inclusion of shade trees in coffee plantations intensively managed requires reducing N fertilization input in order to reduce NO_3^- leaching. Organically managed agroforestry systems were much more nutrient conservative than conventional fertilized coffee systems and less environmentally damaging. Furthermore, incorporating a legume tree stratum into an organic coffee system appeared necessary to obtain an acceptable coffee production without significant increased NO_3^- leaching.

Additional research must be done to identify what efficiencies can be gained from integrating external inputs and tree strata management to increase the speed and volume of nutrient cycles without enhanced nutrient leaching.

AKNOWLEDGEMENTS

The authors thank Verde Vigor SA. and ICAFE for the maintenance of experimental plots, Patricia Leandro from the soil laboratory of CATIE for biomass and soil analysis, the European Commission (INCO project CASCA, ICA4-CT-2001-10071, www.casca-project.com) and the Science and Cultural Cooperation Centre of the French embassy in Costa Rica for supporting part of the operational costs of this research.

REFERENCES

Avila, H., Harmand, J. M., Dambrine, E., Jimenez, F., Beer, J. and Oliver, R. (2004). Dinámica del nitrógeno en el sistema agroforestal *Coffea arabica* con *Eucalyptus deglupta* en la zona sur de Costa Rica. *Agroforesteria en las Americas* 41-42: 83-91.

Babbar, L. I. and Zak, D. R. (1995). Nitrogen loss from coffee agroecosystems in Costa Rica: leaching and denitrification in the presence and absence of shade trees. *Journal of Environmental Quality* 24(2): 227-233.

Beer, J., Muschler, R., Kass, D. & Somarriba, E. (1997). Shade management in coffee and cacao plantations. *Agroforestry Systems* 38(1-3): 139-164.

Cannavo P., Harmand J.M., Zeller B., Dambrine E. Use of the 15N isotope tracing technique for the study of the nitrogen balance in a *Coffea arabica* agroecosystem under *Inga densiflora* shade in Costa Rica. Soil use and management (submitted).

Harmand JM, Avila H, Dambrine E, Skiba U, Renderos Duran RV, de Miguel Magaña S, Oliver R, Jiménez F, Beer J. Nitrogen dynamics, soil nitrate retention and nitrate water contamination in a *Coffea arabica* - *Eucalyptus deglupta* system in Southern Costa Rica. *Biogeochemistry* (submitted).

Imbach, A. C., Fassbender, H. W., Borel, R., Beer, J. and Bonnemann, A. (1989a). Modelling agroforestry systems of cacao (*Theobroma cacao*) with laurel (*Cordia alliodora*) and cacao with poro (*Erythrina poeppigiana*) in Costa Rica. IV: Water balances, nutrient inputs and leaching. *Agroforestry Systems* 8: 267-287.

Reynolds-Vargas, J. S. and Richter, J. D. D. (1995). Nitrate in groundwaters of the Central Valley, Costa Rica. *Environment International* 21(1): 71-79.

Salas, R., Bornemisza, E., Zapata, F., Chaves, V. & Rivera, A. (2002). Absorcion del fertilizante nitrogenado por la planta de café y su influencia sobre la contaminacion de las aguas subterraneas. In: J. Reynolds-Vargas (ed), Manejo Integrado de Aguas Subterraneas. EUNED, San José, Costa Rica, pp 89-104.

An Integrative Approach to Study Environmental Stress Tolerance in *Coffea* sp. – From Leaf to Gene

J. C. RAMALHO[1, 2,*], F.C. LIDON[2], A. E. LEITÃO[1], P. EICHLER[1, 3], P. SANTOS[1, 4], I. P. PAIS[5], P. S. CAMPOS[5], A.P. RODRIGUES[3, 4], M. M. CHAVES[3, 4], M. P. BRANCO[1], A. I. RIBEIRO[1, 2]

[1]Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal/ Inst. Inv. Científica Tropical, Quinta do Marquês, 2784-505 Oeiras, Portugal
[2]Unid. Biotecnol. Ambiental / FCT / UNL, 2829-516 Monte de Caparica, Portugal
[3]Inst. Sup. Agronomia / UTL, Tapada da Ajuda, 1349-017 Lisboa, Portugal
[4]Inst. Tecnol. Química Biológica / UNL, Quinta do Marquês, 2781-901, Oeiras, Portugal
[5]Dept. Fis. Vegetal / EAN / INIAP, Quinta do Marquês, 2784-505 Oeiras
*E-mail: cochichor@mail.telepac.pt

SUMMARY

Environmental constraints disturb plant metabolism and are often associated to yield reductions. Among them, low positive temperatures are of up most importance in tropical plant species, namely in *Coffea* sp., which are known to be cold sensitive. Nevertheless, even in sensitive species some acclimation is possible, leading to different sensitivity degrees. The present multidisciplinary work aims at providing an overview of our group's research potential, considering studies on chilling stress and tolerance in *Coffea* sp., using the photosynthesis as a key marker for plant metabolism and the control of oxidative stress as a decisive feature to an effective acclimation.

INTRODUCTION

Unfavorable temperatures and drought are the major climatic limitations to coffee metabolism and production, being expected to become increasingly important in several coffee growing regions due to the recognized changes in global climate, and because coffee crop has spread towards marginal lands (DaMatta and Ramalho, 2006). Low positive temperatures are known to depress growth, photosynthetic performance and yield (Bauer et al., 1985; DaMatta and Ramalho, 2006; DaMatta et al., 1997; Ramalho ey al., 2003). Among the cell structures, chloroplast is quickly and deeply affected (Kratsch and Wise, 2000) and in chilling-sensitive plants, such as coffee, net photosynthesis ceases almost completely at 5-10 °C (Larcher, 1981). That could be attributed to reductions of stomatal conductance (g_s), loss of photochemical efficiency, increase of damages and reduction of repair processes at the photosystems (PS) I and II (*e.g.*, in D1 protein and pigment complexes), restrictions of electron transport, enzyme activity and carbohydrate metabolism, as well as loss of membrane selectivity (see DaMatta and Ramalho, 2006). Furthermore, chilling exposure for several days might provoke significant after-effects upon rewarming, since CO_2 uptake remains low or completely inhibited (Larcher, 1981).

Due to such sensitivity, the acclimation of the photosynthetic apparatus to low temperatures is of particular importance to plant survival. As reported in coffee that can display some ability to cold acclimate, such process includes an increase of enzyme activities (*e.g.*, from Calvin cycle and sugar metabolism), reinforcement of energy dissipation and antioxidative mechanisms (that maintain Reactive Oxygen Species, ROS, under control), qualitative and

quantitative changes in lipid (*e.g.*, in lipid classes and fatty acid saturation level) and protein membrane composition, altogether contributing to keep metabolic pathways regulation (Campos et al., 2003; DaMatta et al., 1997, Ramalho et al., 2003).

In order to evaluate the cold acclimation ability within the *Coffea* genus, our group is carrying a set of multidisciplinary studies aiming the characterization of the cold and drought tolerance mechanisms, mainly focused in the analysis of oxidative stress impact on the photosynthetic metabolism. With this integrated approach it is expected to screen genotypes that can be adequately used for cultivation in areas prone to low temperature occurrence and for breeding programs.

MATERIAL AND METHODS

Plant Material and Growth Conditions

The experiments were carried out as previously described (Campos et al., 2003) with minor modifications, using 1.5 years old plants from the genotypes *Coffea arabica* cv. Catuaí (IAC 81), Icatu (IAC 2944 – *C. canephora* x *C. arabica*), which are important coffee producers, *C. canephora* cv. Apoatã (IAC 2258) and *C. dewevrei*, which are used in breeding programs. The plants were placed in a growth chamber (700EDTU, ARALAB, Portugal) and subjected successively to 1) a gradual temperature decrease (0.5 ºC/day) from 25/20 ºC to 13/8 ºC (day/night), over 24 days, 2) a 3 day chilling cycle (13/4 ºC), where the plants were subjected to
4 ºC during the night and in the first 4 h of the following morning (thus, concomitantly with light), followed by a rise to 13 ºC applied throughout the rest of the diurnal period, 3) a rewarming period of 6 days at 25/20 ºC, in order to allow recovery. Photoperiod was set to 12 h, RH to 65-70% and PPFD to *ca*. 750 μmol m^{-2} s^{-1}. Determinations were made in the 2 top pairs of mature leaves in 6-8 plants per cultivar.

Assimilation Rates

Determinations followed (21), with minor changes. The net photosynthetic rate, P_n, was measured under photosynthetic steady-state conditions at *ca*. 380 ppm of CO_2, using a CO_2/H_2O porometer (CIRAS I, PP Systems, UK).

Measurements of the photosynthetic capacity, A_{max}, were performed in 2 cm^2 leaf discs, under light (PPFD 800 μmol m^{-2} s^{-1}) and CO_2 (*ca.* 7%) saturating conditions, at 25 ºC, in a Clark-type leaf-disc O_2 electrode (LD2/2, Hansatech, Kings Lynn, UK).

Chlorophyll Fluorescence Parameters and Thylakoid Electron Transport Rates

Chlorophyll fluorescence parameters were measured using a PAM 2000 system (H. Walz, Effeltrich, Germany) on leaf discs placed inside the LD2/2 O_2 electrode, under CO_2 saturating conditions, at 25 °C. The estimation of quantum yield of photosynthetic non-cyclic electron transport, ϕ_e, and the PSII efficiency of energy conversion, F_v'/F_m', were determined under photosynthetic steady-state conditions (PPFD 400 μmol m^{-2} s^{-1}).

Furthermore, the electron transport rates associated to PSII (with participation of Oxygen Evolving Complex – OEC) and to PSI were measured in sub-chloroplast fractions, using an O_2 electrode (LW2, Hansatech), as described in (Ramalho et al., 1999).

Reactive Oxygen Species Production

Hydrogen peroxyde (H_2O_2) determinations were carried out as described in (Sergiev et al., 2001), using 750 mg leaf material. Spectrophotometric measurements were made at Abs_{390nm} and for H_2O_2 quantification a standard curve with known H_2O_2 concentrations was used.

Hydroxyl radical ($OH^{\bullet}$) determinations followed (Babbs and Gale, 1987), using 2 g leaf material. Spectrophotometric measurements were made at Abs_{420nm} and for $OH^{\bullet}$ quantification an extinction coefficient of 11000 M^{-1} cm^{-1} was used.

Antioxidants

Pigment analysis was performed in leaf material (2 cm^2) as in (Ramalho et al., 1997), using a reversed-phase HPLC (with an end-capped, C_{18}, 5 μm Spherisorb ODS-2 column, 250 x 4.6 mm). For identification and quantification of each pigment were used known standards.

Ascorbate evaluation was performed as in (Romero-Rodrigues et al., 1992), with a reversed-phase HPLC similar to that of pigment analysis, but using H_2O at pH 2.2 for elution. Identification and quantification was performed using ascorbate as standard.

Respiratory Enzymes Activity

Activities of pyruvate kinase (PK, EC 2.7.1.40) and malate dehydrogenase (MDH, EC 1.1.1.37) were measured spectrophotometrically, following NADH oxidation at 340 nm for 5 min, as described in (Juszczuk and Rychter, 2002) for PK, and by (López-Millán et al., 2000) for MDH.

Gene Expression Studies

1) cDNA clones corresponding to *cagr1* (glutathione reductase fragment), *carca* (rubisco activase) and *capsaB* (PSI subunit) genes were isolated from subtractive cDNA libraries obtained from coffee leaves (Fernandez et al., 2004). Based on the cDNA sequences, specific primers were designed (data not shown) in order to perform the mRNA expression studies by semi-quantitative RT-PCR as described in (Chen et al., 2005).

Statistical Analysis

A two-way ANOVA (significance level of $P < 0.05$) was used to evaluate differences between the temperatures and between the species, followed by a Tukey test for mean comparison (95% confidence level).

RESULTS AND DISCUSSION

Assimilation Rates

Effects on the photosynthetic machinery can be observed through P_n, which showed strong impacts in all genotypes with the gradual temperature decrease (Figure 1).

At 13/8 ºC Catuaí and Icatu still have P_n values close to 50% of control, but under chilling and in the first 24 h after that they presented negligible values, even lower than those of Apoatã and *C. dewevrei* (that were affected firstly, at higher temperatures). The faster recovery was observed in Icatu, while *C. dewevrei* was not able to recover 7 days after the

chilling exposure. Such negative impact is consistent to the known cold sensitivity of coffee plants (Baueret al., 1985; DaMatta and Ramalho, 2006; Ramalho et al., 2003), but it would arise from different contributions of stomatal and mesophyll impairments among genotypes. In fact, the photosynthetic machinery of Icatu was not deeply affected, since the higher A_{max} reduction occurred at 13/8 ºC (13%). Also, after chilling a non-significant 7% decrease was observed (Figure 1), while the other genotypes suffered reductions of *ca*. 60% (*C. dewevrei*), 45% (Apoatã) and 30% (Catuaí), reflecting different mesophyll impacts and, thus, cold sensitivities, as previously reported (Campos et al., 2003; Ramalho et al., 2003).

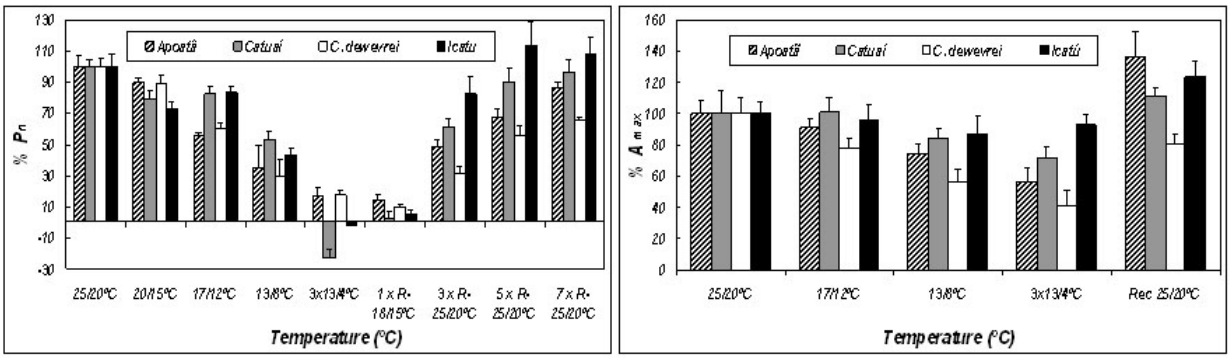


Figure 1. Variation in percentage of the estimation of the net photosynthetic rate, P_n, and the photosynthetic capacity, A_{max}, as compared to their respective control value in μmol m^{-2} s^{-1}. Each value represents the mean +SE (n = 8). Control values (25/20 °C) vary between 3.2-3.7 for P_n and 9.9-14.2 for A_{max}.

Chlorophyll Fluorescence Parameters and Thylakoid Electron Transport Rates

Amongst others, such mesophyll effects may result from different impacts on the PSII photochemical efficiency and electron transport. Strong changes were detected after chilling exposure, with Icatu showing the smallest decreases on F_v'/F_m' (40%) and ϕ_e (45%), as well as in the electron transport rate associated to PSI (13%) and the highest rise in that associated to PSII (250%) (Figures 2 and 3).

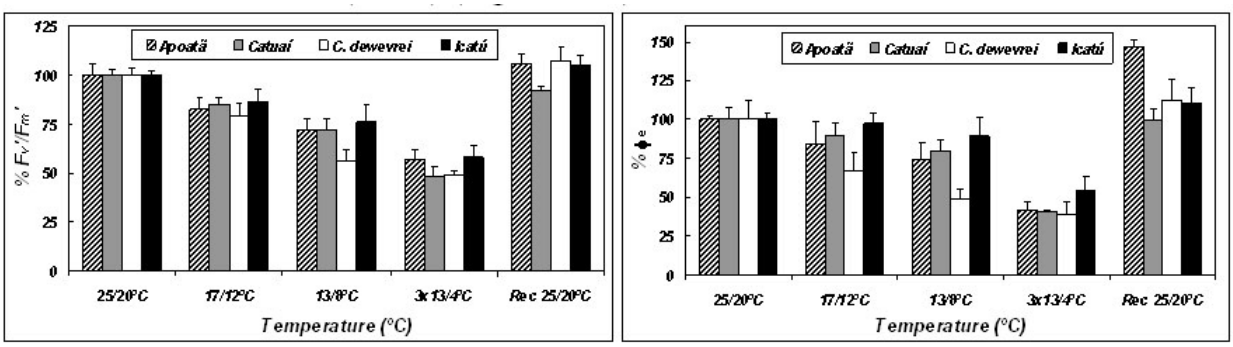


Figure 2. Variation in percentage of the estimation of quantum yield of photosynthetic non-cyclic electron transport, ϕ_e, and the PSII efficiency of energy conversion, F_v'/F_m', as compared to their respective control values. Each value represents the mean +SE (n = 6). Control values (25/20ºC) vary between 0.21-0.31 for ϕ_e and 0.43-0.48 for F_v'/F_m'.

In the case of F_v'/F_m', some of the decrease could be attributable to the presence of photoprotective (dissipative) pigments (see Table 1), thus, reflecting the presence of protective mechanisms instead of damage. Furthermore, to the lower impacts, Icatu usually showed also the higher absolute values of photochemical activity. Contrasting to the

sensitivity of *in vivo* rubisco activity (Ramalho et al., 2003), thylakoid electron transport showed to be quite robust, especially at PSII level where the increased rates suggest the presence of a photoprotective cycle around PSII (except for Apoatã). It is also noteworthy the stronger impact in the electron transport involving PSI when compared to that of PSII. That agrees with several works reporting that PSI is particularly sensitive to cold in tropical plants (Kudoh and Sonoike, 2002; Sonoike, 1999) and with the results obtained in Catuaí submitted to photoinhibitory conditions (Ramalho et al., 1999). Thus, the evaluation of the presence of such sensitivity might be used for cold (or perhaps, to oxidative stress) tolerance selection in coffee plants, since Icatu is indeed the less affected genotype.

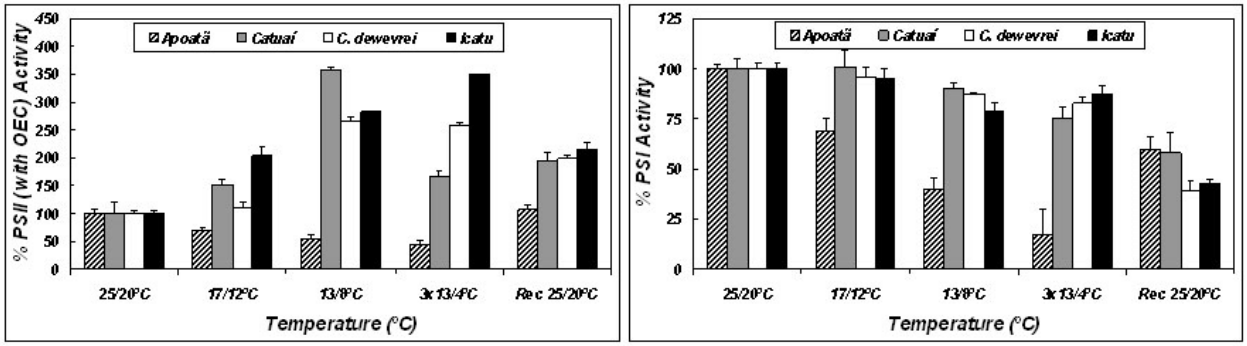


Figure 3. Variation in percentage of the thylakoid electron transport rates associated to PSII (with participation of OEC) and PSI, as compared to their respective control value in $\mu molO_2\ m^{-2}\ s^{-1}$. Each value represents the mean +SE (n = 5). Control values (25/20 ºC) vary between 5-10 for PSII and 82-138 for PSI.

Reactive Oxygen Species and Antioxidants Production

Since under cold the photochemical use of energy absorbed by chlorophyll (chl) is greatly diminished, photooxidative stress may occur due to an accumulation of excited molecules (*e.g.*, ^{3}chl*, ^{1}chl, $^1O_2$) in the pigment bed. Also, the reduction of O_2, mostly at PSI level, would lead to the formation of ROS ($O_2^{-\bullet}$, H_2O_2, $OH^\bullet$) that may cause lipid peroxidation, bleaching of pigments (*e.g.*, in P_{680}), protein degradation (*e.g.*, D1), enzyme inactivation, etc. (9, 18). In this way, the over-expression of antioxidative scavengers such as enzymes (*e.g.*, SOD, APX), hydrophilic (*e.g.*, ascorbate, glutathione) and lipophilic (*e.g.*, zeaxanthin, β-carotene) antioxidants is decisive to cold tolerance (1, 9, 10). In *Coffea* sp. the control of oxidative stress, considering both the production and scavenging of highly reactive molecules, could have contributed to the observed differential impact. In fact, after chilling exposure Icatu and *C. dewevrei* showed the lower production of H_2O_2, while Icatu and Catuaí presented the lower levels of $OH^\bullet$ (Figure 4). Furthermore, under recovering conditions only Icatu and Catuaí presented values below control conditions, while Apoatã and *C. dewevrei* reached levels well above control.

The low levels of ROS in Icatu could be related to the consistent increase of Cu,Zn-SOD and APX activities (involved in the removal of ROS), contrary to what happens in Apoatã and *C. dewevrei* (data not shown). On the other hand, in Apoatã the production of $OH^\bullet$ could provoke lipoperoxidation, contributing to the strong impairment of the electron transport rates associated PSI (that reaches only 17% of the control), since PSI is known as a preferential production site of superoxide radical, that could be transformed in H_2O_2 and, after that, in $OH^\bullet$. Furthermore, the presence of other molecules that may dissipate energy as heat, such as lutein, antheraxanthin and zeaxanthin, would reduce the energetic overcharge in the photosystems, decreasing the probability of ROS production (Table 1).

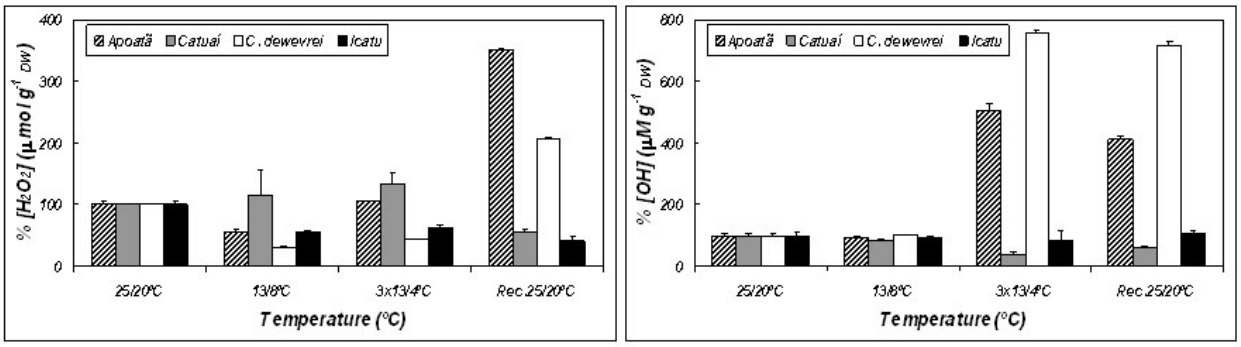


Figure 4. Variation in percentage of the production of some reactive oxygen species in leaves in relation to their respective control value in µmol g^{-1} DW (H_2O_2) and µM g^{-1} DW ($OH^{\bullet}$). Each value represents the mean +SE (n = 5). Control values (25/20 ºC) vary between 8.7-18.4 for H_2O_2 and 200-1350 for $OH^{\bullet}$.

Again, after chilling exposure, Icatu presented the stronger reinforcement (and higher absolute values) of the pigments that integrates the xanthophyll cycle (**V+A+Z**) and of lutein. Moreover, the reinforcement of ascorbate content could help Icatu plants, since it intervenes in the scavenging of H_2O_2 (with APX) and in the transformation of violaxanthin to zeaxanthin. However, that was not decisive, since Apoatã and Catuaí presented also strong increases of this molecule but showed higher cold impacts. Only the complementary reinforcement of protective pigments, ascorbate and scavenging enzymes would explain the lower production of ROS and the lower impact upon chilling in Icatu, including in membrane lipids (Campos et al., 2003).

Table 1. Variation in percentage of some leaf antioxidants in relation to their respective control value in mg g^{-1} DW. Values represent the mean of 5 replicates (SE < 5% mean). Each value represents the mean +SE (n = 5). Control values (25/20 °C) vary between 0.28-0.35 for V+A+Z (violaxanthin+antheraxanthin+zeaxanthin), 0.62-0.94 for Lutein, 1.59-2.10 for Total Carotenoids, 1.37-2.94 for Ascorbate.

	Genotype	Temperature (day/night)				
		25/20°C	18/13°C	13/4°C	3x13/4°C	Rec 25/20°C
V+A+Z	Apoatã	100 b	105 b	106 b	115 b	158 a
	Catuaí	100 c	110 bc	117 bc	123 b	147 a
	C. dewevrei	100 c	132 b	126 b	125 b	152 a
	Icatu	100 c	110 bc	122 b	154 a	161 a
Lutein	Apoatã	100 b	108 b	122 ab	126 a	135 a
	Catuaí	100 b	104 b	109 b	124 a	130 a
	C. dewevrei	100 b	104 b	116 ab	112 b	120 a
	Icatu	100 c	121b	131 b	161 a	147 ab
Tot Carot	Apoatã	100 a	84 b	94 ab	82 b	85 b
	Catuaí	100 a	110 a	109 a	98 a	112 a
	C. dewevrei	100 b	121 ab	108 b	100 b	127 a
	Icatu	100 b	102 ab	111 ab	115 a	104 ab
Ascorbate	Apoatã	100 cd	126 c	223 a	172 b	88 d
	Catuaí	100 c	-	168 ab	187 a	137 b
	C. dewevrei	100 a	88 ab	111 a	99 a	58 b
	Icatu	100 c	105 c	101 c	160 a	128 bc

(a, b, c) - comparison between temperatures within the same genotype.

Respiratory Enzymes Activity

The maintenance of respiratory metabolism is also of great importance, since it allows the production of energy, reducing power and metabolic intermediates that makes possible the repair processes, which are increasingly important under stressful chilling conditions. In cold-sensitive plants, respiration is strongly affected due to the inhibition of several enzymes, which provokes an exponential decline of respiratory rates with decreasing temperatures (Larcher, 1981). In this way**,** the study of the impact on key enzymes of the mitochondrial respiration pathway could give important insights about cold sensitivity. The *in vitro* activity of MDH (Figure 5) decreased under mild cold conditions (18/13 °C) except in *C. dewevrei*. However, by the end of the acclimation period and after the chilling exposure, a general tendency to recover was observed (except in Apoatã), with Icatu showing the highest increase (46% higher than control) and absolute value. Under rewarming conditions MDH activity was similar to that of the control except in Apoatã. *C. dewevrei* showed a slowly, but sustained, increase since the beginning of temperature decrease. However, the values were usually the lowest among the studied genotypes.

The *in vitro* activity of PK decreased also by the middle of the acclimation period (Figure 5). Thereafter, some ability to recover was observed in all genotypes (except Apoatã), as judged by the absence of significant differences after the chilling exposure. By this time Catuaí and Icatu showed the highest values of activity, reflecting a higher respiration potential. During the recovery period PK activity did not present further changes.

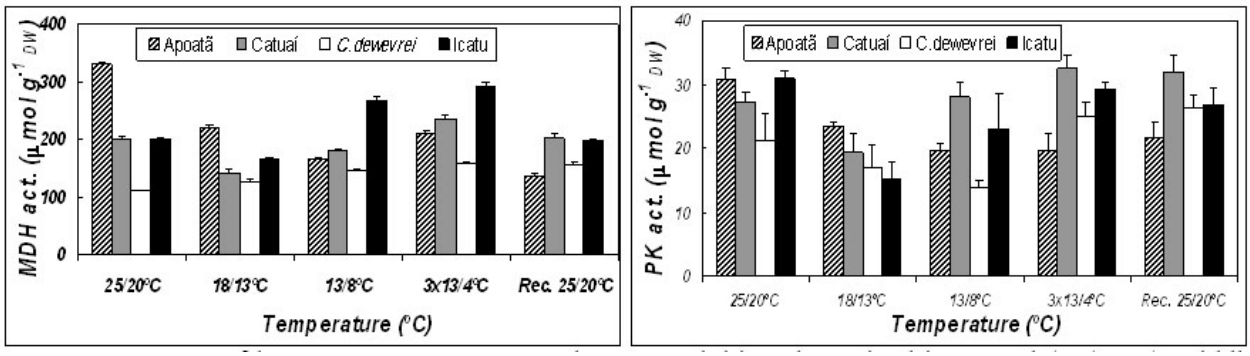


Figure 5. Impact of low temperature on PK and MDH activities, determined in control (25/20 °C), middle (18/13 °C) and at the end of the acclimation period (13/8 °C), after 3 cycles of chilling (13/4 °C) and after 6 days of recovery (Rec 25/20 °C), in the studied Coffea cvs. Each value represent the mean ± SE (n = 4).

The results reflect some sensitivity of the respiratory metabolism (analysed through PK and MDH activities) in Apoatã and a higher tolerance in Icatu and Catuaí. This would constitute a key feature to overcome the stress effects, allowing the cells to obtain energy needed for repair and/or synthesis processes. Furthermore, that is crucial for the maintenance of a balance in sugar concentrations, a feature of utmost importance, since the restoration of the balance between photosynthesis, carbon metabolism and translocation is essential for the expression of a higher cold tolerance (Hurry et al., 2000; Leegood, 1995).

Gene Expression

The control of gene expression in response to cold exposure plays an important role in the maintenance of an efficient cell functioning. Under such conditions, the differential expression of chitinases, lipoxygenase and methalothionein genes has been triggered by cold in coffee (Santos et al., 2005; Ramalho et al., unpublished data). The expression of a

glutathione reductase fragment gene (*cagr1*) shows a stable (constitutive) expression in Apoatã, while in Icatu transcriptional activity is somewhat enhanced upon chilling and recovery (T3 and T4) (Figure 6). This is in accordance with the Icatu low levels of ROS (Figure 4) and with previous results showing that this genotype is the only one with simultaneous increases in Cu, Zn-SOD and APX activities (data not shown). That is likely to reflect a combined increase of the enzymes involved in the protective ascorbate-glutathione cycle and in the photoprotective pigments and ascorbate, all related to the absence of membrane lipoperoxidation (Campos et al., 2003). *C. dewevrei* also shows expression increases after chilling (T3 and T4). However, because of its known cold sensitivity we hypothesise that the production of the protein could have been interrupted or that its action alone was not decisive (since the ascorbate content and the activity of other enzymes from the ascorbate-glutathione cycle did not increase), leading to an increased $OH^{\bullet}$ production (Figure 4), what might explain the rise in membrane lipoperoxidation (Campos et al., 2003).

The expression of Rubisco activase, *carca*, was maintained at constitutive levels in all genotypes, suggesting that the negative impact in the *in vivo* initial Rubisco activity (Ramalho et al., 2003) is not linked to the loss of Rubisco activase, at least at the transcriptional phase.

Concerning the PSI subunit gene (*capsaB*), a cold related production of transcripts was observed in the three studied genotypes, mostly in Icatu. That could be related to the need of *de novo* synthesis due to cold impact on PSI functioning (Figure 3). In fact, Icatu showed the higher expression increase associated to the lower impact in the electron transport rate at PSI. Even so, an insufficient substitution of damaged PSI occurred and/or the damage rate is higher than the repair one, taking into account that at low temperature protein synthesis will be severely slowed down. That was particularly dramatic in Apoatã presenting a severe decrease in the electron transport at PSI level.

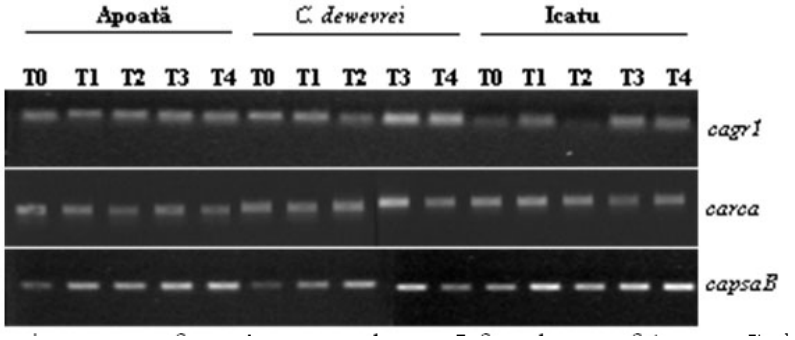


Figure 6. Gene expression pattern of cagr1, carca and capsaB from leaves of Apoatã, *C. dewevrei* and Icatu by RT-PCR, under T0 (25/20 °C), T1 (18/13 °C), T2 (13/8 °C), T3 (3x13/4 °C) and T4 (Rec.25/20 °C) conditions.

CONCLUSIONS

Despite the observation that Apoatã and *C. dewevrei* are firstly affected during the gradual temperature decrease, a first glance analysis could give the idea that chilling strongly affects assimilation similarly among genotypes. However, that reflects, mostly, a strong inhibition of stomatal opening, since mesophyll impairments showed different sensitivities among *Coffea* sp. genotypes, as assessed through, *e.g.*, A_{max}, F_v'/F_m' and ϕ_e, as well as through the impact on the *in vivo* Rubisco activity (Ramalho et al., 2003). The latter does not seem attributable to the shortage of rubisco activase. Icatu displayed a better tolerance to the imposed stress conditions, usually showing lower impacts and prompt and total recoveries, while Apoatã (and *C. dewevrei*) is more affected.

The reinforced activity of the thylakoid PSII electron transport rates (except for Apoatã) at PSII level might indicate the presence of a photoprotective cycle around PSII. On the other hand, the stronger impact observed at PSI level, suggests that such cold sensitivity of PSI could be linked to the production of superoxide radical that, ultimately, could be transformed in $OH^{\bullet}$, with severe lipoperoxidative implications. Again, Icatu showed the lower effects on PSI activity, the higher presence of PSI (*capsaB*) transcripts and the stronger reinforcement in the PSII activity, V+A+Z, lutein and ascorbate contents, as well as in glutathione reductase (*cagr1*) gene expression (and Cu,Zn-SOD and APX activities). That points out for a higher antioxidative potential, justifying the lower ROS (H_2O_2 and $OH^{\bullet}$) detection and, thus, the maintenance of a proper functioning of the photosynthetic (and respiratory) metabolism.

In conclusion, this multidisciplinary approach, using physiological, biochemical, biophysical and molecular biology techniques, allowed the characterization of different cold tolerance potential in the *Coffea* genus, showing that the tolerance of the photosynthetic apparatus is related to the reinforcement of mechanisms that control oxidative stress, either by thermal dissipation of energy or by the scavenging of ROS.

ACKNOWLEDGEMENTS

Thanks are due to Dr.s Joel Fahl, M. Luíza Carelli and Luiz C. Fazuoli (IAC) for access to seed material, Tech. Eng. C. Santiago (EAN/INIAP), P.M. Alves, S. Emidio and C. Lopes for technical help. The work was supported by Fundação para a Ciência e a Tecnologia, through the project POCTI/AGG/43101/2001 (partially financed by the European Fund FEDER) and by the grant SFRH/18361/2004, co-financed by the portuguese PIDDAC program and European Social Fund, under the 3rd framework program.

REFERENCES

Adams III W.W., Barker D.H. (1998) Seasonal changes in xanthophyll cycle-dependent energy dissipation in *Yucca glauca* Nuttall. *Plant Cell Environ*., 21: 501-511.

Babbs C.F., Gale M.J. (1987) Colorimetric assay for methanesulfonic acid in biological samples. *Anal. Biochem*., 163: 67-73.

Bauer H., Wierer R., Hatheway W.H., Larcher W. (1985) Photosynthesis of *Coffea arabica* after chilling. *Physiol. Plant.*, 64: 449-454.

Campos P.S., Quartin V., Ramalho J.C., Nunes M.A. (2003) Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants. *J. Plant Physiol.*, 160: 283-292.

Chen Z.J., Ribeiro A., Silva M.C., Santos P., Guerra-Guimarães L., Gouveia M.M.C., Fernandez D., Rodrigues Jr. C.J. (2003) Heat shock-induced susceptibility of green coffee leaves and berries to *Colletotrichum gloeosporioides* and its association to PR- and hsp70 gene expression. *Physiol. Mol. Plant Pathol.*, 63: 181-190.

DaMatta F.M., Ramalho J.C. (2006) Impacts of drought and temperature stress on coffee physiology and production: a review. *Braz. J. Plant Physiol.*, 18(1): 55-81.

DaMatta F., Maestri M., Mosquim P.R., Barros R.S. (1997) Photosynthesis in coffee (*Coffea arabica* and *C. canephora*) as affected by winter and summer conditions. *Plant Sci.*, 128: 43-50.

Fernandez D., Santos P., Agostini C., Bon M.C., Petitot A.S., Silva M.C., Guerra-Guimarães L., Ribeiro A., Nicole M. (2004) Identification of coffee (*Coffea arabica*) genes

upregulated during the hypersensitive response to the rust pathogen (*Hemileia vastatrix*), *Mol. Plant Pathol.*, 5(6): 527-536.

Foyer C.H., Lelandais M., Kunert K.J. (1994) Photooxidative stress in plants. *Physiol. Plant.*, 92: 696-717.

Hällgreen J.-E., Öquist G. (1990) Adaptations to low temperatures. In: Alscher R.T., Cumming J.R. (Eds.) Stress Responses in Plants: Adaptation and Acclimation Mechanisms. Plant Biology Series, Vol. 12. New York, Wiley-Liss Inc., p. 265-293.

Hurry V., Huner N., Selstam E., Gardeström P., Öquist G. (2000) Photosynthesis at low growth temperatures. In: Raghavendra A.S. (Ed.) Photosynthesis. A comprehensive treatise. Cambridge, University Press, p. 238-249.

Kratsch H.A., Wise R.R. (2000) The ultrastructure of chilling stress. *Plant Cell Environ.*, 23: 337-350.

Kudoh H., Sonoike K. (2002) Irreversible damage to photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. *Planta*, 215: 541-548.

Juszczuk I.M., Rychter A.M. (2002) Pyruvate accumulation during phosphate deficiency stress of bean roots. *Plant Physiol. Biochem.*, 40: 783-788.

Larcher W (1981) Effects of low temperature stress and frost injury on plant productivity. In: Johnson CB (Ed.), Physiological Processes Limiting Plant Productivity, London, Butterworths, p. 253-269.

Leegood R.C. (1995) Effects of temperature on photosynthesis and photorespiration. In: Smirnoff N. (Ed.) Environment and Plant Metabolism - Flexibility and Acclimation. Oxford, Bios Scientific Publishers, p. 45-62.

López-Millán A.F., Andaluz S., Gogorcena Y., Abadía A., De Las Rivas J., Abadía J. (2000) Responses of Sugar Beet Roots to Iron Deficiency. Chances in Carbon Assimilation and Oxygen Use. *Plant Physiol.*, 124: 885-897.

Niyogi K.K. (1999) Photoprotection revisited: genetic and molecular approaches. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 50: 333-359.

Ramalho J.C., Campos P.S., Quartin V.L., Silva M.J., Nunes M.A. (1999) High irradiance impairments on photosynthetic electron transport, ribulose-1,5-bisphosphate carboxilase / oxygenase and N assimilation as a function of N availability in *Coffea arabica* L. plants. *J. Plant Physiol*., 154: 319-326.

Ramalho J.C., Pons T.L., Groeneveld H.W., Nunes M.A. (1997) Photosynthetic responses of *Coffea arabica* L. leaves to a short-term high light exposure in relation to N availability. *Physiol. Plant*., 101: 229-239.

Ramalho J.C., Quartin V., Fahl J.I., Carelli M.L., Leitão A.E., Nunes M.A. (2003) Cold acclimation ability of photosynthesis among species of the tropical *Coffea* genus. *Plant Biol.*, 5: 631-641.

Romero-Rodrigues A., Oderiz L.A., Hernandez J.L., Gandara S. (1992) Comparaison de deux méthodes de dosage par CLHP de l'ácide ascorbique dans *Carica pentagona*. *Sci. Aliments*, 12: 593-600.

Santos P., Machado E., Gouveia M., Lidon F.C.,Nunes M.A., Ramalho J.C., Ribeiro A.I. (2005) Expression analysis of Chitinase coding genes in response to low non-freezing temperatures. *In* Proc. of the X Congresso Brasileiro de Fisiologia Vegetal, XII Congresso Latino Americano de Fisiologia Vegetal, Recife, Pernambuco, Brazil, September 11-16, CD, p. 5.

Sergiev I., Mapelli S., Karanov E. (2001) The effects of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ*., 24: 1337-44.

Sonoike K. (1999) The different roles of chilling temperatures in the photoinhibition of photosystem I and photosystem II. *J. Photochem. Photobiol*., B: Biol., 48: 136-141.

Hydraulic Conductance of Wild Arabica Coffee Populations in Montane Rainforests of Ethiopia

T. KUFA[1] AND J. BURKHARDT[2]

[1]Ethiopian Institute for Agricultural Research, Jimma Research Center, P. O. Box 192, Jimma-Ethiopia
[2]Institute of Plant Nutrition, University of Bonn, Karlrobert-Kreiten-Str.13, D-53115 Bonn, Germany. E-mail: kufataye@yahoo.com

SUMMARY

The study was conducted with the objective to characterize the hydraulic properties of wild Arabica coffee populations in the montane rainforests of Ethiopia. High-pressure flow meter was used to measure hydraulic conductance in root and shoot parts of coffee trees under field conditions. The results showed that coffee trees at the four wild coffee populations did not reveal significant differences in whole-plant, root and shoot hydraulic conductance. Significant vitiations ($P < 0.01$) were, however, detected in main stem hydraulic conductance and specific conductivity. In addition, whole-plant conductance depicted significant variations ($P < 0.05$) within Harenna sites and between coffee trees at Berhane-Kontir and Yayu forests. Hydraulic conductance in the various parts of coffee trees did not alter within sites, except for the significant variations ($P < 0.05$) in the hydraulic conductance of lateral branches and reduced stem length (30 cm long). Accordingly, coffee trees from the drier Harenna forests exhibited significantly high conductance as compared to others. The overall mean whole-plant hydraulic conductance was significantly ($P < 0.05$) and the results followed the order of Harenna (2.69×10^{-4} kg s^{-1} Mpa^{-1}) > Berhane-Kontir (2.30×10^{-4} kg s^{-1} Mpa^{-1}) > Yayu (1.36×10^{-4} kg s^{-1} Mpa^{-1}) > Bonga (1.13×10^{-4} kg s^{-1} Mpa^{-1}). As a consequence, the contribution of root was high in Harenna (45.9%) and Bonga (43.5%) as compared to Berhane-Kontir (37.2%) and Yayu (32.9%). In addition, whole-plant hydraulic conductance showed increasing patterns with the reduction of shoot components. The greatest increment over whole-shoot conductance was measured due to leaf defoliation with the values ranging from 20.8 to 35.0% at Harenna and Berhane-Kontir, respectively. Consecutive removal of fruits and petioles was noticed to enhance hydraulic conductance with the least (5%) and maximum (16.1%) increments over the whole-shoot conductance measured for coffee trees at Yayu and Berhane-Kontir forests, respectively.

MATERIAL AND METHODS

Two experimental coffee trees per sub-site were used for hydraulic measurement. Subsequent to recording all growth parameters, hydraulic measurements were made using high-pressure flow meter (HPFM). In this case, hydraulic conductances in root and shoot parts as well as hydraulic resistance in primary branch segments (Dynamax Inc., Houston, TX, USA) were measured following the procedures adopted by several authors (Martinez et al., 2002; Tyree and Dixon, 1986; Tyree et al., 1993). The flow meter consisted of a water reservoir that could be pressurized with compressed air from a pressure regulator. Water flow rate from the reservoir to the base of the excised plant part was computed from the measured pressure decrease across a capillary tube interposed between the reservoir and root (Tyree et al., 1994). The bases of the main stem and the branch were recut under water and connected to the HPFM. Root and shoot (whole-plant) hydraulic conductance were measured using the

transient method of the HPFM. Measurements were done while the whole-shoot was attached and by consecutive removal of the shoot segments. On the main stem, hydraulic conductivity was measured with the methods described by Sperry et al. (1988). For this, 30 cm long main stem segment (conventional stumping height) was excised under water to prevent air from entering into the xylem. The segment was connected to plastic tubing of the HPFM supplied with degassed and deionized water at a pressure of 0.2 MPa. Root conductance was measured by forcing distilled water into the base of the root system (opposite to the normal direction of water flow during transpiration). The computer connected to the HPFM recorded values of the parameters of the different parts of coffee trees. Analysis of variance (ANOVA) was performed with the default SAS procedures. The means were compared using Tukey´s test at 5% probability level. Graphs of two-way interactions were made with SigmaPlot.

RESULTS

Wild coffee trees from the four wild Arabica coffee populations did not reveal significant differences in whole-plant, root and shoot hydraulic conductance. Significant variations ($P < 0.01$) were, however, detected in main stem hydraulic conductance and stem specific hydraulic conductivity (Table 1). In addition, whole-plant conductance showed significant variations ($P < 0.05$) within Harenna sites and between coffee trees at Berhane-Kontir and Yayu. At Bonga, this was not altered due to sites and coffee trees. Hydraulic conductance in the various parts of coffee plant did not alter within sites, except for the significant variations ($P < 0.05$) in the hydraulic conductance of lateral branches and reduced stem length (30 cm long). Accordingly, Harenna coffees exhibited significantly higher results in contrast to Bonga and Yayu forest. And Berhane-Kontir had intermediate stem results (Table 2). In other words, the magnitude of changes between whole-shoot and stem-cut conductance varied among populations. Consequently, coffee trees from Harenna (41%) and Bonga (54%) depicted high percent increments as compared to Berhane-Kontir (15%) and Yayu (18%) forests. This was positively related to stem size and vegetative growth responses of coffee trees under the heterogeneous forest environments.

At all sites, higher conductance was measured in whole-shoot than root part, though the percent share varied among the coffee populations. As a consequence, the contribution of root was high in Harenna (46%) and Bonga (44%) as compared to Berhane-Kontir (37%) and Yayu (33%) (Figure 1). In addition, whole-plant hydraulic conductance showed increasing patterns with the reduction of shoot components. The greatest increment over whole-shoot conductance was recorded with leaf defoliation with the values ranging from 21 to 35% at Harenna and Berhane-Kontir, respectively. This was in contrast to a slight reduction at Bonga due to leaf defoliation. Moreover, consecutive removal of fruits and petioles was noticed to enhance conductance with the least (5%) and maximum (16%) percent increments over the whole-shoot conductance determined from tree at Yayu and Berhane-Kontir forests, respectively (Figure 2). Coffee trees from Berhane-Kontir and Harenna forest stands revealed high hydraulic conductance both in root and whole-shoot as compared to Bonga and Yayu populations. The overall mean whole-plant hydraulic conductance was significantly ($P < 0.05$) higher at Harenna and Berhane-Kontir as opposed to the lowest value for Bonga forest. Hence, the results (Figure 3) followed the order of Harenna (2.69×10^{-4} kg s^{-1} m^{-2} Mpa^{-1}) > Berhane-Kontir (2.30×10^{-4} kg s^{-1} m^{-2} Mpa^{-1}) > Yayu (1.36×10^{-4} kg s^{-1} m^{-2} Mpa^{-1}) > Bonga (1.13×10^{-4} kg s^{-1} m^{-2} Mpa^{-1}).

Table 1. ANOVA for hydraulic conductance in root and shoot component parts of wild coffee trees (Nested design).

Source	Df	Root	Whole-shoot	Leaf	Petiole/fruit	Branch	Stem-cut
Total	3						
Pop	3	Ns	Ns	Ns	Ns	Ns	**
Site	8	Ns	Ns	Ns	Ns	*	*
Error	12						

Table 2. Variations in hydraulic conductance ($K_h x 10^{-4}$ kg s^{-1} m^{-2} Mpa^{-1}) and stem conductivity (k_h x 10^{-4}kg $s^{-1}m^{-1}$ Mpa^{-1}) of wild coffee trees at the four montane rainforests Ethiopia. Data are means ± SD.

Plant part	Harenna	Bonga	B-Kontir	Yayu	Pr>F
Root system	1.76±0.65	0.62±0.05	1.57±0.73	0.78±0.28	Ns
Whole-shoot	2.21±1.03	0.81±0.26	3.09±1.66	1.60±0.33	NS
Leaves	1.75±0.71	0.98±0.09	2.01±1.57	1.09±0.20	NS
Fruits and petioles	1.59±0.51	0.85±0.31	1.59±0.86	1.01±0.21	NS
Branches	2.49±1.04	1.28±0.62	2.06±0.57	1.30±0.34	NS
Stem cut (30-cm)	6.33±1.22a	2.24±1.24b	3.50±1.00ab	2.40±0.39b	**
Stem conductivity	1.73 ±0.37a	0.90±0.38 b	1.16±0.34ab	0.82±0.26b	**

*Ns = Not significant; *significant at P < 0.05 and **significant at P < 0.01.*

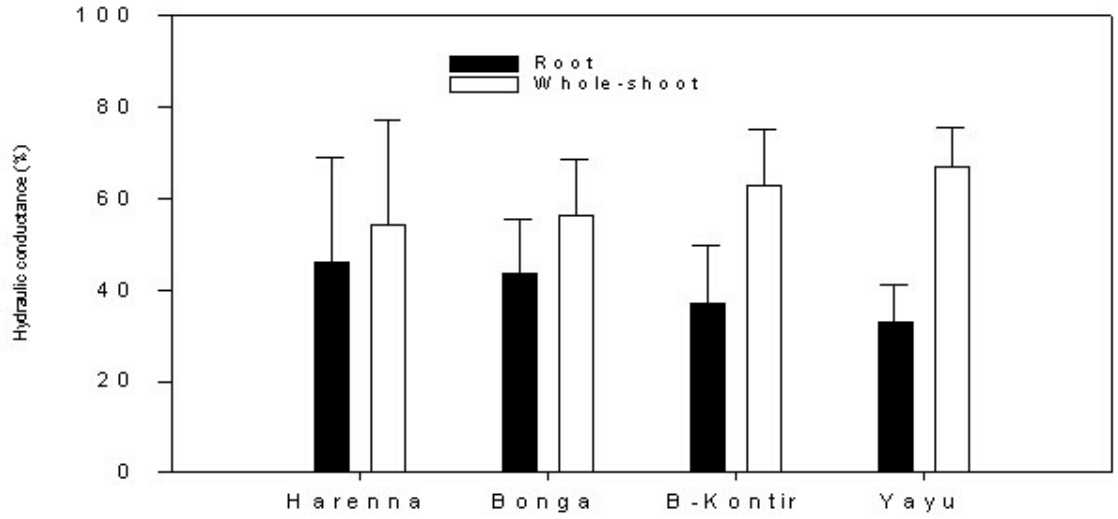


Figure 1. Percent whole-plant hydraulic conductance in root and whole-shoot of coffee trees of the four montane forests

The analysis of variance for hydraulic conductance of root and shoot components also revealed insignificant variations within sites of each population (Table 2). However, relatively high results of root, whole-shoot and leaf conductance were measured for the same sites of each population. These included PIS2, PIIS3, PIIIS3 and PIVS2 when compared to the other respective sites. This pattern was found to shift with the removal of fruits and leaf stalks, maximum conductance measured at PIS3, PIIS3, PIIIS3 and PIVS2. At Yayu, whole-plant conductance decreased from PIVS1 to PIVS3, which may be related to the reduced growth performances of coffee trees with increased shade covers and plant density (Taye et al., 2004). At Bonga, significant site variations were observed in hydraulic conductance due to removal

of all plagiotropic branches and reduced main stem length. Thus, significantly lower values were measured at PIIS1 as opposed to the value at PIIS3. Unlike the variations among populations, within site differences in main stem specific hydraulic conductivity was comparable and the results were in consistence with the stem conductance patterns at Harenna and Bonga. Conversely, it was reduced at Berhane-Kontir (PIIIS3) and increased at Yayu (PIVS3) due mainly to the more and less favoured shoot growths at the two respective sites (Figure 3). The results of main stem diameter and main stem hydraulic conductance showed the same pattern for the wild coffee populations. Consequently, the results were higher for Harenna followed by Berhane-Kontir coffee trees. These were least for Bonga and Yayu coffees (Figure 4).

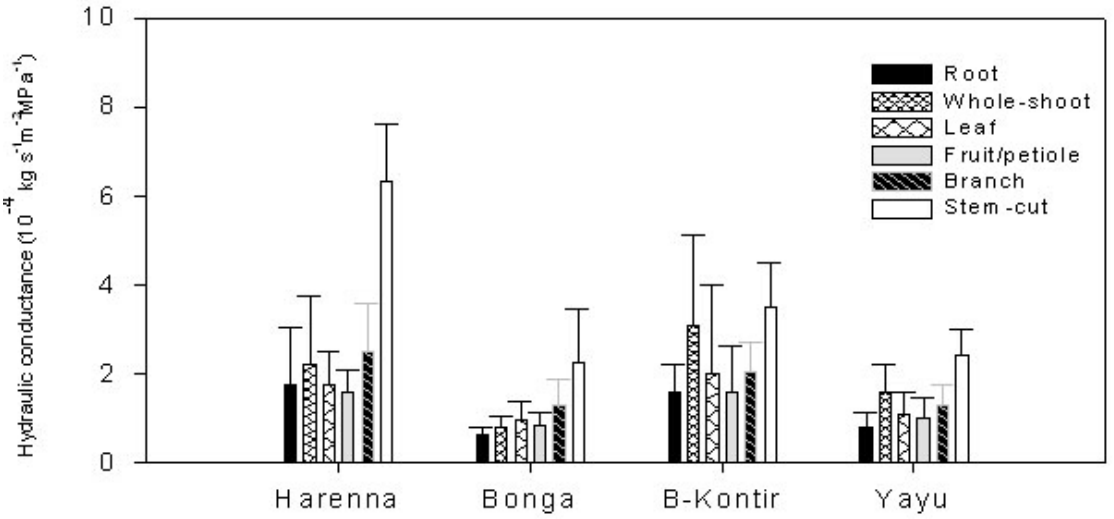


Figure 2. Hydraulic conductance in segments of the wild coffee tree in the four montane rainforests of Ethiopia.

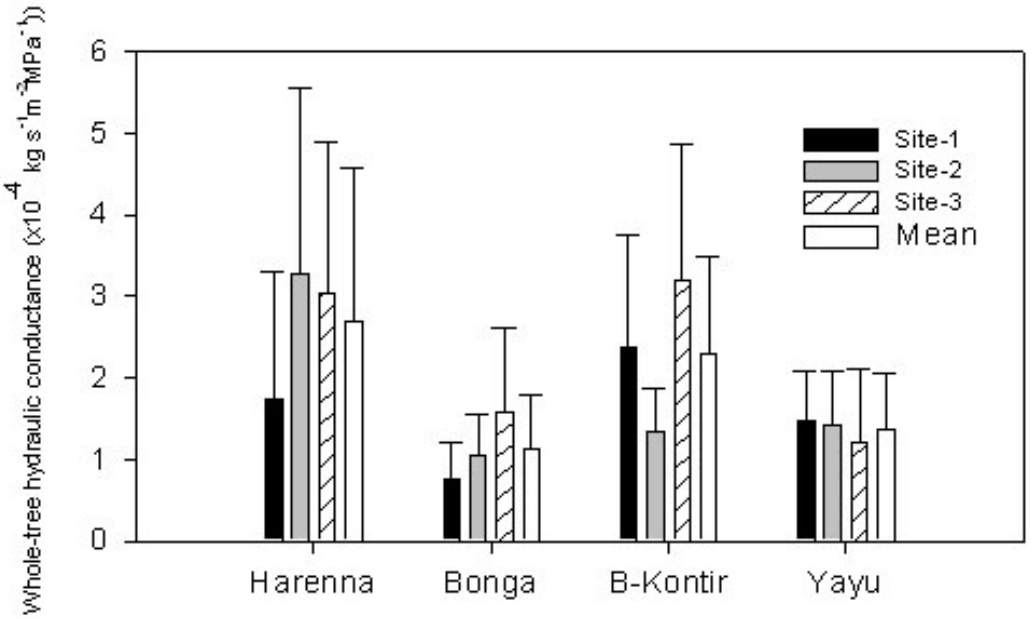


Figure 3. Variations in whole-plant hydraulic conductance of coffee trees within sites of each montane rainforest of Ethiopia.

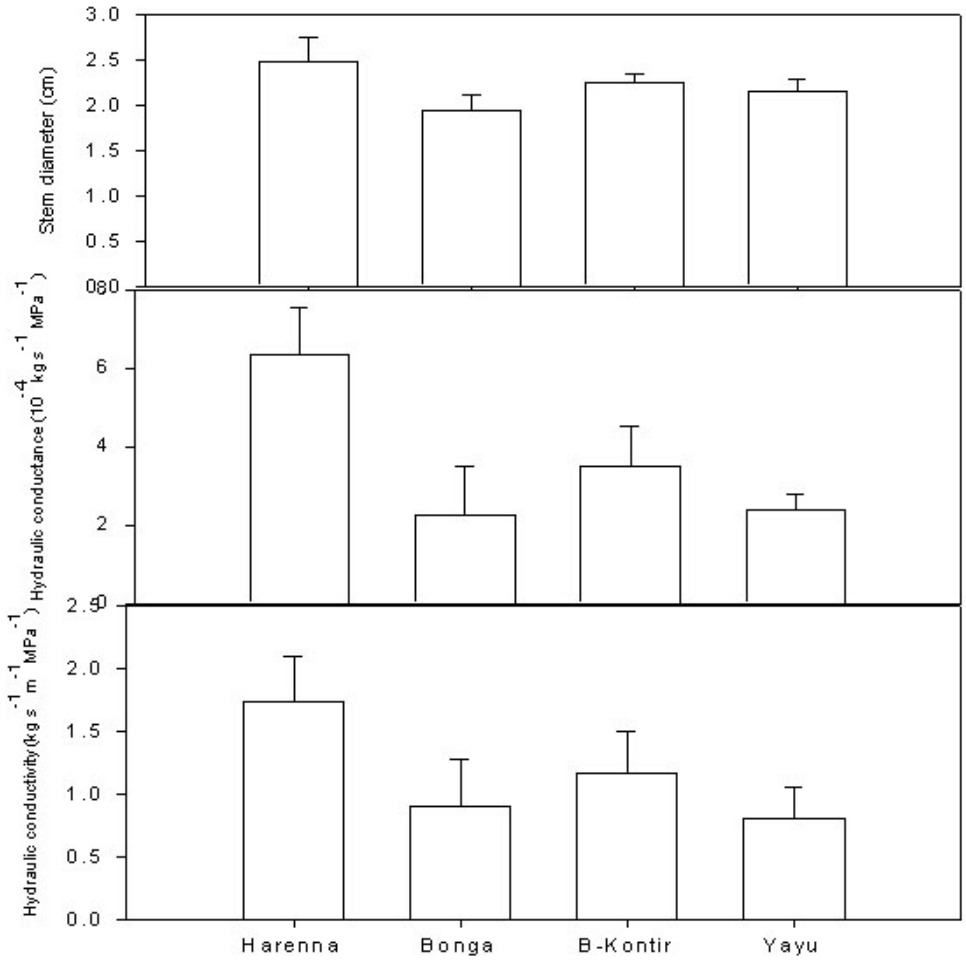


Figure 4. Diameter of main stem cut and hydraulic conductance of wild coffee trees grown at the four montane rainforests of Ethiopia.

DISCUSSION

The difference in the intensity of light interception and photosynthetic rates could probably be attributed to the variations in growth vigor, hydraulic architectural attributes and hence, hydraulic characteristics of wild coffee trees in forest ecology. This is in line with the results of the morphological diversity of the wild Arabica coffee populations (Taye et al., 2004). The differences in water flow patterns within a relatively saturated main stem could suggest the inherent growth variability and impacts of environmental factors, particularly between the geographically distant natural forests. The enhanced water transport in stem segments at Berhane-Kontir and Harenna sites could indicate the high water use rates, although low leaf conductance can limit water use. The mechanism to explain this follows from Ohm´s law analogue for water flow in plants (Tyree, 2003; Yang and Tyree, 1992).

At all the study sites, particularly at Harenna and Bonga the contribution of root conductance to the whole-coffee tree was lower than that of whole-shoot conductance. Consequently, tree hydraulic conductance on either leaf area or dry weight basis might follow the same trend as elucidated by Tyree (2003). The capacity of stem water storage status could demonstrate that the coffee trees might have experienced some degree of drought stress during the past recurrent drought. Therefore, the hydraulic condition of forest grown coffee tree seems to be influenced by multi-variables, which suggests further investigations. The present finding was comparable to other reports on other tropical forest (Borchert, 1994; Lawton, 1984; Sobrado,

2003). According to Lawton (1984), wood density values are the measures of mechanical support and appear to be inversely related to the rates of growth and mortality. Wagner et al. (1998) found that species with heavier mechanical stress have been associated with relatively low stem specific hydraulic conductivity. In addition, the authors have reported twofold higher values of leaf specific conductivity in pioneer compared to forest species. However, Sobrado (2003) reported that specific hydraulic conductance was species-specific independent of the differences in wood density.

CONCLUSIONS

Wild coffee trees of Harenna and Berhane-Kontir rainforests revealed high root and whole-shoot hydraulic conductances as compared to Bonga and Yayu coffee trees. This indicates the diversity in growth architecture, water use efficiency and thus, productivity could vary among and within wild Arabica coffee populations. It can be therefore concluded that wild Arabica coffee populations should be conserved and wisely utilized under their respective original habitats of the montane rainforests of Ethiopia. However, additional investigations are required, among others, on the contributions and influences of climatic variables (altitude, soil, rainfall, temperature) on yield and quality traits of Arabica coffee gene pools in its center of origin and diversity, Ethiopia.

REFERENCES

Borchert R. 1994. Water status and development of tropical trees during seasonal drought. Trees 8: 115-125.

Lawton R. O. 1984. Ecological constraints on wood density in a tropical montane rainforest. Am. J. Bot 71: 261-267.

Martinez V. J., Part E., Oliveras I. and Pinol J. 2002. Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia 133: 19-29.

Sobrado M. A. 2003. Hydraulic characteristics and leaf water use efficiency in trees from tropical montane habitats. Trees 17(5): 400-406.

Sperry J.S. Donnelly J.R. and Meinze F.C. 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell and Environment 11: 35-40.

Taye K., Burkhardt J. and Goldbach H. 2004. Ecophysiological Variability of Forest Arabica Coffee Populations in Hydraulic Characteristics Along a Climatic Gradient in Ethiopia: Morphological and physiological variability. Proceedings of the 20th International Conference on Coffee Science (ASIC), 11-15 October 2004, Bangalore, India, 929-939.

Tyree M.T and Dixon M.A. 1986. Water stress induced cavitation and imbolism in some woody plants. Phsiol Plant 66: 397-405.

Tyree M.T. 2003. Hydraulic limits to tree performance: transpiration, carbon gain, and growth in trees. Trees 17:95-100.

Tyree M.T., Sinclair B., Lu P. and Granier A. 1993. Whole shoot hydraulic resistance in Quercus species measured with a new high-pressure flowmeter Ann Sci For 50: 417-423.

Tyree M.T., Yang S., Cruiziat P. and Sinclair B. 1994. Novel methods of measuring hydraulic conductivity of tree root systems and interpretation using AMAIZED. Plant Physiol. 104: 189-199.

Wagner K.R, Ewers F.W. and Davis S.D. 1998. Tradeoffs between hydraulic efficiency and mechanical strength in the stems of four co-occurring species of chaparral shrubs. Oecologia 117: 53-62.

Yang S. and Tyree M.A. 1993. Hydraulic resistance in *Acer saccharum* shoots and its influence on leaf water potential and transpiration. Tree Physiology 12: 231-242.

Carbon Sequestration in Coffee Agroforestry Plantations of Central America

JM. HARMAND[1], K. HERGOUALC'H[1,2], S. DE MIGUEL[3], B. DZIB[2], P. SILES[2], P. VAAST[1,2]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), TA 10/D, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France
[2]Centro Agronómico Tropical de Investigación y Enseñensa (CATIE), Departamento de Agroforesteria, 7170 Turrialba, Costa Rica
[3]Forest Technology Centre of Catalonia (CTFC), s/n-25280 Solsona, Spain

SUMMARY

The potential of coffee agroforestry (AGF) systems to act as a sink for carbon (C) is of high interest. Coffee farmers consider with interest the conversion of their coffee monoculture into AGF systems as an alternative to face the economical crisis of coffee prices, through diversification (e.g. timber production), production of high quality coffee and payment of incentives for environmental services such as C sequestration. For the last five years, research was undertaken on the potential of shade trees introduced in coffee (*Coffea arabica*) plantations in Central America to increase plant biomass and litter, and hence C sequestration. The work focused on gathering data from selected coffee systems (with or without shade trees) in long term experiments and coffee farms of Costa Rica. Finally a database on C stored in soil and plant biomass of coffee AGF systems in Central America was developed using published information and data collected in experiments and coffee farms. Compared to the amount of C in aerial phytomass (biomass + litter) of 7 year old full sun coffee systems, the total C in aerial phytomass of coffee systems shaded by *Eucalyptus deglupta* (110 shade trees ha^{-1}) or by *Inga densiflora* (280 shade trees ha^{-1}), was multiplied by 2.5. For approximately a ten year period, results from our experiments and published literature showed that the conversion of coffee monoculture to AGF system resulted in an additional mean annual increment in aerial phytomass varying from 1 t C ha^{-1} y^{-1} in the case of regulated shading by *E. poeppigiana*, to (1.7-3.1) C ha^{-1} y^{-1} in the case of shade timber tree. Depending on the derived products (fuel wood for coffee stems and *Inga* species; pallets, logs, etc for timber species) and their life span, various wood production and harvesting scenarios in coffee AGF systems can be considered with respect to C sequestration.

INTRODUCTION

The potential of coffee AGF systems to act as a sink for carbon (C) is of high interest. Coffee farmers consider with interest the conversion of their coffee monoculture into AGF systems as an alternative to face the economical crisis of coffee prices, through diversification (e.g. timber production), production of high quality coffee and payment of incentives for environmental services such as C sequestration.

This report presents some results from research undertaken during the last five years on the potential of shade trees introduced in coffee (*Coffea arabica*) plantations in Central America to increase plant biomass and litter, and hence C sequestration. The work focused on gathering data from selected coffee systems (with or without shade trees) used as long term experiments and inventories of tree cover in coffee farms of Costa Rica. Then a database on C

stored in soil and plant biomass of coffee AGF systems in Central America was developed using published information and data collected in experiments and coffee farms.

MATERIAL AND METHODS

In a few targeted coffee systems, the quantification of aerial tree biomass was generally undertaken developing allometric relationships between diameter at breast height: 1.30 m and stem, branches, leaves or total tree biomass. Random samplings were used to evaluate the biomass of leguminous service trees regularly pruned (*Erythrina poeppigiana*), coffee and litter layer. Dry matter values were multiplied by a conversion factor of 0.48 to obtain C data. For two targeted AGF systems, a full sun coffee plot with the same date of establishment and similar agricultural management as the shaded plot was used as a control. The first studied system was a heavily fertilized coffee plantation receiving 180 kg N ha^{-1} y^{-1} and shaded by *Eucalyptus deglupta* of 7 year old and planted at a density of 110 trees ha^{-1} (De Miguel, 2004). This system was located on a farm (Verde Vigor SA) in the hot low pacific southern zone of Costa Rica (altitude of 600 m, mean annual temperature > 23 °C and average annual rainfall of 2700 mm). The soil was a fine textured Acrisol (Ultisol) derived from sedimentary rocks rich in mafic materials.

The second studied AGF system was a highly fertilized coffee plantation (5000 plants ha^{-1}) receiving 250 kg N ha^{-1} y^{-1} and shaded by *Inga densiflora* of 7 year old and planted at a density of 278 trees ha^{-1}, located on the experimental station of ICAFE (Coffee Institute of Costa Rica) in Barba de Heredia in the Central Valley, a cool medium altitude zone of Costa Rica (altitude of 1180 m, mean annual temperature < 22 °C and average annual rainfall of 2500 mm). The soil was a fine textured Andisol derived from volcanic ash.

In 2003, inventories of tree cover were performed in coffee farms of Costa Rica in order to evaluate tree aerial biomass. The timber tree species studied were *Cordia alliodora* in the hot low-altitude region of La Suiza, *Terminalia amazonia* in the hot low-altitude region of Pérez Zeledon and *Eucalyptus deglupta* in the cool medium-altitude zone of Grecia**.**

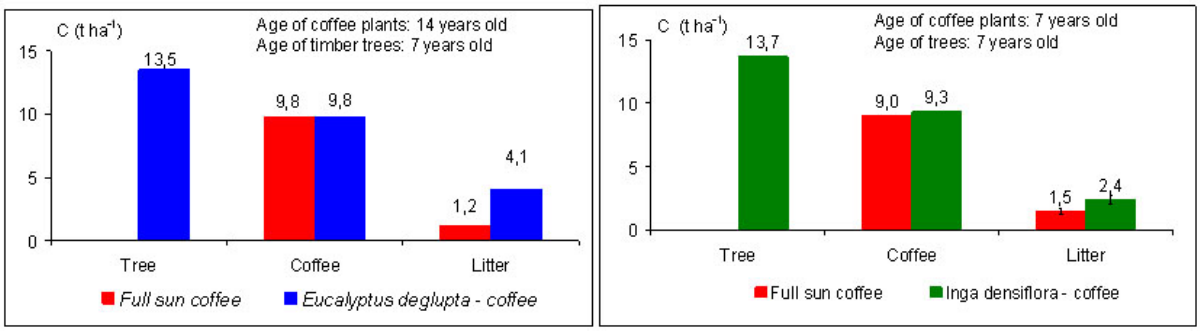
Finally a database on C stocks and accumulation rates was built. It is based on 21 studies located principally in Latin America with reference to 100 different types of coffee plantations.

RESULTS AND DISCUSSION

Compared to the amount of C in aerial phytomass (biomass + litter) in full sun coffee systems (10.5 to 11 t C ha^{-1}), the total C in aerial phytomass in coffee systems shaded by *Eucalyptus deglupta* (110 shade trees ha^{-1}) or by *Inga densiflora* (280 shade trees ha^{-1}), was multiplied by 2.5 (27.4 and 25.4 t C ha^{-1} respectively) after 7 years of inclusion of the shade tree. The shade tree and the litter layer accounted respectively for 82-92% and 8-18% of the additional C in aerial phytomass (+14.9 to 16.4 t C ha^{-1}). These C accumulation values accounted for an additional mean annual increment of +2.1-2.3 t

C ha^{-1} y^{-1} in comparison to coffee monoculture.

Depending on the derived products (fuel wood for coffee stems and *Inga* species; pallets, logs, etc for timber species) and their life span, various wood production and harvesting scenarios in coffee AGF systems can be evaluated with respect to C sequestration (Table 1).



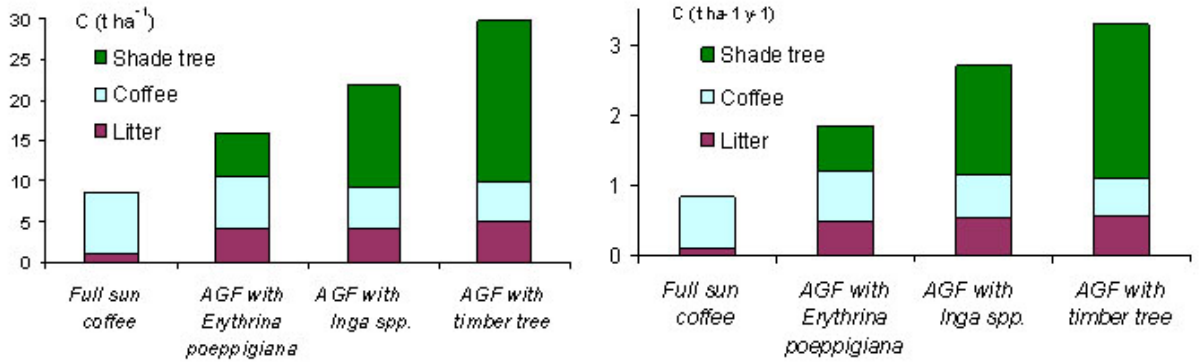
a **b**

Figure 1. Carbon accumulation in aerial biomass and litter (t ha^{-1}) of *Coffea arabica* systems in Costa Rica. a) full sun coffee and shaded coffee with *Eucalyptus deglupta*: b) full sun coffee and shaded coffee with *Inga densiflora*.

Table 1. Carbon fate (t C ha^{-1}) in wood products harvested in 7-year-old coffee systems.

Products	Coffee systems			
	Coffee monoculture	Coffee – *Erythrina poeppigiana*	Coffee – *Inga spp.*	Coffee – *Eucalyptus camaldulensis*
Aerial biomass	10	16	25	27
Fuelwood[1](coffee stems)	4	4	3.6	3.6
Fuelwood[2] (tree stems or branches)	-	-	9	1.4
Pallets [3]	-	-	-	3.4 (5-8 years)
Logs [4]	-	-	-	6.6 (15-25 years)

[1]Coffee stems accounted for 40% of aerial coffee biomass. [2]Stems or branches in the case of Inga, only branches in the case of eucalyptus. [3]With the logging and pallets processing scenarios used in the Verde Vigor farm, 36% of the total eucalyptus stem (70% of aerial biomass) composed the final product with a 5 to 8 year life span.[4]For small constructions 70% of the eucalyptus stem could be used with a 15 to 25 year life span.



Mean age:
10years 9 years 7 years 9 years 10 years 9 years 7 years 9 years

a) **b)**

Figure 2. a) Carbon accumulation (t ha^{-1}) and b) mean annual increment (t ha^{-1} y^{-1}) in aerial phytomass in coffee monoculture and different coffee AGF systems, Synthesis from 21 studies reported in the C data base (16 plots with coffee monoculture, 13 plots with coffee - *E. poeppigiana*, 5 plots with coffee - *Inga sp.* And 138 plots with coffee shaded by timber tree).

Inventories of tree cover performed in coffee farms of Costa Rica showed that the average total aerial biomass was for *C. alliodora*: 37 t C ha^{-1} at a mean density of 184 trees ha^{-1} and 13 years of age (mean annual increment of 2.8 t C ha^{-1} y^{-1}); for *T. amazonia*: 25 t C ha^{-1} at a mean density of 230 trees ha^{-1} and 8 years of age (mean annual increment of 3.1 t C ha^{-1} y^{-1}); for *E. deglupta*: 13 t ha^{-1} at a mean density of 78 trees ha^{-1} and 8 years of age (mean annual increment of 1.7 t C ha^{-1} y^{-1}). Carbon stocks of coffee AGF systems highly depended on shade tree species and their planting density. Mean values from the Carbon database based on 21 studies indicate that 80% of the C system was located belowground in soil organic matter and roots, and 20% aboveground of which at least 50% is in the shade trees.

For a ten year period, mean values from the C data base (Figure 2) shows that the conversion of coffee monoculture to AGF system resulted in a additional mean C increase in aerial phytomass varying from 1 t C ha^{-1} y^{-1} in the case of regulated shading by *E. poeppigiana*, to (2.3-2.6) C ha^{-1} y^{-1} in the case of associated coffee with *Inga* sp. or timber tree.

CONCLUSION

Although change in soil C and GHG emissions (N_2O + CH_4) were not taken into account in the analysis, the value of C-CO_2 sequestration in aerial phytomass (biomass and litter) by the coffee AGF system showed the interest of coffee AGF management for global warming mitigation. For approximately a ten year period, results from our experiments and published literature showed that the conversion of coffee monoculture to AGF system resulted in an additional mean annual increment in aerial phytomass varying from 1 t C ha^{-1} y^{-1} in the case of regulated shading by *E. poeppigiana*, to (1.7-3.1) C ha^{-1} y^{-1} in the case of shade timber tree depending on tree species and their planting density. In addition to maintaining a forest tree cover in the coffee plantation, various wood production and harvesting scenarios in coffee AGF systems can be considered with respect to C sequestration depending on the derived products and their life span.

AKNOWLEDGEMENTS

The authors thank the European Commission (INCO project CASCA, ICA4-CT-2001-10071, www.casca-project.com) and the Science and Cultural Cooperation Centre of the French embassy in Costa Rica for supporting part of the operational costs of this research.

REFERENCES

De Miguel Magaña S., Harmand J.M., Hergoualc'h K., 2004. Cuantificación del carbono almacenado en la biomasa aérea y el mantillo en sistemas agroforestales de café en el suroeste de Costa Rica. Agroforestería en las Américas, 41-42: 98-104.

Photosynthesis and Water Use of Wild *Coffea arabica* Populations along Climatic Gradient *in situ* and under Stress in an *ex situ* Experiment

A. M. BEINING[1], J. BURKHARDT[2]

[1]Center for Development Research (ZEF), University of Bonn, Germany
[2]INRES, Department of Plant Nutrition, University of Bonn, Germany

SUMMARY

Ethiopa is the center of genetic diversity of *Coffea arabica*. The CoCE project (Conservation and use of wild populations of Coffea arabica in the montane rainforests of Ethiopia) aims at assessing the diversity and the economic value of the Ethiopian coffee gene pool and developing concepts of model character for conservation and use of these genetic resources. Thereby it focuses on traits inherent to the wild coffee populations and their possible usefulness for breeders. In our study, we compared the water use of different populations of four wild *Coffea arabica* populations along a rainfall gradient in Ethiopia. Measurements were carried out *in situ*, as well as in an extended experiment at Jimma Agricultural Research Center, where seeds of the four original sites were used to raise seedlings. Measurements tackled all relevant parts of water transport, i.e. soil conditions, the hydraulic conductivity of the root and the shoot system, stomatal control of gas exchange, and the atmospheric demand for water vapour. Water use efficiency *in situ*, measured by isotopes as well as by gas exchange, was found to be higher in the dry than in the wet season, and on dry sites compared to wet sites, thus reflecting the availability of water. Unexpectedly, no correlation with the rainfall gradient was observed neither when measuring the hydraulic system *in situ*, nor when looking at the reaction of seedlings to drought and radiation stress under *ex situ* conditions. Plants from the driest site, Harenna, showed highest transpiration and production. While the root system of Harenna trees was the most extensive compared to the other sites, also the hydraulic system showed the highest efficiency for water transport, and stomatal behaviour was liberal. As a consequence these plants were the most vulnerable ones to drought stress, and eventually they were the first of all to be damaged and to die during an extended drying period. Plants from the wettest site were much more conservative in terms of water use and withstood much longer against drought stress, at the same time having lower productivity. The results showed that the precipitation gradient was not reflected in a simple way by drought stress tolerance of trees. It can be concluded that populations follow different strategies when conserving or spending water under drought stress conditions. Harenna populations might find their way out of serious droughts by putting their main effort into seed production (and thus conserving the population as a whole), while trees from other populations seem to be more oriented to ensure survival of the individuals. The study shows that different wild coffee populations have interesting traits for breeders, many of them still likely to be unknown. The results also underline that conserving the Ethiopian mountaineous rain forests is the only way to conserve these traits.

INTRODUCTION

With abundant evidence that environments have shifted dramatically in climatic conditions in the last decades, drought stress became serious constraints on *Coffea arabica* production worldwide (DaMatta and Ramalho, 2006) Hence, developing crop plants with inbuilt drought

resistance mechanism can be accounted for the most compromising approach to realise sustainable economic production in drought-prone environments and therefore an important goal by breeders targeting these more marginal areas. Despite considerable research on ecophysiology of arabica coffee (Rena et al., 1994; DaMatta and Ramalho, 2006), there is still insufficient knowledge on the mechanisms underlying response to drought. Conventional breeding has met only with little success in the development of drought resistant coffee, mainly constraint by an extreme narrow genetic pool of modern cultivars.

These constraints attract interest to wild relatives of *Coffea arabica* that can be found in the Afromontane rainforests of Ethiopia, its primary center of origin and center of genetic diversity (FAO, 1968). The wild populations occurring there are believed to show high diversity attracting interest as a promising source to increase drought resistance. Due to increasing loss of forest areas natural stands of wild arabica populations are diminishing that result in a permanent loss of coffee genetic resources (Gole et al., 2002). Therefore, action has urgently to be taken to conserve the unique diversity of arabica coffee found in Ethiopia. Therefore, the aim of this study is to provide an in-depth understanding of the ecophysiological diversity in wild populations of *Coffea arabica*, to assign conservation areas of the existing diversity and to give recommendations for optimal concepts of conservation of the endangered wild populations of *Coffea arabica*.

MATERIAL AND METHODS

In order to provide an understanding of the mechanism underlying resistance for water deficit, four distant wild populations of arabica coffee were chosen which span a broad precipitation gradient (Figure 1a).These provenances were used as seed sources for an ex-situ study in Jimma, Ethiopia (Figure 1b). Two irrigation levels as well as two light regimes were imposed over a 17-days period. Throughout the experiment plants survival and physiology were monitored by measuring leaf water relations as well as gas exchange parameters.

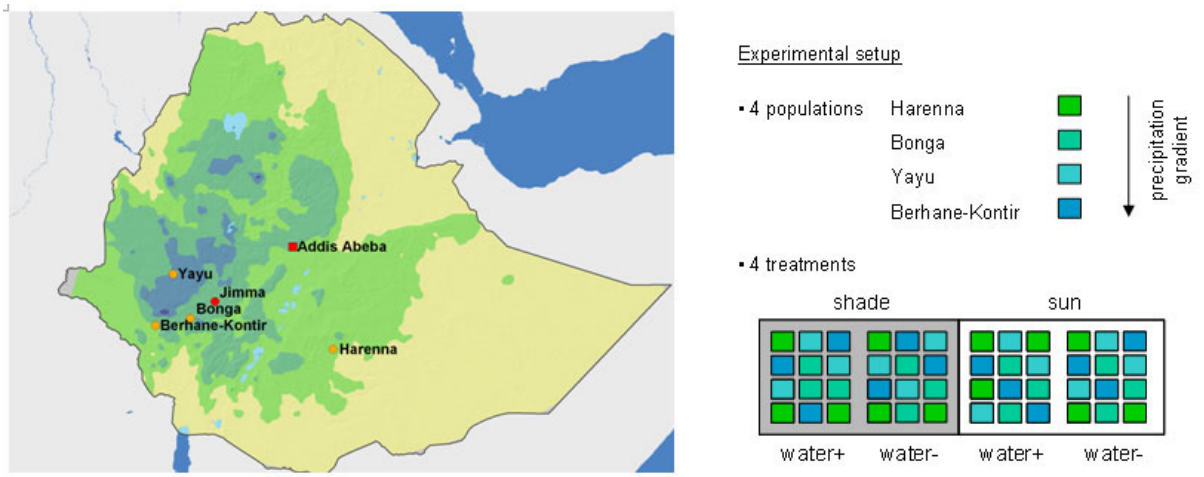


Figure 1. (a) Wild coffee populations across a precipitation gradient, (b) design of the ex-situ study

A 'drought resistance index' (*S*) was calculated for each measured parameter using the relationship $S = (1 - Y_S/Y_P) / (1 - X_S/X_P)$, where Y_S is the mean performances of a certain population under drought stress, Y_P the mean performance of the population under irrigated conditions and X_S and X_P are the mean performances of all populations under these specific treatments, respectively (Fischer and Maurer, 1987).

RESULTS AND DISCUSSION

The selected coffee populations, occupying a broad range of stands with different habitat conditions, showed considerable variability in physiological behaviour.

There was a strong linkage between gas exchange parameters and soil moisture availability (Figure 2). Whereas transpiration rate (Ev) and stomatal conductance (g_s) were maintained at quite high levels during favorable soil moisture levels, a fast and strong decrease was observed after reaching a critical soil moisture content of 20%. This threshold value revealed the maintenance of high leaf water relations under dehydrating conditions as the main drought adaptation strategy in the selected provenances. As a consequence, wild *Coffea arabica* should be accounted as a more water-saving rather than a dehydrating tolerant species. In addition, populations showed to differentiate in their drought response with differences in water use being most significant under sufficient soil moisture conditions. Harenna plants (dry site) maintained gas exchange activity nearly twice as high at treatment start compared to Berhane-Kontir (wet site). Surprisingly, the Harenna provenance showed a more rapid decline in g_s, therefore being more sensitive to soil drying. In contrast, lower transpiration and stomatal conductance of Berhane-Kontir populations at the beginning of the experiment allow to postpone the time of dehydration in contrast to Harenna populations.

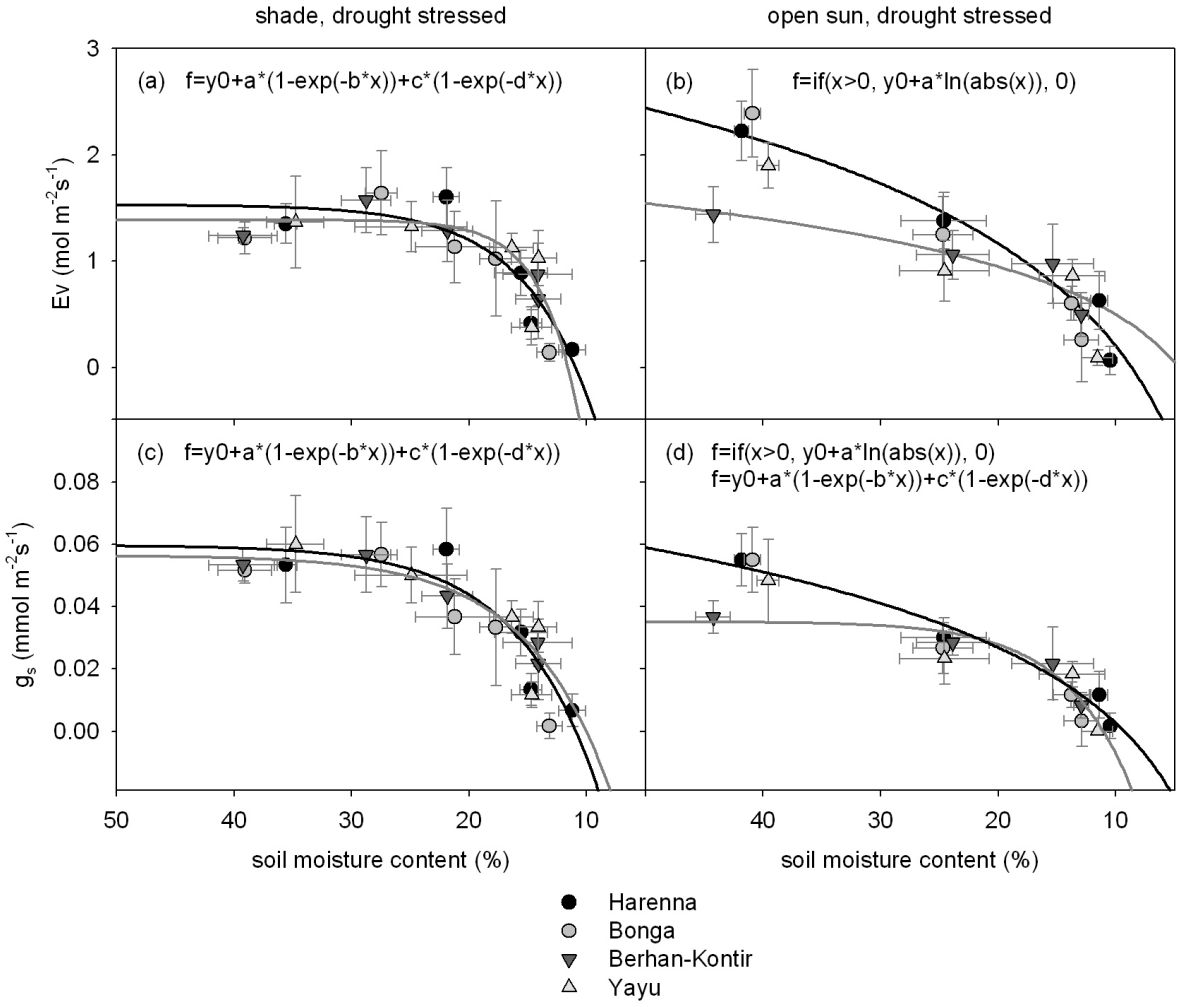


Figure 2. Relationship between daily mean transpiration rate (E, mol $m^{-2}s^{-1}$) and daily mean stomatal conductance to water vapour (g_s, $mmol^{-2}s^{-1}$) to soil moisture conditions in wild *Coffea arabica* populations. Regression lines represent the most different populations (– Harenna; – Berhane-Kontir).

The differences in ecophysiological behavior among populations were also reflected in their specific drought susceptible index (Figure 3). Ranked in increasing order of *S* in leaf water relations, plants from Berhane-Kontir had the lowest, followed by Yayu and Bonga and then Harenna that showed to be most affected by drought stress conditions. Plants originating from Berhane-Kontir sustained unfavorable water conditions during drought by maintaining a relatively high leaf water status (dessication avoidance response), whereas populations from the Harenna populations showed a tendency to a fast depletion of soil moisture followed by a remarkable aggravation of their leaf water relations.

In summary, populations behaviour under common garden conditions could not been predicted from environmental conditions of their specific natural habitats. Plants experiencing high precipitation intensity *in-situ* (Berhane-Kontir) showed good adaptation to drought conditions in the *ex-situ* study whereas the dry site (Harenna) was highly susceptible to a period of water deficit under common garden conditions.

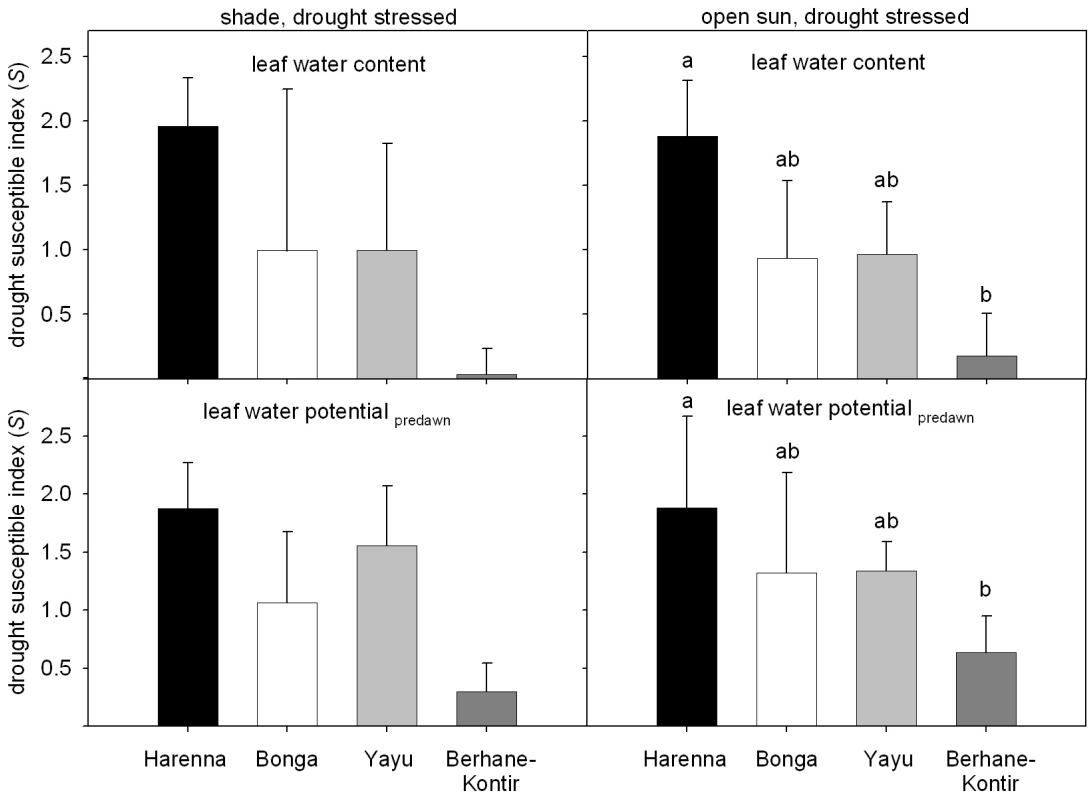


Figure 3. Drought susceptible index (S) of (a) leaf water content, (b) leaf water potential predawn and (c) leaf osmotic potential predawn of wild Coffea arabica populations at the end of drought stress period grown under shade (left panel, after 17 days) and open sun (right panel, after 13 days), respectively. Bars indicate ± SD; n = 3.

CONCLUSIONS

Wild coffee populations show diverse ecophysiological behaviour. However, plants performance measured *ex-situ* could not be predicted from environmental conditions of their natural habitat. Therefore, *in-situ* conservation is needed in order to maintain its ecophysiological diversity found in this study.

REFERENCES

Da Matta, F.M., Ramalho, J.D.C. (2006): Impacts of drought and temperature stress on coffee physiology and production: A Review. Brazilian Journal of Plant Physiology.

FAO (1968): FAO Coffee Mission to Ethiopia, 1964-65. FAO, Rome, Italy.

Gole, T.W., Denich, M., Teketay, D. Vlek, P.L.G. (2002): Human impacts on the *Coffea arabica* genepool in Ethiopia and the need for its in-situ conservation. Chapter 23. In: Ramanatha Rao, V., Brown, A.H.D., Jakson, M.T. (Eds.), Managing Plant Genetic Diversity. PGRI, Rome.

Rena, A.B., Barros, R.S., Maestri. M., Söndahl, M.R. (1994): Coffee. In: Schaffer, B. and Andersen, P.C. (Eds.) Handbook of Environmental Physiology of Tropical Fruit Crops: Sub-Tropical and Tropical Crops. Boca Raton, CRC Press, 1994.

Evaluation of Different Germination Media on Pre-germination Performance of Selected Clones of Robusta Coffee (*Coffea canephora*) in Nigeria

E. A. ADEYEMI AND R. R. IPINMOROTI

Cocoa Research Institute Of Nigeria, P.M.B. 5244, Ibadan, Nigeria

SUMMARY

Five germination media namely: riverbed sand, topsoil, sawdust, sand + sawdust (1:1 ratio) and topsoil + sawdust (1:1 ratio) were evaluated in the pre-germination of seeds of five selected robusta coffee clones (C36, C111, C90 (Quillou variety), T1049 (Java) and M10 (Niaolli)). Percent emergence, seedling vigour and seedling infection (disease) were recorded at 11 and 13 weeks after sowing (WAS). Percent emergence in sawdust (63.2) was significantly ($P < 0.05$) higher compared to other media with values 49.3, 46.1, 42.4 and 36.8 for sand, topsoil + sawdust, sand + sawdust and topsoil respectively. Seedling vigour was significantly better in sawdust while topsoil was found to significantly predispose seedling to infection. Overall performance was best in sawdust and least in topsoil. Clone M10 gave the best seedling emergence, growth vigour and less disease incidence compared to other clones.

INTRODUCTION

Coffee production ranked second to cocoa amongst cash crops in Nigeria. Its propagation is commonly by cuttings across the globe (Cambrony, 1992; Rene, 1992) but due to short supply of cuttings (Adeyemi et al., 2004) as a result of insufficient manpower and lack of technical know-how by farmers, coffee farmers in Nigeria depended mainly on propagation by seed.

Coffee is a slow grower crop and the seeds under-go pre-nursery stage for pre-germination before they are potted in topsoil medium. River sand is the medium used for pre-germination of coffee seeds. Pertinently, river sand is bulky and not readily available all year round and in all places, especially during the raining season when river tide is always high. The bulkiness makes cost of transportation to be high and not affordable by the poor-resource coffee farmers (Agbede and Kalu, 1995).

Sawdust is a common waste of sawmill industry across coffee growing belt in Nigeria. It is used for the pre-germination of kola seeds (Adenikinju et al., 1989) in the pre-nursery stage before they are potted in topsoil filled polythene bags. Presently sawdust is a menace in the sawmill industry and it is been burnt to create space for other generated products and by-products in the wood industry. It will be of interest if sawdust could be converted to some useful agricultural purposes. This would reduce the menace of the waste at the sawmill industry as well as the cost of river sand transportation to nursery sites by farmers. Hence the need to investigate the use of sawdust compared to other pre-germination media for pre-germination of coffee seeds in the nursery.

MATERIALS AND METHODS

Five growth media (river sand, topsoil, sawdust, sand + sawdust and topsoil + sawdust) were prepared into beds in the nursery at 75 cm x 30 cm x 15 cm dimension. The sand + sawdust and topsoil + sawdust mixtures were at 1:1 ratio and the sawdust was cured for 6 months before usage. Coffee beries were harvested from 5 coffee clones {C36, C116, C90 (Quillou variety), T1049 (Java) and M10 (Naolli)} in the germplasm plot. The fruits were depulped in water by hand immediately after harvesting and parchment seeds were air dried for 7 days on racks in the laboratory room. All malformed, small and damaged seeds were removed. The seeds were sown at 2.5 cm x 2.5 cm at 2.5cm depth and 25 seeds were sown per clone per medium in 3 replicates. Watering was done thrice weekly at morning hours of the day.

Seedling emergence and disease counts were carried out and seedling vigour was based on plant diameter values taken with vernier caliper in mm at the soil surface level at 11 and 13 weeks after sowing (WAS). Data obtained were analysed using analysis of variance (ANOVA) and mean differences were separated using Duncan multiple range test (DMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

Seedling emergence was highest in the sawdust medium (Table 1) with 58.4% at 11 WAS and 63.3% at 13 WAS, while it was least in topsoil medium with 34.4% at 11WAS and 36.8% at 13 WAS. Coffee seedlings raised with sawdust had the highest vigour at both 11 and 13 WAS and least in the topsoil medium. Disease incidence was more on coffee seedlings raised with topsoil compared to other growth media.

Table 1. Effect of pre-germination media on performance of coffee seedlings at 11 and 13 weeks after sowing (WAS).

Growth	Emergence (%)		Disease incidence (%)		Plant diameter (mm)	
Media	**11 WAS**	**13 WAS**	**11 WAS**	**13 WAS**	**11 WAS**	**13 WAS**
Sand	44.3_{ab}	49.3_{b}	0.07_{b}	0.07_{c}	5.0_{ab}	8.7
Topsoil	34.4_{b}	36.8_{c}	0.47_{a}	1.27_{a}	3.5_{c}	6.0_{c}
Sawdust	58.4_{ab}	63.2_{a}	0.07_{b}	0.07_{c}	5.8_{ab}	10.1_{a}
Sand + sawdust	38.9_{ab}	42.4_{bc}	0.00_{b}	0.27_{b}	4.0_{bc}	7.7_{bc}
Topsoil + sawdust	45.1_{ab}	42.4_{bc}	0.07_{b}	0.07_{c}	5.0_{ab}	7.3_{bc}

Means with the same letter are not significantly different.

The highest percentage seedling emergence was in clone M10 (Table 2). The value was 48.0% at 11 WAS and 53.7% at 13 WAS. The value was least for clone C90 (Quillou) with 41.3% at 11 WAS and 42.7% at 13 WAS. The seedling vigour was similarly best in clone M10 (Niaolli) at both 11 and 13 WAS and least in clone T1049 (Java). The observed disease incidence on the coffee seedlings was more prevailent on clone T1049 at both 11 and 13 WAS but least on clone C36 at 11 WAS and on clone C90 at 13 WAS.

The sawdust growth medium resulted in significantly ($P < 0.05$) higher coffee seedling emergence compared to the topsoil but not over other media. The better seedling emergence in the sawdust medium may be due to its loose, light and friable nature, while other media were heavy and compacted. The physical properties of the sawdust must have allowed better aeration and adsorption of water sufficient for good germination of the coffee seeds. Sand is loose but heavy and do not possess the ability to retain water adequately for optimum germination and emergence (Bissnnais and Arrouays, 1997). Topsoil could retain sufficient

water but it is heavy and compacted and could not allow adequate aeration needed for germination of coffee seeds. Since coffee requires growth medium with light, loose and friable properties for optimum germination and growth, sawdust with these properties was therefore optimum.

Table 2. Effect of clones on performance of coffee seedlings at 11 and 13 weeks after sowing (WAS)

Coffee Clone	Emergence (%)		Disease incidence (%)		Plant diameter (mm)	
	11 WAS	13 WAS	11 WAS	13 WAS	11 WAS	13 WAS
C36	43.2_a	47.2_a	0.07_a	0.67_a	4.8_{ab}	7.5_a
C111	46.1_a	47.2_a	0.13_a	0.67_a	4.3_{ab}	8.1_a
C90	41.3_a	42.7_a	0.13_a	0.60_a	5.3_a	7.9_a
T1049	36.0_a	44.8_a	0.20_a	1.07_a	3.1_b	7.0_a
M10	48.0_a	53.7_a	0.13_a	0.67_a	5.9_a	8.9_a

Means with the same letter are not significantly different.

Disease incidence was significantly ($P < 0.05$) higher on coffee seedlings raised in topsoil medium compared to other growth media at both 11 and 13 WAS. Topsoil is reported to be high in various kinds of soil microorganisms (Zhon and Everts, 2004), some of which are pathogenic in nature (Zummo and Scott, 1990; Jaime-Garcia and Cotty, 2004). The high organic matter contents of topsoil provide suitable environment and support for myriads of biochemical activities and good multiplication of soil microorganisms.

The better pre-germination performance of coffee in the sawdust growth medium, with less disease incidence compared to other growth media, suggests the need to recommend sawdust for the pre-germination of coffee seeds in the pre-nursery stage of coffee propagation from seeds in the nursery.

ACKNOWLEDGEMENT

The authors are grateful to the Executive Director of Cocoa Research Institute of Nigeria (CRIN) for permission to publish this work.

REFERENCES

Adenikinju,S.A., Esan, E.B. and A.A. Adeyemi (1989). Nursery Techniques, Propagation and Management of Cocoa, Kola, Coffee, Cashew and Tea. Progress in Tree Crop Research (2nd Ed.). pp 1-27.

Adeyemi, E.A., Omolaja, S.S. and A.O. Famaye (2004). The effect of leaf on half node stem cuttings on the propagation of Robusta coffee (*Coffea canephora*) in Nigeria. Proc. ASIC Conf.2004, India. 1038-1041.

Agede, O.O. and B.A. Kalu (1995). Constraints of small – scale farmers in increasing crop yields. Farm size and fertilizer supply. Nigerian Journal of Soil Science. 11, 139-152

Bissonnais, Y. and D. Arrouays (1997). Aggregate stability and assessment of soil crustability and erodibility. II. Application to humic loamy soils with various organic carbon contents. European Journal of Soil Science 48(1): 39-48.

Cambrony, H.R. (1992). Coffee growing. The Tropical Agriculturalist. CTA. Macmillan. 119.

Jaime-Garcia and P. Cotty (2004). Aspergillus flavus in soils and corncobs in south Texas: Implications for management of Aflatoxins in corn-cotton rotations. Plant Disease, 88(12): 1355-1371.

Rene Coste (1992). Coffee. The plant and the product. Macmillan. Pp35.

Zhon, X.G. and K.L. Everts (2004). Suppression of Fusarium wilt of watermelon by soil amendment with Hartry Vetch. Plant Disease, 88(12): 1357-1365.

Accelerated Composting of Coffee Processing Byproducts: an Organic Option for Soil Fertility Management in the Coffee Based Cropping System of Southwestern Ethiopia

S. ENDRIS

Ethiopian Institute of Agricultural Research (EIAR), Jimma Center, P. O. Box 192, Jimma, Ethiopia. E-mail: solomonendris@yahoo.com

SUMMARY

Southwestern Ethiopia is one of the biggest coffee producing regions of the country. A large volume of coffee processing byproducts, mainly coffee pulp and husk, is generated annually from wet coffee processing stations in the region. Increasing environmental concerns and interest in organic coffee production demanded the utilization of such by-products as an alternative source of plant nutrients. This experiment was conducted to address both environmental concerns and organic coffee practice by transforming such by-products into nutritionally balanced compost for use by coffee growers in the region. Two easily and cheaply available organic residues, farmyard manure and leguminous plant materials, were therefore combined in different proportions with coffee pulp and husk. The stabilization period and quality of coffee pulp and husk composts were finally evaluated using standard parameters. Piles of six cubic meters were prepared and monitored regularly. The piles were aerated manually once a week. Compost heaps with organic amendments exhibited longer thermophilic phase and lower C/N ratio, as compared to the control pile containing only coffee pulp or husk. In both coffee pulp and husk composts, piles that contained organic amendments were found better in terms of maturity time and final quality. Therefore, based on the results of this experiment, using organic amendments during coffee byproduct composting is recommended as it shortens the maturity time and improves final quality of the compost produced.

INTRODUCTION

Wet processed (mild) coffee is known to have superior quality than dry processed (hard) coffee, and hence demands higher market price. The central statistical authority (CSA, 2003) reported that in the year 2001 an estimated 32145 tons of coffee has been produced in Jimma zone alone. Moreover, in the year 2003, the Oromia farmers' cooperative union produced 98550 tons of coffee of which 30415 tons was certified organic (Fairtrade foundation producer Profile, 2006). The majority of coffee farmers' cooperatives in the Oromia farmers' cooperatives union are found in southwestern Ethiopia.

The level of awareness and production of organic coffee in Ethiopia is on the increase. As organic coffee is one of the various types of specialty coffees sold at a price higher than mainstream coffees, farmers producing organic coffee are currently encouraged by the better revenue they get. Therefore, there is a very good prospect to increase organic coffee production levels in the region. Hence, cheaply and easily available organic inputs are required to keep the sustainability and feasibility of the sector.

Coffee is known to be a heavy feeder of nutrients and hence extracts a very huge amount of the major nutrients from the soil medium annually (Table 1). Coffee tree requires sufficient

and regular supply of nutrients to produce high yield and good quality beans. Compost prepared from coffee processing byproducts mainly coffee pulp and husk can be a good source of such mineral nutrients. The nutrient content of coffee processing by-products is given below (Table 2). Methods of accelerated composting of coffee fruit waste in Mexico were discussed where a regularly aerated mixture of 40% coffee pulp, 30% sugarcane filter cake, 20% poultry litter and 10% wood chips (bulking agent) resulted in a high quality compost within 50 days (Sanchez et al., 1999). Composting is a deliberate biological and chemical decomposition and conversion of organic or plant refuse and residues for the purpose of producing humus (Muller Samann, 1997). It can also be considered as a waste management strategy, but its fertilizer value differs according to crop and climate characteristics and soil fertility and structure (Rodrigues et al., 1995).

Coffee pulp is usually disposed without any treatment and left to degrade naturally in heaps, with the uncontrolled liberation of noxious odors and nutrient loaded leachate as a consequence. When it is left for natural degradation, it may take 6 to 8 months to achieve a stabilization of the organic matter (Olguin, 1996). It is therefore believed that composting is a feasible and cheap technology in dealing with by-products of coffee processing. Composting of coffee pulp can be done with very low capital investment to produce a very high quality organic fertilizer in a short time than natural stabilization (Olguin, 1996).

Compost produced from coffee processing byproducts mainly *coffee pulp* and *husk* can be used successfully as an organic fertilizer for coffee production. A significant improvement in growth and yield of mature coffees was reported in response to coffee pulp and husk compost application (Chane, 1999).

Table 1. Approximate nutrient uptake by Arabica coffee producing 1t green beans/ha/year.

	N, kg	**P,** kg	**K,** kg
Green beans (1.0 t dry wt)	40	4	45
Pulp + Parchment (1.25 dry weight)	35	7	53
Vegetative growth	60	5	22
Total	135	16	120

Source: Korikanthimath and Hosmani 1998; Mitchell, 1988; Willson, 1985; Wrigley, 1988.

Table 2. Nutrient content of organic residues and manures.

Type	**% Dry matter**			**C/N ratio**
	N	P	K	
Coffee pulp, fresh (India)	2.4	0.5	4.2	
Coffee pulp, composted (India)	2.0	0.2	2.5	
Coffee pulp, composted (Mexico)	3.8	0.4		10
Coffee pulp, fresh (Kenya)	3.7	0.4	6.5	
Cattle manure (India)	0.4	0.2	0.2	

Source: Van Der Vossen, 2004.

MATERIALS AND METHODS

Coffee pulp was collected from wet processing plants in Jimma zone (southwestern Ethiopia). FYM (farmyard manure) was obtained from a dairy farm of Jimma College of Agriculture (Jimma University). Legume plant material (foliage) (LM) was collected from plots in Jimma

research center. Finally the common bulking agent used was top soil (30 cm depth) taken from a farm at the research center. The different ratios of each mixture are given in Table 3 and 4.

Six cubic meter piles were established and managed according to the experimental procedure. The piles were aerated once a week by manual turning. Temperature profile and moisture content of each pile was monitored and recorded throughout the composting process.

A homogenous mixture of samples was taken every week; water content was determined by drying at 105 °C. Organic carbon (Allison, 1965), total nitrogen (Furman, 1975) and pH measurements were done for each sample.

Table 3. Proportion of coffee pulp and each organic residue in each treatment (set I).

Pile type	Treatments					
	1 (Control)	2	3	4	5	6
Coffee pulp, %	90	80	70	80	70	70
FYM, %	0	10	20	0	0	10
LM, %	0	0	0	10	20	10
Top soil, %	10	10	10	10	10	10

Table 4. Proportion of coffee husk and each organic residue in each treatment (set II).

Pile type	Treatments					
	1 (Control)	2	3	4	5	6
Coffee husk, %	90	80	70	80	70	70
FYM %	0	10	20	0	0	10
LM %	0	0	0	10	20	10
Top soil %	10	10	10	10	10	10

RESULTS AND DISCUSSION

Temperature

During the first few days of composting, the temperature of each pile increased steadily (up to around 45 degrees) while the ambient temperature decreased from around 18 to below 15 degrees. After being aerated at the end of the week, an immediate fall in the temperature of each pile was noticed. Compost piles that contained higher proportion of organic residues exhibited higher temperatures along the process till stabilization was achieved. Stabilization of organic matter degradation was achieved in 45 days. From day 9 to 40 the temperature of all piles remained above 25 °C while the ambient temperature was below 20 °C. It is known that a satisfactory temperature is the best indication of successful composting. In general, during composting, temperature climbs sharply at first as the readily available (soluble) compounds oxidize, reaching a temperature of 60 & 70 °C in 1 to 3 days (Muller Samann, 1997).

Moisture profile

The liberated heat in piles with organic residues was higher than in the control pile which was indicated by the higher temperature and low moisture content and a correspondingly lower temperature and higher moisture content of piles in accelerator containing and control plots respectively (Figures 1 and 2). The moisture content of all piles was higher in the second

week than the first. The increase in moisture content in the second week was caused by rainfall. Since the composting was accomplished under natural conditions without roof and other protective structures, higher than the normally expected moisture level was recorded in the second week as a result of higher water input from natural rain. After the second week, moisture level in all compost piles decreased consistently until the ninth week. At the ninth week, piles that contained organic residues had lower moisture content than the control pile indicating the higher evolution of temperature in piles with organic residues that subsequently evaporated more water than in the control pile.

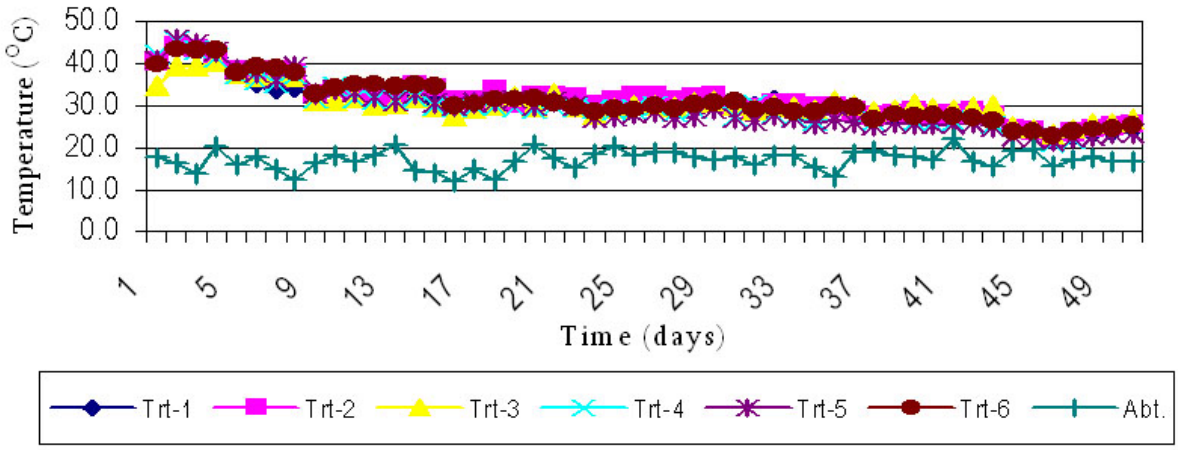


Figure 1. Temperature profile during coffee pulp compostir and with out organic accelerators.

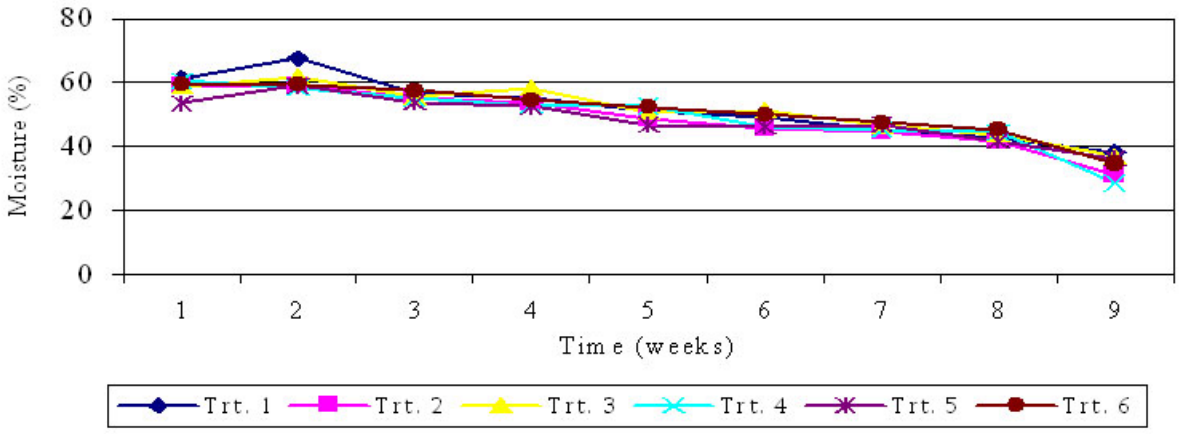


Figure 2. Changes in moisture content during coffe composting with and without organic accelerators

C/N profile and quality of the produced compost

The C/N ratio of all piles decreased at the end of composting. It is known that reduction in the C/N ratio reflects the organic matter mineralization and an adequate evolution of the microbial composting process. The C/N ratio is expected to reach minimum values when mineralization of organic matter has finished. The general quality of the compost in piles with amendments is superior to the quality found in the control pile after 45 days (Table 5), especially, in terms of total nitrogen content and C/N ratio.

Table 5. General quality of coffee pulp compost in piles with different organic residues at the end of composting (49 days).

Parameter	90% Coffee pulp (P1)	80% Coffee pulp + 10% FYM	70% CP + 20% FYM	80% CP+ 10% FYM + 10% LM	70% CP + 20% LM	70% CP + 10% FYM + 10% LM
Moisture %	38.23	30.89	36.97	28.48	36.12	34.59
pH	7.69	7.49	7.50	7.54	7.60	7.51
Tot-N (%)	0.80	0.81	0.90	1.04	1.06	0.83
Organic carbon (%)	7.15	6.71	6.71	5.67	5.62	5.88
C/N	8.5	8.3	7.5	5.5	5.3	7.1

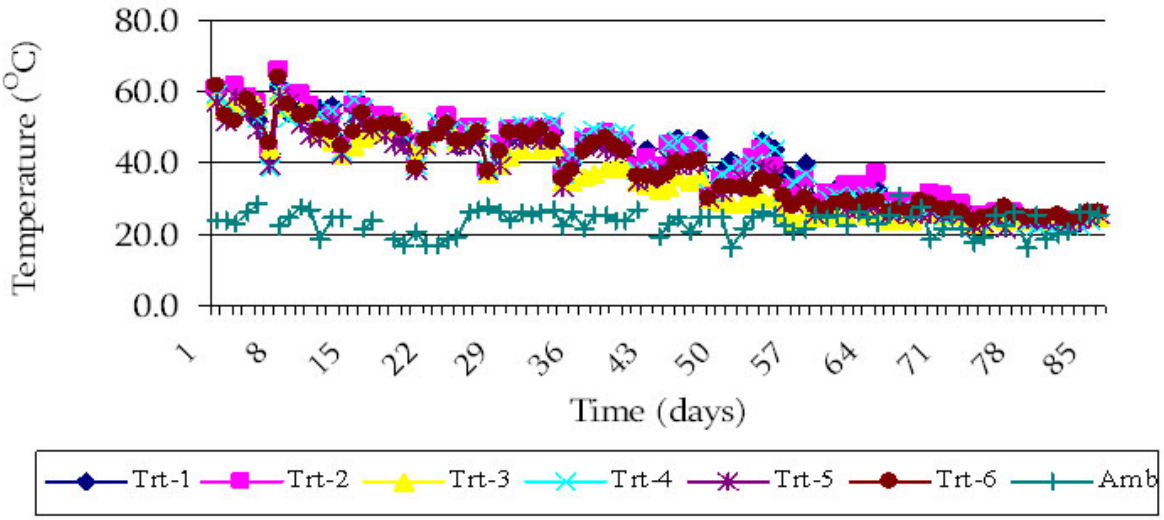


Figure 3. Temperature profile during coffee husk composting with and without organic accelerators.

CONCLUSION

Coffee pulp and husk compost piles with organic accelerators had higher temperature, lower moisture content and higher pH compared with the control. The lower C/N ratio and higher total nitrogen content of accelerator containing compost piles at the end of the composting process indicate the superiority of piles with organic amendments in terms of final quality.

Therefore, in view of the rising demand for organic coffee, coffee processing byproducts which have so far been mismanaged in the region should be converted into a high value organic fertilizer through composting with appropriate amendments. Based on results obtained in this experiment, 70% coffee pulp with 20% FYM was the best combination followed by 80% coffee pulp, 10% FYM and 10% Legume materials. In the case of coffee husk compost, 70% coffee husk, 10% FYM and 10% Legume materials was the best treatment combination. Hence, coffee processing byproducts, mainly *coffee pulp* and *husk*, can be converted into a nutritionally balanced organic fertilizer for coffee production as an integral part of a sound soil fertility management strategy in the region.

REFERENCES

Allison, L.E. (1965) Organic Carbon. In: Black CA et al. (Eds), Methods of Soil Analysis. American Society of Agronomy, Inc., Publisher Madison, WI, USA

Chane, A. (1999) Management of coffee processing byproducts for improved and sustainable coffee production in Ethiopia, PhD dissertation, University of Giesen, Germany.

CSA (2003) Statistical Abstracts, Central Statistical Authority, Addis Ababa, Ethiopia.

Fairtrade foundation producer Profile, Oromia Coffee Farmers Cooperative Union Ltd. (OCFCU), Ethiopia. www.fairtrade.org.uk/downloads/pdf/oromia_profile.pdf. (Accessed on May 15, 2006)

Furman, N.H. (1975) Standard Methods of Clinical Analysis, Krieger, Huntington, NY

Korikanthimath, V.S. & Hosmani, M.M. (1998) Organic recycling of coffee pulp in coffee based cropping systems. *Mysore Journal of Agricultural Science* 32: 127-130

Mitchell, H.W. (1988) Cultivation and harvesting of the arabica coffee tree. In: R.J. Clarke & R. Macrae (eds). *Coffee, vol.4 Agronomy.* Elsevier Applied Science, London & New York. Pp. 43-90

Muller Samann, K.M. (1997) Sustaining growth: soil fertility management in tropical small holdings, Weikersheim, Germany, PP. 347-376.

Olguin, E.J. 1996. Current status and potential of environmental biotechnology in Mexico. In: Moo-Young M et al. (Eds). Environmental Biotechnology: Principles and applications (PP 723-743). Kluwer Academic Publishers, Dordrecht/Boston/London.

Rodrigues, A.M., Ferreira, L.J., Fernando, A.L., Urbano P., and Oliveira, J.S. (1995) Co-composting of sweet sorghum biomass with different nitrogen sources. Bioresource Technology, 54: 21-27.

Sanchez,G., Eugenia, J., Olguin and Gabriel, M. 1999. Accelerated coffee pulp composting. Biodegradation (10): 35-41.

VAN DER VOSSEN, H.A.M. (2004) Organic Coffee Production: Myth or Reality – A review. Proceedings 20th International Scientific Conference, Bangalore, India. ASIC, Paris, France, PP. 960 – 983.

Willson, K.C. (1985) Mineral nutrition and fertilizer needs. In: Clifford, M.N. and Willson, K.C. (Eds.), Coffee: botany, biochemistry and production of beans & beverage, Croom Helm, London, Sydney.

Wrigley, G. (1988) Coffee, Tropical Agriculture Series, Longman Scientific and Technical, Harlow, UK, 639pp.

Response of Arabica Coffee Seedling to Lime and Phosphorus: I. Shoot and Root Growth

A. NETSERE[1], H. GEBREKIDAN[2]

[1]Jima Agricultural Research Center, P. O. Box 192, Jima, Ethiopia
[2]Alemaya University, P. O. Box 138, Dire Dawa, Ethiopia

SUMMARY

Low soil pH and low available P are among the major constraints hampering the productivity of coffee industry in Ethiopian. Yet, information on the potential beneficial effects of lime and inorganic P fertilizer on soil fertility for improved coffee production is virtually absent. Hence, pot experiment was conducted at Jima Agricultural Research Center with the objectives to investigate the effects of lime and P on shoot and root growth of arabica coffee seedlings and establish optimum combination of these agricultural inputs for practical use on Nitosols of Jima area. The treatments were six levels of lime [0, 2.31, 4.62, 6.93, 9.24 and 11.55 g/2.5 kg soil (pot)] and four P rates (0, 250, 500 and 750 mg P/pot) and were laid out in factorial experiment in a randomized complete block design in triplicates. Results depicted that lime and P rates significantly ($p < 0.01$) affected shoot (plant height, stem diameter, and leaf number and area) and root (tap and lateral root length) growth of coffee seedlings. Likewise, the interaction of lime and P significantly ($p < 0.01$) affected only shoot growth. A combination of 0 g lime and 750 mg P/pot and 2.31 g lime and 250 mg P/pot gave the highest, but non significant values of the aforementioned parameters. In conclusion, vigorous and transplantable arabica coffee seedlings could be produced by applying P at a rate of 750 mg P/pot or a combination of low lime (2.31 g lime/pot) and low P (250 mg P/pot) rate. However, the scenario might be quite different after planting in the field. Therefore, further studies are suggested for the future in this area.

INTRODUCTION

Phosphorus is an essential part of nucleoproteins, chloroplast, mitochondria, sugar phosphates, *viz.* adenosine diposphate (ADP), adenosine triphosphate (ATP), nuclicacid, etc, phospholipids and phosphatids that carry the inheritance characteristics of living organism and plays role in cell division, capturing photosyntheticaly active radiation, storage and transformation of energy and stimulation of enzymatic reaction within the living cells of plants (Miller and Donhaue, 1995; Rajan, 2000). Besides it stimulates early root growth, enhances flower initiation and fruit or seed formation bringing about its early ripening (Miller and Donhaue, 1995; Wintgens, 2004).

In contrast, the bulk of soils in coffee growing areas of Ethiopia are classified as Nitosols, which are highly weathered and originated from volcanic rock (Paulos, 1994). The clay fraction of these soils, such as Al and Fe oxides and/or hydroxides, kaolinite and allophane, prone to strong P fixation capacity (Paulos, 1994; Mesfin, 1998). Hence, the proportion of native and/or added P fertilizers that could immediately be available to a crop becomes inadequate and the residues of the fertilizer released very slowly (Brady and Weil, 2002). As a result, P is considered as the second most often deficient nutrient hampering the productivity of coffee industry in the country.

It has been reported that though the application of P fertilizers on such soils has had an enormous impact on plant production, soil management practices, which affect the utilization of P by plants, are equally of a paramount significance for efficient use of native and/or applied P. One of such management practices influencing directly the availability of P is liming. Yet, the advantage of this potential soil fertility management practice has not been utilized for improved coffee production in Ethiopia. The objectives of this study were, therefore, to investigate the effects of lime and P mineral fertilizer on the growth of arabica coffee seedlings and establish optimum combination of these agricultural inputs that give the best growth response under nursery conditions.

MATERIALS AND METHODS

The experiment was conducted at Jima Agricultural Research Center of the former Ethiopian Agricultural Research Organization, Ethiopia. The Center is located at coordinates of 7° 46' N latitude and 36° 0' E longitudes on an altitude of 1753 meters above sea levels. The long-term mean annual rainfall recorded at the Research Center is 1585 mm with mean maximum and minimum temperatures of 26.2 and 11.5 °C, respectively. The predominant soil of the Center on the hilly side is reddish brown *Nitosols* and generally clay dominated and characterized by low available P (Paulos, 1994).

Six levels of lime [0, 2.31, 4.62, 6.93, 9.24 and 11.55 g/2.5 kg soil (pot)] and four P rates (0, 250, 500 and 750 mg P/pot) were factorially combined and laid out in randomized complete block design with three triplicates. Fresh seeds of CBD resistant varities-7440 were sown in black polythene bags of 12 cm wide and 25 cm in length. The pots were field with a fine top soil of 0-25 cm of Nitosols. The soil was well dried and sieved using a 2 mm wire mesh. The different lime rate powdered lime having a $CaCO_3$ equivalent of 97.5% were thoroughly mixed with 2.5 kg of the sieved soil prior to filling in the pots and sowing the coffee seeds, where each experimental consisted of 16 seedlings (pots). These were arranged on nursery beds at 60 cm spacing. The P rates as triple super phosphate (46% P_2O_5 or 20% P) and Urea (46% N) at a rate of 540 mg N/pot were applied in three equal splits, i.e., when the seedlings attained one, three and five pairs of true leaves stages. The seedlings were maintained with the standard nursery management practices until the completion of the study (Tesfaye et al., 2005).

Four seedlings from the inner of each experimental unit were assessed for attributes of shoot [plant height (cm), stem diameter (cm) and leaf number and area (cm^2)] and root [tap and lateral root (cm)] growth, when seven pairs of true leaves observed on good performing seedlings. Intact leaf area was determined using the procedure adapted by Yacob et al. (1995). To this end, the biomass of a seedling was partitioned into shoot and root component. Then the roots were traced on a clean transparent glass placed above a square paper. The length of the root hairs per seedlings was calculated by counting the number of squares covered by individual root hair and multiplied by the length of a single square (0.05). Finally data were analyzed using SAS software program (SAS Inst., 1990). Treatment mean separation was accomplished according to Duncan's Multiple Range Test at $p < 0.01$ probability level.

RESULTS AND DISCUSSION

Shoot (plant height, stem diameter and leaf number and area) and root (tap and lateral root length) growth of coffee seedlings significantly ($p < 0.01$) affected by lime and P rates (Table 1). The highest growth responses for the aforementioned parameters were recorded for seedlings treated with 2.31 g lime/pot, though no variation was detected from seedlings that were not treated with lime. However, the application of lime at rates > 2.31 g/pot decreases

shoot and root growth parameters at a decreasing rate, culminating in the lowest values of the respective parameters at the highest lime rate (11.55 g lime/pot).

Table 1. Shoot and root growth of arabica coffee seedlings as affected by lime and P rates.

Lime and P rates	Shoot growth				Root growth	
	Plant height (cm)	Stem diameter (cm)	Leaf number	Leaf area (cm^2)	Tap root length (cm)	Lateral root length (cm)
Lime rate (g/pot)	**	**	**	**	**	**
0	24.68^{a}	0.40^{a}	11.71^{a}	20.07^{ab}	25.32^{ab}	719.80^{a}
2.31	25.11^{a}	0.41^{a}	11.85^{a}	21.01^{a}	26.96^{a}	729.92^{a}
4.62	21.92^{b}	0.38^{ab}	10.73^{b}	17.96^{bc}	24.68^{bc}	583.42^{b}
6.93	20.64^{b}	0.36^{bc}	10.61^{b}	15.32^{cd}	23.53^{cd}	539.14^{bc}
9.24	17.72^{c}	0.35^{c}	9.77^{b}	13.21^{d}	22.84^{d}	462.49^{cd}
11.55	15.08^{d}	0.30^{d}	8.71^{c}	9.98^{e}	22.69^{d}	403.43^{d}
SE (±)	0.72	0.01	0.26	0.74	0.45	22.53
P rate (mg P/pot)	**	**	**	**	**	**
0	10.91^{d}	0.25^{d}	6.95^{c}	6.14^{d}	22.95^{b}	418.32^{c}
250	20.19^{c}	0.36^{c}	10.81^{b}	15.94^{c}	24.44^{ab}	589.68^{b}
500	24.46^{b}	0.40^{b}	11.74^{b}	19.51^{b}	24.46^{ab}	611.43^{ab}
750	27.89^{a}	0.44^{a}	12.82^{a}	23.46^{a}	25.12^{a}	672.72^{a}
SE (±)	0.59	0.01	0.21	0.61	0.36	18.40
CV (%)	12.04	6.30	8.60	15.80	6.25	13.62

***Significant at 0.01 probability level. Means within a column followed by the same superscript(s) are not significantly different from each other at 0.01 probability level.*

Unlike lime rates, shoot and root growth of the seedlings linearly increased with increasing P rates (Table 1). Accordingly, application of 750 mg P/pot increased plant height, stem diameter, leaf number and area, and tap and lateral root length by 60.9, 43.2, 45.8, 73.8, 8.64 and 80.9%, respectively, over the unfertilized pot.

The better shoot and root growth response of coffee seedlings grown on pots treated with 0 and 2.31 g lime/pot could largely be attributed to the improved chemical properties of the media, specifically high available P (23.8 and 29.6 ppm, respectively), which was eleven times higher than the available P (2.4 ppm) of soils of the study area (data not shown). O the other hand, the decrease in solubility and availability of P, which is caused by the formation of insoluble Ca-P compounds in the soil (Naidu et al., 1990), the deficiencies of Fe, Mn, Zn and B and increased K and Mg retention capacity of soil colloids (Brady and Weil, 2002) might be responsible for the decrease in growth of coffee seedlings when it is grown with the highest lime rate (11.55 g lime/pot).

The least growth response of coffee seedlings in unfertilized pot observed in this study indicate that the soil used for this study is very poor in available P. The results comply with the findings of Taye et al. (1999), who reported stunted shoot and root growth and thus reduced nutrient uptake under relatively nutrient deficient and poor physical media condition. Likewise, Chane (1991) reported that high growth response of coffee seedlings to fertilizer on nutrient poor soils and less to no response in nutrient rich soils. As a result, application of P fertilizers is mandatory on highly weathered, leached and acidic coffee soils of Ethiopia. However, the increase on shoot and root growth with successive increment of P application

observed in this particular investigation apparently seems to be a good indicator of the existence of more room for increase of the parameters by applying P fertilizers at rates above 750 mg P/pot, which awaits further fine-tuning.

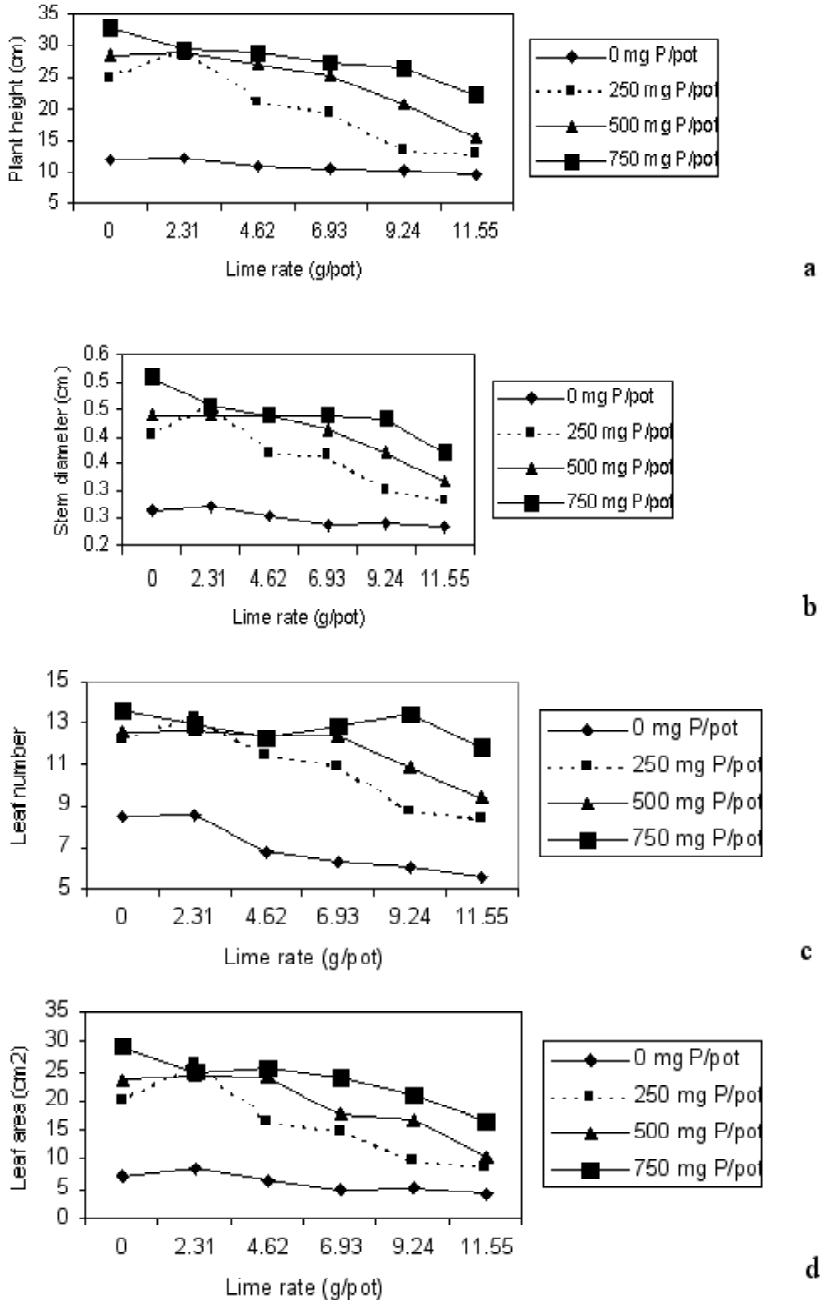


Figure 1. Plant height (a), stem diameter (b) and leaf number (c) and area (d) of coffee seedlings as affected by the interaction of lime and P rates.

In reference to interaction effects, significant ($p < 0.01$) variation were noticed only for shoot growth. Accordingly, the highest but insignificant ($p > 0.05$) growth were recorded for the interaction of 0 g lime and 750 mg P/pot, and 2.31 g lime and 250, 750 and 500 mg P/pot in that order. In contrast, the least and significantly different shoot and root growth were noticed on pots treated with a combination of 0 mg P/pot and increased lime rates (Figure 1). This indicates liming of inherently less fertile coffee soils without P fertilization adversely affect the growth performance of coffee seedlings.

In conclusion, applying P at a rate of 750 mg P/pot or applying low lime (2.31 g/pot) and low P (250 mg P/pot) rate combination produced vigorous and healthy arabica coffee seedlings for field planting at Jimma. But, further studies is required under field condition to evaluate the response of coffee trees to varying levels of lime and P and establish economically optimum levels of these agricultural inputs for major coffee growing agro-ecologies of the country.

REFERENCES

Brady, N. C. and R. R. Weil. 2002. The Nature and Properties of Soil (13th ed.). Pearson Education Inc., Upper Saddle River, New Jersey. 960p.

Chane Abate. 1991. Effects of media composition, volume and nutrition on *Coffea arabica* L. seedlings. M. Sc. thesis, Alemaya University of Agriculture, Alemaya, Ethiopia.

Mesfin Abebe. 1998. Nature and Management of Ethiopian Soils. Alemaya University of Agriculture, Ethiopia.

Miller, R. W. and R. L. Donahue. 1995. Soils in our environment (7th ed.). Prentice Hall Englewood Cliffs, New Jersey. 648p.

Naidu, R., J. K. Syers, R. W. Tillman and J. H. Kirkman. 1990. Lime-aluminium-phosphorus interactions and the growth of *Leucanea leucocephala.* II. Chemical compostion. *Plant and Soil* 126: 1-8.

Paulos Dubale (ed.). 1994. Mineral Fertilizer of Coffee in Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia. 105p.

Rajan, S. S. 2000. Plant Physiology. Anmol Publications PVT LTD., New Delhi, India. 521p.

SAS Institute. 1990. SAS/STAT User's Guide 1990. SAS Institute Inc. Cary, NC.

Taye Kufa, Mesfin Abebe and Paulos Dubale. 1999. Effect of nitrogen and phosphorus and organic fertilizer on growth and development of coffee (*Coffea arabica* L.) seedlings. *In:* African Crop Science Conference Proceedings. 11-14 October 1999, Casablanca, Morocco.

Tesfaye Shimber, Alemseged Yilma, Taye Kufa and Endale Taye. 2005. "Coffee seedlings management and production technology." Amharic version. Ethiopian Agricultural Research Organization (EARO), Addis Ababa, Ethiopia. 17p.

Wintgens, J. N. (ed.). 2004. Coffee: Growing, Processing, Sustainable Production. WILEY-VCH Verlag GmbH and Co. KgaA. Weinheim. 976p.

Yacob Edjamo, Taye Kufa and Alemseged Yilma. 1995. Varietal and age impact on arabica coffee leaf growth parameters at three locations. *In*: Proceedings of the Third Conference of the Agronomy and Crop Physiology Society of Ethiopia. 29-30 May 1997, Institute of Agricultural Research, Addis Ababa, Ethiopia. pp. 38-51.

Response of Arabica Coffee Seedling to Lime and Phosphorus: II. Dry Matter Production and Distribution

A. NETSERE[1], H. GEBREKIDAN[2]

[1]Jima Agricultural Research Center, P. O. Box 192, Jima, Ethiopia
[2]Alemaya University, P. O. Box 138, Dire Dawa, Ethiopia

SUMMARY

A factorial combination of six lime rates [0, 2.31, 4.62, 6.93, 9.24 and 11.55 g/2.5 kg soil (pot)] and four P rates (0, 250, 500 and 750 mg P/ pot) were laid out in randomized complete block design in three replications at Jima Agricultural Research Center. The objectives of the study were to investigate the effect of levels of lime and P on dry matter production of arabica coffee seedlings and its distribution into component parts of the seedlings. Results revealed that both the main effects of lime and P significantly ($p < 0.01$) affected stem, leaf, root and total dry matter production of coffee seedlings. The highest values of these parameters were observed on pots treated with 2.31 g lime and 750 mg P/pot. However, the former was not significantly different from seedlings grown with 0 g lime/pot. Similarly, the interaction of lime and P significantly (either at $p < 0.01$ or $p < 0.05$) affected all the parameters. The highest and non-significant values for the parameters were noticed on pots treated with a combination of 0 g lime and 750 mg P/pot and 2.31 g lime and 250 mg P/pot, respectively. It is, therefore, concluded that coffee seedlings with high dry matter content can be produced by applying P at a rate of 750 mg P/pot or by applying a combination of low lime (2.31 g/pot) and low P (250 mg P/pot) rate. But, further studies should be continued under field condition to investigate growth, yield and quality of coffee to varying levels of lime and P and establish economically optimum levels of these agricultural inputs.

INTRODUCTION

In more than 70% of acid soils of the humid tropics, as those of the Nitosols and other highly weathered and leached soils in the major coffee growing regions of Ethiopia, Al, Fe and Mn toxicity and Ca and Mg deficiencies exist and nearly 100% of the soils are P deficient or have a high P fixing capacity (Sanchez and Salinas, 1981). Consequently, the application of lime to acid soils have been advocated because it displace P from precipitate of Al- and Fe-phosphate, inactivate or precipitate exchangeable and soluble Al and Fe as Al- and Fe-hydroxides, respectively, and increase pH and available P of the soil as well as making the exchange sites more active through the improvement in physico-chemical properties of such soils (Rodrigues et al., 2001; Brady and Weil, 2002). Similarly, Arduino et al. (1993) reported P-fixation due to Al and Fe are best amended with addition of certain rates of lime and repeated application of small quantities of P. However, information on the potential effects of amending acidic soils of the country using lime and P mineral fertilizer in improving soil fertility/productivity and coffee production is virtually absent. This study was, therefore, conducted with the objectives to investigate the impacts of lime and P mineral fertilizer on dry matter production and distribution

into its component parts of arabica coffee seedlings and establish optimum combination of lime and P rate that give seedlings with better dry matter yield for field planting.

MATERIALS AND METHODS

A factorial combination of six levels of lime [(0, 2.31, 4.62, 6.93, 9.24 and 11.55 g/2.5 kg soil (pot)] and four P rates (0, 250, 500 and 750 mg P/pot) were laid out in randomized complete block design in three replications at Jima Agricultural Research Center (7° 46'N, 36° 0'É, 1753 m.a.s.l.) of the former Ethiopian Agricultural Research Organization, Ethiopia. Topsoil (0-25 cm) of Nitosols air-dried and sieved with a 2 mm wire mesh. Powdered lime having a calcium carbonate equivalent of 97.5% was purchased from a local market and it was used as a liming material. The different lime rates were weighted and thoroughly mixed with 2.5 kg of the sieved soil. The blend was then depot in black polythene bags of 12 cm wide and 25 cm long, where a single experimental unit contained 16 seedlings. Seeds of coffee berry disease (CBD) resistant selection- 7440 were sown per pot. The P rates as triple super phosphate (46% P_2O_5 or 20% P) and Urea (46% N) at a rate of 540 mg N/pot were applied in three equal splits, when the seedlings attained one, three and five pairs of true leaves.

The center four seedlings per experimental unit were partitioned into stem, leave and root component, when seven pairs of true leaves observed on a good performing seedlings. These were oven dried at 70 °C for 24 hours to a constant weight and their dry matter measurement (g) was taken separately using sensitive electronics balance. The dry weight of each part was used to determine the dry matter partitioned to stem, leaf and root and total dry matter (stem + leaves + roots dry matter). Finally, the data were subjected to analysis of variance using SAS software program (SAS Inst., 1990). Results were presented as means and were compared using the Duncan's Multiple Range Test at $p < 0.01$ probability level.

RESULTS AND DISCUSSION

Stem dry matter (SDM)

Variations between lime rates were highly significant ($p < 0.01$) for SDM production. Accordingly, lime rate of 0 and 2.31 g produced seedlings with higher but non-significant SDM production, while SDM production was significantly low in pots treated with 11.55 g lime. In contrast, the highest (29.29%) and the lowest (25.27%) stem dry matter distribution was recorded on pots treated with 6.93 and 9.24 g lime in that order (Table 1).

Stem dry matter of coffee seedlings highly significant varied among the P rates. The significant increase in the parameter from 0.15 to 0.99 g could be attributed to the corresponding increase in lime rate from 0 to 750 mg/pot. Similarly, dry matter distributed to stem increased with increasing P rates. Thus, the results ranging between 26.26 to 28.12% were observed on pots treated with 0 and 750 mg P, respectively (Table 1).

Table 1. Dry matter production (g/pot) and distribution (%) of coffee seedlings as affected by lime and P rates.

Lime and P rate	Stem dry matter		Leaf dry matter		Root dry matter		Total dry matter production
	Production	Distribution	Production	Distribution	Production	Distribution	
Lime rate (g/pot)	**		**		**		**
0	0.77^{ab}	27.08	1.46^{ab}	51.28	0.62^{ab}	21.64	2.84^{a}
2.31	0.88^{a}	28.27	1.53^{a}	50.03	0.66^{a}	21.70	3.06^{a}
4.62	0.64^{bc}	26.81	1.21^{bc}	50.94	0.53^{b}	22.13	2.38^{b}
6.93	0.60^{c}	29.29	0.94^{cd}	46.34	0.50^{bc}	23.83	2.04^{c}
9.24	0.43^{d}	25.27	0.86^{d}	50.71	0.41^{cd}	24.04	1.69^{c}
11.55	0.31^{d}	25.80	0.58^{e}	47.41	0.33^{d}	26.79	1.22^{d}
SE (+/-)	0.05		0.07		0.03		0.13
P rate (mg P/pot)	**		**		**		**
0	0.15^{c}	26.26	0.24^{d}	41.57	0.19^{d}	24.44	0.58^{d}
250	0.56^{b}	27.55	1.01^{c}	49.85	0.49^{c}	24.33	2.06^{c}
500	0.76^{b}	27.26	1.40^{b}	50.56	0.61^{b}	22.15	2.76^{b}
750	0.99^{a}	28.12	1.78^{a}	50.83	0.74^{a}	21.02	3.49^{a}
SE (+/-)	0.04		0.06		0.03		0.10
CV (%)	26.23		25.57		21.92		19.51

*** = Significant at 0.01 probability level. Means within a column followed by the same superscript(s) are not significantly different from each other at 0.01 probability level. The interaction of lime and P rates significantly ($p < 0.01$) affected SDM. The highest but insignificant ($p > 0.05$) SDM were observed for seedlings treated with a combination of 0 g lime and 750 mg P/pot, 2.31 g lime and 250 mg P/pot, 0 g lime and 500 mg P/pot, 2.31, 4.62 and 6.93 g lime and 750 mg P/pot and 2.31 g lime and 500 mg P/pot, respectively (Figure 1a). On the other hand, the least and insignificant values for the parameter were noticed for the combination of 0 mg P/pot and increased lime rates.*

Leaf dry matter (LDM)

The use of lime and P as chemical amendments significantly ($p < 0.01$) affected LDM. Accordingly, application of 2.31 g lime/pot gave the maximum dry weight, but statistically not different from 0 g lime/pot (Table 1). However, application of lime above 2.31 g/pot decreased LDM at a decreasing rate, culminating in the lowest value at the highest lime rate (11.55 g/pot). On the other hand, the highest (51.28%) and lowest (46.34%) LDM accumulations were recorded for seedlings grown with 0 and 6.93 g lime/pot, respectively (Table 1).

Leaf dry matter linearly increased with increasing P rates. The lowest (0.24 g) and the highest (1.78 g) values of the parameter recorded for seedlings treated with 0 and 750 mg P/pot, respectively (Table 1). Similarly, total dry matter partitioned to leaf increased with increasing P rates. In aggregate, the result presented in Table 1 and Figure 1b showed 50% of the total assimilates partitioned to the leaf of coffee seedlings grown under the respective main and interaction effects. The results are in accordance of the reports of Rajan (2000) and Taye et al. (2001).

The combined effects of lime and P rates significantly ($p < 0.01$) influenced LDM. The combination of 0 g lime and 750 mg/pot and 2.31 g lime and 250 and 750 mg P/pot gave the highest LDM of 2.32, 1.98 and 1.90 g/pot, respectively (Figure 1b). In contrast, the least and statistically insignificant LDM of 0.31 to 0.14 g/pot was observed on pots received a combination of 0 mg P/pot and 0 and 11.55 g lime/pot. This indicates the adverse effects of liming

inherently less fertile coffee soil without P fertilization. The result also revealed that for each lime rate, application of P accentuated the effect of lime and the effects were greater with increased levels of P addition (Figure 1b). This could be attributed to increase levels of available P (data not shown), which render vigorous shoot growth.

Root dry matter (RDM)

Differences between lime rates were highly significant for RDM production. The highest and non-significant values of 0.62 and 0.66 g of the parameter were recorded from pots treated with 0 and 2.31 g lime in that order. However, lime rates > 2.31 g/pot linearly decreased RDM and declined to the lowest value at the highest lime rate (Table 1). Unlike the above ground parts, dry matter partitioned to roots was highest on pots treated with 11.55 g lime. As a result, the highest (26.79%) and the lowest (21.64%) values were obtained on pots treated with 11.55 and 0 g lime, respectively (Table 1).

The better RDM production on pots received no lime and lime at a rate of 2.31 g/pot attributed to the improved levels of available P (data not shown) which enhanced root growth of the seedlings. The increase in dry matter partitioned to root with increasing lime rate observed in this study attributed to the impaired chemical characteristics of the soil, such as increase in pH and decrease in available P (data not shown). The results comply with the findings Taye et al. (2001), who reported enhanced partitioning of the total assimilate to roots of coffee seedlings under relatively nutrient deficient and poor physical media condition. This concurs to the reports of Marschner (1995), who indicated that root is much stronger sink of the total assimilate under relatively nutrient stressed condition.

Application of P significantly ($p < 0.01$) affected RDM. The highest value of this parameter was recorded for seedlings treated with 750 mg P/pot. Conversely, this parameter was adversely influenced in the absence of P fertilization and hence the lowest value was recorded (Table 1). However, total dry matter partitioned to the root decreased with the increased level of P. The finding confirms the roles of P in promoting shoot and root dry matter of coffee seedlings (Taye et al., 2001; Wintgens, 2004). Likewise, RDM production significantly ($p < 0.05$) influenced by the interaction of lime and P rates. Accordingly, this parameter depressed with the interaction of 0 mg P/pot and increasing lime rates (Figure 1c).

Total dry matter (TDM)

Lime rates significantly ($p < 0.01$) varied for TDM. As a result, the seedlings attained maximum value of 3.06 g/pot of the parameter at lime rate of 2.31 g/pot. However, application of > 2.31 g lime/pot linearly decreased TDM of the seedlings (Table 1). The decrease in dry matter yield observed at the highest lime rate in this study is in agreement with the reports of Naidu et al. (1990), Brady and Weil (2002) and Kidd and Proctor (2001). The possible explanation for this finding has been attributed to the formation of insoluble Ca-P compounds in the soil, deficiencies of Fe, Mn, Zn and B and increased K and Mg retention capacity of soil colloids. These findings invariably illustrated that, depending on the type of crop species, lime rates that only raise the pH to levels neutralize exchangeable Al or reduced it to lower levels increase plant growth and yield (Rodriges et al., 2001).

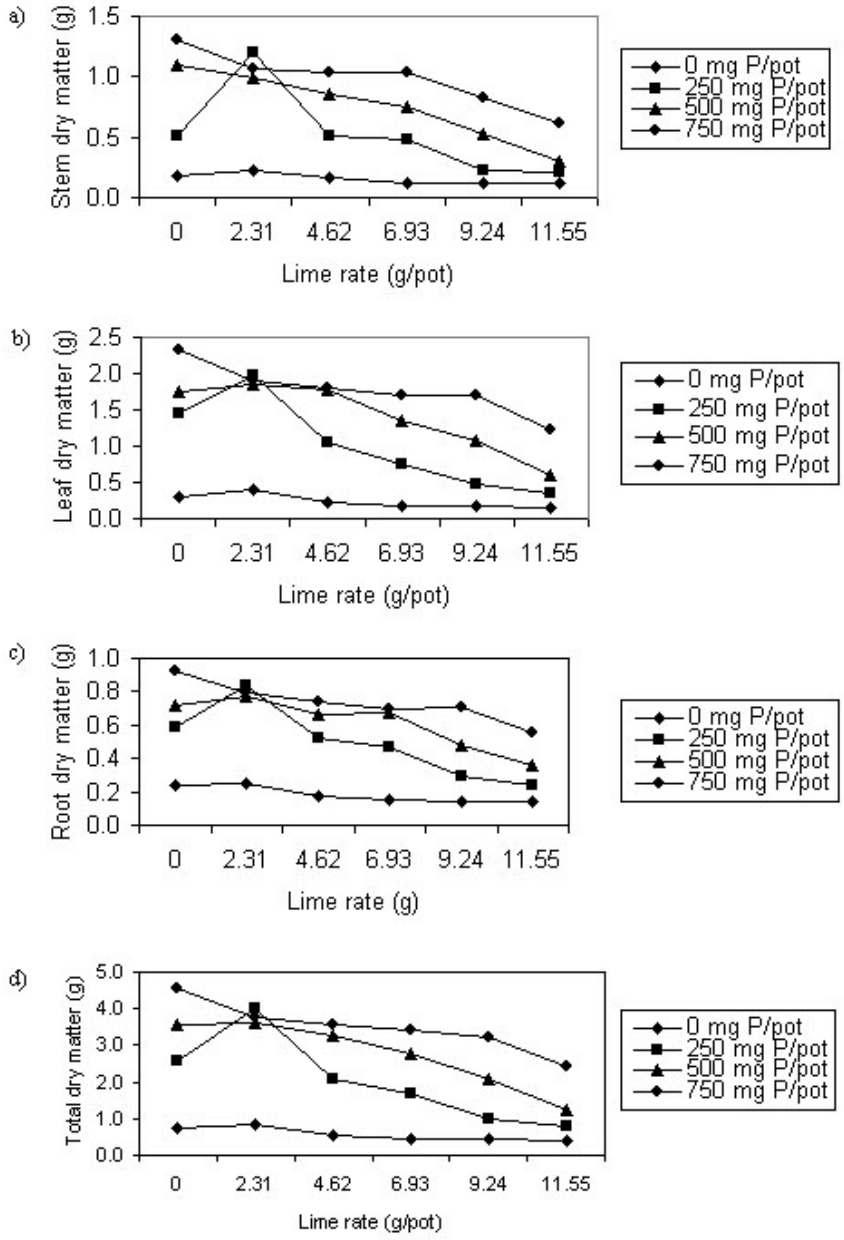


Figure 1. Interaction effects of lime and P rates on stem (a), leaf (b), root (c) and total (d) dry matter production by coffee seedlings.

Application of P at different rate highly significantly affected TDM (Table 1). Accordingly, the results ranged between 0.58 and 3.49 g/pot were recorded for seedlings fertilized with 0 and 750 mg P/pot, respectively. The lowest values of the parameter noticed from P unfertilized pots in the present investigation could be related to the low availability of P in this soil (data not shown). This indicates the benefits of P fertilizer on such inherently infertile coffee soils, a fact, which has been recognized but which awaits quantification of the optimum level through research and further fine-tuning.

In accordance with the main effects, interaction of lime and P rates significantly ($p < 0.01$) affected TDM. At each lime rate, TDM increased with increased levels of P fertilization (Figure 1d). Since the seedlings continued responding significantly in dry matter production up to the

highest P level at each lime rate, it was not certain weather maximum growth of the crop was attained or not.

In conclusion, coffee seedlings with high dry matter content for field transplanting could be grown by applying P at a rate of 750 mg P/pot or by applying low lime (2.31 g/pot) and low P rate (250 mg P/pot) rate combination. However, further studies should be continued under field conditions to investigate growth and yield response of coffee trees and row and cup quality of green beans to different lime and P fertilizer rates and establish economically optimum level of these agricultural inputs for sustainable coffee production in the country by taking into account, *inter alias,* coffee cultivars, seasonal growth and fruiting pattern, management practices and climatic conditions.

REFERENCES

Arduino, E., E. Barberis, B. Badamchian and F. Rooyani. 1993. Phosphours status of certain agricultural soils Lesotho, South Africa. *Commun. Soil Sci. Plant Anal*. 24: 1021-1031.

Brady, N. C. and R. R. Weil. 2002 The Nature and Properties of Soil (13th ed.). Pearson Education, INC. Upper Saddle River, New Jersey. 960p.

Kidd, P. S. and J. Proctor. 2001. Why plants grow poorly on very acid sols: Are ecologist missing the obvious? *Journal of Experimental Botany*. 52(357): 791-799.

Marschner, H. 1995. Mineral nutrition of higher plants (2nd ed.). University Printing House, Cambridge, Great Britain. 889p.

Naidu, R., R. W. Tillman, J. K. Syers and J. H. Kirkman. 1990. Lime-aluminium-phosphorus interactions and the growth of *Leucanea leucocephala.* I. Plant growth. *Plant and Soil* 126: 1-8.

Rajan, S. S. 2000. Plant Physiology. Anmol Publications PVT. LTD., New Delhi, India. 521p.

Rodriges, L. A., H. E. P. Martinez, J. C. L. Neves, R. F. Novis and S. M. Mendonca. 2001. Growth response of coffee tree shoot and root to sub-surface liming. *Plant and Soil* 234: 207-214.

SAS Institute. 1990. SAS/STAT User's Guide 1990. SAS Institute Inc. Cary, NC.

Sanchez, P. A. and G. Salinas. 1981. Low input technology for managing Oxisols and Ultisols in tropical America. *Advances in Agronomy* 34: 280-406.

Taye Kufa, Paulos Dubale and Mesfin Abebe. 2001. Dry matter production and distribution in Arabica coffee as affected by Media components. The 10th Biannual Conference of Crop Science Society of Ethiopia. 19-21 June 2001, Addis Abeba, Ethiopia.

Wintgens, J. N. (ed.). 2004. Coffee: Growing, Processing, Sustainable Production. WILEY-VCH Verlag GmbH and Co. KgaA. Weinheim. 976p.

Yield Response of Forest Arabica Coffee to Ridges and Rejuvenation Methods

A. NETSERE[1], T. SHIMBER[1], T. KUFA[1], E. TAYE[1], W. GEBRESELASSIE[2]

[1]Jima Agricultural Research Center, P. O. Box 192, Jima, Ethiopia
[2]Jima University College of Agriculture and Veterinary Medicine, P. O. Box 307, Jima, Ethiopia

SUMMARY

An experiment was superimposed on forest arabica coffee at Tepi Agricultural Research Sub-center with the objectives to investigate the effects of ridges [tied ridge (closed end ridge), untied ridge (open end ridge) and flat land (without moisture conservation maintained as farmers' practice)] and rejuvenation [topping, agobiado, eskeletamento, decote, layering, clean stumping, ground level stumping and control (not rejuvenated)] methods on boosting and sustaining the productivity of the crop. A split-plot design with three replications, ridges as main-plot and rejuvenation as sub-plot treatments, were used. Mean coffee yield averaged over five cropping seasons significantly ($p < 0.01$) affected by the main effects of both ridges and rejuvenation practices. Tied ridge gave respective yield advantage of 18.96 and 23.56% over untied ridge and the traditional flat land planting. Similarly, topping, agobiado and eskeletamento significantly ($p < 0.01$) out yielded the conventional clean stumping by 43.18, 40.40 and 38.00% and the control by 12.46, 8.35 and 4.67%, respectively. In contrast, the interaction of land preparation and rejuvenation methods did not significantly affect yield. The results indicated that tied ridge, and topping, agobiado and eskeletamento are indispensable for increasing productivity of old coffee orchards in areas like Tepi.

INTRODUCTION

Ethiopian coffee plantations are classified into forest, semi-forest, cottage (garden) and modern plantations (Paulos and Demil, 2000). Forest coffee is a wild type of coffee regenerates spontaneously as an under storey plant growing in Afromotane rain forests (humid forest) of south, west and south western coffee growing tracts of the country. It occupies 10% of the total land covered by coffee in the country, which is estimated to be about 40 000 ha. Although 5-6% of the total 200 000 tons of clean coffee produced annually in the country comes from forest coffee (Ministry of Coffee and Tea Development, MCTD, 1992), its production potential hardly exceeds 200-250 kg ha^{-1} clean coffee (Paulos and Demil, 2000). Exhaustion of coffee trees due to aging, lack of proper rejuvination practices, unregulated tree growth, sloppy land terrain and associated runoff and soil erosion, and moisture deficit are among the major constraints hampering the productivity of the crop (Yacob et al., 1996; Tesfaye et al., 1998).

In original ecology, in tropical high rain forests of south and south western Ethiopia, forest coffee predominantly found on sloppy, rugged and undulating land terrain. In the complex natural habitat, Arabica coffee occurs as under storey plants at the third or forth canopy strata and hence the soil is protected from direct raindrops and runoff. In recent years, however, the accelerated decimation of overhead shade trees for timber and firewood, and the demand for land for food production and other investment purposes has exposed the land to erosion. Besides the observed erratic rain fall distribution, prolonged dry hot period, reduction in

annual precipitation, which may not adequate to satisfy the seasonal crop water requirement and recurrent drought in coffee growing areas of the country adversely affect growth and yield of the crop (Tesfaye, 2006). This conditions necessitates for search of management practices that reduce runoff and soil erosion and harvest rain water. In this line it has been reported that the practice of judicious soil and water conservation undoubtedly increase the productivity of annual crops (Tenaw et al., 2001; Heluf, 2006) and tree crops like coffee (Yacob et al., 1996; Tesfaye et al., 1998). Similarly, Heluf and Yohannes (2001) reported the importance of tied ridge in increasing crop yield by increasing the time for the water to penetrate into the soil.

In its original ecology in the hot humid and cool high lands of south and south western Ethiopia forest coffee population is very aged consisting of trees estimated to be between 40 and 100 years (Personal communication). The trees are very tall (4-6 m) making management practices, such as harvesting of the berries, pruning and training and pesticide spraying, cumbersome to practice. The available research results, however, indicated that stumping the trees in a slant at 30-45 cm height above the ground renovate old coffee orchards and make it productive and manageable (Paulos, 1997; Wintgens, 2004).

Although, the conventional practice for rejuvenating old coffee trees in Ethiopia is stumping, work done elsewhere revealed that coffee trees during cycle conversion can also be rejuvenated and become productive by different rejuvenation practices stumping *viz.* decote, agobiado, topping and eskeltamento (Yilma, 1986; Wintgens, 2004). However, information is not available on the potential benefite of the methods for improved coffee production in Ethiopia. Therefore, the present study initiated with the objectives to evaluate the relative efficiency and effectiveness of ridges and rejuvenation methods and their interaction on the productivity of forest coffee.

MATERIALS AND METHODS

The experiment was superimposed on morphologically identical and uniform age forest coffee trees grown on sloppy land at Tepi Agricultural Research Sub-center. The Center is located at 7° 0'N latitude and 35° 15'E longitude and an altitude of 1200 meter above sea level. The yearly mean rainfall at the site was 1685 mm with respective minimum and maximum temperature of 15.4 and 29.9 °C.

The study was laid down in split-plot design with three replications, where ridges (soil moisture conservation) and rejuvenation methods were assigned to the main- and sub-plots, respectively. The methods of soil moisture conservation consisted tied ridge (closed end ridge) and untied ridges (open end ridge) constructed against the slope of the land and flat land (without moisture conservation maintained as farmers' practices). The ridges were renewed every year right after the onset of the first rain in April.

The rejuvenation treatments were: **1) Eskeletaminto:** all the primary branches are cut back at 15-20 cm from the main stem except those left at the tip of the tree. As a result, new young suckers have been initiated at the bottom of the main stem. When these suckers start to give their first crop, the old stem cut back at a slant at a height of 30 cm above the ground; **2) Agobiado:** bending of the orthotropic branches to the ground and tie it on peg. Then after, branches touching the ground cut back. As a result, many young new suckers have been initiated and grow on top part of the bend stem; **3) Decote:** capping the coffee trees at 1.50 m height above the ground and growth of new suckers below the capping height was checked; **4) Topping:** similar to decote except the height of capping was 2 m; **5) Layering:** roots of the coffee trees digged in one side and then after the main stem of the trees bend and lay it on the

ground; **6) Clean stumping:** cutting back the orthotropic branches of the coffee trees at a slant at a height of 30 cm above the ground level; **7) Ground level stumping:** stumping the main stem at a slant at the ground level; and **8) Control:** not rejuvenated trees as farmers' practices. For treatment number 1 to 7 two vigorous and healthy growing suckers per tree were selected and left for production through out the experimental periods.

Red fresh cherries were harvested from each experimental plot for five consecutive years. The results were multiplied by a factor of 0.166 to convert into clean coffee yield and reported in kg ha^{-1}. The mean yield data were subjected to statistical analysis using MSTAT computer soft ware appropriate to the design and significantly deferring treatment means were separated using Duncan's Multiple Range Test at $p < 0.05$.

RESULTS AND DISCUSSION

Mean coffee yield averaged over five crop seasons significantly ($p < 0.01$) differed due to the effect of ridges (soil and water conservation methods). Among the treatments considered, tied ridge produced yield advantage of 18.96 and 23.56% over untied ridge and flat planting, respectively. However, there was no significant difference ($p > 0.05$) between the yield produced on untied ridge and flat land (Table 1). The results obtained in this study is comparable with the yield advantage obtained from closed end ridges by Heluf (2003) and Tenaw et al. (2001) for annual crops and Yacob et al. (1996) and Tesfaye et al. (1998) for tree crops like coffee. This is also in line with the better growth and yield response of crops observed in drought prone areas of eastern Ethiopia, where small-scale farmers construct band around the crops and/or plant crops in the furrow of ridges (Personal observation and communication). In all cases the higher yield of crops observed for tied ridge is attributed to the higher *in situ* moisture harvesting and retaining capacities as compared to untied ridge and flat land planting. Closed end ridges give more time for rain water to infiltrate into the ground than open end ridge and flat land and, therefore, allow crop plants to use the water that could have been waste as runoff. However, the impact of close ridges in improving crop growth and yield were higher during crop seasons with low total rainfall and/or with poorly distributed rains and prolonged dry hot periods (Heluf, 2003; Heluf and Yohannes, 2001).

Table 1. Effects of interaction of moisture conservation (MC) and rejuvenation methods on yield (kg ha^{-1} clean coffee) of forest coffee at Tepi (five years mean).

Rejuvenation treatment	Clean coffee yield _ MC treatment			**Mean**
	Tied ridge	Untied ridge	Flat land	
Topping	1269	941	806	**1005^{a}(12.64)**
Agobiado	956	999	918	**958^{a}(8.35)**
Eskeletaminto	1132	799	831	**921^{a}(4.67)**
Decote	978	811	727	**839^{a}(-4.65)**
Layering	990	716	618	**775^{ab}(-13.29)**
Clean stumping	709	563	441	**571^{bc}(-53.37)**
Ground level stumping	576	439	471	**496^{bc}(-77.02)**
Control (not rejuvinated)	896	810	927	**878^{a}**
Mean	**938^{a}(23.56)**	**760^{b}(25.31)**	**717^{b}**	

Means within a column or row followed by the same superscript (s) are not significantly different from each other at p < 0.01 probability level. Number in parentheses are percentage of yield increment or reduction over the control.

Similarly, the different rejuvenation treatments significantly ($p < 0.01$) affected clean coffee yield averaged over five crop seasons. On the contrary, the effect was insignificant ($p > 0.05$)

for the interaction of soil moisture conservation and rejuvenation methods. Topping followed by agobiado and eskeletamento gave yield advantage of 12.64, 8.35 and 4.67%; while yield reduction of 4.65, 13.29, 53.71 and 77.02% were noticed for decote, layering, clean stumping and ground level stumping, respectively, as compared to the control plot (Table 1). The findings is in agreement with the results obtained by Yacob et al. (1996), who reported that coffee yield from stumped plots was consistently lower than unstamped (not rejuvenated) ones. Based on the results of the survey work, Getachew et al. (1995) also indicated the reluctance of farmers to accept stumping as a practice promoting the productivity of old coffee orchards in Wollega and Illubabor regions. However, the adoption of the different rejuvenation methods considered in this study is influenced by stem nature of coffee varieties (flexible or stiff), spacing (population density) and age of coffee trees (Yacob et al., 1996), economy and energy of the people who carry out the operation and management system (Yilma, 1996). Thus, agronomically feasible and economically profitable methods should be adopted to renew old coffee orchards.

In conclusion, the findings of the present study shows that coffee yield per unit area could be increased provided that the proper methods of moisture conservation are used. The traditional planting of coffee trees on flat land was inferior to other methods of soil and water conservation (tied and untied ridges) in improving coffee yield. Besides, old and exhausted arabica coffee can be successfully rejuvenated and become productive by different rejuvenation schemes *viz.* topping, agobiado and eskeletamento, which out yield the conventional stumping at least by two fold. However, the efficiency of soil and water conservation and rejuvenation techniques depends on climate, the crop grown and the cropping methods followed. Thus, further investigation should be continued to test these management practices in major coffee growing agro-ecologies of the country and identify technologies that would be agronomically feasible and economically profitable to boost and sustain productivity of old coffee orchards in the country.

ACKNOWLEDGMENTS

This paper is dedicated to the late Mr. Yacob Edjamo, the principal initiator and investigator of the study. The authors also express their thanks to Mr. Debebe Maseresha and Mr. Kassa Getu for their relentless effort in collecting the field data.

REFERENCES

Getachew Olana, Harmen Storck and Mulat Demeke. 1995. Farmers responce to new technologies in coffee production. The small farmers in Ghimbi CIPA, Wollega. Horticultural Systems in Tropics. Working paper series, University of Hannover, Institute of Horticultural Economics, Germany.

Heluf Gebrekidane. 2003. Grain yield response of sorghum (*Sorghum bicolor*) to tied ridges and planting methods on Entisols and Vertisols of Alemaya area, Eastern Ethiopia high lands. *Journal of Agriculture and Development in the Tropics and Subtropics* 104(2): 113-128.

Heluf Gebrekidane and Yohannes Uloro. 2002. Soil and water conservation (tied ridge and planting method) on cultivated lands: The case of eastern Ethiopia. Soil and Water Management Research Program, Alemaya University. 154p.

MCTD.1992. Coffee Statistics Handbook. (1967-1992). MCTD, Addis Ababa, Ethiopia.

Paulos Dubale. 1997. The effect of pruning, weeding and fertilization on the yield of arabica coffee in south western Ethiopia. 17th International Scientific Conference on Coffee (ASIC). 20-25 June, 1997, Nairobi, Kenya.

Paulos Dubale and Demil Tektai. 2000. The need for forest coffee germplasm conservation in Ethiopia and its significant in the control of coffee disease. Proceedings of the Workshop on Control of Coffee Berry Disease (CBD) in Ethiopia. 13-15 August 1999, Addis Ababa, Ethiopia. pp. 125-135.

Tenaw Workayehu, Waga Mazengia, Beirtukan Mekonen, Tolessa Debella, Tesfa Bogale, Berhanu Abate, Hussein Mohammed and Tewodros Mesfin. 2001. Development of appropriate cultural practices for maize production in Ethiopia. Second National Maize Workshop of Ethiopia. 12 - 16 November, 2001.

Tesfaye Shimber. 2006. Growth, water relation, yield and crop quality of arabica coffee in response to water stress and deficit irrigation. Ph. D. Dissertation. University Putra Malaysia. pp. 238.

Tesfaye Shimber, Yacob Edjamo, Alemseged Yilma and Taye Kufa. 1998. Research achivement and transferable technologies in coffee agronomy. Proceedings of the third Technology Generation, Transfer and Gap Analysis Workshop. 12-14 November 1996, Nekemte, Ethiopia. pp. 70-79.

Yacob Edjamo, Tesfaye Shimber, Alemseged Yilma, Taye Kufa, Anteneh Netsere, Takele Negewo, Mohammednur Abachebsa and Bekele Bogale. 1996. Advances in Coffee Agronomy Research in Ethiopia. Proceedings of Inter-Africa Coffee Organization (IACO) Workshop. 4-6 September 1995, Kampala, Uganda. pp. 40-55.

Yilma Yemane Berhan. 1986. Coffee pruning: A review. Proceedings of the First Ethiopian Symposium on Coffee. 20-23 August 1986, Addis Abeba, Ethiopia.

The Nutrient Status of Long Term Fertilized Soils of Coffee Plantation in Southwestern Ethiopia

T. KEBEDE AND Z. MIKRU

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

SUMMARY

Under highly weathered condition over long periods of time and continuous application of TSP, DAP; sulfate of potash, or ammonium formula, soil nutrients undergoes significant changes both in chemical forms and affect the soil properties. The objective of the study was to study the fate of the micro and macro-nutrients in the soil as a result of continued application of N, P, and K fertilizer on coffee plantation. A laboratory investigation was done in Jimma research Center, Ethiopia. The soil type of the area is Eutric nitosol and clay; deep and well drained, with pH of 5-6, medium to high in exchangeable cations. Soil samples were collected from over 15 years, N-P-K fertilizer trial, and several farm management holding fertilizer plots under coffee production. The result of the study indicated that the available P content increased significantly in all fertilized plots compared to unfertilized sites. The availability and concentration of micronutrients in all cases increased with decreased soil pH ranges in critical level. The Cu contents varied 2.7 to 4.5 ppm and showed higher value in the soil. The iron and manganese contents of the soil are high and tend to be excessive range. The high Mn and Fe concentration in soil were certainly due to the low pH of the soil, which all were below pH 6. In continuous application of P fertilizers, soil phosphorus undergoes significant changes both chemical form and its build up was highly observable. In the long-term mineral fertilized plots demonstrate that organic matter highly significantly correlated to Nitrogen, phosphorus and pH soil reaction. Therefore, a continuous supply of organic materials must be added to maintain soil productivity.

INTRODUCTION

Most of the nitrogen fertilizers containing the nitrate form are easily leached. Soils differ widely in their capacity for providing nutrients, depending on the amount of total reserves, on mobilization or fixation dynamics, accessibility of the chemically available nutrients to the roots, etc. (IFA, 1995).Continued application of TSP, DAP; sulfate of potash, or ammonium formula affect the soil properties leading to change in pH. In one or two years application the super phosphates are neutral fertilizers in that they have no appreciable effect on soil pH as have the ammonium containing fertilizers. DAP and MAP, however, contain ammonium, which have an acidic effect on the soil solution.

Phosphate and potash fertilizers are mostly fixed but they may also be leached. Fixation can be complicated where recovery is difficult or it can be easily adsorbed on the surface of the soil in the form of exchangeable. Survey of the soil and foliar nutrient status of the coffee plantations in Coffee Improvement Project (CIP) areas in 1984 indicated that N and P may be low in the soil but the tree may still have normal concentrations (Höfner and Schmiitz, 1994). Paris-Ketting (1987a,b) also found low P and N in soils of Limu Coffee Plantations Enterprise and suggested the application of these fertilizers.

However, continuous application of the same nutrient form for a long time can lead to the occupation of most of the adsorption sites there by creating toxicity problem of the released one. The formula may also contain elements other than the nutrient under study, which will favor the release or fixation of other nutrients. Under highly weathered condition over long period of time and continuous application of fertilizer, soil phosphorus undergoes significant change both in chemical forms and its location in soil profile (Thimma et al., 1991 and Iyengar et al., 1982). The effect of potash application over years significantly increased the amount of K compared to non-fertilized plots with increasing dose of K (Reddy et al., 1993). It is concluded that the applied K has contributed to the luxury consumption of K by the crops and this will still continue to supply K to crops for some more seasons with out reducing yield (Mahendra singh et al., 1982) The purpose of this study was, therefore to study the long-term effect of application of chemical fertilizer on macr-micro nutrient status of the soil. The objective of the present study was to study the fate of the micro and macro-nutrients in the soil as a result of continued application of N, P, and K fertilizer on coffee plantation.

MATERIALS AND METHODS

A laboratory investigation was done at Jima research Center. The soil type of the area is Eutric nitosol and clay; deep and well drained, with pH of 5-6, medium to high in exchangeable cations (Brehanu D.1975, Paulos, 1994). Sixty-four (0-30 cm) soil samples for this study were collected from over 15 years, N-P-K fertilizer trial, and several farm management holding fertilizer plots under coffee production. From these treatments containing different levels of N, P, and K soil samples were collected and studied for the concentration of N, P, K, Fe, Mn, Cu, and Zn. The data were compared with those collected from non-fertilized or intermittently fertilized plots. The changes in concentration of the applied nutrients were also investigated.

The collected soil samples were air-dried, grinded 2 mm meshes. Soil pH was measured 1:2.5 soil H_2O. The available Fe, Mn, Cu, and Zn were analyzed by 0.005M DTPA (Dietylenetriaminepenta-acitic acid) extractant and were read in Atomic Absorption Spectro Photometer (AAS). The total nitrogen and phosphorus were determined colorimetrically using Molybdenum blue and ammonium molybdate method respectively. Potassium was determined by flame photometer. The sources of NPK were Urea, DAP, TSP and Muriate of Potash respectively.

RESULT AND DISCUSSION:

Initially 64 Soil samples were collected from over 15 years fertilized experimental plots. The results of analysis in Table 1 indicated that average soil pH was 4.57. The total nitrogen content was ranged from 0.20 to 0.36% and the average value was 0.25%. The organic carbon content ranges from 2.1% to 3.6 % and the average organic carbon content was found to be 2.96%. The available P content ranges from trace to 22 ppm and the average available P content was 5 ppm. Available P content increased significantly in all fertilized plots compared to unfertilized sites. Unlike N, which is highly liable to mineralization, leaching and volatilization the residual effect and build up of P in the long term fertilized plot is highly observable. In general, the availability of P increased with the high dose of fertilizer in all treated plots.

The analysis of fertilized plots indicated that there were significant effects of the application on the concentration of available P in soil. Particularly P_2 (66 kg^{-ha}) compared with control. Therefore, P fertilization was able to raise the P status of the soil. According to analysis K-fertilization increased potassium concentration of the soil K_2 (134 kg^{-ha}) and K_3 (201 kg^{-ha})

compared with control. This was also confirmed in the earlier work (Paulos Dubale, 1994 and Höfner and Schmiitz, 1994).

Table 1. Some descriptive statistical on indicator parameters of soil samples collected from over 15 years fertilized experimental plots.

Parameters	Mean	Std. Error	Std. Deviation	Variance
Nitrogen (%)	0.25	0.00	0.03	0.00
Organic Carbon (%)	2.96	0.04	0.30	0.09
Phosphorus (ppm)	5.00	0.74	5.90	34.86
pH	4.57	0.06	0.45	0.20

Soils were analyzed for pH, CEC, Organic carbon and NPK (Table 2). As a result it was observed that soil pH in the fertilized soils showed a decreasing trend (acidity increased) and available Phosphorus in fertilized soils increased significantly owing to its low mobility.

Table 2. Physical and chemical properties of long term fertilized and unfertilized plots.

Lab. No	Site Description	PH	N (%)	P (ppm)	K (me/100g)	C (%)	CEC
1	Fertilized 15 to 20 years	6.0	0.18	7.2	0.93	1.8	34.60
2	" " "	5.2	0.22	23.8	1.13	2.7	26.60
3	" " "	4.8	0.21	15.8	0.53	2.8	32.40
4	" " "	4.9	0.21	24.1	0.50	2.5	30.00
5	" " "	5.5	0.27	36.6	1.08	3.0	28.00
6	" " "	4.7	0.22	82.4	0.71	3.9	23.60
7	" " "	5.5	0.21	65.3	0.66	3.5	28.20
8	" " "	6.1	0.22	40.6	0.89	3.5	27.60
9	From unfertilized adjacent fields	5.7	0.22	3.6	0.67	3.6	27.00
10	" " "	6.1	0.18	2.8	0.78	3.3	29.60
S.D		0.53	0.03	26.72	0.24	0.63	3.10
S.E		0.17	0.01	8.06	0.10	0.2	0.98

The total nitrogen content of the soils was generally low to medium 0.18 to 0.27%. The exchangeable potassium in soils was high. However, the level of K did not show significant differences compared to unfertilized sites. CEC value was medium to high and there was no significant different between long-term fertilized and unfertilized plots. This may be due to the relatively high organic matter in this heavy textured soil.

The results of laboratory analysis (Table 3) showed that the availability and concentration of micronutrients in all cases increased with decreased soil pH ranges in critical level. The Cu contents varied 2.7 to 4.5 ppm and showed higher value in the soil. The iron and manganese contents of the soil are high and tend to be excessive range. The high Mn and Fe concentration in soil were certainly due to the low pH of the soil, which all were below pH 6. Therefore, by raising the soil pH the availability of Mn and Fe could be reduced and possibility of its toxic effects eliminated.

From Table 3, it was evident that application of fertilizer increased the availability of micronutrients available Zn, Cu, Fe and Mn in all case compared to unfertilized plots. Similar results had been reported (Prasad. B. nad Jinna N.P, 1982). These results indicated that the solubility of these micronutrients was generally found to increase in decrease soil pH.

Table 3. Status of Micronutrient of soil on long term fertilized fields of coffee.

Lab. No	Site Description	DTPA- Soluble micronutrients (ppm)			
		Fe	Mn	Zn	Cu
1	Fertilized 15 to 20 years	32.8	148.2	2.8	3.2
2	" " "	58.1	124.5	3.3	3.7
3	" " "	46.5	131.5	2.3	3.7
4	" " "	49.8	155.9	2.7	3.1
5	" " "	52.8	101.8	2.9	3.1
6	" " "	63.2	219.1	5.5	4.5
7	" " "	61.9	131.9	3.0	2.7
8	" " "	68.4	172.6	3.3	4.2
9	From unfertilized adjacent fields	32.4	157.2	1.9	3.2
10	" " "	48.9	109.1	2.1	3.3
S.D		3.8	10.8	0.34	0.74
S.E		12.1	34.1	1.1	0.6

Table 4. Correlation cofficient (r) for relationship between organic carbon and available micronutrients, pH and available micronutrients.

	r- value
Organic carbon and Fe	0.674**
Organic carbon and Mn	–0.288*
Organic carbon and Zn	0.248
Organic carbon and Cu	0.131
pH and Fe	–0.159
pH and Mn	0.161
pH and Zn	–0.371**
pH and Cu	0.068

** and ** = significant at p 0.05 and 0.01 respectively.*

Table 5. Correlation cofficient (r) for relationship between organic carbon and available macronutrients, pH and available macronutrients.

	r- value
Organic carbon and Nitrogen	0.621**
Organic carbon and Phosphorus	0.292*
Organic carbon and Potassium	0.161
Organic carbon and pH	–0.354**
pH and Nitrogen	–0.115
pH and Phosphorus	–0.368**
pH and Potassium	0.395**

** and ** = significant at p 0.05 and 0.01 respectively.*

Relation between available micronutrients and organic carbon, pH and available micronutrients are presented in Table 3. It was shown that available Iron positively correlated with organic carbon (OC). The available Manganese was also correlated negatively, however the relation was not significant in Zinc and Copper. Presumably organic matter promotes the availability of these nutrients. Further, the available copper, iron and manganese did not correlate significantly with the soil pH. But, it was negatively significant with zinc.

According to the classification of the limiting value on DTPA soluble Zn, Fe, Cu and Mn, in soil solution the concentrations found to be not below the limiting value. Therefore, deficiencies are rare in soils (Höfner and Schmitz, 1984).

The available micronutrients increased with soil pH and percent organic matter. The total Nitrogen and phosphorus positively correlated with soil organic matter. Exchangeable potassium positively was not correlated with soil organic matter. The available P and exchangeable potassium highly correlated with soil pH; hence, the total nitrogen content did not correlate with soil pH (Table 5).

CONCLUSION

Chemical fertilizers applied long term to the soil can provide crops specific ingredient elements, but not with all essential elements they need. Hence this long-term application of chemical fertilizer might cause some plant nutrients to be depleted and other to be deposited in excess in the soil, and consequently the acidity of the soil increased.

The application of P- fertilizer was effective in every case, while K application had a positive effect only at higher application rate. In continuous application of P fertilizers, soil phosphorus undergoes significant changes both chemical form and its build up was highly observable. In the long-term mineral fertilized plots demonstrate that organic matter highly significantly correlated to Nitrogen, phosphorus and pH soil reaction. Therefore, a continuous supply of organic materials must be added to maintain soil productivity.

RECOMMENDATIONS

The total effect of continuous cropping with various soil fertility treatments have contributed towards improving the macro and macronutrients status of soil. The form and application of Nitrogen fertilizer should be considered the soil reaction with lower pH values below 5. It is advisable to use Ca- containing N and P fertilizers, which are able to correct the soil reaction.

REFERENCES

Brhanu Deble, 1978. Preliminary report for detailed soil survey of Jimma Agricultural Research Station, Station. IAR. Appendex No. 1, Addis Abeba

Fikru Ababa. 1988. Need for soil survey studies. p. 15-20. *In* Desta Beyene (ed.) Proceedings of Soil Science Research in Ethiopia. IAR, Addis Ababa.

Höfner. W. And M. Schmitz 1984. Report on the soil and foliar analysis in CIP areas in socialist Ethiopia. EEC/MCTD, Addis Ababa.

IFA. (International Fertilizer Industry Association). World Fertilizer Use Manual IFA, Paris, France, (1995)

Iyengar, B.R.V., C.S.K. Naik and S.G. Bakre, 1982. Transformation and availability of phosphorus applied to soil under coffee. J. Indian Soc. Soil Sci. 30(3): 285

Mahendra sing, A.P. Singh and S. B. Mittal 1982. Effect of long term fertilization and cropping on the potassium supplay capacity soils. Plant and Soil 65, 375-382

Paris- Ketting, M.T.1987b. Soil survey report of Limu Coffee Plantation Development Enterprise, soil of Kossa coffee State farms. MCTD, Addis Ababa.

Paulos D. 1988. The effect of inorganic fertilizers on the yield of coffee in some areas in Ethiopia. P. 51-59. *In*: Desta Beyene (ed.) Proceedings of Soil Science Research in Ethiopia, 11-14 February 1986. IAR, Addis Ababa.

Paulos Dubale (ed.) 1994. Mineral fertilization of coffee in Ethiopia. Institute of agricultural Research, Addis Ababa.

Prasad B. and Sinha 1982. Chances in the status of micronutrients in soil with longterm application of chemical fertilizer. Plant Soil 64, 437-442.

Reddy H.T., Krishonapp N.C. S. and Violet D' Souzam 1993. Effect of continuous aspplication of potassium on the availability, uptake and drymatter yield of robusta coffeee in red lateritic soils. J. coffee Res. 23 (23): 31-43

Thimma Reddy, Shanmukhappa D.R., Violet D' Souzam, Krishnappa Naaik C.S. and Jayarqama, 1991. Effect of long term application of phosphorus on the availability, form and uptake of P by coffee, J. Coffee Res. 21 (2): 127-134

The Potential Use of a Silicon Source as a Component of an Ecological Management of Coffee Plants

J. CAMARGO MARTINATI[1], R. HARAKAVA[2], S. DIAS GUZZO[2], S. MUI TSAI[1]

[1]Cell and Molecular Biology Laboratory of Agriculture Nuclear Energy Center – (CENA/USP) – Av. Centenário, n.303, P.O. Box 96, Zip Code 13416-00 Piracicaba, SP, Brazil
[2]Phytopathology Biochemistry Laboratory – Biological Institute – São Paulo, SP, Brazil
E-mail: jumarti@cena.usp.br

SUMMARY

Coffee is one of the most important agricultural export commodities in the world and it represents the main export resource for some developing countries. Therefore, the development of new methods of coffee management that improves production without causing any damage to the environment is an attractive alternative for the producers. Much effort has been invested towards the understanding the mode of action of compounds that can induce resistance against several pathogens without injuring the environment. Inasmuch, many researches have considered silicon efficient in order to avoid plant pathogen penetration and development. The purpose of this study was to verify the effect of potassium silicate and calcium/magnesium silicate on the development of coffee seedlings (*Coffea arabica*), cultivar "Mundo Novo" as well as to evaluate the incidence of coffee leaf rust development under greenhouse conditions. The experiment was a completely randomized design with 12 treatments with ten plants per treatment. The treatments were 0, 0.25, 1.25, 2.5, 4 and 5 μM of Si for each source of silicon incorporated into soil. The seedlings were inoculated with a urediniospores suspension of *Hemileia vastatrix* (2mg.mL^{-1}) at the 7th month after planting (six pair of leaves). Evaluations were performed by counting the number of lesions per leaf. The statistical analysis showed that the number of lesions reduced up to 66% in the highest silicon dose when compared to the number of lesions in control plants. Infected plants were found to have a linear decrease of lesions with the increase of silicate concentration. The lowest number of lesions per leaf area was observed in plants that received 5 μM of Si from potassium silicate. This result indicates the use of silicon as an alternative for an ecological management system for coffee disease protection.

INTRODUCTION

Coffea arabica is one of the most important agricultural export products in developing countries. This perennial crop is subject to high losses in potential production due to pests and diseases. The most destructive disease of coffee plants is the Coffee leaf rust, caused by the fungus *Hemileia vastatrix* Berk. Et Br and the yield losses in Brazil have been estimated at 45% if no control methods are taken (Matiello, 1991).

Fungicides provide efficient protection, but their applicability can be jeopardized by adverse environmental effects and by the raising of resistant pathogen strains. Several chemical control strategies for enhancing plant resistance to pathogens have been taken. Such measures provide ecological management allied to the priority of sustainable coffee production. The improvement of plant resistance based on induction of host defense becomes an important option to control the rust disease in coffee plants.

Silicon sources have increasingly been used. Silicon (Si) has long been known to reduce the incidence of fungal diseases in a number of pathosystems (Belanger et al., 2003; Rodriguez et al., 2003; Botellho et al., 2005; Fauteux et al., 2005), but its mode of action in plants remains unclear, although it seems that Si acts on general mechanisms, common to most plant species.

It has been shown that Si could act as an enhancer of plant defense responses or as an activator of strategic signaling proteins. Considered to be biologically active, Si triggers a faster and more extensive plant defense by interacting with several key components of plant stress signaling systems ultimately leading to induced resistance against pathogenic fungi (Fauteux et al., 2005)

Based on their observations with cucumber, Fawe et al. (2001) suggested that the Si bioactivity is compared to the action of secondary messengers of systemic acquired resistance (SAR), Thus, Si would act as a modulator influencing the timing and extent of plant defense responses. The effects of Si on secondary metabolism are achieved only after elicitation, as well as the known activators.

Coffee defense genes, against *H. vastatrix,* have been isolated and identified in resistant plants (Fernadez et al., 2006; Guzzo et al., 2004). These studies were carried out in different periods post fungus inoculation and both of them could demonstrate that the expression of defense genes occurs from the early stages of infection to 72 hours after infection. Those genes were also identified in susceptible coffee plants treated with the elicitor BTH. With these results we could observe that even susceptible coffee plants can react against infection process by enhancing the defense mechanisms through elicitation.

The aim of the present study was to verify the efficacy of silicon sources in reducing coffee leaf rust in susceptible plants in order to establish a new method of disease control.

MATERIAL AND METHODS

Coffee plant

The seeds of *Coffea arabica* cv. "Mundo Novo" (IAC – 388-17-1), susceptible to race II of *Hemileia vastatrix,* used in this study, were donated by Cooperativa Garcafé, Garça SP/Brazil. These seeds were surface sterilized with 2-5% sodium hypoclorite for 15 minutes and were then washed three times in sterile distilled water. The germination occurred in seedling containers filled with sand until cotyledonary leaves were fully developed, about 60 days after planting. Following leaf development, the seedlings were transplanted to plastic vases and kept in greenhouse. All fertilization recommended procedures were performed.

Silicon Treatments

Immediately after the transplant, the coffee seedlings were divided into two groups, according to fertilization treatments: 1- fertilized with calcium/magnesium silicate; and 2- fertilized with potassium silicate. Both groups were completely randomized design in a total of 12 treatments with ten plants per treatment. The treatments were 0, 0.25, 1.25, 2.5, 4 and 5 μM of Si for each source of silicon incorporated into soil

Inoculum and inoculation procedure

Urediniospores of *Hemileia vastatrix* Berk. Et Br., race II were used in the experiments. They were collected from field naturally infected leaves, cv. "Mundo Novo" at Fazenda Santa Elisa

– SP/Brazil. The collection procedure involved scraping off young rust pustules, drizzling the material and wrapping up the spores in polypropylene tubes. The tubes containing the spores, were then stored in constant temperature chamber at –80 °C until the determination of germination rate. Spore batches with less than 15% of germination rate were not used for inoculation procedures.

In order to prepare the inoculation, the selected urediniospores were activated by placing the tube in a water bath at 40 °C for 10 minutes. Spores were suspended in distilled water at a concentration of 2 mg.mL^{-1} and the suspension was maintained homogeneous during all the time of inoculation. Coffee plant seedlings at the seventh month of germination were inoculated with the solution described above. This solution was sprayed on the seedling leaves so that it would entirely cover the abaxial surface with droplets. After the inoculation the plants were placed in a humid dark room at 25 °C for 48 h.

Disease assessment

The evaluation of symptoms was performed by counting the number of lesions per leaf area of each leaf inoculated.

Statistics

Twelve treatments and ten replicates were used. Regression analysis was used to investigate whether silicon concentration and sources affected the number of lesions. In all regression analyses, the percentage variance accounted for (R^2) was used to assess goodness of fit. Analyses were done using SAS ver. 8·2 (SAS Systems, Cary, NC, USA).

RESULTS AND DISCUSSION

The symptoms of coffee leaf rust disease were observed 60 days after inoculation in all inoculated plants. The statistical analysis showed that the number of lesions reduced up to 66% in the highest silicon dose when compared to the number of lesions in control plants. Infected plants were found to have a linear decrease of lesions with the increase of silicate concentration. The lowest number of lesions per leaf area was observed in plants that received 5 μM of Si from potassium silicate (Figures 1 and 2).

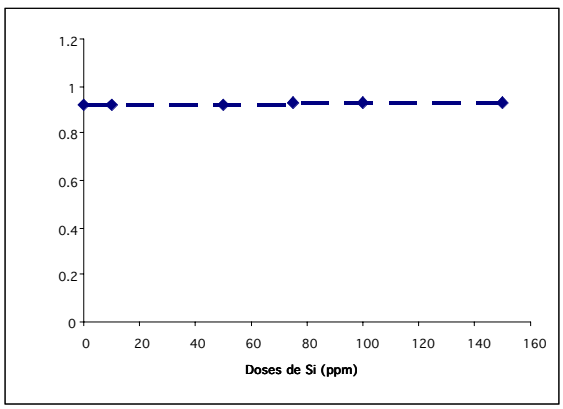


Figure 1. Regression analysis of the total number of lesion in coffee plants treated with calcium/magnesium silicate. Regression curve: 0,9184 + 0,0001 x dose. F value = 0.8272; R2 = 0.987.

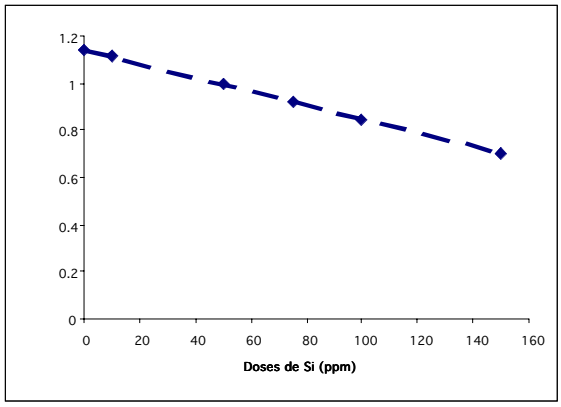


Figure 2. Regression analysis of the total number of lesion in coffee plants treated with potassium silicate. Regression curve: 1,4117-0,00293 x dose. F value = 0.0001; R2 = 0.995.

Results from this study provide strong evidence that silicon amendments mediate an effective protection against *H. vastatrix* in coffee plants. This study confirms the numerous observations on the beneficial role of Si in several plants. However, there is no study regarding the identification of defense genes expressed in coffee plants treated with Si. In a second step of this work, we will identify the defense genes involved in the interaction Coffee – silicon – *H. vastatrix*.

REFERENCES

Bélanger, R.R.; Benhamou, N.; Menzies, J.G. Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (*Blumeria graminis* f.sp. *tritici*). Phytophatology, v.93, n.4, p.402-412, 2003.

Botelho, D. M. S.; Pozza, E. A.; Pozza, A. A. A.; Carvalho, J. G.; Botelho, C.E.;Souza, P.E. Effect of silicon doses and sources on the intensity of the brown eye spot of coffee seedlings. Fitopatologia brasileira, v.30, n.6, p.582-588, Nov./Dec. 2005.

Fauteux, F.; Rémus-Borel, W.; Menzies, J.; Bélanger, R.R. Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiology Letters, v.249, p.1-6, 2005.

Fernadez, D.; Santos, P.; Agostini, C.; Bom, M.C.; Petitot, A.N.; Silva, M.C.; Guerra-Guimarãe, L.; Ribeiro, A.; Argout, X.; Nicole, M. Coffee (*Coffea arabica*) genes early expressed during infection by the rust fungus (*Hemileia vastatrix*). Molecular Plant Pathology, v. 5, n.6, p. 527-536, 2004.

Guzzo, S.D. Biochemical and molecular aspects of systemic acquired resistance in coffee plants against Hemileia vastatrix Piracicaba, 2004, 236p. Tese (Doutorado) – Universidade De São Paulo, Centro de Energia Nuclear na Agricultura CENA/USP. Available on http://www.teses.usp.br/teses/disponiveis/64/64132/tde-24082004-105153

Matiello, J.B. O Café: Do cultivo ao consumo. Coleção do Agricultor – Grãos. São Paulo Editora Globo S.A. 1991, cap. 24, p. 345-363.

Rodrigues, F.A,. Benhamou, N., Datnoff, L.E., Jones, J.B. and Bélanger, R.R. Ultrastuctural and cytochemical aspects of silicon-mediated rice blast resistance. Phytopathology, v.93, p.535-546, 2003.

Geographical Analyses to Explore Interactions between Inherent Coffee Quality and Production Environment

P. LÄDERACH[1], P. VAAST[2,3], T. OBERTHÜR[1], R. O'BRIEN[4], L. D. LARA ESTRADA[3], A. NELSON[5]

[1]International Center for Tropical Agriculture (CIAT), Cali, Colombia, South America, Recta Cali Palmira, KM 18, GIS & Land Use Project. E-mail: p.laderach@cgiar.org
[2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France
[3]Centro Agronómico Tropical de Investigación y Enseñaza (CATIE), Costa Rica
[4]Charles Sturt University, Australia
[5]Joint Research Centre of the European commission, TP 440, Via Enrico Fermi1, I-21020 Ispra (Va), Italy

SUMMARY

In recent years, research has been focusing on the interactions of coffee cup quality and its production environment. Nevertheless, there has been a lack of methods and tools to extrapolate the findings of mainly controlled experiments to wider geographic areas. We present Spatial Decision Support (SDS) elements based on Bayesian Statistics and Geographically Weighted Regression (GWR) methodologies to identify niches, map quality response attributes, and determine their interactions with the environment. To do so, we use two case studies. With the data set of Cauca, Colombia, we introduce the niche concept and test the predictive approaches. With the data set of Nicaragua, we demonstrate the value of spatial analyses assessing the variability of coffee quality response variables and their determining environmental factors.

RÉSUMÉ

Récemment, la recherche sur le café s'est concentrée sur les interactions entre la qualité en tasse du café et l'environnement. Cependant, il manquait des méthodes et des outils pour extrapoler les résultats de la plupart des expériences contrôlées à des aires géographiques plus étendues. Nous présentons un outil basé sur des méthodologies de statistiques de Bayes et de Régression Géographiquement Pondérée (GWR) pour cartographier les caractéristiques de qualité du café et pour identifier leurs interactions avec l'environnement. Pour cela, on utilise deux cas d'étude. Avec les données du département du Cauca au sud de la Colombie, nous introduisons le concept de niche et testons l'outil présenté. Avec les données du Nicaragua, nous démontrons la valeur des analyses spatiales pour évaluer la variabilité des caractéristiques de qualité du café et des facteurs environnementaux qui les dirigent.

INTRODUCTION

Usually, due to high resource inputs, agricultural research is conducted in few experimental sites, findings and generated knowledge is thereafter rolled out and applied to wide areas without taking into account the changes of the environment over space. The development of tools and methodologies for extrapolating findings that are site and environment specific is required. Spatial decision support tools can help to extrapolate findings and identify niches where a specific coffee trait is likely to be found. Niches are clusters of sites with

environmental characteristics that favor product qualities of similar nature. Spatial decision support tools give insights on interactions between species performance and the environment. We use two case studies to demonstrate the utility of geographical analysis. With a data set of Cauca, Southern Colombia, we introduce the niche concept and test a Spatial Decision Support tool and with a data set from Nicaragua, we demonstrate the value of spatial analyses to assess the variability of coffee quality response variables and their determining environmental factors.

METHODOLOGY

A Spatial Decision Support (SDS) tool, that is, a software tool based in Geographical Information Science (GIS) to assist users in decision-making was developed. The tool, CaNaSTA (Crop Niche Selection in Tropical Agriculture) employs Bayesian Statistics. Bayesian methods provide a "formalism for reasoning under conditions of uncertainty, with degrees of belief coded as numerical parameters, which are then combined according to rules of probability theory" (Pearl, 1990). A simple Bayesian model defines prior and conditional probability distributions and combines these to calculate posterior probabilities for each possible outcome. The probability distributions may be derived from data, set by experts or defined from a combination of data and expert opinion.

The CaNaSTA algorithm (O'Brien, 2004) creates conditional probability tables of all predictor variables against response variable categories. In the case of coffee, predictor variables include climate and topographic factors, and the response variable can contain sensorial, physical or biochemical quality attributes. The primary model output is a probability distribution at each location. The probability distribution consists of the probability that the response variable is in each potential state. This information can be used to create maps showing the most likely response value ('Most Likely'). The values in the probability distribution can also be weighted to produce a suitability value ('Score'). Finally, an indicator of reliability (certainty value) can also be displayed as a map ('Certainty'), and can assist in the interpretation of the results. Once locations have been identified where a particular response is likely, further analysis can be carried out to determine which predictor variables are important; a significance indicator is used to

compare the importance of the factors. These factors can be either quality enhancing or reducing, and help with the analysis of specific conditions required for specific coffees.

Geographically weighted regression (GWR) assumes that "...the relationships between variables measured at different locations might not be constant over space" (Fotheringham, 2002). We use GWR to illustrate that the interaction between the environmental factors and coffee quality attributes vary in space. GWR is a spatial statistical method employing moving windows for regression (Fotheringham, 2002) used to describe the spatial variability of coffee quality attributes.

Evidence data used for the predictions and analyses consisted of generated climatic factors with a resolution of 1 km and terrain attributes with a resolution of 90m. Climate layers were generated using WorldClim (Hijmans et al., 2005) and MarkSim (Jones and Thornton, 2000; Jones et al., 2002) data. WorldClim is a global database of climate variables in grid format. The data layers were generated through interpolation of average monthly climate data from 15,000 to 47,000 weather stations during the years 1950 to 2000. Variables generated from WorldClim are annual average precipitation, annual average temperature and dry month per year. MarkSim uses interpolated climate surfaces based on a third-order Markov function. Annual average diurnal temperature range and mean annual solar radiation were generated

using MarkSim. Dew point maps were calculated by the method of Linacre (Linacre, 1977) from the WorldClim dataset. Terrain attributes such as elevation, aspect and slope were generated and mapped from the digital elevation model (DEM) of the Shuttle Radar Topography Mission (SRTM) using geographical information systems (GIS) methodology (Jarvis, 2004).

CASE STUDIES

We present two case studies, one using a data set from the Colombian coffee growing department Cauca and a second one from the Nicaraguan coffee growing departments, Matagalpa, Jinotega, Nueva Segovia and Región Autónoma del Atlántico Norte.

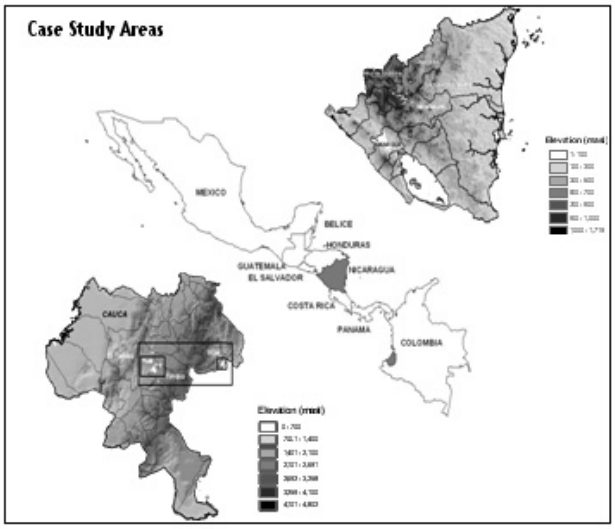


Figure 1. Cauca and Nicaragua case study area. For Cauca the window of the "Driving Factor" analyses is shown: The large rectangle represents the entire study area (775866 ha), the medium the niche of El Tambo-Timbio (160765 ha), and the small the niche of Inzá (16005 ha).

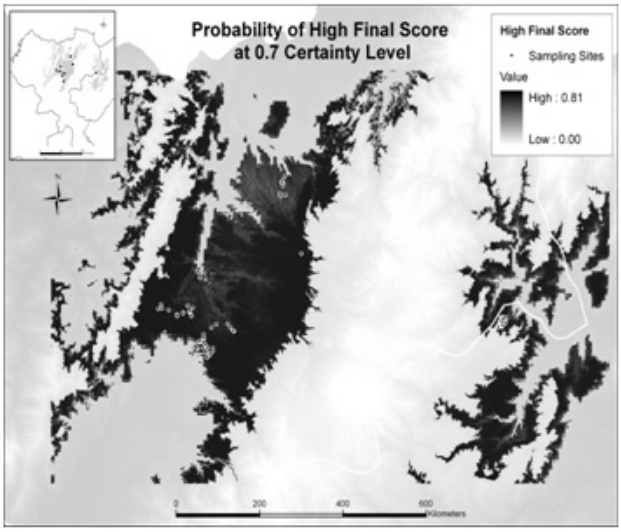


Figure 2. CaNaSTA "Score" analysis; the likelihood of high Final Scores at a 0.7 certainty level are shown. The areas of El Tambo-Timbio and Inzá are identified as potential high value coffee niches.

The Cauca case study data set consist of 88 sample points, 44 from sites in El Tambo-Timbio, 27 from Inzá and 17 from other municipalities. Coffea Arabica was harvested in May and June 2005 in homogeneous and geo-referenced plots of farmers, post-harvest processes were standardized with a mobile processing unit. The sensorial quality was assessed by an international cupping panel in August 2005. The sensorial evaluation was conducted according to SCAA standards and with an adapted cupping form assessing ten sensorial attributes: Fragrance/Aroma, Flavor, Aftertaste, Acidity, Sweetness, Body, Balance, Uniformity, Clean Cup, and Cuppers Score. The probability of High Final Score (Figure 2), the sum of all the sensorial attributes minus the defects, was predicted with CaNaSTA. To validate CaNaSTA three different tests and training sets were used with 25/75, 50/50 and 75/25 percent of the data accordingly to predict and test the model, respectively. Each set was repeated 10 times with randomly chosen predicting and testing sites. The performance of predicting the right quality classes was conducted only on the 70-90 quality class ranges due to the focus on high quality coffees. A quality class refers to 5 scores in the SCAA grading system. With the likelihood ratio chi-square test the dependency of the evidence and predictor scores was tested. The test is using a conformity matrix where the axes represent the evidence and predictor ranges accordingly and the matrix cells the agreement between them. "Driving Factor" analysis was then applied to determine the factors most impacting on sensorial coffee quality. The analysis was conducted on the two different environmental niches and on the entire Cauca data set (Figure 1). The analysis extends of the Inzá niche is of 16 005 ha, the one of El Tambo-Timbio of 160 765 ha, and the Cauca area including all the sampled municipalities of Cauca of 775 866 ha.

The Nicaraguan data set consists of 67 sample points collected and analyzed by Lara-Estrada (2005). The samples, all of Caturra variety, were picked and processed in a standardized way and physical, bio-chemical and sensorial attributes were assessed. The sensorial attributes including Acidity, Body, Bitterness, Aroma, Cuppers preference, Flavor, were determined in the cupping lab of Atlantic SA in Nicaragua. Bio-chemical attributes including Cholorenic Acids, Caffeine, Fat Content, Trigonelline, and Sucrose Content, were assessed by Near Infrared Spectroscopy NIRS in CIRAD, France. "Score" analyses, an indicator of the likelihood to produce high quality, was conducted and combined with the "Certainty" surfaces. The cross correlations between pair surfaces were calculated (Nelson, 2004). GWR analyses were conducted to spatially quantify the impact of the environmental factors on quality attributes.

I RESULTS: NICHE CONCEPT

With the "Score" analyses (Figure 2) the areas of El Tambo-Timbio and Inzá were identified as niches with high probability to produce specialty coffee. CaNaSTA was then validated, comparing prediction and evidence quality scores (Table 1), with the hypothesis being:

H_0 = Prediction and evidence scores are independent
H_1 = Prediction and evidence scores are dependant

Table 1. P values of the likelihood ratio chi-square for the entire area and for the two niches.

	25 / 75	**50 / 50**	**75 / 25**
Cauca	0.43	0.056	0.13
El Tambo-Timbio	0.062	0.051	0.019
Inzá	0.86	0.081	0.014

In El Tambo-Timbio the p-value decreases from 6.2% to 1.9% with increasing amount of prediction points (Table 1). With the 25/75 set, the H_0 is being accepted; prediction and evidence scores are independent. For the 50/50 and 75/25 sets, H_1 can be accepted at a 5.2% confidence interval, prediction and evidence scores are dependent. For Inzá the same is true, with the exception that H_1 for the 50/50 set can only be accepted at a 8.2% confident level, which might be due to little evidence data. When analyzing the entire area no pattern is distinguishable, 50 and 75 percent of the data points are apt to predict the niches at a confidence level of 5.6 and 13% accordingly. These results make a lot of sense, when we recall the CaNaSTA methodology that uses site data and its environmental factor combination to predict entire areas. In the case of Cauca, this implies that sites from Inzá are used to predict qualities in El Tambo-Timbio and vice versa. Therefore, it becomes obvious that the niches cannot be identified at a low confidence level, but the methodology still serves for a general delimitation of niches that can thereafter be refined by adapting the analyses window to niche scale.

Table 2. Quality Enhancing Factors impacting on the Final Score attribute of the niches Inzá, El Tambo-Timbio and the whole Cauca sampling area. In parenthesis the significance indicator c is shown.

Quality Enhancing Factors	Entire Data Set	Inzá	El Tambo-Timbio
Altitude (masl)		1750 -1800 (2.02)	1652-1725 (2.32)
			1725-1798 (2.39)
Average Annual Dew Point (°C)		11.9-12.2 (2.43)	
		12.3-12.6 (2.07)	12.3-12.8 (2.38)
Average Annual Temperature (°C)		17.7-18.1 (2.55)	17.8-18.9 (2.32)
		18-18.4 (2.21)	
Average Annual Precipitation (mm)		1645-1674 (2.2)	
	1760-1934 (2.31)	1587-1616 (2.1)	

Table 3. Quality Reducing Factors impacting on the Final Score attribute of the niches Inzá, El Tambo-Timbio and the whole Cauca sampling area. In parenthesis the significance indicator c is shown.

Quality Reducing Factors	Entire Data Set	Inzá	El Tambo-Timbio
Altitude (masl)	1528-1623 (2.74)		
Slope (Grad)			34.5-40.9 (2.55)
		22.4-25.6 (2.54)	21.6-27.9 (2.10)
Average Annual Dew Point (°C)	12.8-13.5 (2.4)	11.5-11.9 (2.57)	14.3-14.8 (2.00)
Average Annual Temperature (°C)		17.3-17.7 (2.47)	20-21 (2.02)
Average Annual Solar Radiation (Mj/m2/d)			21.8-22.3 (2.32)
Average Annual Precipitation (mm)	1133-1587 (2.78)		
Dry Month per Year (Month / Year)		3 (2.81)	

To illustrate the site specificity of the interactions of environmental factors with quality, the "Driving Factor" analysis was run for the entire data set and for the two niches (Tables 2 and 3) separately. For the entire data set, only 1 enhancing and 3 reducing factors were identified, having a significance value > 2. As stated previously, by running a general analysis, we are

predicting quality areas based on evidence data from distinct environmental conditions and insights of interactions with coffee quality are only of general nature. When analyzing niche by niche, a more detailed set of responsible factors can be obtained.

For both niches, altitude, average annual temperature and average annual dew point play an enhancing role for Final Score quality. The ranges are only slightly different, Inzá permitting lower temperatures and higher altitudes than El Tambo-Timbio. Average Annual Precipitation plays an important enhancing role in Inzá and for the entire data set of Cauca. Slope influences Final Score negatively in both niches. Dew Point ranges below and above the ones identified as enhancing Driving Factors impact negatively, the same is true for Average Annual Temperature. The optimal coffee growing annual average temperature in Inzá is somewhere between 17.7 and 18.4 °C whereas it is slightly higher (17.8-18.9 °C) for El Tambo-Timbio. The results demonstrate variability in the environmental factors that impact on Final Score and the necessity of assessing these factors according to their niches.

II RESULTS: VARIABILITY IN SPACE

Recent studies show the interactions of environmental factors and coffee quality and the correlations between quality attributes for selected study sites: Vaast et al. (2005a) reported no differences in the caffeine content of high and low quality coffees; Avelino et al. (2005) did not find any strong correlation between sensorial characteristics and caffeine, trigonelline, fat, sucrose, cholorenic acids; Vaast et al. (2005a & b) showed that there is a strong relationship between high trigonelline content of coffee beans and higher bitterness and lower acidity of the coffee beverage; Decazy et al. (2003) found a positive relationship between bean sucrose content and coffee acidity and quality; Decazy et al. (2003) found that high bean fat content related to good acidity and beverage preference. Little work has been done on the spatial variability of coffee attributes and their interactions with the environment. The study of Lara-Estrada (2005) was used to put its data and results in a spatial perspective, and to add value to the study. The correlation of the "Score" response maps, for 10 different quality attributes, demonstrate the variability in correlation between the responses (Table 4).

Table 4. Correlation coefficients of response variable pairs (Pref. = Preference, Caff = Caffeine, C.A. = Cholorenic Acids, F.C. = Fat Content, Suc = Sucrose, Trigo = Trigonelline).

	Acidity	Aroma	Bitter	Body	Flavor	Pref.	Caff	C. A.	F.C.	Suc.
Aroma	0.72									
Bitter	-0.22	-0.08								
Body	0.69	0.76	0.17							
Flavor	0.82	0.64	0.06	0.74						
Pref.	0.82	0.74	0.00	0.82	0.90					
Caff	-0.13	-0.03	0.16	0.07	0.07	0.02				
C. A.	0.08	0.08	0.26	0.25	0.12	0.17	0.50			
F. C.	-0.24	-0.21	-0.17	-0.24	-0.24	-0.22	0.28	-0.04		
Suc.	0.33	0.44	0.03	0.42	**0.28**	0.35	-0.10	0.35	-0.23	
Trigo	0.18	-0.04	-0.23	-0.06	0.09	0.03	-0.37	-0.57	-0.31	-0.27

A single figure averaging the correlation of a pair of variables is not always very meaningful. For example, sugar content and flavor are poorly correlated (r = 0.28); only when visualizing the r coefficient on a map, does the importance of the spatial variability become evident (Figure 3) and highly correlated areas (up to r = 1 or r = -1) appear (Figure 3). The spatial correlation window sizes of 3, 5, 9 and 17 grid cells used, translates into 9, 16, 65 and 94 ha

analyzed window; these correspond to farm size (up to 9 ha), groups of farms (up to 16 ha), association (up to 65 ha), and micro-catchments (up to 294 ha). The different resolutions give insight on the scale where correlation patterns emerge, which is valuable information for coffee quality profile identifications and their marketing. The analysis also demonstrates to farmers' associations the strengths and weaknesses of their coffee qualities. Not only do quality responses vary in space, but also the environmental factors and especially their impact on quality. A GWR analysis on the overall importance of the environmental factors impacting on Flavor result in Flavor being significantly dependent on "Number of Average Annual Dry Month" (DM) and "Average Annul Diurnal Temperature Range" (DTR) at a confidence level of 5% and 1%, respectively.

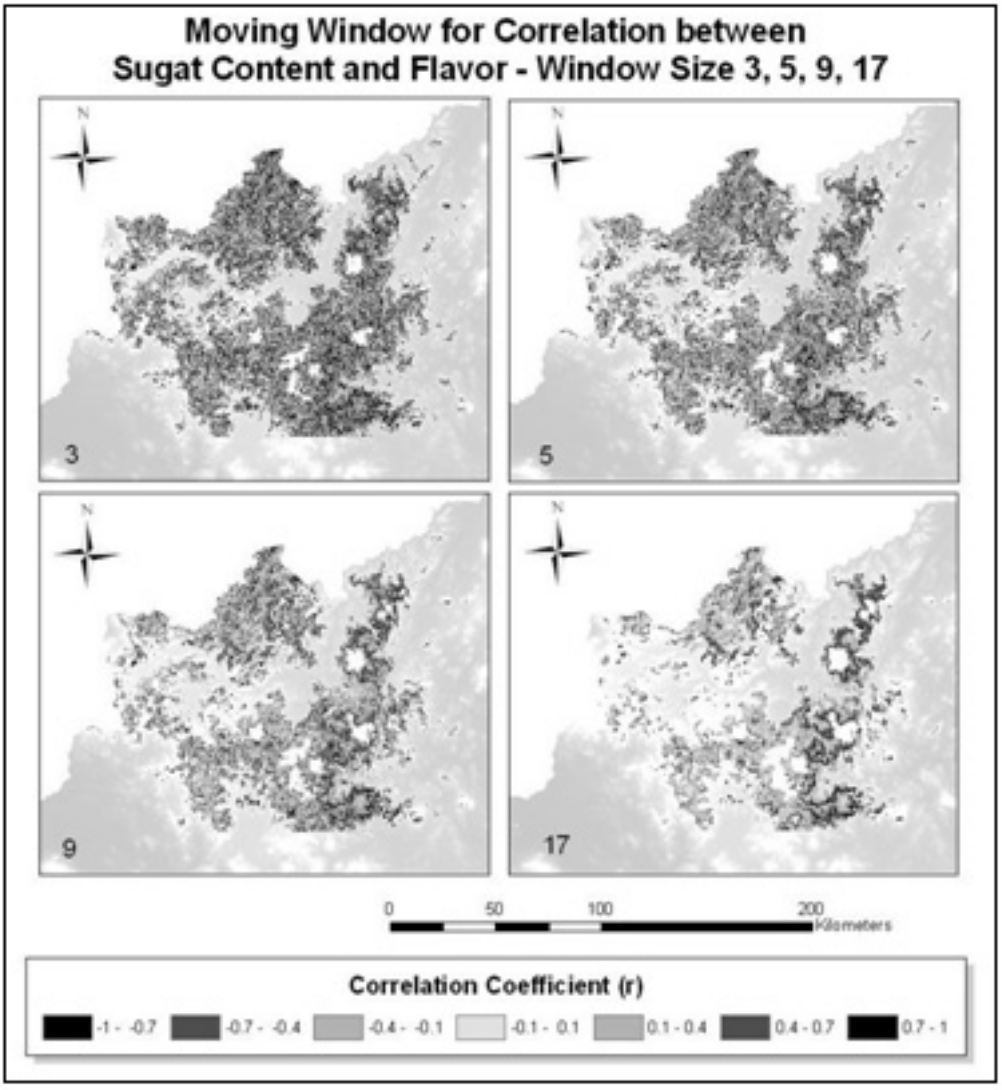


Figure 3. The variability of the correlation between Sugar Content and Flavor at different moving window resolutions.

Even tough these two environmental factors are significant for Flavor; their contribution to each site is distinct. Figure 4 shows the variable impact of DM and DTR on the Flavor quality. Bigger dots are representing a larger impact on Flavor than smaller dots. The impact of these factors is very heterogeneous in space but clusters and forms niches where DM or DTR are more decisive.

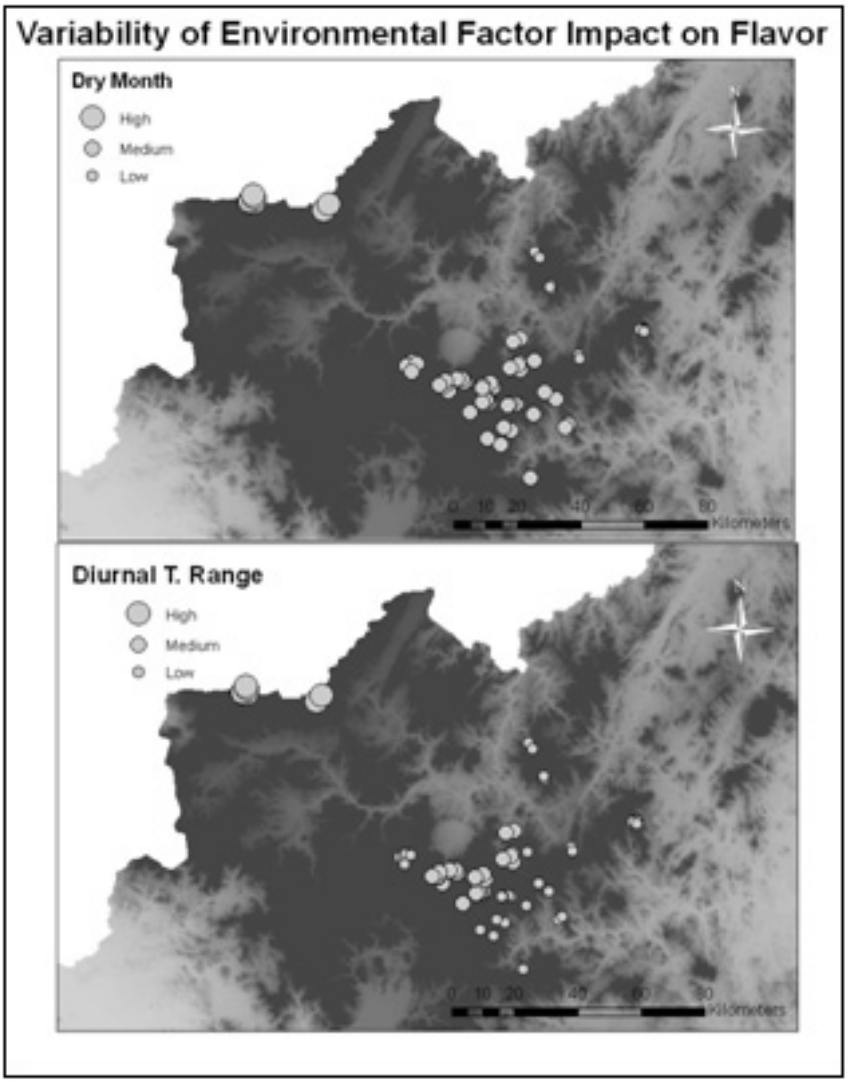


Figure 4. Variability of the impact on Flavor for two decisive environmental factors (dry months and diurnal temperature range). Bigger dots are representing a larger impact on Flavor than smaller dots.

CONCLUSION

CaNaSTA predicts niches likely to produce high Final Score coffee quality values at p = 0.056-0.1 confidence level for a 800 000 ha area in Cauca using only quality data of 88 sites. Within pre-defined niches, quality classes can be predicted at p = 0.019-0.051 confidence levels for the El Tambo-Timbio niche (160 000 ha) and at p = 0.014-0.081 confidence levels for the Inzá niche (16005 ha). The ranges of the factor enhancing or reducing quality in the two niches of Cauca are very different depending on the environmental envelopes predominating in the niche. The importance and utility of SDS tools and Geographical Analyses for assessing the variability of environmental factors and causal quality responses is shown in a case study in Nicaragua. They are very powerful tools to extrapolate point information to surface information. Environmental factors and their impact on quality are very heterogeneous in space. Nevertheless Geographical Analyses allow the identification of niches with similar factor combination.

REFERENCES

Avelino, J., B. Barboza, J.C. Araya, C. Fonseca, F. Davrieux, B. Guyot, and C. Cilas. 2005. Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa Maria de Dota. JSFA, 85:1869-1876.

Decazy, F., J. Avelino, B. Guyot, J.J. Perriot, C. Pineda, and C. Cilas. 2003. Quality of Different Honduran Coffees in Relation to Several Environments. JFS, 68 (7):2356-2361.

Fotheringham, A.S., C. Brunsdon, M. Charlton. 2002. Geographically Weighted Regression the analysis of spatially varying relationships. John Wiley and & Sons Ltd. West Sussex, England.

Jarvis, A., J. Rubiano, Neslon, A. Farrow, A. Mulligan, M. 2004 Practical use of SRTM data in the tropics – Comparison with digital elevation models generated from cartographic data. Working Document no. 198. CIAT, Cali, Colombia.

Jones, P., and P. Thornton. 2000. MarkSim: Software to generate daily weather data for Latin America and Africa. . Agron. J. 93.

Jones, P.G., P.K. Thornton, W. Diaz, and P.W. Wilkens. 2002. MarkSim, Version 1. A computer tool that generates simulated weather data for crop modeling and risk assessment. CIAT CD-ROM series, CIAT, Cali, Colombia.

Hijmans, R.J., S.E. Cameron, J.L.Parra, P.G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J Climatol. 25

Läderach, P. T. Oberthur, N.Niederhauser, H. Usma, L.Collet, H.A.J. Pohlan. 2006. Café especial: Factores, dimensiones e interaciones, in El cafetal del futuro: Realidades y Visiones. (eds) Pohlan, J., L. Soto, J. Barrera, Aachen, Shaker Verlag, 2006, 462pp.

Lara-Estrada, L.D. 2005. Efectos de la altitud, sombra, producción y fertilización sobre la calidad del café (Coffea arabica L. var. Caturra) producido en sistemas agroforestales de la zona cafetalera nor-central de Nicaragua. Master thesis, Centro Agronómico Tropical de Investigación y Enseñanza. Costa Rica.106pp.

Nelson, A. 2004. The spatial analysis of socio-economic and agricultural data across geographical scales: Examples and applications in Honduras and elsewhere, PhD Thesis, University of Leeds, England. pp369.

Linacre, E. 1977. A simple formula for estimating evaporation rates in various climates, using temperature data alone. Agric. Meteorol. 18:409-424.

O'Brien, R. 2004. Spatial Decision Support for Selecting Tropical Crops and Forages in Uncertain Environments. PhD thesis, Department of Spatial Sciences, Curtin University of Technology, Perth, 278pp.

Pearl, J. 1990. Bayesian Decision Methods, in: Readings in Uncertainty Reasoning, SHAFER, G. and J. PEARL (eds.), Morgan Kaufmann, San Mateo, CA, pp. 345-352.

Vaast, P. Cilas, C. Perriot, J; Davrieux, J; Guyot, B; Bolaños, M. 2005a. Mapping of Coffee Quality in Nicaragua According to Regions. Ecological Conditions and Farm Management. In Proceedings of the 20th International Congress on Coffee Research (ASIC) Bangalore, India. p 842-850.

Vaast, P; Van Kanten, R; Siles, P; Dzib, B; Frank, N; Harmand, J; Genard, M. 2005b. Shade: A Key Factor for Coffee Sustainability and Quality. Proceedings of the 20th International Congress on Coffee Research (ASIC) Bangalore, India. p 887-896.

Agrometeorological Model for Monitoring and Predicting Coffee (*Coffea arabica* L.) Productivity in São Paulo State, Brazil

M. B. P. CAMARGO, M .A. SANTOS, M. J. PEDRO JR, J. I. FAHL

Instituto Agronômico (IAC / APTA / SAA), Centro de Ecofisiologia e Biofísica
Campinas, São Paulo State, Brazil – Supported by: PNP&D/Café and CNPq

SUMMARY

A model to reliably monitor and assess agrometeorological impact on coffee yield just before the beginning of the maturation growth stage is proposed. Historical phenological observations and yield data were collected from adult coffee plantations related to the years of 1990/91 to 1999/00 at four different regions of the state of Sao Paulo, Brazil. Basically, the agrometeorological model is based in two parts: first, the model estimates the beginning of the floral induction based on accumulated growing degree days, and a critical rainfall depth. The second part is based on the penalization of the potential crop productivity according to the previous yield and the water stress ratio (ETr/ETp) derived by a 10-day soil water balance during different growth stages. These ratios were weighted by derivation of crop phase yield-response sensitivity coefficients (Ky values), in a multiplicative type model. Also, the model considers penalization for minimum and maximum air temperature. An analysis of the sensitivity coefficients values shows that the model gives higher weight to the water relations during flowering and coffee bean formation phases. This period generally occurs between October and January and it will determine the coffee crop productivity. Model validation for independent years (2000/01 to 2004/05) was satisfactory regardless the region considered. The results support the overall conclusion that the proposed calibrated model shows a good capacity to estimate the coffee productivity. The agrometeorological model looks promising as a tool for monitoring climatic impacts on coffee production.

RESUMO

Um modelo agrometeorológico é proposto visando o monitoramento e avaliação dos impactos meteorológicos sobre a produção de café antes do início da maturação. Observações históricas de fenologia e de produção de grãos foram coletadas de cultivos de café adultos dos anos agrícolas de 1990/91 a 1999/00 para quatro diferentes regiões do Estado de São Paulo. Basicamente, o modelo é dividido em duas partes: primeira, o modelo estima o início da indução floral baseada na acumulação de graus dia, e a quantidade mínima de chuva necessária. A segunda parte do modelo é baseada na penalização da produtividade potencial da cultura de acordo com a produtividade do ano anterior e às relativas aos estresses hídricos (ETr/ETp) derivadas do balanço hídrico ajustados por diferentes coeficientes de sensibilidade da cultura à produtividade ocorridos durante as diferentes fases fenológicas. O modelo considera também penalizações por temperaturas adversas. Uma análise dos valores dos coeficientes de sensibilidade indica que o modelo penaliza com mais intensidade as deficiências hídricas ocorridas durante os períodos do florescimento e da granação dos frutos. Estes períodos ocorrem geralmente entre os meses de outubro e janeiro e que irão determinar a produtividade de café a ser obtida. A validação do modelo utilizando dados independentes (2000/01 a 2004/05) foi satisfatória independentemente das regiões consideradas, apresentando potencial para monitorar os impactos climáticos sobre a produtividade, podendo servir como subsídio aos trabalhos de previsão de safra da cultura do café.

INTRODUCTION

The early estimation of coffee yield is becoming more important for all parties involved in the production and commercialization of coffee. The precision of estimate depends on the different scales, such as by plant, plot, farm, county, regional or national level. Two different approaches to estimate coffee yield can be considered (Cilas and Descroix, 2004): using models of endogenous variables, as different components of the yield that requires the counting on samples of plots on established plants. This approach is known to be more efficient especially for estimating small areas. Another approach using methods based on a model of variations of yield from exogeneous variables, most often climatic, that requires access to reliable meteorological readings from observation points distributed throughout the production zones. The agrometeorological model presented here calls upon this second approach, which the estimates are early desired for estimating larger amplitude, such as county and regional level.

The meteorological elements are the main factors responsible for the oscillations and frustrations of the coffee grain yield in the State of Sao Paulo, Brazil. The relationships between the climatic parameters and the agricultural production are quite complex, because environmental factors affect the growth and the development of the plants under different forms during the growth stages of the coffee crop. Agrometeorological models related to the growth, development and productivity can supply information for the soil water monitoring and yield forecast.

The processes of evapotranspiration and photosynthate production are closely linked (Hanks and Rasmussen, 1982). The processes of photosynthesis become limited when water stress occurs, due to closing of the stoma and reduction in other physiological activities in the plant. Because the transport processes, which make CO_2 available for photosynthesis and allow water to evapotranspiration from the stoma are so closely linked, yield can be reasonably estimated by analyzing the evapotranspiration ratio.

Picini et al. (1999) developed an agrometeorological model for estimating the coffee productivity, based on the mathematical models developed by Stewart et al. (1976), Doorenbos and Kassan (1979) and Rao (1988). These models suggest that the water consumption can be expressed by the water stress ratio between the actual evapotranspiration (ETr) and the potential evapotranspiration (ETp) derived by an soil water balance during different growth stages of the coffee crop, quantifying the effect of the available soil water on the decrease of the final yield.

That model, however penalizes the productivity just for the water stress, and according to Carvalho et al. (2003) should not be considered separately, because, other climatic factors can reduce the productivity, such as adverse air temperatures happened during different growth stages. Solar radiation and relative humidity influence many physiological processes of coffee tree but are not generally thought to play an important role as thermal and rainfall conditions in defining potential yield or ecological limitations for this crop (Damatta and Ramalho, 2006).

An agrometeorological model to reliably monitor and assess agrometeorological impact on coffee yields just before the beginning of the maturation growth stage was proposed by Camargo et al. (2003) based on crop phenology, rainfall conditions and thermal components. The authors mentioned that the model should include the productivity of the previous year due to the interdependence of one year on the subsequent that causes the alternation on grain yield. This happens due to the action of the partition of photosynthetic elements between the

relationships source-drain, due to the reproductive phases and of vegetative growth for the following year, which occurs simultaneously (Camargo & Camargo, 2001). The authors emphasized the need of the calibration of the sensibility coefficients and test of the model for different growing areas.

In view of the preceeding, an agrometeorological study was conducted aiming to adjuste, calibrate and test an agrometeorological model (Camargo et al., 2003) that monitors and assess the quantitative influence of climatic variables, such as air temperature and soil water balance on the coffee crop phenology and yield for different regions of the State of São Paulo, Brazil.

MATERIAL AND METHODS

Historical data obtained from the Instituto Agronomico (IAC) archieves concerning phenological observations and grain yield were collected related to adult coffee plantations, Mundo Novo and Catuai cultivars, for the years of 1990/91 to 1999/00 at four different regions: Franca (latitude: 20º 33'S, longitude: 47º 26'W, altitude: 1026 m above msl), Mococa (latitude: 21º 28'S, longitude: 47º 01'W, altitude: 665m above msl), Campinas (latitude: 22º 54'S, longitude: 47º 05'W, altitude: 674 m above msl), and Marília (latitude: 22º 14' S, longitude: 49º 57' W, altitude: 652 m above msl) of the state of São Paulo, Brazil.

Daily records of air temperature and rainfall were obtained in four standard weather stations located nearby the four regions to estimate the soil water balance.

Basically, the agrometeorological model (Camargo et al., 2003) is based in two parts: first, the model estimates the beginning of the floral induction based on accumulated growing degree days (GDD), and a critical rainfall depth. Phenological observations (Camargo & Camargo, 2001) indicated that floral buds complete maturation and reach dormant stage, being ready for floral induction when the accumulated GDD reaches 1,590, starting from April 1st. After, a rain or irrigation of at least 10 mm is required to break the bud dormancy. Usually the flowering occurs from 6 to 12 days after the increase of the water potential inside the floral buds.

The second part of the model is based on the penalization of the expected potential crop yield in the beginning of each growth year that depends on the scale size of the production area and the biennial yielding pattern. The general model is a productory written as:

$$RLY\ (\%) = [(f\,WD) * (f\,TMIN) * (f\,TMAX)] \qquad [1]$$

where que RLY (%) is the relative coffee loss yield (%) , equal to Ya (actual coffee yield) over Yp (potential coffee yield), *f WD, f TMIN* e *f TMAX* are the penalization factors by water deficit, minimum and maximum air temperatures respectively.

The penalization by water deficit was derived by a soil water balance occurred during different crop growth stages (bud dormancy, floral induction, flowering and coffee bean formation). A soil water balance model (Thornthwaite and Mather,1955) considering 100mm of maximum available water capacity (Camargo and Pereira, 1994) was used to estimate the soil water availability during the growth stages of the crop on a ten-day basis. The water balance model outputs are potential evapotranspiration (ETo), actual evapotranspiration (ETr), water deficit (WD) and water surplus (SUR). ETr represents the water supply and ETp potential water demand of the plant. Thus, the ETr/ETp ratio is an indication of the water

supply in relation to the potential need (Yao, 1969). The ETr/ETp ratio less than the unit, indicates that the crop was submitted to water stress.

$$fWD = \prod \left[1 - \left(Ky \left(1 - \frac{ETr}{ETp} \right) \right) \right] \quad [2]$$

Where π is the productory and Ky values are the crop phase yield-response sensitivity for water deficit coefficients.

These ratios were weighted by derivation of crop phase yield-response sensitivity coefficients (Ky values) in a multiplicative type model. The values of Ky were calculated by adopting successive simulations of the best fitting values between the parameter and the coffee grain yield.

For the penalization by thermal stresses, the model considers penalization for minimum temperature (frost) according to a Gaussian model when the minimum air temperature falls below 2 ºC . For maximum air temperature the model considers a Gompertz model when the number of days beyond the limits of 34 °C during the critical flowering period (usually October) according to Ortolani et al. (1998).

The expected potential yield in the beginning of each growth year depends on the size scale of the considered production area, the previous yield and the biennial yielding pattern according to Santos and Camargo (2006).

The general agrometeorological model can be expressed as:

$$RLY(\%) = \left\{ \left[1 - \left(ky \left(1 - \frac{ETr}{ETp} \right) \right) \right] * \left[1 - f.frost \right] * \left[1 - f.T\max \right] \right\} \quad [3]$$

where RLY is the relative loss yield (%) , equal to Ya (actual coffee yield) over Yp (potential coffee yield) of sacks (60 kg bags.ha^{-1});

The model validation considered independent coffee yield data, for the period of 2000/01 to 2004/05.

Statistics for assessing the model performance besides the coefficient of determination (R^2), includes the d-index of agreement (Wilmott et al., 1985). The d-index is a more sensitive indicator of systematic model error than R^2. Values of the d-index range from 0, for complete disagreement, to 1 for perfect agreement between observed and predicted values. Other procedures used to evaluate model performance were the systematic (Es) and unsystematic (Eu) components of the mean absolute error, a measure of the average magnitude of the differences between the predicted and actual values.

RESULTS AND DISCUSSION

The ten-day water balances, referring to the agricultural years of 1990/91 to 2004/05 allowed to observe periods with water deficits and surpluses during the whole growth stages of the coffee crop. For example, the growing season of 2002/03 showed periods with high water deficits and high air temperatures during October, November, and December, that coincided

with critical growth stages, such as flowering and grain filling, which provoked considerable reductions in the coffee yield.

The crop phase yield-response sensitivity for water deficit coefficients (Ky values) that showed the best fitting values between the parameter and the coffee yield are shown in Figure 1. An analysis of the sensitivity coefficients values shows that this model gives higher weight to the water relations during floral induction period, and especially during the flowering (September-October) and coffee bean formation (November-January) phases. This period generally occurs between September and January and it will determine the coffee crop productivity.

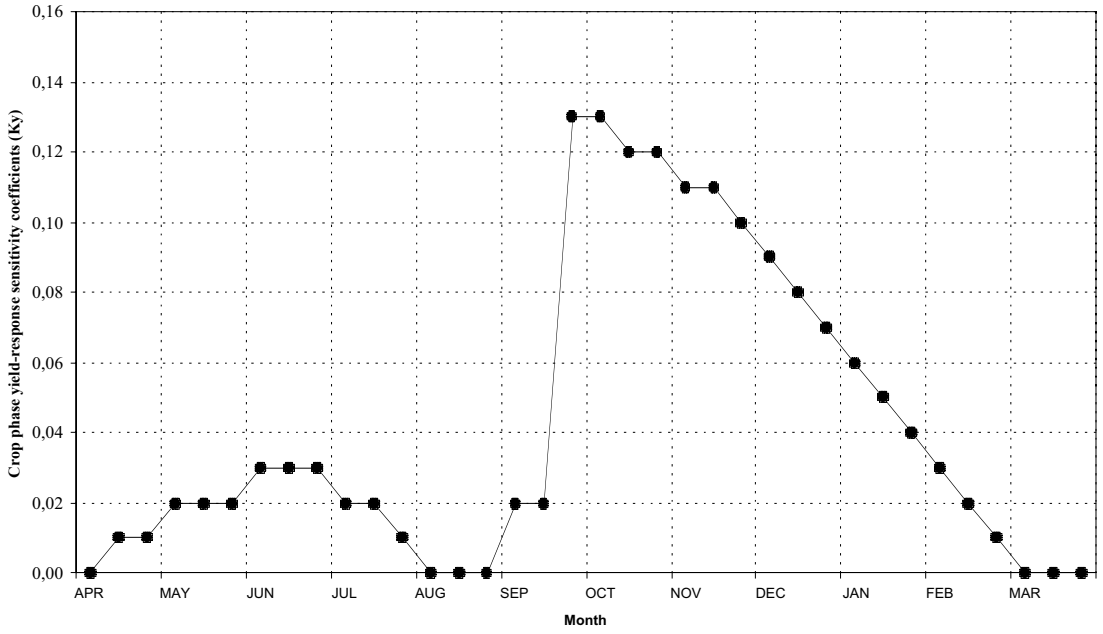


Figure 1. Crop phase yield-response sensitivity water deficit coefficients (Ky values) parameterized for different growth stages (months).

Model validation was satisfactory regardless the region considered. The statistical analysis (Table 1) for predicted and observed coffee productivity reveals a close agreement, with relative high R^2 (0.78 to 0.93), and d-index of agreement (0.73 to 0.90) values for the coffee regions considered. When considered all the regions together, the statistical analysis presented relative high R^2 (0.73), and d-index of agreement (0.91) values. Other measures of model performance, showed relative low values for systematic (4.99 sacks (60kg bags).ha^{-1}) and unsystematic (6.18 sacks (60kg bags.ha^{-1}) components.

Table 1. Statistical results (R^2: coefficient of determination; d: d-index of agreement; Es: systematic error and Eu: unsystematic error (sacks (60 kg bags).ha^{-1})) of the agrometeorological model performance during years 2000/01 to 2004/05 for different regions of the state of São Paulo, Brazil.

Region	d	R^2	Eu	Es
Campinas	0.90	0.78	3.90	3.20
Franca	0.73	0.85	4.50	5.30
Marilia	0.88	0.79	4.85	3.82
Mococa	0.88	0.93	3,80	4.20
All together	0.81	0.73	6.18	4.99

Sources of errors contributing to the differences in estimated and observed coffee yield are not difficult to identify since the agrometeorological model contains parameters whose values are calculated from empirical relationships, such as evapotranspiration values, water balance method, phenological informations, different amplitude level of coffee production etc.

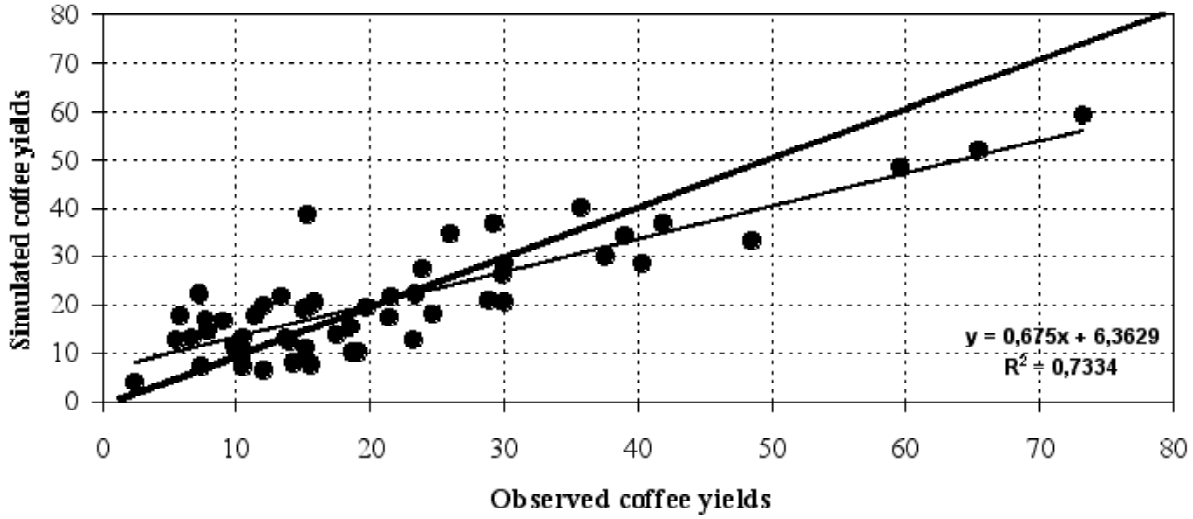


Figure 2. Observed and simulated coffee yields (sacks (60kg bags).ha^{-1}) between 2000/01 to 2004/05 for different regions at the state of São Paulo, Brazil.

Although the coffee productivity estimates showed relative good agreement with observations, these results should be tempered against the limitations. There are some parts of the model that can be improved, such as:

a) Use a water balance model in a daily basis, considering daily evapotranspiration values estimated by Penman-Monteith method. Unfortunately there are few automated weather stations that can support historical data inputs for running this model in the entire Brazilian coffee production region.
b) An error in the empirically derived coffee crop phase yield-response sensitivity coefficients (Ky values), a function of phonological growth stage, may lead to errors in estimating the penalization of the productivity.
c) The penalization by thermal stresses, for minimum temperature (frost) and for maximum air temperature may be improved, especially for maximum air temperature during the critical flowering period.
d) Use this agrometeorological (exogenous) model combined with an endogenous model that considers important biometrics coffee crop information's.

CONCLUSIONS

The results support the overall conclusion that the proposed agrometeorological model shows a good capacity to estimate the coffee productivity in a regional level, using input data that can be found in major coffee production areas in Brazil. The agrometeorological model looks promising as a tool for monitoring climatic impacts on coffee production.

REFERENCES

Camargo, A.P.; Pereira, A.R. Agrometeorology of the coffee crop. Geneve: World Meteorological Organization, 1994. 96p. (Agricultural Meteorology Cam Report, 58).

Camargo, A.P.; Camargo, M.B.P. Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. Bragantia, Campinas, v.60, n.1, p.65-68, 2001.

Camargo, M.B.P.; Santos, M.A.; Pedro Junior, M.J.; Fahl, J.I.; Brunini,O.; Meireles, E.J.L.; Bardin, L. Modelo agrometeorológico de monitoramento e de estimativa de quebra de produtividade como subsidio à previsão de safra de café (*Coffea arabica L.*): resultados preliminares. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, 3., 2003. Porto Seguro, Anais... Porto Seguro: Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café, 2003. p. 75-76.

Carvalho, L.G.; Sediyama, G.C.; Cecon, P.R.; Ramos Alves, H.M. Avaliação de um modelo agrometeorológico para previsão de produtividade de café em três localidades da região sul do Estado de Minas Gerais. Revista Brasileira de Agrometeorologia, Santa Maria, v.11, n.2, p.343-352, 2003.

Cilas, C.; Descroix, F. Yield estimation and harvest period. In: Wintgens, J.N. (Org.) Coffee: growing, processing, sustainable production. Weinheim, Germany. 2004, v.1, p.595-603.

Damatta, F.M.; Ramalho, J.D.C. Impacts of drought and temperature stress on cofeee physiology and production: a review. Brazilian Journal of Plant Physiology, Campinas, v.18, n.1, p.55-81, 2006.

Doorenbos, J.; Kassan, A. H. Yield response to water. Rome: FAO, 1979. 197 p. (FAO Irrigation and Drainage Paper, 33).

Hanks, R.J.; Rasmussen, V.P. Predicting crop production as related to plant water stress. In: Brady, N.C., ed. Crop production and plant water stress. Advances in Agronomy, Madison, 35:193-215, 1982.

Picini, A.G.; Camargo, M.B.P.; Ortolani, A.A.; Fazuoli, L.C.; Gallo, P.B. Desenvolvimento e teste de modelos agrometeorológicos para a estimativa de produtividade do cafeeiro. Bragantia, Campinas, v.58, n.1, p.157-170, 1999.

Ortolani, A.A.; Sentelhas, P.; Camargo, M.B.P.; Pezzopane, J.E.M.; Gonçalves, P.S. Agrometeorological model for the seasonal rubber tree yield. Indian Journal of Natural Rubber Research , New Delli, v.11, n.1&2, p.8-14, 1998.

Rao, N. H.; Sarma, P. B. S.; Chander, S. A simple dated water-production function for use in irrigated agriculture. Agricultural Water Management, Amsterdam v.13, p. 25-32, 1988.

Santos, M.A.; Camargo, M.B.P. Parametrização de modelo agrometeorológico de estimativa de produtividade do cafeeiro nas condições do Estado de São Paulo. Bragantia, Campinas, v.65, n.1, p.173-183, 2006.

Stewart, J. I.; Hagan, R. M.; Pruitt, W. O. Production functions and predicted irrigation programmes for principal crops as required for water resources planning and increased water use efficiency: final report. Washington: U.S. Departament of Interior, 1976. 80 p.

Thornthwaite, C. W.; Mather, J. R. The water balance. Centerton, N. J. 1955, 104 p. (Publications in Climatology. vol 8, n. 1).

Willmot, C. J.; Ackleson, S. G.; Davis, R.E.; Feddema, J.J.; Klink, K.M.; Legate, D.R.; O'donnell, J.; Rowe, C.M. Statistics for the evaluation and comparison of models. Journal of Geophysical Research, Washington. v.90, p. 8995-9005, 1985.

Yao, A.Y.M. The R index for plant water requirements. Agricultural Meteorology, Amsterdam, v.6, n.4, p.259-273, 1969.

Bird Diversity on Coffee Farms in Central Colombia

P. BAKER[1], J. BOTERO[2], G. LENTIJO[2]

[1]CABI, Silwood Park, Ascot, UK
[2]CENICAFE, Chinchiná, Caldas, Colombia

SUMMARY

Coffee farming on the Andean foothills in central Colombia is intensive and much of it is under reduced shade or full sun conditions. Over the last decade various authors and institutes have suggested that sun coffee is little more than a biological desert – drawing attention especially to impoverished bird life – and that coffee farmers should be encouraged to reintroduce shade trees under one of a number of schemes in return for a premium.

This study, part of a Darwin Initiative project funded by the UK government, aimed to objectively study bird diversity and abundance on 80 farms in two municipalities of the central cordillera of Andean Colombia: a) smallholder coffee farmers (Manizales) and b) larger plantations (Palestina), both in the Department of Caldas. Altitude range was between 1300 and 1700 m above sea level, covering biomes classified as tropical lower montane and premontane rain forest (the Holdridge classification).

Abundance and species of birds were counted along walked transects made through the farms. The habitats in which the birds were observed were also recorded, these included: coffee, bamboo groves, gardens, uncultivated (weedy ground), hedgerows, other crops and buildings. A record was made of plant species encountered.

100 species of bird were recorded belonging to 30 families. Most common were Tryrannidae (flycatchers), Thraupinae (tanagers) and Parulinae (wood warblers). Also present were Trochilidae (hummingbirds) and Emberizinae (New World sparrows). Frugivorous families were rather poorly represented. Eleven of the species were intercontinental migrants.

There were only fairly small differences in composition of species between the small farms and the larger estates (66 species in common); abundance was generally higher on the estates. No CITES-listed endangered species were observed and neither were any species in the 'highly vulnerable' category.

The results are discussed in relation to potential ways to further enhance and protect these bird populations and how to involve farmers in this task.

INTRODUCTION: THE COFFEE PROBLEM

In times of low prices a fundamental problem of all commodity production comes to the fore: competition for market share squeezes profits and leads to one of three options for farmers:

- Intensify production – which requires investment that is difficult to find for all but a few and potentially leads to overproduction.
- Diversify production – but in many mountainous coffee agro-systems there are few viable alternatives.

- Add value – through increasing the intrinsic, service or symbolic value of the crop (Daviron and Ponte, 2005).

In this paper we look the latter strategy in the light of a project that took place in the central coffee zone of Colombia (the 'eje cafetero' as it is called there).

ADDING VALUE

Daviron and Ponte (2005) suggest three basic ways to add value to a commodity:

- Intrinsic attributes – i.e. the quality of the product itself, a distinctive taste or other physical attribute.
- Service – where the product is part of a package of activities that customers will pay for (e.g. a café or farm tourism).
- Symbolic – where some other attribute becomes attached to the product, e.g. an environmental or social campaign.

Thanks to the efforts of various NGOs[1] – and it is they, after all, that are in the vanguard of influencing public perceptions – there is much greater awareness of the many possible symbolic attributes that coffee can provide and it is on this last category that this study concentrates.

THE PROJECT

The purpose of our project was to study if or how biodiversity might be able to add some symbolic value to coffee in Colombia, so that any new knowledge might be put to commercial use, to add value to Colombian coffee and thus enhance its economic sustainability through returning more income to farmers and as a consequence helping biodiversity to become more valued by coffee producers and communities.

There have been positive developments in this field in recent years. Rainforest Alliance for example have managed now to certify over 50,000 ha of coffee and a proportion of the coffee produced from in this way is sold with a special seal that guarantees its various environmental attributes, including that the coffee originates from under a diversity of shade trees.

But this is not an easy undertaking for a major coffee enterprise such as the central coffee zone of Colombia. In this region can be found some of the most intensively grown coffee in the world, with up to 10,000 trees/ha and little or no shade tree cover. Indeed this type of coffee culture has been singled out for criticism by Rainforest Alliance and The Smithsonian websites. The latter site includes the following information:

> "… studies in Colombia and Mexico found 94-97% fewer bird species in sun grown coffee than in shade grown coffee. This comes as no surprise since over two-thirds of the birds are found in the canopy of shade plantations and less than 10% are found foraging in coffee plants." [2]

The Rainforest Alliance site makes the following assertion about coffee farms:

> "The conclusions are imperative and unanimous: traditional shaded farms host high

[1] E.g. Fair Trade organisations, organic associations, Rainforest Alliance, Conservation International and others.
[2] http://nationalzoo.si.edu/ConservationAndScience/MigratoryBirds/Fact_Sheets/default.cfm?fxsht=1

levels of biodiversity; the new "full-sun" farms are "biological deserts" with few signs of life." [3]

This presents a problem for Colombia: it is a 'megadiverse' country, one of the most biodiverse on Earth, and it has made important commitments to protect biodiversity by signing the Convention on Biological Diversity that originated from the UN Earth Summit in 1992. And indeed Colombia has made a major effort in recent years to raise the level of biodiversity activities in the country, e.g. by establishing the Humboldt Institute[4]. But at the same time, farmers need to make a living from coffee and the general opinion of Colombian farmers and extensionists there is that a return to shaded coffee would reduce yields and profitability. Even if Colombia did return to shaded coffee in a substantial way, there would be almost no likelihood of gaining a premium of the symbolic type referred to above, since a major increase in supply of such coffee would drive premiums towards zero.

An important additional consideration is that although unshaded coffee has attracted the disapproval of environmentalists, it is still a more appropriate crop to grow than most commercial alternatives, such as annual crops and cattle, both of which increasingly can be seen growing on some steep slopes of the Colombian coffee zone.

Thus there is a conflict: Colombia is both a very important coffee country and a very important biodiversity country. The intersection of these two topics make this matter especially significant.

Can these two factors be reconciled? Can Colombia remain a major coffee producer, can its coffee continue to underpin the economy and relative tranquility of the main coffee growing areas? Or would its sun-coffee status detract from the product over the long term? Could this end up being bad for the overall image of coffee and Colombia? These were some of the questions that occurred to us as we started this project.

PROJECT OPTIONS

If we are to look at protecting or enhancing biodiversity of coffee in Colombia there are a number of practical ways to tackle the issue, none of them without drawbacks. Below are three possibilities:

1. Small scale studies and trials of various levels of biodiverse shaded plots to evaluate the economic, social and environmental advantages of these and commercial potential for the coffee. But there is a scalability problem with this: the commercial niche is still very small, the premiums not very large and possibly contracting (Pratt, 2005), so that the likelihood of large areas of more biodiverse coffee being created that would have a measurable impact on biodiversity in the region, seem unlikely.

2. Evaluation of the role of forest remnants, buffer zones, patches, corridors etc. This has been the approach of Conservation International and other initiatives and has obvious merit, but for practical project reasons (the length of project, its relatively small size) we considered this to be too long-term, and too big a task.

3. Study the mostly unshaded coffee in the central coffee zone which comprises the majority of the coffee produced in the country, measure its deficiencies and look for ways to improve

[3] http://www.rainforest-alliance.org/programs/education/teachers/curriculum/pdfs/conservation-coffee.pdf
[4] www.humboldt.org.co/

it. The difficulty with this approach is that intensive coffee apparently has little biodiversity, so it might be too difficult to restore to an acceptable level.

In this study we picked the third option, mainly because it is the majority situation; we felt that we could not ignore this very large zone and we considered that it had also not been sufficiently well studied. The main objectives of the study therefore were to answer some basic questions:

- How bad is sun/intensive coffee in fact?
- How might we improve it?
- What do farmers think about all this?
- How do we involve them?

From this we hoped to provide new information and knowledge to help decision makers and other stakeholders come to some rational decisions. Outputs of the project include a website with biodiversity information[5] and a field guide as a resource book for extensionists to be published in early 2007. In the rest of this presentation we concentrate on bird diversity as an indicator of coffee biodiversity in general.

PROJECT METHODOLOGY FOR BIRD SURVEYS

We randomly selected 40 smallholder farms and 40 larger intensive farms in two municipalities (municipios of Palestina and Manizales) in the central coffee zone (eje cafetero) of Colombia, lying between 1300 and 1700 m above sea level, covering biomes classified as tropical lower montane and premontane rain forest (according to the Holdridge (1967) classification).

Around each farm observers walked in transects. In all they made 177 visits and made over 2,000 observations. All birds that were seen were recorded, except those in flight, as well as the habitat in which they were situated, e.g. coffee, garden, uncultivated, intensive coffee, semi-shaded coffee, shaded coffee, other crops, bamboo grove (*Bambusa guadua*). An inventory of common plant species was also taken.

RESULTS

Although most farms studied had coffee with little or no shade, all farms were a mosaic of habitats, as can be seen in Table 1.

Table 1. Number of the 80 farms with each category of habitat in the municipalities of Manizales y Palestina.

Municipality	Habitat							
	Uncultivated	Bamboo	Garden	Intensive coffee	Light shaded coffee	Shaded coffee	Hedgrow	Other crops
Manizales	24	33	26	15	19	6	9	1
Palestina	29	32	39	34	6	0	15	9

The birds, if classified according to their basic guild (e.g. forest, woodland, open country) were quite widely dispersed over the various farm habitats (Table 2).

[5] See www.cenicafe.org

Table 2. Total number of bird species observed per farm habitat, according to their guild classification.

	Farm habitat								
Bird guild category	Uncultivated	Bamboo	Garden	Sun coffee	Light shade	Shade coffee	Hedges	Other crops	Buildings
Species in open areas	25	20	25	31	25	22	9	8	21
Woodland species	20	16	13	24	20	13	5	4	4
Forest generalists	8	6	2	7	9	5	3	1	0
Species of bushes/ thickets	16	9	5	13	9	7	5	1	4

A total of 100 species of bird were recorded over the 80 coffee farms. They comprised 30 families the commonest of which are listed in Figure 1. None of the species counted were considered endangered and no very rare forest species typical of Andean montane forest were recorded.

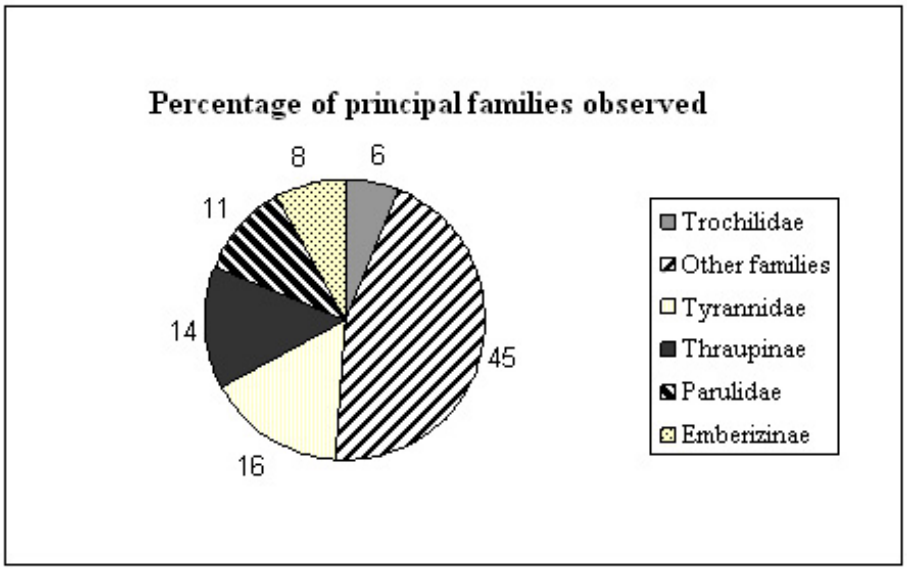


Figure 1. Percentage of principal bird families found in Colombian coffee farms.

Nevertheless, certain species were considered to be of especial interest, including those that are endemic, generalist forest species, migratory, rare or highly vulnerable. 43 of the 100 species fitted these criteria, the commonest of which are *Tangara gyrola, Crypturellus soui, Phaethornis guy* y *Grallaria guatimalensis*. Most of the 43 however are considered quite rare species, including *Heliomaster longirostris, Myiarchus cephalotes* and *Hemithraupis guira.* Eleven transcontinental migrant species were spotted in low numbers (Table 3).

A concomitant study of common plants present recorded 119 species, many in borders, gardens, uncultivated land. Use of herbs for medicinal uses was common by families living on the farms.

Table 3. Presence (1) and absence (0) and total/habitat for migratory species registered in the municipalities of Manizales and Palestina.

Migratory bird species	**Habitat**								
	Uncultivated	Bam-boo	Garden	Sun coffee	Light shade	Shade coffee	Hedge	Other crops	Build-ings
Catharus ustulatus	1	0	0	0	0	0	0	0	0
Contopus sp.	1	0	0	1	1	1	0	0	0
Dendroica fusca	1	0	1	1	1	1	0	0	0
Dendroica aestiva	0	0	1	1	1	0	0	0	0
Mniotilta varia	1	1	0	0	0	0	0	0	0
Oporornis philadelphia	1	0	0	0	0	0	0	0	0
Piranga rubra	1	0	0	0	0	0	0	0	0
Setophaga ruticilla	1	1	0	0	1	0	0	0	0
Tyrannus tyrannus	0	0	1	1	0	1	0	0	0
Vireo olivaceus	0	0	0	0	1	0	0	0	0
Wilsonia canadensis	0	1	0	0	1	0	0	0	0
Total species	7	3	3	4	6	3	0	0	0

CONCLUSIONS

Unshaded 'sun' coffee in Colombia is not a biological desert, as some have claimed. Not surprisingly, many of the birds found were those that are often encountered in altered habitats and there were no very rare birds, none from the CITES list. But amongst the hundred species were a proportion of rare birds were recorded and some that are transcontinental migrants. These latter are of extra significance because it was originally the apparent decline of migratory bird species that helped to launch the intensive study of biodiversity and shade coffee in the 1990s (Rice and Ward, 1996). The rare forest species that would have originally inhabited these slopes when forest covered them were absent however, replaced by a mix of generalist forest species and those more often found in open habitats.

Some guilds of birds were rather poorly represented, for example frugivorous birds. Remarkably perhaps, the smaller and generally less intensive farms did not have a greater diversity of birds.

From other parts of the project (not covered in this paper) we discovered that farmers are interested in biodiversity, though they do not refer to it as such and they are manifestly concerned about the general decline in the quality of their environment, though often revealed more in the decline of mammals that could be hunted, rather than birds themselves. When asked, they also indicated that they would be willing to supply their labour to improving

biodiversity, but not to contribute cash, nor change back to shade coffee unless they could expect a substantial premium for it.

And it is not just birds: another Colombian study shows a high butterfly diversity – 292 diurnal species have been recorded in the central coffee zone (Valencia, 2004) which corroborates the work of this study, suggesting that biodiversity is indeed far from being absent. A major reason for this must be that although much of the coffee is unshaded, the coffee agro-ecosystem is not a monoculture; the study recognized a mosaic of vegetational types that together comprise something that is richer than had been supposed. This, added to the naturally high fertility of the volcanic soils, we suggest is the real reason for the levels of biodiversity encountered.

From all the project information and other sources therefore we come to the following conclusions that might help to improve the level of biodiversity and improve the image of Colombian coffee, that may have been damaged by the accusations of being little more than a 'biological desert'.

1. Major initiatives to move the majority of Colombian coffee over such an extensive area (i.e. more than half a million hectares – 70% of Colombia's coffee is classified as intensive) to return it to shade coffee are probably unrealistic since it is unlikely to result in increased returns to farmers.

2. Farmers are a potential ally in attempts to improve biodiversity. They may be willing to help in practical ways, especially to provide their labour, if they can see that it will not materially affect their coffee production and that it may result in improved numbers of animals and plants.

3. Projects that progressively aim to improve biodiversity, e.g. by planting a range of fruit tree species adjacent to houses or on uncultivated ground may be worth trying, since these might improve diversity of the fruit-eating guild that inhabit tree canopies and as well provide additional nutritional food sources for farm workers.

4. Birds are appreciated by members of all communities and can serve as emblems of biodiversity in general. Initiatives to provide teacher packs, videos, teacher training etc. about these charismatic animals may serve to augment project based efforts to boost diversity.

5. If other efforts to establish and protect forest reserves, or extend and join up any existing corridors were also pursued, such an integrated approach could be the best long term strategy to gradually raise biodiversity without any loss in coffee yield.

6. Current levels of diversity found in coffee, allied to concerted attempts to improve biodiversity, through projects and education could be the basis for a positive marketing message for Colombian coffee; i.e. a more generic approach to the problem rather than a specific farm certification focus. This fits to the history of marketing in Colombia which has focused on branding the coffee through the Juan Valdez® marque. One day perhaps we will discover that the eponymous farmer has a family member who is an ornithologist – perhaps one who even temporarily traps, weighs and rings the migrants to monitor their progress on their long journey northwards.

REFERENCES

Daviron B, Ponte S, 2005. The Coffee Paradox. Zed Books, London & NY, 295 pp.

Holdridge LR, 1967. Life zone ecology. Tropical Science Center, San Jose.

Pratt L, 2005. The market outlook for differentiated coffees. 19th Sintercafé Conference, Costa Rica.

Rice RA, Ward J, 1996. Coffee, Conservation and Commerce in the Western Hemisphere. Washington, DC: Smithsonian Migratory Bird Center/ Natural Resource Defense Council.

Valencia Martínez CA, 2004. Las mariposas diurnas como indicadores biológicos en el cultivo del café. Tésis del grado de Ingeniero Agrónomo. Manizales, Universidad de Caldas, Colombia. 165pp.

Tángara real, *Tangara cyanicollis*

Foto: Grupo Asociativo San Isidro

Coffea arabica Clones from F1 Hybrids in Central America

P. CHARMETANT[1], H. ETIENNE[1], R. SANTACREO[2], B. CISNEROS[2], F. ANZUETO[2], S. GIL[2], E. ALPIZAR[2], B. BERTRAND[1]

[1]CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) TA80/PS3, 34398 Montpellier Cedex 5, France
[2]PROMECAFE, Guatemala (for IHCAFE Honduras, ICAFE Costa Rica, ANACAFE Guatemala, PROCAFE, El Salvador)

SUMMARY

The aim of the breeding programme in Central America is to enlarge the very narrow genetic basis of Bourbon, Caturra, and Catuai, whilst increasing quality and productivity. It is based on heterosis (hybrid vigour = production of fruit-bearing nodes) and genetic diversity obtained by combining dwarf commercial varieties with Sudanese-Ethiopian origins. Somatic Embryogenesis has been developed to multiply vegetatively selected F1 trees. Comparative trials were established in 4 Central American countries. Some clones show a good level of resistance to Coffee Leaf Rust, CBD, and to nematodes. Their products exhibit a noticeable stability (homeostasis) for chemical contents and for cup quality, despite a significant increase in productivity as compared to traditional varieties. The dwarf varieties Caturra, Catuai opened the way to a "green revolution" in coffee growing. It is believed that the use of F1 hybrids, combined with proper strategies, should pave the way to a "double green revolution": economically sustainable coffee growing, but environment friendly as well. Six hybrid clones are expected to be definitely selected within one year, and available for large scale planting within 2-3 years.

CONTEXT AND STRATEGIES

This paper presents the creation and selection of F1 hybrids of *Coffea arabica*, and the expectations they raise in Central America. Coffee was initially introduced to America through the Typica variety. In Central America, variety Bourbon took over from the end of the 19th century. It is now the majority of coffee areas, except for Costa Rica, where the main varieties are Caturra and Catuai. Caturra is a dwarf mutant from Bourbon, and Catuai derives from both Bourbon and Typica through Mondo Novo. The genetic diversity is very narrow (Anthony, Combes et al., 2002). We will consider the tall varieties, mainly Ethiopian varieties as a first genetic group, a major source of diversity. The second genetic group is made of the dwarf varieties Caturra and Catuai.

"Traditional, or conservative strategies" are shaded systems, using low inputs and tall varieties. mainly Bourbon in Central America. The most common strategy generates a low added value, and makes the coffee grower very vulnerable to price fluctuations. In some cases, the combination of know how, good growing conditions and good marketing is conducive to the development of a "fame" that allows a higher added value. However, this is limited to a very small percentage of the coffee area. Both strategies contribute to maintaining the biodiversity, but the persistence of the first one is questioned.

As was then the case for a major food crop like wheat, the development of the dwarf varieties Caturra and Catuai was the catalyst for a "green revolution" in coffee. This was combined

with the removal of shade and with high levels of fertilization. Costa Rica has been widely involved in this mutation. It became a champion for coffee productivity.

Up to now, due to high standards of quality, the profitability has remained good at medium to high elevations. However, the long term durability of this practice is questioned due to its impact on the environment.

One major problem with the cultivated varieties from group 1 and 2 has been their overall susceptibility to major diseases, especially Coffee Leaf Rust in lowlands.

Dwarf varieties, derived from the Timor Hybrid, (HDT) were developed for their resistance to Coffee Leaf Rust. They constitute the third genetic group. Some of them show some resistance to CBD and to nematodes (Bertrand, Anthony et al., 2001). Selections were proposed to the growers in many countries in the world.

Is it now well known that the presence of genes from Coffea canephora is not directly connected to cup quality (Bertrand, Guyot et al., 2005). However, the growing system applied to these varieties (dwarf varieties, full sunshine) may, in some cases, affect cup quality (Bertrand, Etienne et al., 2001).

Figure 1. Final stages of micropropagation using Somatic Embryogenesis: germinated embryos, acclimatization, nursery.

This is why A. Charrier and R.A. Muller proposed to PROMECAFE to make best use of the genetic resources available in Central America by combining in F1 hybrids the potential of cup quality from Group 1, the productivity of Group 2, and/or the resistances from Groups 1 and 3.

ACHIEVEMENTS

Practically, after selecting the progenitors (Groups 1 and 3), the hybrids were created by hand pollination. The progenies were then tested in two factorial trials, one at low elevation, the other one at medium altitude. The trees were assessed individually.

Heterosis can be defined as the superiority of a hybrid progeny over the mean of its parents. For yields, the values range from 10 to 50% in arabica coffee. It is higher for inter-pools hybrids than for intra-pool hybrids. This is in the range found for other autogamous crops.

We found that the production of fruiting nodes, which is directly related to growth, was the main factor of yield increase. The number of fruits per node remained unchanged, but the number of beans per fruit was slightly reduced.

Vegetative propagation of the best hybrid genotypes was made possible thanks to cooperation with CATIE. Indeed, the availability of an efficient multiplication process would allow the rapid mass production of heterozygous materials such as selected *C. canephora* clones and F1 *C. arabica* hybrids. Somatic embryogenesis was developed and thoroughly studied from the lab to the field (Figure 1). Somatic variation was described (Figure 2). It did not exceed 2 to 5% and can easily be detected in the nursery (Etienne, 2003). The development of the technique for this type of crop led to various studies (Etienne, Solano et al., 1998; Etienne-Barry, Bertrand et al., 1999; Etienne-Barry, Bertrand et al., 2002). In particular, the feasibility to apply the bioreactor technology in an industrial micro propagation procedure was discussed in the socio-economic context of coffee growing (Etienne, Dechamp et al., 2006).The time needed until the release of a new variety is greatly reduced –from 20-25 years for genealogic selection to 10-15 years for clone selection (Etienne, 2002; Etienne, Alpizar et al., 2005). Besides, a change of variety is easy and quick.

Figure 2. Examples of somaclonal variations encontered in plants derived from somatic embryos.

From 1999 to 2006 PROMECAFE established a network of comparative trials and observation plots in 4 countries. 19 hybrid clones were compared with commercial varieties (Figures 3,4).

Emphasis was given to cup quality, adaptation, and disease resistance.

Figure 3. Large scale observation field of F1 clones.

Figure 4. Field adaptation trial under shade.

Despite their substancial contribution to the increase of productivity (Figure 5), one important feature of the hybrids has been their stability for cup quality.

Moreover, these varieties may increase cup quality in lower elevations (Figure 6). This is reflected by the chemical composition (fat content). It may be explained by a better homeostasis, due to the combination of complementary genes.

In terms of resistance, the F1 clones present various advantages. Coffee Leaf Rust resistance is a dominant trait. Hybrids of the type without resistance (Group1 x Group2), seem to

compensate their susceptibility by their vigor and by their aptitude to regenerate leaves rapidly.

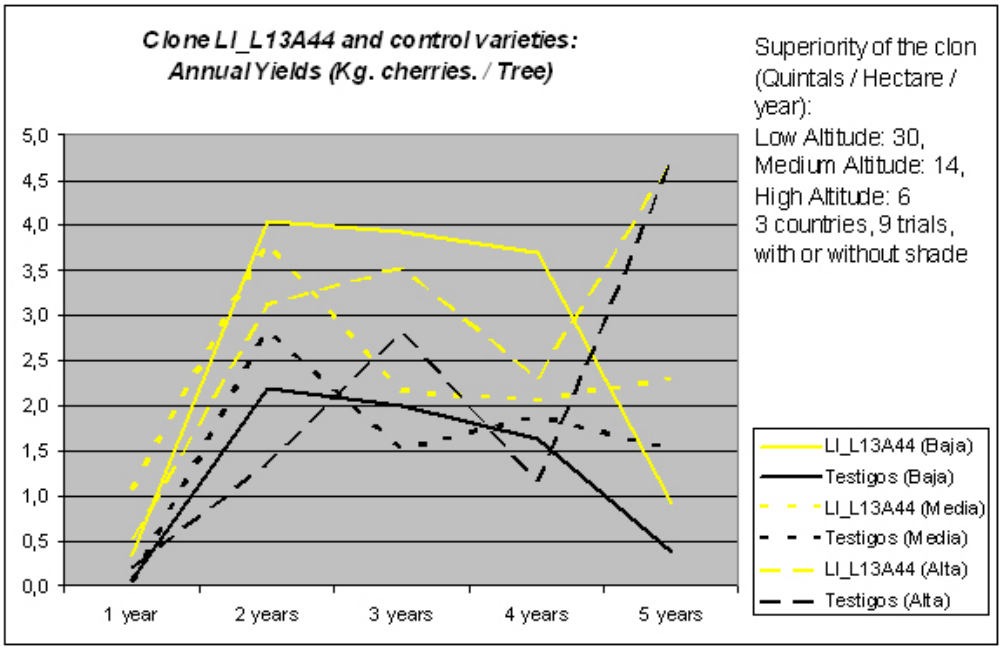


Figure 5. Annual yields, Clone LI_L13A44 vs. commercial varieties at various elevations.

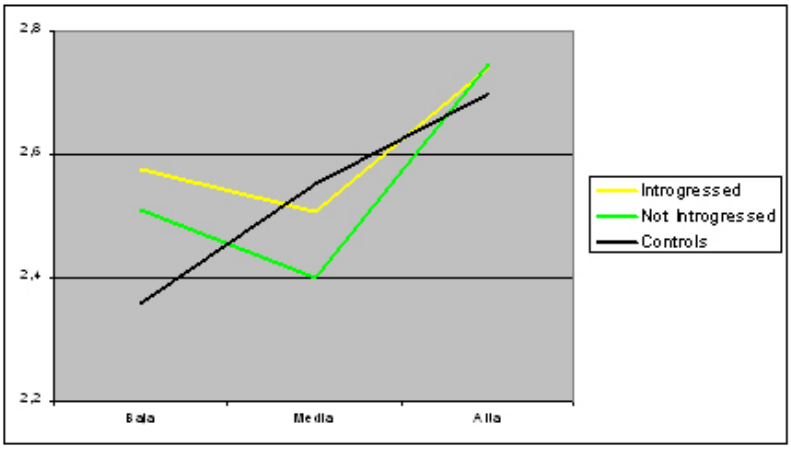


Figure 6. Mean positive atributes in relation with altitude (cup testing, PROMECAFE and ICAFE, 2004, 2005, and 2006 (average).

Nematode resistance is inherited from both introgressed lines and some Ethiopian parents.

Coffee Berry Disease has not been seen in America yet, however, unlike the current varieties, some of the hybrids are partially resistant (tests at Cirad).

EXPECTATIONS

We now refer to the new paradigm of Coffee Industry as exposed in various presentations at this Conference. Coffee based systems may contribute to carbon sequestration and to maintain the biodiversity. The use of the new hybrids, combined with shade or wind brakes, will allow a limited increase in productivity, and will contribute to increase cup quality. This will protect

the biodiversity, yes, but also the economical durability of the system. All this may result in a "double green revolution" for Arabica Coffee.

CONCLUSIONS AND PERSPECTIVES

For *Coffea arabica*, this programme has been the first to make a large use of the diversity available in Latin America. We expect about 6 hybrid clones to be proposed for multiplication within one year. The validation of large scale propagation has started at commercial level for 3 clones. The first plants are expected by 2008. A good collaboration between the various actors will be essential.

AKNOWLEDGEMENTS

We aknowledge the support of all organisations and persons that took part in this adventure. Special mention to the initiators of the programme, Pr. Charrier, the late Dr. Muller and Dr. Eskes, who established cooperation with PROMECAFE/IICA and with CATIE.

Funds were provided by the French Governement and CIRAD, the member countries of PROMECAFE, especially Costa Rica, Guatemala, Honduras and Salvador, and BID/FONTAGRO.

Various research teams were involved. But none of this would have been possible without the constant interaction and support of many Coffee Actors: Producers, Traders, Roasters…

REFERENCES

Anthony, F., M. C. Combes, et al. (2002). "The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers." Theoretical and Applied Genetics **104**(5): 894-900.

Bertrand, B., F. Anthony, et al. (2001). "Breeding for resistance to *Meloidogyne exigua* in *Coffea arabica* by introgression of resistance genes of *Coffea canephora*." Plant Pathology **50**(5): 637-643.

Bertrand, B., H. Etienne, et al. (2001). Growth, production, and bean quality of *Coffea arabica* as affected by interspecific grafting: Consequences for rootstock breeding. Hortscience, 2001; vol. 36; n. 2; p. 269-273; Abstract**:** 269-273.

Bertrand, B., B. Guyot, et al. (2005). The drop of beverage quality caused by *Coffea canephora* gene introgression can be avoided by selection, ASIC 2004-20th International Conference on Coffee Science, Bangalore, India, 11-15 October 2004. 2005; 606-618**:** 606-618.

Etienne-Barry, D., B. Bertrand, et al. (1999). Direct sowing of *Coffea arabica* somatic embryos mass-produced in a bioreactor and regeneration of plants. In: Plant Cell Reports = ISSN 0721-7714. - (1999)vol.19:n°2**:** p.111-117.

Etienne-Barry, D., B. Bertrand, et al. (2002). "Comparison of Somatic Embryogenesis-derived Coffee (*Coffea arabica* L.) Plantlets Regenerated in vitro or ex vitro: Morphological, Mineral and Water Characteristics." Annals of botany **90**(1): 77-85.

Etienne, H. (2002). "Biotechnological applications for the improvement of coffee (*Coffea arabica* L.)." In Vitro Cellular & Developmental Biology Plant **38**(2): 129-138.

Etienne, H. (2003). "Somaclonal variation in *Coffea arabica*: Effects of genotype and embryogenic cell suspension age on frequency and phenotype of variants." Tree Physiology **23**(6): 419-426.

Etienne, H., E. Alpizar, et al. (2005). Agronomic performance and trueness-to-type of *Coffea arabica* hybrids mass-propagated by somatic embryogenesis, ASIC 2004-20th International Conference on Coffee Science, Bangalore, India, 11-15 October 2004. 2005; 897-907**:** 897-907.

Etienne, H., E. Dechamp, et al. (2006). "Bioreactors in Coffee Micropropagation." Brasilian Journal of Plant Physiology **18**(1): 45-54.

Etienne, H., W. Solano, et al. (1998). "Utilization of somatic embryogenesis in liquid media for the mass propagation of F1 hybrids of arabica coffee." Boletin PROMECAFE **XX**: 11-15.

Variability in Yield and Yield Components of Wild Arabica Coffee Populations in the Montane Rainforests of Ethiopia

T. KUFA[1] AND J. BURKHARDT[2]

[1]Ethiopian Institute for Agricultural Research, Jimma Research Center, P. O. Box 192, Jimma-Ethiopia,
[2]Institute of Plant Nutrition, University of Bonn, Karlrobert-Kreiten-Str.13, D-53115 Bonn, Germany. E-mail: kufataye@yahoo.com

SUMMARY

The study was conducted with the objective to compare the variability in yield and yield components of wild coffee trees in montane rainforests of Ethiopia. The results depicted that mature coffee trees showed significant variations for most traits considered at each montane rainforest. Accordingly, the highest mean clean coffee yields were obtained at PIS3 (161.86 g tree^{-1}) and PIIIS3 (149.27 g tree^{-1}), whereas PIS1 (11.24 g tree^{-1}) and PIVS3 (11.51 g tree^{-1}) had the lowest yields. The proportion of crop bearing surface of wild coffee tree was also different at each site. The correlation results depicted significant ($P < 0.001$) positive associations between yield components, indicating the inherent growth relations and the possibility to predict yield potential of wild coffee trees. According to the cluster analysis, wild coffee populations were classified into five broad classes where most of them were grouped according to their geographical areas. Hence, the variations in the architecture and reproductive performances of wild coffee trees could largely reflect the influence of site features. In general, the findings demonstrated the influence of environmental variables on yield performances of wild coffee trees. However, further investigations should continue, among others, to determine the intensity of manipulating the heterogeneous forest environments in a way to improve growth, reproductive efficiency of wild coffee trees and promote sustainable production of high quality organic coffee, while conserving the genetic diversity of wild Arabica coffee populations in the montane rainforests of Ethiopia.
Key words: Ethiopia, rainforest, wild coffee populations, yield and yield components

INTRODUCTION

In Ethiopia, Arabica coffee grows under natural forests and exhibits features typical shade-adapted C_3 plants, occupying a lower to middle stratum of the forest layers. The montane rainforests where wild Arabica coffee populations still exist have more or less evenly distributed precipitation with a dry season lasting for three to four months but not completely without rain (Wrigley, 1988). Forest coffee is a wild coffee type grown spontaneously in the natural forests, which is more or less intact. In Ethiopia, the original ecology of wild coffee gene pool is, however, disturbed and threatened, largely due to the escalating deforestation practices for various purposes. Moreover, the more heterogeneous and dense plant density in the forest ecology has favored several abiotic and biotic stresses to impair the genetic growth and development potentials of coffee trees. Despite this, little or no management practices are being applied to improve the situation and hence, coffee yields remain very low, not exceeding 200 g ha^{-1} (Workafes and Kassu, 2000).

However, there are still unique and high quality coffee types in the montane rainforests of Ethiopia, which have adapted the recurrent drought and other stress pressures. Hence, it is

imperative to study the growth of wild coffee trees under natural forest conditions with the views to provide information on environmentally friendly management options. The purpose of this study was, therefore, to compare the variability in yield and yield components of wild Arabica coffee populations in the montane rainforests of southwest and southeast Ethiopia.

MATERIAL AND METHODS

Study site

The study was conducted in four montane rainforest coffee populations (FCPs) of Ethiopia, namely, Harenna (PI), Bonga (PII), Sheko-Berhane-Kontir (PIII) and Yayu (PIV) with the geographic coordinates varying from 6°23´N, 39°45´E at Harenna to 8°23´N, 35°47´E at Yayu sites. And the altitudes ranged between 1400 (PIIIS1) and 1780 (PIIS1) meters above sea levels. Except Harenna forest of the southeast Ethiopia, the others are found in the more humid southwestern part of the country. That means the study forests are dissected by the Great African Rift Valley and thus varied in rainfall gradients. Again, three study sites (S1, S2 and S3) were selected within each montane rainforest.

Data collection

At each site, nine to twelve experimental coffee trees (5-7 years old) were selected and grouped into three blocks by considering the existing site features mainly moisture and shade gradients. Again, two young primary branches were selected and labelled on each coffee tree and seasonal growth data were collected. Apical dominant ration (ADR), the ratio of main stem height to mean length of lateral branches, was determined according to the procedures described by Parent and Messier (1995) as cited by Robakowski *et al.* (2003). Leaf dimensions (length and width) were also used to estimate the intact leaf area (length x width x 0.66) as described by Yacob et al. (1993). Moreover, thirty coffee beans were sampled and weight with a sensitive balance.

Data analysis

One-way analysis of variance (ANOVA) was computed for the growth parameters of coffee trees. Mean comparison was carried out according to Tukey's Studentized Range at 0.05% probability level using the SAS system (version 8) for windows). Moreover, Pearson bivariate correlation analysis was made between yield and yield attributes of coffee populations. Finally, based on 31 morphological variables cluster analysis was computed using SPSS 11.5 for windows.

RESULTS

Vegetative characters

The results showed that more robust and open coffee types characterized Harenna and Berhane-Kontir forests, while the compact types were more dominant in Yayu sites. Whereas, a more heterogeneous populations with open, intermediate and compact canopy natures were found in Bonga forest. The tallest (333.5 cm) and the shortest (247.6 cm) coffee trees were noticed in Harenna (PIS2) and Bonga (PIIS2), respectively. However, tall, thin and flexible stemmed coffee trees with few plagiotropic branches were found with increased plant density and under dense shadings at all sites. Accordingly, the highest and the lowest main stem diameter at 5 cm above the ground were recorded at Sheko (PIIIS3 = 4.65 cm) and Yayu (PIVS3 = 2.85 cm), respectively. These sites were characterized by the respective wide and

closely spaced coffee trees. There was a significant variation in the mean canopy spread of forest coffee trees. As a result, coffee trees with wide canopy spreads were obtained in Harenna (PIS2) and Sheko (PIIIS3). In contrast, coffee trees with narrow canopy arrangement were recorded at Yayu forests (Table 1). Hence, the results suggest the variability in vigor and growth natures of coffee trees and hence the resistance to the high risks of damage due mainly to wildlife and tree fall in the montane rainforests of Ethiopia.

In addition, the growth nature of primary branches also varied among the wild coffee populations. Consequently, the longest branches with more number of nodes were recorded at the three sites of Berhane-Kontir as opposed to Yayu coffees. The results showed that the number of crop bearing nodes was high for Berhane-Kontir, Bonga, Harenna and Yayu forest coffees in that order (Table 1). On the other hand, Harenna coffee trees had the longest internode. The least number of nodes at PIVS2 and PIVS3 may be attributed to the inherent morphological growth nature or to the dense microclimates. In addition, high number of lateral branches was also recorded at PIS2, PIS3, PIIS2 and PIIIS2. In contrast, the closely spaced coffee trees at PIIS3 and PIVS2 and PIVS3 had the least growth of lateral branches. As a result, single stemmed and tall coffee trees with more non-productive branches, particularly in the lower positions, were found with increased shading. Such modified canopy architecture can also be noticed from the high apical dominance ratio (Table 1) from the most closely spaced and densely shaded coffee trees at Harenna (PIS2 and PIS2) and Yayu (PIVS2 and PIVS3).

Maximum mean leaf number was recorded under relatively moderate shading and coffee density at all sites. The highest leaf number was obtained at PIIS2 (Table 1). In contrast, leaf drop was high in Yayu (PIVS2 and PIVS3) and Berhane-Kontir (PIIIS2). Heavy crop loads on the widely spaced coffee trees at PIIIS3 could also enhanced leaf senescence and subsequent branch die-back. The intact estimated average leaf area was high for Harenna (45.16 to 55.37 cm^2), followed by Berhane-Kontir (37.21 to 40.43 cm^2) and Bonga (28.62 to 36.47 cm^2) sites. In contrast, the smallest leaf area (21.65 cm^2 to 30.09 cm^2) was measured for Yayu trees (Table 1**)**. As a whole, leaf growth (retention or initiation) of varying sizes may also indicates the differences in leaf area index to exploit the natural resources (moisture, light and CO_2) in the forest environments.

Yield components

The results on the number of berries on primary branches showed insignificant differences among the three sites, though the value increased with decreasing coffee density at each forest population. Thus, the highest (49.50) and the lowest (5.56) mean cropping branches were counted at Berhane-Kontir (PIIIS3) and Yayu (PIVS2), respectively (Table 2). Coffee trees had various seasonal changes in crop and non-crop proportions. Hence, maximum crop areas of 33, 26, 28 and 26% at Harenna (PIS3), Bonga (PIIS2), Berhane-Kontir (PIIIS2) and Yayu (PIVS1) forests, respectively (Figure 1). However, the lowest crop bearing surface area was recoded during the spring/summer season at all sites, except Bonga (PIIS2 and PIIS3) and Berhane-Kontir (PIIIS3). This could mainly be due to the varying seasonal availability of the natural resources to favor more young vegetative growths across the study sites. In addition, though crop to leaf ratio did not differ within each montane rainforest, increased values were recorded with decreasing plant density. Hence, high values were obtained at Berhane-Kontir (PIIIS3 = 4.13), Harenna (PIS3 = 4.05), Bonga (PIIS2 = 2.75) and Yayu (PIVS1 = 0.98) with the lowest value of 0.75 was found from the most densely spaced site (PIS1) (Table 2), indicating the enhanced tree vigor and better reproductive performances of wild coffee trees with reduced shadings.

Moreover, significant ($P < 0.05$) difference in the number of fruits and cherry yields were obtained within Bonga sites, though the results did not differ at the other sites. On the other hand, the least fruit and highest leaf defoliation was noticed under dense shades. The highest mean clean coffee yields were obtained at PIS3 (161.86 g tree^{-1}) and PIIIS3 (149.27 g tree^{-1}), whereas PIS1 (11.24 g tree^{-1}) and PIVS3 (11.51 g tree^{-1}) had the least yields. But, coffee yield levels on hectare bases were low at most sites with increased density of coffee trees and shadings. Thus, except the high mean clean coffee yields at PIS3 (1517.44 kg ha^{-1}) and PIIS2 (1002.84 kg ha^{-1}), which may reflect the extent of human interventions. The present low yield levels at the other sites support the work done by Workafes and Kassu (2000). This underlines the need to improve the environment in a way to disfavour the major insects and fungal diseases and maximize the light use-efficiency of coffee trees. Otherwise, soil fertility and moisture conditions were found be to sufficient at all sites during the course of the study (Taye et al., 2004). On the other hand, maximum 100 seed weight was measured for Harenna, Yayu, Bonga and Berhane-Kontir coffee trees in that order (Tables 2 and 3). The results significantly ($P < 0.05$) varied within Bonga sites; there were slight variations within sites at the other populations. The results of crop to leaf ratio and seed weight showed inverse relationships at most sites, which indicate the influence of environment. This particularly reveals the low light intensity, reduced net photosynthetic rate, and constrained reproductive growth of coffee tree (Taye et al., 2002; Tesfaye et al., 2002).

In addition, the linear correlation results depicted significant associations ($P < 0.001$) between all the yield and yield variables (Table 4). The results may demonstrate the inherent trade-offs between vegetative and reproductive growths. This could underline the possibility to estimate coffee yield performances taking into account these variables as Coste (1992) and Wrigley (1988) described. However, stand structure within each production system and hence the extent of competition at the different growth stages may modify these stable and heritable characters, suggesting the need to consider sink-source growth balance in coffee.

From the results of cluster analysis, the 12 wild coffee sub-populations were classified into 4 broad clusters at a 5 cluster distance (Figure 2). Consequently, except for PIS1, PIIS1 and PIVS1, most coffees were grouped according to their geographical areas. The most similarity was found between and within the south-western areas, particularly between Bonga and Yayu sites (PIIS2-PIIS3, PIVS2- PIVS3 and PIIS1- PIVS1). On the other hand, Bonga (PIIS2) and Harenna (PIS1) coffees had the furthest distance (least similarity), reflecting the influence of geographically and environmentally distinct more humid and drier montane rainforests, respectively. This is in line with our report (Taye et al., 2004) on the extent of morpho-physiological diversity of wild Arabica coffee populations in montane rainforests of Ethiopia. In general, the findings could reflect the variability in morphological growth plasticity of coffee trees under limited light conditions and other stresses, which is in agreement with the findings of Robakowski et al. (2003). This corroborates with the works of Taye et al. (2002) and Tesfaye et al. (2002) who reported similar findings under varying close spacing and shade regimes.

CONCLUSIONS

Though Arabica coffee has been evolved under the high plateau of natural forests in south-westrn Ethiopia and thus a shade tolerant plant, its growth and development was found to significantly differ along the prevailing site characteristics. Accordingly, ideal vegetative and reproductive growth was noted with reduced plant density and shadings, though the magnitude differed from site to site. Hence, based on morphological growth variability and plasticity, the wild coffee populations were broadly classified into distinct morphological clusters, mostly following the wide climate ranges between the eastern and western parts from

the Great Rift Valley. As a whole, it can be concluded that the findings would help to target future research and development options, which takes into account the variability in yield and yield related traits of wild Arabica coffee population in the montane rainforests of Ethiopia. However, information on the associations between these traits and coffee quality attributes in relation to environmental variables calls for further investigations, among others.

Table 1. Morphological characters of wild Arabica coffees at different montane rainforest sites of Ethiopia.

Site	Height (cm)	Girth (cm)	CD (cm)	LPB (cm)	NPB/ tree	NN/PB	IL (cm)	ADR	LN /PB	LA (cm^2)
PIS1	289.65	3.12	161.15	43.01	30.04	7.13	6.32	5.79	8.34	45.97
PIS2	333.48	3.50	188.87	45.21	39.59	7.27	6.51	5.96	9.68	45.16
PIS3	282.74	3.48	199.37	48.47	36.11	7.89	7.03	4.95	11.89	55.37
PIIS1	250.91	2.63	164.87	46.83	24.39	7.87	6.15	4.59	9.40	36.47
PIIS2	247.63	3.49	164.64	47.15	34.96	9.43	5.13	4.68	13.24	28.62
PIIS3	279.09	3.13	170.85	48.02	31.80	10.22	4.78	5.42	10.65	32.01
PIIIS1	254.22	4.02	188.47	54.48	31.33	10.84	5.18	4.06	9.90	40.43
PIIIS2	270.07	3.88	181.82	55.89	35.88	10.32	5.52	4.40	11.17	37.21
PIIIS3	258.17	4.65	192.47	52.24	30.93	10.49	5.26	4.05	11.64	39.66
PIVS1	247.85	2.93	157.53	39.11	30.64	7.59	5.24	4.59	10.86	30.09
PIVS2	284.25	2.94	146.81	25.28	25.14	6.69	3.87	7.01	5.84	21.65
PIVS3	268.90	2.85	145.50	31.38	24.90	6.77	4.82	5.98	7.45	28.74
Mean	272.25	3.39	171.86	44.76	31.31	8.54	5.48	5.12	10.01	36.78
SEM	7.04	0.17	5.22	2.61	1.39	0.46	0.25	0.26	0.59	2.68

CD = Canopy diameter, LPB = Length of primary branch, NPB =No of primary branch, NN= No of nodes, IL = Internode length, ADR = Apical dominance ration, LN= Leaf number, LA = Leaf area.

Table 2. Average results of yield and yield components in Arabica coffee accessions grown at various montane rainforest sites of Ethiopia.

Site	No of cropping branch/tree	No of crop/ branch	No of cropping node/branch	No of fruits /node	No of cherry /tree	Cherry weight (g/tree)	Clean coffee (g/tree)	Clean coffee (kg/ha)	Seed weight (g/seed)	Crop to leaf ratio
PIS1	6.56b	7.47	2.89	2.29	59.40	70.70	11.24	133.48	0.19	0.75
PIS2	15.33ab	31.36	3.97	6.41	634.60	761.50	126.41	553.04	0.20	3.14
PIS3	20.11a	39.22	5.00	7.71	826.40	975.20	161.86	1517.44	0.20	4.05
PIIS1	10.33	12.99	2.69b	3.67	136.30b	111.76b	18.55b	208.69b	0.14	1.14
PIIS2	18.83	31.13	5.68a	5.04	678.30a	644.40a	106.97a	1002.84a	0.16	2.10
PIIS3	17.78	29.88	6.17a	4.58	544.50a	555.35a	92.19a	691.43a	0.17	2.75
PIIIS1	11.33	18.05	5.50	3.19b	229.50	222.70	36.96	115.50	0.16	1.96
PIIIS2	15.44	39.28	6.00	6.41a	748.90	621.60	103.18	967.31	0.14	3.17
PIIIS3	18.33	49.50	6.88	6.77a	1045.60	899.20	149.27	466.47	0.14	4.13
PIVS1	11.83	13.07	3.40	3.16	178.60	167.90	27.88	226.53	0.16	0.98
PIVS2	8.78	5.56	1.56	2.16	75.60	83.20	13.81	224.41	0.18	0.96
PIVS3	6.39	3.97	1.04	1.27	62.40	69.30	11.51	280.56	0.19	0.50
Mean	13.42	23.46	4.23	4.39	435.01	431.90	71.65	532.31	0.17	2.14
SEM	1.40	4.39	0.55	0.60	100.82	99.93	16.60	126.14	0.01	0.37

Mean figures followed by the same letter(s) within a column of three sites at each forest unit are not significantly different at 0.05% probability level

Table 3. Yield and yield components of wild coffee trees at the study montane rainforests.

Variable	Harenna	Bonga	B-Kontir	Yayu	Mean	CV (%)	Pr>F
NPBPT	14.00±6.87	15.65±4.63	15.03±3.52	9.00±2.73	13.42	31.19	Ns
NFPB	26.02±16.54	24.67±10.13	35.61±16.04	7.53±4.86	23.46	43.19	Ns
NNPB	3.95±1.06ab	4.85±1.88ab	6.13±0.70a	2.00±1.24b	4.23	31.54	*
NFPN	5.47±2.83	4.43±0.70	5.46±1.97	2.20±0.95	4.39	37.47	Ns
NFPT	506.80±399.15	453.03±282.34	674.67±413.08	105.53±63.62	435.01	53.81	Ns
FCWPT (g)	602.47±472.76	437.17±285.31	581.17±340.06	106.80±53.37	431.90	54.14	Ns
CCPT (g)	99.84±78.75	72.57±47.36	96.47±56.45	17.73±8.86	71.65	54.29	Ns

*Ns = Not significant (P > 0.05), *P < 0.05 probability level. Mean figures followed by the same letter(s) within a row are not significantly different according to Tukey test at 0.05% probability level. Abbreviations: NPBPT = No of productive branches per tree; NFPB = No of fruits per branch; NNPB = No of crop nodes per branch, NFPN = No of fruits per node; NFPT = No of fruits per tree, FCWPT = fresh cherry weight per tree; CCPT = clean coffee per tree.*

Table 4. Correlation values between yield and yield components of wild coffee trees.

Variable	No of crop branches/tree	No of fruits/branch	No of crop nodes/branch	No of fruits/node	No of fruits/tree
No of crop branches/tree	-				
No of fruits/branch	0.91**	-			
No of crop nodes/branch	0.83**	0.87**	-		
No of fruits/node	0.89**	0.94**	0.74**	-	
No of fruits/tree	0.91**	0.99**	0.82**	0.94**	-
Coffee yield (g/tree)	0.93**	0.95**	0.75**	0.96**	0.97**

** = *Correlation is significant at the 0.01% probability level (n = 12; 2-tailed).*

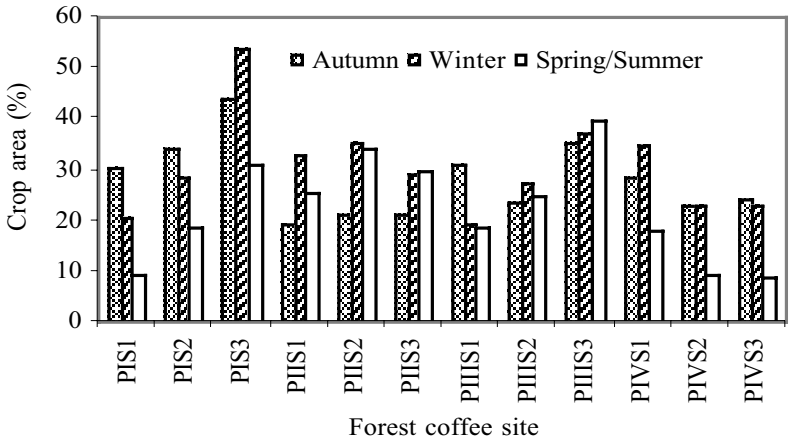


Figure 1. Seasonal variation in proportion of crop bearing area of wild coffee trees at different montane rainforest sites in Ethiopia.

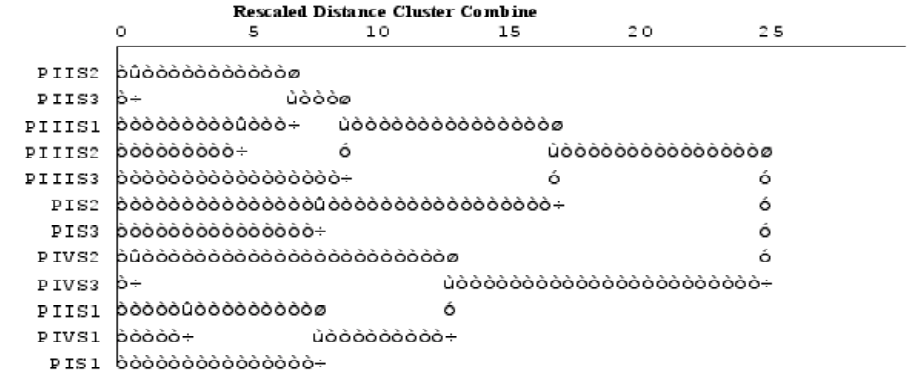


Figure 2. Cluster analysis using average linkage between groups of 12-sites and morphological growth characters of forest Arabica coffees.

REFERENCES

Coste R. 1992. Coffee: The plant and the product. Macmillan, London.

Robakowski P., Montpied P. and Dreyer E. 2003. Plasticity of morphological and physiological traits in response to different levels of irradiance in seedlings of silver fir (*Abies alba* Mill). Trees structure and function, Springer-Verlag 2003.

Taye K., Burkhardt J. and Goldbach H. 2004. Ecophysiological Variability of Forest Arabica Coffee Populations in Hydraulic Characteristics Along a Climatic Gradient in Ethiopia: Morphological and physiological variability. Proceedings of the 20th International Conference on Coffee Science (ASIC), 11-15 October 2004, Bangalore, India, 929-939.

Taye K., Burkhardt J. and Goldbach H. 2004. Ecophysiological variability of forest arabica coffee populations in hydraulic characteristics along climatic gradients in Ethiopia: Moisture dynamics in soil and plant systems under field conditions. Proceedings of the 20th International Conference on Coffee Science (ASIC), 11-15 October 2004, Bangalore, India, 1053-1059.

Taye K., Tesfaye S., Anteneh N., Alemseged Y. and Endale T. 2001. The impact of close spacing on yield of Arabica coffee under contrasting Agro-ecologies of Ethiopia. African Crop Science Journal, 9(2): 401-409.

Tesfaye S., Taye K., Alemseged Y., Anteneh N. and Endale T. 2002. Efficiency of close spacing and light interception in promoting growth and productivity of Arabica coffee in Ethiopia. Proceedings of the 19th International Conference on Coffee Science (ASIC), May 14th-18th, 2001, Trieste, Italy.

Workafes W.T. and Kassu K. 2000. Coffee production systems in Ethiopia. Pp 99-106. *In*: proceedings of the workshop on the control of coffee berry disease (CBD) in Ethiopia. Addis Ababa (*Ghion Hotel*), 13-15 August 1999.

Wrigley G. 1988. Coffee. Tropical Agriculture Series, London, John Wiley and Sons, Inc., NewYork.

Yacob E., MohammedNur A. and Taye K. 1993. Leaf area estimation in CBD resistant Arabica coffee to design a prototype area meter (the third awarded paper of the year). Pp 29-30. Bulletin of the Crop Science society of Ethiopia (CSSE), *SEBIL*, vol., 5, December 1993, Addis Ababa, Ethiopia.

First Industrial Massive Propagation of *Coffea canephora* Through Somatic Embryogenesis Organized in Thailand

S. SIRIPONG[1], J-P. DUCOS[2], C. LAMBOT[2], T. KUNASOL[1], Y. KASINKASAEMPONG[3], P. CHANTANUMAT[3], V. PETIARD[2]

[1]Nestlé Thailand Ltd, Thailand
[2]Centre de Recherche et Développement Nestlé, Tours, France
[3]Chumphon Horticultural Research Center, Thailand

SUMMARY

As well as in some other coffee producing countries, for various economical and technical reasons (IMD report), Nestlé purchases green coffee directly from the growers in Thailand. In parallel Nestlé gives assistance to the small coffee farmers in various domains including selected planting material and agricultural practices. After more than five years of evaluation of various Robusta clones in different countries including Thailand (ASIC 2004), some have been selected for their agronomic, industrial and quality performances. Their large-scale propagation through somatic embryogenesis has been undertaken in collaboration with the Department of Agriculture (DOA). Pregerminated somatic embryos are produced in both CHRC (Chumphon Horticultural Research Center) and R&D Nestlé Tours and developed into plantlets locally. The scaling up of the acclimatization was initiated by the CHRC in 2003 with the objective to produce 200,000 plants/year. Based on this first experience, a new project started in 2005 aiming to produce 1 million plants/year. The project is to distribute 6 millions of plantlets from selected clones over 6 years and therefore to replant up to 10 % of Robusta acreage from Thailand. After one year, the average acclimatization rate already reached up to 45 % and is expected to reach 60% in the coming months. The first commercial plantations made from these clones have shown up to 3-5 times the average field yield for coffee observed in Thailand. After many years of efforts from various research groups in the world, this technology finally reached the practical and industrial stage.

PROJECT BACKGROUND

Coffee production in Thailand is relatively recent, the first fields were planted in the 1960s. The government was not eager to encourage the production of a raw material whose prices were so cyclical. At the time, the country imported more coffee than it exported (6,000 tons vs. 750 tons), negatively tipping the country's balance of payments. The government decided to revise its strategy and began distributing young coffee seedlings for free or at cost price to farmers in the South of Thailand. The strategy was successful, and by the 1970s coffee imports had begun to drop. The maximum national production was reached in 2001 with 85,000 tons of green coffee, but started inexorably to decrease after dropping to 44,000 tons in 2006 (Office of Agricultural Economics, 2006). Thailand is a minor country in terms of coffee production with only 0.72% of the world production and the 22nd rank among producing countries in 2005. The total production area covers around 66,000 ha for about 33,000 coffee producers. The majority of them are small holders with an average plantation between 2 and 2.5 ha. Most of Thailand's production is Robusta coffee, used in making instant coffee. Because of its favorable climate, Chumphon in southern Thailand accounts for half of the production. The strong decrease in national production is the consequence of many factors by which the coffee profitability is maybe the most important one. The comparison of

coffee profitability to other industrial crops is clearly showing that coffee crop is strongly challenged by oil palm and even more rubber plantations (Table 1). The erosion of coffee profitability is affecting the farmers motivation and is affecting the field maintenance: old coffee trees are not replaced, pruning of rejuvenation are not done in time, strong competition with weeds, lack of fertilization program etc… All these problems should be considered as consequences of coffee profitability erosion. The future of coffee in Thailand is closely related to the increase of coffee profitability at farmers level. Nestlé Thailand is the major actor of the coffee sector in Thailand. In 2004, 78% of the national production was bought by Nestlé and locally processed in soluble coffee. Considering the importance of Nestlé in national coffee business, a memorandum was signed in 1999 between the Department of Agriculture and Nestlé. The objective of the memorandum is to define the action of the company to promote the coffee production in Thailand by actions on technical assistance to farmers, introduction of high performing clones and propagation of elite clones. The government clearly stated that it wishes to increase productivity in the existing production zones rather than expanding them.

Table 1. Coffee profitability Vs Palm oil and Rubber.

	Coffee*	Palm oil**	Rubber**
Yield (kg/ha)	750	17100	1770
Cost of production/kg (Bath*)	32.9	1.5	20.4
Cost of production/ha (Bath)	24700	26500	36150
Price/kg (Bath)	47.0	2.8	53.6
Income/ha (Bath)	35250	47280	94750
Profit/ha (Bath)	10550	20730	58600

*48 Baths = 1 Euro. Source: * = Nestle Group Thailand, 2006; ** = Office of Agricultural Economics 2005. Ministry of Agriculture and Agricultural Cooperatives, Thailand.*

OBJECTIVES OF THE PROJECT

The profitability at farmers level can be significantly increased by two main actions:

- a good selling price for the raw material
- to increase the field yield

A fair remuneration of green coffee for the farmer can be achieved by a system of direct procurement. The system of direct procurement in Thailand allows to pay 8 to 10% more to the farmers in comparison to the price guaranteed by the government. This system has also the advantage to maintain a closer relation between the producer and the industrial processor of green coffee and to facilitate by this way the technical assistance program delivered to the farmers. The best way to increase the field yield is to propagate performing genetic materials with a significant better yield in comparison to the local seedlings. Comparative trials with introductions of elite clones of *Coffea canephora*, demonstrated clearly the better field performances of clones compared to the local seedlings. The usage of the somatic embryogenesis system of propagation allowed to define an ambitious objective of coffee propagation in Thailand. It was decided to rapidly propagate on large scale high performing clones to replace old coffee trees. The project aims to propagate 6 millions plants in 6 years to replant up to 10% of Robusta acreage in Thailand.

Somatic embryogenesis was selected as the best option to quickly impact the system of propagation in Thailand avoiding the planting and the management of large wood gardens.

PROJECT IMPLEMENTATION

Even if the project started *sensu stricto* in 2005, it really started 15 years ago with the creation of a large collection of *Coffea canephora* collected worldwide (Pétiard et al., 2004).

The genetic material collected was planted in field in several countries with contrasted environmental conditions. It was certainly the starting point of the project which can be divided in several important steps: creation of collection, multilocal comparative trials, adaptation and scaling up of the somatic embryogenesis technique, local transfer of the somatic embryogenesis technique, conformity control for the plants produced by somatic embryogenesis, system of quality assurance, ex vitro germination process for the somatic embryos, plantlets distribution, technical assistance delivered to farmers. Each of these steps will be briefly described and commented.

Collections

In order to distribute the best planting material to the farmers, it was decided 15 years ago to collect worldwide the main clones of *Coffea canephora* cultivated in the world. A collection, well representative of the diversity was created and planted in the field. The collection was extensively characterized for the biomolecular aspects, the agricultural performances, the biochemical composition, the processing performances and the sensory value of 55 clones (Pétiard et al., 2004). A quite complete description of each clone was obtained leading to the selection of the most interesting ones for their agricultural performances but also for their processing and cup quality characteristics.

Multilocal comparative trials

The first phase of field collection was mainly done in Ecuador where station facilities were available. In order to check the validity of the results, a set of clones was planted in other countries with contrasted environmental conditions. The multilocal trials were often done in a collaborative work organized between the National Institutes and Nestlé (local operational companies and scientific support from R&D). The results obtained for the agricultural performances of the clones in the different countries are given in Table 2.

Based on these results, a first set of clones was selected as candidates for propagation by somatic embryogenesis. The list of selected clones is: FRT 1, FRT 3, FRT 4, FRT 11, FRT 12, FRT 17, FRT 23, FRT 27 and FRT 65. The yield increase between clones and local seedlings in Thailand is fluctuating between 88 and 291 %, giving a strong advantage to farmers using clones instead of local seedlings. It is noticeable that the yield performances in Thailand are much lower to the other countries, especially compared to Ecuador. The environmental conditions of the trial sites are less favorable mainly due to a poor soil fertility and drought stress. FRT 03 has contrasted results between Ecuador and Thailand, this clone is strongly affected by *Colletotrichum gloeosporioides* in Ecuador. Taking into considerations the additional criteria of selection, mainly the cup quality and the ability for somatic embryogenesis, a set of 9 clones was introduced in the process of embryos production.

Table 2. Average green coffee/ha/year of candidate clones (planting years 1999 & 2000).

Clones	Ecuador (4 years)	Philippines (4 years)	Thailand (5 years)	Mexico (5 years)
1	3556	1492	727	1440
3	67	1518	669	--
4	2938	766	616	950
5	3446	522	435	436
7	4100	890	438	--
10	4622	1831	773	--
11	1504	2298	904	1364
12	4353	1294	713	1472
15	3287	1106	463	2031
17	4219	2465	782	1879
23	3603	1706	987*	--
27	3637	1240	1063	1499
65	3699	1233	1279	--
Local control (seedlings)	1500		327	

* *4 years of production for FRT 23 in Thailand.*

Adaptation and scaling up of the somatic embryogenesis technique

During the 90s', three major progress led to the scaling up of somatic embryogenesis of the *Coffea canephora* by reducing the labor cost input: culture of embryogenic cells and torpedo to the cotyledonary stage in liquid media (Zamarripa et al., 1991), pre-germination from the torpedo to the cotyledonary stage by temporary immersion in liquid media (Berthouly et al., 1995), and ex vitro germination by directly sowing cotyledonary stage embryos in the greenhouse (Ducos et al., 1999; Etienne-Barry et al., 1999). Based on these improvements, a process for large-scale production of Robusta somatic embryos was recently implemented at Nestlé R&D Center (Tours). In 2005, the production capacities reached 2.5 M pre-germinated embryos per year (Ducos et al., 2006 submitted). One original development in the process of somatic embryos undertaken at R&D Tours concerns the pre-germination phase with the scaling up of the pre-germination of Robusta somatic embryos performed in 10 L-glass temporary immersion bioreactor (TIB). The torpedo stage is not autotrophic and requires a maturation step to reach the cotyledonary stage which is the earliest stage embryo capable of photosynthesizing (Afreen et al., 2002). This step is performed by regular immersion in liquid media to overcome hyperhydricity. The positive effects of this method have been reported for shoot proliferation, microcuttings and somatic embryogenesis, mainly by using a 1-L commercialized TIB called RITA (Vitropic, FRANCE) (Etienne and Berthouly, 2002). For commercial scale-up, the twin flask system consisting of a pair of bottles connected by a silicone tube is generally preferred because it allows larger vessels, up to 5 or 10 L (Escalona et al., 1999).

Technique transfer

The technique of somatic embryogenesis was transferred from R&D Tours to Chumphon Horticultural Research Center (CHRC) in the frame of two long-term trainings. The first one organized in 2000 aimed to transfer the whole process of embryos production. At this time, the pre-germination was achieved by plating in petri dishes. The second one (2004/05) focused on the technique of temporary immersion in liquid media, with the objective to reduce the cost of embryo production and increase the laboratory capacity.

The local implementation of temporary immersion system is quite challenging and is facing the problem of the availability of the equipment. The usage of horizontal designed bioreactors, consisting of specific disposable plastic bags instead of vessels, should greatly facilitate the local implementation (Ducos et al., 2006).

Conformity trials

In order to validate the propagation technology of *Coffea canephora* via somatic embryogenesis in liquid medium, the clonal fidelity of regenerated trees has been assessed in large scale trials. Large plots of around 5 thousands trees regenerated from 5 to 7 months old embryogenic cell suspension cultures were planted in the Philippines and in Thailand for comparison with control trees derived from *in vitro* axillary budding (microcuttings). For the observed morphological traits and the yield characteristics, no significant differences were seen between the somatic seedlings and the microcuttings-derived trees. It was concluded that this propagation method can be used for large – scale commercial applications without any negative unforeseen consequences for the grower (Ducos et al., 2003). It is noticeable that the standardized procedure applied for the embryos production is free of auxin and the duration of the proliferation phase is limited to 16 months. These two points are considered as critical to maintain the problem of somaclonal variation under control.

System of quality assurance

The industrial production of somatic embryos requires a system to guarantee their quality. A procedure was elaborated to control 3 critical points during the process of production: the absence of contamination with bacteria, molecular fingerprinting and control of the germination capacity. The In vitro production is a guarantee of production without fungal contamination but the presence of pathogenic bacteria (*Xylella fastidiosa* and *Pseudomonas syringae*) needs to be controlled. The mother plants used for leaf explants are systematically controlled with Elisa test for the *Xylella fastidiosa*. The absence of other bacteria is controlled in regular and systematic axenic tests. The in vitro system of production is a guarantee against the main fungal pathogens of coffee. The genetic identity of the clones under production is controlled through sampling of embryos and analysis with specific and polymorphic micro-satellites. The germination capacity of the embryos is systematically controlled on one sample of 250 embryos, randomly taken in each production batch and for each clone. The embryos are sown in controlled conditions to evaluate their germination capacity after 4 months of growing. The quality system allows to supply Thailand with embryos guaranteed on the sanitary, identity and germination capacity aspects.

Ex-vitro germination process for the somatic embryos

A phase of storage of the cotyledonary embryos is organized before their shipment to Thailand. In 2005, R&D-Tours shipped a total of 1.2 million embryos to Thailand for local acclimatization. This phase is sensitive and the embryos are well protected against temperature and humidity stress. All cares are given to make the shipment as short as possible to reduce the risk on the embryos, and usually 4 days are required from door to door. The cotyledonary embryos are sown under plastic tunnel for their conversion to coffee plantlets. The ex-vitro germination protocol consists of acclimatizing the embryos at the cotyledonary stage, without root and leaves. The embryos are planted in a commercial peat media (Klasmann-Deilmann Group) and maintained under confinement for a period of 3 to 4 months. The factors having the major influence on the embryos development were identified as:

- The embryos size has a positive effect on their ability to develop into plantlets. The embryos with a bigger size give better results and consequently only the embryos having well developed cotyledons and a minimal hypocotyle size of 5 mm are considered in the final evaluation.
- The air humidity which should be close to saturation and never less than 90%
- The temperature which should not exceed 35 °C for a long period. Usually a mist system is used to control the temperature by spraying water on the tunnel
- The light intensity which should be higher than 3 kilolux at mid-day. The direct sunlight and the light intensity is regulated by the usage of net shade.
- The control of fungus with regular spraying of fungicides every two weeks.

The average germination rates obtained during the germination control test was 41% but with strong variation between clones and even between batches for the same clone. The percentage of embryos converted into plantlets for each clone is indicated in Table 3.

Table 3. Ex-vitro germination rate of embryos under plastic tunnel (%).

Clones	Germination test (France)	CHRC	Project in Thailand
FRT 1	31		3
FRT 3	21	66	10
FRT 4	72		67
FRT 11	28		5
FRT 12	18		10
FRT 17	39	39	43
FRT 23	38		3
FRT 27	58	63	39
FRT 65	46	38	36

Results are indicating strong variation between clones for the ex-vitro germination rate under tunnel but also strong variation from one site to the other. We suspect that some uncontrolled factors occurring during the process of production can affect the ability of the embryos to grow. It is also noticeable that the percentages of success are systematically better in France and in CHRC (Thailand) where the procedure of acclimatization is routinely used from a longer period. The embryos acclimatization of embryos requires “green fingers” and experience. In order to increase the success rate achieved in this phase, the project will concentrate on the clones with the best germination rates. All cares will also be given to this phase to increase the confinement of embryos having a strong impact on the final rate. The embryos acclimatization requires around 4 months under tunnel in a confined environment. When the plantlets have two pair of leaves they are transferred into plastic bags as usually done in coffee nursery without any particular problems.

Plantlets distribution

When ready for planting, the plantlets are transferred by truck from Bangkok to the coffee area. The trip is organized during the night to avoid stress on the plants. Each clone is transported separately to allow a final planting by rows in the farmer’s plot. The plantlets are sold to the farmers integrated in the system of direct procurement. These farmers are receiving the technical assistance allowing them to plant and grow these coffee trees in the best conditions. The plantlets are subsidized to adjust the selling price at the market level.

In 2006, the project will distribute around 180 000 plants produced at Bangkok nursery. Taking into consideration all the plantlets already produced in the previous years, a total of around 550,000 coffee plantlets will be distributed for the period from 2003 to 2006.

Technical assistance

The distribution of new clones with improved field performances is organized in parallel with the technical assistance. The first objective of the project is not negotiable: new coffee clones are distributed for replacement of old coffee trees and can not be planted into new areas. Then, a package of technical advices are given in order to increase the sustainability of coffee production: intercropping during the first years, shading by fruit trees, cover crop, application of organic fertilizer etc… All cares should be given by the farmers to take all benefit from the new clones potential.

DISCUSSION AND CONCLUSIONS

An ambitious project of propagation started in Thailand in 2005 with the objective to replace 10 % of the actual acreage of coffee. The strong competition between industrial crops for the land profitability is putting pressure on coffee. One way to increase efficiently the coffee profitability is to distribute new clones with much better field performances. It is in this urgent and critical situation that a project of massive propagation of *Coffea canephora* started in 2005. The project is using the technology of somatic embryogenesis for a rapid and massive propagation of selected clones. Embryos are produced in France and in Thailand but germinated locally in Bangkok. The phase of embryo germination is critical having a strong influence on the final number of plants produced. The project will focus on the clones with the best germination rate, trying to improve the procedure and the conditions for the other. The objective to produce millions plants forced to use the process of temporary immersion in liquid media for the embryos pre-germination. This system was adapted to allow larger productions. The embryos quality is regularly controlled for the sanitary aspect, the genetic identity and the germination ability.

The sustainability of coffee propagation using a system of somatic embryogenesis is related to the possibility to produce embryos locally. This will reduce the production cost of embryos and avoid expensive shipments. This is why the technique of temporary immersion in liquid media was transferred to CHRC in the frame of a long-term training.

Massive propagation of coffee by somatic embryogenesis is technically demonstrated. The production cost of the system in comparison with the traditional system of cuttings will be done when the embryos will be routinely produced in Thailand with the system of temporary immersion. Robusta coffee propagation with somatic embryogenesis is particularly interesting to face urgent situations, like the one encountered now in Thailand, when large quantities of improved clone are required in a short delay. The method is also very useful to quickly propagate new clones coming out of a breeding program without passing through the phase of wood gardens.

REFERENCES

Afreen, F., Zobayed, S.M.A. and Kozai, T. 2002. Photoautotrophic culture of *Coffea arabusta* somatic embryos. Annals of Botany. 90:11-19.

Berthouly, M., Dufour, M. Alvard, D., Carasco, C. Alemano, L. and Teisson, C. 1995. Coffee micropropagation in a liquid medium using the temporary immersion technique. P.514-519. In: ASIC (ed.), 16th International Scientific Colloquium on Coffee, Kyoto.

Ducos, J.P., Gianforcaro, M., Florin, B., Pétiard, V. and Deshayes A. 1999. A technically and economically attractive way to propagate elite *Coffea canephora* (Robusta) clones : *in vitro* somatic embryogenesis. P. 295-300. In : ASIC (ed.) 18th International Scientific Colloquium on Coffee, Helsinki.

Ducos, J.P., Alenton, R., Reano, J.F., Kanchanomai, C., Deshayes, A. and Pétiard V. 2003. Agronomic performance of *Coffea canephora* P. trees derived from large-scale somatic embryo production in liquid medium. Euphytica. 131: 215-223.

Ducos, J.P., Chantanumat, P., Vuong P., Lambot, C. and Pétiard, V. 2006. Mass propagation of Robusta clones: disposable plastic bags for pregermination of somatic embryos by temporary immersion. 27th International Horticultural Congress. August 13-19. Seoul. (submitted to Acta Horticae).

Ducos, J.P., Labbé, G., Lambot, C. and Pétiard, V. 2006. Pilot scale process for the production of pre-germinated somatic embryos of selected Robusta clones. In Vitro Cell. Dev. Biol. Plant. Submitted.

Escalona, M., Lorenzo, J.C., Gonzales, B., Daquinta, M., Gonzales, J.L., Desjardins, Y. and Borotto, C.G. 1999. Pineapple (*Ananas comosa* L. Merr) micropropagation in temporary immersion systems. Plant Cell Rep. 18: 743-748.

Etienne, H. and Berthouly, B. 2002. Temporary immersion systems in plant micropropagation. Plant Cell, Tiss. Organ Cult. 69: 215-231.

Etienne-Barry, D., Bertrand, B., Vasquez, N. and Etienne, H. 1999. Direct sowing of *Coffea arabica* somatic embryos mass-produced in a bioreactor and regeneration of plants. Plant Cell Rep. 19:111-117.

Office of Agricultural Economics, 2005 & 2006 Ministry of Agriculture and Agricultural Cooperatives. Thailand.

Pétiard, V., Lepage, B., Lambot, C., Crouzillat, D., Florin, B., Brulard, E., Alvarez, M., Von Rutte, S. Leloup, V. Gancel, C., Liardon, R., Jung, M.L., Rytz, A. and Labbe, D. 2004. Establishement and preliminary agronomic and qualitative evaluation of collections of *Coffea Arabica* and *Coffea canephora* cultivated varieties. P.834-841. In: ASIC (ed.), 20th International Scientific Colloquium on Coffee, Bangalore.

Zamarripa, A., Ducos, J.P., Bollon, H., Dufour, M. and Pétiard V. 1991. Production d'embryons somatiques de caféier en milieu liquide : effets densité d'inoculation et renouvellement du milieu. Café Cacao thé 35 : 233-244

The Potential of Participatory Extension Approaches in Coffee Rejuvenation – Experience from Northern Tanzania

M. H. TEMU, T. NZALLAWAHE, S. MDEMU, J. M. TERI

Tanzania Coffee Research Institute, P. O. Box 3004, Moshi, Tanzania

SUMMARY

Participatory extension approaches in research and extension institutions have been in use since the late 1970s. The approaches were developed after the conventional approaches displayed notable tendencies to underestimate people's potential for action and reaction. As a result, development and dissemination of new technologies failed to benefit smallholder farmers to alleviate rural poverty. This paper gives an outline on the potential of using participatory extension approaches in rejuvenating the coffee industry in Northern Tanzania. Using farmer groups and imparting appropriate knowledge through Farmer Coffee Management School promotes local ownership and empowerment which are necessary conditions for sustained coffee recovery.

INTRODUCTION

The important role farmers can play in agricultural research, development and extension, if only given a chance, has become widely accepted (Chambers et al., 1990; Haverkort et al., 1991; Jiggins and De Zeeuw, 1992). Until recently, agricultural development in rural Africa consisted mainly of farmers and communities being told what to do, often by institutions which had not taken the time to understand their real needs. The results tended to be poor, because rural people did not have any sense of ownership of the ideas imposed on them.

Increased support from governments and development agencies in the area of sustainable development, in particular, has allowed participatory initiatives to bloom in some parts of the world (Cassara, 1995; Rolling and Wagemakers, 1998). It encourages farmers to share their skills and knowledge and promotes innovation and creativity.

For agricultural extension agents, it means fundamental changes in the way they operate. They need to become catalysts and facilitators helping communities achieve their defined and perceived goals (Anandajayasekeram et al., 2001). Participatory tools and methods which enable the implementation of these ideas have become main-stream during the last decade. In efforts to promote farmer-led extension, FAO has developed the now popular Farmer Field School (FFS) as a participatory extension approach.

This paper gives an outline on the potential of using Participatory Extension Approaches (PEA) specifically Farmer Coffee Management School (FCMS) – a modified form of FFS – in rejuvenating the coffee crop in Northern Tanzania, the potential benefits and challenges to be faced before the approaches yield benefits as efficiently as expected.

BACKGROUND AND RECOVERY STRATEGY

The development and economic growth of Tanzania depends heavily on agriculture. Agriculture accounts for about half of the Gross Domestic Product (GDP) providing

employment to about 90% of the rural smallholder population. Agriculture also accounts for over 50% of the country's foreign exchange earnings.

Coffee, a major foreign exchange earner, accounts for more than 20% of the export earnings. 95% of the coffe is produced by more than 400,000 smallholder farmers who depend on it for their livelihoods under diverse and risk prone environments. Further, a total of more than 2.0 million people depend indirectly on coffee for their livelihoods.

Coffee productivity in Tanzania is one of the lowest in the world – at just about 200 kgs per hectare. A multitude of constraints have contributed to the dismal performance of the coffee industry in Tanzania. In an important diverse coffee stakeholders' analysis held in year 2000, the principal constraints to the coffee industry and the priority activities to be urgently undertaken to arrest the situation were identified. Few amongst them were:

- Low productivity and poor quality caused by poor husbandry practices and poor primary processing.
- Notorious diseases especially coffee leaf rust (CLR) and Coffee berry (CBD).
- High production costs, hence unprofitable and low returns to the farmers.
- Aged trees and farmers (hence urgent need to replant with new, disease resistant cultivars as top priority).
-

Faced with this huge task of arresting the above constraints, TaCRI opted for the use of Participatory Extension Approach (PEA) and specifically a modified FFS- FCMS- approach to unlock, package, develop and disseminate the appropriate technologies.

BRIEF INTRODUCTION OF NORTHERN TANZANIA PRODUCTION ZONE

Tanzania is divided into four coffee production zones based mainly on agro-ecological zones and administrative convenience namely: Northern, Southern, Southern highlands and Lake Zone. The Northern Zone consists of 10 administrative districts with almost 70,000 ha under coffee. Ninety five per cent of this coffee area is found just below the slopes of mountain Kilimanjaro and Meru. The districts are endowed with rich, deeply weathered volcanic soils and an annual average rainfall of 1200-1750mm well suited for the best mild Arabica coffees produced in the world. The coffees are of fine taste, special aroma, and full body and flavour all best qualities for best blends.

THE EVOLUTION AND POTENTIAL OF PARTICIPATORY EXTENSION APPROACHES (PEA) IN AGRICULTURE DEVELOPMENT

In Tanzania and in many African countries, a number of approaches have been tested and adopted to improve effectiveness and efficiency of technology dissemination process. Two major approaches of agricultural extension have dominated the landscape of these countries since independence. The "Transfer of Technology" model often failed because of inappropriate technology and/or inadequate "packaging" of the messages (Rolling, 1988). This paved the way for the Training and Visit (T&V) model. Despite the funding and promotion by The World Bank in more than 30 African countries, the T&V model has been found to be ineffective, inefficient and unsuitable (Asiabaka and Mwangi, 2001, Anandajayasekeram et al., 2001) Following these failures the prescription was to change the process by emphasising farmer participation, hence Participatory Approaches (PAs).

Considerable amount of resources have been devoted to group formation, consciousness raising, leadership training and community participation. Recently the concept of Farmer

Field Schools (FFS) has been introduced in Tanzania but with modifications (Lema et al., 2004). Elsewhere, FFS model has also successfully been used in Indonesia in a Sweet-potato IPM project, where FFS protocol for extension purposes and training-of trainers (ToT). was developed (Fliert et al., 1999). FFS was introduced to Kenya in 1995 (Asiabaka and James 1999), and used widely. Challenges for farmer participation in coffee were also reviewed by Williamson (1999).

Basically, the FFS model is a training approach which emphasises learning by doing, and empowering farmers to actively identify and solve their known problems (Van de Fliert, 1998). Empowerment of individuals using participatory approaches is one of the major outcomes (Oakley and Halica, 1991).

THE CONTEXT OF THE PARTICIPATORY EXTENSION APPROACH – FFS – IN NORTHERN TANZANIA

TaCRI is now involved in the process of supporting the rejuvenation programme of the coffee industry. TaCRI has adopted PEA approach to empower voluntary farmer groups to implement on sustainable basis the necessary rejuvenation activities in all the 10 administrative districts in the Northern Zone. In order to suit TaCRI's coffee rejuvenation programme, the classical FFS approach was slightly changed to Farmers' Coffee Management School (FCMS). FCMS makes use of each member's field as a management school in a rotational way, in contrast to FSS where only one field is used as a field school.

The FCMS model applied in this programme is an experiential learning approach aimed at implementing the following coffee rejuvenation activities:

- Facilitate smallholder farmers produce their own Arabica clonal coffee seedlings through vegetative propagation sustainably without external support
- Package old on-shelf coffee husbandry technologies in appropriate formats and disseminate widely to different types of stakeholders
- Reach and enhance human expertise for better coffee management with initial emphasis on rehabilitation of the old abandoned fields.

All these in order to achieve:

- Mass replanting with Coffee leaf rust and coffee berry disease resistant coffee seedlings by 2007.
- Increase productivity from 200gms to 500 gms per tree in 5 years time
- Reduce production costs and increase quality by the use of cost effective appropriate technologies to enhance smallholder incomes. Technologies include weeding, proper pruning, IPM, integrated soil fertility management, mulching, proper irrigation and de-suckering.
- Improve farmers' livelihoods by eradicating poverty.

Before the actual implementation of the FCMS, we made sure that the subsequent programme activities would be owned by the farmers. Two important conditions were put in place:

- Real motivation and enthusiasm within the coffee stakeholders
- Effective community organisations which can support the process and take it forward without continuous external support.

This process started in early 2003 through a wind shield survey on the potentials of rejuvenating the coffee industry in North Tanzania followed by village meetings in all the 10 districts. We were able to build trust, raise awareness in group formation approach, communicate programme objectives, identify and analyse community needs and get feed back to prioritise our stakeholders' needs. This first phase resulted in formation of initial 10 voluntary farmer groups. The next phase involved strengthening of the farmer groups especially ability to cooperate, drafting of their constitutions, democratic election of group leaders, legal registration and opening and operating bank accounts. This also included inviting them to TaCRI's first Open Day where through "look and learn" tour generated more awareness, interest and ideas. This was followed by the third phase where the actual training implementation started.

STEPS IN ESTABLISHING FCMS FOR NORTHEN TANZANIA (SLIGHTLY MODIFIED CLASSICAL FFS)

1. Training curriculum development
2. Training of Master Trainers (ToMT)
3. Training of trainers (ToT)
4. Undertaking FCMS
5. Follow ups by ToT graduands
6. Field days
7. Farmer to farmer training (FtoF)
8. Linking of different farmer training groups

TRAINING INITIATION AND TRAINING OF MASTER TRAINERS (ToMT)

This took place in late 2003 at TaCRI head office where lengthy discussions within the Institute took place. A training curriculum for both farmers and extension workers was developed in collaboration with the MTs. This involved designing of activities, modules, leaflets, posters and other media for training, carrying out pre-testing and revising them accordingly. Once set, the training implementation started.

In order to strengthen linkage with both the respective District Agriculture officers (DADOs) and District Coffee Subject Matter Specialists (DCSMS), imparting PEA methodologies and tools in order to change their top-down approaches and attitudes was top priority.

TRAINING OF TRAINERS (ToT)

Recent experience has shown the positive impact of involving farmers as trainers, and of enhancing farmer networks in order to support FtoF dissemination deliberately (Eveleens et al., 1996; Braun, 1997).

.

In order to facilitate scaling up of FCMS, we undertook an intensive PEA training for the required facilitators. The ToTs involved 10 VEWs who work with the Government conventional extension structure and 20 farmer promoters.(FP). Training took place at District head-quarters where they practiced different coffee husbandry skills, observing regularly different field management practices, worked with the farmers in their fields keenly observing strengths and weaknesses in these fields e.g. pruning systems, conditions of the crop, pests and diseases occurrences and their natural enemies, vegetative propagation, coffee/banana intercropping etc.

The observations were analysed, discussed in small groups, prepared poster diagrams and finally reported to and discussed in the plenary. As part of ToT, the participants conducted their first FCMS under the guidance of a Master Trainer (MT) to gain both facilitation skills and change attitudes from conventional extension to PEAs.

A cooperative behaviour of the MT, flat hierarchies, the group dynamics and the fact that trainers and trainees stay, eat, and learn together contributed to a strong group feeling. This corporate identity provides a joint spirit which is very crucial for the success of the FCMS. Typical characteristics of ToT are:

- Learning is by doing in a typical coffee field.
- Work directly with farmers with lots of discussions
- Ends up in conducting FCMS
- Full of group dynamics and enthusiasm
- Fosters cooperate identity

FARMER COFFEE MANAGEMENT SCHOOL (FCMS)

A FCMS lasts for a whole coffee growing season, involving a group of at most twenty-five farmers in a group in weekly or monthly sessions of, on average three hours. The trainer, who is a VEW or farmer group promoter (FP), is a facilitator of the experiential learning process, not an instructor. Farmers choose their own learning fields in a rotational way amongst members. Different specific subjects relevant for the area and calendar year are discussed. The equipment includes plastic bags, pencils and paper. Each training session contains a set of activities that foster farmers' analysis, decision making and problem solving including:

- Field monitoring in small groups, considering environmental factors, crop development, pest and natural enemy occurrence and interaction, and damage symptoms on the plants.
- Agro-ecosystem analysis, in which drawings of observation data are made, and conclusions about crop status and possible measures are drawn together.
- Presentation to fellow farmers of the agro-ecosystem analyses, and discussion to come to a collective agreement on what crop management measures should be taken or not during the coming week.
- Special topics dealing with locally occurring field problems, or providing opportunities to discover process, is a tool often used by farmers in the management school
- Group dynamics exercises to enliven the school, strengthen the coherence of the group, and make members better aware of the dynamics of group processes.

The process continues in all the farms of the group members making every farm a coffee management school. Even within a single field, conditions can be highly diverse in terms of fertility status, slope, etc. The FCMS model is designed to allow farmers to learn to take coffee management decisions based on their own observations and analyses. This ability often gives them more confidence in their own management skills, which in turn, is reflected in an improved, overall farm management.

The last event in the FCMS is field day. These take one day and are normally organised before the end of the coffee production season. Monitoring and evaluation in a selected farmer group premise where a demonstration plot has been established are done with the following objectives:

- Self evaluation by reflecting on the successes and failures; share knowledge amongst farmers; build confidence through presentations and encourage more farmer to farmer

extension and feed back to extension and research for refining technologies classified by farmers as needing more research.

In order to scale up, promote innovation and sustain the above results of FCMS between and amongst the groups, TaCRI has strongly encouraged competition. TaCRI normally rewards the winners with exchange visits by farmer groups. Some farmers get not only an opportunity to see places but saw what methods other people had devised for example; to cope with inadequate water for irrigation by harvesting rain water.

AREAS OF EMPOWERMENT AND POTENTIAL BENEFITS OF THE PEAs

Participatory approaches trigger several direct and indirect benefits to all stakeholders/ partners involved in the process. Potential benefits and areas of empowerment of participatory approaches have been summarised by DRD (2000).

In the past two and half years, PEA has successfully been applied by stakeholders in North Tanzania with the following key results and milestones:

- Marketing: Four mini-pulperies have been acquired by 4 pioneer groups to facilitate central primary processing to improve coffee quality and fetch premium prices.
- Success of FCMS has spread rapidly. FPs have been invited by other villages and through FtoF extension, 12 new satellite farmer groups have been formed. This has reduced the dependency on VEWs and costs of dissemination as the process is increasingly run by farmers themselves.
- Farmers have developed more confidence to talk and express themselves in public. Three members from pioneer groups contested in the October, 2005 local council elections and worn seats as councillors. They revealed this when asked the secret behind their success.
- Five Savings and Credit societies (SACCOS) have been established in 5 groups as a source of credit to buy coffee inputs and other provisions, i.e. paying school fees.
- Creation of employment: More than 10 FPs are now engaged in improved field lay-out and grafting .Payment of up to $ 0.5. per tree is being charged.
- Tanzania has perhaps been the first country in the world in facilitating farmers through PEA to successfully multiply their own Arabica coffee seedlings through vegetative propagation without external financial resources.

From Figure 1 below, it is worth to note the following interesting cases:

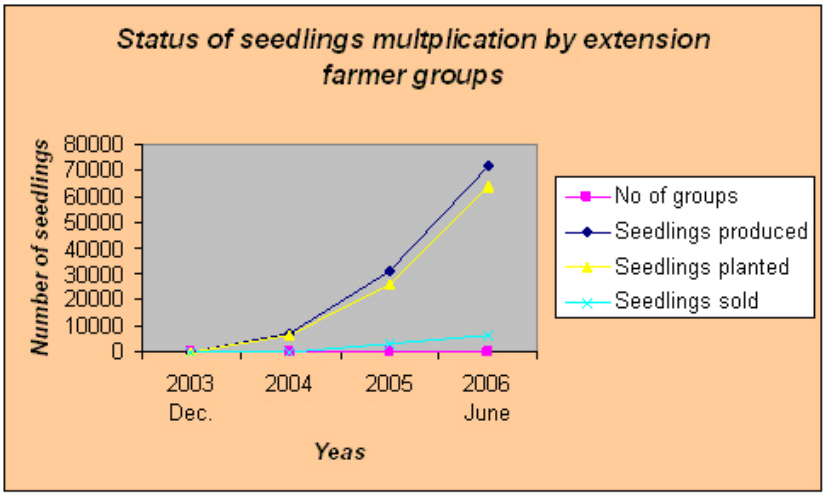


Figure 1. Status of seedlings multiplication by extension farmer groups.

- Extension groups have grown from 10 to 107 in the past two and half years, a growth of more than 1000%. More than 3,000 farmers have been empowered to produce their own coffee seedlings and initiate improvements in their husbandry practices.
- Farmers have generated incomes from selling part of their produced seedlings. Gross income of $10,000 has been earned by 750 farmers.
- The cost of producing the 75,000 seedlings is approximately $ 7,500. This has been absorbed by the farmers through self reliance.

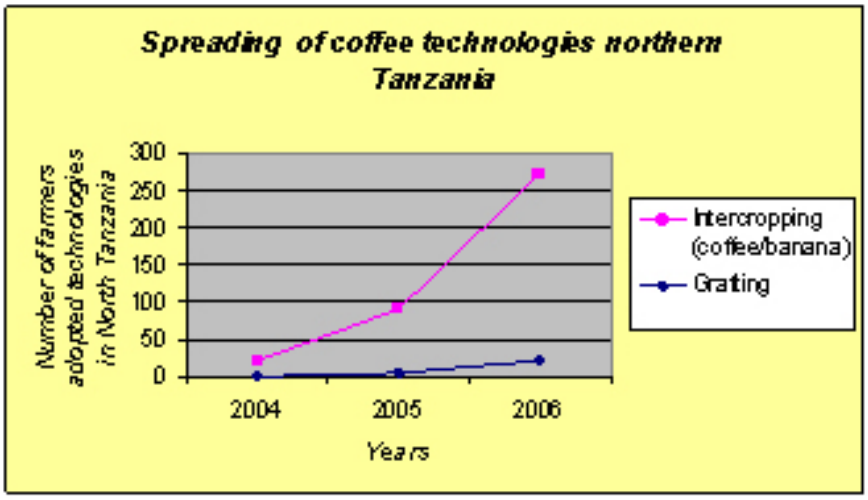


Figure 2. Spreading of coffee technologies in north Tanzania.

From Figure 2 the following is worth noting:

- Rapid spread of technologies from FtoF: Grafting is a new technology of changing the old farms to new varieties. This involves stumping the old trees and then graft with new variety shoots. So far, 22 FPs have adopted fast this technology and are now training other farmers while 1,540 farmers adopted stumping.

CONCLUSION

This paper has outlined the potential and benefits of PEA in rejuvenating the coffee industry in North Tanzania. This has been done through a slightly modified form of FSS namely the FCMS. Through FCMS, farmer groups have been able to successfully multiply vegetatively their own coffee seedlings without external financing through experiential learning. The use of other coffee management technologies has also increased. Scaling-up and farmer-to-farmer extension facilitated formation of more farmer extension groups. FPs have earned employment. Better utilization of resources, reinforces farmers' acceptance of PEA.
For a developing country like Tanzania, there are some challenges to be addressed.

PEA might not always lead to complete success. What is important is that the process is owned by the farmer groups themselves. If there are operational snags, the farmers will re-try innovations to suit their specific conditions through experiential learning. They will no longer wait for an outsider to develop an alternative. Notably, the challenge is to have FCMS replicated fast to more coffee growers in north Tanzania. Also, establishment of a monitoring and evaluation method to assess the socio-economic impact of the process will be opportune.

REFERENCES

Anandajayasekeram, P.; Mweri, A.M, Zishirir, O.J; Odogola, W; Mkuchu, M, and Phiri, M. (2001). Farmer Field Schools: Synthesis of Experiences and Lessons from FARMESA member countries. Harare, Zimbabwe: FARMESA

Asiabaka, C.C. and Mwangi, J.G. (2001). Strategies for effective Extension Services in Africa: Lessons from Kenya Paper presented at the Association of the Third World Scientists, Njoro, Kenya: Ergerton University.

Asibiaka, C.C. and James, B.B. (1999). Farmer Field School for Participatory cassava IPM Technology Development in West Africa. In Renard et al. (eds). Farmers and Scientists in a changing environment: Assessing research in West Africa. Weikersheim, Germany: Margrat Verlag.

Cassara, B. (Ed.). (1995). Adult Education through world collaboration. Malabar, FLA: Krieger.

Chambers, R; Pacey, A. and Thrupp L.A.(Eds.) (1990). Farmer First: Framer Innovation and Agricultural research, London: It Publications

DRD/KIT (2000). Introduction to Client-Oriented Research in Tanzania. Division of Research and Development, Ministry of Agriculture and Food Security, Republic of Tanzania and Royal Institute, Amsterdam, The Netherlands. 12-20.

Hagmann, J, Chuma, E, Mwirira, K., and Connolly M. (1999). Putting Process into Practice: Operationalising Participatory extension. AgREN Network paper 94.1-17.

Oakley P. and Halica (1991). Projects with people: The practice of participation in rural development. International Labour Organisation, Geneva, Swizerland.

Rolling, N. (1999). Extension Science: Information Systems in Agricultural Development. Cambridge: Cambridge University Press.

Rolling, N. & Wagermakers, M.A.E. (1998). Facilitating sustainable agriculture.Cambridge. In Participatory Approaches and Scaling-up.1-12 (Eds Elske van de Fliert, Rini Asmunati & Warsito Tantowijoyo.

Lema, N.M. and Kapange, B. (2004). The Potential of Participatory Approaches in Rural Development. In: Agricultural Technology Development through Participatory Approch. TAP II SUA project , 3-11.

Integrated Sustainable Development of *Coffea arabica* in Vietnam

NGUYEN TU HAI

Ba Vi Coffee Research Central, Vietnam National Agricultural Research Institute

SUMMARY

For the last decade, Vietnam's coffee sub-sector has achieved an impressive development. With an annual output of approximate 800,000 metric tons of green beans, coffee occupies second place in the world coffee production. However, up to 95% of the export volume consists of *C. rubusta* while *C. arabica* accounts for only 4% although potential for growing the later is large and its price is always more attractive. An attempt to extend *C. arabica* to 50,000 ha for the period 1995-2000 has been ended with both success and failure. This paper gives an overview of *C. arabica* development with special focus on its efficiency and sustainability.

ARABIA COFFEE DEVELOPMENT HAS NOT PROGRESSED AS WELL AS EXPECTED

When Catimor arabica coffee was produced, the Vietnamese coffee sector stared developing arabica coffee production with the support of the French Development Organization (AFD). This program has gained numbers of achiecements such as new effective arabica coffee areas in North Western rigion: Son la, Dien bien and the Cental of Vietnam: Nghe an, Quang tri, Thua thien hue or in Western Highlands: Lam dong.

However, the also are areas suffering from heavy losses, leading to the liquidation of thousands hectares of arabica coffee.

The cause of the abovementtioned losses was not related to the technical issue but firstly due to insufficient management from the central to the local level. A new-planting program of 40 to 50 thousand hectares of coffee required economic, financial and technical distribution systems involving numerous organization which could not be fulfilled.

Achievements attained in some areas have shown judiciouness and potentiality of development program of arabica coffee as mentioned below.

Arabica coffee have demonstrated high effectiveness in some areas such as Lam dong, Quang tri, Thua thien hue and North Western Region. Lam dong situates at 11°20 northern latitude, equivelent to geographical position of Costa Rica but futher away from the equator than Colombia. Costa Rica, at the altitude of 1.000 m above the sea level, has produced high quality arabica coffee that is mild and exhibits a good acidity. The soil is vocanic origin and high fertile. Coffee cultivating techniques are based on the aplication of chemical fertilazers, irrigation and cultivated in full sun, which is very similar to the vietnamese coffee cultivation. Coffee from Costa Rica is mainly wet-processed with an avrage productivity of 1.500 kg/ha. In comparison with physical features of Lam dong situated at approximately 1.000 m above sea level, it is possible to produce similar arabica coffee as mild as Costa Rica's, or even a type of coffee that can be compared with Clombia's.

After a period of implementation of arabica coffee development program in North regions, some areas, such as Sonl a, Dien bien, Quang tri, thua thien hue and Nghe an, appear to have potential.

Situated at 22° of Northern latitude and at altitude of 600 m above the sea level, the geographycal position of arabica coffee areas in Son la is visually as similar as arabica coffee areas in Sao Paula of Brazil. Thus, such provinces like Son la and Dien bien are able to produce arabica coffee, of which the quality is in no way inferior to Brazilian coffee. On the other hand, if we have better wet-processed treatment, the quality can even be better than natural coffee of Brazil that is dry-processed production.

Arabica coffee areas in Quang tri: the coffee develops very well, produces high yield and have relatively good quality. The weakness of this areas is that coffee is planted at low altitude, 400m above the sea level. Accordingly, though it is accepted in international market, it somehow affects the quality.

The coffee areas in Nghe an is not sufficiently large but has lots of potentials.

Besides, there are failures in some areas such as Thanh hoa, Yen bai. Those need to be considered carefully in order to learn helpful lessons.

AJUSTMENT OF ARABICA COFFEE'S STRUCTURE TOWARDS A SUSTAINABLE COFFEE PRODUCTION

Continue the development program of arabica coffee and have a policy to establish high quality arabica coffee areas like Lam dong, Son la and Central: Quang tri, Thua thien hue. In case of areas situated above 600m above the sea level, it is necessary to change Catimor variety with original arabica varieties or with hybrids that have better aroma and taste.Agricultural extention practices must be better organized: ripe cherries picking and wet process of arabica coffee must be carried out with correct techniques. The government should consider supporting processing at farmer level, first of all, facilitating drying patios and household pulpers. Besides, scientific and technical issues have an important role in development of the coffee sector. We have had an institute and center of coffee research but the investment, especially arabica coffee research central, has not been sufficient. Research of varieties requires more investment, improving anthropology and facilities in order to keep pace with other countries, such as Brazil, Colombia, India, Indonesia, of which the coffee sector has developed hundreds years before Vietnam.

Some technical points to be considered

On the basis of technical shortcomings in coffee cultivation, we need to concern the following issues:

1. Do not intensify coffee productivity by removing shade trees nor applying with quantity of chemical fertilizers and irrigating water.
2. Protect the invironment and ensure food safety during production and processing. In order to meet the above demends, the below features must be well implemented:
   - Design field so as to constrain erosion as much as possible.
   - Plant shade trees together with other crops in case of coffee fields planting with inferiors coffee varieties.

- Rainforse production and application of organic fertilizers. Combination of animal husbanry and cultivation is required, for instance, combine dairy farming with coffee cultivation.
- Improve pruning of coffee.
- Intensity mulching in order to keep soil moisture in dry season as well as apply a suitable amount of water.
- In aspect of harvesting, only pick ripe but not green cherries. Do not leave cherries over-ripe, dry and defoliated.
- In processing, waste water as well as solid waste needs to be treated well enough not to pollute the environment. Besides, take advantage of processing wastes to apply advanced techniues concluded from PPP projects.

In order to facilitates extension to farmer, coffee sector has set up a summaries fomula: "3 reductions, 3 increases, 1 prevention"

Namely, reduction of chemical fertilizres, of agro-chemicals, of irrigating water.
- Increase shade trees, organic fertilizers and pruning.
- Avoiding picking green or overripe, dry cherries.

Production of high quality coffee beside conventional coffee commodity

Concerning application of certifications required from consumers such as Utz Kapeh, Organic Coffee, Shade-grown Coffee, Bird Friendly certifications etc and after all the others and participate into Fair Trade coffee.

Production of value-added coffee

This is an approach that can bring high economic returns for the coffee sector. Beside, production of value-added coffee also creates favorable conditions for promoting consume of domestic coffee. However, in order to produce value-added coffee plactice, it is necessary to carry out market studies to have a grasp of demend and consuming capacity of the product.

Promotion of coffee consumption in the domestic martket

This is a big program that is costly and requires considerable time. The first reason is that Vietnamese traditionally drinks tea, which is not easy to swich to coffee. In the short term, it is essential to have a particular survey on domestic coffee market in order to set up an approriate plan thereof.

Establishment of co-operatives

Soon establish co-operatives in villages growing coffee, which provides with consulting services for technical issue and processing through the form of a washing station. On the other hands, the co-operatives approach can help farmers to improve marketing of their coffee.

With a right approach for a sustainable coffee production, Vietnamese coffee sector will definity overcomes obstacles of fluctuant international market and bring benefits to millions of coffee farkers which contributes to implementing great objective program for mountainous areas. We hope to achieves objectives of a sustainable development of Vietnamese coffee sector in a better way.

Yield Response of Arabica Coffee to Spacing and Training Methods in Southwestern Ethiopia

E. TAYE, A. NETSERE, T. SHIMBER, T. KUFA AND F. ASFAW

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

SUMMARY

Field experiment was conducted between 1996 and 2006 crop years at Metu Research Station. A morphologically compact Arabica coffee cultivar, 74140 was field transplanted in split plot design; where, seven spacing levels (1.00 m x 1.00 m, 1.05 m x 1.05 m, 1.12 m x 1.12 m, 1.19 m x 1.19 m, 1.29 m x 1.29 m, 1.41 m x 1.41 m and 1.58m x 1.58 m) and two training methods (capping and free growth) were assigned as main and sub-plot treatments, respectively. The result revealed that spacing had significant ($P < 0.05$) impact on coffee yield in 1998/99 crop season. Accordingly, highest and lowest mean clean coffee yields of 2028.87 and 1310.82 kg ha^{-1} were recorded at closest (1.00 m x1.00 m) and widest (1.58 x 1.58 m) spacing, respectively. Coffee yield tended to increase linearly with population in over years analysis and across most of the crop seasons. Moreover, the effects of training methods on coffee yield was also considerable in 1999/00, 2000/01 and 2003/04 crop years, where trees in capped plot out yielded significantly the free growth treatment except in 1999/00 crop year. Significant ($P < 0.01$) difference in coffee yield was observed due to crop years; where highest yields were recorded in 2002/03 and 2004/05. Significant ($P < 0.01$) interaction effect was noted between crop seasons and training methods; indicating the lower yield performance of capped trees during the initial two crop seasons and its subsequent superiority over the free growth treatment in the later years. Though the combined effects of spacing on coffee yield was statistically insignificant more yield advantage were noticed as population density increases. In contrast, training treatment, stem numbers had seldom impact on coffee yield. Therefore, it was concluded that planting compact Arabica coffee cultivar at closer spacing of 1.05 m x 1.05 m had enhanced coffee yield at Metu; indicating the long lasting efficiency of close spacing in mid altitude coffee growing area of southwestern Ethiopia. In contrast, increasing the number of bearing heads of individual coffee tree had low yield advantage at closely spaced plots.

INTRODUCTION

Coffee (*Coffee arabica* L.) is the main agricultural export product of Ethiopia and thus, plays a significant role in the country's economy accounting up to 5% of the growth domestic product (GDP), 10% of agricultural economy, more than 60% of the countries foreign exchange earnings, 10% of the total national income and 12 % of the government revenues. A quarter of the population of the country directly or indirectly depends on coffee through production sale/income, processing, transport and commercial services (Biruk, 2000).

Despite the economic importance and prevailing wide genetic diversity of Arabica coffee in Ethiopia, the national average yield of the crop remains very low, ranges between 450 and 472 kg ha^{-1} of clean coffee (Workafes and Kassu, 2000). Apart from the heavy dependence on traditional and low yielding genotypes, coffee yield is constrained by several unimproved production practices, among which low population density and unregulated number of bearing

heads are among the most common phenomenon noticed. On the other hand, in southwestern coffee growing belts of the country, the existing potential arable farmland is dwindling from time to time owing to the horizontal expansion of cultivation of coffee and other associated crops. Furthermore, the ever escalation of population pressure in the county is another envisaged threat that could ultimately compel farmers to expand cultivation of crops in inaccessible marginal lands. Hence, to overcome such problem and increase yield and productivity of crops, intensification of crops in space is among the potential options, which deserves attention.

In Ethiopia, conventional spacing of Arabica coffee has frequently been considered to be below optimum. In this regard, Taye et al. (2001) indicated that the majority of coffee growing farmers in the country practices the old and traditional cropping patterns, and hence, the limited arable farmland remains less efficiently utilized. With this notion, many research attempts were undertaken in order to generate technologies that could enhance productivity per unit area by considering different crop intensification practices in diverse coffee growing agro-ecologies of the country (Yacob et al., 1996). Accordingly, research attempts in cropping patterns and spacing have been launched on the coffee berry disease (CBD) resistant cultivars with distinct canopy classes (open intermediate and compact) (Yacob et al., 1993). The result showed that a consistent yield increment with the increased population density and number of bearing heads (Yacob et al., 1996). The previous research results have paved the way toward more close spacing by considering training methods, i.e. number of bearing heads of individual coffee trees. The **objective** of this experiment was therefore, to investigate the impact of close spacing and training methods on yield of Arabica coffee under mid altitude coffee growing area of southwestern Ethiopia.

MATERIALS AND METHODS

Field experiment was undertaken for eleven consecutive years between 1996 and 2006 at Metu Research Station that represents the mid altitude coffee growing agro-ecologies of southwestern Ethiopia. The station is located at 36º 0' E longitude and 7º 3' N latitude with an elevation of 1550 m above sea level (Paulos and Tesfaye, 2000). On average it receives 1830.7 mm of annual rainfall. The mean maximum and minimum temperature of the area is 29.6 and 10.6 ºC, respectively. A morphologically compact coffee cultivar-74140 was field transplanted in split plot design with two replications, where seven spacing levels (1.00 x 1.00, 1.05 x 1.05, 1.12 x 1.12, 1.19 x 1.19, 1.29 x 1.29, 1.41 x 1.41 and 1.58 x 1.58 m, respectively representing population density of 10,000, 9066, 7974, 7062, 6010, 5030 and 4006 trees ha^{-1}) and two training methods (capping and free growth) were assigned as main and sub plot treatments, in that order. After field establishment, stems of all young coffee seedlings in the capping plots were cut back at 45 cm above the ground to initiate multiple stems; then only two healthy and vigorous suckers per tree were allowed and maintained to grow through out the course of the experimental periods. On the other hand, coffee trees in the free growth plots were grown on single stem without capping. Except the experimental variables, other routine management practices were timely and uniformly applied to experimental unit as per the recommendation of the area.

In each crop season, red fresh cherry yield was harvested from each experimental unit and weighed separately. The results were multiplied by the factor of 0.166 to convert into clean coffee yield and thus, reported in kilogram per hectare (kg ha^{-1}). The yield data recorded were statistically analyzed using an MSTAT-C computer program (MTSU, 1995). Duncan's Multiple Range Test at $P < 0.05$ probability level were used to compare the difference between means where significant differences were obtained by analysis of variance (Mandefro, 2005).

RESULTS AND DISCUSSION

Coffee yield data of close spacing and training treatments across experimental years are depicted in Table 1. The result revealed that spacing had significant ($P < 0.05$) impact on coffee yield in 1998/99 crop season. Accordingly, yield increased linearly with population density or decreasing spacing. In the year, highest and lowest mean clean coffee yields of 2028.87 and 1310.82 kg ha^{-1} were recorded at closest (1.00 m x1.00 m) and widest (1.58 x 1.58 m) spacing, respectively (Table 1). Except at population density of 5030 trees ha^{-1}, a linearly coffee yield increment with population density was observed in 1999/00 crop season. On the other hand, in the following two crop years, 2000/01 and 2001/202 low and inconsistent coffee yield trend were noted. Accordingly, highest mean clean coffee yield of 2426.42 and 1963.00 kg ha^{-1} were recorded from population density of 9066 and 7974 trees ha^{-1} in 2001/01 and 2001/02 crop years, respectively. A significantly highest mean clean coffee yield of 4491.44 kg ha^{-1} was obtained in the fifth- crop year (2002/03) followed by 2004/05 with mean value of 4457.51 kg ha^{-1} largely due to biennial bearing habit of coffee trees, among others. Accordingly, highest mean clean coffee yield of 4881.63 kg ha^{-1} was recorded from the population density of 7063 trees ha^{-1} followed by 9062 trees ha^{-1} with mean value of 4733.67 kg ha^{-1}. In contrast, lowest yield of 3577.50 kg ha^{-1} was detected at the widest spacing or lowest population density. In the following year, however mean clean coffee yield was reduced significantly perhaps due to exhaustion of coffee tree owing to the heavy crop load in the preceded crop year. In this case, highest mean yield of 4275.66 kg ha^{-1} was recorded from the highest (10, 000 trees ha^{-1}) population density whereas lowest yield of 1481.19 kg ha^{-1} was obtained from 5030 trees ha^{-1} (Table 1). Thus, the current results indicated the long lasting efficiency of close spacing in increasing coffee yield at mid altitude coffee growing area of Metu. The present result is in agreement with findings of Taye et al. (2001). In their work, the increased yield advantage of coffee at close spacing has been indicated under mid altitude condition of Wenago, southern Ethiopia. Mean clean coffee yield increased significantly in the seventh crop year; clearly due to biennial bearing habit of coffee trees, among others. Accordingly, 4457.45kg ha^{-1} clean coffee yield was recorded. In the last crop year, 2005/06 coffee yield significantly reduced due to exhaustion of coffee trees that emanated from heavy crop load in the past crop year, among others. In over years analysis, although yield variations due to spacing treatment were statistically non-significant, a linear yield increase with population densities were observed except for the highest population density, 10,000 trees ha^{-1} where yield reduction was observed. Accordingly 1968.60 kg ha^{-1}, lowest and 2626.10 kg ha^{-1}, highest mean clean coffee yields were noted at population density of 4006 and 9066 trees ha^{-1}, respectively. This result therefore, indicated the possibility of boosting yield and productivity of compact Arabica coffee cultivar by planting at closer spacing or high population density up to the seventh crop years under Metu condition and in areas with similar ecologies elsewhere. The increase in coffee yield with population density in close spaced plots might be due to the efficient utilization of environmental inputs namely: light, moisture and nutrients until the biological optima was attained (Tate et al., 2001)

Training methods had also significantly affected mean clean coffee yield in 1999/00, 2000/01 and 2003/04 crop years. Accordingly, respective mean clean coffee yields of 1945.29 and 2942.04 kg ha^{-1} were recorded in 2000/01 and 2003/04 crop years from capped plots (Table 1 and Figure 1). In comparison with coffee trees grown on free growth, trees on capped plots were inferior in yield performance during the initial (1998/99 and 1999/00 two crop seasons. This is most likely due to the longer juvenile growth stages of the new suckers (stems) of the capped young coffee trees unlike the free growth treatment, which was not capped. Thereafter, it was superior through out the rest of crop years except in 2001/02; where it resulted in lower yield perhaps due to the biennial bearing nature of coffee trees. Though yield responses to training methods were non-significant, higher mean clean coffee yield was

obtained from capped plots both in 2002/03 and 2004/05 crop years. As evidenced in over years analysis, a relatively higher mean clean coffee yield recorded from the coffee trees trained on two stems (capped plots) as compared to single stem, free growth treatment. The combined analyses over years revealed significantly ($P < 01$) interaction effects of training methods with crop year indicating the lower yield performance of capped plots during the initial two crop seasons and its subsequent superiority over the free growth treatment in the later crop years (Figure 1). On the other hand, the interaction of training methods and population density was non-significant across each crop seasons and over years. Further more, though yield variation was non-significant in response to training methods, a relatively higher mean clean coffee yield was noted from capped plots than free growth treatment over years. This finding of course, agree with reports of Yacob et al. (1996) which state that coffee yield linearly increase with increasing number of bearing heads of individual trees. However, as evidenced from the over year analysis increasing the number of bearing heads of individual coffee tree had very low yield advantage under closely spaced coffee plots.

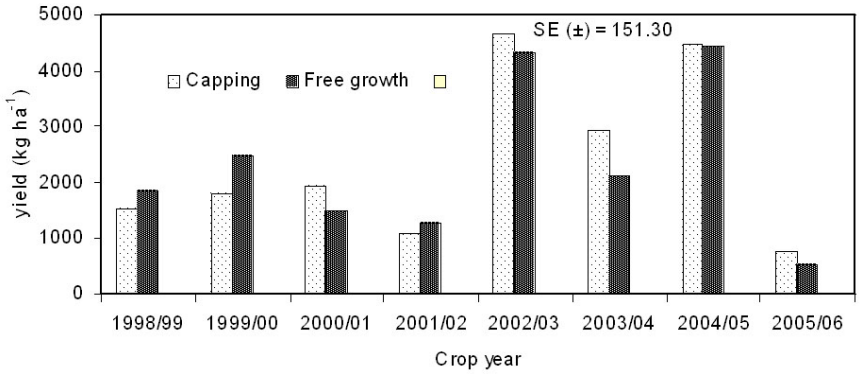


Figure 1. Mean clean coffee yield as influenced by the interaction effect of crop years and training methods at Metu Research Station.

CONCLUSION

Although the analyses of variance revealed non-significant coffee yield responses to population density, closer spacing had a substantial yield advantage over low population densities. Even in the seventh crop season, high mean clean coffee yield recorded at closely spaced plots indicating the long lasting efficiency of close spacing at mid altitude coffee growing area of Metu. Therefore, it was concluded that planting a compact Arabica coffee cultivar at closer spacing of 1.05 m x 1.05 m is recommended until the eighth crop year at Metu. The significant interaction effects observed between crop season and training method indicates the lower yield advantage of capped coffee trees at the initial two crop seasons and its subsequent superiority over the free growth treatment in the later crop years. Increasing bearing heads of individual coffee tree had non-significant yield advantage and thus, allowing more than two orthotropic stems pre tree is not recommended under closely spaced coffee plantations. Further research is crucial to investigate the impact of close spacing and number of bearing heads individual tree on bean and cap quality of coffee.

ACKNOWLEDGMENT

The authors would like to thank staff members of coffee agronomy and physiology research unit of JARC viz. Mr. Shimels H/Wold, Mr. Alemseged Yilma, Mr. Teshome Kumela and Mr. Bekele Bogale for their technical inputs during data summarization and statistical analysis. The authors would also like to acknowledge the late senior coffee physiologist, Mr. Yacob Edjamo for his technical contribution at the initial phase of this work.

Table 1. Mean clean coffee yield (kg ha^{-1}) as influenced by population density and training methods at Metu.

Treatment Population (Trees ha^{-1})		Crop year								
		1998/99	1999/00	2000/01	2001/02	2002/03	2003/04	2004/05	2005/06	Mean
Spacing (m)		*	NS	NS	NS	NS	NS	NS	NS	NS
1.58 x 1.58	4006	1310.82b	1679.71	1530.37	1887.82	3577.50	1648.97	3360.70	753.00	1968.60
1.41 x 1.41	5030	1368.22b	2147.78	1666.28	836.30	4628.95	1481.19	4933.56	593.31	2206.90
1.29 x 1.29	6010	1692.65ab	1833.12	1082.50	990.01	4520.65	2625.10	4998.00	458.22	2275.00
1.19 x 1.19	7062	1702.42ab	1884.50	2126.56	921.32	4881.62	1866.52	4940.24	445.50	2345.61
1.12 x 1.12	7974	1842.31a	2169.69	1381.90	1963.00	4685.22	2608.68	4627.26	664.50	2492.70
1.05 x 1.05	9066	1903.57a	2525.83	2426.42	902.20	4733.67	3231.80	4854.19	431.01	2626.10
1.00 x 1.00	10 000	2028.97a	2697.68	1827.83	727.00	4413.32	4275.66	3488.21	1265.00	2590.40
Mean		**1692.71cd**	**2133.9bc**	**1720.3cd**	**1175.20de**	**4491.7a**	**2533.41b**	**4457.51a**	**658.80e**	
SE (±)		119.290	424.039	340.012	442.61	540.607	595.344	840.828	239.06	175.43
CV (%)		21.69	23.38	23.60	50.23	12.66	15.84	22.28	72.03	24.01
Training methods		NS	**	*	NS	NS	**	NS	NS	NS
Capping		1534.89	1801.44b	1945.29a	1081.70	4666.30	2942.04a	4471.63	780.20	2402.90
Free growth		1850.53	2414.30a	1495.24b	1268.80	4316.83	2124.79b	4443.27	537.30	2312.91
Mean		**1693.14**	**2107.87**	**1720.26**	**1384.15**	**4491.58**	**2533.41**	**4457.45**	**658.75**	
SE (±)		97.217	140.932	108.514	157.31	152.047	107.249	262.129	126.81	484.87
CV (%)		21.69	23.38	23.60	50.23	12.67	15.84	22.27	72.03	24.01

*NS, *, ** = Non-significant and significant at 5% and 1 % probability levels, respectively. Means followed by the same letter within each column are not significantly different at 5% probability level.*

REFERENCES

Biruk Debebe (2000). Remark made on the CBD control workshop. Proceedings of the Workshop on control of coffee berry disease (CBD) in Ethiopia, 13-15. August 1999. Addis Abeba, Ethiopia. PP3-4.

Mandefro Nigussie (2005). Statistical Procedures for Designed Experiments. EARO, Addis Ababa, Ethiopia.

Mihigan Stat University (MSTU) (1995). Users guide to MSTAT-C. MSTU, USA.

Paulos and Tesfaye (2000). Some ecological parameters occurring in the major coffee growing southwestern and southern Ethiopia. 13-15 August 1999, Addis Ababa, Ethiopia. Pp. 107-124.

Taye Kufa, Tesfaye shimmer, Alemseged Yilma, Anteneh Netesre and Endale Taye (2001) The Impact of close spacing on yield of Arabica coffee under contrasting agroecologiesof Ethiopia. African Crop Science Journal. Uganda. Vol: 9:No: 2 Pp. 401-409.

Workafes Woldetsadik and KassuKebede (2000). Coffee production system in Ethiopia. Proceedings of the Workshop on Control of Coffee Berry Disease (CBD) in Ethiopia, 13-15 August 1999, Addis Ababa, Ethiopia. Pp. 99-106.

Yacob Edjamo, Tesfaye shimber Taye kufa,Alemseged yilma,Takele Negewo, Anteneh Netsere and Bekele Bogale (1996). Advance in coffee agronomy research in Ethiopia. In: proceedings of IACO workshop, Kampala, Uganda. 4-6 September 1995. Tenywa, J.S., AdipalaEkwamu and Ogenga-Latigo, M.W. (Eds.), pp, 40-55.

Yacob Edjamo, Tesfaye Shimber, Gebramu Temesgen and Alemseged Yilma (1993).Effects of canopies and hearing heads on density and yields of CBD resistant coffee (Coffee Arabica L) In: Proceedings 15th International ScientificColloquium on coffee, Montpellier, 6-11 June1993.ASIC (Paris, pp.322-328.

Intercropping of Robusta Coffee Trees with Leguminous Plant: Effects on Physical and Chemical Figures of Marketable Coffee and Soil

K. N'GORAN[1], J. G. NEMLIN[1], K. AMANI[1], K. LACO[2]

[1]Centre National de Recherche Agronomique (CNRA), 01 BP 1740 Abidjan, Côte d'Ivoire
E-mail: maxngorank@yahoo.fr
[2]Centre Universitaire Professionnalisé, Cocody, Côte d'Ivoire

SUMMARY

The chemical assessment of Robusta coffee beans reveals that the intercropping of coffee with leguminous plant known as *Gliricidia sepium* and *Albizzia guachepele*, on soils processed with urea treatment or not has a beneficial impact on coffee quality. Analyses showed that most chemical features that are instrumental to the expression of the coffee liquoring qualities evolved positively against the witness processing. One notes in particular, an increase in total acidity, in ashes content, in potassium, in phosphors, in reducing sugars and in proteins. On the other hand, a reduction of the pH, of the fat content and manganese has been observed. Therefore, the intercropping of the Robusta coffee tree with leguminous plant can help to improve the nutritional value of coffee and the liquoring quality of marketable coffee. It can also improve the soil contents for sustainable coffee production.

RÉSUMÉ

L'évaluation chimique des grains de café robusta révèle que l'association culturale du caféier avec les légumineuses arborées, *Gliricidia sepium* et *Albizzia guachepele*, sur des sols traités ou non à l'urée a un apport bénéfique sur la qualité du café. Les analyses ont montré que la plupart des caractéristiques chimiques qui sont à la base de l'expression des qualités organoleptiques du café ont évolué de façon positive en comparaison avec le traitement témoin. On note, en particulier, une augmentation de l'acidité totale, de la teneur en cendres, en potassium, en phosphores, en sucres réducteurs et en protéines. En revanche, une diminution du pH, de la teneur en matières grasses et en manganèse a été observée. Ainsi, l'association caféier robusta aux légumineuses peut-il contribuer à l'amélioration de la valeur nutritive du café et de la qualité organoleptique du café marchand. Elle peut également améliorer le sol pour une production durable du café.
Key words: Coffee Robusta, N-fixing trees, coffee quality

INTRODUCTION

La qualité du café constitue une préoccupation majeure tant au niveau des pays consommateurs que producteurs. Elle peut être influencée par le milieu naturel, les techniques culturales, les variétés cultivées et l'homme qui met en œuvre les itinéraires techniques (Avelino et al., 2001; Guyot et al., 1996; Perriot et al., 2003).

L'intérêt agronomique de l'association du caféier robusta et de deux légumineuses, *Glyricidia sepium* et *Albizzia guachepele*, dans le but de réduire l'apport des engrais minéraux et de mesurer l'effet sur la productivité a été mis en évidence (NGoran et Amani, 2004). Mais les

informations concernant le gain qualitatif et la valeur nutritive du café obtenu au cours de cette association sont encore insuffisantes.

L'objectif de cette étude est de répondre à ces préoccupations en évaluant l'influence de cette association sur les caractéristiques chimiques des grains de café robusta marchand et du sol.

MATERIEL ET METHODES

L'étude a porté sur six échantillons de café prélevés dans l'essai d'association caféier/légumineuses conduit à la station de recherche du CNRA à Divo en Côte d'Ivoire. L'essai comporte 6 traitements et 5 répétitions :

- Traitement 1 (T1) : *Robusta* cultivé seul: variété témoin;
- Traitement 2 (T2) : *Robusta* obtenu après association culturale avec la légumineuse arborée *Glyricidia sepium :* (Gly) ;
- Traitement 3 (T3) : *Robusta* cultivé en association avec la légumineuse arborée *Albizzia guachepele* : (Alb) ;
- Traitement 4 (T4) : *Robusta* cultivé sur sol traité à l'urée à 100 kg/ha;
- Traitement 5 (T5) : *Robusta* obtenu après association culturale avec *Glyricidia sepium* plus sol traité à l'urée à 50 kg/ha: (Gly+Urée) ;
- Traitement 6 (T6) : *Robusta* cultivé en association avec *Albizzia guachepele* plus sol traité à l'urée à 50 kg/ha: (Alb+Urée).

Concernant les analyses physico-chimiques des grains de café, les méthodes suivantes ont été utilisées :

- l'humidité des grains par le passage à l'étuve à 105 °C pendant 24 heures pour atteindre le taux de 12% pour tous les échantillons;
- les cendres selon la méthode BIPEA basée sur l'incinération des grains dans un four à moufle entre 550 et 600 °C pendant 24 heures;
- le phosphore, le potassium et le manganèse dosés par spectrométrie atomique;
- la teneur en acidité déterminée par dosage au moyen d'une solution décimolaire de soude;
- les sucres totaux et réducteurs par la méthode de l'acide 3,5 dinitro-salycilique (DNS) après la détermination de la courbe étalon;
- les matières grasses estimées selon la méthode d'extraction au Soxhlet pendant 6 heures avec un solvant organique;
- les protéines déterminées selon la méthode de Kjeldahl basée sur le dosage de l'azote contenu dans le produit;
- la caféine par la méthode gravimétrique et l'extraction au chloroforme;
- la trigonelline extraite du café par l'eau à 90 °C minimum et décomplexée par addition d'oxyde de magnésium puis séparée et identifiée par HPLC et quantifiée par détecteur ultraviolet.

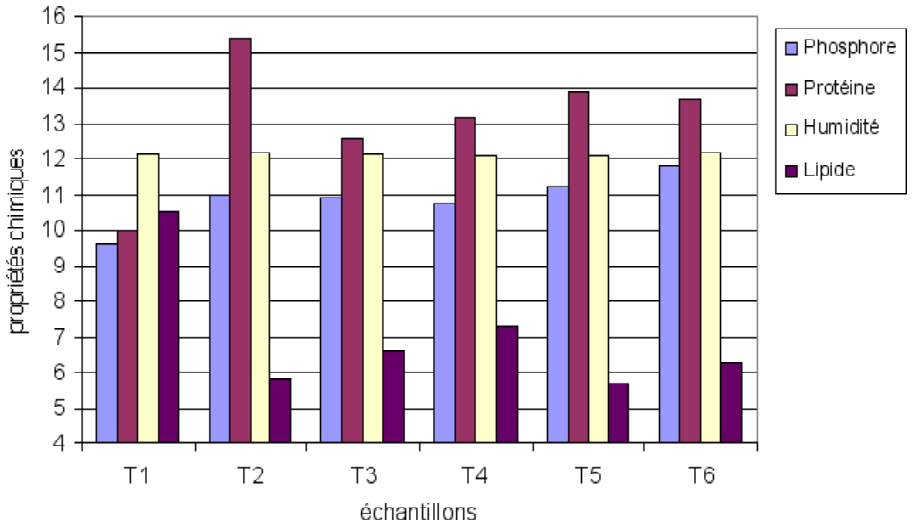
S'agissant du sol, les analyses ont porté sur le carbone, l'azote total, le calcium, le potassium, le magnésium, le phosphore, la somme des bases, la capacité d'échange, le pH.

RESULTATS ET DISCUSSION

Effets sur le café marchand

Les Figures 1, 2, 3, 4 et 5 ci-dessous indiquent:

- une baisse de la teneur en matières grasses, en manganèse et du pH;
- une hausse de l'acidité totale, des cendres, du potassium, du phosphore, des sucres, des protéines et de la caféine.



T1: Robusta témoin; T2: Robusta + glyricidia sepium: (Gly) ; T3: Robusta + albizzia guachepele: (Alb) ; T4: Robusta + urée; T5: Robusta + glyricidia sepium + urée: (Gly+Urée); T6: Robusta + albizzia guachepele + urée: (Alb+Urée).

Figure 1. Propriétés chimiques des échantillons.

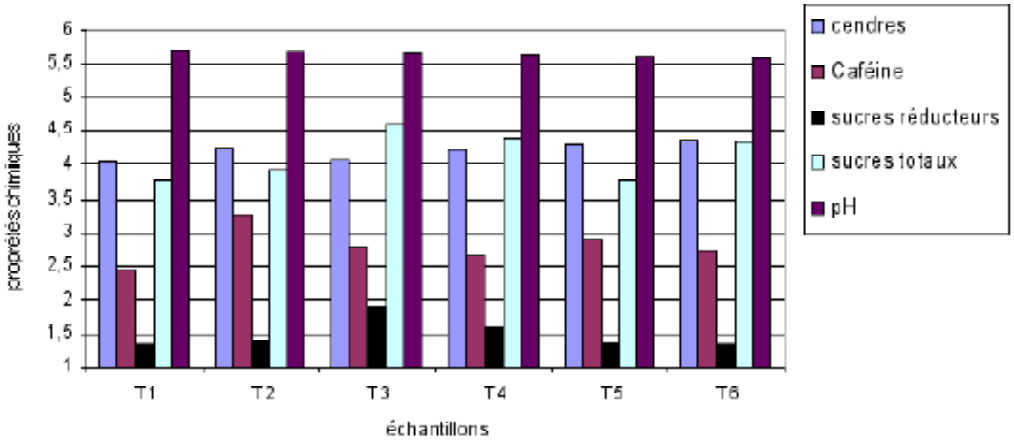


Figure 2. Propriétés chimiques des échantillons.

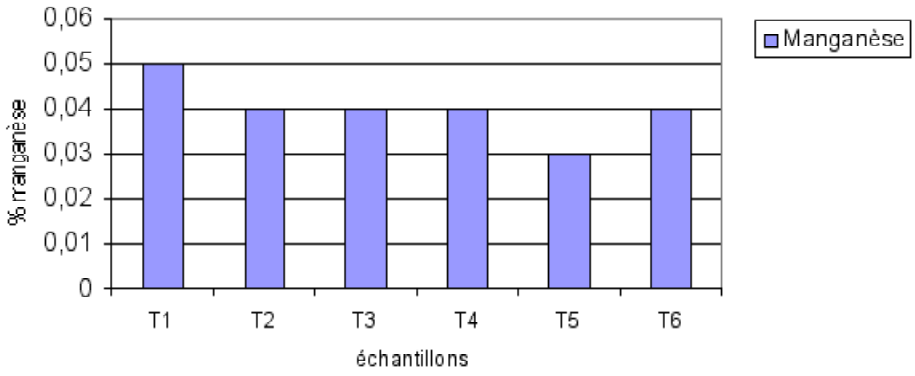


Figure 3. Pourcentage en manganèse des échantillons.

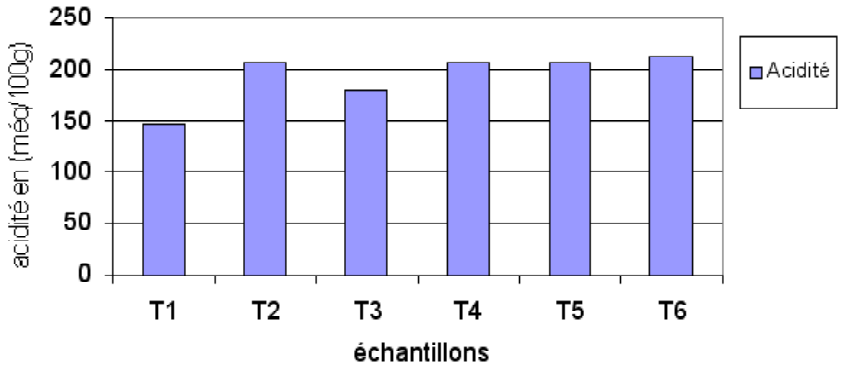


Figure 4. Teneurs en acidité titrable des échantillons.

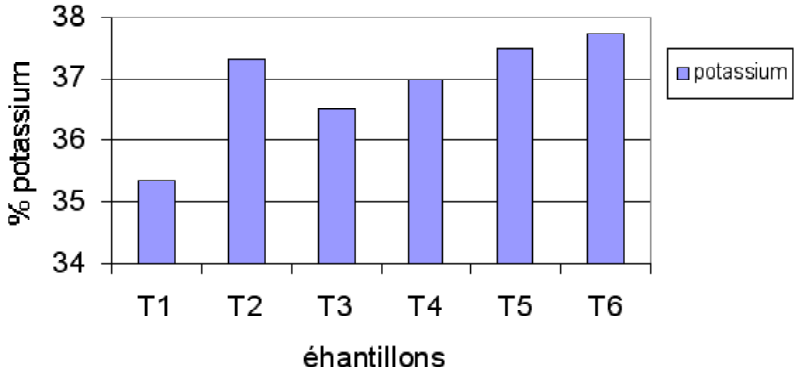


Figure 5. Pourcentage en potassium des échantillons.

Cendres, potassium et phosphore

L'analyse révèle une augmentation de la teneur en cendres, sauf pour l'échantillon T3, du taux de potassium et de phosphore dans les échantillons traités par rapport au témoin sans engrais et sans légumineuses selon le test de Student au risque α de 5%. Les macroéléments comme le potassium et le phosphore font partie des cendres; donc leur augmentation entraîne celle des cendres. Ce qui explique la plus grande valeur en potassium (37,73%) et en phosphore (11,80 mg $P0_4^{3-}$/g) du traitement T6.

La valeur des cendres du témoin (4,03%) est en conformité avec la moyenne trouvée par Coste (1989) qui est de 4%. Les autres taux sont respectivement de 4,25% pour T2, 4,06% pour T3, 4,21% pour T4, 4,29 pour T5 et 4,38% pour T6.

L'échantillon associé à *Albizzia* sur sol traité à l'urée (T6) a une teneur en cendre (4,38%) plus élevée que celui associé à *Albizzia* seul (4,06%). L'urée accentuerait la hausse des sels minéraux dans cette association. Mais avec la légumineuse *Glyricidia,* la hausse n'est pas significative selon le test de Student au risque α de 5%, en passant de 4,25% avec T2 à 4,29% avec T5 (Figure 2).

Ce résultat corrobore avec les observations faites par Snoeck et al. (2000), Gunaratne et Heenkenda (2000), Zaharah et Wan Rashidah (2000), NGoran et Amani (2004) selon lesquelles, l'apport d'éléments nutritifs tels que le potassium, le calcium, le magnésium etc., fait partie de l'effet bénéfique de l'association des caféiers aux légumineuses. Le potassium est l'un des éléments dominant dans les fruits grâce au phosphore qui agit sur la mobilisation

du potassium et de l'azote du sol. L'augmentation de ces éléments minéraux dans les grains de café peut permettre à l'homme de lutter contre certaines carences minérales.

Manganèse (Mn)

Elle diminue significativement selon le test de Student au risque α de 5% par rapport au témoin robusta T1. De 0,05% avec T1, on passe à 0,04% et à 0,03% dans les autres échantillons (Figure 3). Les traitements à l'urée et les associations aux légumineuses ont eu pour effet de diminuer la quantité de manganèse dans les fruits du café. Selon Javillier et al. (1962), Olaru a découvert en 1920 que le manganèse augmentait considérablement la quantité d'azote fixée par les nodules des légumineuses. On peut donc supposer que la diminution du manganèse serait due à l'utilisation de cet élément pour permettre aux légumineuses de fixer l'azote plutôt que de migrer dans les grains de café.

Acidité totale et pH

Le pH de chaque échantillon baisse et leur teneur en acidité augmente significativement selon le test de Student au risque α de 5% par rapport au témoin. Le pH du témoin (5,72) (Figure 5) est proche de celui trouvé par Sivetz et al. (1963), qui indique 5,5 pour le Robusta. Ces deux propriétés chimiques étant liées, la diminution du pH entraîne l'augmentation de l'acidité totale.Les légumineuses seraient responsables de ce résultat qui corrobore celui de Petnga (1986). Pour cet auteur, l'augmentation de l'acidité dans les grains de Robusta traités serait due aux légumineuses arborées et à l'urée qui acidifieraient le sol.

Au niveau de l'acidité, on note que l'urée a un effet positif sur l'association Robusta/Albizzia car l'acidité augmente de 181méq/100g avec T3 à 213 méq/100 g pour T6 (Figure 4). En effet, les valeurs obtenues sont respectivement de 208 meq/100 g pour T2, T4 et T5 contre 146 meq/100 g pour le témoin T1. L'acidité est une caractéristique de la qualité organoleptique recherchée dans le café contrairement au cacao selon Langler et al. (1967); Sivetz (1972); Woodman (1985). Son augmentation constitue donc un gain qualitatif pour le consommateur.

Matières grasses

La baisse significative de la teneur en matières grasses est observée dans les traitements de T2 à T6 selon le test de Student au risque _ de 5% par rapport au témoin robusta dont la valeur de 10,48% se trouve dans l'intervalle de 9 à 14% indiqué par Coste (1989) pour les variétés Robusta.

D'après Javillier et al. (1962) les lipases végétales renferment du calcium. L'augmentation des éléments nutritifs du café tel que le calcium, pourrait entraîner celle des lipases qui sont responsables de l'hydrolyse des matières grasses produites. La synthèse des lipases en grande quantité peut être à l'origine de la diminution de la matière grasse dans les échantillons de café associé.

Les matières grasses ou lipides complexes sont des esters d'acide gras et de divers alcools dont l'élaboration procède d'abord de l'activation des acides gras.

Cette activation a lieu en présence de co-enzyme A (CoA) sous forme réduite selon Javillier et al. (1969). La réduction du CoA se fait par le zinc et l'acide acétique (Javillier et al., 1964). On peut donc supposer que les légumineuses ont diminué la quantité de zinc à apporter aux

caféiers en entraînant la baisse de CoA réduit, la baisse de l'activation des acides gras et donc celle des matières grasses complexes.

Cette baisse est considérable avec les échantillons cultivés en association sur sol traité avec ou sans urée : on passe successivement de 10,48% du témoin à 5,68% pour T5, 5,76% pour T2, 6,25% pour T6, et à 6,57% pour T6 (Figure 1).

La diminution de la matière grasse peut constituer un avantage pour la conservation post-récolte du café car, d'après Coste (1989), l'oxygène de l'air agit sur la matière grasse entraînant un goût de rance très désagréable. Cette diminution règle donc partiellement ce phénomène de rancissement ; surtout que la matière grasse du café ne peut pas être utilisée comme une huile comestible à cause des nombreuses substances insaponifiables qu'elle renferme.

Glucides

La quantité de sucres totaux des échantillons traités est significativement plus élevée selon le test de Student au risque α de 5% que celle du témoin robusta qui est de 3,75%. La valeur du témoin est comprise dans l'intervalle 3,7% à 7,1% donné par Coste (1989) pour le Robusta.

L'analyse décèle une différence significative au risque α de 5% entre la valeur de T2 (3,93%) et T5 (3,67%) d'une part, et d'autre part, entre celles de T3 (4,60%) et de T6 (4,34%) (Figure 2). La légumineuse *Albizzia* (T3) et le traitement urée (T4) ont eu un effet positif sur les sucres totaux. En ce qui concerne les sucres réducteurs, leur quantité a aussi augmenté de façon significative par rapport au témoin au risque α de 5%. Mais les échantillons T5 (1,36%) et T6 (1,35%), sont identiques au témoin avec un taux de 1,33% (Figure 2).

D'après Javillier et al. (1969), la biosynthèse des sucres se fait en présence d'ATP et d'ions magnésium. On peut donc supposer que l'augmentation du magnésium dans les caféiers grâce aux légumineuses serait à l'origine de la hausse des sucres. Cette hausse peut contribuer à la diminution de l'amertume du café (Picard et al., 1984; Vithzum, 1976).

Protéines

Les résultats obtenus avec les échantillons associés aux légumineuses sont élevés par rapport à celui du témoin qui est de 10%. Cette valeur s'inscrit dans l'intervalle des teneurs en protéines indiqué par Anonyme (1991), qui est de 10 à 12% et est en conformité avec celle de Payen citée par Pochet (1990), qui est de 10 g/100 g de café.

L'augmentation de la teneur en protéines dans les échantillons traités est due aux légumineuses qui enrichissent le sol en azote, élément important dans le métabolisme de croissance du caféier. Il est un élément constitutif des protéines, et sa hausse entraînerait celle des protéines et tous les composés azotés tels que les alcaloïdes.

Le caféier associé à une légumineuse bénéficie de l'azote atmosphérique fixé par l'intermédiaire des émondes déposées aux pieds du caféier et par les échanges dans le sol entre les systèmes radiculaires. En effet, les légumineuses sont capables de fixer et transformer l'azote atmosphérique en composés nitrés qui constituent des éléments fertilisants pour le caféier. Le caféier associé à *Glyricidia* (T2) a le plus fort taux en protéine (15,37%) mais ce taux baisse à 13,88% pour T5, 13,69% pour T6, 13,16% pour T4, 12,56% pour T3 et à 10% pour le témoin (Figure 1). Le traitement café associé à *Albizzia* seul (T3) apporte moins de protéines aux grains de caféier

L'augmentation des taux de protéines peut améliorer la qualité organoleptique des cafés traités car les produits de dégradations des protéines telle que la trigonelline sont responsables du développement de l'arôme du café lors de la torréfaction (Coste, 1989). Les taux de trigonelline sont plus élevés dans les traitements avec légumineuses que dans le témoin et l'urée seul: 60.5% pour T2, 57.5% pour T3, 53% pour T5, 59% pour T6 contre 50.5% pour le témoin et 49.5% pour l'urée.

Caféine

On note une hausse significative selon le test de Student au risque α de 5% de la teneur en caféine dans les échantillons avec urée ou associés aux légumineuses par rapport au Robusta témoin qui a une valeur de 2,44% (Figure 2). Ce taux s'inscrit dans l'intervalle des taux de caféine cité par Coste (1989) qui est de 1 à 2,5% selon le matériel végétal; en effet, certains Robusta peuvent même dépasser le taux de 3% en caféine. L'augmentation est due à l'apport de l'azote au caféier, azote ayant servi à la formation de ce principal alcaloïde: la caféine.

On note un effet négatif de l'urée sur l'association *Robusta/Glyricidia*; car la teneur de la caféine du café associé à *Glyricidia* (T2) passe de 3,28% à 2,88% pour le traitement *Glyricidia* + urée (T5) ; la différence est significative selon le test de Student au risque α de 5%.

L'augmentation de la caféine peut constituer un avantage d'une part, pour des industries pharmaceutiques qui l'utilisent comme un tonicardiaque et, d'autre part, pour une certaine catégorie de consommateurs qui préfèrent la force du café Robusta par rapport à l'Arabica

Effets sur le sol

Le tableau 1 indique la différence entre les taux et teneurs en éléments minéraux du sol. On peut noter que les valeurs des traitements avec urée et légumineuses (T2 à T6) sont plus élevées que celles du témoin (T1) à l'exception du phosphore.

L'application des émondes aux pieds des caféiers améliore le sol en notamment en carbone, en azote et en bases échangeables. La capacité d'échange est également améliorée ; les réserves en éléments nutritifs pour les plantes deviennent très importantes.

Toutefois, on observe une forte utilisation du phosphore du sol par les caféiers du fait du faible apport de cet élément par les légumineuses (NGoran et Amani, 2004); de même, les caféiers utilisent une partie du potassium du sol, notamment dans les traitements avec Albizzia (T3 et T6) et urée (T4) qui apportent peu de potassium dans le système sol-plante.

Table 1. Eléments chimiques du sol: différence entre les traitements de T2 à T6 et le témoin.

Trait.	C%	Nt %	Ca	Mg	K	P	S	T
T2	0.34	0.04	1.42	0.31	0.05	– 8.8	1.80	2.0
T3	0.13	0.02	2.31	0.22	– 0.01	– 7.4	1.34	0.91
T4	0.12	0.002	0.92	0.12	– 0.04	– 10.2	1.01	1.46
T5	0.068	0.01	0.42	0.27	0.04	– 11.4	0.66	0.79
T6	0.07	0.01	– 0.046	0.11	– 0.03	– 0.124	– 0.04	0.46

CONCLUSION

L'association du caféier Robusta aux légumineuses telles *Glyricidia sepium* et *Albizzia guachepele* peut contribuer à l'amélioration de la valeur nutritive et de la qualité organoleptique du café du café marchand. Les résultas indiquent que *Glyricidia sepium* apporterait plus de protéines, de cendres, d'acidité et potassium qu Albizzia guachepele. Ce type d'association est donc important à la fois pour l'industriel et le monde agricole car elle contribue à améliorer le sol et assurer une production durable du caféier.

REFERENCES

Avelino J., Perriot J.J., Pineda C., Guyot B., Cilas C., 2001. Vers une identification de cafés-terroir au Honduras: caractérisation physique, phytotechnique et biologique des caféières honduriennes. In XIX^{e} colloque scientifique international sur le café, Triestre, Italie, 14- 18/05/2001. Paris, France, ASIC.

Coste R., 1989. Cafés et caféiers. Ed G P Maisonneuve & Larose, Paris 358p.

Gunratne W.D.L., Heekenda A.P. – 2000 – Green manure (*Gliricidia sepium*) effects on growth and yield of coffee and quantification of nitrogen recovery using ^{15}N dilution. Nuclear Techniques in integrated plant nutrient, water and soil management*, IAEA-SM-363/7* p26-31.

Guyot B., Gueule D., Manez J. C., Perriot J.J., Giron J., Vilain L., 1996. Influence de l'altitude et de l'ombrage sur la qualité des cafés Arabica. *Plantations, Recherche, Développement*, 3 (4) : 272-283.

Javillier M., Polonovski M., Florkin M., Boulanger P., Lemoigne M., Roche J., Wurmser R., 1962. Traité de biochimie générale. Éd Masson et C^{ie} tome II. P 400 et p 403.

Javillier M., Polonovski M., Florkin M., Boulanger P., Lemoigne M., Roche J., Wurmser R., 1964. Traité de biochimie générale. Éd Masson et C^{ie} tome II. p 526.

Javillier M., Polonovski M., Florkin M., Boulanger P., Lemoigne M., Roche J., Wurmser R., 1969. Traite de biochimie générale. Éd Masson et C^{ie} tome III. p 447 et p 451.

Langler, J. E., Libbey, L. M. and Day, E. A., 1967 Journal of Agricultural Food Chemist. 15, 386-91

Ngoran K., Amani K., 2004. Legume trees for best quality of coffee production in Côte d'Ivoire, ASIC, Bangalore, India.

Perriot J.J., Ribbeyre F., Montagnon C., 2003. Les qualités d'un café. In Cafés: terroirs et qualités, CIRAD, 153 p.

Petnga E., 1986. Analyse multidimensionnelle de la classification qualitative du café *Robusta*. Thèse de doctorat 3é Cycle Université des sciences et Techniques de Languedoc, 106p.

Picard H., Guyot B., Vincent J. C., 1984. Etude des composés stéroliques de l'huile du café (*C. Canephora*). *Café Cacao Thé*, Paris, vol 28, 4^{e} éd, p. 47-62.

Pochet P., 1990. La qualité du café de la plantule à la tasse. *A.G.C.D.*, Bruxelles, n° 21, 78 p.

Sivetz M., 1972. Food Technology (Chicago), 26 70-7

Sivetz M., 1963. Coffee processing technology ed Avi Pubushing Company, Inc, Volume two p165-168.

Sivetz M., and Foote Elliott, 1963. Coffee processing technology. Ed Avi Pubushing Company, Inc, Volume one.p.232-233.

Snoeck D, Zapata F, Domenach A M. – 2000 – Isotope evidence of transfer of nitrogen fixed by legumes to coffee trees. *Biotechnol. Agron. Soc. Environ*. 4 (2), p. 95-100.

Vithzum O. G., 1976. Chemie und Bearbeitung des Kaffees, in Kaffees und Caffeine. Springer-Verlass ed., Berlin, p. 3-38.

Woodman, J. S., 1985.The role of acids in infusions. In Coffee Chemistry, Elsevier Applied Science Publishers, London and New-York, vol. 1, 266-89.

Zaharah Ar., Wan Rashidah Wak. – 2000 – The potential of fresh leaves to improve acid-sol infertility. Nuclear Techniques in integrated plant nutrient, water and soil management, *IAEA-SM-363/68P* p.436-437

Emergence and Growth of Arabica Coffee Seedlings as Influenced by Some Pre-sowing Seed Treatments

T. KUFA AND A. YILMA

Ethiopian Institute of Agricultural Research, Jimma Research Center, P. O. Box 192, Jimma, Ethiopia. E-mail: kufataye@yahoo.com

SUMMARY

A factorial experiment arranged in randomised complete block design with three replicates was used to undertake the experiment at Jimma Agricultural Research Centre (JARC) nursery site. The objective of the study was to examine the contributions of some pre-sowing seed treatments for enhanced coffee seed germination and subsequent seedlings growth. For this, removal of parchment and soaking coffee seeds in pure cold water (each at two levels) were examined. The results indicated that the initial stage coffee seed emergence was significantly influenced due to the main parchment effect with the maximum results obtained from sowing clean coffee seeds. Soaking treatment had also improved emergence and growth, though the results did not vary. The interaction effect was comparable, although maximum average values for seedling emergence and subsequent shoot and root growths were recorded due to sowing of clean coffee seeds and water soaking. In general, pre-sowing seed treatments were noted to have different amplitudes on seed emergence and thus, put the most imprints on the latter growth stages of coffee seedlings.
Keywords: Coffee seed, germination, soaking, parchment coffee

INTRODUCTION

Seed physiologist describe four germination stages: 1) hydration or imbibitions, during which water penetrates into the embryo and hydrates proteins and other colloids, 2) the formation or activation of enzymes, leading to increased metabolic activity, 3) elongation of radicle cells, followed by emergence of the radicle from the seed coat (germination proper) and 4) subsequent growth of the seedlings. The seed covering layers can interfere with the penetration of water and oxygen or both and they can prevent emergence of the radicle by acting as a mechanical barrier (Salisbury and Ross, 1992). Germination process is complete when nutrition no longer depends upon reserve materials, but is autotrophic. By this time, the root has secured a hold in the soil the cotyledons or the primary leaves are unfolded and the seedlings attained independence growth and ensure plant establishment. According to Larcher (2003), the duration of germination is the time elapsing between hydration of the seed and the appearance of the radicle and its rate is the percent increase in germinating seeds per unit time.

Coffee seed consists mainly of endosperm, which is coved by endocarp (parchment) and seminal tegument-silver skin. It contains starch, fat, reducing sugars, saccharides, tannins, caffeine and water. There is an embryo a radicle and cotyledons (Coste, 1992). The water content of coffee seed should be gradually reduced under shade and it should not be dropped below 18 per cent during storage, most probably indicating an intermediary behaviour of coffee seeds between an orthodox and recalcitrant seeds.

In Ethiopia, coffee seeds are prepared by careful hand pulping and the parchment remains intact, mainly to protect the seed/bean from the high external damages. But, the two-layered seed coats can act as barriers to inhibit water absorption and aeration. Depending on the external internal, eco-physiological and environmental conditions and the growth phase of coffee seedling usually takes long time. This is particularly a case in point at high altitude coffee growing areas where it takes about 3 months for coffee seeds to germinate and emerge above the soil surface with the subsequent slow seedling growth rates (more than a year) to attain the normal transplanting stage. Thus, it needs high costs encored at the early nursery stages alone, which is beyond the reach of most subsistent coffee farmers.

Moreover, it is also not uncommon to observe poor and irregular stand establishment in modern coffee plantations, largely due to the variations in seed characteristics and early growth performances at nursery level. To compensate this, more number of seedlings, usually double of the actual required, is being produced at most coffee nurseries, escalating costs of purchasing coffee seeds as well as post-sowing nursery operations. For the breaking of seed dormancy, a variety of mechanisms including scarification may be effective (Larcher, 2003; Salisbury and Ross, 1992). In Ethiopia, research information on possible ways of improving the slow germination rate of coffee seeds is lacking. The objective of this study was, therefore, to investigate on the influence of parchment removal and soaking coffee seeds in cold pure water on seedling emergence and growth of coffee seedlings at Jimma Research Center, southwest Ethiopia.

MATERIAL AND METHODS

The study was conducted at Jimma Agricultural Research Center (7° 46’ N latitude and 36° 0’ E longitude), southwest Ethiopia. It is located within the Tepid to cool humid highlands agro-ecological zone of the country at an altitude of 1750 meters above sea level. The site receives high amount of rainfall with a long-term mean of 1595 mm per annum distributed into 173 days. The driest season lasts between December and January. The mean maximum and minimum temperatures are 25.9 and 11.2 °C, respectively, the coldest month being December. The predominant soil of the Center is *Eutric Nitosols.*

A 2 x 2 factorial experiment arranged in complete randomised block design with three replicates of 16 pots per plot was employed. In this case, parchment removal (un removed and removed/ clean coffee) and soaking in pure cold water (un soaked and soaked for 24 h) were studied as pre-sowing treatments. Fully ripe red and healthy coffee cherries were harvested from the lowland Sheko (Berhane-Kontir) natural forest and prepared for sowing. Because, coffee accessions from this area shown significantly delayed germination and seedling growth as compared to the other coffee populations evaluated under JARC environment. To minimize the risks of germination, two seeds were sown on April 02, 2004 in a black black diothene 200-gauge polythene bags (12 cm x 24 cm) filled with the ideal potting mixes of topsoil and well-decomposed coffee compost at the respective volume of 1:3 (Taye et al., 2002).

Based seedling growth stages, water was applied at an intervals of every 2, 4 and 8 days within a week from sowing to emergence, emergence to appearance of true leaf stage and thereafter, respectively (Taye et al., 2002). The seedlings were grown under a moderate shade level of about 50% light interception. All the other post-sowing nursery practices were also adhered to the previous recommendations of the centre (IAR, 1996). Coffee seedlings started to emerge after 12 weeks or 46 days after sowing (DAS) and emergence and subsequent growth stages (soldier, butterfly, true leaf pairs) were recorded at an interval of a week. Thinning to one seedling per pot was made after about 5 months when seedling with different pairs of true leaf stages stated to be recorded. When the seedling attained transplanting stage

with six pairs of true leaves after 8 months after sowing, two seedlings were sampled for the extension and destructive shoot and root parameters. Relative growth rate (RGR) was calculated for the dry weights measured over the two-months. The experiment was undertaken between April 2004 and January 2005. Finally, the data were analysed using a SAS (version 8) package and the analysis of variance (ANOVA) for each growth stage and treatment means were compared according to Tukey's at 5% probability level.

RESULTS AND DISCUSSION

Seedling emergence

The results revealed that the removal of parchment significantly promoted mean emergence of coffee seedlings as compared to the parchment seeds (Table 1; Figure 1). Consequently, significant variations in average germination were noted for the early times (between 12 and 20 weeks after sowing) with the highest mean difference of about 3-folds were recorded in the 15-weeks after sowing (Figure 1). Then after, the differences revealed a decreasing trend and disappeared during the latter seedling ages. The ripe healthy and well-formed coffee seeds may germinate as soon as it is harvested if it is placed in a satisfactory environmental condition such as humidity, heat and aeration (Coste, 1992; Wrigley, 1988). In contrast, no significant difference was recorded between the un soaked and soaked coffee seeds, which perhaps associated to the short soaking time (24 h) in the cold pure water to imbibe the required amount of water to bring a difference. However, soaked coffee seeds had maximum rate of emergence, particularly during the early stages after sowing (Table 1; Figure 1). This could be explained in terms of the enhanced water imbibitions and subsequent physiological processes. Coffee seed absorbs water and its internal action triggers the metabolic and enzymatic processes that induce germination and germination rate depends on the interaction of temperature, moisture, the aeration of the soil environment and its pH (Coste, 1992; Wrigley, 1988).

Table 1. Mean (±SD) for weekly per cent emergence of coffee seedlings as influenced by the main pre-sowing treatments.

Week after sowing	Removing parchment			Soaking in water		
	Unremoved	Removed	Difference	Un soaked	Soaked	Difference
12	1.04±1.62	44.79± 21.35	43.75**	15.63±18.75	30.21±33.58	14.58
13	6.25± 7.12	53.65± 12.09	47.40***	23.96± 25.36	35.94± 28.55	11.98
15	25.52± 8.48	78.13± 10.08	52.60***	49.48± 25.65	54.17± 34.10	4.69
17	46.88± 17.57	87.50± 9.27	40.63**	67.71± 19.33	66.67± 31.79	1.56
19	63.54± 18.92	88.02± 8.71	24.48*	79.17± 12.29	72.40± 24.80	6.77
20	76.05± 13.06	90.63± 5.93	14.58*	86.98± 6.38	79.69± 16.15	7.29

, ** and * within each row shows significant mean differences at P≤0.05, P≤0.01 and P≤0.001, respectively.*

Similarly, treatment interaction displayed no significant effects on subsequent seedlings growths. However, soaking reduced mean emergence days for parchment and clean coffee seeds, reflecting the more favored seed germination process, which calls for detailed research of coffee seed physiology. According to Coste (1992), seed and environmental factors can influence seed germination. On the whole, the findings would provide insights on the possibility to enhance germination and seedling growth as per the field-planting calendar of each agro-ecological zone. However, it could be necessary to further continue and search for some other practical seed treatments including prolonged soaking in hot and cold-water treatments for the varying seed sizes and wet and dry seasons.

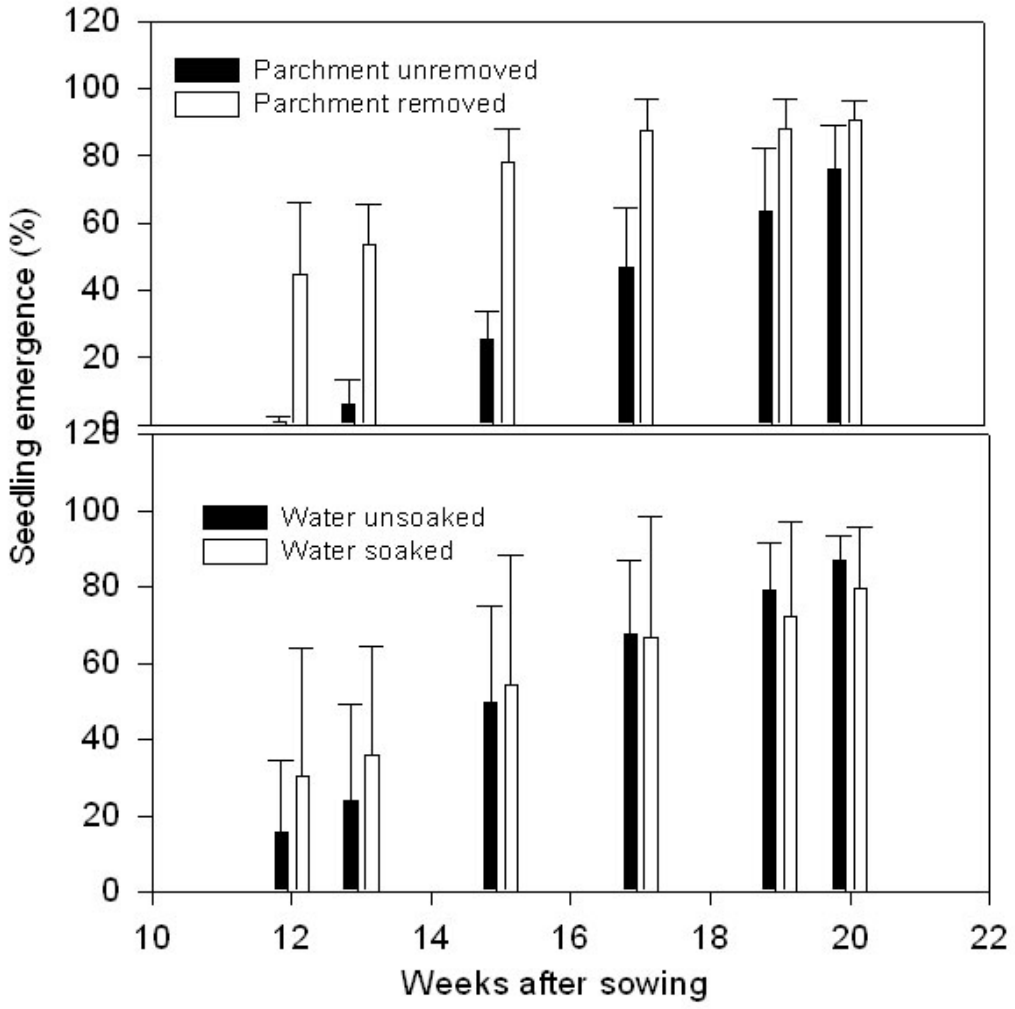


Figure 1. The effect of pre-sowing seed treatment on rate of seedling emergence in Arabica coffee accession.

Seedling growth

Similar to seedling emergence, pre-sowing treated seeds had significantly high values of enhanced growth performances. Accordingly, the seedlings significantly differ in the growth of the first and second true leaf pairs due to the main and interaction in 39 weeks after sowing (about 5 months). However, only parchment removal showed significant variations until the growth of second true leaf pair recorded in 55-weeks after sowing. Thereafter, no significant difference due to either the main or interaction was noted. However, about 53% of the seedlings raised from clean coffee seeds had attained the transplanting stage with 6 pairs of true leaves within 8 months after sowing (Table 2a). At this time, about 54% of the seedlings from soaking treatments (Table 2b) attained the 5^{th} pairs of true leaf stage. In other words, parchment removal was found to enhance seedling growth and shortens the nursery time to produce vigorous and transplantable seedlings by about a month as compared with the other pre-sowing treatments. Besides, parchment removal has an advantage to select normal and healthy coffee beans, which may be affected by insects and diseases and hence, would help to maximize uniform seedling growth.

Table 2. Mean (±SD) for weekly growth stage (%) of coffee seedlings as influenced by the levels of two pre-sowing seed treatments.

a) Parchment treatment

Week after sowing	Parchment un removed				Parchment removed (clean coffee)			
	Emerging	Soldier	Butterfly		Emerging	Soldier	Butterfly	
12	33.33±51.94	0.00±0.00)			89.23±10.83	10.77±10.83		
13	38.89±35.52	27.78±31.03			28.24±12.37	71.76±12.37		
15	46.39±16.28	53.61±16.28			18.15±7.33	81.85±7.33		
17	24.48±10.85*	75.52±10.85			4.47±4.80	94.99±4.81		
19	16.24±3.90**	83.77±3.90			0.58±1.41	97.57±4.47		
20	5.49±4.52*	91.56±5.38	2.95±2.38**		0.58±1.41	78.15±10.97	21.27±11.34	
22	3.58±5.29	85.29±9.14**	11.13±10.04**		0.62±1.51	58.46±6.41	40.93±5.80	
24	0.70±1.70	69.33±8.85**	28.72±8.71**		0.00±0.00	43.90±8.26	56.10±8.26	
	Soldier	Butterfly	1st leaf pair	2nd leaf pair	Soldier	Butterfly	1st leaf pair	2nd leaf pair
27	29.64±11.75	70.36±11.75	0.00±0.00*		15.35±11.32	72.72±9.53	11.94±7.71	
28	16.12± 6.01	80.67±8.51	3.22±3.00**		7.64±7.43	51.29±10.64	41.08±7.95	
29	9.24±5.24	69.36±7.81**	21.40±7.96		5.34±7.90	31.24±5.90	58.05±15.96	
31	4.26±4.09	56.93±12.77	38.81±12.24	0.00± 0.00	5.34±7.90	15.97±5.84	57.01±12.44	21.68±10.99
33	4.32±4.19	35.70±24.92	53.58±26.58	6.41± 4.86	3.43±4.87	2.04±2.24	40.70±17.94	53.84±16.84
	1st leaf pair	2nd leaf pair	3rd leaf pair	4th leaf pair	1st leaf pair	2nd leaf pair	3rd leaf pair	4th leaf pair
39	39.58±16.14***	58.33±17.97***			2.08±3.22	94.79±4.70		
41	22.92±16.61*	77.08±16.61			0.00±0.00	85.42±16.14		
44	84.38±8.62***	14.58±8.54***			22.92±21.89	77.08±21.89		
48		37.50±24.04*	48.96±39.21*			2.08±3.23	89.58±9.41	
51		87.50±7.91***	10.42±7.57***			23.96±17.86	73.96±15.01	
55		40.63±11.00	56.25±15.81	0.00±0.00		6.25±10.46	75.00±18.96	18.75±22.36
		4th leaf pair	5th leaf pair	6th leaf pair		4th leaf pair	5th leaf pair	6th leaf pair
62		23.81±11.66	69.05±12.51	3.57±3.91*		9.52±20.03	38.10±27.35	51.19±33.63

, ** and * indicate significant mean differences within a row of each week after sowing at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.*

b) Soaking treatment

Week after sowing	Water unsoaked				Water soaked			
	Emerging	Soldier	Butterfly		Emerging	Soldier	Butterfly	
12	64.00±49.70	2.67±4.14			58.57±46.59	8.10±12.33		
13	24.51±22.68	42.16± 34.93			42.62±30.29	57.38±30.29		
15	40.55±22.14	59.46±22.14			24.00±11.94	76.01±11.94		
17	13.66±11.83	86.34±11.83			15.30±15.47	84.17±15.02		
19	9.53±9.58	90.48±9.58			7.29±8.34	90.86±7.60		
20	3.69±4.62	88.76±7.50	7.56±8.28		2.38±3.81	80.96±12.82	16.67±14.74	
22	0.64±1.57	78.08±16.05**	21.28±16.97		3.56±5.29	65.67±14.38	30.77±18.04	
24	0.00±0.00	60.55±12.99*	38.85±13.47*		0.70±1.70	52.68±18.12	45.96±19.55	
	Soldier	Butterfly	1st leaf pair	2nd leaf pair	Soldier	Butterfly	1st leaf pair	2nd leaf pair
27	21.13±11.14	74.15±9.48	4.73±8.40		23.87±16.13	68.93±11.22	7.21±8.40	
28	11.94±8.01	69.83±19.76	18.23±18.94*		11.82±8.38	62.12±16.65	26.06±23.17	
29	9.11±8.13	48.70±22.79	42.19±24.54		5.47±5.00	51.03±21.03	37.26±22.52	
31	6.98±7.61	33.85±19.18	49.97±10.06	9.32±11.91	2.62±3.24	39.05±28.63	45.97±19.82	12.36± 15.98
33	5.13±5.24	8.99±7.40	56.00±25.37	29.88±30.95	2.62±3.24	28.74±31.89	38.27±17.20	30.38± 26.42
	1st leaf pair	2nd leaf pair	3rd leaf pair	4th leaf pair	1st leaf pair	2nd leaf pair	3rd leaf pair	4th leaf pair
39	15.63±12.96*	82.29±10.77*			26.04±29.69	70.83±30.79		
41	7.29±8.31	84.38±8.62			15.63±21.92	78.13±21.92		
44	55.21±32.21	44.79±32.21			52.08±42.14	46.88±43.08		
48		21.88±30.30	70.83±35.06			17.71±20.32	67.71±37.17	
51		58.33±36.59	39.58±32.76			53.13±38.07	44.79±40.20	
55		21.88±12.96	69.79±8.31	8.33±17.53		25.00±27.67	61.46±26.64	10.42±20.03
		4th leaf pair	5th leaf pair	6th leaf pair		4th leaf pair	5th leaf pair	6th leaf pair
62		10.72±10.84	53.57±28.84	33.33±37.43		22.62±21.39	53.57±25.45	21.43±31.94

, **, * = significant mean differences within a row of each week after sowing at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.*

Table 3. Morphological growth parameters (means±SD) of coffee seedlings as influenced by pre-sowing seed treatments.

Growth character	Parchment removal		Water soaking	
	Unremoved	Removed	Unsoaked	Soaked
Seedling height (cm)	28.02±6.16	28.77±2.89	27.33±3.24	29.46±5.78
Man stem girth (cm)	0.46±0.08	0.49±0.05	0.46±0.04	0.48±0.08
No of true leaf pair	7.00±0.32	7.50±0.89	7.00±0.71	7.50±0.63
No of nodes/seedling	8.25±0.42	8.67±0.75	8.42±0.66	8.50±0.63
Internode length (cm)	3.40±0.72	3.28±0.26	3.24±0.40	3.43±0.64
No of leaves/seedling	13.83±0.98	15.50±2.43	13.83±1.17	15.50±2.35
Leaf area (cm^2)	18.06±4.84	17.00±1.84	17.43±3.03	17.63±4.28
Leaf dry weight (g)	1.84±0.59	2.25± 0.47	2.02±0.35	2.07±0.74
Specific leaf area (g cm^{-2})	10.07±1.66	7.91±2.06	8.81±1.55	9.18±2.71
Stem fresh weight (g)	4.13±1.82	5.11±1.68	4.41±1.26	4.83±2.24
Stem dry weight (g)	1.04±0.45	1.34± 0.40	1.14±0.29	1.25±0.57
Root dry weight (g)	0.70±0.22	0.77±0.16	0.72±0.19	0.74±0.20
Tap root length (cm)	22.28±1.11	21.60±1.16	22.04±1.41	21.84±0.92
No of lateral roots	61.50±9.25	57.67±4.78	60.42±5.87	58.75±9.00
Length of lateral root (cm)	7.05±1.70	7.76±0.89	7.89±1.58	6.91±0.95
Total dry matter (g)	3.58±1.22	4.36±1.01	3.88±0.78	4.06±1.50
Leaf water content (%)	75.83±2.00	75.80±0.52	75.64±1.83	75.99±0.90
Shoot dry weight (g)	2.88±1.03	3.60±0.86	3.15±0.63	3.32±1.30
Root to shoot ratio	0.25±0.04	0.22±0.02	0.23±0.04	0.24±0.04
Root volume (cm^3)	4.04±1.46	4.25±1.26	3.83±1.45	4.46±1.19
Leaf partitioning (%)	51.96± 2.00	51.86± 1.34	52.57± 2.03	51.25± 0.80
Shoot partitioning (%)	80.33± 2.68	82.25± 1.53	81.57± 2.30	81.00± 2.49
Root partitioning (%)	19.68± 2.68	17.76± 1.53	18.43± 2.30	19.00± 2.49
RGR (g $month^{-1}$)	0.58±0.26	0.74±0.20	0.62±0.21	0.70±0.31

On the other hand, the later morphological growth characters did not show significant differences between the main and interaction effects. As a result, only the main-effect average values of the extension and destructive growth parameters were presented (Table 3). Inhere, maximum results for most of the above-and below-ground growth results were obtained for coffee seedlings raised from parchment removed and water soaked seeds. In this case, relatively maximum shoot and root dry weights and relative growth rate were obtained due to the same treatment effect. In addition, most other extension and destructive parameters were also high for seedlings produced from clean seeds and water treated seeds. However, sowing clean coffee seeds resulted in slight reduction of leaf surface area, tap root length, number of lateral roots and leaf water content. The accumulation and partitioning of total dry matter yields into the different seedling parts also varied under the two main treatments and their adaptation under stress environments could vary accordingly. In general, the findings revealed vigorous seedling growth due to the removal of seed coats and water soaking. This corroborates with the notion of many authors (Cambrony, 1992; Coste, 1992; Wrigley, 1988). Taye et al. (2004) also reported the existence of great diversity among wild Arabica coffee populations in morphological and seed characteristics. Further research on seed characteristics and morphogenesis of coffee seedlings remain to be sought to ensure the production of uniform and high quality coffee seedlings for maximum field establishments. Coffee seeds for sowing must have the basic information, among others, on germination rate, purity percent, variability time and other handling practices. Hence, focused attentions should be given to generate these information for the genetically diverse Arabica coffee materials in Ethiopia.

CONCLUSIONS

Removing the hard seed covers and soaking in water showed remarkable effects to improve emergence and growth rate of Arabica coffee seedlings. Above all, careful removal of the parchment immediately before seed sowing was noted to significantly facilitate both the initial seedling emergence and growth performances. It can be, therefore, concluded that depending on the nursery and field calendars, these pre-sowing practices can be scaled-up to similar areas with the views to shorten the nursery time, reduce associated costs and help to produce high quality coffee seedlings. It is, however, imperative to include more levels of the present treatments and consider detail seed and seedling physiological processes for the varying Arabica coffee populations and agro-ecological zones of Ethiopia.

REFERENCES

Cambrony H. R. 1992. Coffee growing. CTA/Macmillian, London.

Coste R. 1992. Coffee: The plant and the product. CTA/Macmillian, London.

IAR (Institute of Agricultural Research). 1996. Recommended Production Technologies for Coffee and Associated Crops. Research Extension Task Force of Jimma Research Centre. Addis Ababa, Ethiopia.

Larcher W. 2003. Physiological Plant Ecology: Ecophysiology and stress physiology of functional groups (4th ed.). Springerr-Verlag Berlin Heidelberg, NewYork.

Salisbury F. B and Ross C.W. 1992. Plant Physiology (4th ed). Wardsworth Learning, Inc., Thomson Asia Pte Ltd., Singapore.

Taye K, Tesfaye S. and Alemseged Y. 2002. Influence of media mixture and watering frequency on seed germination and seedling growth of Arabica coffee in Ethiopia. Proceedings of the 19th International Conference on Coffee Science (ASIC), May 14th-18th, 2001, Trieste, Italy.

Taye K., Burkhardt J. and Goldbach H. 2004. Ecophysiological Variability of Forest Arabica Coffee Populations in Hydraulic Characteristics Along a Climatic Gradient in Ethiopia: Morphological and physiological variability. Proceedings of the 20th International Conference on Coffee Science (ASIC), 11-15 October 2004, Bangalore, India, 929-939.

Wrigley G. 1988. Coffee. Tropical Agricultural Series. Longman and Scientific Technical, John Wiley and Sons, Inc., New York.

Effect of Inorganic and Organic Nutrient Sources on Growth, Dry Matter Yield and P-Uptake of Coffee (*Coffea canephora* L.) Seedlings

R. R. IPINMOROTI[1], L. A. ADEBOWALE[1], M.O. OGUNLADE[1], G. O. IREMIREN[1] AND G. O. ADEOYE[2]

[1]Cocoa Research Institute of Nigeria, PMB 5244, Ibadan, Nigeria
E-mail: ipinmag@yahoo.com
[2]University of Ibadan, Ibadan, Nigeria

SUMMARY

The performance of Coffee (*Coffea canephora* L.) seedlings supplied with organic fertilizer materials as alternative nutrient sources was compared to that supplied with combination of nitrogen, phosphorus and potassium (NPK) and no fertilizer (control) at Ibadan (7° 10^{1N} and 3° 52^{1E}), Nigeria. The organic materials viz: Cocoa pod husk (CPH), kola pod husk (KPH), Coffee husk (CFH), Tea fluff (TFF), Pace-setter grade A (PSA) and Pace-setter grade B (PSB) were significantly better than the control and were either superior or comparable with the NPK for optimal coffee seedling performance for all the growth parameters and dry matter yield (DMY) considered. The organic nutrient sources enhanced P-uptake of between 8.23-36.08% compared with the treatments of NPK and control.

INTRODUCTION

Decline in soil fertility is a major problem contributing to the low yield of crops in most tropical and sub-tropical regions of the world (Gutteridge and Shelton, 1994). Nigerian soils, like other tropical soils, lack adequate plant nutrients and organic matter (Ogunwale et. al., 2002). They could not support sustainable optimal crop production over a long period without fertilizer addition. Most of the coffee farms in Nigeria are established on medium to low fertility status (Obatolu, 1991). Over the years, coffee farmers have been recording low berry yield. The farmers are poor and do not have the resources to make investments to replenish their farm soils. This problem is aggravated by the fact that inorganic fertilizes are beyond the reach of the farmers due to high cost and uncertainty accessibility (Agbede and Kalu, 1995). Coffee seedlings have been reported to respond favourably to organic fertilizer amendment to soils. Green manures, farmyard manures, wastes from processed coffee in form of compost and mulch amongst others, have been reported to be beneficial to coffee production in Nigeria and other parts of the world (Obatolu, 1991; Maisonneuve, 1992). To alleviate the farmers' low productivity therefore, this investigation was conducted to look inward to the use of some nutrient rich farm wastes and locally blended organic fertilizers compared with inorganic fertilizer for the growth of coffee seedlings in Nigeria.

MATERIALS AND METHODS

Thirty-two plastic pots, each filled with 10 kg soil, were planted to 2-month old coffee seedlings of similar sizes. Inorganic fertilizers – NPK (15:15:15); farm wastes – kola husk, coffee husk, cocoa husk, tea fluff; locally blended organic fertilizers – pacesetter grades A and B, and the control (no fertilizer) were imposed as treatments. The fertilizers were applied to supply 28 gm N $plant^{-1}$ $year^{1}$. All treatments were in 4 replicates and set up in the

greenhouse under completely randomized design (CRD). The pot contents were watered twice weekly to 70% field capacity. Growth parameters – plant height, girth, leaves, branches and leaf area were monitored for 12 months. Plants were uprooted, washed, oven dried and dry matter yield taken. The harvested plants were sectioned into leaf, stem and root, milled and P contents determined colorimetrically using Vanado – Molybdate method. Results were statistically analyzed and mean differences were separated using LSD at $P < 0.05$.

RESULTS AND DISCUSSION

The soil is low in the N, P, K and Mg levels (Table 1) compared with the critical levels of 0.9 gkg^{-1}N, 6.0 $mgkg^{-1}$P, 0.4 and 0.8 $cmolkg^{-1}$ K and Mg, calculated for soils suitable for coffee in Nigeria (Egbe et al., 1989). This clearly suggests the need for fertilizer addition to auguement for the native soil fertility for better coffee performance. The low soil nutrient content indicated why most coffee plots in Nigeria are performing below expectation in berry yield over the past few decades (Obatolu, 1991). The organic fertilizer materials contain plant nutrients in varying amount. This suggests differences in the quantity of the organic materials that would be needed to supply equal amount of Nha^{-1}. While the organic materials contain Ca and Mg in addition to N, P and K, the inorganic fertilizer is deficient in this.

Table 1. Some nutrient contents of fertilizers used.

Properties	Soil	Cocoa husk	Kola husk	Coffee husk	Tea fluff	Pacesetter grade A	Pacesetter grade B	NPK (15:15:15)
N (%)	0.12	0.95	2.73	1.75	2.90	2.58	1.46	15
P $mgkg^{-1}$	4.60	0.15	0.88	0.14	0.30	1.1	1.03	15
K $cmolkg^{-1}$	0.63	3.96	2.58	3.74	2.155	0.68	0.60	15
Ca $cmolkg^{-1}$	1.60	0.77	3.61	2.11	1.53	0.36	0.36	–
Mg $cmolkg^{-1}$	0.36	0.33	0.50	0.41	0.62	0.11	0.11	–

Fertilizer treatments were significantly superior to the control in all the coffee seedlings growth parameters and dry matter yield (DMY). The organic fertilizers were either superior or comparable with the NPK (reference fertilizer) in these parameters (Table 2). The kola and coffee husks resulted to highest plant height values, while coffee husk was superior to other fertilizers in the resultant plant girth values. The NPK resulted to more number of branches by the coffee plants compared with organic fertilizers. Plant branches that resulted from NPK treatment were however shorter in length compared with those due to organic fertilizers. Since the branches are the points of berry production (Maisonneuve, 1992), the organic fertilizer treated coffee plants with longer branch lengths, would result to more points of berry production than those treated with NPK. The kola husk and NPK treatments resulted to the highest number of leaves. These values were however not significantly different from those due tea fluff, pacesetter grades A and B treatments. The number of leaves produced was on the other hand not in concomitant with the leaf area $plant^{-1}$. Coffee husk with the least number of leaves resulted to the highest leaf area of 2847.2 cm^2. The leaf area values due to the fertilizer treatments were more than twice greater than values obtained for control plant.

The mean DMY differences amongst the fertilizer types were not significant over one another. However, the values indicated that kola husk, tea fluff and coffee husk were superior to the NPK, while cocoa husk, pacesetter grades A and B values were similar and comparable with the NPK value. The organic fertilizers P up-take values were between 8.26-25.53% and 17.56-36.28% better than the NPK and the control treatments respectively. However, the NPK value was significantly better than the control. The P-uptake of 39.3-53.6 mg P (90-120 mg P_20_5) $plant^{-1}$ were similar in values to value reported by Maisonneuve (1992). Therefore,

under high cost and scarcity of inorganic fertilizers as is the situation in Nigeria presently, the organic fertilizer materials could be used to replace NPK as alternative sources of nutrients for better coffee plant performance.

Table 2. Effect of fertilizer treatments on growth parameters, DMY and P up-take.

Treatment	Height (cm)	Girth (cm)	Leaves	Branches	Leaf Area (cm)	DMY (g/plant)	P up-take g/plant
Cocoa husk	60.5	0.98	39.0	4.50	2348.3	32.3	45.2
Kola husk	72.3	1.00	45.8	5.30	2530.7	39.9	53.6
Coffee husk	71.3	1.08	30.8	4.00	2847.2	36.1	51.8
Tea fluff	57.8	1.00	41.0	5.30	2649.4	37.5	50.6
Pace-setter grade A	65.7	1.00	40.3	4.30	2480.6	33.8	46.9
Pace-setter grade B	59.9	1.03	40.5	5.50	2382.7	33.8	51.3
NPK	64.0	1.03	45.5	5.80	2601.1	35.3	42.7
Control	54.0	0.93	30.5	4.50	1150.0	22.5	39.3
LSD (0.05)	5.71	0.05	6.25	NS	253.0	10.1	3.0
CV (%)	18.4	14.4	22.4	38.4	45.9	30.3	12.1

LSD = Least significant difference, CV = Coefficient of variation.

ACKNOWLEDGEMENT

The authors are grateful to the Director/Chief Executive of Cocoa Research Institute of Nigeria for permission to publish this work.

REFERENCES

Agbede, OO. and Kalu, BA. 1995. Nigeria Journal of Soil Science. Vol.11: 139-152.

Egbe, NE, Ayodele, EA and Obatolu, CR. 1989. Progress in Tree Crop Research: Pp28-38.

Gutteridge,RC and Shelton, AM. 1994. The role of forage tree legumes in cropping and grazing systems. In Forage Tree Legumes in Tropical Agriculture. CAB International, UK. Eds. R.C. Gutteridge and A.M. Shelton. Pp 125-133.

Maisonneuve, EL.1992. Coffee: The plant and the product. Macmillan, London. Ed. C.Rene. Pp 60-119.

Obatolu, CR. 1991. Ph.D Thesis, University of Ibadan, Nigeria. Pp276.

Ogunwale, JA, Olaniyan, JO and Aduloju, MO. 2002. Moor Journal of Agric. Research. Vol.34 (2): 147-154

Influence of an Albizzia Shade Tree on Soil Chemical Properties, Coffee Plant Growth and Yields in Kenya

J. K. KIMEMIA

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

SUMMARY

Shade and shade trees play a crucial role in the management of coffee in the tropics. Shade trees do moderate soil and temperatures while the litterfall acts as mulch besides providing nutrients on decomposition. Where trees of the *Legumisae* family are used, the nitrogen contribution is significant. A trial was carried out in Ruiru, Kenya in plot of Arabica coffee SL28, with a mature *Albizzia schimperiana* shade tree. Soil chemical characteristics and coffee yields were recorded in a 30m transect from the base of the shade trees in 2004-2005.

INTRODUCTION

Coffee, which is a major cash crop in Kenya, is grown by both small-scale farmers (55%) and large-scale farmers (45%). The original coffee plantings were done under shade from various trees mainly *Grevellea robusta, Albizzia spp,* Cordia *abysinica* among others (Njoroge and Kimemia, 1993). The trees could be either planted or derived from natural regeneration (Rao et al., 1998). Roskoski et al. (1982) found that when Inga jinicul was planted as shade tree in a coffee plantation it added over 40 kg N/ha that was 53% of the average amount of nitrogen applied annually. Krishnamurthy (1989) reported that shade trees contributed about 100 kg nitrogen, 35 kg phosphorous and about 45 kg potassium per ha per year. Nitrogen fixation in situ and leaf fall are the two important processes through which nitrogen and other nutrients are added to the soil (Venikateswarlu et al., 1990). The addition of the organic matter besides providing nutrients improves the soil structure and water holding capacity. This in turn improves the coffee tree growth and yields. In the absence of natural shade system, it would be both difficult and uneconomical to provide the same amounts of organic matter through use of manures as through the use of natural shade (Raghuramulu, 2005).

MATERIALS AND METHODS

The trial was sited at Coffee Research Station Ruiru (1 06'S, 36 45'E, 1620 m above sea level and an average rainfall of 1100 mm, most of it falling between April and September). The soils at the site are humic nitosols.

Arabica coffee cultivar SL28 planted in 1989 was used. A mature *Albizzia schimperiana* shade of about 30 meters tall and a canopy spread of 15 meters was used (Figure 1). Soil samples were taken at 0-15 cm and 15-30 cm depths and analyzed for nitrogen, calcium, phosphorus, potassium and manganese.

Data on coffee tree height, diameter, primary branch length (fourth primary from the tree top), number of primaries, and number of nodes on the fourth primary and leaf area (second opened leaf on the fourth primary from the top) were recorded at the beginning of the trial and one year later. Ripe coffee was picked from two trees at 2.5 m intervals away from the shade

tree, weighed and processed after every picking. The trial was laid in a completely randomized block with three replicates.

RESULTS

The shade tree did not significantly influence the soil pH which was within the recommended levels for coffee. Potassium declined significantly away from the shade tree with high Potassium levels being recorded below the tree crown (Table 1). Potassium and calcium declined by 27.5and 40% from the shade tree to the open area respectively (Table 1).

Table 1. Effect of *Albizia* shade tree on top soil chemical properties.

Distance from shade tree(m)	pH	K (m.e%)	Ca (m.e%)	Mn (m.e%)	P (ppm)
2.5	5.5abcd	1.60a	1.34a	1.51a	68.0a
5.0	5.0abcd	1.59a	1.21ab	1.36ab	61.0ab
7.5	4.88cd	1.32ab	1.04bc	1.10ab	22.0c
10.0	4.80d	1.10b	0.96bc	1.26ab	23.0c
12.5	4.90bcd	1.07b	0.94bc	1.28ab	26.5c
15.0	5.07abcd	1.24ab	0.94bc	1.22ab	24.0c
17.5	5.10abcd	1.21ab	0.83c	1.17ab	19.5c
20	5.13abcd	1.19ab	0.86c	1.12ab	17.5c
22.5	5.27ab	1.26ab	0.98bc	1.12ab	16.5c
25	5.25abc	1.12b	0.94bc	1.15ab	19.0c
27.5	5.25abc	1.19ab	1.11abc	1.10ab	30.5bc
30	5.25abc	1.20ab	1.21ab	1.04b	27.0c
32.5	5.30a	1.16b	1.09abc	1.00b	23.5c
Mean	**5.1**	**1.25**	**1.08**	**1.19**	**29.1**

Means followed by the same letter down the column are not statistically significant according to Turkey's Honestly Significant Difference $P \geq 0.5$.

There were significantly high levels of manganese below the tree crown (Table 1). At a distance of 32m, the Manganese levels declines by 34%. Phosphorous declined significantly from 68 ppm at 2.5m to 23.5 ppm at 32m, a decline of 65%.

The shade tree did not affect significantly the coffee tree height and diameter which averaged 67.85 cm/yr and 1.32cm/yr respectively. However, the trees at the 2.5m had significantly higher primary extension than those at 32m away (Table 2). The shade tree significantly reduced the number of bearing primary branches per tree (Table 2). The coffee tree yield increased by 236% from the base of the shade tree towards the open area (Table 2).

DISCUSSION

The level of nutrients was observed to be high under the tree canopy as compared to the open area. Rao et al. (1998) reported improved soil fertility under the tree crown. The increase can be as high as 117% organic N (Depommier et al., 1992). Generally there was fertility gradient with fertility decreasing from the tree base the edge of its crown and beyond. The fertility improvement was on the topsoil (0-15cm). The improvement in soil fertility could be attributed to biological nitrogen fixation, in case of leguminous trees like Albizzia. Nutrients are taken from the subsoil by the deep roots and accumulated on the surface through litter fall (Buresh and Tian, 1997). The area under the tree crown had the highest concentration. The extensive lateral root spread can also take nutrients from the surrounding area and eventually

concentrate them under the crown (Rao et al., 1998). A large number of birds were observed to be nesting on the tree. The excreta could also contribute to higher nutrient levels under the tree (Tejwan, 1994).

Table 2. Effect of *Albizia* shade tree on coffee plant growth parameters.

Distance from shade tree (m)	Tree height cm/yr	Coffee tree diameter growth cm/yr	No. of bearing primary branches	Primary branch extension cm/yr	Coffee cherry yield Kg/tree
2.5	69.5 ab	1.24 b	25 cd	68.6 a	1.9 a
5.0	71.38 ab	1.34 b	37 bcd	54.99 b	1.38 a
7.5	67.72 ab	1.08 b	23 d	49.22 bc	1.27 a
10.0	67.38 ab	1.33 ab	34 ab	49.80 bc	4.20 a
12.5	73.68 ab	1.25 b	23 d	41.08 cd	4.68 a
15.0	74.50 ab	1.05 b	35 ab	42.3 cd	4.07 a
17.5	58.08 b	1.18 b	29 abcd	39.62 cd	3.65 a
20.0	77.50 a	1.64 ab	36 a	42.33 cd	7.47 a
22.5	68.88 ab	1.30 b	30 abcd	45.85 bcd	6.90 a
25.0	65.13 ab	1.28 b	36 a	44.99 bcd	6.22 a
27.5	62.38 ab	1.22 b	36 a	43.15 bcd	5.43 a
30.	63.25 ab	1.91 a	36 a	46.5 bcd	6.00 a
32.5	62.75 ab	1.31 b	37 a	34.47 d	6.63 a
Mean	**67.85**	**1.32**	**32**	**46.23**	**4.52**

Means followed by the same letter down the column are not statistically significant according to Turkey's Honestly Significant Difference P ≥ 0.5.

In this study a high accumulation of phosphorus was observed under the tree crown. This phosphorus accumulation could have been aided by symbiotic association with endomycorrhizae (Tomlinson et al., 1995). The coffee cherry yield was reduced drastically under the tree crown. The increased soil fertility did not result in increased coffee yields. Crop yield declines, relative to yields in open, treeless fields have been noted under canopies of large, evergreen and unmanaged trees (Rao et al., 1989). Competition for light is the greatest contributor to the reduction in yields (Kessler, 1992). There is also bound to be competition for water and nutrients. There could also have had allelopathic effects as allelochemicals are present in almost all tree parts (Rice, 1984). The reduction in coffee yields reduces the advantages of the shade trees in coffee. However as there is growing market for shade coffee, it is crucial to retain the shade trees but manage the competition through tree pruning. The belowground competition is however limited to tree choice; those with deep and compact root system and also devoid of allelopathic effects. More study on the effect of scattered shade trees on coffee is recommended.

ACKNOWLEDGEMENTS

The assistance of Agronomy staff is hereby acknowledged. The paper is published with kind permission of Director of Research, Coffee Research Foundation.

REFERENCES

Buresh R J and Tian G. (1997). Soil improvement by trees in sub-Sahara Africa. *Agroforestry Systems* 38: 51-76.

Depommier D, Janodet E and Oliver R 1992. *Faidherbia albida* parks and their influence on soils and crops at Watinoma, Burkina Faso. In: Vandenbeldt R J (ed) *Faidherbia albida* In West African Semi Arid Tropics, pp111-116 ICRISAT Patencheru A.P. India

Kessler J J. 1992. The influence of karate (*Vitellaria paradoxa*) and nere (*Parkia biglobossa*) trees on sorghum production in Burkina Faso. *Agroforestry Systems* 17: 97-118.

Krishnamurthy R.W. 1989. Nutrition management in plantation crops. *J Plantation Crops* 6:403-411

Njoroge J. M. and J. K. Kimemia (1993). Current intercropping observations and future trends in Arabica coffee Kenya. *Outlook in Agriculture* 22: 43 - 48.

Raghuramulu Y. 2005. Significance of shade for sustainable coffee production in India. *Indian Coffee* LXIX No. 9 :21-24

Rao M R., P K R Nair and C K Ong. 1998. Biophysical interactions in tropical agroforestry systems. *Agroforestry Systems* 38: 3-50.

Rice, E L. 1984 Allelopathy. *Academic* Press, New York, USA

Roskoski, J.P., Montano, C. Van Kessel and G. Castillega. 1982. Nitrogen fixation by tropical woody legumes: potential source of soil enrichment. *In Graham P.M. and Haris, S.C. (eds). Biological nitrogen fixation technology for tropical agriculture*. CIAT, Cali, Columbia pp 447-454.

Tejwan K.G 1994. Agroforestry in India. Oxford &IBH Publishing Co Pvt Ltd, New Delhi India

Tomlison H., Teklehaimont Z, Traore A and Olapade E 1995. Soil amelioration and root symbioses of *Parkia biglobossa* (Jacq.) Benth in West Africa. *Agroforestry Systems* 30: 145-159.

Veniktaswarlu, B., Korwar G.R and R.P. Singh 1990. Studies on nitrogen fixation and nutrient addition by *Leucaena leucocephala* in a semi arid alfisol. *Leucaena Research Reports* 11:65-67.

Evaluation of Berry Yield from Clonal and Seedlings Materials of *Coffea canephora* Pierre ex Froehner in Different Geometry and Clonal Combinations

A.O. FAMAYE, A. A. OLOYEDE AND K. AYEGBOYIN

Cocoa Research Institute of Nigeria, P.M.B. 5244, Ibadan, Oyo State, Nigeria – Tunmos. E-mail: 2010@yahoo.com

SUMMARY

Field experiment was conducted in 1999 at Cocoa Research Institute of Nigeria CRIN Headquarters Ibadan to evaluate the berry yield of coffee clones and seedling materials of different combinations and geometry. There were six treatments viz: (i) all seedlings (control) (ii) triangular arrangement of seedlings and cuttings, (iii) all cuttings of different clones, (iv) alternate rows of cuttings and seedlings, (v) alternate stand arrangement of cuttings and seedlings and (vi) half cuttings and seedlings arrangement of quillou and Java varieties. There were thirty stands per treatment planted at 3.1m apart with land area of 192m^2 per treatment. The plots were laid out in a randomized complete block design with three replicates. Result obtained from fresh berry weight showed that treatment (vi) half cutting and half seedlings arrangement was of the least berry yield (530kg/ha) yield (1487.2 and 1310.4 kg/ha wet and dry berry yield respectively) cutting followed by alternative rows of cutting and seedlings treatment (iv) The same trend was followed by the dry coffee berry in all the treatments Treatment with mixture of clones (iii) in the study further underscores the need for combinations of several clones of *Coffea canephora* for optimal yield.
Key word: Coffee, clones, cutting combination, geometry, yield

INTRODUCTION

Coffee cultivation is receiving attention in Nigeria because of the current administration effort in cash crop production. However, Nigerian coffee farmers are oblivious of husbandry practices such as the required spacing/density, geometry etc. *Coffea canephora* Pierre ex Froehner is known to be self-incompatible (Opeke, 2005). Observation on farmers' farms in Nigeria shows very low yield as a result of planting seedlings of the same genetic constitution. (Coste, 1992) has reported combination of different clones of *Coffea canephora* to improved yield. (Pochet and Flemal, 2001) also corroborated this fact.The objective of the study is to increase yield by exploiting different geometry, clonal and seedling combination.

MATERIAL AND METHODS

This study was carried out 1999 at the Headquarters of Cocoa Research Institute of Nigeria (CRIN) Ibadan located at Latitude 7°10°N, Longitude 3°52°E and altitude of about 122 m above sea level. Varieties of coffee canephora use were obtained from seed garden of cocoa Research institute of Nigeria where the cuttings and the berries as planting materials were obtained. The one-node cuttings were prepared, raised in the Central nursery of the Institute. Seedlings were similarly raised in the nursery. Plantain was used as shade crop planted at a spacing in between two stands of coffee. The transplanting was done between May and June 1999.The experimental Design was Randomized Complete block (RCBD) Six treatments replicated three times. The Treatments are: All seedlings (control),triangular arrangement of

seedling and cuttings, all cuttings of mixed clones, alternative rows of cuttings and seedlings, alternative stands arrangement of cutting and seedling stands Half cutting and seedling arrangement.

S S S S S	C C C C C	C C C C C	C C C C C	C S C S C	C C C C C
S S S S S	C C C C S	C C C C C	S S S S S	S C S C S	C C C C C
S S S S S	C C C S S	C C C C C	C C C C C	C S C S C	C C C C C
S S S S S	C C S S S	C C C C C	S S S S S	S C S C S	S S S S S
S S S S S	C S S S S	C C C C C	C C C C C	C S C S C	S S S S S
S S S S S	S S S S S	C C C C C	S S S S S	S C S C S	S S S S S
(i)	(ii)	(iii)	(iv)	(v)	(vi)

Legend: S = coffee seedling of quilluo and Java varieties; C = cuttings of quilluo and Java varieties.

Figure 1. shows the field layout of the trial.

RESULT AND DISCUSSION

The result obtained is as shown in Table 1. Analysis of variance revealed that significant difference existed between the treatments. The order of performance is as shown below 1487.2 > 1414.4 > 1071.2 > 873.6 > 811.2 > 530.0 kg/ha green bean yield value. Yield of 1487.2 and 1414.4 kglha obtained in treatment (iii) and (iv) underscores the suitability of the geometry explored at increasing yield as against the average yield of 700 kg/ha on farmers farm (Omolaja, 2003). A yield of between 1000-2000 kg/ha has been reported to be good by (Pochet and Flemal, 2001).

Table 1. Coffee Mean berry yield (kg/ha) within the various treatments.

Treatment	Wet weight	Dry weight
I	873.6	738.4
II	1071.2	811.2
III	1487.2	1310.4
IV	1414.4	1289.6
V	811.2	644.8
VI	530.4	416.0
Mean	1031.3	868.4
LSD (5%)	368.5	359.6

The dry weight followed similar trend, the result obtained in this study buttressed the need to have as many clones together to enhance higher yield in a well-defined geometry and combination/s.

ACKNOWLEDGEMENT

The authors acknowledge the Executive Director (CRIN), for the permission to publish this paper.

REFERENCES

Coste R. (1992): Coffee. The plant and the products. Macmillan published. 328pp.

Omolaja, S.S. (2003): Characterization of Nigerian Robusta coffee (*Coffea canephora* Pierre ex.Froehner) Germplasm and determination of factors controlling Compatibility.Ph.D. Thesis.202 pp

Opeke, L.K. (2005): Tropical Commodity Tree Crops. Spectrum Published. 503 pp

Pochet, P and Flemal, J (2001): Coffee (beverage and stimulant crops. In: Crop Production in Tropical African Romaine (H.Raemaekers Ed). pp921-959.

Effectof Organic Fertilizers on Nutrient Uptake *Coffea canephora* Seedlings

M. A. DANIEL[1] AND A. O. OBI[2]

[1]Cocoa Research Institute of Nigeria P.M.B.5244, Ibadan
[2]Obafemi Awolowo University, Ile-Ife, Nigeria

SUMMARY

The effects of three organic fertilizers *Chromoleana odorata*, cowdung and *Pennisetum purpureum* were evaluated on the nutrient uptake of coffee *canephora* seedlings in the greenhouse. The organic fertilizers were applied at 0, 5 and 10 tha^{-1} to coffee seedlings. The treatments were arranged in a complete randomized design replicated four times. The results showed that, *C odorata* treated seedlings showed higher nutrient uptake than cowdung and *P. purpureum* at 10 tha^{-1} ($P \leq 0.05$). Combination of *C. odorata* with either of the two organic fertilizers increased nutrient Uptake of Coffee seedlings and the soil chemical properties. Therefore, 10 tha^{-1} of *C. odorata* and cowdung mixture was found to be better for coffee seedling nutrient uptake.
***Key word**: C. odorata*, *p. purpureum*, cowdung, *coffea* seedlings, nutrient uptake

INTRODUCTION

Coffee is one of the most important agricultural produce traded worldwide. Second largest legally traded commodity in the world market (Melissa, 2002). This is why Sara Lee (2003) noted the essence of enhancing its productivity since this would be economically rewarding to the producing countries. Coffee production in Nigeria and else where in the world is mainly a cash crop that is in the hands of low-income small-scale farmers. Coffee is known to be a high nutrient-demanding crop. To ensure sustainability of production, there is need to enhance its potentials by improving the fertility status of the soil (Obatolu, 1990). The high cost and the scarcity of inorganic fertilizers calls for other readily available, cheaper and environmentally friendly alternative ways. Evolution of ways to increase production of "Organic Coffee" through the use of sustainable farming techniques, using organic manure and wastes as organic fertilizers had always been considered a better option (F.A.O, 1984). This study was set out to evaluate growth response of *Coffea canephora* seedlings in the greenhouse. Field trials are suggested.

MATERIALS AND METHOD

The greenhouse study was carried at Cocoa Research Institute of Nigeria, Ibadan. (7°10′ N, 3°52′ E) at an altitude of 122 m, classified as Alfisol Oba series. The climate in Ibadan is characterized as tropical with dry season as from early November to March while the raining season runs from the end of March to November. The total annual rainfall of about 1100 mm with two peaks in June and September. The temperature ranges between 20 °C and 28 °C for the year, relative humidity ranges between 50% and 80% throughout the year. Three organic materials used for this study were *Chromolaena odorata* (Siam weed), *Pennisetum purpureum* (elephant or napier grass) and Cowdung were collected fresh, sun – dried and milled. Bulk soil sample was collected at a depth of 0-30 cm air-dried, crushed to pass through a 2 mm sieve and routine analysis carried out. Ripe coffee berries were harvested

from mature *Coffea canephora* tree (Quillo variety) at the Research Plot during the 2004/2005 season, de-pulped and pre-nursed. Five kilogrammes of air-dried, sieved soil were weighed into each of 5-litre (PVC) plastic pots having five drainage holes at the bottom. Three rates, namely, 0, 5 and 10 tha^{-1} each of the organic materials corresponding to 0, 11.16 and 22.32 g per 5 kg soil were applied in various combinations. Twenty-seven treatments were obtained from a factorial combination of the organic fertilizers and replicated four times. Bare root method of transplanting was used. Growth parameters were measured every two weeks; plant heights, leaf area, number of leave and stem diameter. Total sampling of the seedlings was done at the end of the study. The seedling in each pot was uprooted, washed and separated into shoot and root with a secatuer. The harvested plant materials were weighed and chopped to 5 cm size and oven-dried at 68 °C for 48 hrs. The oven-dried plant materials were milled using stainless steel hammer mill and analyzed.

RESULTS AND DISCUSSION.

Application of each of the organic fertilizers and their combinations at 5 tha^{-1} improved the nutrient uptake of coffee seedlings (Table 1). There were significant increases in the uptake of N, P, K, Ca and Mg by coffee seedlings in all the treatments applied. The highest P uptake was obtained where all the three organic fertilizers were combined at the first rate of 5 tha^{-1} of application. Potassium uptake by coffee seedling was also enhanced by the addition of the organic fertilizers. Uptake of K was highest where cowdung alone was added and this may not be unrelated to the K content of cowdung which was the highest amongst the organic materials.

Uptake of Ca by coffee seedlings was significantly influenced by the addition of the organic fertilizers both when added singly or in combination with each other. The highest uptake of Ca was obtained in pots treated with the combination of cowdung and *Chromolaena odorata*. This was significantly higher than Ca uptake in all the other treatments. The organic fertilizers either added singly or in combination with one another significantly enhanced magnesium uptake by coffee seedlings. The highest uptake of Mg was obtained where cowdung alone was added while the least was where the three organic fertilizers were combined. Generally, the Magnesium uptake by the plant was the least of all the nutrients considered. This may be connected with the fact that Mg is required in lower quantities by plants than the other nutrients under consideration this might been due to the fact that Mg is readily translocated and also involved in the activation of many enzymes.

The addition of the organic fertilizers two-fold (10 tha^{-1}) did not double the nutrients uptake of the seedlings but considerably increased the N, P and K uptake. (Table 1). The effect did not show any consistent pattern within the columns of each of the elements considered. Uptake of N at this rate (10 tha^{-1}) was significantly improved by the addition of organic fertilizers. The highest N uptake of 82.55 mg that was six times that of the control was obtained where the three organic fertilizers were added. Uptake of P was significantly improved by the addition of each and various combinations of the three organic fertilizers. A value of 48.82 mg, representing the highest uptake of P obtained from the combination of cowdung with *Pennisetum purpureum* and the addition of the three organic fertilizers. The organic fertilizers in all their combinations significantly improved the uptakes of the (K, Ca & Mg). The uptake of Mg was the least among the bases as was also obtained at the first rate (5 tha^{-1}) of addition.

Table 1. Effect of each and different combinations of three organic fertilizers on nutrient uptake of coffee seedlings (mg pots^{-1}).

Treatments	N	P	K (mg)	Ca	Mg
C_0D_0 P_0	16.66d	30.32c	25.53e	27.04c	8.81c
C_1	79.51b	38.08a	36.27d	46.64b	16.77b
D_1	68.87c	43.83a	57.74a	42.98b	19.27a
P_1	90.75a	47.02a	49.96b	53.54a	16.84b
C_1 D_1	99.05a	41.24b	40.09c	58.48a	16.65b
C_1 P_1	62.90cd	42.53ab	39.13c	42.72b	17.24a
D_1 P_1	80.97b	39.01b	42.21c	53.52a	16.41b
C_1 D_1 P_1	53.49d	38.08a	44.82bc	44.88b	15.19b
C_2	66.70c	43.83a	61.64b	46.64b	17.96b
D_2	77.18b	47.02a	44.03d	42.98b	18.74ab
P_2	69.26c	41.24b	63.33b	53.54a	20.06a
C_2 D_2	70.55b	42.53ab	99.85a	58.48a	15.26c
C_2 P_2	73.66b	39.01b	35.04e	42.72b	13.62d
D_2 P_2	71.56b	48.82a	54.83c	53.52a	23.08a
C_2 D_2 P_2	82.55a	48.82a	30.42f	44.88b	11.41d

*C= Chromoleana odorata; D = Cowdung; P = Pennsetum purpureum. 0 = Control; 1 = 5 tha^{-1}; 2 = 10 tha^{-1}. *Mean within the same column, followed with same letters are not significantly different from each other at 5% level of significant (New Duncan's Multiple Range Test).*

CONCLUSION

A significant correlation was observed between the soil N and content of the coffee seedlings. But for P, all the nutrients considered showed significant correlation. The study shows that, both nutrient of the soil and uptake of coffee seedlings were significantly enhanced with the addition of each of the three organic fertilizers applied either singly or in combination with one another at the various rate but more at 10 tha^{-1}. Nutrient uptake by coffee seedlings and soil nutrient contents were significantly improved by the addition of the three organic fertilizers

ACKNOWLEDGEMENT

The authors are grateful to the Director of CRIN for the permission to publish the paper.

REFERENCES

Mellissa, J. (2002): Coffee, a rellaying point for fair trade. In: Coffee, the Environment and Labour.National Catholic Reporter, Dec. 27, 2002.

Obatolu, C.R and Agboola,A.A (1990): Evaluation of different sources of organic materials for their Manurial properties. Soil Science Society of Nigeria. 13p In: Soil Organic Matter Dynamics and Sustainability of Tropical Agriculture. (Ed.)

Sara-Lee,(2003): Sustainable coffee production Through sustainable farming techniques in poor countries. The Environment And Conservation. 116p.

Soil Survey Staff (1976): Soil Taxonomy: A Basic System for classification and making interpreting of soil surveys. USDA Soil Conservation Services. Washington D.C.

Effect of Amended Growth Media on the Production of *Coffea canephora* Seedlings in the Nursery

E. A. ADEYEMI AND M. A. DANIEL

Cocoa Research Institute of Nigeria. P.M.B5244, Ibadan, Nigeria

SUMMARY

Five growing media namely; topsoil sawdust (cured) topsoil + sawdust (1:1 ratio), topsoil + sawdust + N.P.K at two levels: 60:30:30 and 30:15:15 kg/ha were used in raising pre-germinated two-leaf stage coffee seedlings in the nursery. The assay of the growth media was taken prior to the experimentation. Treatments were arranged in randomized complete block design in four replicates. Agronomic data were taken at two months interval for six months on plant height, number of leaves, leaf area and stem diameter. Data obtained were analyzed using analysis of variance (ANOVA) and means separated with Duncan's Multiple Range Test (DMRT). Results indicated that, topsoil was not significantly different ($P \leq 0.05$) from topsoil + sawdust (1:1 ratio) and topsoil + sawdust + N.P.K 60: 30:30: kg/ha in all growth parameters measured. Least performance was observed in 100% sawdust, which was significantly different from other treatments. Topsoil + sawdust (1:1 ratio) could therefore be preferred in the raising of coffee seedlings thereby reducing the amount of topsoil that will be excavated from the field annually.

INTRODUCTION

Coffee is one of the most important Agricultural produce traded worldwide and a relying commodity for trade fair in the international market. Coffee is the largest legally traded commodity in the world market next to mineral oil (Mellisa, 2002), Coffee production in Nigeria dates back to the early 1950's (Daniel, 2005). Two main varieties cultivated in Nigeria are *Coffea arabica* and *Coffea canephora.* The raising of coffee seedlings with a lot of top soil to fill polythene pots has always pose problems in terms of costs, bulkiness and continual scrapping of the top soil for such purpose has rendered some lands marginal in soil fertility level. Sawdust is a waste product in the sawmill industries with but high potential for agricultural purpose if properly cured will improve the soil fertility status (Warrall, 1978). The paper evaluated the suitability of mixing sawdust with topsoil and amended with N.P.K. 15:15:15 as an alternative to the use of sole topsoil in raising coffee seedlings in the nursery.

MATERIALS AND METHODS

The nursery study was conducted at Ibadan (7^0N$3^0$52'E) in the year 2004/2005. The soil used was sandy clay loam and has been classified as Oba series (Smyth and Montgomery, 1962). Two materials (Topsoil and sawdust) were used singly and in equal ratio combination without and with N.P.K.15: 15:15 addition at two levels. The sawdust was cured for six months before use. The five growth media investigated were topsoil (TS), sawdust (SD) combination of the topsoil and sawdust in 1:1 ratio (TSSD) and addition of N.P.K fertilizer at 60:30:30 (TSSD1) and 30:15:15 (TSSD2) kg/ha to the mixture. All the growth media were analyzed for their nutrient content prior to use. Two leaf stage of *C. canephora* seedling, that were pre-germinated in conventional sand medium were transplanted bare rooted into two-kilogram of each of the growth media in polythene pot. N.P.K 60:30:30 and 30:15:15 at 108 g and 54 g respectively were dissolved each in 40 mls of water and applied in two splits at two and six

weeks after transplanting. Agronomic data on plant height, number of leaves, leaf area and stem diameter was taken bi-monthly from two to six months. Data obtained were analyzed using analysis of variance (ANOVA) and means were separated with Duncan's Multiple Range Test (DMRT).

Table 1. Assay of growth media.

Media	PH (H_2O)	Total. N (g/kg)	Avail.P mg/kg	Exch. K Cmol/kg	Ca Cmol/kg	Mg Cmol/kg	Org. C (g/kg)
TS	6.20	12.30	72.00	0.36	0.36	0.36	10.80
SD	-	1.00	0.06	0.03	0.02	0.04	46.00

Table 2. Effect of growth media on *coffea canephora* seedlings.

Growth media	Plant Heght (cm)	No of leaf	Leaf Area (cm)	Stem Diameter (mm)
2 MAP				
TS	7.25ab	5.25a	4.08b	0.25b
SD	7.13b	5.50a	3.10b	0.30ab
TSSD	7.48ab	5.25a	3.28b	0.33a
TSSD1	7.93a	6.00a	9.10a	0.30ab
TSSD2	7.63ab	6.25a	4.10b	0.25b
4 MAP				
TS	8.80a	7.50a	3.70a	0.43a
SD	8.53a	6.75a	6.40a	0.28a
TSSD	10.0a	7.50a	3.68a	0.30a
TSSD1	8.85a	6.50a	5.55a	0.28a
TSSD2	8.35a	6.50a	6.28a	0.25a
6 MAP				
TS	9.83ba	12.50a	14.15a	2.95ba
SD	9.10b	12.25a	14.75a	2.55c
TSSD	10.58a	13.00a	13.03	3.03a
TSSD1	10.15ab	13.00a	15.63a	2.50a
TSSD2	9.13b	12.25a	14.75a	2.75bc

Means within the same column, followed with same letters are not significantly different from each other at 5% level of significant (Duncan's Multiple Range Test). TS = Top soil; SD = Sawdust.

RESULTS AND DISCUSSIONS

At two months after transplanting (MAT), the combination of topsoil, sawdust and N.P.K fertilizer at 60.30:30 kg/ha (TSSD1) produced coffee seedling with highest plant height value of 7.93cm, which was only significantly different ($P \leq 0.05$) from pure sawdust medium (Table 2). Leaf area of seedling raised in TSSD1 was significantly different from all other treatments. The better performance in plant height and leaf area could be an adhered advantage to the seedling in the production and assimilation of photosynthate materials that could result in a vigorous seedling. The mixture of topsoil and sawdust (TSSD) medium produced seedling with stem diameter 0.33 mm, which is significantly different from topsoil (TS), and the combination of topsoil, sawdust and NPK fertilizer at 30:15:15 (TSSD2). Seedling performance at 4 MAT was not significantly different in all the growth media investigated. The TSSD resulted to the highest plant height (10.0 cm) and least in TSSD2

(8.35 cm).The number of leaf was similarly highest due to TSSD and TS media (7.50).The leaf area was highest in SD media (6.40 cm^2) and least in TSSD (3.68 cm^2) while stem diameter value was highest in TS (0.43 mm) and least in $TSSD_2$ (0.25 mm). The value were however not significantly different. Six months after transplanting TSSD medium produced seedling of highest value (10.58) in plant height and stem diameter (3.03 mm) which was significantly different ($P \leq 0.05$) from seedling raised in SD and TSSD2 growth media (Table 2) but compared favorably with the conventional topsoil medium for coffee seedling production. This finding agrees with earlier finding of Adeyemi (2000) who reported that Coffea seedling raised in a combination of topsoil and sawdust in 1:1 ration was not significantly different from seedling raised in topsoil medium. The poor performance of coffea seedling in pure sawdust medium could be as a result of the wide C:N ratio of 1:46 which must have immobilized the N thus impeded the growth of the seedling (Brady, 1999) (Table 1) (Brady, 1999).

CONCLUSION

The mixture of topsoil + sawdust (cured) at the ratio of 1:1 with out fertilizer amendment could be recommended instead of topsoil in raising *Coffea canephora* seedling in Nigeria.

ACKNOWLEDGEMENT

The authors are grateful to the Executive Director of Cocoa Research Institute of Nigeria for the permission to present this paper for publication.

REFERENCES

Adeyemi E.A (2000) Evaluation of different growing media fo raising coffea canephora (Pierre Ex.Froehner L) seedlings in the Nursery – preliminary result.Bulletin of Science association of Nigeria. Vol. 23 (2000) 133-135.

Brady, N.C (1999) The nature and properties of soils 12th edition. pp124-230.

Chapman, H.D.(1965) Cation exchange,In method of soil analysis Part 2,P891-901.C.A.Black (Ed) Monograph No.9 AM.Soc.Agron,Madison Wisconsin.U.S.A.

Daniel, M.A (2005) The effect of three organic fertilizers on the growth and nutrient uptake of coffee canephora seedlings.M.Sc Thesis Awowolo Obafemi Universty. Ile – Ife, Nigeria.

Worrall, R.J (1978) The use of composted wood waste as a peat substitute in Acta horticulture no 28 pp 79-86.

Physiological Response of Coffee to Different Shading Regimes

S.R. STEIMAN[1], T. W. IDOL[1], H C. BITTENBENDER[1], L. D. GAUTZ[1], M. C. JACKSON[2]

[1]University of Hawaii, Honolulu, HI, USA
[2]Hawaii Agriculture Research Center, Aiea, HI, USA

SUMMARY

Artificially shaded coffee was measured on various ecophysiological parameters. After less than a year of shading (one fruiting cycle), shaded coffees showed higher photosynthetic rates, different leaf chemical composition and better adaptative traits to water stress. Growth as determined by node production was not different.

INTRODUCTION

As a shade-tolerant species, coffee responds well to full sun. However, full sun typically contains more light than is necessary for optimum growth and health of the plant. Understanding how coffee responds to abiotic sources of shade may help in the development of shade management practices in Hawaii.

MATERIALS AND METHODS

Coffea arabica cv Typica trees were grown, in situ, in Kunia, Hawaii in the U.S.A. After the first major flowering in 2005, trees were grow in full sun or shaded with either 40% Aluminet shade cloth, 40% black shade cloth or a kaolin spray-on shade (Surround WP).

Nodes on lateral branches that produced flowers in 2006 were counted. Leaf temperatures were measured using an infrared thermometer between 11 am and 12 pm. Leaf stable C isotope ratios were measured with an elemental analyzer connected to an isotope-ratio mass spectrometer. Chlorophyll per gram of leaf was estimated by dividing SPAD measurements by specific leaf area. Photosynthesis measurements were taken with a CIRAS-1 Portable Photosynthesis System between 9 am and 12 pm. One year after the start of the experiment, leaf tissues were analyzed with an elemental analyzer for N and ICP emission spectrophotometer for P, K, Ca, Mg, B, Cu, Fe, and Zn.

RESULTS

- Nodal growth was not affected by shade (Table 1).
- Shade treatments significantly reduced leaf temperature 3-5 °C (Table 1).
- Chlorophyll per gram of leaf was higher in the black and Aluminet treatments than the sun and kaolin treatments (Table 1).
- Leaf photosynthesis was significantly greater in kaolin shade than in full sun (Table 2).
- Leaf nutrient concentration was significantly different for K, Ca, Mg and Zn (Table 3).

Table 1. Node growth, leaf temperatures, carbon isotopes and chlorophyll/g leaf[a].

Treatment[b]	Nodes	Temp (°C)	13C (o/oo)	Chl/g
Alu	12a	33b	-26.4ab	9342a
Blk	12a	32b	-26.6b	9613a
Kao	13a	34b	-25.3a	8199b
Sun	12a	37a	-25.9ab	8084b

[a]Different letters within a column and within a location are significantly different at p = 0.05; [b]Alu = 40% Aluminet shade cloth; Blk = 40% black shade cloth; Kao = kaolin; Sun = no shade.

Table 2. Photosynthetic rate of sun and shaded coffee[ab].

Treatment	μmol CO2/m^2/s
Kao	14.2a
Sun	8.3b

[a]Different letters within a column are significantly different at p = 0.05; [b]Kao = kaolin; Sun = no shade.

Table 3. Leaf nutrition 1 year after start[ab].

Treatment[c]	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
Alu	2.70	0.15	1.58a	0.56b	0.43b	75	92	7a	11	32
Blk	2.71	0.14	1.55a	0.63ab	0.46ab	77	118	7a	10	34
Kao	2.75	0.15	1.32b	0.76a	0.52a	50	141	5b	10	40
Sun	2.62	0.14	1.33b	0.68ab	0.49ab	61	133	5b	8	37

[a]Different letters within a column are significantly different at p = 0.05. Columns without letters have no significant differences; [b]N, P, K, Ca and Mg measured in % dwb, Fe, Mn, Zn, Cu and B are ppm; [c]Alu = 40% Aluminet shade cloth; Blk = 40% black shade cloth; Kao = kaolin; Sun = no shade.

DISCUSSION

While 40% shade does not affect node production, it does affect other areas of coffee physiology. Leaf temperature, leaf structure (chlorophyll/g leaf) and leaf function (photosynthesis) were all improved by the presence of shade. In addition, the carbon isotope ratios and leaf temperatures suggest a trend towards higher water use efficiency in shaded systems. The differences in leaf composition after just one fruiting season demonstrate that shaded plants interact differently than sun plants with respect to plant nutrition. All these factors affect growth and production of coffee as well as adaptation to stressful conditions. In the long term, these differences may play a role in the health and sustainability of modernized production systems.

Since the artificial shade was applied after the first major flowering, it is likely that the amount of shade-time experienced by the coffee was too short to illustrate true life-time differences. Long term shade adaptation will become prevalent in future years and these differences may very well become larger and/or more distinct.

Research funded by USDA TSTAR Grant Award no. 2003-34135-14033

Shade Coffee in Hawaii – The Impact of Light on Coffee Quality

S. R. STEIMAN[1], H. C. BITTENBENDER[1], T. W. IDOL[1], L. D. GAUTZ[1], M. C. JACKSON[2]

[1]University of Hawaii, Honolulu, HI, USA
[2]Hawaii Agriculture Research Center, Aiea, HI, USA

SUMMARY

Artificially shaded coffee and coffee underneath macadamia trees was measured on various aspects of coffee quality. Cup quality varied only slightly in coffee beneath macadamia trees. Yields were lower in shaded systems and green bean sizes were slightly larger than full sun coffee.

INTRODUCTION

Shade coffee is a prominent product in the current specialty coffee market as ecological and social considerations appeal to consumers. However, quality is often neglected in discussions of shade coffee. Various measures of coffee quality from trees grown under different shade regimes were examined.

MATERIALS AND METHODS

Coffea arabica cv Typica trees were grown, in situ, in Kunia, Oahu and in Kona, Hawaii, U.S.A. In Kona, trees were grown either in full sun or underneath macadamia nut trees. After the first major flowering in 2005, replicates of full-sun trees were shaded with 40% Aluminet shade cloth. The coffee beneath the macadamia trees had been growing there for 14 years. At the same time in Kunia, trees grown in full sun were subjected to either 40% Aluminet shade cloth, 40% black shade cloth, a kaolin spray-on shade (Surround WP) or grown in full sun.

The treatments were harvested, processed and roasted in the same fashion. Cherries were picked when mature until the trees were completely harvested. Green bean yields in Kunia were actual weights of green bean; Kona green bean yields were estimated from the harvested cherry weight. Green beans were sorted by hand to remove defects, peaberries and broken pieces. Floaters were removed after pulping. All samples were sorted using standard screen sizes based on 64^{ths} of an inch (0.4 mm) in the range of 13-20. A trained panel of cuppers rated the intensities of 7 attributes in the brewed cup.

RESULTS

- Shade tended to depress green bean yields (Table 1).
- Cupping quality at both locations did not differ among treatments for most characteristics (Table 2).
- The macadamia shaded coffee had lower body than the sun treatment, but not the Aluminet treatment.
- Macadamia shaded coffee had less aftertaste than the Aluminet treatment, but not the sun coffee.
- Shaded coffees had slightly larger beans than sun coffee (Figure 1).

- There were no significant differences among treatments for floaters, defects, peaberries or broken beans.

Table 1. Green bean yields and values[a].

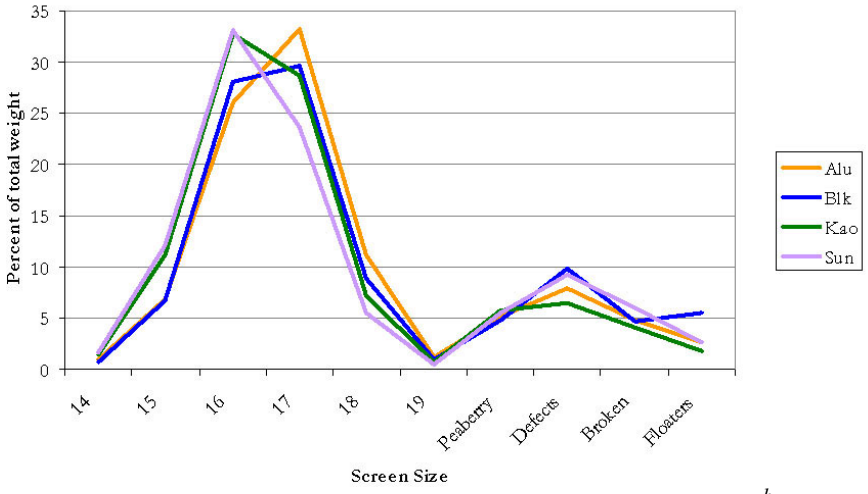
Location	Treatment[b]	Kg/ha	Value/ha[c]
Kunia 1800 trees/ha	Alu	860ab	$6,100
	Blk	800b	$5,400
	Kao	1600a	$11,300
	Sun	1400ab	$9,500
Kona 2740 trees/ha	Mac	1200b	$13,200
	Alu	2500ab	$27,500
	Sun	3500a	$38,500

*[a]Different letters within a column and within a location are significantly different at p = 0.1. [b]Alu = 40% Aluminet shade cloth; Blk = 40% black shade cloth; Kao = kaolin; Sun = no shade; Mac = macadamia trees. [c]Value = current price/grade * percent in grade * green bean/ha.*

Table 2. Cupping scores using a 0-10 rating of attribute intensity[a].

Location	Treatment[b]	Dry Aroma	Wet Aroma	Acidity	Flavor	Sweetness	Body	Aftertaste
Kunia	Alu	5.8	5.2	4.0	5.3	2.6	4.8	4.2
	Blk	5.8	4.9	4.1	4.9	2.4	4.7	4.1
	Kao	5.7	5.4	4.3	5.0	2.3	4.6	3.6
	Sun	5.5	5.0	3.9	5.4	2.4	5.2	3.9
Kona	Alu	5.9	5.3	4.8	5.1	2.5	4.8ab	4.1a
	Mac	5.9	4.8	4.8	4.7	2.5	4.3b	3.2b
	Sun	6.3	5.3	4.6	5.2	2.4	5.1a	3.8ab

[a]Different letters within a column and within a location are significantly different at p = 0.05. Columns without a letter do not have significance between treatments. [b]Alu = 40% Aluminet shade cloth; Blk = 40% black shade cloth; Kao = kaolin; Sun = no shade; Mac = macadamia trees.



[a]Sizes 13 and 20 were not included as they composed < 1% of the total. [b]Alu = 40% Aluminet shade cloth; Blk = 40% black shade cloth; Kao = kaolin; Sun = no shade

Figure 1. Green bean screen sizes and off-grade characteristics at Kunia[ab].

DISCUSSION

Shading coffee had few significant differences on these measures of quality. However, related studies show that shading does affect coffee chemistry (PC396) and ecophysiology (PA170). The cup quality differences under the macadamia trees suggest that the microenvironment they create or the biological interaction between the trees and the coffee are more important than simply reducing light intensity.

The fact that this is the first harvest in this pruning cycle for these trees may help explain the similarity in the yields. Future harvests may show greater differences. Furthermore, an average yield over the course of an entire pruning cycle is the best measure of yield for a production system.

Since the artificial shade was applied after the first major flowering, it is likely that the amount of shade-time experienced by the coffee was too short to discern distinct differences. Shading effects on quality may require an entire growth season or longer for quality effects to manifest.

Research funded by USDA TSTAR Grant Award no. 2003-34135-14033

Risk Management in Coffee Production in Kenya with Uncertain Rainfall

J. N. MUTUA

Coffee Research Foundation, P O Box 4, 00232 Ruiru, Kenya
E-mail: crf@kenyaweb.com

SUMMARY

Coffee production in Kenya takes place in an unpredictable and highly variable environment. In dealing with this uncertainty, three principles are applied ranging from agriculture to economics and policy issues, controlling external sources of variability, maintaining a meaningful diversity and maintaining flexibility. A considerable proportion of coffee farmers in Kenya lack a comprehensive risk management policy and the approach associated with uncertainties in rainfall has been on ad-hoc basis. A sustainable coffee production system requires being in equilibrium with the available soil moisture. This paper identifies the coffee management practices influenced by rainfall and the decision making processes (strategic, tactical and operational) that should be put in place to ensure that farmers are able to reduce risks associated with uncertainties in rainfall. The product will be of immediate benefit to resource poor coffee farmers in all coffee agro-ecozones.

INTRODUCTION

About 35% of year-to-year crop yield fluctuations are attributed to climate while fertilizer and disease contribute 18% and 12% respectively (Doorenbos and Christian, 1979). Interactions between climate and diseases, fertilizers and cultivars also contribute significantly to the year-to-year fluctuations in yield

There has been a declining trend in coffee production in some coffee growing areas in Kenya. A combination of factors have attributed to this negative trend but the most common of them all is the apparent increase in production costs and risks resulting from unfavourable weather conditions and insect pests and disease incidences

Climatological records show that in Kenya, recurrences of above and below normal rainfall anomalies and other extreme rainfall events are common in all rainfall time series **(**Le Houerou et al., 1993**).** Some of these anomalies are relatively small while others are very severe. It is therefore necessary for the coffee farming community to put in place strategies to counter these anomalies hence reduce their effect on coffee production.

Coffee production in Kenya takes place in three different rainfall zones namely: the South Eastern areas, the highlands East of the Rift Valley, highlands West of the Rift Valley and the Lake Basin. There are large year-to-year variations in precipitation levels within these regions (Figure 1). These variations are inter related with the natural and external variations occurring within the entire global scale systems (Lomas, 1999).

Above normal rainfall on the other hand may lead to increased moisture content which though necessary for proper growth may sometimes lead to soil nutrient imbalance which might lead to leaching of nitrogen and associated minerals, increased pH, production of flush growth

leading to misallocation of nutrients, significant increase in certain insect pests and diseases. Planning of appropriate risk management programme in order to mitigate its effects is therefore essential (Lomas, 1999).

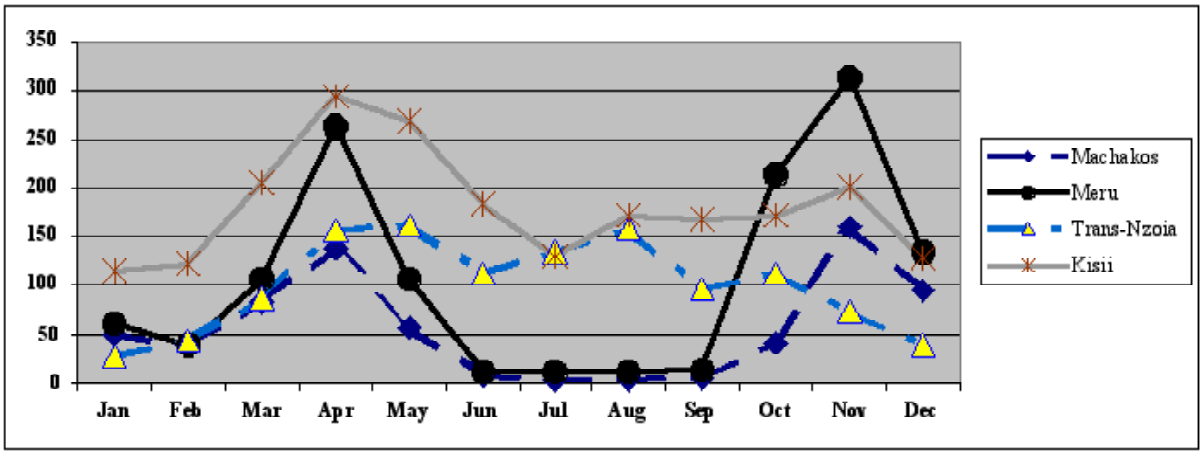


Figure 1. Mean monthly rainfall four major coffee zone in Kenya with South Eastern coffee Zone (Machakos), Highlands East of Rift Valley zone (Meru) Highlands West of Rift Valley Zone (Trans Nzoia and Lake basin Zone (Kisii).

RISK MANAGEMENT APPROACHES

Coffee establishment

Coffee should be established within the delineated coffee belts where microclimatic conditions are close to optimal requirements. These areas have specific rainfall amounts and distribution patterns, which coupled with other geographical and climatic, and factors make them suitable for coffee production. Farmers establishing coffee outside belts are likely to encounter risks associated with rainfall uncertainty.

Choice of Cultivars

Commercial coffee cultivars grown in Kenya have specific environmental conditions in which they perform well and their levels of resistance/torelance to diseases and moisture stress are varied (Table 1). The choice of cultivars in relation to the specific coffee zone reduces risks particularly those associated with rainfall deficits.

Canopy Management

Above normal rainfall is often coupled with increased flush growth in the coffee canopy thereby leading to misallocation of nutrients to unproductive plant parts. To mitigate this farmers should undertake handling and desuckering to remove unwanted suckers and branches continuously after every two months.

Irrigation

The Critical water requirement stage in Coffee (berry expansion) in some coffee zones (South eastern and parts of Highlands East of Rift valley) takes place during the months of January and February during dry or depressed rainfall periods. Irrigation as a management tool is often required to mitigate this. Well-researched irrigation suitable for large estate farmers and basin irrigation for small holder farmers are available for adoption

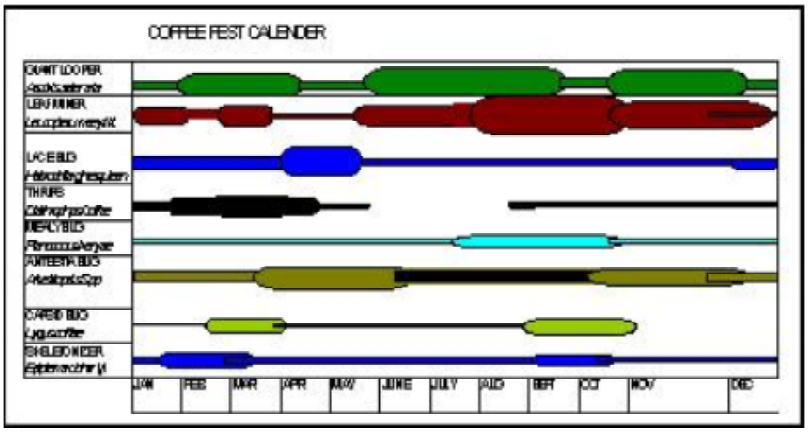
Fertilizer application

Above normal rainfall would lead to leaching of nutrients causing nutrient imbalance which in turn affects soil PH thereby impeding uptake of nutrients by tree roots. Depending on the anticipated duration of rainfall, it is necessary to reduce or increase nitrogen splits.

Insect pest management

Depending on upon intensity and spread, rains can either aggravate or subdue insect pest in coffee. For instance coffee green scales, coffee leaf miners and coffee thrips with low rainfall intensities while giant loopers increase with increased rainfall intensities. Understanding rainfall patterns and its influence on insect pest dynamics will enables the farmer take control measures before insect pest population level reaches economic threshold. Figure 2 shows changes in insect pest dynamics with time

Table 2. Changes in coffee insect pest dynamics with time.



Disease management

The major coffee diseases in Kenya i.e. Coffee Berry Disease, Coffee Leaf Rust and Bacterial blight of coffee are directly influenced by rainfall amounts and duration. The spray programmes formulated for control of these diseases are based on the number of rainy days and the extent of wetness. Altering spray applications in regard to duration of wetness and use using recommended fungicide stickers is a corrective measure towards reducing the risks of disease occurrence

Weed management

Weeds in coffee are best-controlled two weeks after onset of rains when weeds are at two leaf stage. Weed management by use of herbicides and/or weed slashing during periods of using prolonged rainfall would reduce the number of weedings and therefore labour costs. During periods of depressed rainfall, deep forking as a weed management should be avoided in order to reduce moisture loss via evaporation.

Coffee processing

The bulk of coffee processing in most coffee zone in Kenya takes place during the periods of high rainfall (October and November). This creates high risks associated with rewetting which

causes with quality deterioration. Investing in mechanical driers and recommended coffee drying materials. e.g. Nylex and Hessian cloth would held reduce the risks.

Waste Management and water re-circulation

During dry periods adequate water may not be available for coffee processing in such periods water recirculation in the coffee processing units would reduce risks associated with water shortage.

CONCLUSION

The above factors, either singly or in varying combinations affect coffee production greatly and this calls for the government and the governed to put in place measures to reduce risks associated with weather uncertainties

ACKNOWLEDGEMENTS

I wish to thank the CRF officers who have made it possible for me to compile this paper.

The paper is published with the kind permission of the Director of Research.

REFERENCES

Doorenbos, O.T and K R Christian., (1979). Yield Response to water. FAO irrigation and drainage. Paper 33. Food and Agriculture Organisation of the United Nations, Rome.

Le Houerou H N., G F Popov and L. See.,(1993). Agro-bioclimatic classification of Africa, Agrometeorology Series working Paper, Number 6, FAO Rome Italy

Lomas J. (1999): Vulnerability and awareness – the case of Agricultural drought reducing susceptibility by consciousness of the consequences, Sub-forum on Science and Technology in support of Natural disaster reduction, Geneva – Switzerland

Intercropping *Coffea canephora* Pierre ex Froehner with Food Crops at Establishment Stage in Nigeria

A. A. OLOYEDE, A. O. FAMAYE

Cocoa Research Institute of Nigeria – P.M.B. 5244, Ibadan, Nigeria

SUMMARY

Coffee was intercropped with selected food crops at establishment stage in Nigeria in 2001. The five crop combinations investigated were: coffee/sweet potato/maize, coffee/cassava/ maize, coffee/cassava, coffee/cocoyam/okra/pepper and coffee sole. The above combinations were laid out in a Randomized complete block design (RCBD) in three replicates. Results on vegetative growth between 2001 and 2003 respectively showed coffee in cocoyam/okra/pepper mixture to be the best in height (59.91.83.8, 116.8) girth (0.065, 1.2, 2.5) number of leaves (18.4, 114. 301.8) , Leaf area (69.63,, 134.90 & 183.70 and as well as number of branches (2.0, 14.4 & 47.8). The lowest value was however obtained from coffee/cassava combination by the third year of cultivation- height (45 cm), girth (1.5), number of leaves (101.3), Leaf area (78.84 cm^2 and braches 914.3). Yields from the food crops component are: Cocoyam (6.0 t/ha); cassava in coffee/cassava/maize (24.8 t/ha), cassava in coffee/cassava (23.7 t/ha; in coffee/sweet potato/maize (2.04 t/ha), pepper (1.22 t/ha) and okra (1.70 t/ha). Coffee clean bean yield by 2005 range between 257.5-315 kg/ha with cocoyam/pepper/okra combination producing the highest. Land equivalent Ratio (LER) indicated beneficial effect of the intercrops.

Keywords: Coffee, intercropping, Growth, Yield, Food crop

INTRODUCTION

Coffee is an important export crop traded globally. ICO (2003) reported 119.32 million bags to have been produced globally. It is a major foreign exchange earner for Brazil, Colombia, Cote d'Ivoire, Indonesia, Mexico, Costa Rica and Kenya (Montagnon et al., 1998). The two *Coffea* species of economic importance viz robusta and arabaica are grown in Nigeria. However robusta consistitute more than 90% of coffee production in Nigeria. Survey by (Obatolu and Osajuyigbe, 1984) revealed that coffee is produced by peasant farmers who seldom intercrop it with food crops. It takes between 3-5 years before coffee attains economic berry production. Before this period is attained there is the need for farmers to derive alternative source/s of revenue for sustainability. Intercropping, which is defined as the planting of two or more crops simultaneously on the same piece of and may be the remedy. Intercropping coffee with food crops has been reported in Kenya (Njoroge, 1992). Also results obtained from intercropping experiment on Arabica coffee revealed the suitability of intercropping *Coffea arabica* with maize (Okelana, 1982). The effort of this work is to investigate the possibility of intercropping *Coffea canephora* with some selected food crops in Nigeria.

MATERIAL AND METHODS

This experiment was conducted at the Headquarters of Cocoa Research Institute of Nigeria, CRIN, Ibadan, located on Latitude, 7° 10^1N, longitude 3°52^1E and altitude of 122 metres

above sea level. The rainfall is tropical with dry and rainy seasons with average temperature of 31 °C. The average relative humidity is 79%.

The experiment was Randomized Complete Bloc Design (RCBD) with three replications. The five treatments investigated were: (i)Coffee/sweet potato/maize(ii)Coffee/cassava/ maize(iii) Coffee/cassava(iv)Coffee/cocoyam/okra/pepper(v)Coffee sole. Mounds were constructed for cassava cocoyam and sweet potato at interval of 1 metre. The spacing and plant population for the various crops are as indicated above.

Table 1. Crop spacing and plant population.

Crop	Spacing©	Population/ha
Coffee	3 x3	1,111
Sweet potato	1 x 0.45	22,222
Cassava/cocoyam	1 x 1	10,000
Maize	1 x 1 (2 per stand)	20,000
Okra	1 x 0.5	40,000
Pepper	1 x 0.5	40,000

RESULTS AND DISCUSSION

Table 2 shows the mean growth parameters of coffee between 2001 to 2003 as influenced by various crop combinations. Coffee/cocoyam/okra/pepper gave the best vegetative growth in all the combinations. Indicate values height, girth, Leaf Area etc. This is in agreement with the works of Okelana (1982) that reported beneficial effect of intercropping arabica coffee with selected food crops. This indicates that the different arable crops combined give good architectural arrangement that create conducive environment for coffee growth, particularly as obtained in Coffee/cocoyam/okra/pepper combination. This was in consonant with earlier work report for cocoa by Adeyemi (1989). Yield of coffee green beans and land equivalent ratio are shown in Table 3. The results followed similar trend as recorded for vegetative growth. These results indicate that it is possible profitably to intercrop young coffee with some annual food crops. Opoku-Ameyaw et al. (1999) has reported the economic advantage of intercropping. The land equivalent ration (LER) followed similar trend. The lower values recorded under cassava and potato treatments could be ascribed to high nutrient demand by cassava and potato in the combination/s This study has revealed that intercropping with arable crops is possible especially when coffee is intercropped with cocoyam, okra and pepper.

Table 2. Cumulative means of growth parameters of coffee (2001-2003).

	2001			2002			2003		
Treatment	HT	GT	LA	HT	GT	LA	HT	GT	LA
	cm		cm^2	cm		cm^2	cm		cm^2
A	55.3	0.60	58.8	84.2	1.3	98.4	62.2	2.0	115.7
B	49.3	0.55	55.2	73.1	1.1	129.2	81.3	2.4	130.7
C	48.2	0.54	54.4	76.4	1.1	128.7	45.0	1.5	78.8
D	59.9	0.65	69.6	83.8	1.2	134.9	116.8	2.5	183.7
E	56.6	0.66	48.1	69.9	1.2	100.4	69.5	1.8	139.4
LSD (5%)	5.72	0.07	9.11	7.34	0.10	20.07	30.86	0.48	43.76

Table 3. Mean clean bean yield of coffee (kg/ha) LER for 2004 and 2005.

	Yield			LER		
Treatment	2004	2005	Mean	2004	2005	Mean
A	60.5	257.5	159	0.93	0.85	0.89
B	64.0	280.0	172	0.99	.93	0.96
C	63.4	267.5	165.5	0.98	0.88	0.93
D	70.3	315.0	192.7	1.08	1.04	1.06
E	64.8	302.5	183.7	-	-	--
LSD (P = 0.05)	4.11	27.53	13.60	-	-	--

A = Coffee/sweet potato/maize; B = Coffee/cassava/maize; C = Coffee/cassava; D = Coffee/ cocoyam/okra/pepper; E = Sole coffee.

REFERENCES

Adeyemi, A.A (1989): Progress in tree crops research. Pp224.

ICO (2003): International coffee market situation. Pp11.

Montagnon , C., Leroy, T and Eskes, A.B. (1998): Varietal improvement of *Coffea canephora* 11, breeding programmes and their results. Plantations recherché developpement2:89-98.

Njoroge, J.M. (1992): Studies on fertilization, plant density, pruning, replacement methods of established coffee and intercropping food crops with *Coffea arabica* L cv Ruiru !!.Ph.D Thesis, University of Nairobi, Kenya.

Obatolu, C.R and Osajuyigbe, O (1984): A survey report of the coffee growing areas of Kwara State, CRIN Technical Bulletin No 10. Pp13

Okelana, M.A.O (1982): Cocoa Research Institute of Nigeria Annual Report. pp67.

Omolaja, S.S. (2003): Characterization of Nigerian robusta coffee (*Coffea canephora* Pierre ex Froehner) germplasm and determination of factors controlling compatibility. Ph.D thesis University of Ibadan. Pp2002.

Opoku-Ameyaw, K., Oppong, F.K, Amoah, F.M, Osei-Bonsu, K.(1999): Preliminary investigations into the use of intercropping for weed management in young coffee in Ghana. ASIC 99 Books of Abstracts.

Analysis of Coffee Output Variations as Correlate of Weather Variables in Nigeria

R. A. SANUSI[1], M. O. ADEJUMO[2] AND T. R. SHITTU[3]

Cocoa Research Institute of Nigeria, P. M.B. 5244, Idi-Ayunre Ibadan, Oyo-State. Nigeria

SUMMARY

Rain-fed husbandry has remained the most established practice of crop cultivation in Nigeria. In spite of this, evaluation of crop-yield-weather relationship is not given a well deserved attention. In view of the fact that these variables play a key role in determining crop yield, this study evaluated weather effect on Nigerian coffee output for the period 1991-2005. Secondary data (from sources such as the international coffee organization) were obtained and analysed with Parvin model of decomposing total yield variation into technology and weather effect. The linear trend model was chosen as the lead equation for the Parvin model using statistical and econometric criteria. Also key parameters estimates were significant, the correlation coefficient (r) was 0.731 and F – stat = 14.62 ($p < 0.01$). Analysis revealed that if the weather was (consistently) "ideal", bean yield could have been 14.22% higher than obtained while if (consistently) "unideal", bean yield could have been 14.78% lower than obtained during the period under review. Therefore, attention need be paid to weather influence on crop production by policy makers and scientists to achieve the policy objective of resuscitated agro-allied driven (Nigerian) economy.

Key words: coffee, weather, Parvin model, rain-fed husbandry

INTRODUCTION

The roles of agriculture in the economic and social life of human beings cannot be overemphasized particularly due to income earned from agriculture by individuals and government. Government earns income from agriculture through exports and this make the export sub-sector a key sub-sector of the economy. For instance, over 60% of Nigeria's foreign earnings was from agricultural exports in the early days of independence (Shittu, 1997; Fashina, 1999; Oduwole, 2004; Sanusi and Oluyole, 2005). This fact, in addition to others, made Nigerian agricultural scientists to continuously undertake research on all aspects of (Nigerian) agriculture while the government addresses issues relating to agriculture via fiscal and monetary policy design and implementation. All these efforts (of government and scientists) can be regarded as effesctive through "controllable" factors such as technology, material inputs and management. However, a single "uncontrollable" factor that can considerably reduce the impact of these efforts is weather. This is because weather variables are major factors influencing variations in crop yields, soil characteristics as well as crop and animal distributions. Furthermore, weather can relegate all other factors especially the social and economic factors to relatively minor positions (Akintola, 1983) or magnify their deleterious effect. For example, the severe frost experienced by Brazil in 1994 led to a 10% drop in world coffee production and a price rise of about 118% (Dubois, 2006). Unfortunately, the influence of weather variables on crop yields is given less attention in many developing countries including Nigeria (Akintola, 1983, Akintola, 2000). This study becomes quite pertinent in view of the fact that one of the variants of weather (probably the most important) which is rainfall, as reported to exhibit some inconsistencies in the forest belt of Nigeria in year 2000, thereby heightening the fear of a reduction in yield of tree crops

(including coffee). Hence, this study examines the influence of weather variables on coffee yield in Nigeria.

METHODOLOGY

Secondary data sourced from International Coffee Organization (ICO), Food and Agricultural Organization (F.A.O), Federal Department of Agriculture (FDA) of the Federal Ministry of Agriculture and Rural Development in Nigeria were used for analysis in this study. The analytical technique employed was Parvin model of decomposing total yield variation into technology and weather effects (Parvin, 1973). In a simplistic form, the model reveals the overall effect of composites of weather factors on (crop) yield. The model is as enumerated below.

The procedures adopted in executing relevant computations are as follows –

A. Yield data were regressed on time variable to obtain a linear, quadratic, and cubic trend equation;
B. The trend equation was used to predict yields.
C. Deviations of actual yields from predicted yields were then obtained..
D. The effect of "ideal" weather conditions was synonymous with "ideal" crop yield which was supposed to occur in the year when weather had its largest positive effects on crop yields.
E. The effect of "un-ideal" weather conditions was synonymous with "un-ideal" crop yield, which was supposed to occur in the year when weather had its largest negative effects on crop yields.
F. The potential loss in yield as a result of less-than-ideal weather conditions was estimated as the difference between the "ideal" weather yield and actual yield, expressed as a percentage of actual yields;
G. The potential gain in yield as a result of better-than-unideal weather conditions was estimated as the difference between the "un-ideal" weather yield and actual yield (adjusted "un-ideal weather yield), expressed as a percentage of actual yield.

Step 1 - estimation of trend equation(s):

a. Linear trend – $Y_1 = \alpha_t + \beta_t T_t + \mu_t$ ……..……..(i)
b. Quadratic trend – $Y_1 = \alpha_t + \beta_{t1} T_{t1} + \beta_{t2} T^2_{t2} + \mu_t$ …..…….……..(ii)
c. Cubic trend – $Y_1 = \alpha_t + \beta_{t1} T_{t1} + \beta_{t2} T^2_{t2} + \beta_{t3} T^3_{t3} + \mu_t$ …….(iii)

where:

α_t & β_t = parameter estimtes;
Y_1 = trend yield in year t; T = 1 – 15 years; μ_t = stochastic disturbance term.

Step 2 - estimation of the effect of weather on cocoa yield:

$Y^* = Y_t - Y_1$ ……..…….…….(iv)

where:

Y^* = weather effect on (cocoa) yield; Y_t = observed level of yield in year t;

Step 3 - estimation of the effect of "ideal" and "un-ideal" weather conditions on cocoa:

a. $Y_i = Y_t + \max. (Y^*)$ ……..……..(v)

where:

max. (Y*) = "ideal" weather effect; Y_i = adjusted "ideal" weather effect on trend yield; Y_t & Y* = as defined previously.

b. $Y_u = Y_t + \min. (Y^*)$ ……..……..(v)

where:

min. (Y*) = "un-ideal" weather effect;
Y_u = adjusted "un-ideal" weather effect on trend yield; Y_t & Y* = as defined previously.

Step 4 - Computation of the adjusted weather differential:

a. $Y^{\bullet} = Y_l - Y_i$ ……..……..……(vi)

where:

$Y^{\bullet}$ = adjusted ("ideal") weather differential; Y_l & Y_i = as previously defined.

b. $Y^I = Y_l - Y_u$ ……..……(vii)

where:

Y^I = adjusted ("un-ideal") weather differential; Y_l & Y_i = as defined previously.

Step 5 - Computation of the adjusted weather effects as percentages of actual (observed) yields:

a. $Y^{\circ} = [Y^{\bullet}(Y_t^{-1})]100$……(viii)

where:-

Y° = index (percentage) of ("ideal") weather differential; $Y^{\bullet}$& Y_t = as previously defined.

b. $Y^v = [Y^I(Y_t^{-1})]100$ ……(ix)

where:-

Y^v = index (percentage) of ("un-ideal") weather differential; Y^I & Y_t = as previously defined.

RESULTS AND DISCUSSION

Table 1 revealed that the trend variable was significant (P<0.01) which means that the variable (an embodiment of technological input, improved soil and husbandry practices) was a key determinant of coffee yield during the period under consideration. Also, the correlation coefficient was 0.731 implying that 73.1% of variations in coffee yield were attributable to

variations in the trend value. A significant (P<0.01) F-value (of 14.62) means that the model best fit the data.

Table 1. Linear – trend model result.

Variable	Estimate	Standard Error
Constant	33.43***	2.07
t	0.87***	0.23
r	0.731	–
F-value	14.62***	–

*** *Sig. at 1%.*

Table 2 shows that if the weather was consistently "ideal", the yield realizable could have been higher than the actual yield while the reverse could have been the case if the weather was consistently "unideal". In actual fact, the yield could have been 17.12% higher than the actual yield realized in 1995 and 1996. This implies that Nigeria could have earned additional US$971,065.50 (₦131,093,842.50[1]) and US$728,511.00 (₦98,348,985[1]) from coffee exports in 1995 and 1996 respectively. Furthermore, for the entire period (1991-2005), coffee bean yield could have been 14.22% higher than the actual yield realized if the weather was consistently "ideal" This means that Nigeria could have earned an additional US $568,22.50 (₦76,709,767.50[1]).

Table 2. "Ideal" and "Unideal" weather effect on coffee yield in Nigeria (1991-2005).

x	Years	Yi	Yt	Yt - Yi	Yi + YL	Yi + YS	Y* (%)	Y** (%)
1	1991	34	34.3	0.3	39.65	28.13	16.62	(17.26)
2	1992	34	35.17	1.17	39.65	28.13	16.62	(17.26)
3	1993	40	36.04	3.96	45.65	34.13	14.13	(14.68)
4	1994	39	36.91	-2.09	44.65	33.13	14.49	(15.05)
5	1995	33	37.78	4.78	38.65	27.13	17.12	(17.79)
6	1996	33	38.65	5.65	38.65	27.13	17.12	(17.79)
7	1997	40	39.52	-0.48	45.65	34.13	14.13	(14.68)
8	1998	45	40.39	-4.61	50.65	39.13	12.56	(13.04)
9	1999	37	41.26	4.26	42.65	31.13	15.27	(15.86)
10	2000	48	42.13	-5.87	53.65	42.13	11.77	(12.23)
11	2001	43	43.00	0	48.65	37.13	13.14	(13.65)
12	2002	49	43.87	-5.13	54.65	43.13	11.53	(11.98)
13	2003	42	44.74	2.74	47.65	36.13	13.45	(13.98)
14	2004	44	45.61	1.61	49.65	38.13	12.84	(13.34)
15	2005	45	46.48	1.48	50.65	39.13	12.56	(13.04)

1 US$1= ₦135

However, if the weather have been consistently "unideal", the yield could have been 17.79% lower in 1995 and 1996 (Table 2). This implies that Nigeria could have lost US$1,008,876.90 (₦136,198,381.50)[1] and US$756,877.80 (₦102,178,503.00)[1] in 1995 and 1996 respectively. For the entire period (1991-2005), coffee yield could have been averagely lower by 14.78% i.e. Nigeria could have lost US$590,345.90 (₦ 79,696,696.50)[1] in coffee export earnings.

[1] US$1= ₦135

CONCLUSION AND RECOMMENDATION

The result of this study shows that weather variables can significantly affect the yield of coffee in Nigeria and consequently the earning of the country from coffee export. The export earnings (or losses) computed through this analysis could have been higher if the country realizes her full potentials in the coffee sub-sector. Hence, in view of the fact that efforts of the Nigerian Government have been directed to economic diversification through agriculture, it becomes imperative for policy makers, government and scientists to pay more attention to the influence of weather on crop production. This will go a long way in helping to achieve the policy objective of the Nigerian government of a resuscitated agro-allied driven economy.

REFERENCES

Akintola, J. O. 1983. An analysis of the effects of agro-climatological factors on food crop yield in Ibadan area of Oyo State. Unpublished Ph.D. Thesis, Department of agricultural economics, University of Ibadan, Ibadan, Nigeria.

Akintola, J. O. 2000. Forecasting food crop yields from meteorological variables. *Journal of Rural Economics and Development*. Vol. 14, No. 1. pp.24-33.

Dubois, P. 2006. Improving market conditions for coffee producers: the experience of the ICO. Paper presented at the conference of the committee on trade and development of world trade organization (WTO). Geneva, Switzerland. 11th May 2006. 4-9 .

Fashina, A. B. 1999. Sustainable tree crop production to support the manufacturing sector to boost Nigeria's foreign exchange earnings. Information paper presented at the International Seminar on "Support for manufacturing to boost non-oil export earnings using agricultural raw materials" organized by Ebun Industries Ltd, Sheraton Hotels and Towers, Abuja, Nigeria.

Oduwole, O. O. 2004. Adoption of improved agronomic practices by cocoa farmers in Nigeria: a multivariate Tobit analysis. Unpublished Ph.D. thesis, Department of agricultural economics and extension, Federal University of Technology, Akure, Nigeria.

Sanusi, R. A. and Oluyole, K. A.2005. An analysis of cocoa production and export in Nigeria (1930-2003). Bulletin of Science Association of Nigeria. Vol. 26. 146-154.

Shittu, T. R. 1997. Price response analysis of selected cash crops in Nigeria: the case of cocoa, cashew, rubber. Unpublished M.Sc. project report, Department of agricultural economics, University of Ibadan, Ibadan, Nigeria

Technology Transfer and Adoption of Coffee Coppicing in Kogi State, Nigeria

A. E. AGBONGIARHUOYI, O. O. ODUWOLE AND R. A. SANUSI

Cocoa Research Institute of Nigeria (CRIN) Idi-Ayunre, P.M.B. 5244, Ibadan, Oyo State, Nigeria

SUMMARY

Coffee farms in Nigeria are largely old plantations and have been virtually abandoned due to marketing problems. Consequently, this neglect led to low productivity as well as diseases and pests attack. A technique of improving abandoned and unproductive coffee farms is coppicing, and this study examined the adoption level of coppicing by coffee farmers in Kogi State. Forty coffee farmers from fifteen main coffee producing communities of Kabba-Bunu Local Government Area (LGA) in Kogi State were randomly sampled. Data were sourced and analysed using descriptive statistics and multivariate probit model. Forty-three percent of the farmers were above 60 years of age and 45% had no formal education. Ninety percent of the farmers were aware of coppicing technology and 82.5% claimed to have adopted it. Farm size (0.37) was the major significant factor influencing the probability of coffee coppicing adoption ($p < 0.1$). The probability of a farmer adopting coppicing technology did not increase proportionately with farm size since adoption – farm size elasticity was 0.09. Adoption of coffee coppicing technology could be enhanced through improved farmer – extension – research linkage as well as implementation of appropriate government policies.
Keywords: coffee, coppicing, rehabilitation, adoption

INTRODUCTION

Coffee is an important commodity crop, which is second to oil in Africa and in World trade (Cambrony, 1992). It has a significant and unique socio-economic contribution in terms of foreign exchange revenue for developing countries. Historically, coffee has been one of the few crops that small holders could grow to escape poverty (Federal Ministry of Commerce, 1999). However, production is fast decreasing due to declining market prices that have now become a serious concern for stakeholders in the coffee sub-sector (Surendra, 2002; Interafrican Coffee Organization 2003 and Sanusi et al., 2004). Farmers are also experiencing low production from their farms as a result of old age of coffee trees (Famaye and Ibiremo, 2001). Over the last ten years, Nigerian coffee was 15,900 tons (Interafrican Coffee Organization, 2003).

In improving the productivity of farmers and income earning potentials of coffee, coppicing technology- an agronomic method of rehabilitating old trees was developed by Cocoa Research Institute of Nigeria (CRIN). Coppicing is practiced as a result of long stem height, aging, low productivity, abandonment, pest and disease attack and this was introduced to farmers in Kabba, Kogi State more than eight years ago (Famaye and Ibiremo, 2001; Opeke 2005). Hence, there is a need to investigate its usage among farmers. The main objective of this study is to determine the level of adoption of coffee coppicing technology by farmers in Kabba, Kogi State. Specifically, the objectives are to describe coffee farmers' personal characteristics and ascertain the factors determining adoption of coffee coppicing technology.

METHODOLOGY

Study Area

The study was conducted in Kabba-Bunu Local Government Area of Kogi State. It was purposively chosen because the area is the major producer of coffee (robusta) in Nigeria (Federal Ministry of Commerce, 1999). Respondents were randomly selected across fifteen localities in the study area. Sixty questionnaires were administered but only 40 were used due to consistency and adequacy of information.

Data Analysis

Descriptive statistics such as frequency distribution and percentage, as well as multivariate probit model were used to analyse data for the study.

Different variable categories namely farmer, Farm, Coppicing and Constraint specific variables were regressed on adoption level. Significant variables within each category were pooled together and regressed on adoption level.

Probit Model

$$C_d = gV_i + \varepsilon_I \quad [1]$$

where:

g = Vector of unknown coefficients;
C_d = Variable that indexes the adoption of coffee coppicing technique.
V_i = Vector of explanatory variables;
ε_i = Stochastic error term

The explanatory variables (V_i) used in the probit model analysis are age (years), Farming Experience (years), Educational Level, Farm Size (Ha), and Source of obtaining Coffee Coppicing information. Others are Coppiced Plants growing better than Uncoppiced, yield, Risky Practice of Coppicing, Constraint of coppicing-weak extension linkage and underlying principles of coppicing technique.

RESULTS AND DISCUSSION

Awareness, adoption and reasons for coffee coppicing

Most of the respondents (90.0%) were aware of coffee coppicing technique and (82.5%) claimed to have adopted it (Table 2). From Table 2, farmers' coppiced coffee trees because of old age (87.5%), long stem height (47.5%) and low productivity (35.0%). This means that coffee trees in the study area had become less productive.

Table 1. Awareness, adoption and reasons for coppicing.

Variable Categories	Awareness		Adopter		Coppicing Reasons		
	Yes	No	Yes	No	Age	Stem height	Productivity
Percentage	90.0	10.0	82.5	7.5	87.5	47.5	35.0

Source: field survey, 2004.

Table 2. Coffee farmers' personal characteristics.

Variables	Categories	Frequency	Percentage
Age (years)	31-40	2	5.0
	41-50	8	20.0
	51-60	13	32.5
	above 60	17	42.5
Experience (years)	Less than 5	2	5.0
	6-15	4	10.0
	16-25	11	27.5
	26-35	7	17.5
	36-45	14	35.0
	above 45	2	5.0
Educational Level	No formal Edu.	18	45.0
	Primary	5	12.5
	Secondary	3	7.50
	Tertiary	14	35.0
Farm size (Ha)	1-2	9	22.5
	3-4	15	37.5
	5-6	8	20.0
	above 6	8	20.0

Source: field survey, 2004.

Farmers' Personal Characteristics

The findings in Table 2 revealed that 42.5 percent of the respondents were above 60 years. This means that old people were more involved in coffee production in the study area. Forty percent had coffee-farming experience of 36-45 years and above 45 years. Hence coffee respondents were highly experienced in coffee farming. The educational level of coffee farmers indicates that 45% had no formal education while most of them (55.5%) had undergone one form of formal education or another. An appreciable proportion (40.0%) cultivates (coffee) farm of 5-6 hectares. Therefore, a good proportion of farmers' plots could be said to be above small-holdings.

Table 3. Probit model result for coppicing technology adoption.

Variable	**Estimate**	**Standard Error**	**Probability**
Intercept	-6.8353	10.6172	0.5197
Age (years)	0.0223	0.1264	0.8599
Farming Experience	-0.0546	0.8343	0.5126
Educational Level	0.5288	0.6055	0.3825
Farm Size	0.3655*	0.2098	0.0816
Information Source	-0.5623	0.6099	0.3566
Coppiced and Uncoppiced	3.8235	3.3514	0.2539
Yield	3.3401	2.2729	0.1417
Risk	3.2790	3.5720	0.3597
Constraints	-1.4964	2.1581	0.4881
Principles	-2.1240	1.8602	0.2535
Log Likelihood	-7.9556	-	-

*Significance: *10%, **5%, ***1% (source: Field Survey, 2004).*

Probit model Analysis

Table 3 revealed that the significant variable determining the probability of coppicing adoption was farm size ($p < 0.1$). This means that whether a farmer adopts coppicing or not depends on farm size. The farm size elasticity estimate was 0.09, implying that the probability of a respondent adopting coppicing technology did not increase proportionately to farm size. The probability of a respondent being an adopter of coppicing technology was predicted with equation [1] to be 95.9%.

CONCLUSION

Most farmers were aware and adopted coffee coppicing. Farm size was the major significant factor affecting the probability of coppicing adoption. Adoption of coffee coppicing could be enhanced through improved farmer-extension-research linkage and implementation of appropriate government policies.

ACKNOWLEDGEMENT

We are grateful to the Executive Director, CRIN for the permission granted to publish this work. Our thanks are due to Dr. Aigbekaen E. O. (CRIN) and Dr. Akinbile L.A. (University of Ibadan) for their useful comments.

REFERENCES

Cambrony, H.R. (1992): The Tropical Agriculturist- Coffee Growing.CTA, Macmillan Publishers, Netherlands. Pp. 41-44.

Famaye O.A and Ibiremon O.S (2001): Coffee Rehabilitation and Routine farm management. In: CRIN Coffee production Technology Training Manual. Sponsored by the Federal Department of Agriculture (FDA) ISSN: 0794-6456. Pp.11-13.

Federal Ministry of Commerce FMC, (1999): Final Report of the Technical committee on the Nation-wide survey on coffee production. Garki, Abuja. Pp. 3-4.

Interafrican Coffee Organisation. (2003): International Coffee market situation. Document No. 343/03/E. P.3.

Opeke, L.K. (2005): Tropical commodity Tree crops.2nd edition. Spectrum books limited, Ibadan. Pp. 84-86.

Sanusi, R.A., Oduwole, O.O. and Lawal, J.O. (2004). Impact of coffee marketing problems on Coffee production on Nigeria. In: proceedings of the 20th International Conference on Coffee Science held in Bangalore, India. 11th-15th October 2004. Pp. 1030-1033. http://www.asic-café.org

Surendra, K. (2002): Coffee in Africa. Impacts and Issues of Low price crisis. Discussion Paper for ministerial conference held in Malabo, Equatorial Guinea, October 2002. Prepared for Inter African Coffee Organisation, Pp. 12-18.

Preliminary Assessment of Smallholder Coffee Value Chains in Specific Regions of Kenya, Uganda and Rwanda

A. T. AITHAL, F. PINARD

ICRAF-CIRAD, Kenya

SUMMARY

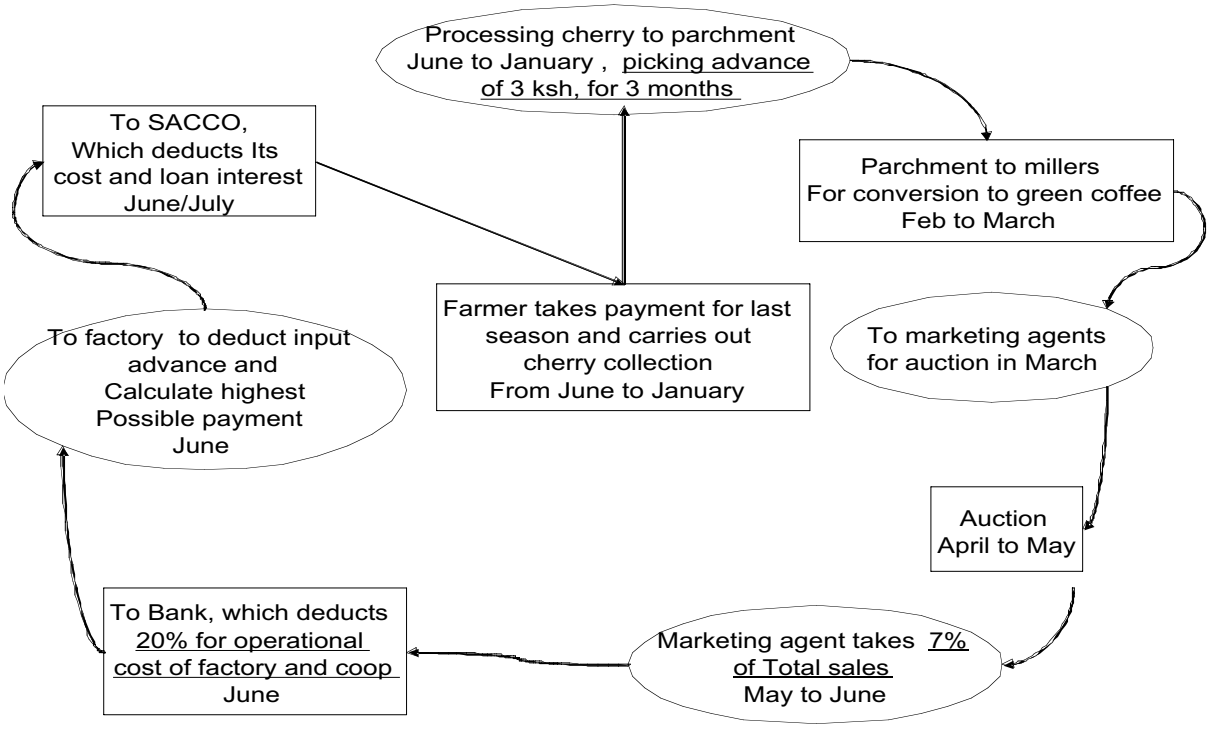
Comparing smallholder farmers and the coffee value chains between the open market systems of Uganda, Rwanda and the controlled cooperative system of Kenya; preliminary results suggest a similar profile of the small holder coffee producer, regardless of the difference in the market chains and production systems. As expected, the market chains in the open market systems are much smaller, with faster payment schedules and choice on the type of product to sell. The controlled auction/cooperative system allows the ownership of the coffee to stay with farmers till sold at the auction, and prices are higher, but the delay in payment and distribution of income along the chain has adverse effects on what the farmer actually gets. Perception of good quality coffee varies from farmer to farmer, and payment doesn't change with the quality produced though farmers in Mt. Kenya get prices based on the quality of the coffee sold by the factories.

INTRODUCTION

The objective of the study is to understand the link between the quality of the coffee produced and the farm gate revenue for it, to assess the viability of using quality promotion as strategy to increase smallholder coffee farmer incomes. The study is being carried out in pilot sites in three countries, Lake Kivu region of Rwanda, Mt Elgon region of Uganda and Mt Kenya region of Kenya; to capture the different market structures and farmer profiles, through working with one representative farmer group in the Arabica growing areas of each country. The research protocol is structured primary data survey of between 40 to 60 farms through stratified sampling in the each of the selected pilot sites.

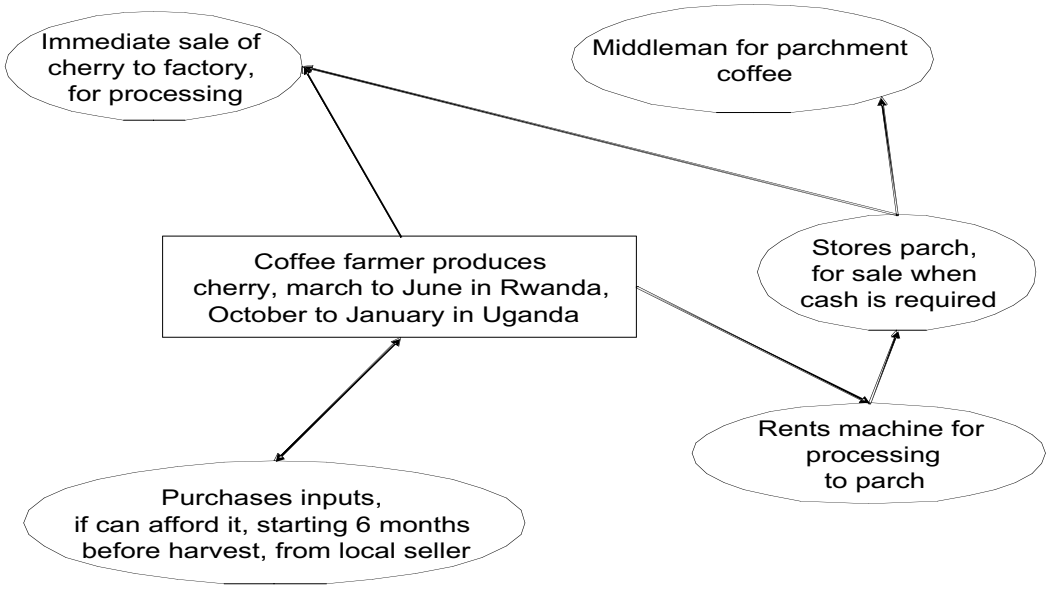
From what is seen on the field, the current cooperative structure in Kenya is as follows. A cooperative has between 1 and 17 factories, each covering between 200 and 500 farmers. The farmer is registered to the cooperative, but deals with factories. The farmer produces cherry between June and January on a weekly basis during the start of the season and daily during the peak; and sends it to the factories for processing. The farmer is given a receipt for each time that cherry is bought to the factory which includes the name, cooperative number and the amount produced. The farmer also has access to inputs from their factories, on credit. The factory then processes the cherry into parchment and stores it till all the cherry available, is processed. The parchment is then sent to one of the three authorized millers/marketing agents, who then process is further into green coffee, before selling it for the factories at the auction. Once the coffee is sold, the miller, after deducting own costs, makes the payment to the cooperative; which in turn deducts the costs of running a cooperative and all the factories, before arriving at the price per kilo of coffee. This amount differs from factory to factory, within the cooperative. The price per kilo of coffee is then used to calculate payment due to each farmer, the cost of input taken by each farmer is then deducted, and the final payment is then credited into the farmer's account. This whole process takes a year on average.

Table 1. Farmers in a controlled auction/cooperative system.



SACCO = Savings and Credit Cooperative

Table 2. Farmers in open market systems.



The foreseeable advantages of this system are that the price per kilo of cherry is based on the auction price received; there is scope for producing good coffee, as all processing is centralized; the cooperative provides support to farmers in terms of inputs on credit, trainings and loans; and the farmer owns her/his coffee till it is sold at the auction. On the other hand, there are issues with the distribution of income along the chain; input support, which makes farmers take as much input as they can and fall into a debt trap; farmers don't have any

control on the timing of the payment received; and all farmers within a factory get the same price per kilo of coffee regardless of the quality of coffee produced.

Rwanda and Uganda, both of which have liberalized open market economies have short chains for coffee. The farmer produces, sells to either a middleman, cooperative (Rwanda) or a factory close by; gets paid on the spot, and has the choice of who to sell to. It is also seen that farmers in this system sell two products, coffee cherries and parchment coffee. Most buyers prefer to set up small processing units in specific catchment areas and buy cherries; to ensure control on processing. Most farmers prefer to sell parchment, as it can be stored till such a time as cash income is required. Farmers also try to wait till a good price is reached in the market for parchment, before selling it, though it is observed that this doesn't work all the time due to poor market information. It is found that some cooperatives in Rwanda are fair trade certified, but the preliminary survey shows that farmers get similar prices from the cooperative or the factory, and the premium doesn't trickle down to the farmers.

The foreseeable advantages of this system are that farmers are able to sell either coffee cherry or parchment, depending on their financial circumstances; they have control over the timing of sale; payments are immediate; and sometimes farmers have the choice of selling different buyers for higher prices. On the other hand, there is no support for the farmer in terms of inputs or loans; there is no control of quality, as farmers each process their own coffee, in their own way depending on access to procuring material; the farmer loses ownership of the coffee as soon as the product is sold; and farmers need access to market information to make better use of the open market system, which is unavailable

COFFEE PRODUCTION PROFILE

The data collected shows some interesting comparisons between the smallholder coffee producers in each country.

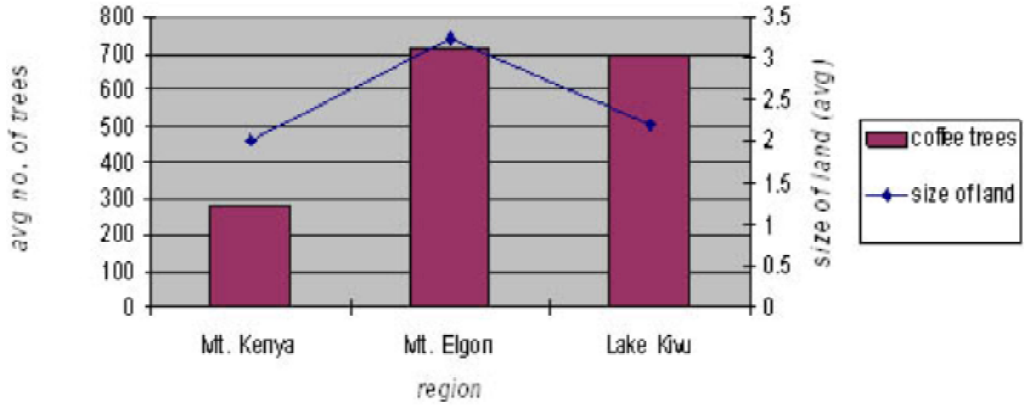


Figure 1. Coffee trees and land size. Shows that is no direct link found between land size and number of coffee trees on farm. Probable reasons are Govt policy on removal of coffee trees in Mt. Kenya, advent of coffee berry and in Mt. Elgon and higher returns on other cash crops in Lake Kivu.

Based on primary data, the income and expense per kilo of coffee is computed, and then, to see what the net gain per kilo is for farmers, a ratio is arrived at using income per kilo/expense per kilo –1. Based on this, it is seen, within the sample that Mt Elgon farmers have the highest profitability with coffee, followed by farmers from Lake Kivu, while farmers interviewed in Mt. Kenya have a negative ratio of –0.25, and make net loses. Probable reasons for a negative

ratio are higher costs due to accessing inputs on credit, and higher cost of hired labour, as shown by Figure 4.

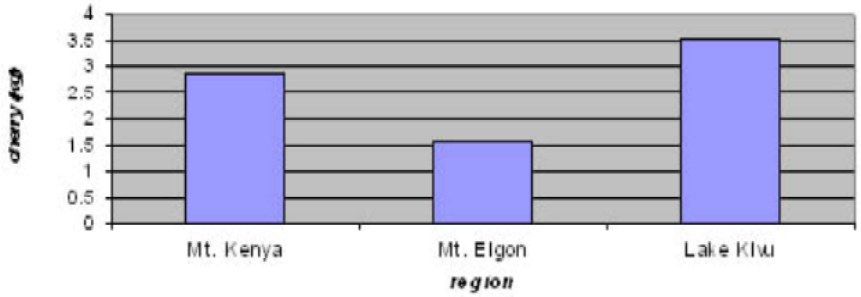


Figure 2. Cherry production per tree. Shows that kilos of cherry produced is higher in Lake Kivu, where very little chemical input is put and most farming is organic, as compared to a heavy input application system in the Mt. Kenya region.

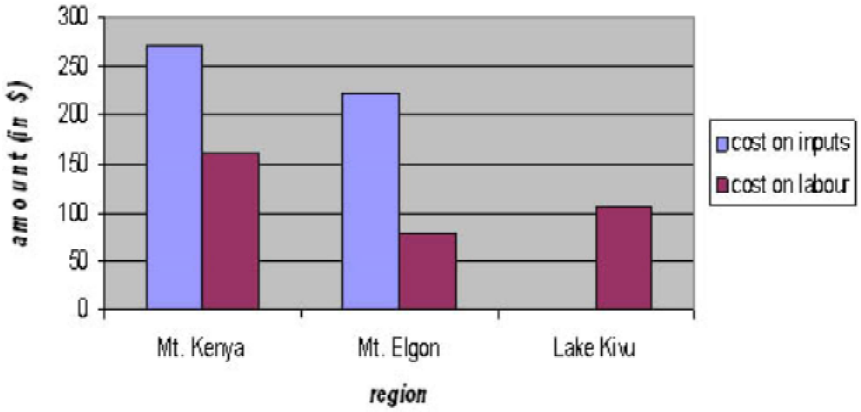


Figure 3. Coffee expense, share on imputs and labour. Shows that farmers in Mt. Kenya spend the most on inputs and hired labour, while those in Mt Elgon spend more money on chemicals compared with on hiring labour The farmers in Lake Kivu only spend on hired labour, as they get free pesticide from the Govt, though not on time, and cannot afford chemicals.

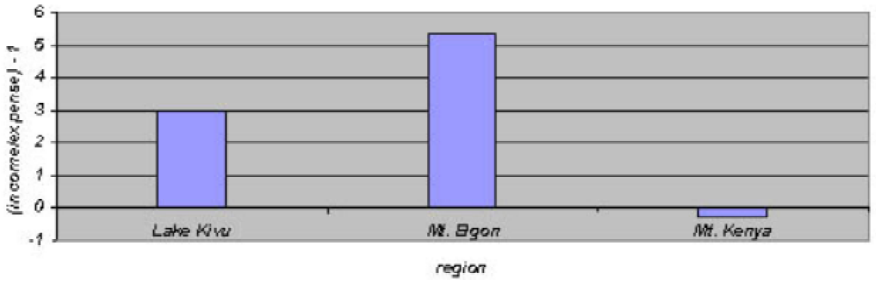


Figure 4. Ratio of Net Gain per kilo of coffee.

COFFEE AND THE FARMER

The profile of the coffee farmer is found to be similar in all three countries and within the 2 market systems. Farm sizes are under a hectare, subsistence farming is the norm and farmers are shifting or willing to shift to other cash crops that have lower costs and higher returns as compared with coffee. All farmers interviewed have other off farm activities that actually

generate income for the family. As is shown by Figures 5 and 6, share of coffee expense is highest and share of coffee income lowest for farmers in Kenya.

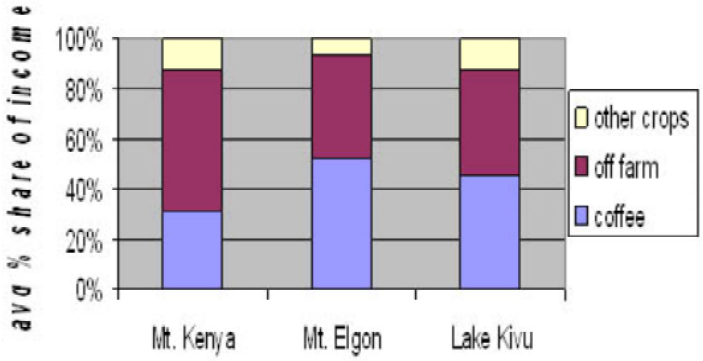


Figure 5. Coffee share of total household income.

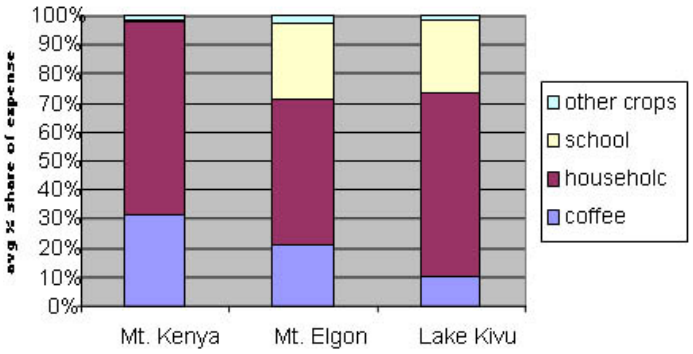


Figure 6. Coffee share of total household expense (in $).

In terms of farmer appreciation of coffee quality, less than 1% of the farmers interviewed drink coffee, so for the farmers, quality is a factor of chemical inputs in Kenya; Post harvest processing in Uganda; and time of harvest in Rwanda. All farmers agree that production methods, especially pruning of the coffee trees is very important to get good quality coffee.

CONCLUSION

Based on the first primary survey in the selected sites and point data collected, it is possible to arrive at some conclusions. Market systems in Lake Kivu and Mt. Elgon have faster payment schedules as compared with farmers in Mt. Kenya. Farmers in Mt. Kenya sell cherry, while farmers in Lake Kivu and Mt. Elgon have the choice of selling either cherry or parchment coffee. Farmers in Kenya have support from the coffee cooperatives in terms of access to inputs and money on credit. Market chains in all three regions promote higher production of coffee, as payment is based on how much is produced, though Mt. Kenya farmers get payment based on quality of coffee produced by the factories.

Analysis of Coffee Consumption in Nigeria

R. A. SANUSI, J. O. LAWAL AND T. R. SHITTU

Cocoa Research Institute of Nigeria, Idi-Ayunre, P.M.B 5244, Ibadan, Nigeria

SUMMARY

Coffee production has been faced with crisis over the years both in Nigeria and the world at large. However, some origin countries are vigorously pursuing increased internal consumption of coffee. This study hereby evaluates coffee beverage (CBV) demand in Nigeria. A multistage sampling technique was adopted in collecting the data used in the study. The first stage involved a random selection of nine out of twenty-four States in South and North-central Nigeria. These are Abia, Edo, Kaduna, Kwara, Lagos, Niger, Ondo, Osun, and Oyo States. In the second stage, twenty local government areas (LGAs) were randomly sampled in the selected States. The last stage employed the use of a systematic random sampling of one hundred households from each LGA. A total of 2,000 households were sampled. However, 1,413 questionnaires, representing 70.7% response rate, were used for the analysis. Data collected with the aid of structured questionnaire included household size, age, gender, etc and were analysed using descriptive statistics and multivariate Tobit model. Average household size was 5 people while average age of household head was 33.1 years. Male-headed households, constituting 54.8% of the entire respondents, had an average monthly CBV demand of 150.5g while female-headed households had average monthly CBV demand of 125.6 g. CBV household budget share was ₦43.43 (US$0.32) i.e. 0.40%. The factors that significantly influenced the demand for CBV were marital status, price of cocoa beverage, own price of CBV, and price of tea beverage ($p < 0.1$). Concerted efforts need be made to popularize CBV demand so that efforts on increased coffee consumption could yield the desired dividend of improved coffee production in Nigeria.
Keywords: coffee, demand, budget share, Tobit model

INTRODUCTION

The production of coffee has been bedeviled with several crises over the years and these reflect in low producer prices for coffee in the international market (Sanusi et al., 2004). Some of the problems include unfavorable weather conditions, loss of quality and contamination of coffee bean. It has been pointed out that increased domestic coffee consumption in origin countries need be encouraged to assist in the sustainability of the coffee sub-sector of the world economy (Sanusi et al., 2004; Osario, 2006; Dubois 2006).

The advantages inherent in this effort are many. According to Osario (2006), the benefits of encouraging coffee consumption in origin countries include: a means of achieving a balanced global (coffee) market, provision of alternative market for (coffee) farmers, increasing producers' awareness of consumer preferences and leading to greater appreciation by (coffee) growers of the value of quality, provision of incentive for significant foreign investment and promoting the development of small and medium enterprises such as regional roasters and coffee houses. As elegant as these highlights are, the need for information on the level of coffee demand by consumers who are the ultimate users of the product of the efforts on increased domestic consumption as well as the factors that determine their demand for value added coffee cannot be overemphasized. Therefore, this study examined coffee beverage (CBV) demand in Nigeria.

METHODOLOGY

A multistage sampling technique was employed in collecting the data used in the study. The first stage involved a random selection of nine out of twenty-four States in South and North-central Nigeria. These are Abia, Edo, Kaduna, Kwara, Lagos, Niger, Ondo, Osun, and Oyo States. In the second stage, twenty local government areas (LGAs) were randomly sampled in the selected States. The last stage employed the use of a systematic random sampling of one hundred households from each LGA. A total of 2,000 households were sampled. However, 1,413 questionnaires, representing 70.7% response rate, were used for the analysis.

Data collected with the aid of structured questionnaire included household size, age, gender, etc and were analyzed using descriptive statistics and multivariate (truncated) Tobit model. The Tobit model used is as stated below:

$$Q_t = \theta L_i + \rho_i \text{ if } \theta L_i + \rho_i > 0 \quad [1]$$

$$Q_t = 0 \text{ if } \theta L_i + \rho_i \leq 0 \quad [2]$$

where:

Q_t = quantity of CBV;
θ = vector of unknown parameters;
L_i = vector of explanatory variables;
The explanatory variables (L_i) included in the Tobit model are:-
TMI = total monthly (household) income (₦);
FDEXP = food expenditure (₦);
NFDEXP = non-food expenditure (₦);
PCCB = average price (₦) of cocoa beverage (CCB);
PCFB = average price (₦) of coffee beverage (CFB);
PTB = average price (₦) of tea beverage (TBV);
RGN = region of respondent (D = 1 if South, D = 0 if North-central);
SOC = secondary occupation (D = 1 if yes, D = 0 if otherwise);
HHZ = household size;
TTL = title of respondent (D = 1 if titled, D = 0 if otherwise);
AGE = age of household head (in years);
MST = marital status of household head (D = 1 if married, D = 0 if otherwise);
GDR = gender of respondent household head (D = 1 if male; D = 0 if female);
YSS = years spent schooling by household head (as a proxy for educational status);
YRS = years spent in residence by respondent household head;
FDC = food-stuff decision maker (D = 1 if household head, D = 0 if otherwise);
ρ_i = random error term.

RESULTS AND DISCUSSION

The average age of household head was 33.1 years. Male-headed households, constituting 54.8% of the entire respondents, had an average monthly CBV demand of 150.5 g while female-headed households had average monthly CBV demand of 125.6 g. Implying that male-headed household demand for CBV more than female-headed households. The budget share of CBV in household budget was ₦43.43 (US$0.32) i.e. 0.40%. Average household size was 5 people. This means that per capita CBV expenditure in the study area was very low, amounting to ₦8.69 (US$0.06).

Table 1 revealed the factors that significantly influenced the demand for CBV. Household income is a significant determinant of CBV demand ($p < 0.05$). This implies that the income-earning capacity of households is vital in demand for CBV; hence any socio-economic variable or policy that affects household income will significantly affect coffee consumption. This becomes more crucial in view of the fact that food expenditure and CBV demand had a significant and direct relationship ($p < 0.01$) i.e. the higher the household food expenditure, the higher the CBV demand. Furthermore, the price of CBV as well as CCB and TBV had significant relationship with CBV demand ($p < 0.01$, $p < 0.1$, $p < 0.01$ respectively). Also, their parameter estimates had the expected *a priori* signs i.e. CBV is a normal good having falling demand with price increase. Hence, CBV companies need to take this fact into cognizance in product promotion strategies. However, while CCB had a negative relationship with CBV, TBV had a direct relationship with CBV. This suggests that CBV is complementary to CCB but substitute TBV in household budget. This is not far fetched since CBV and TBV are both stimulants while CCB is a (nutrition/) energy beverage according to Wellman (1961).

Table 1. Tobit Result for Coffee Beverage Consumption in Nigeria.

Variable	Estimate	SDE
Intercept	–25.3510***	6.9737
TMI	–0.00003**	0.00002
FDEXP	0.0030***	0.0005
NONFDEXP	–0.00006	0.0001
PCCB	–0.1960*	0.1136
PCFB	–0.7532***	0.1599
PTB	3.4325***	0.9561
RGN	–2.5319**	0.0447
SOC	–1.7741	1.8896
HHZ	0.4789	0.2935
TTL	0.0278	0.4019
AGE	–0.0458	0.0916
MRT	5.8697***	1.9272
GDR	4.7863***	1.4934
YSS	0.3685**	0.1814
YSR	–0.0098	0.0717
FDC	0.6324	0.7355
Sigma	27.1521***	0.5108
Log Likelihood	–6669.9147	–

Respondents in the South demanded less of CBV than those in the North-central ($p < 0.5$) while male-headed households demanded more of CBV than female-headed households ($p < 0.05$) and households with married heads demanded more of CBV than households with single heads ($p < 0.01$). Also, households with educated heads had higher demand for CBV than households with uneducated heads ($p < 0.05$). This implies that enlightenment as a result of education is factor in CBV demand. Therefore, marketing strategies to be employed by CBV firms need pay attention to these facts.

CONCLUSION

Male-headed households, constituting 54.8% of the entire respondents, had an average monthly CBV demand of 150.5g while female-headed households had average monthly CBV demand of 125.6g. CBV household budget share was ₦43.43 (US$0.32) i.e. 0.40%. The

factors that significantly influenced the demand for CBV were marital status, price of cocoa beverage, own price of CBV, and price of tea beverage ($p < 0.1$). Concerted efforts need be made to popularize CBV consumption through appropriate policies so that efforts on increased coffee consumption could yield the desired dividend of improved coffee production in Nigeria. This will not only have social and economic benefits but health benefit as well.

REFERENCES

Dubois, P. 2006. Improving market conditions for coffee producers: the experience of the ICO. Paper presented at conference of World Trade Organization committee on Trade and Development. 11th May 2006. Geneva.

Osario, N. 2006. A review of coffee market situation. An address to Excorcafe conference. 3rd-5th May 2006. Mexico.

Sanusi, R. A.; Oduwole, O. O. and Lawal, J. O. 2004. Impact of marketing problems on coffee production in Nigeria. In *proceedings of 20th ASIC colloquium*. India.

Wellman, L. F. 1961. *Coffee: botany, cultivation, and utilisation*. *World crops' book*.

Polumin, N. (ed.). Leonard hill (books) Ltd, London (Pub.); Interscience publishers Inc., New York (pub.).

Prospects for Promotion of Local Coffee Consumption in Context of Increment of Manufactured Value Added Product in Africa

J. R. ONZIMA

Interafrican Coffee Organization (IACO), BP v. 210, Abidjan, Côte d'Ivoire (West Africa)

SUMMARY

Coffee is becoming the most popular and widely consumed beverage in Africa. Its awareness and popularity have increased despite controversial issues in context of its beneficial as well as harmful effect on human health. An annual output of 14.5 to 20.7 million bags is produced by the 25 member countries of IACO on an estimated 4.55 million hectares. The 5-10% of 440 million vulnerable demographic populations depends on coffee as their livelihoods. According to the bench mark estimates, it is envisaged that local consumption and coffee manufacturing process may absorb 20 to 30%, approximately 3.5 to 6.0 million bags annually to reduce production within 10 to 20 years in Africa. African countries have developed socio-economic potential to consume 2.05 to 2.42 million bags, equivalent to 125,000 to 187,000 metric tonnes per year. This is just 16% of 15.24 millions bags being produced in cropping year 2004/2005. At least 1,000 metric tonnes of coffee is consumed every year in following countries: Angola, Ethiopia, Guinea, Kenya, Democratic Republic of Congo, Nigeria and Uganda. Insignificant amount of coffee is consumed by member countries of Equatorial Guinea and Benin. Over 91,000 tonnes of Arabica coffee is manufactured locally per annum and consumed in Ethiopia, giving an average of 1.52 to 2.0 kg per head in a year. Between 1996 and 1998, the trends of coffee consumption appeared to be relatively rising, competing against beverages. The average means consumption increased by 120,000 bags per year. Findings of studies carried out in main six coffee producing countries: Cote d'voire, Cameroon, Burundi, Kenya, Uganda and Tanzania showed that more than 13,744,000 families depend on coffee for their livelihoods. Subsequently, it is economically viable to invest in coffee production as well domestic consumption including manufacturing as an added value product in Africa. Compressive Reports by the defunct APROMA Consultancy Service indicated that the most of Robusta origins are strong, neutral and sometimes mild can be used as fillers for blending purpose to make Arabica type or known as "pseudo" coffee product. The variable characteristics of origins depend on different coffee varieties, soil-climatic conditions, altitude, processing and also on farmers' socio-economic farming practices. Currently, there is a proposal designed to conduct scope studies to determine variable or identical factors in Africa in terms of establishment of factors of coffee roasting and grinding process. The result of scope will give recommendations for the origins, which can be substituted easily, while different coffees are being blended to create unique and attractive taste brands with conspicuous and eligible trade marks. Therefore, this review paper highlights initiatives taken by Interafrican Coffee Organization (IACO) and African Coffee Research Network (ACRN) including the member countries as well as other organizations to disseminate favourable socio-economic factors on coffee consumption with regards to African common origins, traceability, local blends and value-added products.

INTRODUCTION

Coffee is becoming gradually one of the most popular and widely consumed beverages in Africa. Its awareness and popularity have increased despite controversial issues in context of

its beneficial as well as harmful effect on human health. From 1970s to 1980s, an annual output of 14.5 to 20.7 million bags is produced by the 25 member countries of IACO on an estimated 4.55 million hectares. The 5-10% of 440 million vulnerable demographic populations depends on coffee as their livelihoods.

Table 1. Production of Potential Member countries of Africa – Export 2003-2005.

Member countries	Sub-region	Ranking	Average @ bag 60 kg, million	Revenue – million US Dollars
Ethiopia	East Africa (A)	1	2.511	286.3
Uganda	East Africa (A+R)	2	2.680	146.8
Cote d'Ivoire	West Africa (R)	3	2.193	119.7
Kenya	East Africa (A)	4	0.756	87.6
Cameroon	West Africa (A+R)	5	0.734	42.9
Tanzania	East Africa (A+R)	6	0.359	76.3
Burundi	East Africa (A)	7	0.355	40.2
Rwanda	East Africa (A)	8	0.345	39.3
Guinea –Conakry	West Africa (R)	9	0.322	17.6
Zambia	East Africa (A)	10	0.119	13.6
Total			10.600	870.3
Average			1.060	87.03

The result of statistical analysis (Table 1) during 2003-2005 has shown that only 10.6 million bags can be produced by ten (10) potential productive member countries in Africa. Ethiopia is currently leading under recently improved production programme in Africa. Ethiopia earned an estimated revenue of 286.3 million US Dollars. Considering the bench mark figures of export, the average production for the last three years stands at 1.060 million bags.

REASONS AND ANALYSIS OF COFFEE CONSUMPTIONS IN CONTEXT OF VALUE ADDED PRODUCTS

According to the bench mark estimates, it is envisaged that local consumption and coffee manufacturing process may absorb 20 to 30%, approximately 3.5 to 6.0 million bags annually to reduce over supplying within 10 to 20 years in Africa. African countries have developed socio-economic potential to consume 2.05 to 2.42 million bags, equivalent to 125,000 to 187,000 metric tonnes per year. This is just 16% of 15.24 millions bags being produced in cropping year 2004/2005.

At least 1,000 metric tonnes of coffee is consumed every year in following countries: Angola, Ethiopia, Guinea, Kenya, Democratic Republic of Congo, Nigeria and Uganda. Insignificant amount of coffee is consumed by member countries of Equatorial Guinea and Benin. Over 91,000 tonnes of Arabica coffee is manufactured locally per annum and consumed in Ethiopia, giving an average of 1.52 to 2.0 kg per head in a year. Between 1996 and 1998, the trends of coffee consumption appeared to be relatively rising, competing against beverages. The average means consumption increased by 120,000 bags per year. Findings of studies carried out in main six coffee producing countries: Cote d'Ivoire, Cameroon, Burundi, Kenya, Uganda and Tanzania showed that more than 13,744,000 families depend on coffee for their

livelihoods. Subsequently, it is economically viable to invest in coffee production as well domestic consumption including manufacturing as an added value product in Africa.

Compressive Reports by the defunct APROMA Consultancy Service indicated that the most of Robusta origins are strong, neutral and sometimes mild can be used as fillers for blending purpose to make Arabica type or known as "pseudo" coffee product. The variable characteristics of origins depend on different coffee varieties, soil-climatic conditions, altitude, processing and also on farmers' socio-economic farming practices. Currently, there is a proposal designed to conduct scope studies to determine variable or identical factors in Africa in terms of establishment of factors of coffee roasting and grinding process. The result of scope will give recommendations for the origins, which can be substituted easily, while different coffees are being blended to create unique and attractive taste brands with conspicuous and eligible trade marks.

Therefore, this review paper highlights initiatives taken by Interafrican Coffee Organization (IACO) and African Coffee Research Network (ACRN) including the member countries as well as other organizations to disseminate favourable socio-economic factors on coffee consumption with regards to African common origins, traceability, local blends and value-added products.

- IACO and African Coffee Research Network (ACRN) with their members and collaborating institutions had organised at least three conferences/workshops on "Coffee Private Sector Development", "Poverty Reduction in context of strengthening the regional projects" and the Regional Ministerial Conference on Coffee Crisis, which were held between 2001 and 2003; and objectives were to develop a "Framework" for integration and development of coffee industry on sustainable basis as well as answer needs of international markets;
- To refocus on Promotion of Local Consumption, manufacturing for the value added products (VAP) –CAFÉ AFRICA PROJECT in order reduce surplus, create employment and improve livelihoods of rural farmers;
- To explore opportunities for manufacturing "added – value product in terms of capital investments so as to transformation of green coffee through economic market studies (national, regional and international levels) using e.g. AU/NEPAD, ADB,AFREXIMBANK,ICO,EU,CFC, roasters assistance – Coffee Alliance and the donors;
- To incorporate the private sector development into sustainable development in context of regional and international coffee industry matters and both ICO and IACO are playing a leading role to achieve socio-economic targets;
- To encourage diversification of production systems at sustainable basis level; to access the niche markets and disseminate regularly the market research information;
- To emphasize the significance of coffee productivity and good marketable quality parameters with focus on income generation through feasible technology application and transfer through research and development and as well answer consumer demands.

The IACO endeavours to keep close co-operation between partners of ICO, international organizations, roasters, and consumers in coffee producing member countries in project development and achieving the synergies and finding solutions to routine and seasonal international coffee crisis.

Under south-south cooperation and trade agreement, the African Coffee producing countries in Africa must make a "break through" and initiate to invest in economic feasible manufacturing of current large amount of green coffee being produced for raw agricultural

commodity export into value added products for the market outlets within its region. A position paper was prepared on a framework on how to promote coffee consumption by establishing Promotion Centres in South Africa, Morocco, Tunisia, Egypt, Algeria, Libya and the Sudan with technical assistance of ACRN. Many private sectors will be involved including major ministries of Commerce/Trade and Ministry of Foreign Affairs in non-coffee producing countries, but consuming in Africa.

The Interfrican Coffee Organization has strongly refocused on training and improving human resource development. In collaborations with CIRAD and other partners, it has trained over fifty (50) participants in skilful preparation and cupping programme. Meanwhile twenty (20) women course participants successfully completed their courses and obtained the Certificates of Attendance in Promotion and Preparation of different coffee products as designed in market outlets.

Table 2. Coffee Growers' – Price Payments 2001-2004 (US cents/lb).

Member countries	Type of Coffee	2001	2002	2003	2004		Avarage
Kenya	Colombian	69,39	67,67	41,07	71,01	249,14	62,29
Tanzania	Colombian	34,86	25,93	24,78	23,2	108,77	27,19
	Other Milds						
Burundi	Other Milds	32,19	29,17	24,88	27,36	113,6	28,4
Malawi	Other Milds	169,59	237,6	185,62	164,65	757,46	189,37
Cameroon	Other Milds	19,09	24,56	35,27		78,92	26,31
Madagascar	Other Milds	12,92	14,5	35,9	46,19	109,51	27,38
Rwanda	Other Milds	23,26	17,88	24,88	29,6	95,62	23,91
Uganda	Other Milds	53,43	53,41	58,16	74,29	239,29	59,82
	Naturals						
Ethiopia	Naturals	43,78	26,88	35,94	48,51	155,11	38,78
	Robustas						
Angola	Robustas	21,07	10,71	6,61	9,8	48,19	12,05
Congo (DRC)	Robustas	81,03				81,03	81,03
Cameroon	Robustas	21,11	16,05	22,26		59,42	19,81
Central African Republic	Robustas	13,21	11,94	18,06	20,17	63,38	15,85
Cote d'Ivoire	Robustas		13,44	17,71	17,72	48,87	12,22
Gabon	Robustas	47,88	44,58			92,46	46,23
Madagascar	Robustas	10,53	11,14	19,72	16,68	58,07	14,52
Togo	Robustas	15,3	18,27	22,17	21,91	77,65	19,41
Tanzania	Robustas	5,21	4,3	6,72	6,7	22,93	5,73
Uganda	Robustas	21,93	25,49	41,24	52,54	141,2	35,3
Avarage[Mean]	Avarage-Mean	38,1	36,31	36,53	42,02		37,28

Source: ICO Website Statistics September 2005.

Last five years ago, coffee prices had basically slumped, the composite price was low and it had registered 46 cts/lb ($1.02 per kilogram); which was drastically low and consumers were only paying "peanut" prices to farmers due to over supply and increased production. As indicated in Tables 2 and 3, many farmers (growers) received significantly very low farm-gate prices for their exports. Miserably, the lowest paid registered approximately 6.61 cts/lb.

Table 3. Categories of Member Countries according to Payments to Coffee Growers. Cropping Years 2001-2004.

No.	Five (5) Potential Member Countries with Poorest and Lowest Payment to Growers	Type of Coffee	Average Price paid to Growers (cts/lb) in USD	No.	Five (5) Potential Member Countries with better and Highest Payment to the Growers	Type of Coffee	Average Price paid to Growers (cts/lb) in USD
1.	Tanzania	Robusta	5.73	1.	Malawi	Arabica	189.37
2.	Angola	Robusta	12.12	2.	Kenya	Arabica	62.29
3.	Côte d'Ivoire	Robusta	12.22	3.	Uganda	Arabica	59.82
4.	Madagascar	Robusta	14.52	4.	Ethiopia	Arabica	38.78
5.	Central African Republic (CAR)	Robusta	15.85	5.	Burundi	Arabica	28.40
	Average [Mean]		***12.07***		***Average [Mean]***		***75.73***

Source: ICO Website – 2005 and IACO/ADB Report 2003.

PRICE DIFFERENTIAL BETWEEN ROBUSTA AND ARABICA

Price Differentials between Robusta and Arabica Coffees have proved statistically variable, depending on marketing situation (demand, supply stocks) seasonal cropping systems and output of marketable good quality, earning premium prices and blending combinations in concept of using Robusta coffees as "fillers" by roasters and manufactures.

(a) July 2002 to January 2004, the average Robusta Prices again increased above the differential prices;
(b) January 2004 to January 2005, the average Robusta prices at this period again dropped below the differential prices.

(i) due to changes in socio-economic in coffee industry and taking into account the impact of liberalisation, privatization and global trade, IACO/ACRN with its member countries and collaborating institutions is reforming and reformulating its policies and functions, refocusing on to satisfy the needs of all stakeholders, especially the producers (farmers) donors, and consumers through capacity building process;
(ii) Coffee business in terms of world trade is more than fifty five ($55.0 billion USD), unfortunately Africa only earns revenue below 2.0 USD, which is below 5.0%. The impact of revenue is causing severe poverty, depraving the basic human needs and affecting livelihoods of 5-10 million rural farmers in Africa. This phenomena has caused endless coffee crisis known as "*bitter and unscrupulous coffee trade";*
(iii) As observed in many monthly reports, ICO is mobilising resources with its stakeholders to facilitate promotion of coffee consumption and it had expanded the generic consumption in Russia and Chine and many other countries;
(iv) .The significance of coffee quality improvement according to Resolution 420 was observed, diversification including Sustainable development in context of environment protection as well as observation of the Kyoto Protocol Coffee-agricultural commodity trade agreement

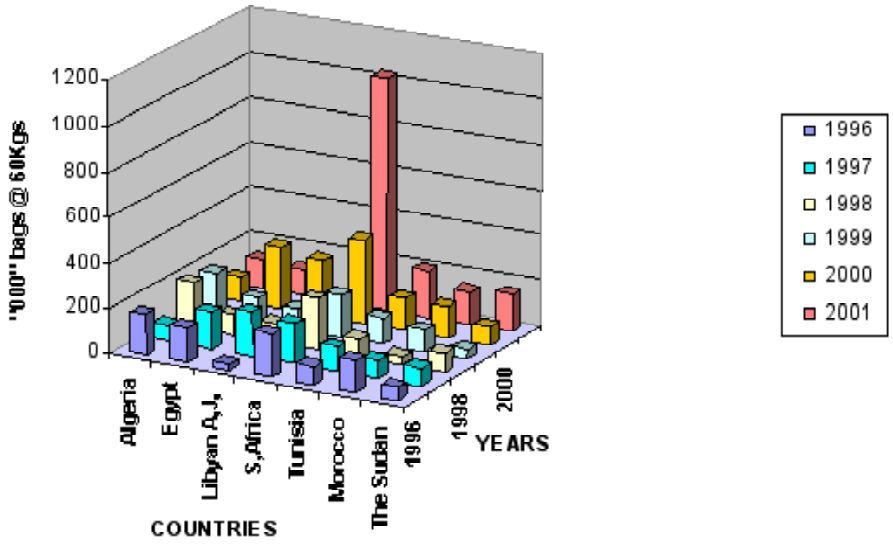


Figure 1. Coffee consumption by non producing countries in Africa. 1996-2201 "000" bags@60 kg (green Robusta & Arabica types).

According to the bench mark estimates, it is envisaged that local consumption and coffee manufacturing process may absorb 20 to 30%, approximately 3.5 to 6.0 million bags annually to reduce production within 10 to 20 years in Africa. According to ADB Appraisal report (2003), African countries have developed potential to consume 2.05 to 2.42 million bags, equivalent to 125,000 to 187,000 metric tonnes per year. This is just 16% of 15.24 millions bags being produced in cropping year 2004/2005.

Table 4. Analysis of Value Added Products – Exports of Roasted Coffees (1998-2003), million bags @ 60 kg.

Years	All Producing Countries	African Countries	% of African to all producers
1998	0.617	0.020	3.0
1999	0.159	0.050	3.1
2000	0.119	0.050	5.1
2001	0.079	0.010	2.0
2002	0.135	0.009	1.0
2003	0.114	0.002	0.0
Total	1.223	0.132	14.2
Average-Mean	0.223	0.022	2.3

Source: ICO Statistics & IACO Market Report 2005

A number of producers are currently engaged in increasing process of all ground, roasted and soluble coffees for consumption purposes and local or regional exports. In Africa, (Table 4) the average of roasted coffees for six (6) years amounted to 0.22 million bags, which is equivalent to 2.3 % to all coffee producers in the world.

At least 1,000 metric tonnes of coffee is consumed every year in following countries: Angola, Ethiopia, Guinea, Kenya, Democratic Republic of Congo, Nigeria and Uganda. Insignificant amount of coffee is consumed by member countries of Equatorial Guinea and Benin. Over 91,000 tonnes of Arabica coffee is manufactured locally per annum and consumed in Ethiopia, giving an average of 1.52 to 2.0 kg per head in a year. Between 1996 and 1998, the trends of coffee consumption appeared to be relatively rising, competing against beverages

and the average mean consumption increased by 120,000 bags per year. Findings of recent studies carried out in main six coffee producing countries: Cote d'voire, Cameroon, Burundi, Kenya, Uganda and Tanzania showed that 13,744,000 families depend on coffee for their livelihoods. Subsequently, it is economically viable to invest in coffee production as well domestic consumption including manufacturing as an added value product in Africa.

Compressive Reports (1998-2000) by the defunct APROMA Consultancy Service and Common Fund for Commodities (CFC), indicated that the most of Robusta origins are strong, neutral and sometimes mild can be used as fillers for blending purpose to make Arabica type or known as "pseudo" coffee product. The variable characteristics of origins depend on different coffee varieties, soil-climatic conditions, altitude, processing and also on farmers' socio-economic farming practices. Currently, there is a proposal designed to conduct scope studies to determine variable or identical factors in Africa in terms of establishment of factors of coffee roasting and grinding process. The result of scope will give recommendations for the origins, which can be substituted easily, while different coffees are being blended to crate unique and attractive taste brands with conspicuous trade marks. Similar to European counterparts like Kraft, Jacobs, Nestle, Elita Poliska, at least 2-4 innovative African small-scale enterprises have initiated the exchange of their products "origins" at a regional level.

INNOVATIVE APPROACHES OUT OF MAINSTREAM COFFEES

The Eastern and Central African member countries of East African Fine Coffee Association (EAFCA), private sector organization have initiated vigorous programme for improvement coffee fine quality in context earning premium prices, increasing income; and improving livelihoods.

Annually, more than six hundred (600) participants take part and witness the official opening ceremonies. Under EAFCA's training programme, many obtained the Certificates of Attendance in Promotion fine coffee and exhibition different coffee value added products, cupping as designed in market outlets.

Some of common the origins or pure "Robusta or Arabica" or mixtures (blends) are: Ethiopian Coffee, Kenyan Blue Mountains, Uganda-Bugishu Local, Côte d'Ivoire – Java, Burundi – OCIBU, Cameroon – Philiber 70% Arabica & 30% Robusta, Café-Gabon, Guinea Ziama – 100% robusta, Madagascar – Taf Café Moulu, Rwanda-OCIRA, Angola – Cafengoli, Togo – Danyi Dzigbegan, and EAFCA – Kahawa Malumu and the Zambian .At least a number of these conspicuous labelled and aromatic coffees are often commercialised through national and local market outlets in form of ground, roasted beans as well as soluble coffees.

Large coffee producers like Kenya, Uganda, Tanzania, Côte d'Ivoire have already initiated small-scale production of diversified Value-Added-Products (VAP) such as Gourmet & Speciality Coffee, Arabusta Coffee, Decaffeinated Coffee and Organic Coffee. Many of these products are not consumed locally, but many earn premium prices on world markets. Many international reports have indicated that Brazil, Colombia and India are investing significantly in promotion of coffee consumption of their own crops by 12-66%.

INDICATORS OF SOCIO-ECONOMIC BARRIERS IN COFFEE CONSUMPTION AND MANUFACTURING

- It is noticed that the Northern developed Governments and the Private Sector have been unwilling to contemplate any support to local consumption, just because oversupply means good business for political powerful trans-national companies and roasters;

- The developed countries impose import taxes and tariff duties on manufactured coffee products from least developed agricultural producing countries; and this requires consultations under WTO arrangements;
- The consumer habits in the developed countries may not be easily adjusted to the new coffee blends emerging from least developing countries;
- Low incomes and purchasing power of working class (civil society) in urban population in developing countries;
- Many producers have incoherent and inconsistent policies in collaboration with multi-corporations in agricultural and investment coffee development programmes which don't set priority in value added products.

However, dynamic activities of coffee consumption through "kiosks" or coffee shops are being communicated through magazines, radio programmes, television, cultural or sports events. There are plans to target the youth in educational institutions especially the African Universities. Recent scientific reviews by Verbalt University of USA, ASIC, ICO and ACRN with its NARS including the associated member provided "scientific proof" and positive effects of coffee consumption. Coffee consumption is very beneficial and not detrimental, but nutritious to human health. Caffeine content reinforces the human brain capacity.

CONCLUSIONS

i. It is economically viable to invest in coffee production as well domestic consumption including manufacturing as an added value product in Africa.
ii. To explore opportunities for manufacturing "added – value product in terms of capital investments so as to transformation of green coffee through economic market studies (national, regional and international levels) using e.g. AU/NEPAD, ADB,AFREXIMBANK,ICO,EU,CFC, roasters assistance – Coffee Alliance and the donors.
iii. The significance of coffee quality improvement programme, diversification including Sustainable development in context of environment protection may partly increase local farm-gate prices under private sector development and current liberalization.

REFERENCES

Appraisal Report of IACO/ADB, 2003, Abidjan, Cote d'Ivoire.

Coffee in Africa, Impacts and Issues of Low Prices Crisis, Ministerial Conference, Malobo, Equatorial Guinea, October 2002.

EAFCA, Annual Report, Kampala, Uganda, 2005.

ICO Market Reports, Letter from Executive Director, January –July 2006.

The African Coffee Research Network –ACRN , The Proposal for Development of 5-Year Network Programme, July 2001,revised [1] September 2003.

Rationalizing Control of Coffee Tree Diseases

J. AVELINO

CIRAD, UR Maîtrise des Bioagresseurs des cultures pérennes, TA 80/02, Avenue Agropolis, 34398 Montpellier Cedex 5, France

SUMMARY

The risk of an epidemic developing results from the pathogen, its host, the environment, human intervention through the cropping system, and their interactions. Disease is conventionally represented by a tetrahedron, each extremity of which is occupied by one of these factors. However, few parasite management systems are based on all these elements at the same time. For instance, cropping system is rarely taken into account. Yet, it will be shown that all the components of the tetrahedron need to be taken into account for optimum parasite management and durable control, especially the cropping system. Indeed, with knowledge of the physical, technical and biological attributes of a plantation, it is possible to assess the extent of the epidemic risk to which it is exposed, and deduce control recommendations which are both in relation to that risk and adapted to the cropping system. Some considerations on coffee rust, American leaf spot of coffee, coffee berry disease, tracheomycosis and crespera are given.

INTRODUCTION

The main challenge facing agriculture is maintaining its ability to produce in the long term. Today, its sustainability is threatened by disruptions to ecological balances, and the resulting economic and social crises. In this paper, some considerations on coffee tree diseases are proposed within a context of sustainable agriculture, emphasizing cropping practices as an important component of sustainability and durable control. First, the basic concepts of durable disease control will be presented, emphasizing cropping practices, based on examples taken from crops other than coffee. Next, those concepts will be applied to coffee rust caused by *Hemileia vastatrix*, and American leaf spot of coffee, caused by *Mycena citricolor.* Lastly, the considerations will be extended to other coffee pathogens: *Colletotrichum kahawae* (CBD), *Gibberella xylarioïdes* (tracheomycosis), and *Xylella fastidiosa* (crespera).

PESTS, DISEASES AND AGRICULTURE SUSTAINABILITY

The history of agriculture has a vast amount of disasters caused by the uncontrolled development of pests and diseases. Crop failures, famine, emigration are some of the consequences of such developments. The Irish Potato Famine caused by *Phytophthora infestans*, which was responsible for potato blight in Ireland between 1846 and 1851, is one of the best known cases. Another example, the Phylloxera, due to *Daktulosphaira vitifolia*, caused serious losses in the European grapevines from 1863 to the beginning of the twentieth century. On coffee, we can mention coffee rust in Ceylon, or tracheomycosis in Africa. Also worth mentioning is the rice brown spot epidemic, caused by *Cochliobolus miyabeanus* which was responsible for two million deaths in Bengal in 1942. The increasing use of pesticides, as crop intensification proceeded in industrialized countries, allowed them to avoid such disasters after World War II. Yet, intensive pesticide use lies behind major negative externalities, with human health problems, poisoning and diseases of users and consumers, pollution, a reduction in the populations of beneficial organisms, the emergence of secondary

diseases, or pathogen resistance to pesticides (Zadoks, 1992). These negative externalities are only partly avoided by using resistant varieties. The emergence of secondary diseases and loss of resistance are common. The loss of effectiveness of a control method is obviously detrimental to agro-ecosystem sustainability.

INTEGRATED PEST MANAGEMENT (IPM), SUSTAINABILITY AND DURABLE CONTROL

To compensate for the vulnerability of agro-ecosystems in the face of pests and diseases, it is therefore important not to bank everything on a single control method. Indeed, durable protection of agro-ecosystems requires the integration of a set of control methods in an overall strategy, in which cropping practices can play an important role. That is precisely the IPM principle. Indeed, IPM, as defined by FAO, is "the careful integration of a number of available pest control techniques that discourage pest population development" (www.fao.org). IPM increases the sustainability of agro-ecosystems by applying ecofriendly methods, by reducing pesticide use, through greater social stability given the group work involved in this strategy, and also through economic stability, due to less dependence on pesticides, which are usually costly. But it can also be said that IPM increases agro-ecosystem sustainability by promoting durable control, because it decreases the probabilities of pesticides resistance appearance, and resistance breakdown (major genes) or resistance erosion (quantitative). Indeed, by limiting the size of the pathogen populations using an appropriate combination of control methods, and especially cultural control, the evolutionary potential of a pathogen could be reduced, as the number of mutations is proportional to the size of the population (McDonald and Linde, 2002). This observation has given rise to a new resistance management concept, Integrated Avirulence Management (Aubertot et al., 2006), which consists in combining gene deployment strategies, to limit selection pressure on the pathogen, and different control methods, particularly cultural control, to reduce the size of parasite populations. This concept could be extended to quantitative resistance too.

THE DISEASE TETRAHEDRON

The reason it is possible to incorporate a multiplicity of methods into a durable control strategy is that a disease is the outcome of multiple factors, and of their interactions, against which it is possible to act in different ways. Indeed, the existence and severity of a disease are determined by the effects of a host, a pathogen, an environment, and their interactions. Disease is diagrammatically represented as a triangle. In 1979, Zadoks and Schein proposed a modification to that representation by adding a component: the actions of man, i.e. the cropping system which is able to affect the incidence and severity of diseases by its action on the host, the pathogen and the environment. The triangle became a tetrahedron following the inclusion of that fourth component. The host component concerns types of resistance, complete and partial. It also involves physiological, morphological, or even architectural aspects. The parasite component concerns the biology of the organism. It also concerns genetic aspects, virulence and aggressiveness. The environmental component concerns the climate (primarily wetness, temperature, irradiation, wind), but also the soil, its chemical and physical characteristics, its biology, climate or topography. The crop management component brings into play all the agricultural practices that affect the disease.

RATIONALIZING CONTROL OF PESTS AND DISEASES

Rational pest and disease control therefore means coherently integrating different control methods into a control strategy that is ecofriendly and respects natural balances, by reducing pesticide use. That also means applying a control strategy adapted to each plantation to its

biological, agro-ecological, and agro-socio-economic contexts, as opposed to a standardized and general recommendation. Indeed, by knowing the physical, technical and biological attributes of a plantation, it is possible to assess the extent of the epidemic risk to which one is exposed, and thereby deduce control recommendations that are both proportionate to that risk and adapted to the cropping system. That amounts to defining different recommendation domains.

IMPACT OF CROPPING PRACTICES ON ANNUAL CROPS DISEASES

The favourable or unfavourable effects of cropping practices on diseases are particularly well documented for annual plants in temperate or cold countries, such as cereals, certain legumes, and some oil crops. Krupinsky and co-workers (2002) showed how the cropping system, through its effects on the parasite, host and environment, helped to minimize the parasite risks of the main diseases on those plants in North America. Crop rotation, which enables the decomposition of infectious residues by planting non-susceptible species for a certain number of cycles, is particularly effective against soil-borne pathogens or pathogens that survive on crop residues. Crop rotation also makes it possible to maintain rich microbial activity in the soil that is antagonistic to those pathogens. Reduced tillage, compared to deep tillage, has the same favourable effect on microbial populations. That technique might also alter parasite development through its effect on the pedoclimate, by increasing moisture and buffering soil temperatures. Reduced tillage is also found to be conducive to leaf diseases due to conservation of the primary inoculum on the surface. High planting densities, nitrogen fertilization and irrigation, which increase foliage density, tend to encourage leaf diseases, by favouring propagule capture, but also by creating microclimatic conditions that are more conducive to their development. Other practices directly affect the pathogens, such as seed treatments, or the removal of volunteers and weeds that might serve as inoculum reservoirs. Lastly, yet further practices affect diseases particularly through the host. Obviously, there is the use of resistant varieties, but also varieties with less compact architecture, hence less conducive to disease development. Staggering planting dates may also make it possible to avoid the severest infection periods. Adequate nutrition, particularly with oligo elements, can also improve physiological resistance.

COFFEE RUST

There is less information on the effects of the cropping systems on tree crops diseases, and especially on coffee tree diseases. That lack of information does not mean that the effects are negligible. To be persuaded of that, one merely has to conduct an exercise on coffee rust. To understand the effects of the cropping system on that disease, it is necessary first of all to understand in what way the microclimate and the plant affect the life cycle of the fungus (Avelino et al., 2002). The main factors known to have, or assumed to have, effects on the life cycle of the fungus are rainfall and wind, which affect spore dispersal, leaf area which plays a role in spore capture, light which affects germination, leaf wetness which affects fungus germination and penetration in the leaf, temperature which affects germination, penetration and tissue colonization, fruit load and soil moisture, which affect penetration and colonization, or even stomatal density, which should logically affect penetration and sporulation, since the fungus penetrates and sporulates via a stoma. Obviously, resistance arise once close relations are established between the coffee tree and the fungus.

Although the effects of the cropping practices on coffee rust have been little documented, it is possible to say that some cropping practices are bound to influence the development of that disease. Indeed, they act on the microclimate and host factors which, themselves, act on the

life cycle of the fungus (Avelino et al., 2002). To illustrate that, the example of shade can be taken. The effects are complex and often antagonistic.

- Shade trees are able to intercept rainfall. If rainfall is moderate, the raindrops do not reach the coffee trees and do not take part in spore release and dispersal. In this case, shade has an unfavourable effect. If rainfall is heavy, the shade trees form gutters, where water builds up into larger drops. The number of impacts on the coffee trees is therefore reduced (which has an unfavourable effect on spore release and dispersal), but the impacts may be greater in some places of the plantation (which conversely has a favourable effect).
- Shade intercepts wind, which is detrimental to spore dissemination.
- When shade is not too dense, it increases the leaf mass and life span of the leaves, which is conducive to the capture of disseminated spores and the life span of lesions.
- Shading decreases the amount of light that reaches the coffee canopy, and reduced irradiation favours urediniospore germination
- Shade generally helps to keep humidity in the plantation and buffers temperatures, which is conducive to the infection process.
- Shading limits berry production during a given year, but stabilises yield levels over the successive years. Without shade, yields and coffee rust epidemics follow a biennial rhythm; with successive high and low levels. With shade, the biennial rhythm is not so marked, as yields do not reach very high levels. However yields seem to be always sufficient to render coffee leaves susceptible enough to infection.
- Stomatal density seems to be lower under shade.

It therefore appears obvious that shade has an effect on leaf rust, but it is difficult at first glance to say in which way it has an effect, as the antagonistic effects that exist are so many. The complexity is even greater when the cropping system is considered as a whole, a sum of cropping practices (Avelino et al., 2002).

To gain a clearer understanding of the impact of cropping systems on coffee rust, and particularly of shade, we conducted a survey in Honduras between 94 and 97 (Avelino et al., 2002; 2006). The survey was conducted in plots planted with dwarf varieties susceptible to leaf rust, such as Caturra or Catuai. These are varieties still widely used, as they are appreciated by the markets. It was seen that the effects of the cropping system on coffee rust were closely linked to their effects on yields, especially in the intensive system with little shade. High yields went hand in hand with high incidences and low yields were accompanied by low incidences. In plots with dense shade, as expected, yields did not reach very high levels, and leaf rust incidence did not reach levels as high as in full sunlight, but it was never low either. It could even be very high when the trees had a fruit load of more than 200 fruiting nodes, a fruit load that remains very modest. These results suggest that shade has negative effects on leaf rust by keeping yields at low levels, whilst conversely favouring leaf rust once production reaches a certain threshold, probably by favouring spore germination. This observation helps to shed light on the controversy existing as to the effect of shade on leaf rust. Indeed, some authors have reported low attack intensities under shade, whilst others have reported high incidences. Those different results can be explained by the interaction of yield with shade.

The result of that survey is a segmentation tree that can be used to define risk domains and to rationalize coffee rust control (Avelino et al., 2006). The tips of the tree branches group together plots that were similar for certain environmental, cropping practices, or coffee tree characteristics. In each group, the plots were subjected to similar leaf rust attacks. The groups can therefore be considered as different risk domains with regard to the disease. Local characteristics specific to each plantation were particularly well linked to the intensity of

coffee rust epidemics, whereas regional factors such as rainfall appeared to be of secondary importance. The yield and the number of leaves of the coffee trees were positively linked to epidemic development. Soil pH and fertilization were negatively associated with epidemic development. Shade, when it did not limit yield, probably affected the microclimate in such a way that coffee rust incidence increased. Altitude was a serious constraint in disease development.

In terms of rational leaf rust control, several uses can be made of the tree. It can be used to draw up control recommendations in line with the risks entailed. For example, the greater the risk, the more intense the chemical control will have to be. It can also be used to propose cultural control methods which, under certain conditions, could reduce the epidemic risk. For instance, a slight reduction in shade in plantations with a high productive potential, but which will not be fertilized, would make it possible to move down from a high risk situation to a medium risk situation. This tool also makes it possible to more effectively match recommendations to the agro-socio-economic conditions of the farmer. For example, farmers who do not apply fertilizers are probably modest producers who will not invest in chemical control. Cultural control and genetic control would doubtless be more appropriate in their case.

AMERICAN LEAF SPOT DISEASE

M. citricolor is a gemmiferous basidiomycete fungus confined to the American continent from which it originates. It attacks coffee trees causing high yield losses in Central America, and especially in Costa Rica. It is found on branches, leaves and fruits, and causes fruits and leaves fall. The infectious unit is the product of a mass of compact hyphae in the form of a pinhead, whose head, the propagule or gemma, comes away in contact with water. The production of carpophores is quite rare and usually occurs on dead leaves, which suggests that any participation of basidiospores in the epidemic is negligible. It is difficult to control this disease. There does not seem to be any effective genetic resistance. However, degrees of susceptibility are found. For example, the varieties derived from the Timor Hybrid seem to be much more susceptible than Caturra or Catuai. Current control methods are fungicide-based. There are few effective fungicides and control is expensive. Consequently, everything needs to be done to reduce the use of those products (Muller et al., 2004).

The results of a survey we conducted in Costa Rica in 2002 and 2003 show that attack levels for this disease were dependent on the environment and on cropping practices. We performed a Partial Least Square via Spline functions regression analysis, a kind of non-linear multivariate regression (Avelino et al., submitted). The most influential variables were in relation to plot topography (altitude, slope aspect and inclination). Low and high altitudes were unfavourable for *M. citricolor* development. Slopes favoured its development, but eastern facing slopes were clearly less affected than the others, probably because they received more sunlight in the morning (the afternoon is often cloudy). Then, came the variables that characterized the cropping system or the coffee tree (distance between rows, shade percentage, height of the coffee trees, annual number of fertilization rounds, type of shade). Rows of coffee trees spaced very wide apart gave better ventilation of the plantation. In addition, this practice probably reduces the possibilities of row by row contamination. A large number of fertilization rounds, were unfavourable conditions for *M. citricolor.* Conversely, tall coffee trees with dense shade appeared to provide moisture conditions propitious to fungus development. The most favourable shade for the disease was that provided by fruit trees or forest species which are difficult to manage.

These results suggest it will be possible to draw up a map of epidemic risks that takes into account topographical factors. Recommendations will then be made for planting densities and shading practices depending on risks due to the topography. In risk zones, an attempt will be made to increase the distances between coffee planting rows, to reduce shade and avoid shade made of fruit trees and forest species. These practices should facilitate chemical control, or even improve the expression of any partial resistance (Avelino et al., submitted).

COFFEE BERRY DISEASE

The active form of the fungus, the form that causes damage, develops during the rainy season, on fruits ranging in age from 8 to 20 weeks after flowering, between the rapid expansion and pericarp growth and endosperm formation. The severity of the disease comes from the coincidental occurrence of a particularly susceptible berry stage and a climatic period propitious to infection. After that age, infection causes another type of lesion, a dry lesion or scab, that is analogous to a resistance reaction. In the dry season, the fungus survives on mummified berries, or even on other aerial organs of the plant (Muller et al., 2004). This disease is difficult to control. Chemical control is highly restrictive. It requires numerous fungicide applications per year. It is therefore expensive. Genetic control is obviously a good solution, but the resistance in Arabica only appear to be partial and bring few genes into play (Do Ceu Silva et al., 2004). Their durability is therefore exposed. In this pathosystem, as in the case of *M. citricolor*, durable control may rely on using cultural techniques to reduce pathogen pressure, facilitate chemical control, and improve the expression of partial resistance.

To gain a clearer picture of how the cropping system affects this disease, as with leaf rust, it is useful to take a look at the different stages in the life cycle of the fungus and to see which factors are known to have an effect, or assumed to have an effect, on that cycle (Muller et al., 2004). Conidia are primarily dispersed by splashing, hence probably over quite short distances. The successful deposition of conidia on fruits must therefore logically be linked to the quantity of fruits, and the proximity of those fruits to each other. Moisture affects conidia germination and fungal penetration in the fruit. The temperature probably has an effect throughout the infection process. Temperatures of around 22 °C are conducive to germination (Nutman and Roberts, 1960). The fruit stage is very important for its colonization by the fungus. Lastly, resistance is also involved at that stage of the cycle.

As in the case of rust, shade can be examined to illustrate the effects of cropping practices. Shade effects on CBD, through its effects on rainfall, temperatures, wetness and fruit load, seem to be very similar to those on leaf rust. However, at least two additional effects can be indicated. First, in high altitudes, where CBD is common, shade can help to avoid dew formation on the coffee trees which would be detrimental to conidia germination and penetration. Moreover, with shade, fruits take longer to ripen and probably all stages of fruit development are extended, particularly the period of cherry susceptibility. In that respect, shade would appear to have a similar effect to the altitude. It should also be noted that, with or without shade, the larger the fruit load is, the longer the ripening stage takes. That is probably also true for all the fruit development stages (Vaast et al., 2006). Given the antagonistic effects of shade on CBD cycle, it is very difficult to see at first glance what impact shade has on the disease. Moreover, there are some contradictory observations. Actually, it would not be surprising to see that, as in the case with rust, the different results obtained could be explained by interactions between factors, like shade with altitude or shade with yield.

Other practices probably have effects on CBD. Using dwarf varieties ought to favour CBD development due to the strong aggregation of fruits in those varieties. Irrigation during the dry

season makes the fruit develop sooner, thereby avoiding the period most propitious to CBD development. Moreover, it is conventionally recommended to remove mummified berries in order to control the primary inoculum (Muller et al., 2004). Pruning also makes it possible to reduce attack levels somewhat, maybe because it removes infected tissues, and maybe because it improves ventilation in the plantation (Phiri et al., 2001).

These relations suggest that, as for rust and Mycena already discussed, there are ways of rationalizing CBD control. There are clearly some cultural control possibilities. But there are, above all, different risk domains, which obviously depend on the environment, but also on the plant and the cropping system. These different risk domains should lead to different control recommendations.

TRACHEOMYCOSIS

This disease affects the xylem of the coffee tree. The disease is very serious. It led to the disappearance of *Coffea excelsa* in the 40 s and 50 s. After virtually disappearing at the beginning of the 60 s, it is once again causing serious problems today on *C. canephora* and *C. arabica*, in central Africa and East Africa. Little is known about how the environment and the host affect the parasite. It is not really known how to control this disease. We are therefore a long way from being able to rationalize control (Do Ceu Silva et al., 2006; Rutherford, 2006).

However, it is believed that risk conditions exist for the development of this disease. For example, in Ethiopia disease incidence seems to depend on regions, suggesting environmental effects (Girma et al., 2001). Cropping practices also seem to affect disease development. Intensive plantations may be more subject to this sort of problem (Rutherford, 2006; Girma et al., 2001) due to the uniformity of the planting material used, high planting densities, and systematic application of pruning techniques that might facilitate tree contagion.

Today, research is resolutely geared towards identifying resistance (Do Ceu Silva et al., 2006; Rutherford, 2006). That solution promises to be effective in view of the probably low genetic diversity of the fungus which suggests a low evolutionary potential (Adugna et al., 2005). However, this aspect needs to be investigated deeply, because if it were high, it would risk jeopardizing the durability of the resistance to be brought into play. That is quite a realistic eventuality, when considering that the fungus is capable of both sexual reproduction, with ascospores, which can result in the creation of new genotypes, and asexual reproduction, with microconidia and macroconidia, which can result in the multiplication of these genotypes to a high frequency. Concerning the genotype flow, it depends on the distance of propagule dissemination which remains mostly unknown. However, human activities contribute to spread propagules to medium distances through the transportation of infected material. Moreover, the size of populations is probably large, insofar as the fungus is able to proliferate in soil, in living and dead tissues. For instance, perithecia continue to form long after trees have been killed. In that case, it would be a matter of combining resistance with other control techniques intended to reduce the size of populations and genotype flow.

CRESPERA

Lastly, one final pathosystem, a complex one, that of the xylem bacterium *X. fastidiosa*, transmitted to coffee trees by leafhoppers and which causes the disease known as "crespera" in Costa Rica and leaf scorch in Brazil (Rodríguez et al., 2001). *X. fastidiosa* is also the causal agent of PD, the Pierce Disease of grapevine, and of the CVC, the Citrus Variegated Chlorosis. The symptoms described in Costa Rica are as follows: leaf malformations, very

short internodes, aborted flowers and fruits, which obviously causes a serious reduction in yields (Rodríguez et al., 2001).

For any substantial development of the disease, the bacterium needs to multiply in great quantities inside the plant, and the vector needs to be present and multiply too (Almeida et al., 2005). In this case, the disease tetrahedron is transformed into a pyramid, with the incorporation of a new organism, the vector which, like the bacterium, is obviously sensitive to the environment, the host and the cropping systems. Actually, the system is much more complicated because there are several vectors, several pathogen host plants, and several vectors host plants. *Xylella* epidemics can be managed based on disruptions of the interactions among all these factors. The current management strategy investigated is to combine multiple tactics in order to interrupt, even partially, several interactions, which is more realistic and at lower risk than a management strategy based on a single tactic (Almeida et al., 2005).

CONCLUSION

Cropping systems partly determine the disease risk, by acting directly on the parasite, or indirectly through the plant and the environment. Moreover, if control is to be durable, it has to combine a large number of methods making it possible to act at different levels of the disease tetrahedron. Reductionist visions of disease control, either with pesticides alone or even with resistance alone, will not guarantee the sustainability sought. Cropping practices can play a substantial role in control, even if, taken individually, they may not always lead to major reductions in attack levels. It is their accumulation and their integration in a set of control methods that generally make it possible to reduce the impact of diseases. This paper suggests that it is possible to develop cropping systems that naturally limit disease levels. Even if it is difficult to transform a cropping system deeply and rapidly, a total system approach to disease management is probably the best way to get a durable control, due the multiple effects of the cropping systems on parasites. Moreover, this approach should facilitate the control through specific methods, that can be more rapidly implemented when necessary.

Developing cropping practices or cropping systems that limit disease levels is a complicated task. First, in-depth knowledge of the epidemiology of pathogens is obviously required to identify potential management options. Next, there is a problem of scale for the evaluation of these options. What is found in the necessary small factorial experiments is probably not the reflection of what happens at large scale, like plantation or regional scale. This partly explains the interest of combining factorial experiments and surveys. Finally, transforming a cropping system is not easy. There are socio-economic factors that have to be taken into account. Behind each system there is a producer with his production objectives, his economic capability, his knowledge, his risk perception. In other words, the producer himself, as decision maker, and not anymore the pathogen, is at the centre of the problematic.

REFERENCES

Adugna G., Hindorf H., Steiner U., Nirenberg H.I., Dehne H.W., Schellander K. 2005. Genetic diversity in the coffee wilt pathogen (Gibberella xylarioides) populations: Differentiation by host specialization and RAPD analysis. Journal of Plant Diseases and Protection. 112: 134-145.

Almeida R.P.P., Blua M.J., Lopes J.R.S., Purcell A.H., 2005. Vector transmission of *Xylella fastidiosa*: applying fundamental knowledge to generate disease management strategies. Annals of Entomological Society of America. 98: 775-786.

Aubertot J.N., West J.S., Bousset-Vaslin L., Salam M.U., Barbetti M.J., Diggle A.J., 2006. Improved resistance management for durable disease control: A case study of phoma stem canker of oilseed rape (*Brassica napus*). European Journal of Plant Pathology 114: 91-106.

Avelino J., Cabut S., Barboza B., Barquero M., Alfaro R., Esquivel C., Durand J.F., Cilas C. Topography and crop management are key factors for the development of American leaf spot epidemics on coffee in Costa Rica. Phytopathology (submitted).

Avelino J., Willocquet L., Savary S., 2002. Effects of crop management patterns on coffee rust epidemics. Plant Pathology. 53: 541-547.

Avelino J., Zelaya H., Merlo A., Pineda A., Ordoñez M., Savary S., 2006. The intensity of a coffee rust epidemic is dependent on production situations. Ecological modelling. 9 7: 431-447.

Do Ceu Silva M., Varzea V., Guerra-Guimarães L., Azinheira H.G., Fernandez D., Petitot A.S., Bertrand B., Lashermes P., Nicole M., 2006. Coffee resistance to the main diseases: leaf rust and coffee berry disease. Brazilian Journal of Plant Physiology.18: 119-147.

Girma A., Hulluka M., Hindorf H. 2001. Incidence of tracheomycosis, *Gibberella xylarioides* (*Fusarium xylarioides*), on Arabica coffee in Ethiopia. Journal of Plant Diseases and Protection. 108: 136-142.

Krupinsky J.M., Bailey K.L., McMullen M.P., Gossen B.D., Turkington T.K., 2002. Managing plant disease risk in diversified cropping systems. Agronomy Journal. 94: 198-209.

Lewis W.J., van Lenteren J.C., Sharad C. Phatak, Tumlinson J.H. 1997. A total system approach to sustainable pest management. Proceedings of the National Academy of Sciences USA. 94: 12243-12248.

McDonald B.A., Linde C., 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology 40: 349-379.

Muller R.A., Berry D., Avelino J., Bieysse, D. 2004. Coffee diseases. *In* : Wintgens J.N. (Ed) Coffee: growing, processing, sustainable production: A guidebook for growers, processors, traders, and researchers. Wiley-VCH, Weinheim , p. 491-545.

Nutman F.J., Roberts F.M., 1960. Investigations on a disease of *Coffea arabica* caused by a form of *Colletotrichum coffeanum* Noack. 2. Some factors affecting germination and infection, and their relation to disease distribution. Transactions of the British Mycological Society. 43: 643-659.

Phiri N.A., Hillocks R.J., Jeffries P., 2001. Incidence and severity of coffee diseases in smallholder plantations in northern Malawi. Crop Protection. 20: 325-332.

Rodríguez C.M., Obando J.J., Villalobos W., Moreira L., Rivera C., 2001. First report of *Xylella fastidiosa* infecting coffee in Costa Rica. Plant Disease. 85: 1027.

Rutherford M. A., 2006. Current knowledge of coffee wilt disease, a major constraint to coffee production in Africa. Phytopathology 96: 663-666.

Vaast P., Bertrand B., Perriot J.J., Guyot B., Génard B., 2006. Fruit thinning and shade improve bean characteristics and beverage quality of coffee (*Coffea arabica* L.) under optimal conditions. Journal of the Science of Food and Agriculture. 86: 197-204.

Zadoks J.C., 1992. The costs of change in plant protection. Journal of Plant Protection in the Tropics 9: 151-159.

Zadoks J.C., Schein R.D., 1979. Epidemiology and plant disease management. Oxford University Press, 427 p.

Disease Situation in Wild *Coffea arabica* of Ethiopia with Emphasis on the Coffee Leaf Rust, *Hemileia vastatrix*

H. HINDORF[1] AND A. ZERU[2]

[1]Institut für Pflanzenkrankheiten, Nussallee 9, D-53115 Bonn
E-mail: h.hindorf@uni-bonn.de
[2]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center,
P. O. Box 192, Jimma/Ethiopia

SUMMARY

At four habitats (Harenna in the Bale Mountains in southeast, Bonga, Berhane-Kontir and Yayu in southwest) of Ethiopia with indigenous Afromontane rainforests including wild coffee (*Coffea arabica*) disease assessments of coffee leaf rust (*Hemileia vastatrix*), coffee berry disease (*Colletotrichum kahawae*) and coffee wilt disease (*Gibberella xylarioides*) were carried out during 2003 and 2006. Coffee leaf rust occurred in all field sites with annual variations in frequency and intensity. The disease frequency ranged between 2 and 96%. Spore samples collected on leaves could be identified comparing morphological characteristics and classified into the species *H. vastatrix* (Ritschel, 2005). The mean sizes of uredinospores measured 31.6 mµ in length and 21.2 mµ in width. Scanning electron microscopic photos represent typical spore formations of the wild coffee population. Race specification revealed in races II, III and X confirmed by Varzea (pers. comm.). The coffee berry disease was present in 2005 on 18.6% of the trees at Harenna, 40% at Bonga, 6% at Berhane-Kontir and 26.3% at Yayu. The coffee wilt disease appeared on 9.7% of the trees at Harenna, 6.4% at Bonga, 2.4% at Berhane-Kontir and 16.9% at Yayu, respectively.
Key words: *Hemileia vastatrix*, *Coffea arabica*, wild coffee, disease frequency and intensity, morphology, Ethiopia

INTRODUCTION

World-wide Ethiopia is known as a country of great diversity for wild plants and agricultural crops due to favoured climatic, soil, topographic and water conditions. Of course the best known and most important cash crop coffee (*Coffea arabica*) achieved an outstanding influence in international agriculture and has its roots in the former Province of Kaffa in the southwest of the country. Ethiopia is not only the centre of origin for Arabica coffee, but the country also provides a great diversity in the last remaining rainforests, which could serve as a gene-pool for further breeding improvements. Coffee played for centuries an important role in the society of the Ethiopian people and represented a crop for social, economical, political and ecological sustainability. Therefore coffee achieves high attention in the country and will be always protected even under difficult economic conditions concerning prices on the world market at presence. The Ethiopian highlands favour with its congenial climatic conditions, fertile soils, sufficient and cheap labour capacities and good management better yields and high quality with best aromas and natural flavours. More than 50% of the coffee production Ethiopians consume on their own, preparing and honouring the crop in a ceremony unique in the world.

For centuries Ethiopian coffee selections proved to be resistant or tolerant against many diseases and pests. Grown under indigenous shade trees coffee selections adopted to stand

drought conditions and developed a certain tolerance against diseases. The few occurring pathogenic organisms and parasites facilitated best hosts for antagonists, hyperparasites and natural enemies. Coffee leaf rust (CLR) seems to be present in Ethiopia since coffee has been grown, but the quarantine disease in that country never achieved such an importance like in Asia in the 19th century (Hindorf et al., 2004).

Due to human influences during the 1970s two major diseases were introduced to the country: coffee berry disease (CBD) and coffee wilt disease (CWD) causing tremendous losses (Hindorf, 1998). Coffee Berry Disease (CBD), *Colletotrichum kahawae* causing anthracnose on fruit pulps was firstly reported on coffee berries in Kenya in 1924 and thereafter distributed by infected material to surrounding countries all over East-, South- and Central-Africa (Hindorf, 1998). The disease was introduced to Ethiopia in 1970 (Mogk, 1971) and has since been distributed throughout all coffee growing areas in the country causing here the highest losses and partly eradication of coffee. In Kenya breeding the resistant variety Ruiru 11 has solved the CBD problem locally (Omondi et al., 2001). In Ethiopia tolerant selections distributed regionally to farmers proved to control CBD at time.

Coffee Wilt Disease (CWD), *Gibberella xylarioides* (*Fusarium xylarioides*) or tracheomycosis caused in the 20s century eradicating losses to coffee species such as *C. excelsa*, and recently to *C. canephora* in Central- and East-Africa. In Ethiopia the disease was firstly reported on Arabica coffee in 1971 being introduced into the state owned farms in the south-west of the country (Mogk, 1971; Kranz and Mogk, 1973). Recent outbreaks with an alarming fast distribution in large scaled farms, the forest coffee and even in the wild populations demand high attention of growers and researchers. The fungal population was studies genetically by Adugna et al. (2001; 2005) in a number of samples from most areas of coffee cultivation, so far not including wild coffee populations.

MATERIAL AND METHODS

Disease assessments were carried out during 2004 and 2006 in four habitats with larger areas of the last remaining rainforest in the southeast and southwest of Ethiopia in an altitude range of 1 200 to 1 800 m: Harenna (Province Bale), Bonga (Province Kaffa), Berhane-Kontir (Province Banj Maji) and Yayu (Province Illubabor). Per habitat two field sites were selected not at all being cultivated by men and grown naturally. People only used these sites for picking coffee and spices during harvesting time end of the year.

The disease frequency (DF in %) for coffee leaf rust (CLR) was scored by counting diseased and healthy trees (100 trees per experimental field site). The disease intensity (DI in a scale ranging from 1-4) was scored on 100 infected branches in the experimental field site using the following scale: class 1 = no disease; class 2 = one rust pustule/leaf; class 3 = two rust pustules/leaf and class 4 = three and more rust pustules/leaf. The DI was calculated using the formula:

$$\mathbf{n_1 + n_2 \times 2 + n_3 \times 3 + n_4 \times 4 \,/\, n_1 + n_2 + n_3 + n_4}$$

n_1 = number of leaves in class 1, n_2 = number of diseased leaves in class 2, etc.

During 2003 and 2004 rust samples were brought to Germany for further diagnostic investigations on the morphology of uredinospores. Spore sizes could be measured and scanning electron microscopic (SEM) photos were taken by Dr. Anja Ritschel, Institute of Botany, University of Tübingen/Germany. In May 2005 leaf samples with active sporulating

rust pustules were collected and sent to Dr. Vitor Varzea of CIFC, Oeiras/Portugal for further investigations on races of *H. vastatrix*.

The frequency of the coffee berry disease (CBD) and coffee wilt disease (CWD) was assessed visually by counting 100 or 30-50 diseased or healthy trees per field site, respectively.

RESULTS

In all field sites of the four habitats CLR could be detected more or less frequent depending on seasonable variations. No significant differences of the disease frequency (DF) in lower (1 200 m) or higher altitudes (1 800 m) could be observed (Table 1). The infection concentrated on younger leaves of tips of secondary branches. The intensity (DI) was scored only on diseased trees showing the number of pustules on the first completely developed 4 leaves from the tip of secondary branches (Table 2). Leaves scored in class 4 showing al least 3 pustules and up to 30 had no chance to survive, they dropped lateron. The majority of leaves in the wild coffee produced only very few pustules, so that a serious loss in leaf area could be ignored. In older lesions quite common the hyperparasitic fungus *Verticillium hemileiae* could be observed prohibiting a further production and distribution of uredinospores. The actual loss in leaves was caused partly only by CLR and more often by a drought stress or insect damage like common leaf miner (*Leucoptera* spp.), serpentine leaf miner (*Liriomyza trifolii*) and skeletonizers (*Epiplema dohertyi*).

Table 1. Disease frequency (DF, %)* during 2003 and 2006 of coffee leaf rust in wild coffee.

Habitat and site	Altitude (m)	9.2003	1.2004	4.2004	8.2004	4.2005	1.2006
I Harenna 2	1 580				32,0	92.0	95.0
I Harenna 3	1 610				38,0	100	96.0
II Bonga 1	1 805	74,0			32,0	60.0	69.0
II Bonga 3	1 750	24,0	52,0		88,0	20.0	80.0
III Berh.-Kont. 2	1 200	54,0	70,4	20,0	24,0	48.0	98.0
III Berh.-Kont. 3	1 320	68,0	70,8	18,9	2,0	16.3	53.0
IV Yayu 1	1 530	53,3				82.0	99.0
IV Yayu 2	1 590	69,8		74,3	96,0	70.0	100

100 trees per site.

Table 2. Disease intensity (DI)* during 2003 and 2006 of coffee leaf rust in wild coffee.

Habitat and site	Altitude (m)	9.2003	1.2004	4.2004	8.2004	4.2005	1.2006
I Harenna. 2	1 580				1.54	2.06	3.49
I Harenna 3	1 610				1.76	2.75	3.65
II Bonga 1	1 750	1.02	3.04		2.42	1.88	1.96
II Bonga 3	1 805	2.17			1.81	1.79	2.53
III Berh.-Kont. 2	1 200	1.90	3.13	1.92	2.06	2.26	3.33
III Berh.-Kont. 3	1 320	1.79	2.95	1.94	1.11	1.93	1.56
IV Yayu 1	1 530	1.76				3.15	2.90
IV Yayu 2	1 590	2.15		2.32	3.00	2.95	3.22

**Class 1: no disease; class 2: one rust pustule/leaf; class 3: two rust pustules/leaf and class 4: three and more rust pustules.*

Spore measurements of *H. vastatrix* resulted in typical length and width of the uredinopores with average sizes of 31.61 x 21.19 mμ (Table 3). Comparing these results with defined samples from known selections like Arba Gugu or Harrar from Jimma/Ethiopia or from other countries like Indonesia and Colombia the spores showed an identical morphology (Table 3). From all field sites SEM photos were taken and partly presented in Figures 1-5.

Table 3. Uredinospore sizes of *Hemileia vastatrix* (µm).

Habitat and site	Coll. date	Length	Width	Variations
I Bale Mountains 1	8. 2004	33.7	22.1	31-36 x 21-23
II Bonga 3	1. 2004	30.3	18.9	29-33 x 18-20
II Bonga 3	5. 2004	31.8	23.3	27-37 x 20-26
II Bonga 2 (1-BA2)	11.2003	30.5	21.2	29-32 x 20-23
II Bonga 2 (3-6)	11.2003	30.1	19.8	28-31 x 18-21
II Bonga 2 (2-9)	11.2003	30.0	20.5	28-32 x 20-22
II Bonga 2 (1-BA5)	11.2003	30.9	20.4	30-33 x 20-22
II Bonga 1	5.2004	32.7	23.7	29-36 x 21-26
III Sheko 3	1.2004	32.1	19.9	30-34 x 19-21
III Sheko 3	5.2004	34.5	23.6	32-40 x 21-26
III Sheko 2	1.2004	33.2	19.5	30-36 x 18-21
III Sheko 2	5.2004	29.7	21.9	26-33 x 17-25
IV Yayu 1	5.2004	30.3	22.7	27-34 x 20-25
IV Yayu 1	11.2003	30.4	20.4	29-32 x 20-22
IV Yayu 2 (8)	11.2003	31.2	19.8	30-33 x 19-20
IV Yayu 2	5.2004	34.4	21.3	31-38 x 19-23
mean	2003/04	31.61	21.19	26-40 x 17-26
Selection Arba Gugu	9.2003	30.7	20.5	30-32 x 19-22
Selection Harrar	9.2003	31.1	20.3	30-33 x 20-21
Blawan/Indonesia	4.2003	32.4	20.0	30-35 x 18-22
Calarcá/Colombia	1998	30.8	21.0	29-33 x 20-22

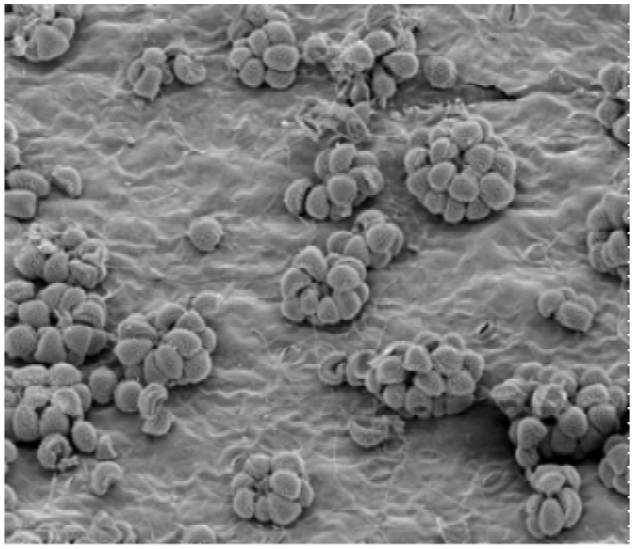


Figure 1. Lower coffee leaf site with rust pustules from Berhane-Kontir from Sheko.

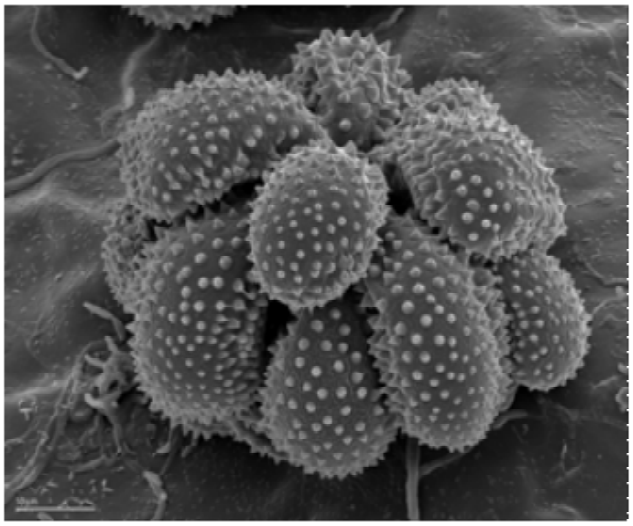


Figure 2. Uredosorus from Harenna.

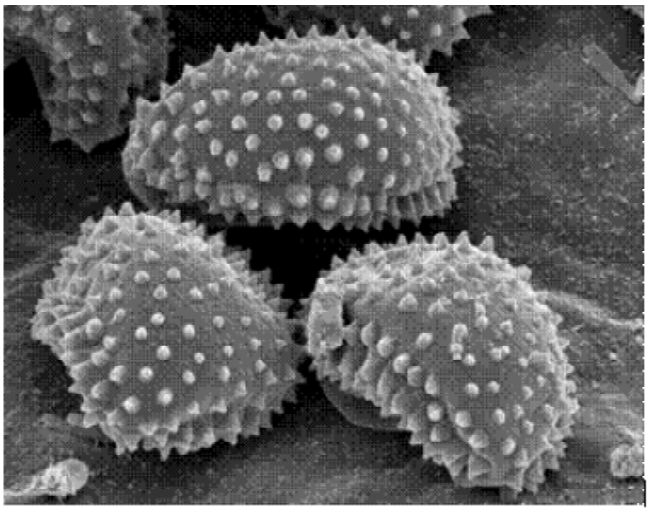


Figure 3. Three lemon shaped uredinospores from Yayu.

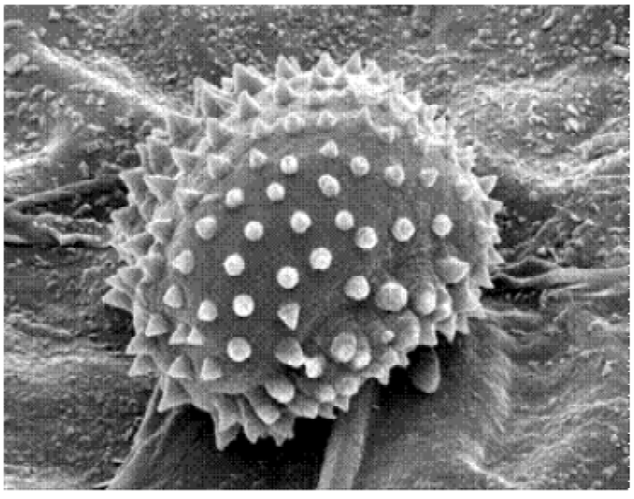


Figure 4. Single spiny uredinospore from Berhane-Kontir.

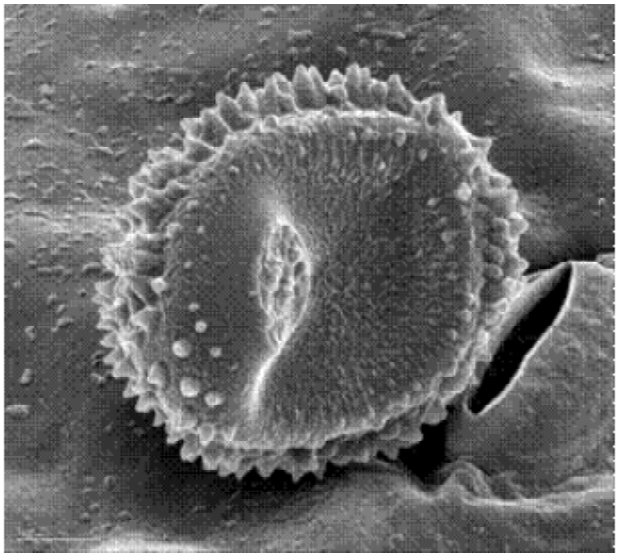


Figure 5. Uredinospore from Harenna starting germination into stoma.

Table 4. Frequency of CBD in wild coffee (2005).

Habitat	Sample site*	Altitude (m)	CBD frequency (%)	Habitat	Sample site*	Altitude (m)	CBD frequency (%)
I Harenna	1	1683	30.0	III Berh.-Kont.	1	1711	10.0
	2	1715	40.0		2	1707	20.0
	3	1556	1.0		3	1185	0
	4	1674	50.0		4	1078	0
	5	1532	0		5	1053	0
	6	1451	0	**Mean**			**6.0**
	7	1420	0	SD			8.9
Mean			**18.6**	IV Yayu	1	1482	30.0
SD			21.2		2	1721	30.0
II Bonga	1	1893	6.0		3	1495	0
	2	1872	0		4	1475	0
	3	1845	8.0		5	1469	40.0
	4	1775	10.0		6	1404	30.0
	5	1568	8.0		7	1493	30.0
	6	1663	40.0		8	1675	50.0
Mean			**40.4**	**Mean**			**26.3**

**Number of samples: 30-50 trees/site.*

In a preliminary experiment CIFC could identify rust races from a few samples collected at Bonga and Berhane-Kontir. The following races could be detected: at Bonga 1 race III (v1,5), at Bonga 3 race III (v1,5) and race X (v1,4,5), at Berhane-Kontir 2 race II (v5).

CBD and CWD could be assessed in the 4 habitats only in 2005. The lowest amount of CBD infected trees were scored at Berhane-Kontir (Table 4). In three sites of 100 trees each no CBD was present and in two sites 10 and 20% of the trees were infected, respectively. The highest infection rate occurred at Bonga with 20-60% infected trees in all sites. In general CWD could be observed in all habitats very rarely, but the disease was present in such remoted habitats (Table 5). Again the field sites at Berhane-Kontir seemed to be less infected.

In two sites of 30-50 trees each no wilted trees occurred and in three sites 2-6% of the trees were killed by the disease. The highest infection rate was scored at Bonga with 14-30% dead trees in 4 sites.

Table 5. Frequency of CWD in wild coffee (2005).

Habitat	Sample site*	Altitude (m)	CWD frequency (%)	Habitat	Sample site*	Altitude (m)	CWD frequency (%)
I Harenna	1	1683	0	III Berh.-Kont.	1	1707	2.0
	2	1715	6.0		2	1180	6.0
	3	1516	12.0		3	1080	0
	4	1531	10.0		4	1070	4.0
	5	1519	8.0		5	1053	0
	6	1476	16.0	**Mean**			**2.4**
	7	1298	16.0	SD			2.6
Mean			**9.7**	IV Yayu	1	1477	16.0
SD			5.7		2	1475	20.0
II Bonga	1	1780	6.0		3	1404	30.0
	2	1775	0		4	1471	14.0
	3	1568	8.0		5	1435	18.0
	4	1660	10.0		6	1446	0
	5	1525	8.0		7	1493	20.0
Mean			**6.4**	**Mean**			**16.9**
SD			3.5	SD			9.0

**Number of samples: 30-50 trees/site.*

CONCLUSIONS

Coffee (*Coffea arabica*) as a qualified beverage with a great demand in many countries and coffee leaf rust (*Hemileia vastatrix*) as a quarantine pathogen causing high losses have attracted world-wide high attention. Ethiopia as the source of origin for perhaps both, host and pathogen, plays an important role in science either for breeders or pathologists (Meyer, 1965). Coffee leaf rust occurs in Ethiopia in nearly all areas and under all growing types like wild, forest, garden and plantation coffee not following a certain altitude preference as in Kenya. After collecting uredinospores from wild coffee in 4 different rainforest areas of Ethiopia morphological characteristics proved that all samples could be identified as *H. vastatrix* (Ritschel, 2005). But so far the disease did not influence the production seriously. Several reasons could be responsible for that situation: First of all, since fungicides were never used, the hyperparasite *Verticillium hemileiae* occurs quite frequently and is able to reduce the inoculum to a certain threshold. Secondly the race spectrum might exist of less aggressive races. Since the work of Wondimu et al. (1987) on race specification not much has been done in the Ethiopian rust population. Therefore our investigations on races from wild coffee will result in new aspects (Varzea, pers. comm.). Thirdly some selections grown under commercial purposes originating in Ethiopia produced a reasonable resistance against the disease. And finally, the differing caffeine content from tree to tree and organ to organ can be a factor of establishing the pathogen too (Biratu et al., 1996; Medeiros et al., 1990). For further genetical, morphological and phytopathological investigations on the host and pathogen the remaining ecosystems of rainforest/wild coffee in Ethiopia urgently need to be protected. With international help there exists a strong effort to develop an agro-ecosystem

which preserves the natural rainforest including the wild coffee, but allows people to share the benefits of products in that habitat like coffee, spices and fruits.

ACKNOWLEDGEMENT

The author kindly thanks the German Ministry for Education and Research (BMBF) for financial support and the Ethiopian Institute of Agricultural Research (EIAR) for providing working facilities. Dr. Anja Ritschel, Institute of Botany, University of Tübingen/Germany carried out morphological measurements of the spore collection and took the SEM photos. Dr. Vitor Varzea, CIFC Oeiras/Portugal is at time conducting greenhouse experiments to identify the races of the *H. vastatrix* population collected from wild coffee in Ethiopia.

REFERENCES

Adugna, G.; Mengistu, H. and H. Hindorf: Incidence of tracheomycosis, *Gibberella xylarioides* (*Fusarium xylarioides*) on Arabica coffee in Ethiopia. J. Plant Diseases and Protection **108**,136-142,2001.

Adugna, G.; Hindorf, H.; Steiner, U.; Nirenberg, H.I.; Dehne, H.-W. and K. Schellander: Genetic diversity in the coffee wilt pathogen *(Gibberella xylarioides*) population: Differentiation by host specialization and RAPD analysis. J. Plant Diseases and Protection **112**,134-145,2005.

Biratu, T.; Omondi, C.O. and H. Hindorf: Caffeine content in relation to resistance of *Coffea arabica* L. to coffee berry disease (*Colletotrichum coffeanum* Noack). J. Plant Diseases and Protection **103**,15-19,1996.

Hindorf, H.: Current diseases of *Coffea arabica* and *C. canephora* in East Africa causing crop losses. Med. Fac. Landbouww. Univ. Gent **63**,861-865,1998.

Hindorf, H.; Kassahun, Y. und A. Ritschel: Krankheitssituation in der Wildpopulation von Kaffee (*Coffea arabica*) in Äthiopien unter besonderer Berücksichtigung des Kaffeerostes (*Hemileia vastatrix*). Mitt. BBA **396**,388,2004.

Kranz, J. and M. Mogk: *Gibberella xylarioides* Heim et Saccas on Arabica coffee in Ethiopia. Phytopath. Z. **78**,365-366,1973.

Medeiros, M.A.P.X.; Guedes, M.E.M. and M.L. Barros E Sousa: Has caffeine a role in the resistance of coffee to the orange rust? Proc. 13th Coll. ASIC, 733-744,1990.

Meyer, F.G.: Notes on wild coffee from south-western Ethiopia. Econ. Bot. **19**,136-151,1965.

Mogk, M.: Report on the coffee disease survey in Ethiopia. IAR, Jimma/Ethiopia, 1971.

Omondi, C.O.; Ayiecho, P.O.; Mwang'ombe, A.W. and H. Hindorf: Resistance of *Coffea arabica* cv. Ruiru 11 tested with different isolates of *Colletotrichum kahawae*, the causal agent of coffee berry disease. Euphytica **121**,19-24,2001.

Ritschel, A.: Monograph of the genus *Hemileia* (Uredinales). Bibliotheca Mycologica Vol. **200**. E. Schweizerbart, Borntraeger and Cramer Science Publishers, Stuttgart, Germany, pp. 132,2005.

Wondimu, M.; Mengistu, H. and C. Rodrigues jun.: Distribution of races of *Hemileia vastatrix* and physiologic resistance groups of *Coffea arabica* in Ethiopia. Eth. J. Agr. Sci. **9**,25-39,1987.

Tracheomycosis (*Gibberella xylarioides*) – A Menace to World Coffee Production: Evidenced by Cross Inoculation of Historical and Current Strains of the Pathogen

A. GIRMA[1], J. FLOOD[2], H. HINDORF[3], D. BIEYSSE[4], S. SARAH[5], M. RUTHERFORD[2]

[1]Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, P. O. Box 192, Jimma, Ethiopia. E-mail: gadugna@yahoo.com
[2]CABI Europe (UK), Silwood Park, Buckhurst Road, Ascot, Berks, SL5 7TA UK
[3]Institut für Pflanzenkrankheiten, Universität Bonn, Nussallee 9, D-53115 Bonn, Germany
[4]CIRAD UMR BGPI TA41/K Av. Agropolis, 34398 Montpellier Cedex 5, France
[5]CABI Africa Regional Centre, P O Box 633-00621 Village Market, Nairobi, Kenya

SUMMARY

The objectives of this study were to determine regional diversity of the coffee wilt pathogen *Gibberella xylarioides* (*Fusarium xylarioides*) strains collected from *Coffea spp*. in different countries of East and Central Africa, and to test reactions of various coffee collections to the pathogen and thereby demonstrate its pathogenic threat to world coffee production. In this regard, three independent sets of cross inoculation experiments were conducted using strains of *G. xylarioides* recently isolated from *Coffea arabica* in Ethiopia (G3P22) and *C. canephora* in Uganda (CAB003), DR Congo (RDC002) and Tanzania (TZ008, TZ009). In addition, the infectivity of a historical strain collected from *C. excelsa* in the 1960s from Central African Republic (DSMZ62457) was tested in comparison to an isolate from the same host species in the recent tracheomycosis outbreak in Uganda (OUG152). Seedlings were raised in humus substrate mixed with vermiculite from seeds obtained from *C. arabica* in Ethiopia, Kenya and Costa Rica; *C. canephora* from DR Congo and Ivory Coast; and *C. liberica*. Between 25 and 30 seedlings/cultivar were inoculated at fully expanded cotyledon leaf stage by the standard stem inoculation method with spore suspension of each strain adjusted to 2 x 10^6 conidia per ml. Seedlings treated with sterile water were included as a control. The inoculated seedlings were then kept in a growth room with a 12 hr light/dark cycle at 25 °C temperature at CIRAD, Montpellier. The number of infected (dead) and healthy seedlings was recorded fortnightly following disease development and finally percent seedling death was computed and analyzed. Re-isolations were also made to prove host-pathogen compatibility. Results showed that the Ethiopian Arabica strain G3P22 (IMI375909) severely infected (killed) seedlings of all of six Arabica cultivars obtained from Ethiopia, Kenya, and Costa Rica (severity ranging from 30 to 94%) but did not cause wilt symptoms on any of the Robusta lines. Conversely, the strains isolated from Robusta coffee, CAB003 (IMI392263), TZ008 (IMI392678), TZ009 (IMI392679) and RDC002 (IMI392268), were pathogenic to all eight Robusta coffee lines. The historical strain from Excelsa, DSMZ62457 (IMI127629) killed seedlings of two *C. liberica* lines, T-1984 and T-1872, with 80 and 94% deaths, respectively. This strain also induced some symptoms in *C. arabica* (37%) and *C. canephora* (15%) and thus the Excelsa strain has a compatible interaction with all three *Coffea* spp.. The strain isolated more recently from Excelsa, OUG152 (IMI392681), was pathogenic specifically to *C. canephora* seedlings, confirming its pathogenic fitness over time to this host. *C. liberica* showed susceptibility to strains CAB003 (95%) and G3P22 (21%). In conclusion, these results indicate host specificity of *F. xylarioides* such that strains isolated from Canephora attack *C. canephora* and strains from Arabica induce disease on *C. arabica.*

These results also confirms susceptibility of most of the commercial *Coffea* spp. which indicates that tracheomycosis is a potential threat to the world coffee production. Although the disease is currently limited to East and Central Africa where it is attacking both Arabica and Robusta coffees, these findings warrant establishment of a strong quarantine policy against this disease by every coffee producing country in the world.

INTRODUCTION

Tracheomycosis or coffee wilt disease (CWD) is a typical vascular disease incited by the fungus *Gibberella xylarioides* (*Fusarium xylarioides*) that attacks almost all species of coffee. Its first record went back to the 1920s in a plantation of *Coffea excelsa* in the Central African Republic (CAR), but many hectares of *C. excelsa* and *C. canephora* throughout West and Central Africa were decimated by this disease in the 1950s (Muller, 1997; Flood and Brayford, 1997). The same disease attacking some of these cultivars was reported in the Democratic Republic of Congo (DRC) in about 1948 where the disease quickly spread and became a serious problem across the country. It was further extended to the west, damaging Robusta coffee in Ivory Coast, before being recorded in Guinea in 1958 although earlier occurrence was suspected by Kranz (1962). Kranz (1962) noticed varietal differences in Robusta coffee, with Kouillou and Game lines being susceptible whereas Lulla and Kissi exhibited resistance to the disease in this country. Also, a Robusta line (named INEAC) remained free from infection. Kouillou was one of Robusta lines entirely wiped out by the disease during the outbreak in Ivory Coast in the early 1950s. It was emphasized by Kranz (1962) that CWD was introduced to Guinea with planting material of Kouillou obtained from nurseries in Ivory Coast. At around the same time, some lines of Robusta coffee were imported from the then Belgian Congo (DRC) into Ivory Coast and these appeared to exhibit certain levels of field resistance. Conversely, some lines of *C. excelsa* showed disease tolerance in Ivory Coast but were completely susceptible in CAR.

Since the late 1980s, tracheomycosis has reemerged as a major threat to coffee production causing serious losses of Robusta and Arabica coffees in various countries of East and Central Africa. Extensive biological surveys have shown CWD to be present in 31.4% of the 5505 visited farms across the region (CABI, 2003; Oduor et al., 2005). Highest disease incidence (90.3%) was found in Uganda with average severity 44.5%). Much lower levels were observed in Tanzania (overall incidence of 2.2%) where outbreaks of the disease have only been observed more recently. The disease was observed only in north Kivu and Oriental Provinces of DRC where the national average incidence and severity were 26.5% and 17.8% respectively. Incidence and severity of CWD in Ethiopia were 27.9% and 3.0%, respectively. CWD was not observed in Rwanda, Cameroon and Cote d'Ivoire during this survey. Socioeconomic data obtained during the survey revealed that income from coffee has declined by over 50% in the three affected countries and those annual losses at a national level attributable to CWD ranged from US$ 9,644,279 in Uganda to US$ 3,750,976 in Ethiopia and US$ 197,551 in Tanzania.

CWD has been observed only on Robusta coffee in DRC, Uganda and Tanzania, and on Arabica coffee in Ethiopia (Girma et al., 2001; CABI, 2003) suggesting some form of host specialization in the pathogen population. The reappearance and wide scale damage caused by the disease also suggests that there may have been a shift either in the host and/or pathogen populations. In this paper the regional diversity of the coffee wilt pathogen *Gibberella xylarioides* (*Fusarium xylarioides*), encompassing strains available from historical and current collections from *Coffea spp*. in West, East and Central African countries, was determined; reactions of various *Coffea* spp. to the pathogen were investigated in order to demonstrate its threat to world coffee production.

MATERIALS AND METHODS

Cross inoculation experiments were undertaken in three independent trials which evaluated *G. xylarioides* (*F. xylarioides*) strains from historical and current collections representing almost all of the regions where tracheomycosis has been a serious problem (Table 1). The reactions of the three *Coffea* spp. from various countries were tested at CIRAD, Montpellier, France.

RAISING COFFEE SEEDLINGS

Seeds from the cultivars/lines of the respective *Coffea* spp. were sown directly in a humus (Neuhaus)/ vermiculite substrate, mixed at a 1:2 ratio, in sterile plastic boxes in the glasshouse with regulated temperature of 25 ± 3 °C and relative humidity of about 80% from fog system. Optimum moisture level of the substrate was maintained for seed germination, emergence and seedling growth throughout the study period.

INOCULUM PREPARATION AND INOCULATION

Selected fungal strains were first retrieved on SNA (Nirenberg 1976) and then cultured on PDA for 10 days under light and dark cycling conditions for micro- and macro-conidia production. Inoculum suspension from each of the isolates was prepared by scraping from the agar surface in distilled sterile water, and inoculum concentration of the suspension was adjusted to approximately 2 x 10^6 conidia per ml (van der Graaff and Pieters, 1978; Girma and Mengistu, 2000; Girma et al., 2005).

Table 1. *G. xylarioides* strains of recent and historical collections from various *Coffea* spp. used in the inoculation experiments

Strain	IMI[1] accession no.	*Coffea* sp.	Geographic origin	Year of isolation
G3P22	IMI392680	*C. arabica*	Ethiopia	1997
CAB003	IMI392263	*C. canephora*	Uganda	1997
TZ008	IMI392678	*C. canephora*	Tanzania	2003
TZ009	IMI392679	*C. canephora*	Tanzania	2003
RDC002	IMI392268	*C. canephora*	DR Congo	1992
OUG152	IMI392681	*C.excelsa*	Uganda	2002
DSMZ62457	IMI127629 (BBA62457)	*C. excelsa*	Central African Republic	1955
CBS258.52	IMI392674	*Coffea* sp?	Ivory Coast	1951
CBS749.79	IMI392675 (BBA62721)	*C. canephora*	Guinea	1963
ATCC15664	IMI392676	unknown	Unknown	unknown

[1]IMI = International Mycological Institute, ATCC = American Type Culture Collection, Manasas, VA, USA; BBA = Biologische Bundesanstalt fur Land- und Forstwirtschaft, Berlin-Dahlem, Germany; CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.

The stem nicking inoculation procedure was initially used on young coffee seedlings (at fully expanded cotyledon leaf stage) being preferred to older seedlings (6-9 months old) for easy and efficient inoculation. Seedlings (25-30 seedlings/isolate) were nicked with scalpels dipped in the suspension of each strain, while 3-5 seedlings were treated with sterile water as a control. For comparison, a group of 6-8 month old seedlings of *C. arabica* (Caturra line) and *C. canephora* (Nemaya line) were each inoculated (4 seedlings/isolate) with conidial

suspension of strain G3P22 and CAB003 using a stem injection method. This involved injecting 20 µm of suspension, held in a syringe, into the lignified part of the stem above the cotyledon leaves. Inoculated seedlings were maintained in a growth room with a 12 hr light/dark cycle at 25 °C. The number of healthy and infected (dead) seedlings (i.e., developing characteristic wilting symptoms) was recorded at two weeks interval over a period of six months. Finally, samples from symptomatic and asymptomatic seedlings were collected for re-isolation and proof of host-pathogen compatibility.

RESULTS

In trial 1, Arabica isolate G3P22 (IMI392680) from Ethiopia was shown to be aggressively pathogenic to seedlings of the two *C. arabica* cvs. K7 and SL28 from Kenya, with 90 and 94% respectively of seedling deaths by termination of the trial (Table 2). Historical strain DSMZ62457, isolated from *C. excelsa* in CAR also infected seedlings of these cultivars (58 and 63% deaths, respectively). Robusta isolates CAB003 and TZ009, obtained from recent CWD outbreak areas in Uganda and Tanzania respectively, attacked the four *C. canephora* lines from DR Congo and Ivory Coast with seedling mortality ranging from 14.3 to 80.6%. Neither of these isolates induced symptoms in Arabica coffee. A number of the Robusta seedlings inoculated with the historical strain also developed symptoms (13.3% death). Similar host-pathogen combinations were observed on older seedlings inoculated with the above strains.

Table 2. Seedling deaths (%) of *Coffea arabica* and *C. canephora* inoculated with *Gibberella xylarioides* strains (Trial 1).

Coffea spp.	Coffee	Country	*G. xylarioides* strain			
	cultivars/line	of	Arabica strain	Canephora strain		Excelsa strain
		origin	G3P22 (E**)	CAB003 (U)	TZ009 (T)	DSMZ62457(C)
C. arabica	K7	Kenya	90.0	0.0	0.0	58.3
	SL 28		93.8	0.0	0.0	62.5
C. canephora	LR/R1P2(7)	DR Congo	0.0	61.3	34.5	13.3
	LR/R1P3(17)		0.0	52.9	80.6	6.9 *
	LR/R1P4(25)	Ivory Coast	5.4 *	73.3	61.9	5.6 *
	TR CI17/37		0.0	20.6	14.3	0.0

**Infection not confirmed by re-isolation (seedling death caused by other factors); **E = Ethiopia, U = Uganda, T = Tanzania, C = Central African Republic*

In Trial 2, Arabica isolate, G3P22, specifically infected seedlings of the two Arabica coffee *cvs*. 7454 and 74165, obtained from Ethiopia, but did not induce symptoms on *C. canephora* lines (Table 3). Conversely, these Arabica cultivars were not infected by any pathogen strains from Robusta coffee, namely CAB003 (IMI392263), TZ008 (IMI392679) and RDC002 (IMI392268). Strain OUG152 (IMI392681), isolated from symptomatic Excelsa coffee at Kituza, Uganda, caused up to 65% seedling death in the *C. canephora* lines but did not induce wilting on the Arabica coffee.

In Trial 3, the Arabica isolate, G3P22, induced severe symptoms in seedlings of the two *C. arabica* accessions known as Yemen/Java and E-238 received from Costa Rica with 91 and 78% seedling death, respectively (Table 4). Seedlings of *C. liberica* lines, T-1984 and T-1872, from the same country were susceptible to the historical strain, DSMZ62457, and the

current Robusta isolate, CAB003. The characteristic wilting symptoms appeared on these strain-coffee line combinations within less than 30 days of inoculation, and most of the seedlings collapsed at cotyledon stage. Accession T-1872 also exhibited moderate susceptibility to the Arabica isolate. In all cases, control seedlings showed no wilting symptom. Successful re-isolation of *F. xylarioides* from plant tissues of the majority of inoculated seedlings confirmed the host-pathogen compatibility.

Table 3. Seedling deaths (%) of *Coffea arabica*, and *C. canephora* inoculated with *G. xylarioides* strains (Trial 2).

Coffea spp.	Coffee	Country	*G. xylarioides* strain				
	cultivars/line	of	Arabica strain	Canephora strain			Excelsa strain
		origin	G3P22 (E**)	CAB003 (U)	TZ008 (T)	RDC002 (C)	OUG152 (U)
C. arabica	7454	Ethiopia	53.3	0.0	0.0	0.0	0.0
	74165		30.0	0.0	0.0	0.0	0.0
C. canephora	TR CII7/1	Ivory Coast	4.0 *	63.0	48.1	59.3	65.4
	TR CI17/16		8.3 *	21.7	38.5	46.2	40.9

**Infection not confirmed by re-isolation (seedling death caused by other factors; **E = Ethiopia, U = Uganda, T = Tanzania, C = Democratic Republic of Congo*

Table 4. Seedling deaths (%) of *Coffea arabica*, *C. canephora* and *C. liberica* inoculated with *G. xylarioides* strains (Trial 3).

Coffea spp.	Coffee	Country	*G. xylarioides* strain		
	cultivars/line	of	Arabica strain	Canephora stain	Excelsa strain
		origin	G3P22 (E**)	CAB003 (U)	DSMZ62457 (C)
C. arabica	Yemen/Java	Costa Rica	90.9	0.0	4.2 *
	E-238		77.8	0.0	37.0
C. canephora	KR 10/7	DR Congo	0.0	51.9	15.4
	KR 15/4		0.0	42.3	0.0
C. liberica	T-1984	Costa Rica	5.0 *	100	80.0
	T-1872		21.1	95.8	94.4

**Infection not confirmed by re-isolation (seedling death caused by other factors; **E = Ethiopia, U = Uganda, T = Tanzania, C = Central African Republic*

Arabica coffee collections of 120 accessions, introduced and established in three replicated plots (16 trees/plot) in the field at Gera, Ethiopia, were seriously devastated by CWD after about 10 years (Table 5). Subsequently, 50 surviving accessions were replanted in two blocks (10 trees/plot) for the second time in the same field, on which the disease developed a year after transplanting and damaged the young trees in the manner observed previously. The average tree deaths during the former and the latter epidemics were 56.8 and 37.3%, respectively.

Table 5. CWD incidence (mean %) on some International collections of Arabica coffee in the field at Gera, Ethiopia.

Coffee Lines	Description	Incidence (% tree death)*		Mean
		Batch I (1981-1991)	Batch II (1992-2001)	
79201	Typica	36.7	60.0	48.4
79203	Surinam	50.0	60.0	55.0
79205	Murta	27.0	35.0	31.0
79206	S.1 Arbagougou	9.1	35.0	22.1
79208	Mizan Teferikela	66.7	45.0	55.8
79210	Mundo Novo	38.9	75.0	57.0
79211	S-2-B Enneria	77.8	35.0	56.4
79212	Nicaragua	100.0	40.0	70.0
79214	Togokuma	80.0	10.0	45.0
79215	Dilla (Melville)	81.1	15.0	48.1
79216	Dale (Melville)	53.3	10.0	31.6
79217	S-17 Yirgalem	100.0	25.0	62.5
79218	Rume Sudan	100.0	40.0	70.0
79219	Java noun	57.7	40.0	53.4
79220	Kenya	100.0	35.0	64.1
79221	Kouti	66.7	30.0	52.5
79222	N Kougam	93.	20.0	41.6
79223	Martinique	50.0	30.0	40.0
79224	Barbarida	21.0	45.0	33.0
79225	Las Palmas	50.8	75.0	62.9
79226	Puerto Rico	69.0	25.0	47.0
79229	S-134-S12-Kaffa	16.7	35.0	25.8
79230	Bourbon	50.0	10.0	30.0
79232	Sumatra	76.2	30.0	53.1
79233	Blue mountain	72.2	30.0	51.1
79238	S-2129 Agaro	41.7	30.0	35.8
79239	Gaudaloupe	41.7	30.0	35.8
79242	Ainamba Kaffa	50.0	60.0	55.0
79243	Badabuna-Jimma	16.7	40.0	28.3
79245	Bourbon Vermelho	50.0	50.0	50.0
79249	K7 (Arabica)	66.7	45.0	55.8
79252	Mysore	74.6	45.0	59.8
79256	Mokka	31.1	80.0	55.6
79260	San Ramon	34.9	35.0	34.9
79262	Bourbon Mayaguez	52.4	15.0	33.7
79267	Reunion	95.2	25.0	60.1
79269	Colombia	65.2	35.0	50.1
79270	Pretoria	58.3	55.0	56.6
79280	F-59 local	38.1	35.0	36.6
Mean		56.8	37.3	47.1

**Cumulative coffee tree death for the years indicated.*

DISCUSSIONS

The three cross inoculation trials showed that, with the exception of the historical strain, host specialization occurs in the *G. xylarioides* population. Those strains collected from *C. arabica* were pathogenic to Arabica coffee cultivars obtained from Ethiopia, Costa Rica and Kenya. The strains isolated from *C. canephora* trees in the recent outbreak countries of DR Congo, Uganda and Tanzania were aggressively pathogenic to all the lines of *C. canephora* collected in Ivory Coast and DR Congo. Girma at al. (2005) reported a similar host specific interaction where 10 Arabica isolates were specifically pathogenic to seedlings of five *C. arabica* cultivars but non-pathogenic to *C. canephora*, and *vis-versa*

The recent strain OUG152 (IMI392681) isolated from *C. excelsa* in the clonal garden at Kituza, Uganda, also induced disease in *C. canephora* lines but did not affect *C. arabica*. Excelsa strains, OUG151, OUG154 and OUG155, isolated from the same host species known as "bwamba" showed varying levels of seedling death on *C. canephora* (1331) (D. Bieysse, *unpublished data*). On the other hand, the historical Excelsa strain, DSMZ62457 (IMI127629), originally collected from *C. excelsa* trees in Central African Republic in 1955, induced typical CWD symptoms and exhibited a wide range of infectivity on *C. arabica*, *C. canephora* and *C. liberica*. Pathogenicity testing of other historical strains ATTC15664 (IMI392676) and CBS749.79 (IMI392675) collected from *C. canephora* in Ivory Coast, and CBS258.52 (IMI392674) isolated from an unknown *Coffea* sp. in Guinea caused death of 10, 30 and 35% of plants of *C. canephora* (1406) respectively (D. Bieysse, *unpublished data*). These results suggest that historical strains collected during the 1960s remained aggressively pathogenic to *Coffea* spp. after 40-50 years of preservation. Although this finding needs to be supported by testing on seedlings of the same host species from which it was originally isolated, the infection of Excelsa coffee trees was reported in the field. As already hypothesized by Girma et al. (2005), the compatibility and infection of *C. canephora* by Excelsa strains implies that the *G. xylarioides* population presently causing CWD outbreaks in DR Congo, Uganda and Tanzania, may have arisen from older populations in Central African Republic or, alternatively, that a separate divergent population is evolving on *C. excelsa* in nature. Based on the results of host-pathogen interaction accompanied by RAPD analysis, Girma et al. (2005) introduced the epithet *formae speciales*, *Gibberella xylarioides* f. sp. *abyssiniae* (anamorph: *Fusarium xylarioides* f. sp. *abyssiniae*) for strains attacking only *Coffea arabica* and confined to Ethiopia; and *Gibberella xylarioides* f. sp. *canephorae* (anamorph: *Fusarium xylarioides* f. sp. *canephorae*) for strains specifically pathogenic to *C. canephora* and *C. excelsa.* Based on molecular studies Rutherford (2006) grouped strains of the fungus obtained since re-emergence of the disease into variants 'A' and 'C'. More broadly, Geiser et al. (2005) reported that strains of *G. xylarioides* belong to the African clade of *G. fujikuroi* species complex.

The present cross inoculation results also indicate that Arabica accessions in other parts of the world are highly susceptible to the CWD pathogen, while the long term field observations at Gera (Ethiopia) also suggest that almost all of the introduced Arabica accessions were susceptible to the disease. Although the level of host reaction observed in the local coffee germplasm was variable, Caturra and Cataui lines also showed high levels of susceptibility response to *G. xylarioides* both in seedling tests and in the field (Girma et al., 2001, 2005; Girma and Chala, in press). Indian lines S1934, S288, S795, S947 and S952 were particularly susceptible in the field (van der Graaff and Pieters, 1978). Arabica cultivars such as Kents, S795, S2828, Cauvery (Catimor) in India; SL28, Catimor and Ruiru 11 with high levels of resistance to CLR and CBD and higher yield potential have been replacing the traditional varieties in Kenya, Colombia, Brazil, and Central American countries (van der Vossen 2005)

but could be susceptible to CWD and should be tested for their susceptibility/ resistance to this disease.

Many coffee diseases have a world wide distribution. Coffee leaf rust (*Hemileia vastatrix*), for example, has spread to all coffee producing countries since its catastrophic outbreak on Arabica coffee in Ceylon (Sri Lanka) between 1868 and 1888. Coffee berry disease (*Colletotrichum kahawae*) has also caused heavy crop losses since 1922 but remains confined to Africa. The reemergence of tracheomycosis in DR Congo and its very rapid spread to Uganda and then to Tanzania is another threat to the coffee industry. *G. xylarioides* produces stromatic perithecia containing large number of viable ascospores in the field that, with high germination rates (90-95%), can disseminate to new areas through various agencies (Girma et al., 2005; Flood, 1997). Any part of infected coffee trees (roots, stems and branches) can serve as survival organ and sources of inoculum for infection (Girma et al., 2001).

As experienced in other soil borne diseases and vascular pathogens, long term and successful management of tracheomycosis will be only effective through the deployment of resistant coffee cultivars/lines and in some countries in Africa affected by the disease breeding programmes are underway. Screening a wide range of accessions as well as wild *Coffea* may result in some resistance to the disease being identified. In addition to developing CWD resistant cultivars and designing efficient breeding programmes for the future of coffee growing in the regions of Africa that are currently affected by the disease, it is also necessary to raise awareness about effective quarantine measures in countries neighbouring the affected countries and even beyond. Given that much of the Arabica germplasm worldwide is susceptible to the disease, then an effective quarantine policy needs to be established by coffee producing countries to reduce the risks of spread of this disease.

ACKNOWLEDGEMENT

We are very grateful to the Department for International Development (DFID) for providing financial support for this research, undertaken at CIRAD, Montpellier. We would like to thank B. Janzac, K. Bonnemayre, V. Roussel, O. Trochme for their welcoming assistance while conducting the experiment.

REFERENCES

CAB International (CABI). 2003. Surveys to assess the extent and impact of coffee wilt disease in East and Central Africa. Final technical report. CABI Regional Centre, Nairobi, Kenya. 49 pp.

Flood, J. and Brayford, D. 1997. Reemergence of Fusarium wilt of coffee in Africa. *In*: Proceedings of 17th International Scientific Conference on Coffee Science (ASIC), 20-25 July 1997. Nairobi, Kenya. Pp 621-627.

Geiser, D. M., Lewis Ivey, M. L., Hakiza, G., Juba, J. H. and Miller, S. A. 2005. *Gibberella xylarioides* (anamorph: *Fusarium xylarioides*), a causative agent of coffee wilt disease in Africa, is a previously unrecognized member of the *G. fujikuroi* species complex. *Mycologia* 97:191-201.

Girma A., H. Hindorf, U. Steiner, H. I. Nirenberg, H-W. Dehne. 2005. Genetic diversity in the coffee wilt pathogen (*Gibberella xylarioides*) populations: Differentiation by host specialization and RAPD analysis. *In*: Proceedings of 20th International Scientific Conference on Coffee Science (ASIC), 11-15 July 2004, Bangalore, India. pp 1222-1230.

Girma, A. and Hindorf, H. 2001. Recent investigation on coffee tracheomycosis, *Gibberella xylarioides* (*Fusarium xylarioides*) in Ethiopia. *In*: Proceedings of 19th International Scientific Conference on Coffee Science (ASIC). 14-18 May 2001. Trieste, Italy.

Girma, A. and Mengistu, H. 2000. Cultural characteristics and pathogenicity of *Gibberella xylarioides* isolates on coffee. Pest Mgt. Journal of Ethiopia **4**: 11-18.

Kranz, J. 1962. Coffee diseases in Guinea. FAO Plant Protection Bulletin **10 (4):** 107-109.

Muller, R. A. 1997. Some aspects of past studies conducted in Western and Central Francophone Africa on coffee tracheomycosis. *In*: Proceedings of the first regional workshop on coffee wilt disease (tracheomycosis). 28-30 July 1997. International Conference Centre, Kampala, Uganda. (Hakiza, G. J., Birkunzira, B., Musoli, P., eds.). Pp 18-30

Oduor, G., N. Phiri, G.J. Hakiza, M. Abebe, T. Asiimwe, D.L. Kilambo, A. Kalonji-Mbuyi, F. Pinard, S. Simons, S. Nyasse, I. Kebe. 2005. Surveys to establish the spread of coffee wilt disease, *Fusarium* (*Gibberella*) x*ylarioides*, in Africa. *In*: Proceedings of 20th International Scientific Conference on Coffee Science (ASIC), 11-15 July 2004, Bangalore, India. pp 1252-1255.

Rutherford, M. A. 2006. Current knowledge of coffee wilt disease, a major constraint to coffee production in Africa. *Phytopathology* 96:663-666.

Van der Graaff, N. A. and Pieters, R. 1978. Resistance levels in *Coffea arabica* L. to *Gibberella xylarioides* and distribution pattern of the disease. Neth. J. Pl. Path. 84: 117-120.

Van der Vossen, H.A.M. 2005. Organic coffee production: Myth or reality - a review. *In*: Proceedings of 20th International Scientific Conference on Coffee Science (ASIC), 11-15 July 2004, Bangalore, India. pp 960-983.

Characterization of *Colletotrichum kahawae* Diversity

A. LOUREIRO[1], V. VÁRZEA[1], L. GUERRA-GUIMARÃES[1], A. RIBEIRO[1], M.C. SILVA[1], B. BERTRAND[2]

[1]Instituto de Investigação Científica Tropical (IICT) – Centro de Investigação das Ferrugens do Cafeeiro (CIFC), Quinta do Marquês, 2784-505 Oeiras, Portugal
[2]Centre De Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), 2477, Avenue de val Montferrand, BP 5035, Montpellier, France

SUMMARY

A major threat to the production of *Coffea arabica* in Africa is the coffee berry disease (CBD), caused by the fungus *Colletotrichum kahawae* Bridge and Waller. Crop losses can reach more than 50% if no control measures are applied. The characterization of *C. kahawae* diversity has been carried out at morphocultural, pathogenic, biochemical and molecular levels. Morphocultural studies made in 13 *Colletotrichum* isolates confirmed the results obtained by other authors where differences in fungal growth rates at different temperatures were found. Preliminary pathogenic and aggressiveness tests (made with isolates grown at different temperatures) revealed that the growing temperature of the fungal isolate is a factor that interacts with its ability to infect the green berries. Thus, some isolates grown at 10ºC and 15 °C were more aggressive than the isolates grown at 22 ºC. Previous molecular studies using RAPD, ITS and IGS techniques did not show polymorphism within these isolates. The isoenzymatic characterization of the same isolates, based on the activity of esterase, acid and basic phosphatase and peroxidase was done using the techniques of isoelectric focusing electrophoresis (IEF) and polyacrylamide gel electrophoresis (PAGE). For both techniques it was possible to detect a high esterase activity with many bands per isolate while the activity of basic and acidic phosphatase, and peroxidase was lower, showing few bands per isolate. However all the enzymes revealed polymorphisms. The extent of isoenzymes polymorphisms found in *C. kahawae* provide a useful tool to study the genetic and diversity of this fungus.

INTRODUCTION

Coffee is one of the most valuable primary products in world trade. Its cultivation, processing, trading, transportation and marketing provide employment for millions of people worldwide. In many of the world's Least Developed Countries, exports of coffee account for a substantial part of their foreign exchange earnings (ICO – International Coffee Organization – http://www.ico.org/coffee_story.asp).

Colletotrichum kahawae sp. nov. (Waller and Bridge, 1993) is responsible for coffee berry disease (CBD) in Arabica coffee at high altitudes, in Africa. This disease can induce crop losses of more than 50% if no control measures are taken. Traditional methods for identification and characterization of *Colletotrichum* spp. have been based on morphological differences (colony color, size and shape of conidia, growth rate, presence or absence of setae, etc) (Freeman et al., 1998). However, the high variability of *Colletotrichum* spp. under culture conditions makes this criteria not adequate for reliable differentiation among the species (Afanador-Kafuri et al., 2003; Freeman et al., 1998). For other part, the knowledge of the genetic diversity of the populations is necessary to understand how populations will evolve in response to different control strategies (MacDonald, 1997). DNA-based molecular markers such as species-specific polymerase chain reaction (PCR) primers, random amplified

polymorphic DNA (RAPD) and other methods have increased the potential to detect and measure variability among individuals (Afanador-Kafuri et al., 2003; Freeman et al., 1998; Sreenivasaprasad et al., 1993). On the other hand, isoenzymes analysis have been also used to study variation among several species of C*olletotrichum* (Bonde et al., 1991) to differentiate closely related species within *Fusarium* (Mohammadi et al., 2004) and to detect genetic diversity within and among nematode species (Andrés et al., 2001). We propose to study *C. kahawae* diversity at pathogenic, molecular and biochemical levels.

MATERIAL AND METHODS

Growth rate

The growth rate of *C. kahawae* isolates from Angola (Ang 6), Ethiopia (Eti 17), Malawi (Mal 2), Rwanda (Rua 1), Tanzania (Tanz 1), Kenya (Que 2, Que 48, Que 70, Que 71, Que 72), Cameroon (Cam 1), Zimbabwe (Zim 1) and one isolate of *C. gloeosporioides* from China (Chi 1) was recorded from cultures grown on malt extract agar (Oxoid) plates. The isolates were incubated in darkness at different temperatures (10, 15, 20, 25, 30 and 35 ºC) and the diameter of the colony was recorded daily during 8 days (8 replicates and 2 measures per replicate). Data were analysed using analysis of variance and Scheffe's multiple range test.

Aggressiveness - green berries inoculation

Green berries of the variety Caturra were inoculated with the same isolates referred above (grown at 10, 15, 20 and 25 °C), according to the technique described by Van der Vossen et al. (1976) with slight modifications. The green berries were placed on trays lying down on a nylon sponge and then inoculated with a conidia suspension (2 x 10^6/ml). Covered trays were placed in a Phytotron 750 E at 22 ºC incubated the first 24 h in the dark and then kept with a photoperiod of 12 hours. The lesion diameter was measured 10 days after inoculation.

DNA extraction and PCR amplification

Eight *Colletotrichum* isolates of the 13 studied above were used for the diversity study, namely, Ang 6, Eti 17, Mal 2, Rua 1, Tanz 1, Que 2, Que 48, Zim1 and Chi1. Three techniques were performed - RAPD-PCR (random amplified polymorphic DNA polymerase chain reaction), ITS (internal transcribed spacer) and IGS (intergenic spacer). The total DNA was extracted following the method of Raeder and Broda (1985). RAPD amplifications were carried out in 25µl reaction mixtures containing 50 ng of genomic DNA, 1X PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 3mM $MgCl_2$, 0.2 mM dNTP's, 25 ng ten base primers (OPA-1,2,4; OPC-3,4,7-9; OPD-1-12,15-20, OPF-1-20 Operon Technologies), 1unit (U) Taq DNA polymerase. The PCR mixtures were subjected to a 2 min denaturation at 94 ºC, 40 cycles of 1 min at 94 ºC, 1 min at 35 ºC, 2 min at 72 ºC and a final extension step of 5 min at 72 ºC on a Thermal cycler (PTC-100, MJ Research). Universal PCR primers were used for amplification of the ITStotal, ITS1, ITS2 and IGStotal, IGS1 and IGS2 regions between the small and large nuclear rDNA. Amplifications were carried out in 25µl reaction mixtures containing 50ng of genomic DNA, 1 X PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTP's 25 pmol/µl Primer forward, 25 pmol/µl Primer reverse, 1unit (U) Taq DNA polymerase. The PCR mixtures were subjected to a 2 min denaturation at 94 ºC, 30 cycles of 1min at 94 ºC, 1 min at 48/50 ºC, 2 min at 72 ºC and a final extension step of 5 min at 72 ºC on a Thermal cycler (see above). All PCR products (15µl) were checked on 1% (W/V) agarose gels supplied with 0.1µgml^{-1} EtBr (Sigma).

Amplification products of ITS and IGS regions were purified with the High Pure PCR Product Purification kit (Roche). For digestion, performed during 3h at 37 ºC, 1µg of DNA, reaction buffer, 1unit enzyme and H_2O MiliQ were joined in a final volume of 10 µl. The enzymes tested were *Bam* HI, *Eco* RI, *Hae* III, *Hha* I, *Hind* III, *Sal* I, *Sma* I. Digestion products (5 µl) were checked on 1% (W/V) agarose gels as described before.

Isoenzymes

Protein extraction

Enzymatic extracts were obtained from the 13 isolates referred above. The fungi were grown in 50 ml of liquid medium (malt extract 30% and peptone 5%) for 10 days at 25 ºC without agitation. Mycelium was collected on Whatman nº1 filter paper by vacuum filtration and washed several times with dH_2O and then homogenised with acetate buffer (0.05 M, pH 4.5) with a porcelain mortar and pestle. The homogenate was centrifuged at 13000 rpm for 1 hour at 4º. The supernatant was collected, dialyzed against water at 4 ºC over night and concentrated in polyethylene glycol (6000). The extract obtained was stored at –80 ºC until used for electrophoresis. The protein content of each sample was determined according to the Bio-Rad protein assay kit.

Electrophoresis

Laemmli (1970) discontinuous system with 4% (w/v) stacking and 10% (w/v) resolving polyacrylamide gels and by isoelectric focusing electrophoresis (IEF) in a vertical slab according to Robertson et al. (1987), with 5% (w/v) polyacrylamide gel and 2% ampholytes (various pH). After running, the gels were incubated in different substrate solutions according to the enzyme under study: esterases, acid and basic phosphatases and peroxidase (Vallejos, 1983).

Data analysis

Data matrices from enzyme patterns (by IEF) were formed by identifying the presence (1) or absence (0) of a particular band. A genetic similarity matrix based on Jaccard[1] coefficient was calculated. A phenogram based on the estimate similarity coefficients was constructed by UPGMA (unweighed pair group methods analysis) using the computer software package NTSYS-pc version 2.02 (Rohlf, 1997).

RESULTS AND DISCUSSION

Growth rate

The incubation temperature affected the growth rate of *Colletotrichum* spp. (Table 1). For all the isolates studied the higher growth rate was observed at 25 ºC. At 25 ºC, 30 ºC and 35 ºC it was possible to separate *C. kahawae* from *C. gloeosporioides* being the best temperature to distinguish them 30 ºC. As it can be observed on Table 1, only isolate Chi1 (*C. gloeosporioides*) had the ability to grow at 35 ºC. These results confirmed the results obtained by other researchers (Chen, 2002; Hindorf et al., 1997; Manga, 1999).

[1] $S_J=a/(a+b+c)$ where *a* are bands present in both isolates being compared, *b* are bands present only in the first isolate and *c* are bands present only in the second isolate.

For each temperature, it was possible to find differences between *C. kahawae* isolates. The analysis of variance made showed significative differences between isolates, temperatures and also interaction between temperature x isolates (Table 2). It is important to note that the performance of isolates could be specific and need to be defined for each one.

Table 1. Growth rates of *C. kahawae* and *C. gloeosporioides* isolates in MEA, at different temperatures.

Isolates	Growth rate (mm)					
	10 ºC	**15 ºC**	**20 ºC**	**25 ºC**	**30 ºC**	**35 ºC**
Ang 6	1,8±0,1 bcde	2,7±0,2 bc	6,1±0,4 de	9,4±0,5 f	2,5±0,8 bc	0 a
Cam 1	2,4±0,2 e	2,9±0,1 bc	7,1±0,2 fg	9,2±0,2 f	2,3±0,3 b	0 a
Eti 17	2,3±0,4 e	3,2±0,7 bc	5,6±0,2 bcd	7,3±0,5 cde	4,0±0,2 efgh	0 a
Mal 2	1,9±0,6 cde	3,5±0,6 c	6,4±0,2 ef	8,1±0,2 e	3,6±0,2 efgh	0 a
Rua 1	2,3±0,6 de	3,0±0,3 bc	5,6±0,4 cd	7,6±0,2 cde	4,1±0,3 fh	0 a
Tanz 1	1,2±0,2 abc	1,0±0,4 a	5,5±0,7 bcd	7,9±0,4 de	0,9±0,1 a	0 a
Zimb 1	2,4±0,3 e	3,2±0,4 bc	5,9±0,2 de	8,1±0,1 e	3,8±0,3 efgh	0 a
Que 2	1,8±0,2 bcde	3,0±0,3 bc	6,6±0,3 ef	8,0 ±0,1 e	2,8±0,1 bcd	0 a
Que 48	1,2±0,6 ab	1,2±0,2 a	4,2±0,5 a	7,1±0,4 bcd	3,1±0,3 cdef	0 a
Que 70	2,1±0,6 de	2,5±0,2 b	4,8±0,1 ab	6,3±0,3 ab	3,4±0,2 defg	0 a
Que 71	1,6±0,2 abcd	2,5±0,1 b	5,0±0,4 abc	6,3±0,4 a	3,3±0,3 cde	0 a
Que 72	1,0±0,2 a	1,0±0,3 a	4,5±0,2 a	7±0,5 abc	2,7±0,2 bcd	0 a
Chi 1	2,3±0,2 de	3,5±0,4 c	7,5±0,1 g	10,5±0,4 g	9,1±0,3 i	1,3±0,4 b

In each column, the mean values followed by the same letter did not differ significantly according to the Scheffe's test (P≤0.05). (x± SD) mean ± standard deviation.

Table 2. Analysis of variance for *C. kahawae* isolates growth rates, in MEA, at different temperatures (10, 15, 20, 25 e 30 °C).

	df	**Mean Square**	**F**
Isolates	11	13.44	105.3***
Temperature	4	572.23	4482.8***
Temperature x Isolates	44	3.37	26.39***
Residual	406	0.1277	

****Significantly different (P ≤ 0.001).*

Aggressiveness

Aggressiveness tests were performed in green berries with isolates grown at different temperatures. Due to the reduced number of repetitions it was not possible to make statistic analysis.

The growing temperature of *C. kahawae* isolates was a factor that interacted with its ability to infect (aggressiveness) the green berries (Figure 1). Almost all of the isolates showed more aggressiveness in green berries when grown at 15 ºC. Some exceptions were Tanz 1 and Mal 2 that presented a higher lesion diameter when grown at 25 ºC. At 30 ºC the majority of isolates were less aggressive than when grown at other temperatures.

It's known that CDB occurs mainly in higher altitudes in a relatively cool and wet climate (Firman and Waller, 1977). We tried to understand and correlate the aggressiveness of different isolates (from different geographic origins) with their growing temperature.

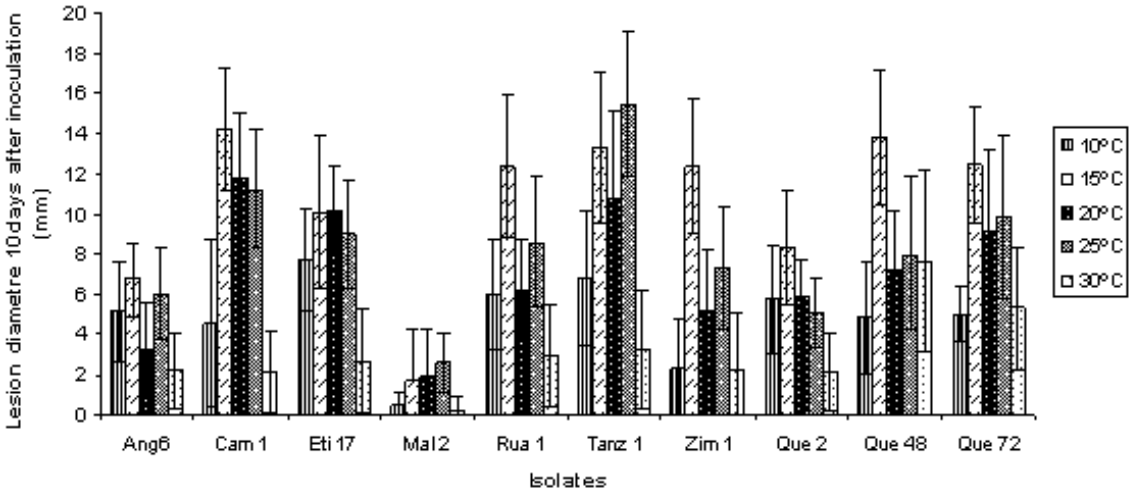


Figure 1. Lesion diameter (mm) obtained 10 days after inoculation for each isolate and temperature studied. Number of green fruits/isolate/temperature = 50.

RAPD analysis and RFLP's of ITS and IGS region

DNA from all the isolates of *C. kahawae* tested gave rise to identical RAPD patterns for the 49 primers used. However *C. gloeosporioides* showed a different pattern. Similar results were obtained by other researchers (Manga, 1999; Sreenivasaprasad et al., 1993). The restriction fragment length polymorphism (RFLP's) of ITS (ITStotal, ITS1 ITS2) and IGS (IGStotal, IGS1 and IGS2) regions from *C. kahawae* isolates also revealed identical digestion profiles for all the isolates studied. However, more recently, studies using microsatellite primers clearly separated *C. kahawae* from other *Colletotrichum* species and two geographical populations (Cameroon and East Africa) were established among *C. kahawae* isolates (unpublished data, INCO – project ICA4-CT-2001-10008).

Isoenzymes analysis

Using PAGE and IEF electrophoresis techniques it was possible to detect polymorphisms in all the systems analysed. Among the isoenzymes studied esterase showed the highest activity with many bands per isolates. Conversely the activity of acid and basic phosphatases and particularly peroxidase was lower, showing few bands per isolate (data not shown). Cluster analysis on isoenzyme data from esterase, acid and basic phosphatases and peroxidase patterns, obtained by IEF electrophoresis technique, clearly separates the isolate of *C. gloeosporioides* (Chi1) from the isolates of *C. kahawae* with a similarity coefficient of 0.30 (Figure 1). Among *C. kahawae* isolates Cam1 (from Cameroon) seemed to be the more distantly related with the others showing a similarity coefficient of 0.38 (Figure 2). This isolate shows a specific morphocultural characterization as well as high pathogenicity (aggressiveness) comparatively with isolates from other regions (Várzea et al., 1999). When isoenzymes were analysed separately (data not shown) basic phosphatase seemed the most discriminant because it clearly separated *C. gloeosporioides* from *C. kahawae* (similarity coefficient 0.15) and it also separated Cam1 isolate from the others *C. kahawae* isolates (similarity coefficient 0.39). So, basic phosphatase appeared as a promising biochemical

marker. Moreover other isolates and enzymatic systems must be tested in the future to obtain biochemical markers that could be used to separate the isolates according to their aggressiveness and geographic localization.

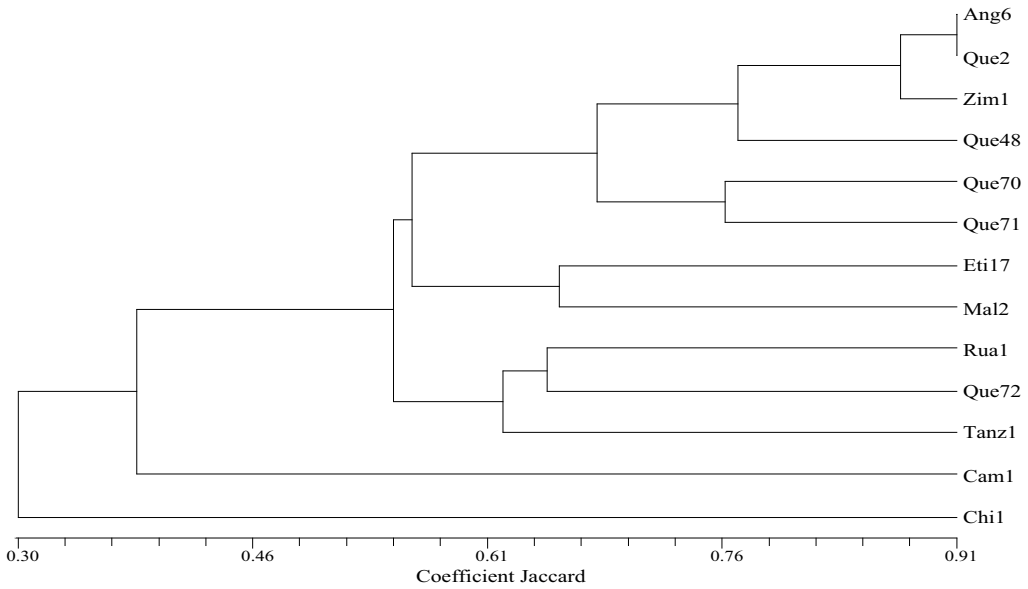


Figure 2. Phenogram resulting from cluster analysis based on similarity matrix of four enzymes (esterase, basic and acid phosphatase and peroxidase) for 12 isolates of *C. kahawae* and 1 of *C. gloeosporioides* using UPGMA and Jaccard similarity coefficient (NTSYS software package).

ACKNOWLEDGEMENT

This work was partially funded by the EU Project ICA 4 – CT – 2001 – 10008 and by FCT fellowship POCI-2010/ SFRH/ BD/ 16684/ 2004

REFERENCES

Afanador-Kafuri L, Minz D, Maymon M, Freeman S (2003). Characterization of *Colletotrichum* isolates from tamarillo, passiflora, and mango in Colombia and identification of a unique species from the genus. *Phytopathology* 93: 579-587.

Andrés MF, Romero MD, Montes MJ, Delibes A (2001). Genetic relationships and isozyme variability in the *Heterodera avenae* complex determined by isoelectrofocusing. *Plant Pathology* 50: 270-279.

Bonde MR, Peterson GL, Maas JL (1991). Isozyme comparisons for identification of *Colletotrichum* species pathogenic to strawberry 81:1523-1528.

Chen Z (2002). Morphocultural and pathogenic comparisons between *Colletotrichum kahawae* and *Colletotrichum gloeosporioides* isolated from coffee berries. *PhD thesis,* Universidade Técnica de Lisboa, Instituto Superior de Agronomia.

Freeman S, Katan T, Shabi E (1998). Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. *Plant Disease* 82: 596-605.

Firman ID, Waller JM (1977). Coffee berry disease and other *Colletotrichum* diseases of coffee. *Phytopathological papers* 20. CAB international. 53pp

Hindorf H, Biratu T, Omondi CO (1997). Correct identification of the pathogen *Colletotrichum kahawae* causing coffee berry disease (CBD). *Proceedings of 17th International Conference on Coffee Science (ASIC)* Nairobi, Kenya. pp 599-603.

Laemmli UK (1970). Cleavage of structural proteins during the essembly of the head of bacteriophage T4. *Nature* 227: 680-685.

MacDonald BA (1997). The Population Genetics of Fungi: Tools and Techniques. *Phytopathology* Vol 87, Nº 4, 448- 453.

Manga B (1999). Etude de la diversité de *Colletotrichum kahawae* responsable de l'a antracnose des baies et characterisation de la résistance du caféier arabica à cet agent pathogène. *PhD Thesis,* Université Montpellier, France.

Mohammadi M, Aminipour M, Banihashemi Z (2004). Isozyme analysis and soluble mycelial protein pattern in iranian isolates of several formae speciales of *Fusarium oxysporum*. *J. Phytopathology* 152: 267-276.

Robertson EF, Dannelly HK, Mallory PJ, Reeves HC (1987). Rapid isoelectric focusing in a vertical polyacrylamide minigel system. *Analytical Biochemistry* **167**: 290-294.

Raeder U. & Broda P. (1985). Rapid preparation of DNA from filamentous fungi.

Letters in Applied Microbiology 1: 17-20.

Rohlf FJ (1997). Numerical taxonomy and multivariate analysis system. Exeter Publishing, New York.

Sreenivasaprasad S, Brown AE, Mills PR (1993). Coffee berry disease pathogen in Africa: genetic structure and relationship to the group species *Colletotrichum gloeosporioides*. *Mycol. Res*. 97 (8): 995-1000.

Waller JM, Brigde PD, Black R, Hakiza G (1993). Characterization of the coffee berry disease pathogen *Colletotrichum kahawae* sp. nov. *Mycol. Res.* 97 (8): 989-994.

Vallejos EC (1983). Enzyme activity staining in isoenzymes in P*lant Genetics and Breeding, Part A* – S. D. Tanksley and T. J. Orton (Editors). Elsevier Science Publishers B.V. Amsterdam: 469-516.

Van der Vossen HAM, Cook RTA & Marakaru GNW (1976). Breeding for resistance to coffee berry disease caused by *Colletotrichum coffeanum* Noack (sensu Hindorf) in *Coffea arabica* L. I. Methods of pre-selection for resistance. *Euphytica* **25**: 733-745.

Várzea VMP, Rodrigues Jr. CJ, Silva MC, Pedro JP, Marques DM (1999). High virulence of a *Colletotrichum kahawae* isolate from Cameroon as compared with other isolates from other regions. In: *Proceedings of the 18th International Conference on Coffee Science*. Helsinki, Finland. Abstract, A131.

Antagonistic Interaction between *Epicoccum nigrum* and *Colletotrichum kahawae*, the Causal Agent of Coffee Berry Disease

L. GUERRA-GUIMARÃES[1], H. G. AZINHEIRA[1], A. C. MARTINS[1], M. C. SILVA[1], E. K. GICHURU[2], V. VÁRZEA, B. BERTRAND[3]

[1]Instituto de Investigação Científica Tropical (IICT) - Centro de Investigação das Ferrugens do Cafeeiro (CIFC), Quinta do Marquês, 2784-505 Oeiras, Portugal
[2]Coffee Research Foundation (CRF), Ruiru P. O. Box 4, Kenya
[3]Centre De Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), 2477, Avenue de val Montferrand, BP 5035, Montpellier, France

SUMMARY

Colletotrichum kahawae Bridge and Waller, the causal agent of Coffee Berry Disease (CBD) in Africa, may cause up more than 50% of losses, if no chemical control measures are adopted. The use of antagonists is a potential non-hazardous alternative to control this disease. Several microorganisms (*Bacillus macerans*, *Phoma*, *Epicoccum nigrum*, *Aspergillus niger*, *Penicillium citrinum*, *Cladosporium* sp.) were isolated from phylloplane of arabica coffee leaves and fruits collected in Kenya coffee plantations. *E. nigrum* was selected for further studies due to its inhibitory effect on *C. kahawae* growth both in gelose media and in detached green fruits. *E. nigrum* grown in Roux bottles showed a stationary phase between 21-24 days after incubation, with a maximal dry weight at 31 days. The filtrates of *E. nigrum* obtained, at different incubation times, caused a slight reduction of *C. kahawae* mycelium growth and in conidia germination *in vitro*. The treatment of green coffee berries with *E. nigrum* filtrates, at the time of inoculation with virulent isolates of *C. kahawae* suspensions, showed that conidial germination and appressoria formation was also reduced and consequently, the number of lesions was lower and its appearance was delayed around 2 days. Different biological tests showed that the filtrates had no phytotoxicity. The biologically active compounds obtained from filtrates of *E. nigrum* were extracted with chloroform and separated by thin layer chromatography (TLC). After TLC, two zones showed fungitoxic activity for *Cladosporium cucumerinum*. For the identification of the inhibitory compounds different testes are currently under study. In the field, treatment of green berries with *E. nigrum* achieved good control of this disease when compared to levels observed with copper based fungicide.

INTRODUCTION

Coffee berry disease caused by *Colletotrichum kahawae* J.M. Waller & P.D. Bridge is the major threat to economic Arabica coffees in Africa. It has been reported that the damages caused by this disease may reach more than 50%, if chemical control is not used (Waller 1985). This show how important is to develop alternative control methods, namely the biological control. The use of antagonists is a promising way to the development of efficient systems for plant protection without disturbing the equilibrium of ecosystems. Several microorganisms were isolated from phylloplane of Arabica coffee leaves and fruits collected in Kenya coffee plantations (Gichuru, 2005). In the present work, an isolate of *Epicoccum nigrum* was selected to investigate the way the fungi itself and the compounds it produces inhibit the growth of the isolate Z1 of *C. kahawae in vitro*, *in vivo* and in field trials.

MATERIAL AND METHODS

Plants and microorganisms

C. kahawae isolate Z1 (from Zimbabwe), *Epicoccum nigrum* (from Coffee Research Foundation, Kenya), green berries from Catimor variety and leaves of tobacco (*Nicotina tabacum* var. Samsun) and tomato (*Lycopersicum escutentum* var. Marmande) were used for antagonism tests.

Inhibitory effect of *C. kahawae* mycelium growth by *E. nigrum in vitro*

Mycelium of *C. kahawae* and *E. nigrum* were placed in opposite edges of PDA Petri dishes. As control, PDA Petri dishes were only inoculated with *C. kahawae*. The incubation was made in a Phytotron 750 E during 12 days, at 22 ºC.

Inhibitory effect of *C. kahawae* growth by *E. nigrum in vivo*

Detached green berries (Catimor) were inoculated with a 5µl of *E. nigrum* suspensions ($2x10^6$ conidia /ml), jointly with a 5µl conidia suspension of *C. kahawae* ($2x10^6$ conidia/ml). As control green berries were inoculated only with *C. kahawae*. The green berries were placed on plastic trays on a nylon sponge and covered with a plastic bag**.** The trays were incubated in a Phytotron 750 E during 12 days at 22 ºC, with a fotoperiod of 12 hours.

E. nigrum growth profile

E. nigrum was grown as stationary culture in Roux flasks containing 150 ml of Czapek-Dox medium pH 5.6. Roux flasks were incubated at 21-22 ºC, in the dark and withdrawn at 3, 7, 10, 14, 17, 21, 24, 28, 31 and 35 days. At each incubation time the mycelium was removed by filtration and used for mycelia dry weight. The obtained culture filtrates were used for different analysis.

Interaction between *E. nigrum* culture filtrates and *C. kahawae* mycelium growth *in vitro*

E. nigrum culture filtrates (for each incubation time) with 2% agar were sterilized at 120 ºC. From the obtained medium, 15 ml were pipetted into Petri dishes, inoculated with *C. kahawae* isolate and incubated during 8 days at 22 ºC. The percentage of inhibition of the *C. kahawae* mycelium growth was evaluated.

Interaction between *E. nigrum* culture filtrates and *C. kahawae* mycelium growth *in vivo*

Detached green berries were treated with *E. nigrum* culture filtrates jointly with conidia suspension of *C. kahawae* ($2x10^6$ conidia/ml) in a proportion of 1:1. As control green berries were inoculated only with *C. kahawae*. The green berries were placed on plastic trays on a nylon sponge and covered with a plastic bag**.** The trays were incubated in a Phytotron 750 E at 22 ºC, with a fotoperiod of 12 hours. The symptoms were evaluated at 6, 10 and 15 days after inoculation.

Phytotoxic tests

The phytotoxic activity of *E. nigrum* culture filtrates (for each incubation time) was tested on cuttings of young tomato plant (Graniti and Procacci, 1966; Ballio et al., 1969) and on infiltrated tobacco leaves (Király et al., 1970).

Isolation and identification of biologically active compounds produced by *E. nigrum*

The culture filtrates were extracted with chloroform and separated by thin layer chromatography (TLC) using different eluent systems. a) chloroform:methanol (90:10); b) ethyl acetate:toluene:acetic acid (70:30:1) and c) petroleum ether:acetone (70:30). After running TLC silicagel plates were observed at UV radiation (254 and 366nm) and each silicagel plate was sprayed with a different dyeing reagent solution, namely FeCl 10% in etanol, Liebermann – Burchard reagent, Godin reagent, KOH 5% in etanol, vanillin – sulphuric acid, ninhydrin and Neu's reagent to detect the presence of tannins, steroids, saponins, anthraquinone, complex phenols and high alcohols, amines and flavonoids, respectively. Another set of TLC silicagel plates was tested for antifungal activity with *Cladosporium cucumerinum*, according to the procedure described by Allen & Kuc (1968).
Field trials: the results presented concerns to experiments carried out during 2005 in Coffee Research Station, Ruiru, Kenya. To test the field activity of *E. nigrum*, the fungus was grown for 2 weeks on liquid malt extract media incubated at room temperature and gently agitated twice a day. For field application, the liquid cultures were filtered with a double layer of muslin cloth and sprayed to bearing branches of the susceptible commercial cultivar SL28. Commercial copper based fungicide (Copper Nordox: *cuprous oxide*) was used as chemical control. The fungus and the fungicide were applied twice per season: in April (as the berries expand) and one month later. These treatments were coincident with the beginning of the disease period. The field test plots consisted of one bearing branch per tree on six trees. One non-treated branch on each tree was monitored as control.

RESULTS AND DISCUSSION

Caracterization of the inhibition of *C. kahawae* growth *in vitro* and *in vivo*

After incubation it was possible to observe the inhibitory effect of *E. nigrum* on the mycelium growth of *C. kahawae* in PDA Petri dishes (Figure 1a). Twelve days after inoculation the development of symptoms was inhibited in detached green berries inoculated with *C. kahawae* jointly with *E. nigrum* (Figure 2a).

***E. nigrum* growth profile**

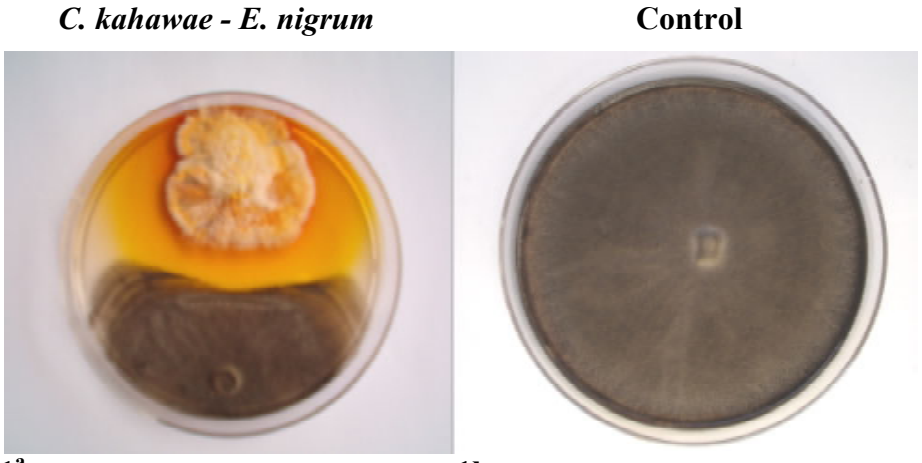
In the growth profile of *E. nigrum* (mycelium dry weight) in culture conditions the stationary phase was obtained around 21-24 days after incubation, with a maximal dry weight of 4g at 31 days. After stationary phase medium conditions were not favourable to the growth and the biomass started to decrease (data not shown).

Interaction between *E. nigrum* culture filtrates and *C. kahawae* mycelium growth *in vitro*

The inhibitory effect of *E. nigrum* culture filtrates for all the incubation time was measured on the colony development of isolate Z1 of *C. kahawae.* Culture filtrates of *E. nigrum* caused a slight reduction of *C. kahawae* mycelium growth with an average inhibitory effect of 27% (Figure 3).

Interaction between *E. nigrum* culture filtrates and *C. kahawae* mycelium growth *in vivo*

The treatment of green coffee berries with a 24 days *E. nigrum* culture filtrate, at the time of inoculation with conidia suspension of *C. kahawae*, showed that conidial germination and appressoria formation was slightly reduced. Consequently, the number of lesions was lower and its appearance was delayed around 2 days (Figure 4).



1[a] 1b

Figure1. a) Inhibition of *C. kahawae* mycelium growth by *E. nigrum* in PDA Petri dishes. b) Control – *C. kahawae* mycelium growth in PDA Petri dishes.

2a **2b**

Figure 2. a) Inhibition of symptoms development in detached green berries inoculated with *C. kahawae* jointly with *E. nigrum;* b) Control – Symptoms observed in detached green berries inoculated with *C. kahawae*

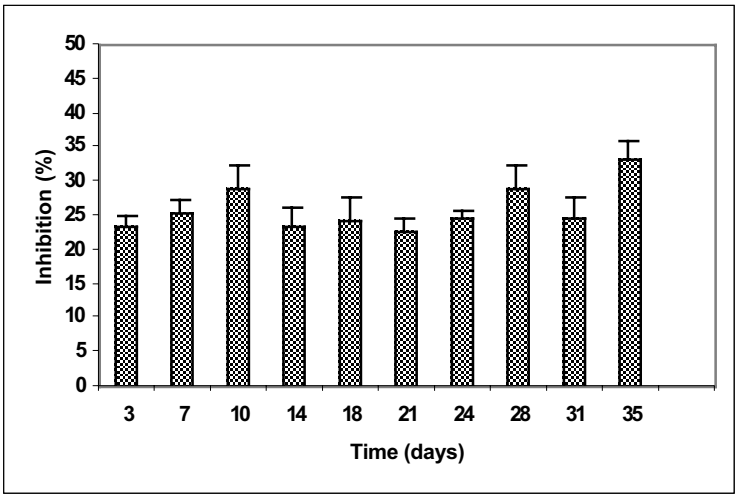


Figure 3. Percentage of the inhibition of colony development of isolate Z1 of *C. kahawae* by *E. nigrum* filtrates with different incubation time.

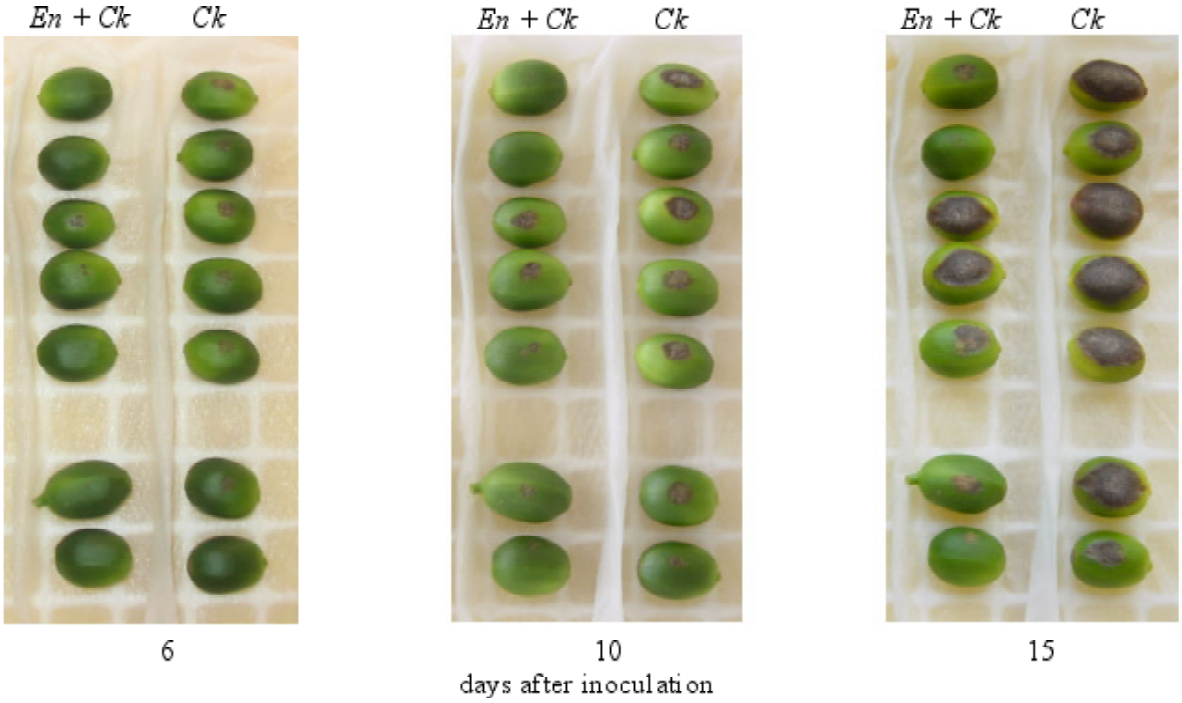


Figure 4. Inhibition of symptoms development in detached green berries inoculated with *C. kahawae* jointly with *E. nigrum* culture filtrates (*En* + *Ck*). Control – Symptoms observed in detached green berries inoculated with *C. kahawae* (*Ck*).

Phytotoxicity tests

The intensity of symptoms observed on cuttings of young tomato plant used to test the toxic activity of *E. nigrum* culture filtrates, was a slight leaf curl with stem wilted. This intensity of symptoms was observed for all the filtrates studied (data not shown). No toxin activity was observed on diluted *E. nigrum* culture filtrate. Tobacco leaves infiltrated with *E. nigrum* culture filtrates didn't show significant phytotoxic activity (data not shown).

Isolation and identification of biologically active compounds produced by *E. nigrum*

Twenty four days *E. nigrum* culture filtrates were extracted with chloroform (CLO) were used in thin layer chromatography (TLC). Among the different eluent system mixtures studied the ethyl acetate:toluene:acetic acid (70:30:1) was the one which provided a good separation of the compounds present in the extract.

TLC plates were sprayed with different dyeing reagents solutions to determine the class of the compounds present. Positive results were obtained concerning the presence of tannins, steroids, saponins, anthraquinone, complex phenols and high alcohols.

Another set of TLC plates tested for antifungal activity (sprayed with a *C. cucumerinum* conidia suspension) showed the presence of two inhibitory zones (white spots on a grey/brownish background composed of spores and mycelium). These zones were not detected with UV radiation (254 and 366nm) and have Rf (relative mobility factor) values around 0.3 and 0.6 (Fig. 5). However the application sample place that shows also antifungal activity has UV radiation (254) and presents some pigmentation. Further studies are needed to determine which classes of compounds have antifungal activity.

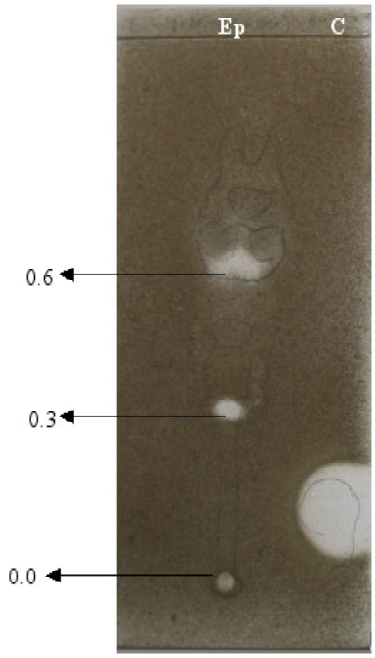


Figure 5. Chromatogram of *E. nigrum* sample extracted with chloroform, run with ethyl acetate:toluene:acetic acid (70:30:1) solvent system and tested for antifungal activity with *C. cucumerinum* (antifungal compounds appear like white spots on a grey/brownish background composed of spores and mycelium). Ep: *E. nigrum* sample; C: control (caffeine). Application of 30 µl of sample per chromatogram.

Field trials

There was a good control of the disease by *E. nigrum* liquid cultures comparatively to levels observed after copper treatments and in non treated branches (control) (Figure 6). The amount of the disease increased at the end of July, i.e. two months after the last treatment, because the weather remained favourable to the disease. In the dry month of September the disease was reduced, but it increased again during the second annual rainfall season. Further studies will focus on the longevity action of the *E. nigrum* liquid cultures as well as its accumulated effects over time.

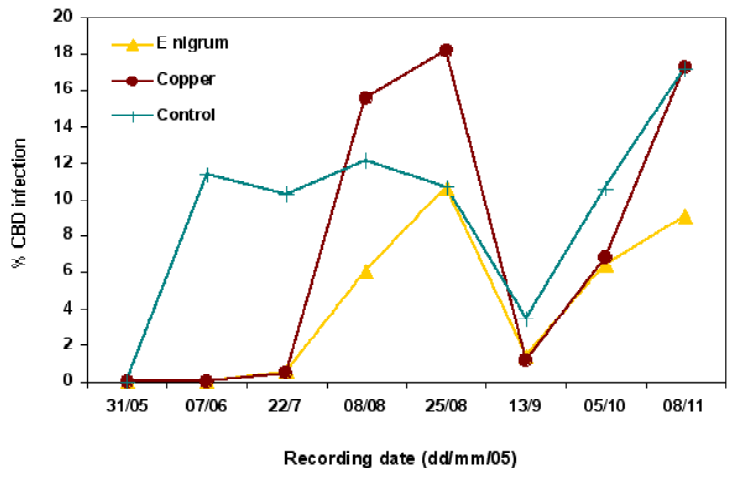


Figure 6. Percentage CBD infection on green coffee berries sprayed with an isolate of *E. nigrum* at Coffee Research Station, Ruiru, Kenya.

ACKNOWLEDGMENTS

The technical assistance of João Matos and CRF staff is acknowledged. This work was partially funded by the EU Project ICA 4 – CT – 2001 – 10008

REFERENCES

Allen E.H. and Kuc J. (1968). α-Solanine and α-chaconine as fungitoxic compounds in extracts of irish potato tubers. *Phytopathology* **58**: 776-781.

Ballio A., Bottalico A., Buonocore V., Carilli A., Di Vittorio V., Graniti A. (1969). Production and isolation of aspergillomarasmin B (lycomarasmic acid) from cultures of *Colletotrichum gloesporioides* Penz. (*Gloeosporium olivarum* Alm.). *Phytopathologia Mediterranea* **8**: 187-196.

Gichuru E.K. (2005). Laboratory screening of some saprophytic coffee surface microflora antagonistic to *Colletotrichum kahawae. Agronomie Africaine* **17**: 73-79.

Graniti A. and Procacci R. (1966). Attività tossiche sistemiche di *Gloeosporium olivarium* Alm. Agente delle «lebbra» del olive. Proceedings of *1º Congresso Unione Fitopatologica Medit*erranea, Bari-Napoli, Italy. Pp 104-112.

Király Z., Klement Z., Solymosy F., Voros J. (1970). *Methods in Plant Pathology*. Akadémiai Kiadó, Budapest, 509 pp.

Waller J.M. (1985). Control of coffee diseases. In: Coffee: Clifford M.N., Willson R.C. (eds), Botany, Biochemistry and Production of Beans and Beverage, pp.219-229. Croom Helm Ltd.

(*S*)-2–Hydroxy-3-Decanone as Sex Pheromone for Monitoring and Control of Coffee White Stem Borer (*Xylotrechus quadripes*)

JAYARAMA, M. V. D'SOUZA[1], D. R. HALL[2]

[1]Central Coffee Research Institute, Coffee Board, Coffee Research Station-577117, Chikmagalur Dist, Karnataka, India
[2]Natural Resources Institute, University of Greenwich at Medway, Chatham, Meritime, Kent

SUMMARY

Coffee White Stem Borer (*Xylotrechus quadripes)* Chevrolat (Coleoptera: Cerambycidae) is a major pest of coffee arabica in India causing great economical loss in terms of production and destruction of mature plants. Chemical and mechanical methods of controlling this dreaded pest though available, their use is restricted on account of the harmful nature of the pesticides and laborious techniques. Therefore, sex pheromone system for the control of White stem borer in coffee has been developed. The male borer is found to attract the females through chemical secretion in the field. This compound was isolated, identified and chemically characterized to be (*S*)-2-hydroxy-3-decanone. The compound has been successfully synthesized in the laboratory. Bioassay studies using this synthetic compound established that female borers could be effectively attracted and trapped. Field experiments conducted for over 8 years in India demonstrated that synthetic male sex pheromone could be efficiently used as an attractant to trap female borers in the field. Methods of trapping have been standardised. Use of sex pheromone in controlling the pest can greatly reduce the use of hazardous chemicals thereby lessening the environmental contamination. This paper deals mainly with the isolation, identification, chemical synthesis of male sex pheromone of coffee white stem borer, laboratory bioassay and results of field experiments.

MATERIALS AND METHODS

Collection of Pheromone

The crude pheromone was collected from the virgin male white stem borer by whole body wash, container wash and air entrainment techniques. The collected materials were column chromatographed on a glass column filled with florisil for various fractions and all the fractions were analysed in GC on fused silica capillary columns coated with polar CP Wax 52CB or non polar CP Sil 5CB. Enantiomeric purity was measured by GC analysis on a cyclodextrin column (CP-Chiracil-Dex CB, 25 m X 0.32 mm i.d) operated isothermally at 130 °C.

EAG

Male collected every fractions were subjected for electro antenno graphic (EAG) studies using the freshly cut female antenna to identify the responsive components by a method described by Cork et al. (Chromatography and Isolation of insect hormones and pheromones, 1990 and Hummel, 1984).

Linked GC-EAG

Studies were conducted simultaneously recording EAG response from female antennae to GC column effluent from the body washes, container washes or entrainment body volatiles to find out the active components accurately. Carlo Erba 5300, 6000 Mega series GC instruments were employed in the study.

Chemical identification

Bio active components were then identified, chemically characterized using GC, ^{1}HNMR (JEOL EX270 at 270MHz), ^{13}C NMR (67.8 MHz), UV-VIS (Beckman DU), IR (Bio Rad 760 FTIR and Perkin Elmer) and GC-MS spectral analysis. HRGC 5160 mega series GC with WAX 52 (30 m X 0.25 mm i.d) and Ms Finnigan MAT 700 Ion Trap detector were employed for linked GC-MS analysis.

The structure and absolute configuration of the identified components were established by standard comparative methods.

Synthesis

The bio active compounds were synthesised from (*S*)-ethyl lactate or (R)-ethyl lactate by a short, general route suitable for large scale production.

Laboratory Bio assay

Laboratory bioassay studies were conducted using virgin male and female beetles and synthetic pheromone components to know the bio efficacy of the synthetic pheromone components in eliciting the response from the female borers. Various techniques like wind tunnel, alfactometer, cage method, pit fall, swastika bioassay, trailing and straight tube bio assay chambers were employed in the study. Bio assay studies were conducted between 9th and 12th hours of the photophase in a laboratory where sufficient light and air circulation is provided so that it simulates the field conditions.

Field studies

Field trials were conducted in India and in Yunnan, China (Nestle Scientists, Singapur) with different types of pheromone dispensers (polyethylene sachets, 2.5 cm X 2.0 cm X 120 μ thick and polyethylene vials, 40 mm height X 9 mm dia of 1 ml capacity) and traps (polypropylene sticky cross vane, delta, sticky plane sheet and funnel type) placed at different heights. The traps of different colours like white, yellow, blue and red are used to know whether the insects have any special preference towards colour. Field trials were conducted in South Indian Coffee fields for 8 continuous years from 1997 during the borer flight season (two times in a calendar year during Mar-June and Sept-Jan) to 2001. Nestle scientists based at Singapur conducted field trials in Gonglang 2 coffee base, Daheishan and Nestle E & D farm, Jinghong during 2000-01 seasons. The pheromone (50-100 mg) were loaded in polyethylene sachets or vials (sealed) and hung in appropriate places on the traps. The traps were placed at different heights in the field namely 1, 1.5, 2, 3 and 4 meters above the ground level and at a distance of 70 feet apart from each other in white stem borer infested coffee fields. Required number of control traps without pheromone and with live males (in small nylon mesh cage) was also installed in the test fields. The trap catches were recorded periodically.

RESULTS AND DISCUSSION

The EAG response of the female white stem borer antennae recorded for 1 micro ml of dichloromethane, 2 m.ml of male volatile, 2 m.ml of female volatile, 2 m.ml of male container or body wash is shown in the Figure 1. Number of EAG's recorded for various fractions showed that male volatile elicited a greater EAG response from the female antennae. The females responded to little extent to the male body and container washed EAG preparations. The experiments conducted using female volatiles did not elicit any EAG response either from male or female. The male fractions failed to elicit any EAG response from the males. This has clearly indicated that males attract the females through a chemical communication.

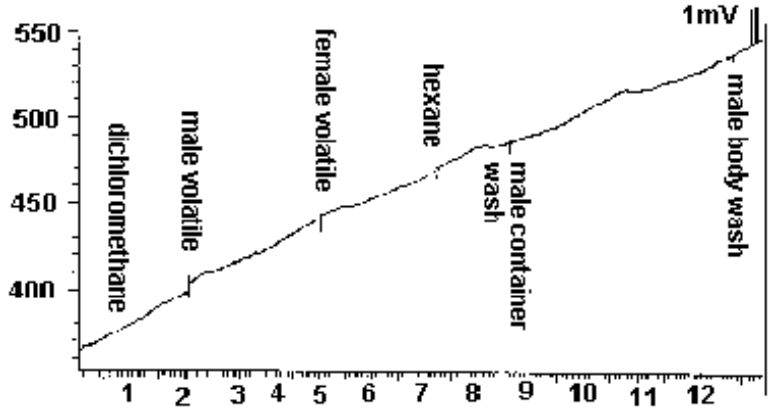


Figure 1. EAG response curve for various fractions by female *X. quadripes.*

All the fractions were then examined on GC linked to EAG. The GC-EAG recordings using female beetles cut antenna showed two active major components (P-I and P-II accompanied by a minor third component (P-III) on a polar GC column. The response to the component P-I is significantly higher than P-II, which in turn is better than to P-III (Figure 2). None of the above three components were present in the similar collections from female beetles. GC analysis of male volatiles on a polar column showed that component P-I was produced at rates up to 2 μg /h/beetle over a 24 h period assayed by comparison of peak area with that of a 1-octen-3-ol standard. However, in the male body and container washed fractions only P-I and P-II components were noticed and these were totally absent in the female collections. The analyses of the ratio of P-I **:** P-II in volatile collections varied between 1.0 **:** 0.14 and 1.0 **:** 0.75 suggesting that these two components undergo inter-conversion under the conditions of analysis.

Linked GC-MS analysis was performed to identify the components. The analyses show that components P-I and P-II in the male fractions have the similar mass spectra indicating a possible molecular weight of 172. The molecular weight assignments are then confirmed by taking chemical ionisation, CI (isobutane) mass spectra (Figure 3). The TIC of GC-MS of male volatiles, EI spectra of P-I and P-II are shown in figure 4. The comparison of the EI mass spectrum with those reported by Kunwahara, et al. (1987) for 2-hydroxy-3-octanone and 3-hydroxy-2-octanone (the pheromone compounds of *Xylotrechus chinensis* Chevrolat , grape borer), suggested that P-I and P-II components to be the 10-carbon homologue ($C_{10}H_{20}O_2$) such as 2-hydroxy-3-decanone and 3-hydroxy-2-decanone. The spectrum of P-I has a prominent ion at m/z 127, corresponding to that in the spectrum of 2-hydroxy-3-octanone at m/z 99. The spectrum of P-II has ion at m/z 111 and 129 corresponding to those at m/z 83 and 101 in the spectrum of 3-hydroxy-2-octanone. The IR spectrum has shown OH and C=O absorptions at 3400 and 1710 cm-1 respectively. The NMR spectra were also consistent with

the proposed structure in comparison with ^{1}H (Bel-Rhlid et.al,1989) and ^{13}C (Mori and Otsuka, 1985) NMR spectra of 2-hydroxy-3-octanone. The ^{1}H NMR spectrum showed a singlet at 2.20 corresponding to $C\underline{H}_3$ –C=O in structure of P-II integrates roughly 7% relative to $C\underline{H}_3$ –CH- OH in the P-I .This is an indication of some isomerization of P-I to P-II. The configuration of the component P-I was established to be (S) by comparison of its retention time with those of the synthetic enantiomers in GC analysis on a Cyclodextrin column and ^{1}H NMR spectrum in the presence of a chiral shift reagent. The components P-I and P-II were thus identified to be (*S*)-2-hydroxy-3-decanone and (*R*)-3-hydroxy-2-decanone.

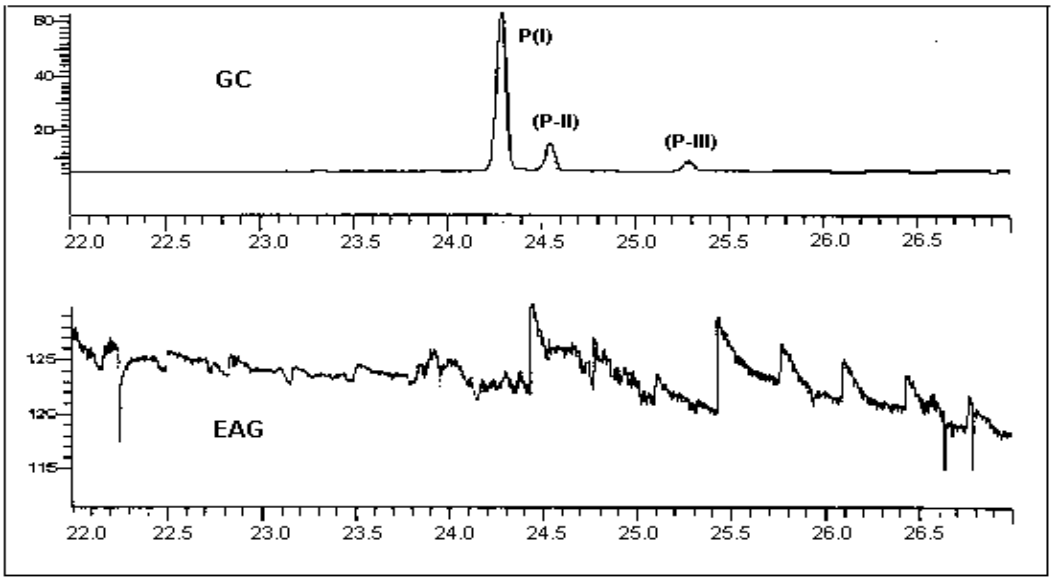


Figure 2. Linked GC- EAG analysis of male volatiles against female *X.quadripes* on polar gc column.

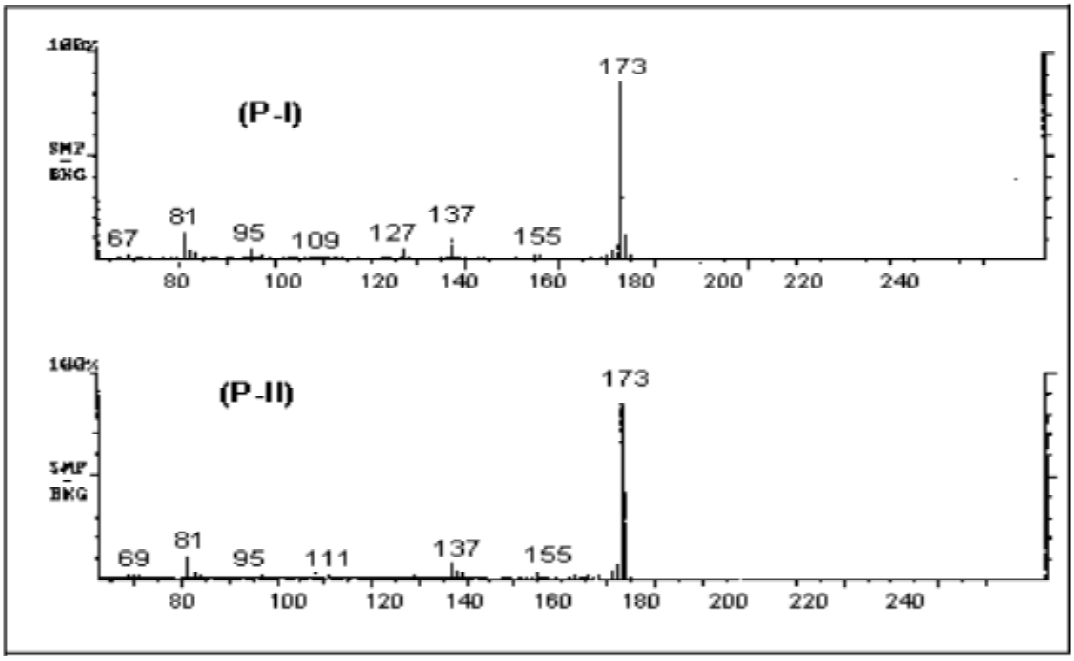


Figure 3. CI spectra of pheromone components (P-I) and (P-II).

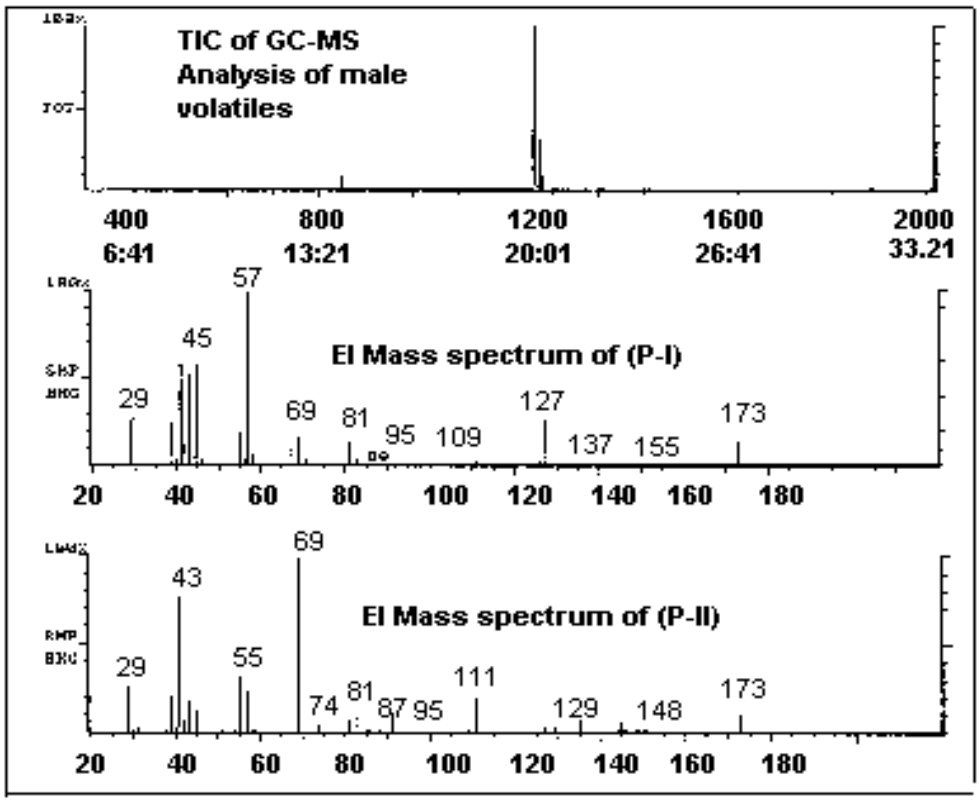


Figure 4. TIC of GC-MS and EI spectra of pheromone components.

The third component was also examined and it was found to be 2,3-octane diol from the data of EI,CI mass spectra and the retention time on polar and non polar GC columns. The structures of all the components are shown in Figure 5.

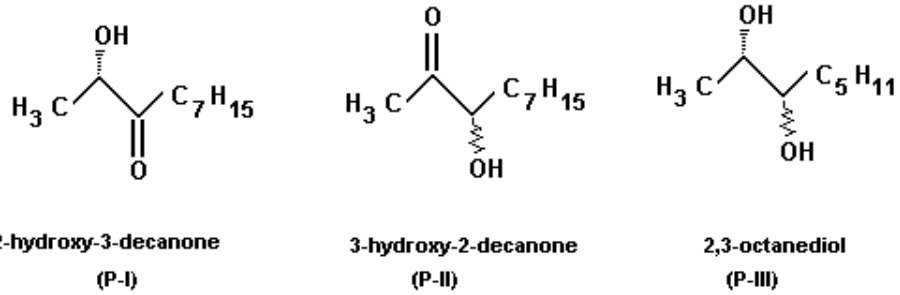


Figure 5. Structures of Coffee Male WSB Pheromone components identified.

Laboratory Synthesis

The P-I and P-II pheromone components were conveniently synthesized in the laboratory following a short general route suitable for large scale preparation from (*S*) and (*R*) ethyl lactate.

The EAG response studies were conducted using linked GC-EAG employing natural and synthetic pheromones of coffee white stem borer. It was found that (*S*) component elicited significant response from the female antenna while (*R*) has elicited a small response but positive. But the third component was very poor in eliciting response from the females. The analysis of natural and synthetic enantiomers of 2-hydroxy-3-decanone on a cyclodextrin GC

column (i.s = decyl acetate) clearly demonstrated that both the natural and the synthetic compounds are the same Figure 6.

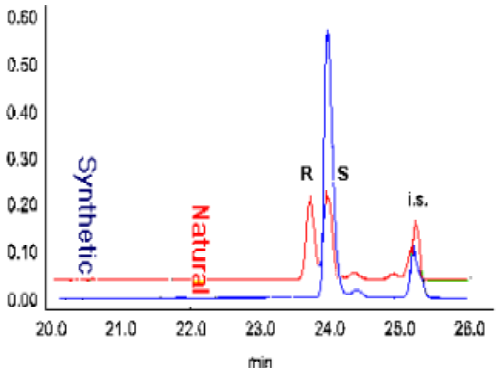


Figure 6. Analysis of natural and synthetic enantiomers of 2-hydroxy-3-decanone on a cyclodextrin GC column (i.s= decylacetate).

Laboratory Bio Assay Study

The laboratory bioassay studies conducted using the live males, synthetic (*S*)-2-hydroxy-3-decanone, (*R*)-3-hydroxy-2-decanone and 2,3-octanediol as sex attractants against the virgin female coffee white stem borer beetles in wind tunnel bioassay chamber has clearly established that synthetic pheromone was successful in eliciting quick response from the female beetle. The order of positive response observed against the females is (*S*) ≅ Male > (*R*). The 2, 3-octanediol failed to elicit any response from the females. The combinations of *S* and *R* pheromones were also tried and it was found that the response is in the order (*S*) > (*SR*) > (*R*). Similar studies conducted using pit fall and cage bio assay chambers have also more or less revealed the same result. However, though positive results were observed, the results from these bio assay studies were inconsistent probably due to internal saturation of the chamber with synthetic pheromone and inconsistent air flow in the chambers confusing the test beetles leading to erratic response. Hence more advanced Swastika and Straight tube bio assays were tried. The results accrued from these experiments proved beyond doubt that synthetic pheromone can be successfully used for attracting the female white stem borer. The female borers were attracted very specifically to the synthetic pheromone especially to (*S*) form significantly which is very similar to the response shown to virgin males by females in the bioassay chamber.The (*S*) form of pheromone was the most active component among the other one that is (*R*) while the third component is not active. The (*S*) component behaved exactly similar to that of a live male beetle against female in eliciting quick positive response. The results are depicted in Figures 7 and 8.

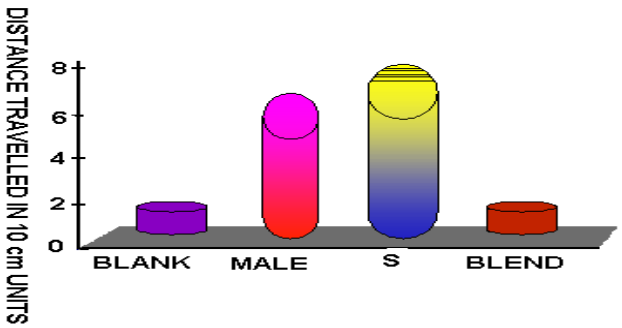


Figure 7. Attraction of females to male, pheromone and pheromone blend in straight tube bio assay tube.

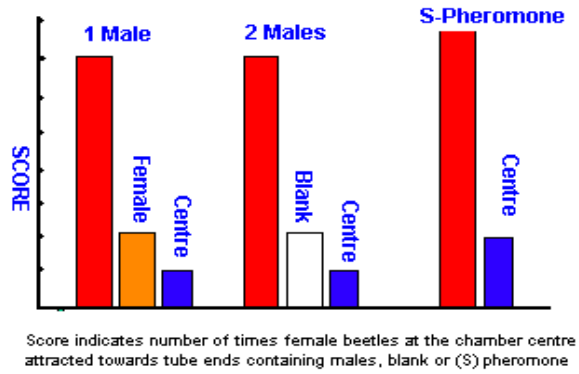


Figure 8. Response of female beetles to male, blank and pheromone in swastika bioassay chamber.

Field Trials

Among the pheromone dispensers studied, it was found that polyethylene vials loaded with 100 mg of synthetic pheromone were the best and pheromone release from these vials were continuously consistent (20-30 µg/h) and lasted for about 3-4 months under the field conditions.

Among the different traps studied, sticky cross vane traps were found to be the most effective when placed at a height of 1.5 meters. Beetles did not show any special preference to any coloured traps. Hence white polypropylene sticky cross vane traps (50 cm X 25 cm X 3 mm thick) were used in the field studies.

In the preliminary field studies conducted in different agro climatic zones during the first year of field study to standardize trap design, trap height, distance, longevity of of pheromone, active component of pheromone, it was found that (*S*)-2-hydroxy-3-decanone was the most effective and efficient pheromone component compared to (*R*), and (*RS*) combinations as (*S*) pheromone caught more number of female *X. quadripes* beetles. The results of field studies further supported the results obtained from laboratory bio-assay studies that (*S*)-2-hydroxy-3-decanone is the most active pheromone component present in male *X. quadripes* beetle which effectively attracts the females. Similar results were obtained from the experiments conducted in Yunnan, Chinese coffee fields. The results of the field experiments are given in tables 1 to 4.

It was also noticed that male and other Cerambycid species were also caught in baited lures but their numbers are non significant compare to the total females trapped. This is the first report of 2-hydroxy-3-decanone and 3-hydroxy-2-decanone as male produced sex pheromone components of Cerambycid beetles. However, 8 and 6 carbon analogue sex pheromones of other cerambycid related species have been reported by various authors (*X. Pyrrhoderus*, Sakai et al., 1984; *X.Chinensis*, Kuwahara et al., 1987; Iwabuchi et al., 1987 and Leal et al., 1995 for *Anaglyptus subfasciatus* Pic) but all of them were very short range in activity. The field experiments have shown that 2-hydroxy-3-decanone has a long range activity and suggests that trapping the female coffee white stem borers with synthetic pheromone baited traps in the field is possible. The number of catches however varied depending on the prevailing field and environmental situations. It was inferred that the catches were poor during the rainy days, cloudy and very high temperature (above 35 °C) situations irrespective of infestation levels. One of the problems noticed during the field experiment is that the sticky traps were prone to catch the falling debris from the shade trees thereby lowering the effective catch area and hence frequent cleaning of the trap surface is necessary. When this pest is

present and causes significant damage at very low population levels, even very low catches in the traps assumes significance. The levels of catches give an indication to the pest population in the field which can be utilized to take up necessary other remedial measures in time. The field experiments were continued over larger areas even today. But the results are not presented in the present study. Our 8 years of experience in field trapping of borers using synthetic pheromone conclusively proved that this technique has a greater potential as one of the most eco-friendly control measures as it was found that the pheromone baited traps can trap 25 to 60% of the emerged populations in the field when the traps are placed in strategic locations. The pheromone traps caught very large number of flying borers (more than 60% of the emerged beetles) when the infestation level is very high in the field. Further fine tuning of the techniques in terms of trap design, may lead to further improvement in the trap catches which can effectively prove to be an eco-friendly technique to monitor and control CWSB as we are sliding towards environmentally safe techniques to protect our nature and to control pests in the mean time without the use of hazardous chemicals.

In the light of the foregoing discussions, it was inferred that synthetic male sex pheromone [(*S*)–2-Hydroxy-3-Decanone] of coffee male white stem borer has the potential to attract the female beetles in the field. Hence (*S*)-2-hydroxy-3-decanone could be used as sex attractant for female coffee white stem borer and the pheromone technique as one of the eco-friendly tools to monitor and control this dreaded pest. The use of perfume (pheromone) instead of poison(pesticides / insecticides) will be the key to an eco friendly pest control measures as the pheromone technology would appear to offer a great promise in the IPM of coffee white stem borer.

Table 1. Field trap catches of beetles in pheromone baited traps.

Pheromone source	**March-June (62days observation)**		**Sept-December (102days observation)**		**Total**	
	Female	Male	Female	Male	Female	Male
R	**2**	3	**8**	1	**10**	4
RS	**7**	4	**15**	2	**22**	6
S	**13**	2	**44**	2	**57**	4
Male	**-**	-	**6**	-	**6**	-
Control	**2**	6	**3**	4	**5**	10
Total	**24**	15	**76**	9	**100**	24

Locations 4, Trap height 1- 4 meters, 8 traps/location.

Table 2. Field trap catches of beetles in pheromone baited traps*.

Pheromone source	**March-June (81days observation)**		**Sept-December (70 days observation)**		**Total**	
	Female	Male	Female	Male	Female	Male
R	**1**	4	n.t	n.t	**1**	4
RS	**11**	3	n.t	n.t	**11**	3
S	**57**	2	**45**	5	**102**	7
S+ss	n.t	n.t	**6**	2	**6**	2
Control	n.t	n.t	**1**	0	**1**	0
Total	**69**	9	**52**	7	**121**	16

*n.t = not tested during the period. *16 locations during Mach-June period & 22 locations during Sept-Dec with 8. Traps per location. Trap height 1.5 meters, Trap= sticky cross vane.*

Table 3. Field trap catches of beetles in pheromone-baited traps*.

Pheromone source	March -June (87days observation)		Sept-December (39 days observation)		Total	
	Female	Male	Female	Male	Female	Male
S	**112**	11	**126**	24	**238**	35
Control	**1**	6	**31**	13	**32**	19
Total	**113**	17	**157**	37	**270**	54

*n.t = not tested during the period. *12 locations during Mach-June period, 8 traps/location & 76 locations during Sept-Dec with 4 traps/location, Trap height 1.5 meters, Trap= sticky cross vane.*

Table 4 : Field trap catches of beetles in pheromone-baited traps at Chinese fields (2000-01).

Pheromone source	May-June				Total	
	Gonglang 2 coffee base		Daheishan coffee base			
	Female	Male	Female	Male	Female	Male
S	**16**	21	**7**	7	**23**	28
Control	-	-	-	-	-	-
Total	**16**	21	**7**	7	**23**	28

Number of baited traps 8 and control 8 in Gonglang while in Daheishan 3 baited and 3 control. Trap= sticky cross vane and dispenser = vial.

REFERENCES

Bel-Rhlid R., Fauve A. and Veschambre H. (1989). Synthesis of the pheromone components of grape borer *Xylotrechus pyrroderus* by microbial reduction of an alpha-diketone. *Journal of Organic Chemistry*, **54**, 3221-3223.

Hummel H.E. and Miller T.A. (1984), Techniques in Pheromone research, Springer - Verlag, N.Y. Berlin Heidelberg Tokyo.

Iwabuchi K., Takahashi J. and Sakai T. (1987). Occurrence of 2,3-octanediol and 2-hydroxy-3-octanone, possible male sex pheromone in *Xylotrechus chinensis* Chevrolat (Coleoptera: Cerambycidae). Applied Entomology and Zoology, **22**, 110-111.

Kuwahara Y., Matsuyam S. and Suzki T. (1987). Identification of 2,3-octanediol, 2-hydroxy-3-octanone and 3-hydroxy-2-octanone from male *Xylotrechus chinensis Chevrolat as possible pheromones (*Coleoptera: Cerambycidae*). Applied Entomology and Zoology*, **22**,25-28.

Leal W.S., Shi X., Nakamuta K., Ono Mikio and Meinwald J. (1995). Structure,stereochemistry and thermal isomerization of the male sex pheromone of the long horn beetle *Anagalyptus subfasciatus.* Proceedings of the National Academy of Sciences USA, **92,** 1038-1042.

McCaffery A.R. and Wilson I.D. (1990). Chromatography and isolation of insect hormones and pheromones, Plenum press, Newyork and London.

Mori K. and Otsuka T. (1985). Synthesis of (2S,3S)-2,3-octanediol and (S)-2-hydroxy-3-octanone, the male pheromone of the grape borer*, Xylotrechus pyrrodeurs, Tetra Hedron*, **41**, 553-556.

Sakai T., Nakagawa Y., Takahashi J., Iwabuchi K. and Ishii K. (1984). Isolation and identification of the male sex pheromone of the grape borer, *Xylotrechus pyrrodeurs* Bates (Coleoptera : Cerambycidae). *Chemistry Letters*, 263-264.

Genetic Variability of *Hypothenemus hampei* (Ferrari) in Colombia and Development of Molecular Markers

P. BENAVIDES[1], J. J. STUART[2], F. E. VEGA[3], J. ROMERO-SEVERSON[4], A. E. BUSTILLO[1], L. NAVARRO[1], L. M. CONSTANTINO[1], F. E. ACEVEDO[1]

[1]National Coffee Research Center, CENICAFE, Chinchiná, Colombia. Research co-sponsored by the Colombian Ministry of Agriculture and the Colombian Institute for the Development of Science and Technology Colciencias
[2]Department of Entomology, Purdue University, West Lafayette, IN, U.S.A.
[3]Insect Biocontrol Laboratory, U. S. Department of Agriculture, Agricultural Research Service, Beltsville, MD, U.S.A.
[4]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, U.S.A.

SUMMARY

The genetic variability of the coffee berry borer (*Hypothenemus hampei*) in Colombia was evaluated using the amplified fragment length polymorphism (AFLP) technique. Sixty *H. hampei* DNA samples from 16 coffee producing areas throughout Colombia were analyzed. We performed AFLP´s with these DNA samples using the two most polymorphic selective primers from a previous investigation. The results revealed very low genetic variation in the Colombian samples but enough polymorphism to perform a phylogenetic analysis. The findings indicated that there were multiple *H. hampei* introductions into Colombia probably from Peru, Ecuador, and Brazil. There was a main line of *H. hampei* present in every coffee-producing area in Colombia that spread throughout the country, perhaps due to coffee pickers moving within coffee regions and transporting infested coffee berries or adult *H. hampei* females. We also found that a line of *H. hampei* present in Costa Rica matched the fingerprint profile of this main line in Colombia, thus raising the possibility that the line of *H. hampei* in Costa Rica originated from Colombia. We designed sequence tagged sites STS markers from AFLP polymorphisms to answer some issues related to the biology and ecology of *H. hampei*. The design of co-dominant markers using Genome Walking and Single Stranded Conformational Polymorphism SSCP will be also of enormous relevance for a better understanding of the biology and ecology of *H. hampei.*
Key words: AFLP, SSCP, DNA Fingerprinting, broca del café, Coffee, insect-pest, molecular markers

INTRODUCTION

The beetle *Hypothenemus hampei* (Ferrarri) is the most destructive insect-pest of coffee and now threatens coffee crops worldwide (Le Pelley, 1968). This insect originated in Central Africa (Baker, 1999). *Hypothenemus hampei* increases production costs, decreases yield, and reduces commercial value of coffee beans (Benavides and Arévalo, 2002; Benavides et al., 2002).

Hypothenemus hampei was introduced to the American continent in 1913 imported in seeds from Congo or Java to Brazil (Bergamin, 1946). During the next half century, this insect spread to most coffee-producing countries in South America. It was introduced into Colombia in 1988, presumably from Ecuador (Bustillo et al., 1998). *Hypothenemus hampei* is currently present in all the regions where coffee is grown in Colombia, and annually infests up to 13%

of the total coffee production (Federacafé, personal comm. 1997). How this insect pest spread throughout Colombia is unclear. The presence of *H. hampei* was first documented at the Southwest of Colombia in 1988 (Bustillo, 1990). *Hypothenemus hampei* continued spreading from the south to the north in the following year, but in 1990 the insect was found at the middle of the country more than 400 miles away from the first reported location. It was hypothesized that *H. hampei* was introduced through the commercial trade of food from Ecuador, and then spread throughout the country on food or tools coming from infested areas (Bustillo, 1990).

Hypothenemus hampei disperses in the field by being physically transported (carried by wind or water, or being moved by human agencies) or by flying from one region to another (Baker, 1984; Sánchez, 1985; Bustillo et al., 1998; Castro et al., 1998; Moreno et al., 2001). Considering the geographical barriers and long distances between coffee producing areas in Colombia, dispersion by human agencies might be the main means of long-distance dispersal. Factors such as the illegal trade of agricultural products between coffee regions or between coffee producing countries might have contributed to this phenomenon since illegal trade bypasses normal agricultural inspection regulations and procedures.

Hypothenemus hampei completes most of its life cycle inside the berry, except for the short period of time that females fly in search of new berries to infest. Adult females emerge from infested berries and colonize new berries where oviposition takes place. Each female lays an average of 74 eggs. The total life cycle requires about 28 days (24.5 °C), and females live up to 150 days (Bergamin, 1943).

The reproductive behavior of *H. hampei* ensures a high degree of inbreeding. A biased sex ratio was estimated to be 10 females for each male (Bergamin, 1943). Females mate with their flightless male siblings while still inside the berry, so they leave the infested berry already fertilized. Although cytological examination of somatic cells in males proved that they were diploid, males failed to express paternally derived alleles and then transmitted only their maternally derived chromosomes, a condition termed functional haplo-diploidy (Brun et al., 1995).

Molecular techniques are currently available to establish relatedness among organisms and distinguish individuals within or between populations with genetic markers. Amplified Fragment Length Polymorphism (AFLP) can detect dominant loci throughout the genome. AFLP's can be used to establish relatedness between samples of genomic DNA, or to find genetic markers to identify phenotypic traits or genetic loci (Vos et al., 1995). AFLP polymorphic fragments often can be converted into co-dominant markers, which allow genetic characterization of individuals for a specific locus (Meksen et al., 2001).

After estimating the genetic variability and biogeography of a worldwide collection of *H. hampei* (Benavides et al., 2005), a new set of objectives were proposed in order to corroborate the multiple introduction nature of different lines into Colombia. The objectives of this experiment were to estimate the genetic variability of *H. hampei* in Colombia, to identify the relationship between Colombian lines and other lines found in Latin America, and to locate exclusive polymorphisms within Colombia that could be used in the development of molecular markers. Molecular markers could be used in further experiments aimed at tracing dispersion patterns in the field, determining reproductive behavior and the genetics of this insect. Consistent with our hypothesis, we found a low genetic variability of *H. hampei* in Colombia and evidences of multiple introductions into the country. AFLP also detected polymorphisms from isolated geographical regions, which were used to design site-specific molecular markers.

MATERIALS AND METHODS

Insect samples

We collected samples from several coffee producing areas in Colombia in order to determine the biodiversity and genetic relatedness of *H. hampei* within. Sixty-six samples were obtained from 16 geographical areas (departments) that grow coffee in Colombia (Table 1). Each sample consisted of all stages of *H. hampei* within single coffee beans and presumably represented the genotype of the offspring of a single female. Infested coffee beans were collected in the field and were dissected in the laboratory.

Table 1. Location of *Hypothenemus hampei* samples collected in Colombia.

Department	n	Latitude/Longitude
Antioquia	7	6.15N/75.60W
Cundinamarca	5	4.50N/74.20W
Caldas	6	4.98N/75.62W
Caqueta	1	1.61N/75.62W
Cauca	1	2.42N/76.61W
Cesar	8	10.05N/73.25W
Huila	4	2.21N/75.65W
Magdalena	2	11.26N/74.19W
Meta	3	4.15N/73.64W
Nariño	3	1.20N/78.80W
Norte de Santander	2	7.91N/72.51W
Quindio	1	4.53N/75.69W
Risaralda	9	4.88N/75.62W
Santander	5	7.13N/73.14W
Tolima	3	4.92N/75.08W
Valle	6	4.28N/75.93W

Genomic DNA was extracted from each sample using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA). DNA quality was then examined by agarose gel electrophoresis and DNA concentration was determined using a Hoefer Scientific TKO 100 Mini-fluorometer. Genomic DNA samples were maintained at –70 °C.

DNA Fingerprinting

AFLP analysis was performed using the AFLP Analysis System II for small genomes developed by Life Technologies (GIBCO Invitrogen Corporation, Carlsbad, CA) and ^{33}P-ATP end labeled primers as recommended by the manufacturer with minor modifications. Each sample of *H. hampei* genomic DNA (~125 ng) was completely digested with the restriction endonucleases *Mse*I and *Eco*RI. Double stranded DNA adapters provided in the kit were ligated to the ends of the DNA fragments. Preselective primers were complementary to the core of the adapter sequences. The new DNA fragments were then pre-amplified and selective amplification was performed using two primer combinations (E-AT combined with M-CTC and M-CTG). These two combinations previously proved to detect great numbers of polymorphisms (Benavides et al., 2005). Amplified fragments were separated by gel electrophoresis for 2 h at 50 V in 6% Long Ranger (FMC Bioproducts, Rockland, ME) denaturing polyacrylamide gels. After electrophoresis, the gels were dried and exposed to Biomax MR (Kodak Rochester, NY) X-ray film for 48 to 72 hours for autoradiography.

DNA polymorphic bands were visually detected and excised from the polyacrylamide gel then kept at –70ºC for further analysis.

Conversion of AFLP polymorphisms into STS markers

Amplified fragments of DNA were excised from the polyacrylamide gel. The DNA was eluted into approximately 50 µl of TE buffer (10 mM Tris-HCL, 1 mM EDTA). Then 5 µl of the eluted DNA was re-amplified using the selective primer combinations that originally produced the polymorphic band. The re-amplified fragments were cloned using the TOPO TA Cloning Kit for Sequencing (GIBCO Invitrogen Corporation, Carlsbad, CA) and pGEM®-T Easy vector System I (Promega, Madison, WI). Briefly, these techniques allow the direct insertion of PCR products into a plasmid vector for sequencing. The *Taq* polymerase in the PCR adds a single deoxyadenosine to the 3' ends of PCR fragments. The vector contains a single overhanging 3' deoxythydimidine residue that allows PCR inserts to ligate with the vector. The cloned plasmids were then transformed into *E. coli* cells in order to select recombinants. Cells were incubated overnight in pre-warmed LB plates with 50 µg/ml of kanamycin. About 10 colonies were selected and cultured overnight in LB medium containing 50 µg/ml of kanamycin. The selected *E. coli* colonies were placed in a plate and sent to the Genomic Center at Purdue University for the isolation and sequencing of the plasmids. Sequencing was performed through automated fluorescent DNA BigDye terminator using an ABI-3700 Automatic Sequencer (PE Applied Biosystems). BLASTX and BLASTN sequence analysis (Altschul et al., 1994) were used to compare the sequences with other sequences in the GenBank database.

These sequences were used to design site-specific primers to amplify genomic fragments from those samples in which polymorphisms were detected. Samples in which polymosphisms were present were used as positive controls, while samples in which polymorphisms were absent would amplify the alternative allele. PCR was performed on a PTC 100 thermocycler (MJ Research, Watertonwn, MA). DNA from populations with presence and absence of polymorphisms were used in a 25 µl PCR containing 15 pmol of each site-specific primer, 10 nM Tris-HCl pH 9.1, 50 nM KCl, 2.5 mM $MgCl_2$, 0.1% Triton X-100, 2 mM of each dNTP and 1U of *Taq* polymerase. The PCR product was electrophoresed through a 1.0% agarose gel in 1X TBE.

Fragments that amplified both present and absent bands, were electrophoresed in an SSCP gel in order to reveal the polymorphisms. Those polymorphisms in which the nature of their genetics difference were not possible to observe using the above mentioned techniques, were used as template to design primers during a Genome Walking procedure (GenomeWalker®, Promega, Madison, WI). After getting larger fragments in both 5' and 3' ends, new primers were designed and polymorphic DNA samples tested again using the same principles and procedures as looking for STS´s.

Genetic analysis

The banding patterns associated with each selective primer combination and each sample were visually analyzed and converted into digital fingerprints. Each fragment position was scored as '1' if the fragment was present and as '0' if it was absent. To avoid artifacts, bands that were not recognized by 2 independent observers were eliminated from the analysis.

Digital fingerprints were used to calculate the percentage polymorphism for insect populations within the country, as well as to infer population genetic structure and perform phylogenetic analyses. The percentage polymorphism for insect populations within the

country was determined as the proportion of the polymorphic bands to the total number of fragments generated by AFLP in all samples within the country.

Population genetic structure was inferred using an analysis of molecular variance (AMOVA, Arlequin v2.0) (Excoffier et al., 1992). The analysis used all the fingerprints generated with all the samples within the country. Locations were treated as populations under the null hypothesis of no population substructure. All locations were tested as one group. Geographical subdivision of a population is called population substructure and it is usually measured by the fixation index Φ_{ST} that quantifies the inbreeding effect of population substructure (Wright, 1921). Species that are spread over large geographical areas are usually divided into subpopulations (Hartl and Clark, 1997; Hanski, 1999). In general, mating between organisms within the same subpopulation would be more likely than mating between organisms in different subpopulations (Hartl and Clark, 1997). If we consider an Φ_{ST} =0 as no genetic divergence, we can follow the qualitative guidelines for the interpretation of population substructure given by Wright (Wright, 1978).

For the phylogenetic analysis, we performed a bootstrap using the *Phylogenetic Analysis Using Parsimony* software (PAUP 4.0b10 Altivec) (Swofford, 1998) under the Neighbor Joining algorithm. We used 2000 re-samplings and 10 000 replications with the total number of pair-wise character difference distance setting. No outgroup was used for this analysis. Furthermore, in order to determine the origin of *H. hampei* fingerprints that were introduced into Colombia, results from this experiment were phylogenetically compared to the fingerprints obtained in previous investigations that aimed to detect genetic diversity from *H. hampei* samples collected in most coffee producing countries in Latin America and the Caribbean islands (Benavides et al., 2005).

RESULTS AND DISCUSSION

Insect samples

A total of 66 samples of *H. hampei* that covered 16 coffee producing areas within Colombia were collected (Table 1). Genomic DNA was isolated from dissected *H. hampei* individuals preserved in absolute ethanol. We dissected an average of 29.3 ± 18.2 individuals per berry that yielded 4.3 ± 1.8 µg of genomic DNA per sample. We correlated the number of individuals of *H. hampei* dissected in each single bean and the amount of DNA obtained from these samples (Table 2). We found no statistical correlation between these 2 variables (*significance F* = 0.92 > 0.05). Although an average of 20 to 40 individuals yielded the most DNA, 1 to 20 individuals were sufficient to isolate enough DNA to perform genetic analysis.

Table 2. Isolation of genomic DNA from *Hypothenemus hampei* samples collected in Colombia.

H. hampei	DNA per sample (µg)	Standard deviation	Min.	Max.
1 – 20	3.9	1.5	1.0	6.6
21 – 40	4.4	1.8	1.5	8.2
41 – 60	5.7	2.3	4.0	9.3
61 – 80	3.7	1.5	2.0	5.0

DNA Fingerprinting

AFLP amplified 60 fragments of DNA when the 2 highest polymorphic primer combinations were used (Benavides et al., 2005). We found only 7 polymorphic bands (11.7% of the total bands generated by AFLP), which indicated a very low genetic variability within populations of *H. hampei* in Colombia (Figure 1). The band patterns observed in each of the 66 samples generated only 8 fingerprints (Table 3). Fingerprint COL01 comprised 76% of the total sample size and grouped all geographical areas that were tested within Colombia. These results are indicative of a main line distributed in all departments within Colombia; however, there were more lines present in specific geographical areas. We found 4 other fingerprints (COL02, COL03, COL04, and COL05) that were unique for 4 different regions and which suggested independent introductions into Cesar, Caldas, Valle, and Risaralda. The remaining 3 fingerprints, COL06, COL07, and COL08, comprised samples from 4 departments: Caldas, Risaralda, Antioquia and Nariño. These results were a possible indication of a shared line among these departments. The sharing of food trade and exchange of labor among coffee farms could explain the relationship among samples from Antioquia, Risaralda, and Caldas within these 3 neighboring departments. The relationship between samples from Nariño with other regions could be better explained by the exchange of labor that occurs along the country.

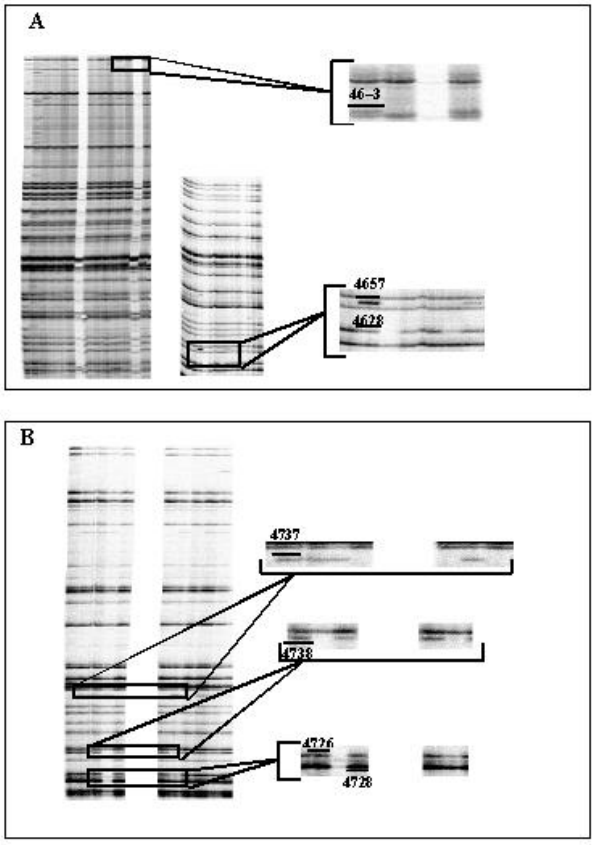


Figure 1. DNA fingerprints generated with primer combination E-AT/M-CTC (A) on samples Risaralda 08, Tolima 02, 03, Antioquia 01, 02,03, and Cesar 01, 02, 03, 04, 05, 07 (A1); and samples Risaralda 08, 05, 06, 07 08, 09, Caldas 06, Antioquia06, 07, Nariño02 and 03 (A2). DNA fingerprints with primer combination E-AT/M-CTG (B) on samples Risaralda08, Tolima02, 03, Antioquia02, 03, Cesar01, 02, 03, 04, 05 and 07. Two lanes in AFLP gel A1 did not amplified DNA fragments properly, one between samples Antioquia 03 and Cesar 01 and the other between samples Cesar 05 and 07. The 7 polymorphisms found within Colombian samples are indicated.

Table 3. AFLP Fingerprints generated with 2 primer combinations on 66 Colombian samples of *Hypothenemus hampei*.

Fingerprint	n	Sample	Equivalent Fingerprint in Latin America	Equivalent samples in Latin America
COL01	50	Antioquia01-05	FP02	CR02
		Cundinamarca01-05		
		Caldas01-04		
		Caqueta01		
		Cauca01		
		Cesar01-03,05,08		
		Huila01-04		
		Magdalena01-02		
		Meta01-03		
		Nariño01		
		Norte Santander01-02		
		Quindio01		
		Risaralda01,03-04		
		Santander01-05		
		Tolima01-03		
		Valle01-04,06		
COL02	2	Cesar04,07	FP27	Unique to Colombia
COL03	1	Caldas06	FP06	BR04,06 JA01, 02 EC01, 02, 04, 05 NI01, 03, 04 HO01, 04, 06, 07
COL04	1	Valle05	FP03	BR02, 07, 14
COL05	1	Risaralda07	FP05	Control. Unique to Colombia
COL06	2	Caldas05	FP28	Unique to Colombia
		Risaralda02		
COL07	5	Antioquia07	FP15	BR01, 03, 05
		Nariño02		PE01, 02, 05, 06, 10
		Nariño03		SA01
		Risaralda05		
		Risaralda06		
COL08	4	Antioquia06	FP12	PE03, 04, 07, 09
		Caldas07		
		Risaralda08		
		Risaralda09		
TOTAL	66			

Hypothenemus hampei was first detected inside Colombia in Nariño, which is located at the Southwest of the country at the border with Ecuador (Bustillo, 1990). Harvesters from Nariño travel yearly to the middle of the country where about 60% of the Colombian coffee is produced (Antioquia, Caldas, Risaralda, Quindio and Valle). These harvesters travel that long a distance (above 400 miles) motivated not only because salaries are higher in these regions but also because their main coffee harvest period is at a different time. More specifically, the main harvest has finished at the south by the time the harvest period starts in the middle of the country. We hypothesized that one line of *H. hampei* (COL07) might come to Risaralda and

Antiqouia carried by harvesters from the Southwest, which could also explain the presence of the line COL01 along the country. The exchange of labor is our strongest hypothesis to explain the dispersion of *H. hampei* within Colombia.

A phylogenetic analysis of the samples from Colombia revealed 3 clusters (Figure 2). Fingerprint COL01 formed the first isolated group. A second cluster indicated closer relatedness between fingerprints COL02 (Cesar) and COL06 (Caldas and Risaralda). A third cluster grouped Caldas, Risaralda, Valle, Antioquia and Nariño, which were contained in the remaining 5 fingerprints. Although this neighbor-joining analysis was exhibiting some relatedness among fingerprints, it was not relevant for revealing the origin of the introduced samples. The variation among samples was probably the result of multiple introductions and not the result of evolutionary events such as random mutations.

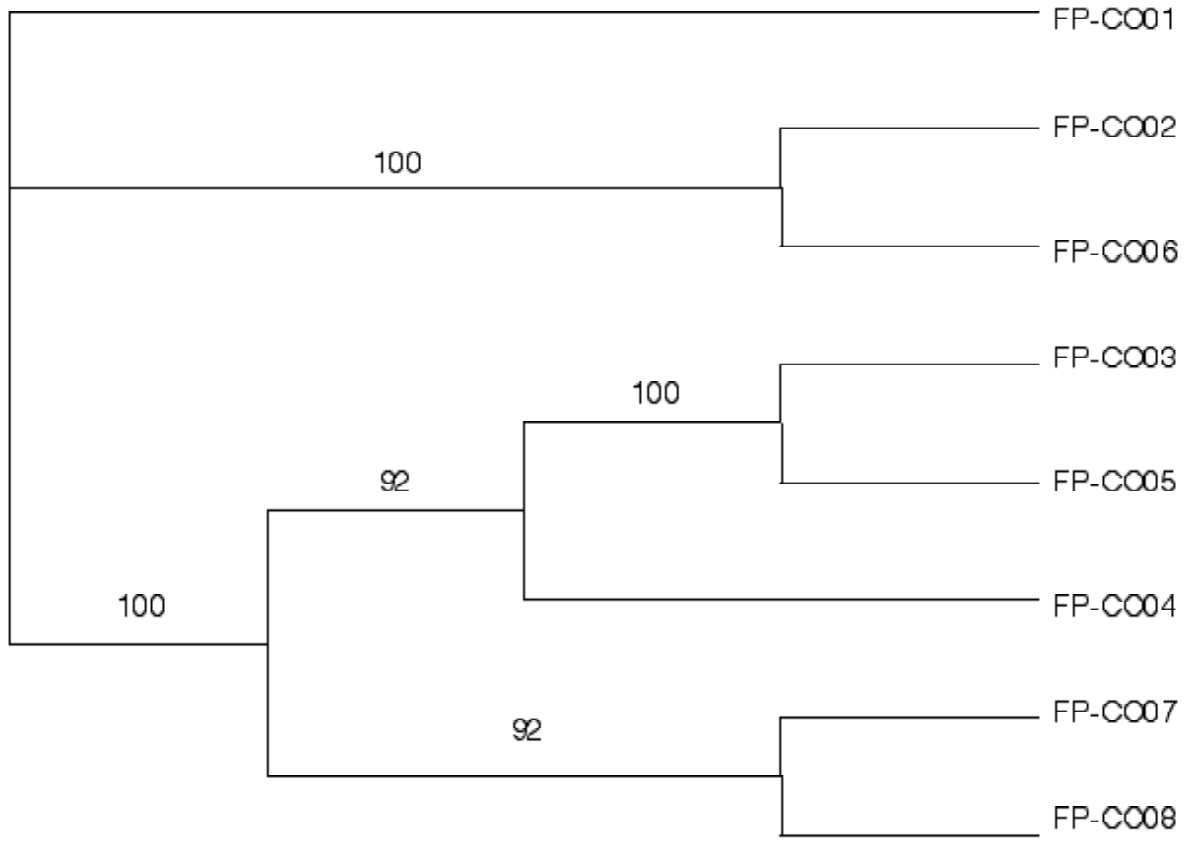


Figure 2. Bootstrap under Neighbor Joining Distance Analysis with the data obtained for the Colombian fingerprints generated by the primer combinations E-AT/M-CTC and E-AT/M-CTG. Fingerprint COL01 comprised 76% (50) of the total number of samples collected and was represented in all coffee producing departments in Colombia. Fingerprint COL02, COL03, COL04, and COL05 were exclusively found in the departments of Cesar, Caldas, Valle, and Risaralda respectively. Fingerprint COL06 contained Caldas and Risaralda; COL07 Antioquia, Nariño, and Risaralda, and COL08 Antioquia, Caldas and Risaralda.

In order to estimate population substructure on *H. hampei* in Colombia, we performed an analysis of molecular variance (Table 4). Samples Caqueta01, Cauca01 and Quindio01 were excluded from this analysis since only one sample was collected in each department and, therefore, variance within populations could have been altered. We found 92% of the variance due to difference among locations and 8% due to difference within locations, results expected due to inbreeding. According to Wright's Φ_{ST} parameters (Wright, 1978), a value between

0.05-0.15 is an indication of moderate genetic differentiation. Therefore, *H. hampei* in Colombia evidenced moderate genetic differentiation probably as a result of recent introductions from diverse populations.

Table 4. Hierarchical analysis of variance for 13 departments in Colombia with no grouping.

Variance		d.f.	Variance	% total	*p**	*Φ*-statistics
Among locations	σ_a^2	12	0.018	8.32	0.09	
Within locations	σ_c^2	50	0.202	91.68	0.00	$\Phi_{ST} = 0.083$
Total		62	0.220			

Furthermore, in order to determine the origin of *H. hampei* fingerprints introduced into Colombia, we compared Colombian fingerprints with those previously found in all Latin American countries and the Caribbean islands (Benavides et al., 2005) (Table 3). An interesting finding related fingerprint COL01, which grouped the majority of the Colombian samples, with the fingerprint FP02 that was unique for Costa Rica. This result suggested that Colombia was probably the main source for the introduction of *H. hampei* into Costa Rica. We observed 3 fingerprints unique to Colombia and not related to any other country. We believe that these samples that generated unique fingerprints could have been present in other geographical areas, but were not sampled during this experiment. Fingerprint FP06 that was found in Brazil, Jamaica, Ecuador, Nicaragua, and Honduras, was also found in Caldas06; and fingerprint FP03, which was present in Brazil, was also present in Valle05. These results suggested that there may have been more than one single introduction of *H. hampei* into Colombia, and perhaps Colombia has been the source of later introductions into other Central American countries.

Fingerprint FP15 present in Brazil, Peru and El Salvador, and the Peruvian fingerprint FP12 were found in Colombia in the departments of Antioquia, Nariño, Caldas, and Risaralda. These results suggested that some lines were introduced from different origins and later dispersed throughout the country. Although the introduction of *H. hampei* was reported into Colombia directly from Ecuador to Nariño (Bustillo, 1990), the genetic analysis indicated no link between these 2 points. A clearer link was detected between samples from Antioquia, Risaralda, and Nariño and samples from Peru. The fact that *H. hampei* was reported first in Nariño is not a strong indication that this region was the initial source of this insect in Colombia. *Hypothenemus hampei* could have been introduced first into Antioquia from Peru and then transported to Nariño by the exchange of labor between coffee regions within the country, or even from Peru directly to Nariño and then carried to Antioquia by the same means. The presence of the Peruvian fingerprint FP12 into Colombia strengthens this hypothesis; therefore, *H. hampei* was more likely initially introduced from Peru into Colombia, and the mechanism of this introduction could be hypothetically explained by the trade of illegal drugs in the Americas (Figure 3).

An AMOVA with all Colombian and Latin American samples was performed (Table 5). We found similar results in this analysis as with those obtained when worldwide countries were analyzed. Great genetic differentiation was found in *H. hampei*.

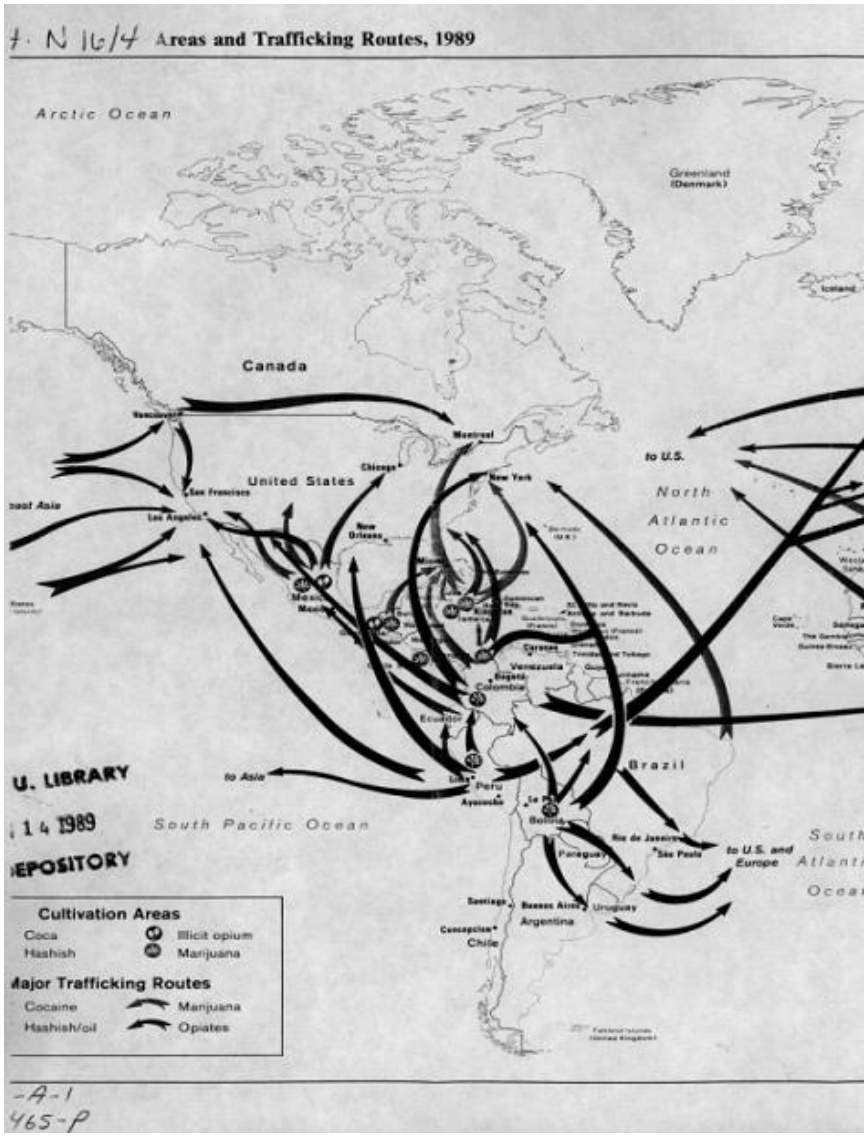


Figure 3. Areas and trafficking routes of illegal crops (C.I.A. 1989). Observe clear routes coming from Peru straight to the south portion of Colombia and to Ecuador. There is also a route to Jamaica from Colombia.

Table 5. Hierarchical analysis of variance for Colombian and Latin American samples with no grouping.

Variance		d.f.	Variance	% total	p*	Φ-statistics
Among locations	σ_a^2	18	0.178	44.12	0.00	
Within locations	σ_c^2	96	0.225	55.88	0.00	Φ_{ST} = 0.4412
Total		114				

Conversion of AFLP polymorphisms into STS markers

In order to design molecular markers, AFLP polymorphisms were converted into site-specific primers. Ten polymorphic bands were excised from the AFLP acrylamide gels; therefore, those polymorphisms were cloned and sequenced. We obtained AFLP fragments between 60 and 578 total bases (Figure 5). Site-specific primers were designed with these sequences and PCR was performed on samples where AFLP polymorphisms were absent and present (Figure

4). Site-specific primers 4742, 4753, 4754, and 4628 showed amplification on AFLP present samples and partial amplification on AFLP absent samples. We believe that this partial amplification is due to the nature of the mutation detected in these samples with these primer combinations. A single mutation in the *Mse*I or *Eco*RI restriction site would allow partial amplification when using the site-specific primers. Only the site-specific primer 46-3 revealed presence and complete absence patterns in present and absent samples. These results suggested the lack of one of the restriction sites sequence in the AFLP absent sample, or perhaps an inversion of a large fragment that could have not been amplified by PCR. Complete amplification of DNA fragments in absent samples was observed when site-specific primers 4756, 4737, 46-2, 46-6, and 4657 were used. Primers 46-2 and 46-6 were present in the AFLP of all Colombian samples, except for a few samples where their presence was not clear. The results obtained after this experiment allowed us to corroborate the presence of these 2 fragments in all Colombian samples. The amplification of fragments in AFLP absent samples when using primers 4756, 4737, and 4657 can also be explained as the presence of a single point mutation in the sequence of one of the restriction sites, and the successful amplification through PCR.

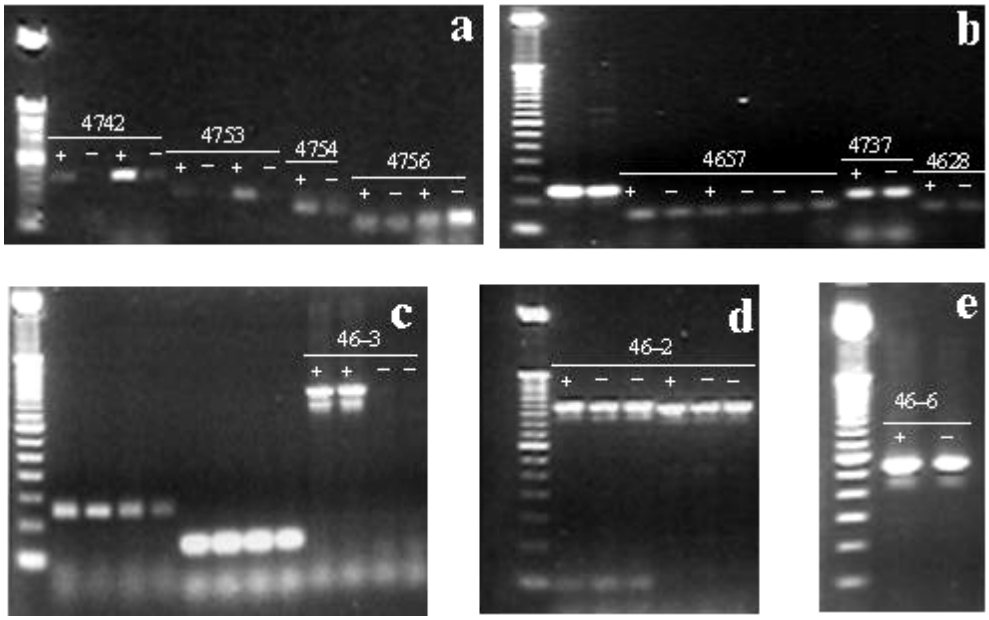


Figure 4. PCR using site-specific markers. First lanes indicate a 50 bp marker. We obtained partial amplification of negative samples when primers 4742, 4753, 4754, and 4628 were used. Complete absence on the alternative allele with primer 46-3 and complete amplification of negative samples with primers 4756, 46-2, 46-6, 4657, and 4737.

Even though we did not find a marker that revealed co-dominance, the site-specific primer 46-3, unique for only 2 samples within Colombia and located in the geographical area of Cesar, showed dominance and can be used as a genetic marker in field experiments aimed to trace dispersion patterns in the field. To corroborate this information, the primer combinations designed to amplify fragment 46-3 was used in a PCR with all Colombian populations (Figure 5). The results showed the amplification of this particular fragment in the 2 initially amplified populations, and complete absence in the rest of the samples from Colombia. Unfortunately, this polymorphic band was not possible to be recovered from the original population once in Colombia due perhaps to genetic drift events, the presumed nature of the polymorphism as being a transposon, or the presence of genomic contamination from insect symbionts.

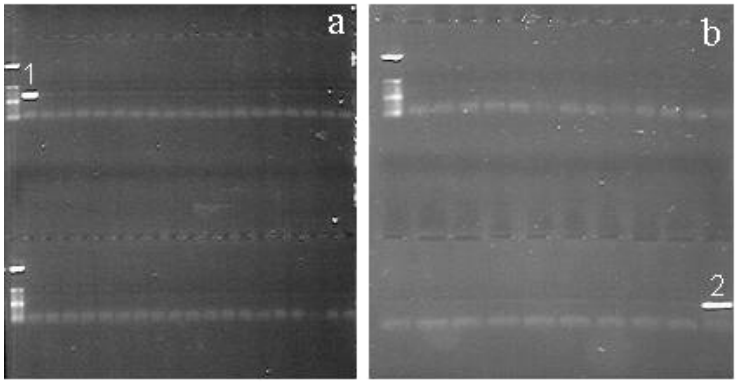


Figure 5. PCR with primers designed to amplify allele 46-3. Two positive Colombian samples, 1 in gel a and 2 in gel b (1:Cesar04 and 2:Cesar07) were used with 59 other Colombian samples (38 samples in figure a and 23 samples in figure b). amplification occurred only in the 2 original samples.

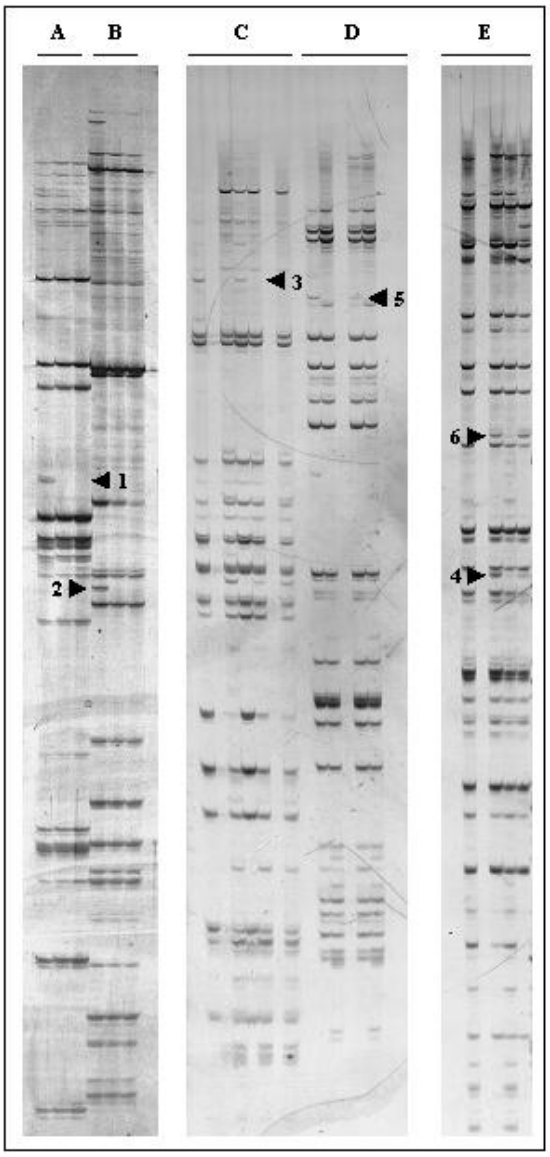


Figure 6. New AFLP polymorphisms detected in field Colombian *H. hampei* populations. Five new sets of primer combinations (A to E) were selected using the four most polymorphic *H. hampei* Colombian samples.

Furthermore, we developed six new STS markers from field *H. hampei* samples (Figure 6). After recovering the polymorphisms from the populations used in AFLP, we designed a new set of primers based on the AFLP fragment sequences. The fragments were further amplified on the present and absent populations used during the AFLP technique (Figure 7). Those *H. hampei* samples that amplified the fragment in both the present and the absent populations, were electrophoresed in an SSCP gel and allowed us to identify the nature of the polymorphic STS *Hha*STS2 and *Hha*STS5 (Figure 8).

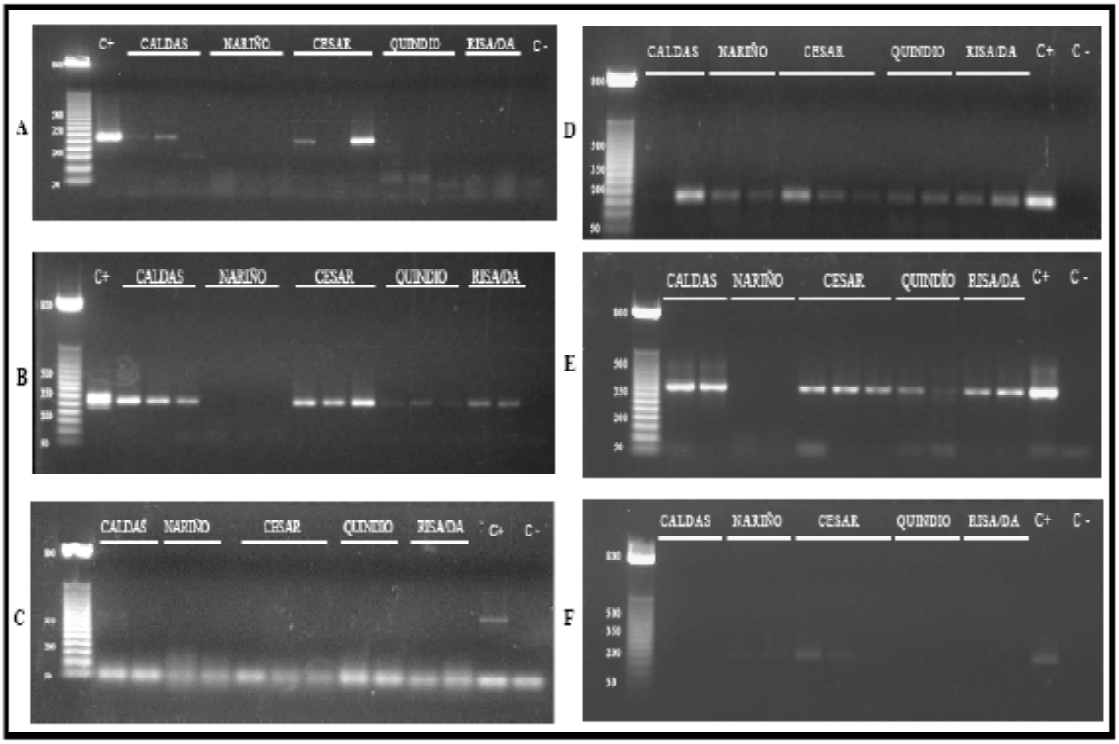


Figure 7. PCR using site-specific markers. First lanes indicate a 50 bp marker. We obtained amplification of negative samples when primers *Hha*STS2 (B), *Hha*STS4 (D) and *Hha*STS5 (E) were used. Partial amplification on the alternative allele with primer *Hha*STS1 (A) and *Hha*STS6 (F); and no amplification with *Hha*STS3 (C). C^+ and C^- were the positive and negative controls for the PCR respectively.

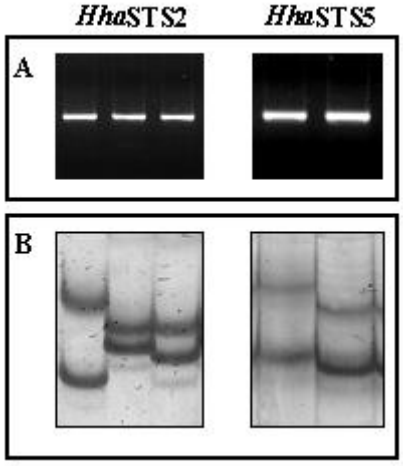


Figure 8. Electrophoresis on 2% TBE agarose gel (A) and SSCP (B) of present and absent *H. hampei* populations SSCP. SSCP allowed us to detect the different alleles with the STS *Hha*STS2 and *Hha*STS5.

*Hha*STS2 polymorhic alleles were further sequenced and aligned. This alignment showed a highly polymorphic region of four nucleotides and a deletion of 39 bases into one allele. The nature of this polymorphism was clearly revealed. We are currently working on the STS *Hha*STS5 in order to find the sequence of this new polymorphism.

We believe that these new co-dominant molecular markers can be used now as tools for studying the inheritance of genetic traits in *H. hampei*, as well as the dispersal patterns used by this insect in the field.

REFERENCE

Altschul, S. F., M. S. Boguski, W. Gish, and J. C. Wootton. 1994. Issues in searching molecular sequence databases. Nature Genetics 6: 119-129.

Baker, P. S. 1984. Some aspects of the behavior of the coffee berry borer in relation to its control in southern Mexico (Coleoptera: Scolytidae). Folia Entomológica Mexicana 62: 9-24.

Benavides, M., F. E. Vega, H. Romero, A. Bustillo, and J. Stuart. 2005. Biodiversity and biogeogaphy of an important pest of coffee, the coffee berry borer, *Hypothenemus hampei* (Ferrari)(Coleoptera: Curculionidae: Scolytinae). Ann. Entomol. Soc. Am.

Benavides, P., and H. Arévalo. 2002. Manejo Integrado: una estrategia para el control de la broca del café en Colombia. Cenicafé 53: 50-59.

Benavides, P., A. E. Bustillo, E. C. Montoya, R. Cárdenas, and G. Mejía. 2002. Evaluación de los métodos de control cultural, químico y biológico en el manejo integrado de la broca del café. Revista Colombiana de Entomología 28: 247-253.

Bergamin, J. 1943. Contribuicao para o cohnecimento da biologia da broca do cafe *Hypothenemus hampei* (Ferrari 1867) (Col. Ipidae). Arq. Inst. Biol., Sao Paulo 14: 31-72.

Bergamin, J. 1946. A broca do cafe no Brasil. Bol. Supt. Serv. Cafe (Sao Paulo) 33: 21-22.

Brun, L. O., J. Stuart, V. Gaudichon, K. Aronstein, and R. H. ffrench-Constant. 1995. Functional haplodiploidy: a mechanism for the spread of insecticide resistance in an important international insect pest. Proc. Natl. Acad. Sci. U. S. A. 92: 9861-9865.

Bustillo, A. E. 1990. Perspectivas de manejo integrado de la broca del café en Colombia, pp. 106-118, Seminario sobre la broca del café. Sociedad Colombiana de Entomología, Medellin, Colombia.

Bustillo, A. E., R. Cárdenas, D. Villalba, P. Benavides, J. Orozco, and F. J. Posada. 1998. Manejo integrado de la broca del café *Hypothenemus hampei* (Ferrari) en Colombia. Cenicafé, Chinchiná, Colombia.

Castro, L., P. Benavides, and A. E. Bustillo. 1998. Dispersión y mortalidad de *Hypothenemus hampei* dutante la recolección y beneficio del café. Manejo Integrado de Plagas 50: 19-28.

Excoffier, L., P. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Hanski, I. 1999. Metapopulation Ecology. Oxford University Press, Oxford.

Hartl, D. L., and A. Clark. 1997. Principles of population genetics. Sinauer Associates, Inc., Sunderland, Massachusetts.

Le Pelley, R. H. 1968. Pests of Coffee. Longmans, Green & Co., Ltd., London.

Meksen, K., E. Ruben, D. Hyten, K. Triwitayakorn, and D. A. Lightfoot. 2001. Conversion of AFLP bands into high-throughput DNA markers. Molecular Genetic Genomics 265: 207-214.

Moreno, D., A. E. Bustillo, P. Benavides, and E. C. Montoya. 2001. Escape y mortalidad de *Hypothenemus hampei* en los procesos de recolección y beneficio del café en Colombia. Cenicafé 52: 111-116.

Sánchez, R. 1985. Biología de la broca del café (*Hypothenemus hampei* Ferr.), pp. 97-104. *In* P.-. Anacafé [ed.], Curso sobre Manejo Integrado de Plagas del Cafeto, con énfasis en la Broca del Fruto (*Hypothenemus hampei* Ferr.). Promecafé - Anacafé, Guatemala.

Swofford, D. L. 1998. Phylogenetic Analysis Using Parsimony computer program, version 4. By Swofford, D. L., Sunderland, Massachusetts.

Vos, P., R. Hogers, M. Bleeker, M. Rreijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Wright, S. 1921. Systems of mating. Genetics 6: 111-178.

Wright, S. 1978. Evolution and the genetics of populations. Variability within and among natural populations. University of Chicago Press, Chicago.

Histochemical Differences in *Coffea* Genotypes Resistant and Susceptible to the Coffee Leaf Miner

D. RAMIRO, O. GUERREIRO-FILHO

Instituto Agronômico (IAC), Centro de Café 'Alcides Carvalho', Avenida Barão de Itapura, 1481, CP 28, CEP 13001-970, Campinas (SP), Brazil

SUMMARY

The leaf miner *Leucoptera coffeella* is the major pest of coffee culture. The Coffee Breeding Program of the Instituto Agronômico (IAC) has been transferring, through traditional crossings, genes that confer the resistance to the leaf miner from the species *C. racemosa* to the susceptible species *C. arabica.* The main objective of this study was to characterize leaf tissues, at histological and biochemical level from the parental species *C. racemosa* and *C. arabica,* and also from their hybrids exhibiting different resistance levels. Results reveal that there are significant differences in leaf tissue thickness between parental species *C. arabica* and *C. racemosa.* However, in hybrids analysis no such difference could be observed between resistant and susceptible progenies, suggesting that the anatomical differences of parental genotypes may not be related to coffee resistance mechanisms to *L. coffeella.* Results of biochemical analysis demonstrated that the activity of peroxidase (POD) was the only one affected by the attack of the leaf miner, and no other differential response was observed between resistant and susceptible hybrid progenies. ln this case, the activation of POD was related to the insect damage rather than to resistance mechanisms. The activity of polyphenol oxidase (PPO) was increased in *C. racemosa* leaves upon leaf-miner attack. Concentration of phenols and clorogenic acid were significantly higher in leaves of *C. arabica* and hybrid progenies, but were reduced after insect infestation. In the other hand, in *C. racemosa* leaves an increase of clorogenic acid levels was observed in the presence of larvae, at four days after eclosion. However, average concentration of phenols and clorogenic acid was similar among resistant and susceptible hybrid progenies. Also, all hybrid progenies showed similar protein levels and same pattern of PPO activity. The results obtained in this work suggest that the phenolic oxidation catalyzed by PPO and POD is not direct1y related to coffee defense mechanisms against *L. coffeella.*

Key-words*:* Coffee, *Coffea racemosa*, pest resistance, *Leucoptera coffeella*, oxidative enzymes, palisade parenchyma

INTRODUCTION

The leaf miner, *Leucoptera coffeella* (Guérin-Méneville, 1842) (Lepidoptera: Lyonetiidae), the main insect pest of coffee plants in Brazil, is also considered to be an important primary pest in other coffee-producing countries. Both of the commercially important species produced in the world, *C. arabica* L. and *C. canephora* Pierre, are susceptible to the insect. Leaves attacked usually fall and depending on the defoliation intensity yield loss may account for 50% of the coffee production (Paulini et al., 1976).

A strategy for the development of cultivars resistant to leaf miners has been the transfer of resistance genes from the wild species *C. racemosa,* via successive back crosses to *C. arabica*. Though it is known that resistance to this pest is conferred by two complementary and dominant genes, little is known about the biochemical nature of the resistance (Guerreiro-

Filho et al., 1999). The main objective of this study was to characterize leaf tissues, at histological and biochemical level from the parental species *C. racemosa* and *C. arabica,* and also from their hybrids exhibiting different resistance levels.

MATERIAL AND METHODS

Analyses were carried out in leaves of health mature coffee plants maintained under field conditions at the Experimental Center of Instituto Agronômico (IAC), Campinas (SP), Brazil. These plants could be divided in four groups: 1) *C. arabica* (susceptible); 2) *C. racemosa* (resistant); 3) susceptible hybrids; and 4) resistant hybrids. Histological analysis were performed in leaf transverse cuts, and included measurements of superior and inferior cuticles thickness, total palisade and spongy parenchyma, total leaf thickness, and percentage of the palisade parenchyma in total leaf mesophyll. The biochemical analyses consisted of comparative analyses between infested and uninfested leaves, in different stages of insect development, of the concentrations of total soluble phenols and chlorogenic acid (5-caffeoylquinic acid), and the activities of the oxidative enzymes peroxidase (POD) and polyphenol oxidase (PPO). A chromatographic profile of the phenolic compounds was obtained with a Shimadzu HPLC, equipped with a diode array detector.

RESULTS

Histological Analysis

Results reveal that there are significant differences in leaf tissue thickness between parental species *C. arabica* and *C. racemosa.* However, in hybrids analysis no such difference could be observed between resistant and susceptible progenies, suggesting that the anatomical differences of parental genotypes may not be related to coffee resistance mechanisms to *L. Coffeella* (Table 1; Figure1).

Table 1. Mean thickness of leaf tissues in *Coffea arabica* and *C. racemosa*, and in two hybrids derived from crosses between them, expressing different levels of resistance to the coffee leaf miner (*Leucoptera coffeella*).

Groups	SC (µm)	SE (µm)	PP (µm)	SP (µm)	IE (µm)	IC (µm)	PPT (%)	TLT (µm)
Coffea arabica	2.65b	19.97c	48.43a	162.48a	13.43c	2.11a	22.99c	249.06a
Coffea racemosa	3.93a	36.42a	57.14a	91.71c	22.13a	2.24a	38.45a	213.57bc
Resistant hybrids	2.89b	23.68b	47.18a	109.26bc	17.79b	2.11a	30.10b	202.91c
Susceptible hybrids	3.35ab	24.29b	59.28a	129.77b	17.35b	2.19a	31.37b	236.23ab

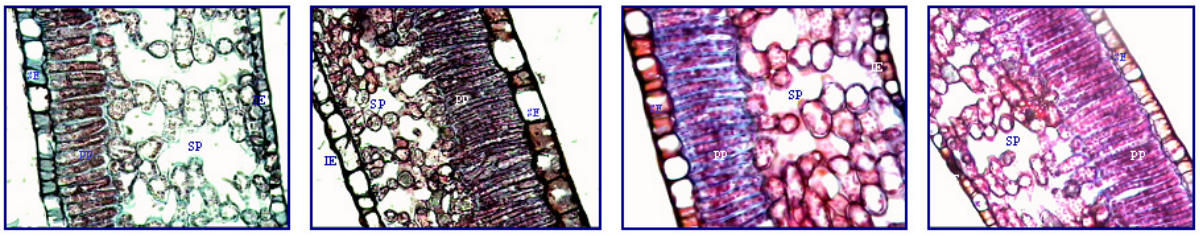


Figure 1. Leaves transversal cuts in *Coffea arabica* and *C. racemosa* plants, and in two hybrids derived from crosses between them. SE: superior epidermis; PP: palisade parenchyma; SP: spongy parenchyma; IE: inferior epidermis.

Biochemical analyses

Feeding by the coffee leaf miner did not significantly alter the phenol levels in the leaves, either on the damaged side of the leaves, or on the side from which the eggs were removed, in each group of plants (Table 2). The HPLC analysis did not reveal qualitative differences in the phenolic compounds in the infested leaves compared to uninfested leaves (data not shown).

Table 2. Mean concentrations of phenolic compounds (mass equivalents - mg - of chlorogenic acid per g fresh leaf) in leaves of *Coffea arabica* and *C. racemosa* and of two hybrids derived from a Cross Between these species.

Groups	UL	USL	IL	Mean*
C. arabica	60.9 ± 10.9	49.2 ± 7.1	48.8 ± 4.6	52.9 a
C. arabica x C. racemosa (S)	53.2 ± 13.9	45.7 ± 5.4	45.1 ± 5.5	48.0 a
C. arabica x C. racemosa (R)	52.5 ± 2.1	49.9 ± 3.8	51.0 ± 2.9	51.2 a
C. racemosa	24.9 ± 9.6	24.2 ± 10.3	20.6 ± 7.8	23.0 b
Mean**	47.9 A	42.2 AB	41.5 B	-

Mean values for groups (*) and treatments (**) when followed by the same letter are not significantly different (Tukey 5%); (S) susceptible; (R) resistant; (UL) uninfested leaves; (USL) uninfested side of the leaf; (IL) infested side of the leaf.

The leaf contents of chlorogenic acid of the species were significantly different in all treatments. Uninfested leaves of *C. racemosa* had approximately six times less chlorogenic acid than *C. arabica* and the hybrid populations, which did not differ significantly from each other (Table 3). The chlorogenic acid concentrations showed a similar tendency to that observed for total phenols in the hybrids and in *C. arabica*, i.e. a reduced content in the infested tissues. On the other hand, opposite to what was observed for total phenols, *C. racemosa* responded to leaf miner attack with a 66.6% increase in chlorogenic acid concentration.

Table 3. Mean concentrations of chlorogenic acid (µg/g fresh leaf) in plants of the species *Coffea arabica* and *C. racemosa*, and in two hybrids derived from crosses between them, expressing different levels of resistance to the coffee leaf miner (*Leucoptera coffeella*).

Groups	UL	USL	IL
C. arabica	2092 ± 411 a A	1522 ± 229 b A	944 ± 202 c B
C. arabica x C. racemosa (S)	1949 ± 337 a A	1674 ± 197 b A	1409 ± 182 b A
C. arabica x C. racemosa (R)	1935 ± 201 a A	1833 ± 178 a A	1559 ± 222 a A
C. racemosa	355 ± 18 b B	431 ± 164 b B	591 ± 126 a C

The values for each group followed by the same capital letter and treatment followed by the same small letter were not significantly different (Tukey 5%); (S) susceptible; (R) resistant; (UL) uninfested leaves; (USL) uninfested side of the leaf; (IL) infested side of the leaf.

POD activities, measured soon after the leaves were collected, were higher in *C. arabica* and the resistant progeny than in the susceptible hybrid. Lowest activities were observed in *C. racemosa*, which had up to 15 times less activity.

Under insect infestation, the groups responded differently to treatments 1 DAE and 4 DAE. There was no induction of POD activity due to insect attack in any of the populations, in the evaluations made at 1 DAE (Table 4). At 4 DAE, leaf miners provoked a 72.4% increase in

POD activity in *C. arabica* and 89.6% in the susceptible hybrids. POD activity induced by *L. coffeella* was positively correlated with the type of lesion provoked by their feeding activity (data not shown). In these plants the damage caused by insect feeding is characterized by a massive and localized destruction of a large number of palisade cells (Ramiro et al., 2004).

Table 4. Mean activity of peroxidase (δa_{470}/hour/μg protein) in recently collected, infested and uninfested coffee leaves, one or four days after eclosion of the leaf miner larvae.

Groups	RCL	1 DAE		4 DAE	
		UL	I	UL	I
C. arabica	0.569±0.116 b A	0.363±0.088 c B	0.397±0.078 c B	0.566±0.111 b A	0.976±0.240 a A
Susceptible hybrids	0.212±0.049 c B	0.457±0.090 b AB	0.432±0.129 b B	0.355±0.091 b B	0.673±0.194 a B
Resistant hybrids	0.676±0.097 a A	0.591±0.118 a A	0.627±0.192 a A	0.550±0.183 a A	0.720±0.144 a B
C. racemosa	0.047±0.013 c C	0.065±0.020 bc C	0.097±0.022 b C	0.155±0.024 a C	0.168±0.052 a C

The values for each group followed by the same capital letter and treatment followed by the same small letter were not significantly different (Tukey 5%); DAE = days after eclosion of the leaf miner larvae; (RCL) recently collected leaves; (UL) uninfested leaves; (I) infested leaves.

PPO was significantly induced by the insect feeding activity only in *C. racemosa,* at 4 DAE (Table 5).

Table 5. Mean activity of polyphenol oxidase (ΔA_{470}/hour/μg protein) in recently collected, infested and uninfested coffee leaves, one or four days after eclosion of the leaf miner larvae.

Groups	RCL	1 DAE		4 DAE	
		UL	I	UL	I
C. arabica	0.155±0.028 aB	0.128±0.015 aB	0.132±0.029 aB	0.155±0.043 aB	0.153±0.045 aB
Susceptible hybrids	0.075±0.023 aC	0.055±0.019 aC	0.070±0.023 aC	0.080±0.025 aC	0.106±0.029 aC
Resistant hybrids	0.068±0.017 aC	0.057±0.020 aC	0.074±0.027 aC	0.048±0.015 aC	0.095±0.028 aC
C. racemosa	0.303±0.077 bA	0.350±0.104 bA	0.366±0.054 bA	0.336±0.087 bA	0.521±0.176 aA

The values for each group followed by the same capital letter and treatment followed by the same small letter were not significantly different (Tukey 5%); DAE = days after eclosion of the leaf miner larvae; (RCL) recently collected leaves; (UL) uninfested leaves; (I) infested leaves.

CONCLUSIONS

The results suggest that there is no relation between the leaf anatomical characteristics and the resistance to the leaf miner. Assuming that the resistant hybrids inherited the two resistance genes from *C. racemosa* (Guerreiro-Filho, 1994), the results obtained here for phenolic

content and activity of the enzymes POD and PPO are not a strong evidence for their participation in a direct defence mechanism of coffee against *L. coffeella*.

REFERENCES

Ramiro, D.A., Guerreiro-Filho, O.; Mazzafera, Paulo. (2006). Journal of Chemical Ecology, 32: 1977-1988.

Ramiro, D. A., Guerreiro Filho, O., Queiroz Voltan, R. B. E Matthiesen, S. C. (2004). Bragantia, 63(3):363-372.

Guerreiro-Filho, O., Silvarolla, M.B., Eskes, A.B. (1999). *Euphytica*. 105:7-15.

Paulini, A.E., Matiello, J.B., Paulino, A.J. (1976). In: Congresso Brasileiro de Pesquisas Cafeeiras, 4., 1976, Caxambu. Resumos... Rio de Janeiro: IBC – GERCA. p.48-49.

Biodiversity of Root Knot Nematodes, *Meloidogyne* spp., on Coffee in Central America

L. VILLAIN[1], J. L. SARAH[1], A. HERNANDEZ[2], P. CHARMETANT[1], B. BERTRAND[1], F. ANTHONY[3], P. TOPART[3], P. LASHERMES[3], F. ANZUETO[4], R. M. D. G. CARNEIRO[5]

[1]Cirad, Montpellier, France
[2]Procafé, Santa Tecla, El Salvador
[3]IRD, Montpellier, France
[4]Anacafé, Guatemala City, Guatemala
[5]Embrapa – Recursos Genéticos e Biotecnología, Brasilia, Brazil

SUMMARY

A survey was carried out in different coffee production regions of Central America (CA) in order to assess the biodiversity of root knot nematodes (RKN), *Meloidogyne* spp., parasitizing coffee tree roots in this region. Populations extracted from roots were reared on tomato and coffee plants before realizing their diagnostic by isozyme electrophoresis (esterase phenotype, Est). *M. exigua* (Est E1, Rm: 1.5) appeared as the RKN with larger distribution on coffee in CA with large presence in Honduras, Nicaragua and Costa Rica. This species was also observed in just one sample from Guatemala. The predominance of *M. paranaensis* (Est P1, Rm: 1.32 and P2, Rm: 0.9, 1.32) on coffee in Guatemala was confirmed. This RKN has not been detected in others countries of CA. The recently described species on coffee in El Salvador, *M. izalcoensis* (Est I4 = Sa4, Rm: 0.86, 0.96, 1.24, 1.30), seems to be largely scattered in the south-eastern region of Izalco. The presence of *M. arabicida* (Est AR2, Rm: 1.20, 1.40) was confirmed in Turrialba valley in Costa Rica where it seems to be confined. Est I1 phenotype (Rm: 1.0), characteristic of *M. incognita*, was found for one population from El Salvador, one from Guatemala and several from three different regions of Costa Rica. These are the first based on esterase diagnostic reports of *M. incognita* on coffee in CA. *M. arenaria* (Est A2, Rm: 1.20, 1.30) was observed in several farms in the south-western region of Izalco in El Salvador. The new phenotype, Sa2 (Rm 1.20, 1.32), was also observed in the same region. *M. hapla* (Est H1, Rm: 1.1) was observed at two highland sites, one in Guatemala and the other one in El Salvador. New reports of *M. mayaguensis* (Est M2, Rm: 0.79, 1.08) on coffee in CA were observed: one from Costa Rica and the other one from Guatemala. Est A1 (Rm: 1.30), characteristic of *M. arenaria* and other species, was observed for one population from Guatemala, apparently very pathogenic on coffee. A large inter-specific diversity of RKN was observed on coffee in CA.

RESUMEN

Se realizó un monitoreo de la biodiversidad de los nematodos agalladores, *Meloidogyne* spp. parásitos de las raíces de cafetos en diferentes regiones de producción de Centroamérica (CA). La poblaciones extraídas de las raíces fueron criadas en tomateros y cafetos antes de realizar su diagnóstico por electroforesis isoenzimática (fenotipo esterásico, Est). *M. exigua* (Est E1, Rm: 1.5) apareció como el nematodo agallador más distribuido en CA con una amplia presencia en Honduras, Nicaragua y Costa Rica. Esta especie fue también observada en una sola muestra de Guatemala. La predominancia de *M. paranaensis* (Est P1, Rm: 1.32 and P2, Rm:0.9, 1.32) en café en Guatemala fue confirmada. Esta especie no fue detectada en

ningún otro país de la región. La especie recién descrita en café, en El Salvador, *M. izalcoensis*, (Est I4 = Sa4, Rm: 0.86, 0.96, 1.24, 1.30), parece ser ampliamente distribuida en la región suroeste de Izalco. La presencia de *M. arabicida* (Est AR2, Rm: 1.20, 1.40) fue confirmada en el valle de Turrialba donde parece ser confinada. El fenotipo, Est I1 (Rm: 1.0), característico de *M. incognita* fue encontrado en una población de El Salvador, una de Guatemala y varias poblaciones originarias de tres diferentes regiones de Costa Rica. Estas observaciones son los primeros reportes de *M. incognita* en CA basados en el diagnóstico esterásico. *M. arenaria* (Est A2, Rm: 1.20, 1.30) fue observada en diferentes fincas de la región suroeste de Izalco, en El Salvador. El fenotipo, Sa2 (Rm 1.20, 1.32), fue observado en esta misma región. *M. hapla* (Est H1, Rm: 1.1) fue observada en dos sitios de altitudes elevadas, uno en Guatemala y el otro en El Salvador. Nuevos reportes de *M. mayaguensis* (Est M2, Rm: 0.79, 1.08) en café en CA fueron constatados: uno en Costa Rica y otro en Guatemala. Se observó el fenotipo Est A1 (Rm: 1.30), característico de *M. arenaria* y otras especies, para una población colectada en Guatemala y aparentemente muy patogénica sobre café. Se observó una amplia diversidad inter-especifica de nematodos agalladores parásitos de café en CA.

INTRODUCTION

A survey was carried out in different coffee production regions of Central America in order to assess the biodiversity of root knot nematodes (RKN), *Meloidogyne* spp., parasitizing the roots of coffee trees in this region.

MATERIALS AND METHODS

Populations extracted from roots were reared on tomato and coffee plants before realizing their diagnostic by isozyme electrophoresis (esterase phenotype, Est).

RESULTS AND DISCUSSION

Eleven esterase phenotypes were observed (Table 1, Figure 1). ***M. exigua*** (Est E1, Rm: 1.5) appeared as the RKN with larger distribution on coffee in CA with presence in Honduras, Nicaragua and Costa Rica. This species was also observed for one isolate from Guatemala. The recently described species, ***M. izalcoensis*** (Est I4 = Sa4, Rm: 0.86, 0.96, 1.24, 1.30), on coffee in El Salvador, seems to be largely scattered on coffee in the south-western massif of Izalco volcano. This species has not been found yet in any other region in El Salvador or in any other CA country. The predominance of ***M. paranaensis*** (Est P1, Rm: 1.32 and P2, Rm: 0.9, 1.32) on coffee in the South Western of Guatemala was confirmed. The Est P2 appeared as the most common esterasic phenotype in this country. However, the Est P1 was found at one site in two much closed farms. This species has not been detected yet in any other country in CA. Corky root symptoms caused by this species are very impressive. ***M. arabicida*** (Est AR2, Rm: 1.20, 1.40) seems to be confined to Turrialba Valley in Costa Rica. Root symptoms are similar to those caused by *M. paranaensis*. Est I1 phenotype (Rm: 1.0), characteristic of ***M. incognita***, was found for one population from El Salvador and one from Guatemala. It was also detected on several populations from three different regions in Costa Rica. These are the first based on esterase diagnostic reports of *M. incognita* on coffee in CA. Est A1 (Rm: 1.30), characteristic of *M. arenaria* and other species, was observed for one population from Guatemala. This population is under genetic and morphologic studies in order to conclude on its taxonomic statute. Attention should be given to this population since root symptoms are very severe even on *C. canephora* rootstock. New reports of ***M. mayaguensis*** (Est M2, Rm: 0.79, 1.08) in CA were observed: One from one coffee tree in Costa Rica and the other from only few coffee trees in Guatemala. ***M. hapla*** (Est H1, Rm: 1.1) more adapted to temperate

climates was observed in highlands, at one site in Guatemala and at the Salvadorian massif of Izalco Volcano. In the same region, presence of the before reported new phenotype Sa2 (Rm 1.20, 1.32) was confirmed. ***M. arenaria*** (Est A2, Rm: 1.20, 1.30) was observed twice, at South-East of El Salvador and South of Guatemala.

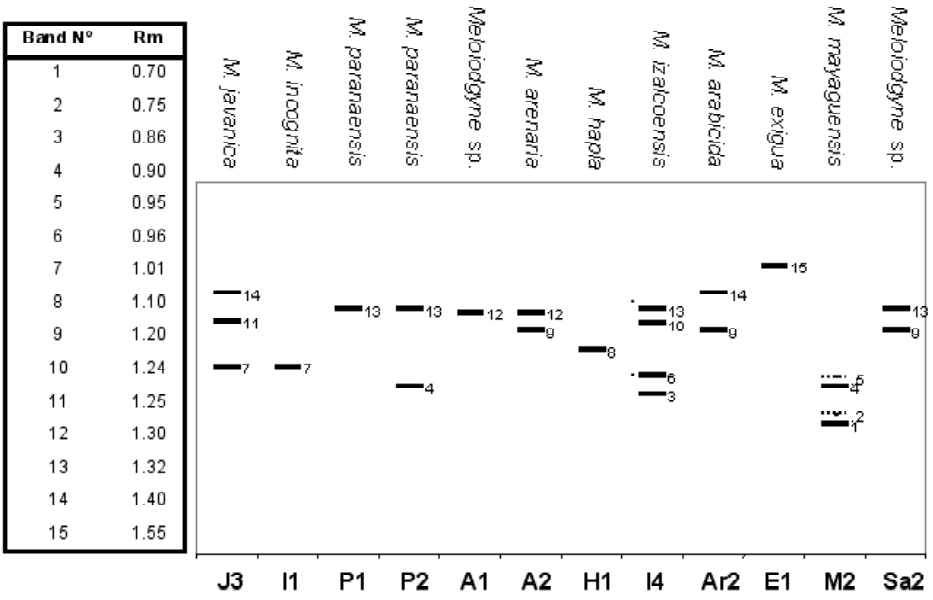


Figure 1. Comparative diagram of the esterase phenotypes observed among the studied RKN isolates.

CONCLUSION

A large interspecific diversity of RKN was observed on coffee in CA. Taxonomic studies must be carried on for new esterasic phenotypes. Pathogenicity and distribution on coffee must be specified for sporadic new reports such as for *M. mayaguensis*. Surveys should be carried on to be more representative of all coffee production regions. Contrary to *M. exigua*, species such as *M. paranaensis*, *M. izalcoensis* or *M. arabicida* seem to have a remarkably restricted area of distribution at CA scale. This fact may be related with the extremely mountainous relief of the region recognized in other respects as a biological corridor where influences from both North and South America converge. Surveys in neighbouring regions, also coffee producers, should be carried out.

Table 1. RKN isolates collected on coffee in CA with their corresponding observed esterase phenotype and species diagnostic.

Country	Region	Province	County	Isolate N° (Farm N°)	Esterase Phenotype	Species Diagnostic
GUATEMALA	Northern Highlands	Alta Verapaz	Santa Cruz V.	1	M2	*M. mayaguensis*
				1	H1	*M. hapla*
	Central Highland	Chimaltenango	Acatenango	1	E1	*M. exigua*
	Pacific South	Escuintla	Palin	8 (2)	P1	*M. paranaensis*
				2 (1)	A2	*M. arenaria*
		Suchitepequez	San Francisco Z.	1	P2	*M. paranaensis*
				9 (2)	M1	*M. incognita*
			El Palmar	6 (1)	A1	*Meloidogyne* sp.
		San Marcos	La Reforma	6 (1)	P2	*M. paranaensis*
				1	A1	*Meloidogyne* sp.
			San Rafael P.C.	13 (1)	P2	*M. paranaensis*
EL SALVADOR	Pacific South West	Sonsonate	Izalco	1	SA2	*Meloidogyne* sp.
				13 (13)	I4	*M. izalcoensis*
			Huiscoyolate	3 (3)	I4	*M. izalcoensis*
			Las Lajas	1	I4	*M. izalcoensis*
				1	M1	*M. incognita*
			Los Naranjos	1	H1	*M. hapla*
			San Isidro	1	H1	*M. hapla*
			Talcomunca	1	H1	*M. hapla*
				1	I4	*M. izalcoensis*
			Teshcal	3	I4	*M. izalcoensis*
			Tunalmiles	4	I4	*M. izalcoensis*
	Pacific S.E.	Usulutan	Santiago de Maria	2 (1)	A2	*M. arenaria*
HONDURAS	Central East	El Paraiso	El Paraiso	5 (5)	E1	*M. exigua*
			Trojes	4 (4)	E1	*M. exigua*
NICARAGUA	Pacific South	Carazo	-	4 (4)	E1	*M. exigua*
COSTA RICA	Pacific Nortth	Guanacaste	Hojancha	1	M2	*M. mayaguensis*
	Northen plains		Tilaran	1	I1	*M. incognita*
				1	I1	*M. incognita*
	Central Valley	Alajuela	Palmares	1	I1	*M. incognita*
		Heredia	Heredia	3 (1)	E1	*M. exigua*
		Cartago	Juan Viñas	2 (1)	AR2	*M. arabicida*
				2 (1)	E1	*M. exigua*
			Turrialba	3 (3)	AR2	*M. arabicida*
				1	E1	*M. exigua*
	Pacific South	San José	Perez Zeledon	4 (4)	I1	*M. incognita*
				1	E1	*M. exigua*

Esterase Activity and Adhesion during the Early Stages of *Hemileia vastatrix* Differentiation

H. GIL AZINHEIRA[1], L. GUERRA-GUIMARÃES[1], M. D. C. SILVA[1], V. VÁRZEA[1], C. RICARDO[2]

[1]Instituto de Investigação Científica Tropical – Centro de Investigação das Ferrugens do Cafeeiro (CIFC), Quinta do Marquês, 2784-505 Oeiras, Portugal
[2]Instituto de Tecnologia Química Biológica, Ap. 127, 2781-901 Oeiras, Portugal

SUMMARY

Hemileia vastatrix Berkeley and Broome is the causal agent of coffee leaf rust, the major disease of *Coffea arabica* plants. The fungus infects the lower surface of the leaves where it produces large, orange colonies of uredosori leading to premature leaf fall and yield losses.The initiation of the dycariotic phase of *H. vastatrix* on coffee leaves, as with other rust fungi, involves specific events including adhesion to the host surface, urediospore germination, appressorium formation over stomata, penetration and inter- and intracellular colonization. Cytological and biochemical techniques were used to investigate the adhesion process of *H. vastatrix* to coffee leaves and oil collodion membranes. Urediospores adhesion assays showed the relevance of extracellular matrix in this process. However the residual level of adhesion observed in washed urediospores (without extracellular matrix) may result from passive interactions acting simultaneously with an active process. The use of oil collodion membranes, that induced urediospores germination and apressoria development in host absence, allowed the analyses of protein composition and some enzymatic activities during these early steps of rust differentiation. Results obtained, showed an increase of total esterase activity both in extracellular matrix of urediospores and after appressoria formation. Isoenzymatic profiles obtained using the techniques of isoelectric focusing electrophoresis (IEF) and polyacrylamide gel electrophoresis (PAGE) support these data and seem to suggest the involvement of esterases in *H. vastatrix* adhesion process.

INTRODUCTION

The interaction between *H. vastatrix* and coffee leaves begins with uredospores adhesion to the leaf surface and the differentiation of infection structures as a result of a sophisticated host surface recognition system.

Adhesion to the host, an essential step to the successful establishment of pathogenesis (Knogge, 1998), prevents fungal displacement by wind and/or water and ensure a close contact with the host surface. It had been demonstrated the involvement of cutinases, esterases, and other unspecific factors, in the adhesion of spores of bean rust *Uromyces viciae-fabae* to *Vicia faba* (Deising et al., 1992; Mendgen, 1997).

The aim of this work was to study the involvement of esterase activity in the adhesion of uredospores during the early stages of *H. vastatrix* differentiation in the host leaves and collodion membranes.

MATERIAL AND METHODS

Fresh uredospores of *H. vastatrix* (culture 2191) were inoculated on the lower surface of young coffee leaves (*Coffea arabica* L.) and on collodion membranes, as described by D' Oliveira & Rodrigues (1961).

Collodion membranes were prepared according to Heath & Heath (1976).

Uredospores in batch of 100mg were washed by agitation with 5ml of water containing 0.02% of Tween 20 or PMSF (Benzylsulfonyl fluoride – serine-esterases inhibitor) 1 mM. The wash liquid was then collected by filtration and the uredospores were dried.

In adhesion assays dry uredospores were dusted onto leaves and collodion membranes. Leaves or membranes were incubated in a dark humidity chamber at 24 ºC. After intervals of 0, 6, 18 and 24 h inoculated sites were washed by delivery 1 ml of water from a height of 5 cm. After washing, uredospores that remained on the surface were counted and present as a percentage of uredospores originally present.

Uredospores germination and apressoria formation were evaluated following the technique describe by Silva et al. (1985).

Esterase activity on the surface of uredospores was assay by the method in which indoxyl acetate was used as substrate for non-specific esterases (Barnett and Seligman, 1951).

Esterase activity in early stage of *H. vastatrix* differentiation were assayed after extraction (Azinheira, 2005) by the hydrolysis of p-nitrophenyl butyrate at 405 nm (Deising et al., 1992). PAGE and isolectric focusing electrophoresis was used to compare esterase profiles from germinated and non-germinated spores, and germinated spores with apressoria. The bands with esterase activity were visualized according method of Nave and Sauhey (1968).

RESULTS AND DISCUSSION

Uredospore adhesion

The washing procedure with Tween or PMSF (serine-esterares inhibitor) resulted in a decrease of the fungus ability to adhere to the leaves (Table 1) and to the collodion membranes (results not shown). The background level of adhesion of washed uredospores may be due to physical interactions and does not depend on the esterase activity.

Table 1. Percentage of uredospores adhering to the cuticle of the coffee leaf surface.

	Adhesion (%)			
	0h	**6h**	**18h**	24h
Fresh (unwashed spores)	89±10.6 a	89±12.1 a	83±7.0 a	**61±17.2 a**
Tween washed spores	33±20.4 b	33±14.2 b	45±12.2 b	**52±21.3 a**
PMSF washed spores	**40±3.0 b**	**40±1.4 b**	**57±8.1 b**	**55±2.2 a**

In each column values followed by the same letter do not differ significantly according Tukey HSD test ($P < 0.05$).

Washing uredospores decreased the germination rates but the percentage of uredospores that differentiate appressoria did not changed comparatively to unwashed spores, suggesting that

esterases from the spore surface are involved in the adhesion and in the germination but not in appressoria formation (Table 2).

Table 2. Percentage of germinated uredospores and appressoria formation 24 h after inoculation in coffee leaves.

	Germination (%)	Apressoria (%)
Fresh (unwashed spores)	77±7.4 a	**56±1.7 a**
Tween washed spores	46±15.9 b	**45±12 a**
PMSF washed spores	**49±11.3 b**	**42±5.3 a**

In each column values followed by the same letter do not differ significantly according Tukey HSD test ($P < 0.05$).

Esterase activity

Localization on uredospores surface

The inoculation of uredospores on glass slides with gelatine containing indoxyl acetate allowed the localization of esterase activity by the formation of indigo blue crystals in the places of enzymatic activity. Glass slides were observed 3h after inoculation. In all the uredospores tested indigo blue crystals were detected in the intracellular space. On the surface of uredospores washed (with Tween or PMSF) a great decrease on the indigo blue crystals was observed, on the contrary to what was observed in fresh spores (unwashed) where more than 75% showed extracellular esterase activity (Figure 1).

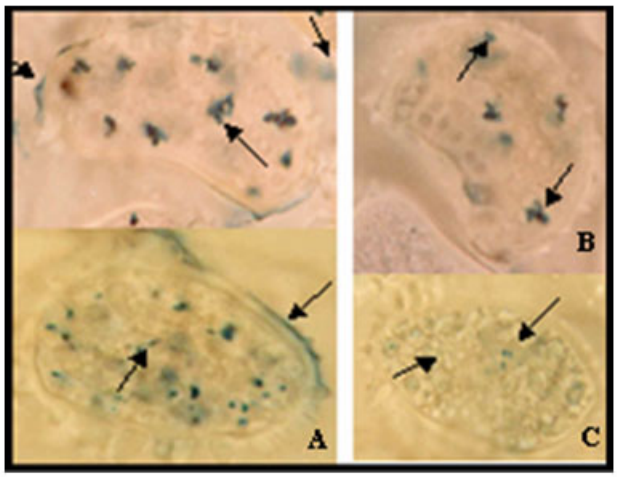


Figure 1. Cytological localization of esterase activity in fresh uredospores (A) washed with 0,02 % Tween 20 (B) and washed with PMSF 1 mM (C) (x1550).

Total activity in early stages of differentiation

The higher esterase activity was observed in the extracellular fraction of non-germinated uredospores (EF) and in uredospores that had differentiated apressoria (DU) (Figure 2).

Isoenzimatic profiles in early stages of differentiation

Isoelectric focusing and native polyacrylamide gels showed distinct esterase profiles suggesting the involvement of different isoforms during uredospore's adhesion to the host surface and appressoria formation (Figure 3).

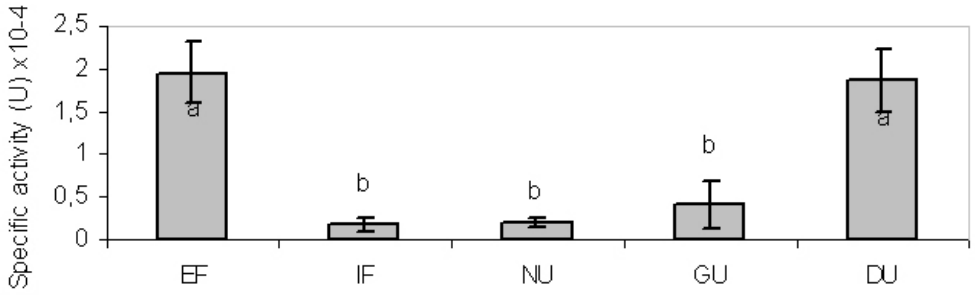


Figure 2. Esterase activity in protein fractions extracted from non-germinated uredospores and in the early stages of differentiation. Determination with *p*-nitrophenyl butyrate as substrate. One unit was defined as the amount of protein necessary to hydrolyzed 1 mmol of substrate in 1h at 25 °C. Vertical bars with the same letter do not differ significantly according Fisher MDS test (P < 0.05). (EF extracellular uredospores fraction; IF intracellular uredospores fraction; NU non-germinated uredospores total fraction; GU germinated uredospores; DU uredospores with differentiated apressoria).

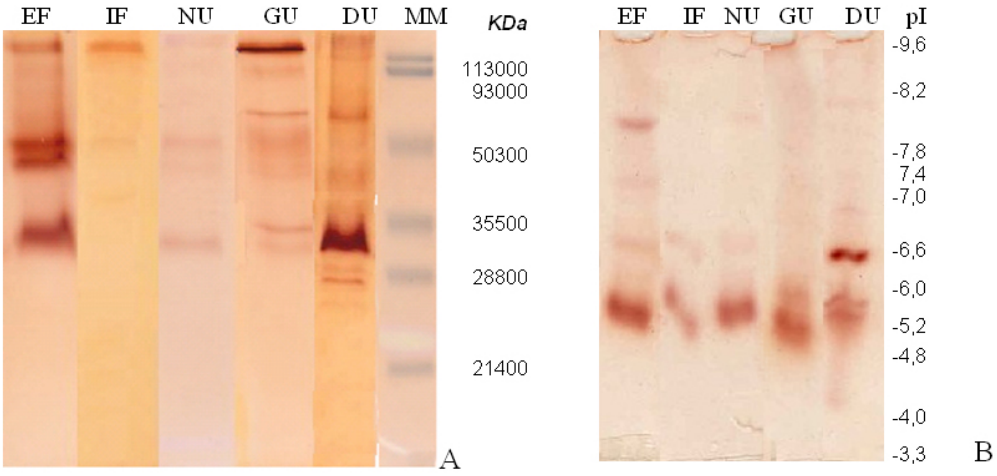


Figure 3. Esterase isoenzimatic profiles in protein fractions extracted from non-germinated uredospores and in the early steps of differentiation, separated by (A) native polyacrylamide gel electrophoresis and (B) isoelectric focusing (pH [3-10]).

Esterases activity seems to be involved in the adhesion of *H. vastatrix* uredospores to coffee leaves or to collodion membranes as well as in the differentiation of appressoria. These results are consistent with observations made with *Uromyces viciae-faba* and *Chalara elegans* (Deising et al., 1992; Wattimena, 2001) in which cutinases and esterases released from spores appear to be associated with the phenomenon of adhesion.

The background level of adhesion observed in washed uredospores suggested that the mechanism of adhesion of the fungus to plant surface may also involve a passive non-specific process based in physical properties; similar results have also been described for other interactions (Epstein et al., 1987; Deising et al., 1992).

REFERENCES

Azinheira HG (2005) "Estudos celulares, bioquímicos e moleculares da diferenciação de *Hemileia vastatrix* Berk & Br" UTL/ISA PhD Thesis, Lisboa,163p

Barnett RJ and Seligman AM (1951) *Science*. **114**, 579-582.

D'Oliveira B and Rodrigues Jr. CJ (1961) *Revista do Café Português*, **8**, 5-50.

Deising H et al. (1992) *Plant Cell*, **4**, 1101-1113.

Epstein L et al. (1987). *Physiological Molecular Plant Pathology*, **30**, p.373-388.

Heath IB and Heath MC (1976) *J. Cell Biol.*, **70**, 592-607.

Nave EB AND Sauhey VK (1986) *J. Plant Physiol.* **125**, 451-465.

Silva MC et al. (1985) In: Proceedings of the 11th International Scientific Colloquium on Coffee, Lomé, 11-15 February, ASIC (Paris), 635-645.

Silva MC et al. (2006) *Braz. J. Plant Physiol.,* 18, 119-147

Wattimena SC (2001) MsS Simon Fraser University, Canada, 79p.

Characterization of Histological Effects of *Meloidogyne incognita* Infection in Resistant and Susceptible *Coffea arabica* Genotypes

É. V. S. A. BARROS[1], P. M. COSTA[1], A. C. M. M. GOMES[1], R. FALCÃO[1], V. F. RIBEIRO[1], M. T. S. EIRA[1], A. A. PEREIRA[2], M. NICOLE[3], D. DE SÁ[3], M. F. G. FERNANDEZ[1], R. M. D. G. CARNEIRO[1]

[1]Embrapa – Recursos Genéticos e Biotecnologia, 708849-970 Brasília, DF, Brasil
[2]EPAMIG, CRZM, 36570-000 Viçosa, MG, Brasil
[3]IRD - UMR DGPC 34394 Montpellier Cedex 5, France

SUMMARY

Root-knot nematode infection is responsible for many losses in coffee crop infested areas. The *C. canephora* species is a source of genetic resistance, but difficult to be directly used by breeders in the allotetraploid, long life cycle of *C. arabica* plant, which is the most cultivated species and the most susceptible to nematodes at the same time. We studied tissue cuttings from two resistant (H 419-5-4-5-2 progeny of the Paraiso cultiva and UFV 408-28 of the Hibrido de Timor cultivar) and one susceptible (catuai Vemelho IAC 15) coffee genotypes of *C. arabica* regarding *M. incognita* development. The resistance of those genotypes was previously confirmed for *M. exigua* and *M. incognita*. Here we present the plant response at histological level upon infection with a highly pathogenic population of *M. incognita* race 1. We observed the penetration, migration and establishment of feeding sites during a period of five days, corresponding to the early stages of the nematode parasitic cycle. The infected root sections were analyzed by optical microscopy under UV light in order to detect the autofluorescence related to aromatic compounds. Accumulation of phenolic compounds was registered next to nematodes bodies penetrating the epidermis of resistant roots. Migrating nematodes were also observed inside the vascular system of resistant plants, surrounded by necrotic cells. Neither necrosis nor autofluorescence of phenolic residues were recorded in the susceptible plant, while nematodes were present and started feeding. Corresponding portions of the root tissue cells and nematodes presences were revealed by toluidine blue staining. Giant cells formation was not observed in the early stages of infection in one of the resistant genotypes, while the other resistant plant allowed initial formation of feeding sites. At the same time, highly vacuolated and dense cytoplasm containing cells were observed in the susceptible plant. Results indicated that the mechanism of resistance involved the hypersensitive response that should be blocking the *M. incognita* development in the resistant cultivars. Confirmation of the histopathological responses of contrasting genotypes will be useful to future ESTs libraries construction.

INTRODUCTION

The coffee business is one of the most traditional and financially important activities of Brazilian agribusiness: 40% of the and the second major consuming market world coffee consumer. A variety of coffee plants are available in Brazil from conventional genetic breeding programs, although it takes a long time to create a new cultivar (Dufour et al., 2000). There is great agronomic interest in the improvement of cup quality, resistance or tolerance to biotic (nematodes, pests and diseases) and abiotic stresses (Etienne et al., 2002).

With a high limitation in application of chemical pesticides, the importance in controlling the resistance in host plants is growing up. Investigations on the molecular basis of the plant–pathogen interactions of parthenogenetic nematodes like *M. incognita* are focused on the characterization of *Meloidogyne* secretions, (a)virulence factors and nematode responsive plant genes. Studies of the molecular events and regulatory mechanisms involved in this parasitic relation should allow the development of target-specific strategies to limit crop damage by these pathogens (Abad et al., 2003).

Nematodes are phytoparasites that cause severe damage to coffee roots by gaining nutrients simplistically from the phloem via the giant cells, which causes a strong sink effect that reduce the plant production. Nematodes from genus *Meloydogine* (root-knot) have a larvae form (juvenile 2) of free life in soil. After entering the root in the zone of elongation, typical root galls are formed on the root of susceptible plants when sedentary adult females establish a feeding site and produce a large mass of eggs that initiate a new cycle. The most injurious species to coffee crops in Brazil are: *M. incognita*, *M. paranaensis* and *M. exigua.*

There is a co-evolution of secreted effector proteins by plant pathogens and plant proteins, characterized genetically as gene-for-gene resistance (Flor, 1971), that specifically recognizes nematodes and other phytopathogens. In the presence of a cognate R and effector association, resistance is activated, resulting in the initiation of defense signaling and host resistance.The absense of paired interaction results in the disease (Chisholm et al., 2006). The resistance response causes programmed cell death (PCD) around the infection site (the hypersensitive response, HR), as well as tissue reinforcement and antibiotic production (McDowell and Woffenden, 2003). Nematode juveniles surrounded by necrotic cells fail to develop and die. The response can occur early, preventing parasite penetration or migration, or later, inhibiting the development of giant cells and suppressing parasite development and multiplication. The HR is locally accompanied by the production of phenolic compounds (Caparolino et al., 2005).

We studied the morphological differences among three *C. arabica* cultivars innoculated with *M. incognita*. Tissues were observed by optical microscopy either under UV light or after toluidine blue staining. The histological observations evidenced phenolics accumulation related to resistant genotypes.

MATERIALS AND METHODS

Biological material

We used 3 month-old *C. arabica* plants from resistant H 419-5-4-5-2 (PA) and UFV 408-28 (MG) and susceptible Catuaí vermelho – IAC 15 (CV) coffee genotypes tested with *M. incognita* race 1.

Penetration experiment

About 10 plants from each coffee genotype were inoculated with a water suspension containing 10.000 J2. We collected secondary roots at 1, 2, 3, 4, and 5 days after soil infestation (dpi). Root systems were washed and 20 tips were fixed to posterior inclusion, sectioning (4 μm), staining with toluidine blue and microscope observation, as described by Pegard et al. (2005). The remaining roots were stained with acid fucsin (Byrd et al., 1983).

RESULTS AND DISCUSSION

The aim of this study was to compare *M incognita* penetration and subsequent development in two resistant and one susceptible coffee genotypes. We examined approximately 8,640 root sections of the three genotypes. Penetration of J2 were observed at the maximum number in 6 dpi sections, both in susceptible and resistant genotypes.

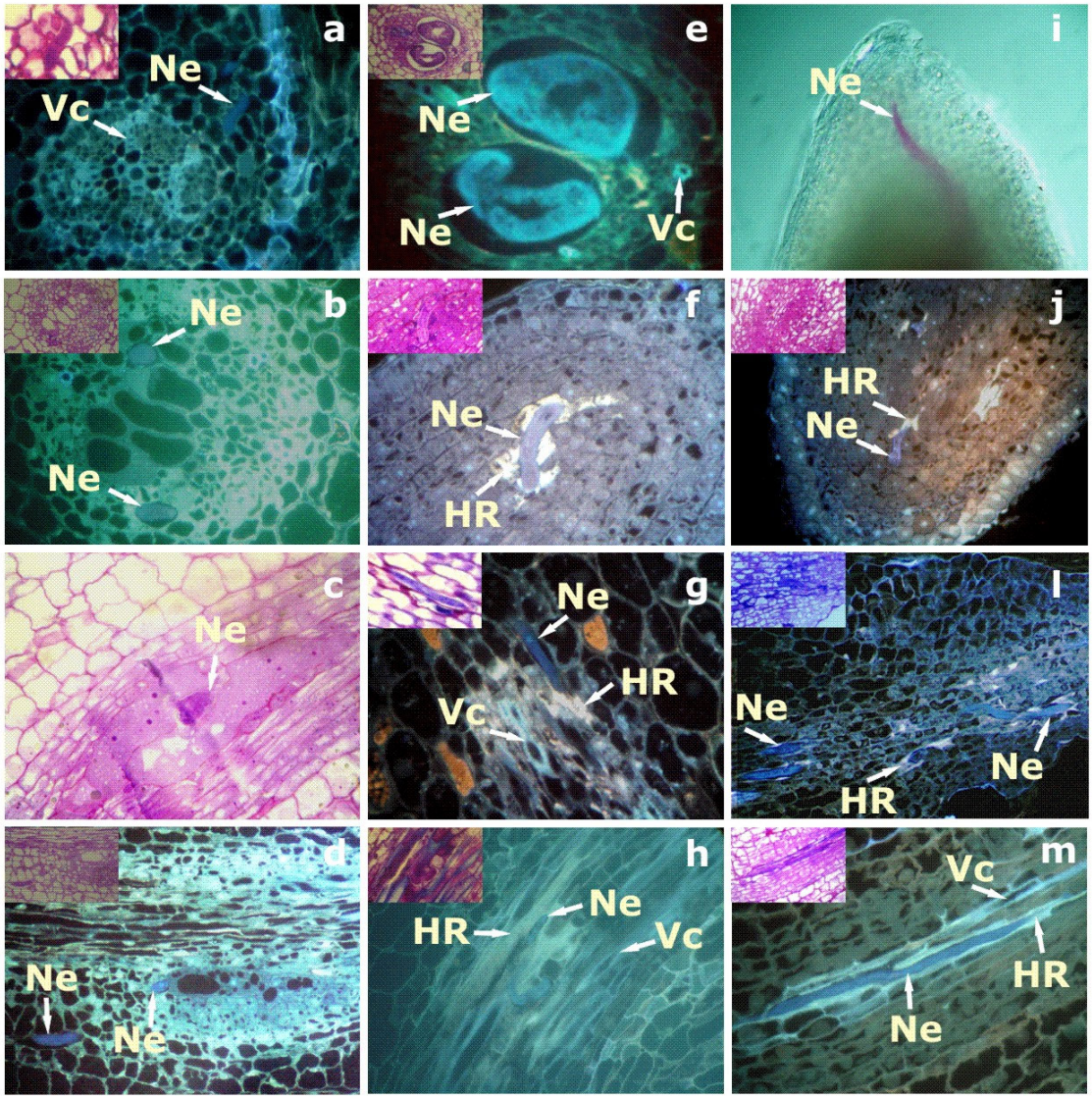


Figure 1. *C. arabica* roots from susceptible (CV) and resistants (PA and MG) inoculated with *M incognita*. Sections were observed under UV light and then stained (reduced images) with toluidine blue (except by letter h): (a) Penetration through cortex towards the vascular cylinder (CV 2 dpi); (b) Nematodes at J3 next to giant cells showing a high density cytoplasm with large vacuoles (CV 6 dpi); (c) Multinucleated cells at feeding site (CV 7 dpi); (d) numerous cells forming a gall (CV 8 dpi); (e) two J4 almost at the end of the endoparasite cycle (CV 34 dpi); (f) penetration in the root apex. Phenolic accumulation at cells surrounding the nematode (PA 6 dpi); (g) necrosis around nematode migrating to the vascular cylinder (PA 7 dpi); (h) vermiform nematode surrounded by dead cells (PA 14 dpi); (i) penetration in the root tip (MG 3 dpi); (j) cell death blocking penetration(MG 6 dpi) and (l) migration (MG 7 dpi); (m) HR reaction around J2 near a vascular cylinder (MG 8 dpi). Ne = nematode, HR = hypersensitive reaction, vc = vascular cylinder.

PERSPECTIVES

Continue morphological studies of CV until 42 dpi and include 4 dpi and 10 dpi sections. Observed non infected tissues of the resistant plants.

As an alternative to transgenic approaches, naturally occurring, defense-inducing compounds could be directly applied to crops (McDowell and Woffenden, 2003)

REFERENCES

Byrd, J. D. W., Kirkpatrick, J., and Barker, K. R. (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* **15**, 142-143.

Etienne, H., Anthony, F., Dussert, S., Fernandez, D., Lashermes, P., and Bertrand, B. (2002). Biotechnological applications for the improvement of coffee (*Coffea arabica* L.). *In Vitro Cell. Dev. Biol.-Plant* **38**, 129-138.

McDowell, J. M., and Woffenden, B. J. (2003). Plant disease resistance genes: recent insights and potential applications. *TRENDS in Biotechnology* **21**, 178-183.

Pegard, A., Brizzard, G., Fazari, A., Soucaze, O., Abad, P., and Djian-Caporalino, C. (2005). Histological Characterization of Resistance to different Root-Knot Nematode Species Related to Phenolics Accumulation in *Capsicum annuum*. *Phytopathology* **95**, 158-165.

Supported by: Embrapa, Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café-CBP&D/Café and FAP-DF.

Preliminary Studies on Sources of Resistance in *Coffea arabica* L. to Coffee Leaf Miner, *Leucoptera caffeina* Washbourn

E. MENDESIL, C. ABDETA

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

SUMMARY

Fourteen *Coffea arabica* L. cultivars were evaluated for their resistance to coffee leaf miner, *Leucoptera caffeina* Washbourn (Lepidoptera: Lyonetiidae) in the lath-house at Jimma Agricultural Research Center, Ethiopia. Percentage leaf damage was significantly lower ($P < 0.05$) on cultivars 7454, Desu, Ababuna and Melko-CH2 than on 7440, Meoftu and 744. Number of eggs laid were significantly lower ($P < 0.01$) on 754, Melko-CH2, 74140, 7454, Gawe, Dessu, 7440 and 75227 than on Catimor J-21 and Catimor J-19. However, there was no significant difference ($P > 0.05$) in the number of larvae developed on leaves of coffee cultivars tested. The results of the present study indicated the existence of antixenosis resistance in *C. arabica* L. cultivars against *L. caffeina*. The result also suggests further evaluation of the promising cultivars in order to identify resistant cultivars which can be used as a component of IPM of *L. caffeina*.

INTRODUCTION

Coffee leaf miner, *Leucoptera caffeina* Washbourn (Lepidoptera: Lyonetiidae) is one of the most important insect pests of coffee in Ethiopia. There are two species of coffee leaf miner attacking coffee leaves in Ethiopia, namely *L. meyricki* Ghesquière and *L. caffeina* Washbourn (Crowe and Tadesse, 1984; Million, 1987). The later species is the most important which is commonly occurring in shaded coffee, while the other species is of minor importance in Ethiopia probably due to the fact that coffee is grown under shade which is not conducive to the normal development of this species (Million, 1987; Crowe, 2004). Heavy infestation of leaf miner was observed in different coffee growing areas of the country (IAR, 1984; IAR, 1986; Million, 2000), causing heavy fall of leaves during severe infestation. Moreover, it has also been observed to damage coffee seedlings. Studies conducted at Agro Sub-Center indicated that, percentage leaf damage due to this insect ranged from 2.2-55, with average infestation of 13% (IAR, 1984; IAR, 1986). The wide genetic diversity of *C. arabica* in Ethiopia prompted an interest to find sources of resistance to *L. caffeina* which can be used as a component of IPM of this pest. In this study, fourteen *C. arabica* cultivars were evaluated for resistance to *L. caffeina* in the lath-house.

MATERIALS AND METHODS

Fourteen *C. arabica* L. cultivars, which include three hybrids (Melko-CH2, Gawe and Ababuna), were screened for the resistance against *L. caffeina* in the lath-house at Jimma Agricultural Research Center (JARC), Jimma, Ethiopia during 2003-2004. Seedlings were grown in the nursery using the standard nursery practices as recommended by Yacob et al. (1996). Six months old healthy seedlings were transferred to the lath-house constructed with fine wire mesh. *Leucoptera caffeina* infested leaves were collected from the field and reared in the laboratory. The newly emerged moths were released into seedlings in the lath-house.

The experiment was arranged in Randomized Complete Block Design with four replications consisting six seedlings per cultivar. Number of infested leaves, number of eggs and larvae per seedling were recorded at weekly interval during April to August. The data was subjected to analysis of variance using MSTAT-C computer program. Means were separated using Duncan's Multiple Range Test (DMRT) at P = 0.05.

RESULTS AND DISCUSSION

Percentage leaf damage was significantly lower ($P < 0.05$) on cultivars 7454 followed by Dessu, Ababuna and Melko-CH2. On the other hand, the highest percentage damage was recorded on cultivars 7440, Meoftu and 744 (Table 1). There was highly significant ($P < 0.01$) cultivar preference in oviposition. The lowest number of eggs was laid on cultivars 754, Melko-CH2, 74140, 7454, Gawe, Dessu, 7440 and 75227, while the highest on Catimor J-21 and Catimor J-19 (Table 1). As shown in Figure 1, significantly ($P < 0.05$) the lowest frequency of oviposition was observed on 754 followed by 7440, 74140 and Ababuna, while the highest on Catimor J-21 and Gesha. There was no significant difference ($P > 0.05$) in the number of larvae developed on leaves of coffee cultivars tested. However, the lowest mean number of larvae developed on Dessu (3.25), while the highest on Catiomor J-21 (16.20 (Table 1). It was noted that, only 26.93% of the larvae were developed from the total eggs laid. This could be due to reduction in egg hatching or larval mortality as a result of antibiosis effects on *L. caffeina*.

Table 1. Percentage damaged leaves, mean number of eggs and larvae of *L. caffeina* on *C. arabica* cultivars at Jimma, Ethiopia.

Cultivars	Damage	Eggs	Larvae
754	3.68 bcd	14.00 c	4.50
Meoftu	5.54 ab	26.35 bc	12.42
Catimor J-21	4.67 abcd	38.49 a	16.20
Melko-CH2	3.37 cd	14.18 c	8.25
Gesha	4.54 abcd	23.24 bc	9.06
Catimor J-19	4.66 abcd	30.95 ab	11.62
744	4.93 abcd	19.64 bc	5.87
Gawe	3.96 abcd	14.44 c	3.67
7440	5.87 a	16.11 c	4.62
Dessu	2.96 cd	15.69 c	3.25
74140	3.78 bcd	14.23 c	4.00
Ababuna	3.13 cd	20.90 bc	8.00
7454	2.86 d	14.41 c	7.17
75227	4.10 abcd	16.61 c	4.25
Mean	4.14	19.94	7.35
CV	29.00	39.00	50

Means followed by the same letter (s) within a column are not significantly different at P < 0.05, DMRT.

The results of the present study revealed that, cultivars 7454, Dessu, Melko-CH2, 754 and 74140 were more resistant for *L. caffeina* than the remaining cultivars as demonstrated by the lower mean number of eggs laid and percentage leaf damage. Host selection of the insect may depend on the suitability of the host (cultivar) for its feeding and reproduction. From the present study it was evident that, variation in the level of resistance was observed among the cultivars tested in terms of percentage damaged leaves and preference for oviposition, which indicate the existence of antixenosis resistance. Nevertheless, preference for oviposition may

be more expressive parameter of antixenosis resistance as compared to the percentage damaged leaves, which showed only small difference among the cultivars tested.

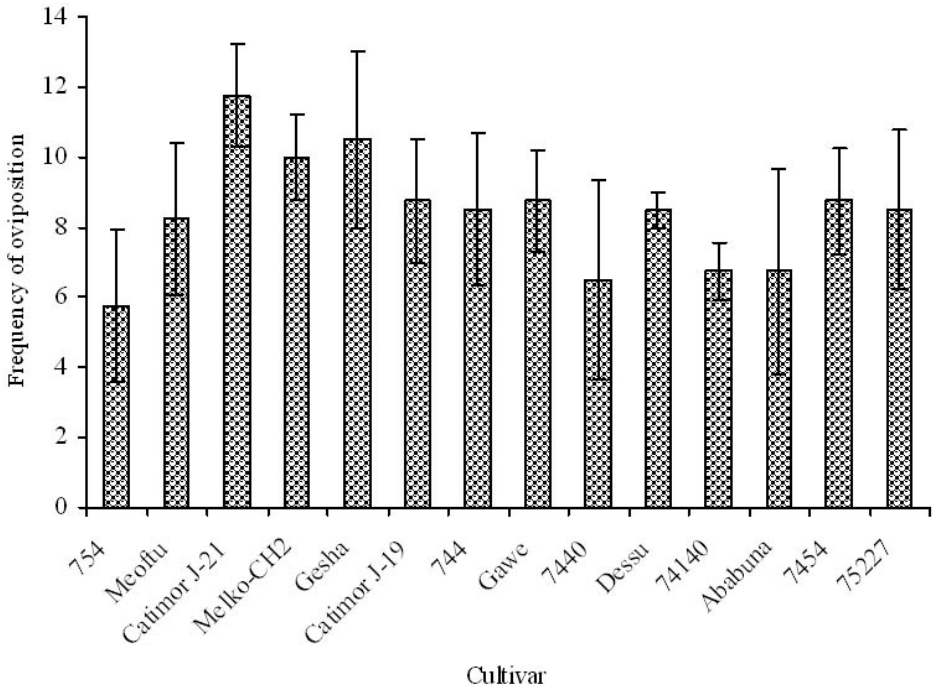


Figure 1. Oviposition frequency of *L. caffeina* on leaves of *C. arabica* cultivars. Vertical bars indicate ± SEM.

Matos et al. (2001) reported the existence of antixenosis resistance in *C. canephora* and *C. congensis* for the resistance to coffee leaf miner, *Perileucoptera coffeella*. Moreover, in Brazil coffee trees resistant to this insect have been developed using genes from the *C. racemosa* (Guerreiro-Filho et al., 2000). The basis of antixenosis mechanism can be morphological or chemical (Dent, 1991). The lighter green color predominantly found in *C. canephora* leaves was reported as contributing factor for the low oviposition frequency of *P. coffeella* on this plant (Cardenas, 1981 cited in Matos et al., 2001). On the other hand, caffeine, the major alkaloid found in coffee does not have a protective role in coffee against leaf miner, *P. coffeella* as reported by Guerreiro-Filho and Mazzafera (1999). This indicated that, resistance in coffee may be attributed to other biochemical factors as well as morphological features of the plant.

It is suggested that, these promising cultivars which showed good level of resistance to the insect should further be evaluated both in the lath-house and under field conditions. It is also worth to screen more cultivars in order to find useful sources of resistance to *L. caffeina* and to use them as component of integrated management of this insect pest.

ACKNOWLEDGMENTS

The experiment was financially supported by the Ethiopian Institute of Agricultural Research (EIAR). The authors are grateful to Mr. Teklemariam Erge for his assistance during the experiment.

REFERENCES

Crowe, T. J. 2004. Coffee Pests in Africa. In: Wintgens, J.N. (Ed.). Coffee. Growing, Processing, Sustainable Production, Wiley-Vch Verlag GmbH & Co. KgaA, Weinheim, pp. 441-445

Crowe, T. J. and Tadesse G. 1984. Coffee Pests in Ethiopia: Their Biology and Control. Addis Ababa, IAR, 45 pp.

Dent, D. 1991. Insect pest management. CAB International, UK, 604 pp.

Guerreiro-Filho, O and Mazzafera, P. 1999. Caffeine does not protect coffee against the leaf miner *Perileucoptera coffeella*. In: Proceedings of the 18th International Conference on Coffee Science, ASIC, Helsinki, Finland.

Guerreiro-Filho, O, Medina-Filho, H.P., Fazuoli, L.C., Goncalves, W. 2000. Development of coffee trees resistant to leaf miner. In: Sera, T., Soccol, C.R., Pandey, A., Rossos, S. (Eds). Coffee Biotechnology and Quality. Proceedings of the 3rd International Seminar on Biotechnology in the Coffee Agro-Industry, Londrina, Brazil. Kluwer Academic Publisher, The Netherlands, pp. 219-227.

IAR. 1984. Department of Coffee Progress Report for the Period 1982-1983, IAR, Jimma, 129 pp.

IAR. 1986. Department of Coffee Progress Report for the Period 1983/1984, IAR, Jimma, 149 pp.

Matos, J.W., Ramiro, D. A., Goncalves, W., Guerreiro-Filho, O. 2001. *Coffea canephora* and *C. congensis* have antixenosis resistance to the coffee leaf miner. In: Proceedings of the 19th International Conference on Coffee Science, ASIC, May 14th-18.th Trieste, Italy.

Million A. 1987. Insect pests of coffee with special emphasis on Antestia bug, *Antestiopsis intricata,* in Ethiopia. Insect Science and its Application 8: 977-980.

Million A. 2000. Significance of Arthropod Pests of Coffee in Ethiopia In: Proceedings of workshop on the control of CBD in Ethiopia. 13– 5 August 1999, Addis Ababa, pp. 66 - 71.

Yacob E., Tesfaye S., Alemseged Y., Taye K., Anteneh N. and Takele N. 1996. Advances in Coffee Agronomy Research in Ethiopia. In: J. S. Tenywa, Adipala Ekwamu & M. W. Ogengu-Latigo (Eds). Proceedings of Inter-Africa Coffee Organization (IACO) Workshop. 4-6 September, 1995, Kampala, Uganda, pp. 40-55.

The Developmental Biology, Morphometrics and Damage Assessment of *Epicampoptera strandi* Bryk. Sub sp. *glauca* Hmps. (Lepidoptera: Drepanidae) on Robusta coffee in Nigeria

F. A. OKELANA, J. C. ANIKWE

Entomology Division, Cocoa Research Institute of Nigeria, P.M.B. 5244, Ibadan, Nigeria

SUMMARY

Some aspects of the biology and body morphometrics of the tailed caterpillar, *Epicampoptera strandi* as well as the damage inflicted on leaves of *Coffea canephora* were investigated in the laboratory at ambient temperature and relative humidity. Freshly laid field-collected egg pillars of *Epicampoptera strandi* reared in the laboratory had a mean eclosion period of 4 days. The larval stages passed through five instars with a mean total larval period of 18 days while the mean development period from egg to adult was 28.8 days. The life stages were each measured and described while the damage caused by each instar larva was reported. The last instar larva was found to be the most destructive and consumed an average percentage leaf area of 88.4% per day. The growth ratio for each instar larva was calculated and the head capsule measurements increased by an average factor of 1.7. The short developmental period, coupled with high fecundity and pattern of egg-laying as observed from this work makes *E. strandi* an important economic insect pest of robusta coffee in Nigeria.

INTRODUCTION

The robusta coffee, *Coffea canephora* Pierre ex, Froehner (Rubiaceae) is one of the widely cultivated coffee species in the middle belt and southern states of Nigeria. It accounts for about 94% of coffee export from Nigeria (Williams, 1989). It is a more vigorous and more productive cultivar than the arabica coffee (Coste, 1992). Presently, it is gaining ground in the world market because of its high solubility and hence it is used for the production of instant coffee (Le Pelley, 1978). However, coffee yield is not yet at its optimal level because of insect pest infestation, notably among which is the defoliator – *Epicampoptera species*.

Okelana (1989) reported the *Epicampoptera* species as a major insect pest of robusta coffee in Nigeria. The two species recorded are *Epicampoptera strandi* Bryk. Sub sp. *glauca* and *Epicampoptera andersoni* Tams. Sub sp. *glauca*. Of these two species *E. strandi* is more destructive, occurring almost exclusively during the early rains (March-August) with a peak in July (Idowu, 1971) and causing enormous defoliation and consequent decrease in yield of coffee (Okelana, 1985).

It is generally known that the larval stage of *E. strandi* is the damaging stage of the moth. The caterpillars defoliate coffee leaves leading to a marked reduction in photosynthesis and consequent drop in berry production (Okelana, 1989). This study becomes imperative in order to design an appropriate and ecologically sound control strategy for the pest.

MATERIALS AND METHODS

Developmental Biology Studies

Field-collected 'egg pillars' on leaves of *C. canephora* were incubated in covered plastic bowls (13x8x6 cm), bearing a 3cm diameter hole, covered by a fine nylon mesh (30 mesh/cm) on each of the four sides of the plastic container. The base of the bowl was lined with moistened cotton wool covered with filter paper. The surface of the leaf bearing the eggs was placed facing upwards. Soon after hatching, 40 pairs of newly emerged larvae were separately transferred onto freshly cut coffee shoots dipped in water in a conical flask (250 ml). The shoots were held in place in a flask and fixed firmly with the aid of cotton wool at ambient laboratory temperature of 27±2 °C and 70-88% relative humidity. The coffee shoots were changed on a daily basis between the hours of 7.00 and 9.00 hrs GMT. The flask (with shoot and larvae) was placed in a rectangular white container to facilitate detection of the cast headcapsules. Older larvae were given more shoots. In the first batch, 20 newly emerged larvae were taken out everyday for morphological description and measurement of the cast headcapsules. In the second batch, each of 20 larvae was demobilized in ethyl acetate fumes and measured using a micrometer attached to a stereo-binocular microscope. Morphometric parameters such as the body length, abdominal width, and the width of vertex were measured. These data were subjected to appropriate one-way analysis of variance and mean separation using Turkey's Honestly Significant Difference ($P < 0.05$) (Gomez and Gomez, 1984). The growth ratio was determined for each larval instar by dividing the mean width of vertex of one instar with the one preceding it (Ewete, 1990; Emehute and Odiete, 1991).

Damage Assessment Studies

Ten day-old larvae of *E. strandi* were introduced separately onto freshly plucked leaves of robusta coffee, which were changed daily in the Laboratory. Leaf Area Damage was assessed via the use of a Leaf Area Meter (Model C1 – 202). Plucked coffee leaves were scanned with the Area Meter before being fed to the insects and 24 hours after being fed to the insects. The area of leaf defoliated was estimated as the difference between the areas of leaves before and after feeding. The equivalent damaged area of leaf was finally expressed as a percentage of the mean area of whole leaf

$$\text{Percentage Area of Damaged Leaf} = \frac{\text{Mean damaged leaf Area}}{\text{Mean whole leaf Area}} \times \frac{100}{1}$$

RESULTS

Each pillar consists of 6-48 eggs. Eggs of *E. strandi* were usually laid in form of curved pillars ranging from one to ten per batch. The egg pillars are attached to the leaves at its base by means of a waxy adhesive. Each unit (egg) of the pillar is barrel shaped having a mean length of 1.02 ± 0.09 mm while the width was 0.61 ± 0.05 mm (Table 1) with a rough and tough chorion. Most times, the egg pillars were encountered on the lower surface of the leaves and occasionally found on the upper surface when infestation levels are high. The incubation period of the egg stage lasted 4 days on the average.

Table 1. Body morphometrics, width across head capsule (mm ± SE), growth ratio and duration of developmental stages of *Epicampoptera strandi* on *Coffea canephora*.

Growth stage	Sample Size (n)	Length (mm)	Width (mm)	Tail (mm)	Width of vertex (mm)	Growth Ratio	Duration of Stages (days)
Egg	20	1.02±0.03	0.61±0.02				4
1st instar larva	20	3.49±0.38	0.35±0.02		0.34±0.01e		4
2nd instar larva	20	7.55±0.64	0.68±0.05		0.56±0.02d	1.65	3
3rd instar larva	20	11.25±0.71	1.09±0.11	3.3	1.08±0.02c	1.93	3
4th instar larva	20	24.50±1.13	3.87±0.30	11.2	1.93±0.03b	1.79	4
5th instar larva	20	34.70±1.20	5.50±0.31	13.7	2.74±0.03a	1.42	4
Pupa	20	20.70±0.40	5.40±0.27				6.8
Adult (Male)	20	18.43±0.06	4.01±0.02				
Adult (Female)	20	20.00±0.08	6.43±0.4				
Mean Growth Ratio						1.70	

Means followed by the same letter in the same column are not significantly different (p>0.05) Turkey's Honestly Significant Difference (HSD).

Newly emerged larvae were black in colour and actively motile. The first instar larva has a mean body length of 3.49 ± 0.37 mm and body width of 0.35 ± 0.02 mm with head capsule measurement of 0.34 ± 0.04 mm. The determination of the different larval instars and their corresponding developmental period were assessed by searching for the cast head capsule (vertex) in the rectangular container after moulting. The damage effected on coffee leaf at this stage was very minimal as the larvae were found to scrape the upper epidermal layer of coffee leaves. Only 1.41% of leaf was consumed during the four days duration of the first instar (Table 2). The second instar larva is 7.55 mm long with a body width of 0.68 mm. The larval colour changed from black to green at this stage and the swollen knob on the dorsal part of the thorax had become distinct. Eating of the coffee lamina from the edge ensued from this stage. The larva consumed 4.35% of leaf surface per day at this stage. The second and third instars lasted three days each. By the third instar, a distinct tail was observed emanating from the lower end of the abdomen and the body is now multi-coloured with yellow markings on green background. The third instar has a mean body length of 11.25 mm, body width of 1.09 mm and a mean tail length of 3.3 mm. Mean of 25.6% of leaf was consumed per day by the third instar. The fourth and fifth instars lasted four days each. Whereas the fourth instar larva is 24.5mm long with a mean vertex of 1.93mm, the fifth instar measured 34.7mm in length with a mean vertex of 2.74 mm while the tail lengths are 11.2mm and 13.7mm respectively. The mean growth ratio was 1.70 (Table 1). Mean percentage leaf area of 63.2% was consumed per day by the fourth instar larva while the last instar larva consumed the highest value of 88.4% in a day (Table 2).

Table 2. Mean percentage leaf area consumed by each larva instar of *Epicampoptera strandi.*

Larva Stage	Sample size (n)	Leaf area consumed (cm^2)	Range of leaf area consumed (cm^2)	Leaf area consumed (%)
1st instar	10	3.78	0.74-8.32	1.41
2nd instar	10	12.10	8.61-16.70	4.35
3rd instar	10	18.67	17.22-26.45	25.60
4th instar	10	24.74	26.10-30.84	63.20
5th instar	10	32.50	31.67-34.55	88.40

Just before pupation, the multi-coloured last instar larva rolled itself inside the coffee leaf. The pupa was dark brown in colour and the pupal duration lasted 6.8 days on the average. The pupa measured 20.7 mm in length with a mean body width of 5.4 mm. The adult male appeared similar to the female in colour and body markings but smaller in size. The adult male measured 18.43 mm in length with body width of 4mm while the female is 20 mm long with body width of 6.43 mm. The adult moth has a dusty brown colour covered with scales.

DISCUSSION

E. strandi derived its common name from morphological characteristics observed from the third instar, hence it is called the tailed caterpillar. *E. strandi* is also commonly referred to as the coffee leaf roller and this is because the fifth instar larva rolls itself inside the coffee leaf just before pupation.

Table 1 shows that the mean time required for *E. strandi* to complete its development from egg to adult on leaves of robusta coffee in the laboratory is 28.8 days. This short developmental period coupled with its high fecundity and mode of egg-laying as observed from this study and corroborated by Okelana (1989) makes the insect an economic pest, which deserves immediate attention.

The head capsule measurements increased by an average factor of 1.7. This is in slight variance with Dyar's law (Wigglesworth, 1965; Ewete, 1990; Emehute and Odiette, 1991). However, the growth ratio between the fourth and fifth instar increased by a factor of 1.42 and this agreed with Dyar's law, which states an increase of 1.4. It was relatively easier to separate the larval instars accurately on the bassis of head capsule measurements than using other morphometric parameters.

The fifth instar larva was the most destructive with 88.4% of leaf area consumed in a day. This is in agreement with the observations made by Okelana (1989) that the last instar larva is the most destructive and capable of eating not just the leaf lamina but the entire coffee leaf especially during severe infestation leading to the yield of the shrub being affected for several years. And since the feeding stages are exposed on the leaves most of the time, control with the use of natural pesticides that have contact and/or antifeedant properties should be explored along with natural enemies of this pest.

ACKNOWLEDGEMENT

We are grateful to the Director, Cocoa Research Institute of Nigeria, for the permission to publish this paper. Many thanks to Mr. M.A. Olayiwola and Mrs. M.A. Akande for their technical assistance.

REFERENCES

Coste, R (1992): Coffee, the Plant and the Products. The Macmillan Press Ltd 328pp.

Emehute, J.K.U. And Odiete, W.O. (1991): Some aspects of the biology of *Conchyloctenia nigrisparsa* Boh. (Coleoptera: Chrysomelidae), a sweet potato defoliator in Southern Nigeria. *Nigerian Journal of Entomology* 12:25-34.

Ewete, F.K. (1990). The Immature Stages and Chaetotaxy of *Acrea terpischore* L. (Lepidoptera: Nymphalidae). *Journal of African Zoology* 104:191-199.

Idowu, O.L. (1971): Report on work done on coffe entomology as a Research Officer-in-Training (1968-1977) at the CRIN, Ibadan. 24p. (Unpublished).

Okelana, F.A. (1985): Oviposition pattern, hatching and parasitism of eggs of *Epicampoptera strandi* Bryk. Sub. Sp. *Glauca* Hmps (Lepidoptera: Drepanidae) on coffee in Nigeria. Café, Cacao Thé, Number 4, Pp. 273-276.

Okelana, F.A. (1989). Bio-ecology and control of insect pests of coffee. In: *Progress in Tree Crop Research,* 2nd edition, CRIN, Ibadan, Nigeria. Pp. 152-165.

Williams, J.A. (1989): Coffee Breeding in Nigeria. In: *Progress in Tree Crop Research*, 2nd edition, CRIN, Ibadan, Nigeria. Pp.127-140.

Wiggles Worth, V.B. (1965): The Principles of Insect Physiology, 6th Edition, London, Methuen. 741pp.

Studies on Nematodes in Coffee Soils of Nigeria: Survey of Plant Parasitic Nematodes Associated with Coffee

O. A. FADEMI AND S. B. ORISAJO

Cocoa Research Institute of Nigeria – P.M.B. 5244, Ibadan, Nigeria
E-mails: tayofademi@yahoo.com and sambukki@yahoo.com

SUMMARY

A survey of the coffee experimental fields and germplasm plots of the Cocoa Research Institute of Nigeria (CRIN) in Ibadan, Nigeria in 2005. Plant–parasitic nematode genera occurring in approximately 50% or more of the fields were *Meloidogyne, Pratylenchus, Radopholus, Scutellonema, Tylenchus and Xiphinema. Meloidogyne spp.* had the greatest frequency of occurrence (60.5%) and could be regarded as the major nematode problem of coffee in Nigeria. Generally, frequency of occurrence for each of the other nematode was below 20%, with *Radopholus* spp. leading the less frequent (i.e. "minor") nematodes. Nematode association profile in this study is at variance with reports from other coffee-growing parts of the world where *Pratylenchus* spp. is ranked as the major nematode problem of coffee. This paper discusses the need to expand the scope of the survey to major plantations with a view to updating information on nematode genera associated with coffee with additional focus of ranking the nematodes in order of importance based on pathogen city studies and ultimate development of management strategies for nematodes.

INTRODUCTION/WORLD PERSPECTIVES

Nematodes are an important cause of damage and yield loss to field crops, coffee inclusive. However, due to the hidden nature of nematode association with crops, commensurate attention is rarely given to this pest when protective measures for field crops are being considered.

As part of an exercise to properly position implication of nematodes in yield losses to food crops, the International Meloidogyne Project coordinated a worldwide survey involving 75 countries in 1987. Information from this survey showed that losses due to nematodes range from 3.3% to 20.6%, with average of 10.7% and 14.0% for life-sustaining crops (LSC) and economically important crops (EIC) respectively (Table 1). Generally, losses arising from nematodes to LSC and EIC in the developed countries are lower than losses suffered by these crops in the developing countries – 12.6% to 7.0% and 16.5% to 10.5% for LSC and EIC respectively. Averaging both categories together, losses for the 40 crops in the developed countries were estimated to be 8.8% compared to 14.6% for developing countries.

Monetary losses due to nematodes on 21 crops, six (including coffee and cocoa) of which are EIC were, as at 1987, estimated at US$77 billion annually (Table 2). Interestingly, the bulk of world's coffee is produced in the developing countries, highlighting the need for a re-direction of research efforts on coffee (and cocoa) in these countries. Coffee and cocoa are two of the five crops that the Cocoa Research Institute of Nigeria (CRIN) is mandated to work on. The others are cashew (*Anacardium occidentale*), tea (*Cammelia sinensis*) and kola (*Kola nitida and K. acuminata*).

Table 1. Summary of estimated annual yield losses due to damage by plant-parasitic nematodes – World basis.

Life Sustaining Crops	Loss (%)	Economically Important Crops	Loss (%)
Banana	19.7	**Cacao**	10.5
Barley	6.3	Citrus	14.2
Cassava	8.4	**COFFEE**	**15.0**
Chickpea	13.7	Cotton	10.7
Coconut	17.1	Cowpea	15.1
Corn	10.2	Eggplant	16.9
Field bean	10.9	Forages	8.2
Millet	11.8	Grape	12.5
Oat	4.2	Guava	10.8
Peanut	12.0	Melons	13.8
Pigeon pea	13.2	Misc. others*	17.3
Potato	12.2	Okra	20.4
Rice	10.0	Ornamentals	11.1
Rye	3.3	Papaya	15.1
Sorghum	6.9	Pepper	12.2
Soybean	10.6	Pineapple	14.9
Sugarbeet	10.9	**Tea**	8.2
Sugarcane	15.3	Tobacco	14.7
Sweet potato	10.2	Tomato	20.6
Wheat	7.0	Yam	17.7
Average	10.7	**Average**	14.0

Source: Sasser and Freckman, 1987.

On a worldwide basis, the ten most important nematodes were reported to be *Meloidigyne* (1375), *Pratylenchus* (782), *Heterodera* (606), *Ditylenchus* (251), *Globodera* (244), *Tylenchulus* (233), *Xiphinema* (205), *Radopholus* (170), *Rotylenchulus* (142), and *Helicotylenchus* (122).

Coffee is an important cash crop worldwide. It is cultivated on over 3 million farm units, most of which are small agricultural enterprises. *Coffea arabica* accounts for 75% of the world coffee exports, and produced in 60 countries, mostly in South and Central America, while *Coffea canephora* accounts for approximately 25% (Anon, 1985), mostly concentrated in Africa and Asia. In Nigeria, Arabica coffee, (*Coffeae arabica*) is found in the Mambilla Plateau, North-Eastern Nigeria, while the Robusta coffee, *Coffeae canephora* is planted in the South West.

Table 2. Estimated annual losses due to nematodes for selected world crops.

Crops	Number of Estimates per Crop	FAO Production Estimates (1000 mT)	Estimated Price per mT (US$)	Estimated Yield loss by Nematodes (%)	Estimated Monetary loss Due to Nematodes (US$)
Banana	78	2,097	431	19.7	178,049,979
Barley	49	171,635	102	6.3	1,102,926,510
Cassava	25	129,020	90	8.4	975,391,200
Citrus	102	56,100	505	14.2	4,022,931,000
Cocoa	13	1,660	2,584	10.5	450,391,200
COFFEE	**36**	**5,210**	**3,175**	**15.0**	**2,481,262,500**
Corn	125	449,255	147	10.2	6,736,129,470
Cotton**	85	17,794	2,160	10.7	4,112,549,280
Field bean	70	19,508	544	10.9	1,156,746,300
Oat	37	43,355	99	4.2	180,270,000
Peanut	69	20,611	416	12.0	1,028,901,000
Potato	141	312,209	152	12.2	5,789,403,696
Rice	64	469,959	342	10.0	16,072,597,800
Sorghum	53	71,698	119	6.9	588,712,270
Soybean	91	89,893	282	10.6	2,687,081,500
Sugar beet	51	293,478	37	10.9	1,183,596,774
Sugarcane	65	935,769	115	15.3	16,464,854,000
S. potato**	67	117,337	219	10.2	2,621,073,906
Tea	16	2,218	2,807	8.2	510,562,300
Tobacco	92	6,205	2,996	14.7	2,732,756,460
Wheat	89	521,682	159	7.0	5,806,320,660
TOTAL					**$77,698,508,015**

***Cotton = cotton (lint only), S. potato = Sweet potato. Source: Sasser and Freckman, 1987.*

ROOT-KNOT NEMATODES, *Meloidogyne spp*.

Many genera and species of nematodes have been associated wilt coffee in many countries causing great losses to the coffee farmers and to local economy of such coffee producing countries (Campos et al; 1990). Although other nematode genera are implicated with coffee, the root-not nematodes of the genus *Meloiclogyne* are more widely distributed throughout the world in coffee plantations. *Meloidgyne exigua* has been found in all major coffee producing states in Brazil (Campos and Melles, 1987), Venezuela (Florez and Yepez, 1969), Columbia, Nicaragua and Bolivia (Gomez, 1980; Vega, 1982; Bridge et al., 1982).

Meloidogyne incognita was reported attacking coffee in Guatemala (Chitwood and Berger, 1960), Ivory Coast (Luc and de Guiran, 1960) Tanzania (Whitehead, 1969), Jamaica (Hutton et al., 1982) and India (Kumar, 1984). *M. africana* is widespread, while *M. megadora* is found in Angola and Uganda (Whitehead, 1968a; 1969). *Meloidogyne* infections on coffee have also been found in Zimbabwe (Way, 1981)

Most information on the economic importance of root-knot nematodes comes from Brazil where for over a hundred years the areas of coffee cultivation have migrated across the country due to the pressure of nematode damage. In Colombia, *M. exigua* and *M. javanica* have caused an estimated loss of US$800 million per year on coffee (Barriga, 1976). Although there is no information available in Tanzania on the actual yield losses caused by nematodes, it is estimated that yield losses of trees severely infested with the African coffee

root-knot nematodes will be in the region of 20% in optimum conditions; extending to the point of non–productivity in sever cases (Bridge, 1984). The stress to which trees are subjected to because of nematode damage will also cause pre mature fruit drop, twig die-back and defoliation, nutrient deficiency symptoms and stunted growth.

LESION NEMATODES, *Pratylenchus* spp.

The lesion nematodes, *Pratylenchus* spp. known to occur on coffee are *Pratylenchus coffee, P. brachyurus, P. goodeyi, P. pratensis and P. loosi*. For a long time, *P. brachyurus* was the only *Praylenchus* species known to infect coffee in South America (Lordello, 1972). Later, *P. coffeae* was found in Dominican Republic (Schieber and Grullon, 1969), Costa Rica (Figuerua and Pertaza, 1982), and Brazil (Monteriro & Lordello, 1974). *P. coffeae* also occurs on coffee in India (Palanichemy, 1973), Southeast Asia, Barbados, Mozambique and Tanzania (Whitehead, 1969a; Bridge, 1984), Madagascar and Indochina (Whitehead, 1968a). In Java, it is rated a very damaging pest of coffee and also considered a predominant species in Salvador (Palanichamy, 1973).

P. brachyurus has been found in many regions in Brazil (Lordello and Mello Filho, 1969; D'Antonio et al., 1980; Campos and Lima, 1986) in West Africa and Peru (Whitehead, 1968b). In Sao Paulo and Mina Gerais States of Brazil, *P. brachyurus* was more widespread than *P. coffeae*, occurring in 20% of samples collected around coffee trees (Gonzalez et al., 1978; D'Antonio et al., 1980). *P. pratensis* has been reported from one locality in South India Whitehead (1968b) and *P. loosi* from Ceylon Whitehead (1968a). *P. goodeyi* occurs on coffee Tanzania (Bridge, 1984).

Roots of coffee infected by *P. coffeae* turn yellow, then brown and most lateral roots are rotten. Infected plants look stunted and have few small chlorotic leaves (Whitehead, 1969b) Severely infested plants may die prematurely. In the field, the symptoms may occur in patches with reduced yield according to the disease severity. *P. brachyurus* causes reduced plant and root growth, shedding of leaves and nutritional deficiency (Lordello, 1984). The influence of infestations of *P. goodeyi, P. Lossi and P. pratensis* on coffee growth is not known.

OTHER NEMATODE PARASITES OF COFFEE

Among other species of nematodes parasitic to coffee, *Rotylenchulus reniformis* has caused greatest damage to this crop. In the Philippines, *R. reniformis* attacked *Coffea arabica*, *C. robusta* and *C. excelsa* with equal severity (Valdez, 1968). In India, it is an important parasite of *C. arabica* (Anon, 1966). R. reniformis is reported also from coffee seedlings in a commercial nursery in Brazil (Lordello, 1980) and is also recorded on coffee in New Guinea, Fiji Tonga and Southern Samoa (Bridge, 1988).

Whitehead (1968b) commented on the great importance of *Radopholus similis* to coffee in Java and reported that this nematode genus is harmful to coffee in that country, and ranked second in importance to *Pratylenchus coffeae.* Volvas (1987) reported on the widespread occurrence of *Trophotylenchulus obscurus* as a pest of coffee in Sao Tome, West Africa.

Many other parasite nematode species belonging to the genera *Caloosia, Criconemella, Discocriconemella, Helicotylenchulus, Hemicriconemoides, Hoplolaimus, Longidorus, Ogma, Scutellonema, Trichodorus, Tylenchorhynchus and Xiphinema* have been found associated with coffee plants (Whitehead, 1968a,; 1969; Lordello, 1972; Bridge et al., 1982; Bridge,

1984; Bridge and Page, 1984). However, information on pathogenicity, damage, yield loss and possible control measures of nematodes attacking coffee is still largely lacking.

COFFEE NEMATODES: NIGERIA

Six species of plant-parasitic nematodes were recovered from the soil samples taken around coffee trees. Sampling was done during the wet season in all cases over a period of 48 months. A total of 160 samples spanning through the entire four coffee germplasm plots in the Cocoa Research Institute of Nigeria, a National Agricultural Research Institution (NARI) with coffee as one of her mandate crops.

The frequency of occurrence of the nematodes recovered is expressed as a percentage of samples examined (Table 1). *Meloidogyne incognita* is the most abundant followed by *Radopholus similes, Pratylenchus brachyurus, Xiphinema nigeriense, Trichodorus monohystera* and *Scutellonema brachyurus* respectively.

Table 3. Frequency of occurrence of nematodes associated with coffee in Nigeria.

Nematode	Frequency of Occurrence (%)
Meloidogyne incognita	60.5
Pratylenchus brchyurus	7.4
Radopholus similis	16.3
Scutellonema brachyurus	3.4
Trichodorus monohystera	5.8
Xiphinema nigeriense	6.6

Even though relatively few surveys have been done in Africa to produce a good picture of the distribution of nematodes in different countries where coffee is grown, the data available suggest that many species of *Meloidogyne* occur in coffee soils (Luc et al., 1990). The outcome of this survey confirmed the ubiquity of root-knot nematodes. It accounted for 60.5% of the nematode populations in the soil samples collected. Next to the root-knot nematodes, *Radopholus similis* is next in abundance. This is probably so because plantains and banana are commonly used as shade plants in coffee plantations at establishment.

CONCLUSION

Pratylenchus coffeae and *Radopholus similis* are the principal nematodes affecting coffee in sub-tropical and tropical coffee growing areas, and are responsible for substantial losses. *Meloidogyne sp.* and *Radopholus sp.* are of local importance, while reports from other countries indicated that *Meloidogyne paranensis* (Carneiro et al., 1996), *M. thames* (Kumar, 1984), *Pratylenchus coffeae* (Monteiro and Lordello, 1974), *Rotylenchulus reniformis* (Valdez, 1968) and *Teophotylenchulus obscurus* (Volvas, 1987) are also implicated in yield losses arising from coffee-nematode association. There is urgent need to scale up level of research on association of nematodes with coffee, in particular expanding survey to capture coffee-nematode association in major plantations, assess coffee productivity in the plantations and relate same to nematode status in soils/rhizospherical soils in such plantations. Additional research is needed to establish the pathogenicity of the nematodes already associated with coffee (and others that may emerge from the expanded survey) in order to rank them along the line of losses they could inflict on coffee. Studies on control options is also desirable, even at this initial stage so as to have at hand, a basket of options from which to pick by the time the need for control arises.

REFERENCES

Bridge, J.1984. Coffee nematode survey of Tanzania: Report on a visit to examine plant parasitic nematodes of coffee in Tanzania, February/March, 1974. Commonwealth Institute of Parasitology, 22p.

Bridge, J. 1988. Plant parasitic nematode problems in the Pacific Islands. *Journal of Nematology*, 20: 173-183.

Bridge, J. and S. L. Page. 1984. Plant nematode pests of crops in Papua New Guinea. *Journal of Plant Protection in the Tropics*, 1: 99-109.

Carneiro, R. M. D. G., R. G. Carneiro, I. M. O. Abrantes, M. S. N. A. Santos and R. A. Almeida, 1996. *Meloidogyne paranaensis* n.sp.)Nemata: Meloidogynidae) a root knot nematode parasitizing coffee in Brazil. *Journal of Nematology* 28: 177-189.

Chitwood, B. G. and C. A. Berger. 1960. Preliminary report on nemic parasites of coffee in Guatemala with suggested and interim control measures. *Plant Diseases Reporter*, 44: 841-847.

Kumar, A. C. 1984. Resistance in coffee to *Meloidogyne* spp. and occurrence of intersexes in *M. thames*. *Nematologica,* 30: 108-110.

Monteiro, A. R. and L. G. E. Lordello. 1974. Encontro do nematoide *Pratylenchus coffeae* atacando cafeeiro em Sao Paulo. *Revista de Agricultura*, *Piraciceba* 49: 164.

Palanichamy, L. 1973. Nematode problems of coffee in India. *Indian Coffee*, 37: 99-100.

Valdez, R. B. 1968. Stubby roots of coffee seedlings caused by *Rotylenchulus reniformis*. *Philippines Agriculture* 51: 672-679.

Volvas, N. 1987. Parasitism of *Teophotylenchulus obscurus* on coffee roots. *Revue de Nematologie*, 10: 66-68.

Impact des Facteurs Environnementaux sur les Populations de Scolytes (*Hypothenemus hampei* Ferrari) (Coleoptera: Scolytidae)

K. WEGBE[1], C. CILAS[2], C. ALAUZET[3], B. DECAZY[2]

[1]ITRA/CRA-F BP 90 Kpalimé Togo. E-mail: wegbepascal@yahoo.fr
[2]CIRAD-CP, 34398 Montpellier France. E-mail: christian.cilas@cirad.fr
[3]Université Paul Sabatier Toulouse – France

SUMMARY

The coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae) is acknowledged to be a serious pest in coffee plantations worldwide. This pest was long controlled by insecticides such as endosulfan. Reports on insecticide resistance of the pest have encouraged researches on ecological control strategies that preserve the natural enemies of the berry borer and the environment. The present study was undertaken in Togo (one of the originate country of the beetle) in attempt to promote agroecological management of coffee plantations. The work was carried out over 2 years in 79 coffee plantations distributed on the whole coffee production area. A survey in these plantations permitted to collect qualitative data (age of plantation, beginning of harvest, number of sanitary harvest…). Measures allowed having quantitative data (quantity of rain fall, number of rainy days, quantity of light, soil chemistry, number of attacked fruits, number of residual berry borer…). The analysis of these data consisted in one hand to search for relationships between the different qualitative factors and the attack rates, and in second hand to make analysis of variance with the quantitative factors. Results revealed that environmental factors such as altitude, number of rainy days, coffee stand age, presence of shade and essentially the incomplete harvesting of infested berries from the previous harvest season were the major factors that explain the level of infestation in coffee plantations.
Key words: coffee**,** *Hypothenemus hampei*, agroecological management, Togo

RÉSUMÉ

Le scolyte des fruits du caféier *Hypothenemus hampei* Ferr. (Coleoptera: Scolytidae) est le ravageur le plus dommageable dans les caféières. Les lacunes de la lutte chimique et de la lutte biologique contre ce ravageur ont ouvert la voie à la recherche sur l'étude de l'influence des facteurs environnementaux et phytotechniques sur la dynamique des populations du ravageur en vue de proposer une gestion agroécologique des caféières. Les travaux ont été conduits au Togo dans 79 plantations caféières paysannes réparties sur toute la zone de production. Une enquête réalisée dans ces plantations a permis de collecter les données qualitatives (âge de la plantation, début de récolte, nombre de passage de récolte…). Des mesures et des comptages ont permis d'obtenir des données quantitatives (pluviométrie, nombre de jours de pluie, quantité de lumière,…). L'analyse de ces données a consisté à rechercher des liens entre les différentes variables et les taux d'attaque. Les résultats obtenus ont montré qu'en dehors du nombre de jours de pluies et de l'altitude qui sont des facteurs généraux qui influent sur les niveaux d'infestation, les facteurs tels que la variété de caféier cultivée (Niaouli ou Robusta), l'âge de la plantation, le taux d'ombrage et les scolytes résiduels d'intercampagne conditionnent également les niveaux d'infestation des caféières.
Mots clé: café, *Hypothenemus hampei*, gestion agroécologique, Togo

INTRODUCTION

Le scolyte des fruits du caféier (*Hypothenemus hampei*) est le principal ravageur dans les caféières. Les attaques du ravageur sont plus importantes dans les pays où il a été introduit accidentellement. Dans les pays d'origine (Afrique), les attaques sont moindres, mais on peut observer quelque fois des pullulations massives entraînant des pertes de production non négligeables (Wegbe et al., 2003). La lutte chimique menée contre ce ravageur avec l'endosulfan s'est vite confrontée à des phénomènes de résistance (Brun et al., 1987). De même, la lutte biologique entreprise dans les années 1990 dans les pays de l'Amérique Latine n'a pas donné les résultats escomptés (Barrera, 1994; Damon, 2000). L'échec de ces deux types de lutte menés séparément a conduit à réaliser des études pour comprendre les relations entre les facteurs environnementaux, phytotechniques et la dynamique des populations du scolyte dans les caféières.

L'objectif de cette étude est d'identifier les facteurs clé qui favorisent les pullulations de scolytes et d'en tenir compte dans les propositions de gestion agroécologique des caféières

MATÉRIEL ET MÉTHODES

Soixante dix neuf plantations en production de différents types (Robusta ou Niaouli, âgée ou jeune, recepée ou non recepée…) et d'aspects différents possible (ombragée ou non ombragée, désherbée ou enherbée…) réparties sur 3 zones agroclimatiques (Kpalimé, Kpélé et Dayes) ont été choisies au hasard pour l'étude.

L'étude a consisté en la conduite d'une enquête auprès des producteurs et à la réalisation de comptages et mesures dans les plantations pour compléter les données.

L'enquête

L'enquête a été réalisée grâce à un questionnaire administré sur les 79 plantations pendant 2 campagnes agricoles successives (2001-2002 et 2002-2003). Elle a consisté en la collecte de données relatives à la variété, à l'âge de la plantation, au début de récolte, au nombre de passages de récoltes, de récoltes sanitaires, d'égourmandage et de désherbages.

Comptages et mesures

Insectes résiduels

Des prélèvements de 250 fruits secs perforés d'intercampagne tombés ont été effectués entre mars et avril dans chacune des plantations et les insectes émergés (scolyte et parasitoïdes) ont été collectés et dénombrés chaque après-midi jusqu'à l'arrêt des émergences.

Pluviométrie et nombre de jours de pluie

Sur chaque parcelle, un pluviomètre a permis de collecter la quantité de pluie. Le nombre de jours de pluie a été également noté. La collecte a été faite sur 10 à 11 mois (de janvier jusqu'à la date de récolte).

La lumière

Le pourcentage de lumière sous chaque caféier a été mesuré avec un luxmètre.

Chimie du sol

Un prélèvement de sol (taraudage à 20 cm de profondeur) a été effectué en mai 2003. Par parcelle, un échantillon composite a été constitué à partir des 15 sous échantillons. L'analyse a porté sur la mesure du pH, le dosage de l'Azote, du Potassium, du Phosphore et de la matière organique.

Taux d'attaque

Un échantillonnage systématique en quinconce de 30 caféiers par plantation (Remond, 1996) suivi du choix d'un rameau fructifère dans la partie médiane du caféier (Barrera, 1994) a permis de déterminer le taux d'attaque de chaque plantation. Ce taux est calculé comme suit: **P/(S+P) x 100** avec **S** le nombre de fruits sains et **P** le nombre de fruits scolytés.

Les différentes données collectées ont été analysées avec les logiciels SAS (version 8.2) et STATISTICA (version 5.1).

RÉSULTATS

Les résultats obtenus sont présentés dans les Tableaux 1 et 2.

Tableau 1. Résumé des analyses de variances.

Variables		Nombre de plantations	Nombre de tiges	Taux d'attaque (%)	p
Altitude (m)	813 m 270 m	30 20		33,66a 7,76b	0,008
Variété	Niaouli Robusta	4 14		19,5a 5,4 b	0,0004
Nb récoltes sanitaires	1 2 3	23 15 13		42,0a 7,0b 8,1b	0,000
Lumière (lux)	lum < = 62 62 < lum < = 76 lum > 76		146 153 145	61,8a 51,4c 56,4b	0,0003
Age des plantations	âge < = 19 19 < âge < = 23 âge > 23		209 149 88	51,6b 63,0a 56,7b	0,0001

Les moyennes suivies des mêmes lettres dans les colonnes ne sont pas significativement différentes (Test de Student - Newman - Keuls au seuil de 5 %).

Ces résultats montrent que le taux d'attaque est plus important sur les plateaux (813 m) que dans les plaines (270 m) (Wegbe, 2004). De même, il est plus élevé dans les plantations de Niouli (variété locale) que dans celles de Robusta. Le taux d'attaque est inversement proportionnel au nombre de récoltes sanitaires mais proportionnel à l'âge des plantations. Ces résultats en outre montrent globalement que les plantations les plus ombragées sont les plus attaquées par le scolyte (Tableau 1).

Tableau 2. Corrélation entre le nombre de jours de pluie, les scolytes résiduels d'intercampagne et le taux d'attaque.

Variables	Nombre de plantations	Minimum	Maximum	Moyenne	r	p
Nb. jour de pluie	51	63	149	95,1	0,82	0,000
Scolyte	62	0	1168	181,66	0,82	0,0001

r = coefficient de corrélation, p = probabilité, corrélation significative quand p < 0,05.

Le Tableau 2 indique que les niveaux d'infestation sont liés au nombre de jours de pluie et au nombre de scolytes résiduels dans les parcelles avant les nouvelles fructifications.

DISCUSSION ET CONCLUSION

Cette étude a montré qu'en dehors du nombre de jours de pluie et de l'altitude (agissant par la température) qui sont des facteurs généraux qui influent sur les niveaux d'infestation, certains facteurs locaux liés aux plantations jouent un grand rôle dans la réinfestation et le maintien du scolyte dans les plantations. C'est ainsi que les plantations mal récoltées seront plus attaquées et que les vieilles plantations constitueront des foyers d'infestation.

L'ombrage intense crée un microclimat favorable au développement et à la multiplication rapide du scolyte.

Malgré la pénibilité de l'opération de récolte sanitaire et la main d'œuvre importante qu'elle requiert, elle est impérative pour réduire les attaques de scolyte à des niveaux raisonnables. Pour le choix de la variété, des études restent encore à faire pour identifier des variétés plus tolérantes aux attaques de scolyte.

Bien que nous n'ayons pas trouvé un lien significatif entre les taux d'attaque et les autres pratiques culturales, l'application de ces dernières devra renforcer les effets de la variété tolérante et des récoltes sanitaires. C'est ainsi qu'un désherbage bien fait permettra de mieux réaliser la récolte sanitaire, de même, des caféiers bien égourmandés offriront un environnement favorable à l'action des auxiliaires (*Cephalonomia stephanoderis* et *Phymastichus coffea*) mais défavorable au scolyte.

La culture d'une seule variété (Robusta), une récolte soigneuse, un recepage régulier des caféiers et l'élagage périodique des arbres d'ombrage permet de créer un environnement favorable à l'activité des ennemis naturels (*Cephalonomia stephanoderis* et *Phymastichus coffea*) et de ce fait de minimiser les attaques du scolyte.

L'intégration de ces pratiques agroculturales dans une bonne stratégie de lutte doit permettre de développer une gestion agroécologique des caféières contre le scolyte des fruits.

REMERCIEMENTS

Les auteurs remercient la Banque Mondiale à travers le Projet National de restructuration des Services Agricoles (PNASA) au Togo, CEREXAGRI, le CIRAD-CP, l'Université Paul Sabatier de Toulouse III, Dr ILLY et le comité d'organisation de l'ASIC 2006 pour leur aide de toutes natures

RÉFÉRENCES BIBLIOGRAPHIQUES

Baker P. 1999. The coffee berry borer in Colombia. Final report of the DFID-CENICAFE-CABI Bioscience, Ascot, UK 143 p.

Barrera J.F. 1994. Dynamique des populations du scolyte des fruits du caféier, *Hypothenemus hampei* (Coleoptera: Scolytidae) et lutte biologique avec le parasitoïde *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae) au Chiapas, Mexique. Thèse de Doctorat de l'Université Paul Sabatier de Toulouse, France, 301p.

Borbon-Martinez O. 1989. Bioécologie d'un ravageur des baies de caféier, *Hypothenemus hampei* Ferr.(Coleoptera:scolytidae) et de ses parasitoïdes au Togo. Thèse de Doctorat de l'Université Paul Sabatier de Toulouse, France,185 p.

Brun L.O. Ruiz J. L. 1987. Detection of endosulfan resistance in coffee berry borer *Hypothenemus hampei (*Ferr.) (Coleoptra: Scolytidae) in New Caledonia. *Int. Conf. on Pesticides*

Decazy B. 1989. Le scolyte du fruit du caféier *Hypothenemus hampei* Ferr. Considération sur la lutte intégrée contre ce ravageur *in* 13ème Colloque de l'Association Scientifique Inernational du Café. Paipa (Colombie) 21-25 août 1989, p 655-665 *in tropical Agriculture*, Kuala Lumpur, 23-25 sept.1987.

Giordanengo P. 1992. Biologie et éco-éthologie et dynamique des populations des grains de café, Hypothenemus hampei Ferr. (Coleoptera: Scolytidae), en Nouvelle Calédonie. Thèse, Université de Rennes I, 109 p.

Rémond F. 1996. Mise au point de méthodes d'échantillonnage pour estimer les attaques des fruits du caféier par le scolyte (*Hypothenemus hampei* Ferr.). Thèse, Université de Montpellier II, 278 p.

Ticheler J.H. 1961. Etude analytique de l'épidémiologie du scolyte des graines de café, *Stephanoderes hampei* Ferr., en Côte d'Ivoire. Mededelingen Landbourshogeschool Wageningen, 61: 1-49.

Wegbe K., Cilas C., Decazy B., Alauzet C. & Dufour B. 2003. Estimation of production losses caused by the coffee berry borer (Coleoptera: Scolytidae) and Calculation of an economic Damage threshold in Togolese Coffee plots. *J. Econ. Entomol*. 96(5): 1473-1478 (2003).

Wegbe K. 2004. Contribution à la gestion agroécologique des scolytes (*Hypothenemus hampei* Ferr. (Coleoptera:Scolytidae) dans les caféières du Togo. Thèse de doctorat de l'Université Toulouse III, France, 148 p.

Reaction of *Coffea arabica* Genotypes to *Meloidogyne* spp.

R. M.D.G. CARNEIRO[1], E. V.S.A. BARROS[1], W. GONÇALVES[2], A. A. PEREIRA[3]

[1]Embrapa – Recursos Genéticos e Biotecnología, CP. 02372, 708849-970 Brasília, DF, Brazil
[2]IAC, Seção de Café, 13001-970 Campinas, SP, Brazil
[3]EPAMIG, CRZM, 36570-000 Viçosa, MG, Brazil

SUMMARY

Among the most damaging root-knot nematode species, *Meloidogyne exigua*, *M. paranaensis*, *M. incognita*, *M. arabicida, M. izalcoensis* and *M. mayaguensis* exert the main agronomic constraint on coffee-growing areas in Latin America countries. The resistance reaction to those six species was studied on a new genotype (MGH 419-5-4-5-2 progeny of the Paraíso cultivar) derived from *C. arabica* x *C. canephora* (Timor Hybrid) x Catuaí (IAC 30) hybridization in an experiment under green-house conditions. The cultivar IAC 144 (Catuaí vermelho) was used as control for susceptibility to *Meloidogyne* spp. The plants were inoculated with 6.000 eggs/plants and the experiment was in a completely randomized design, replicated 10 times for each *Meloidogyne* species and genotype. Evaluation was 240 days after inoculation and the reproduction factor (RF= Final population/6000) was used as the parameter to evaluate the resistance. The number of galls and egg masses was not recommended as a good parameter for resistance evaluations because the symptoms of damage caused by the *Meloidogyne* species on coffee are variable. *M. exigua* caused typical rounded galls mostly on new roots and egg masses are produced in the cortex under the root epidermis. *M. incognita, M. paranaensis* and *M. arabicida* caused swelling roots, peeling and cracking of cortical parts of the root tissue. The egg masses are produced outside (*M. incognita*) or under the root epidermis (*M. paranaensis* and *M. arabicida*). No symptoms were observed for *M. mayaguensis*. *M izalcoensis* caused very small galls, mostly on the extremity of new roots. Egg-masses are produced outside the roots in large quantities. The cultivar IAC 144 was susceptible (FR>1.0) to all studied *Meloidogyne* spp., except for *M. mayaguensis*. This nematode isolate used in this experiment seems to be a weak parasite for coffee. The Paraíso genotype was resistant to *M. exigua* and *M. incognita* (FR<1) and susceptible to *M. paranaensis*, *M. arabicida* and *M. izalcoensis*. Considering the intraspecific variability of *M. incognita* and *M. exigua*, more studies will be necessary to confirm the resistance of Paraíso genotype.

INTRODUCTION

Meloidogyne spp. have a substantial economic impact on the production in almost all coffee-producing regions in many countries (Campos and Villain, 2005). In Brazil and Central America six species are known to cause serious damage: *M. exigua* in Brazil and Costa Rica (Campos and Vilain, 2005), *M. paranaensis* in Brazil and Guatemala (Carneiro et al., 2004), *M. incognita* in Brazil, Guatemala, El Salvador, Costa Rica, Nicaragua, Cuba (Campos and Villain, 2005), *M. arabicida* in Costa Rica and *M. izalcoensis* in El Salvador (Carneiro et al., 2005) and *M. mayaguensis* in Cuba (Campos and Vilain, 2005). Considering this great diversity of species, it is of prime importance to assess the specific pathogenicity of the *Meloidogyne* species in the different areas, so as to implement efficient integrated management strategies. Thus, the present study investigated the pathogenecity of six main species of *Meloidogyne* on coffee on two selected *Coffea arabica* genotypes.

MATERIAL AND METHODS

The work used 6 species of *Meloidogyne*, four from Brazil: *M. exigua* (Lavras, MG, coffee), *M. paranaensis* (Londrina PR, coffee), *M. incognita* (Avilândia, SP., coffee) and *M. mayaguensis* (Petrolina, PE, guava) and two from Central America: *M. arabicida* (Costa Rica, coffee) and *M. izalcoensis* (El Salvador, coffee).

The *Coffea arabica* genotypes are from Instituto Agronômico de Campinas (IAC 144 , 'Catuaí vermelho') and Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG (MGH 419-5-4-5-2, a progeny of the Paraíso cultivar, derived from *C. arabica* x *C. canephora,* Timor Hybrid**).** The cultivar Catuaí was used as control for susceptibility to *Meloidogyne* spp. and the new genotype Paraíso was previously found to be resistant to *M. exigua* in greenhouse and field conditions (Gonçalves, W. pers. inform.).

One experiment was undertaken in a greenhouse (25-30 °C). The plant arrangement was completely randomized and replicated 10 times for each *Meloidogyne* species and genotype. Two months after transplanting i.e. when they had at least two pairs of leaves, coffee plants were inoculated with 6,000 eggs/plant extracted by Hussey & Baker's method (1973) and counted in Peters slides. Evaluation was 240 days after inoculation and the reproduction factor (RF = Final population/6,000) were used as the parameter to evaluate the resistance. The galling and egg-masses index (Taylor and Sasser, 1978) was used as a second parameter to evaluate resistance to *Meloidogyne* spp.

RESULTS AND DISCUSSION

There were no significant differences among the six *Meloidogyne* species for the vegetative parameter plant height (Table 1). The weight of fresh roots was significantly higher when coffee Catuaí was infested with *M. exigua,* which causes several elongated galls of up to 1cm of diameter (Table 1). *M exigua* caused typical rounded galls, mostly on new roots and egg masses were produced in the cortex under the root epidermis. *M. incognita, M. paranaensis* and *M. arabicida* caused swollen roots, peeling and cracking of cortical parts of the root tissue, all of which very difficult to quantify. The egg masses were produced outside (*M. incognita*) or under the root epidermis (*M. paranaensis* and *M. arabicida*). No galls were observed for *M. mayaguensis*. *M izalcoensis* caused very small galls, mostly on the extremity of new roots. Egg-masses were produced outside the roots in large quantities (Figure 1).

The number of galls and egg masses was not recommended as the only parameter for resistance evaluations because the symptoms of damage caused by the *Meloidogyne* species on coffee are variable and very difficulty to quantify. Based on these findings, the best parameter is number of eggs per plant or the reproduction factor (RF) used in this paper.

Meloidogyne mayaguensis from guava was unable to develop on cv Catuaí (Table 1), confirming the observations by Hernandez et al. (2004 b). This confirms that coffee is not a good host of *M. mayaguensis* from guava. This species is one of the main coffee pests in Cuba (Rodriguez et al., 1995), indicating that the isolate from guava is probably different from the Cuban isolate from coffee.

Meloidogyne exigua reproduced very well on cv Catuaí Vemelho (RF = 98.2), and no galls and no reproduction were observed on cv Paraíso (RF = 0.9). The same occurred with *M. incognita* (cv Catuaí, RF = 15.0 and cv Paraíso, RF = 0.6). The other three species *M. paranaensis* (cv Catuaí, RF = 17.3 and cv Paraíso, RF = 47.3), *M. arabicida* (cv Catuaí, RF =

19.8 and cv Paraíso, RF = 86.3) *and M. izalcoensis* (cv Catuaí, RF = 30.6 and cv Paraíso, RF = 71.9) reproduced very well in both cultivars (Table 1).

Considering the intraespecific variability among isolates of *M. incognita* and *M. exigua* (Carneiro et al., 2004; Hernandez et al., 2004 a, b), more studies will be necessary to confirm the resistance of Paraíso genotype.

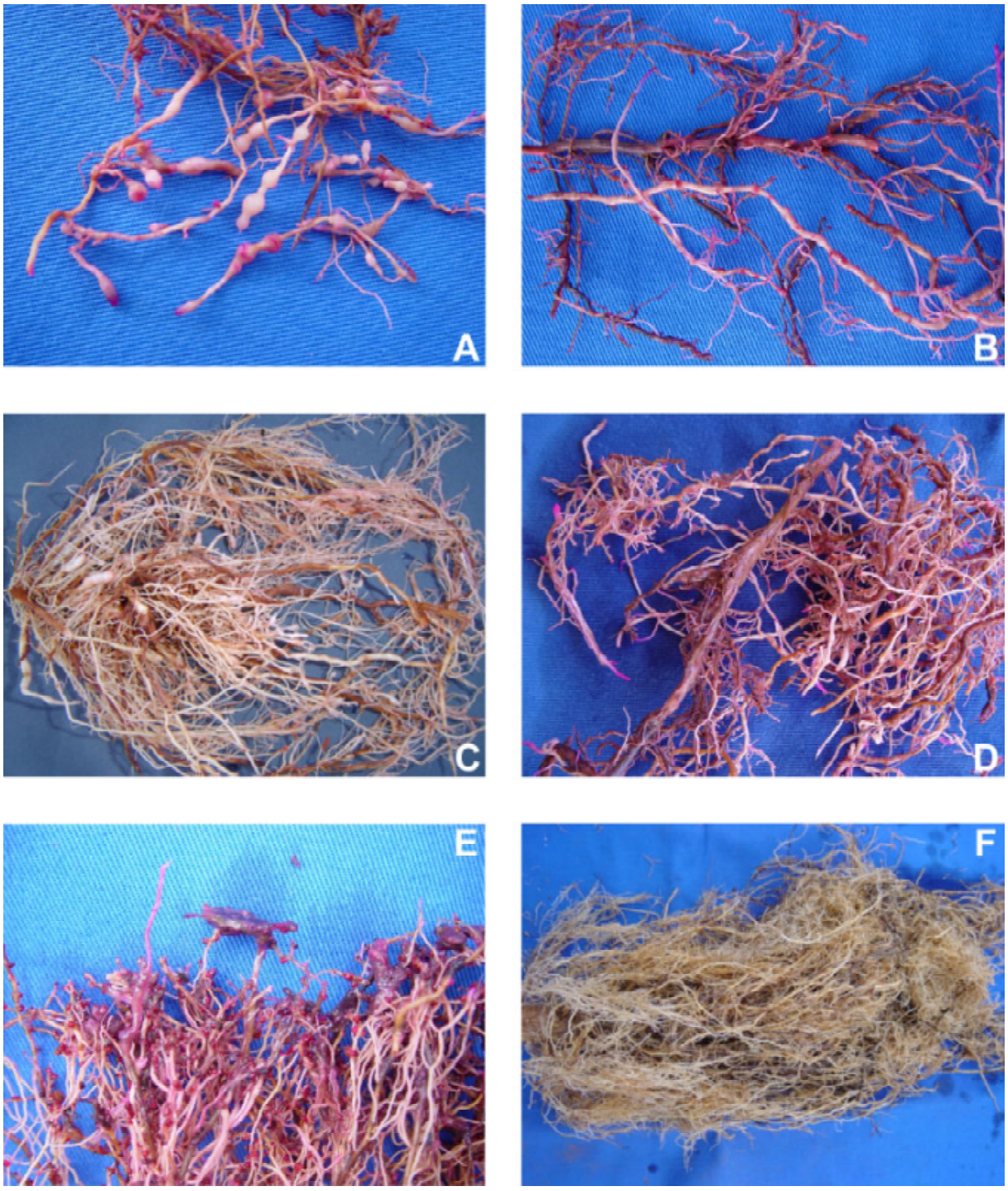


Figure 1. Symptoms caused by *Meloidogyne* spp. on *Coffea arabica* roots of cv. Catuaí Vermelho: A) *M. exigua*, B) *M. incognita*, C) *M. paranaensis*, D) *M. arabicida*, E) *M. izalcoensis.* F) *Coffea arabica* root of cv Paraíso (resistant) inoculated with *M. exigua.*

Table 1 Reaction of two genotypes of *Coffea arabica* to *Meloidogyne* spp.

Species of root-knot nematode and esterase phenotype (Est)	*C. arabica* cv Catuaí Vermelho IAC 144			*C. arabica* cv. Paraíso		
	Height of plants	Fresh Root weight	RF[a]	Height of plants	Fresh Root weight	RF *
M. exigua (Est 1)	49.0	81.0 b **	98.2 d (S)	54.2	43.8 a	0.9 a (R)
M. incognita (Est I1)	43.2	15.0 a	16.1 b (S)	44.8	40.2 a	0.6 a (R)
M. paranaensis (Est P1)	37.5	16.1 a	17.3 b (S)	42.3	35.1 a	47.3 b (S)
M.arabicida (Est Ar2)	38.0	16.4 a	19.8 b (S)	54.8	52.1 a	86.3 d (S)
M. izalcoensis (Est S4)	44.6	20.3 a	30.6 c (S)	50.5	38.8 a	71.9 c (S)
M. mayaguensis (Est M2)	33.7	13.3 a	0.5 a (R)	54.0	45.1 a	0.9 b (R)

**RF = Final population / 6000 egg. The plants with RF > 1 were considered susceptible (S) and RF < 1.0, resistant (R). **lower case letters indicate that arithmetical means differ according to the Scott and Kenett (1974) test at P < 0.05*

REFERENCES

Campos, V.P., Villain, L. (1995). Nematode parasites of coffee, cocoa and tea. In: Luc, M., Sikora, R.A. and Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture.* CAB International, Wallingford, UK, pp. 529-579.

Carneiro, R.M.D.G., Almeida, M.R.A., Gomes, A.C.M.M. and Hernandez, A. (2005a). *Meloidogyne izalcoensis* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitizing coffee in El Salvador. *Nematology* 7 (6):819-832.

Carneiro, R.M.D.G., Tigano, M. S., Randig, O., Almeida, M.R.A and Sarah, J.L. 2004. Identification, Characterization and Diversity of *Meloidogyne* spp. (Tylenchida, Heteroderidae) on coffee from Brazil, North and Hawaii. Nematology 6: 287-298

Hernandez, A., Fargette, M. and Sarah, J.L. (2004a). Characterization of *Meloidogyne* spp. (Tylenchida, Meloidogynidae) isolated from coffee plantations of Central America and Brazil. Nematology 6 (2):193-204.

Hernandez, A., Fargette, M. and Sarah, J.L. (2004 b). Pathogenecity of Meloidogyne spp. (Tylenchida: Meloidogynidae) isolates from Central America and Brazil on four genotypes of coffee. Nematology 6 (2):205-213.

Hussey, R.S. and Barker, K.R. (1973). A comparison of methods of collecting inoculum of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter* 57, 1025-1028.

Rodriguez, M.G., Rodriguez, I. and Sanchez, L. (1995). Especies del genero *Meloidogyne* que parasitan el cafeto en Cuba. Distibución geográfica y sintomatologia. Revista de Protección Vegetal, 10, 123-128.

Taylor, A.L. and J.N. Sasser. (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh:North Carolina state University Graphics. 111p.

Preferential Oviposition of Leaf-miner for Erecta Coffee Genotype

H. P. MEDINA-FILHO, O. GUERREIRO-FILHO, D. A. RAMIRO, R. BORDIGNON

Centro de Café Alcides Carvalho, Instituto Agronômico (IAC), Av. Barão de Itapura, 1481, 13020-902, Campinas, SP, Brazil. E-mail: medina@iac.sp.gov.br

SUMMARY

The dominant mutation erecta (*Er Er*) of *Coffea arabica* affects the angle of plagiotropic fruiting branches changing them from the normal 66° to 26° and consequently determining an upright growth and a more compact plant habit. Breeding programs have tried to exploit this variation transferring *Er* allele to commercial lines aiming at the reduction of the canopy projection area of plants thus allowing for closer spacings. On the course of the investigations, some good yielding segregants in the field apparently presented higher infestation of leaf-miner (*Leucoptera coffeella*). In the present experiment four individuals representing homozygous F_3 *Er Er* progenies were compared in randomized block design to four homozygous *er er* individuals from different F_2 individuals of the same original F_1 plant *Er er*. Eighteen-months old plants were caged and subjected overnight to oviposition of insects. Eggs laid in the adaxial surfaces of the leaves were then recorded for all leaves of the plants. A total of 1486 eggs were recorded in 82 leaves (18,12 eggs/leaf) of *Er Er* genotypes and 867 in 80 leaves (10,84 eggs/leaf) of normal *er er* genotypes. Average oviposition in erecta plants was higher in both ortotropic and plagiotropic branch leaves although statistically significant only for the latter. Oviposition was higher in the second and third leaf pairs of plagiotropic branches and on the leaves of the middle part of the ortotropic branches. The preference of *L. coffeella* for *Er Er* genotypes could be determined either by a more attractive position of the leaves for oviposition, by linkage of *Er* to an unknown attractive factor or by a pleiotropic effect of the *Er* allele. Further experiments are under way in order to ascertain the underlying cause of the preference of leaf-miner adults for oviposition in erecta plants.

INTRODUCTION

The botanical variety erecta (*Coffea arabica* L. var. *erecta* Ottoländer) was first reported by Cramer (1913) in Java, latter found in Puerto Rico, El Salvador and, in São Paulo, Brazil, it was encountered in a old collection at the end of 19th century, subjected to botanical description and genetic analysis (Krug et al., 1939; Krug and Carvalho, 1951). Erecta phenotype is conditioned by a completely dominant allele *Er*.

Coffee plants have dimorphic branches (Cook, 1911) with angles of insertion of the lateral plagiotropic ones usually ranging from 58° to 75° in different varieties (Krug et al., 1939). Erecta plants have plagiotropic branches growing upright (11° to 41°) being 26° the average angle formed with the orthotropic branches (Figure 1). Plants are thus more compact with denser foliage that theoretically would be more appropriate to be planted at close spacings and in windy areas (Medina Filho et al., 1984). In the course of a breeding program aiming at the development of new lines associating the alleles mokka (*mo*), caturra (*Ct*) and erecta (*Er*)

Among several cultivars in that study and latter investigated in the whole germplasm of arabica collection at Campinas, mokka was the only *C. arabica* with reduced infestation in the field. In the course of the aforementioned breeding program, the low rates of infestation were again observed in some plants (Figure 1). However, in lab conditions with leaf discs in no-choice tests, Guerreiro-Filho (1987) has shown that the damaged area by one caterpillar in cv. Ibairi is similar to the observed in other susceptible cultivars. The present investigation had the objective of investigating the oviposition of adult insects in *mo mo* genotype comparatively to the correspondent normal *Mo Mo*.

Figure 1. From left to right: Cage used for oviposition experiments. Eggs of leaf-miner oviposited on the adaxial surface of coffee leaf. Recombinant plant *mo mo* (mokka) and caturra (*Ct Ct*) displaying reduced infestation in field condition. Fruits and beans of normal plants (above) and mokka (below).

MATERIAL AND METHODS

Ten randomly chosen individuals of genotype *mo mo* (mokka) and 10 *Mo Mo* (normal) from F_3 progenies originated from different F_2 plants of a single *Mo mo* F1 hybrid of Ibairi (*mo mo*) x Catuaí (*Mo Mo*) were selected to be tested as to oviposition of adult insects of leaf-miner. Insects were raised in confinement at 27 ± 3 °C, 80% relative humidity (Guerreiro-Filho et al., 1992) supplemented with 10% sucrose solution (Nantes and Parra, 1978). One leaf of each eighteen-month old plant was removed and exposed overnight to oviposition of insects inside a cage (Figure 1). Eggs laid on the upper surface of leaves (Figure 1) were recorded observing the leaves under a 100X magnifier. The experiment was set up in randomized block design with 10 replicates and performed twice, first with high then with low infestation intensity in the cage. Data of oviposition in leaves of *mo mo* and *Mo Mo* plants were subjected to Analysis of Variance (Little and Hills, 1978) using Minitab software version 14.

RESULTS AND DISCUSSION

Data of oviposition on leaves of normal and mokka plants are shown in Table 1. As in some blocks the number of eggs was less than ten, chiefly in the low infestation experiment including one with no eggs at all, recorded counts were transformed to $\sqrt{x + 0,5}$ before the analysis of variance. In the experiment with high level of infestation ($F = 11,48$; $p = 0,008$), oviposition (Figure 1) occurred 2,2 times more intensively on the normal (*Mo Mo*) (μ=107,2 eggs/leaf) than in mokka (*mo mo*) leaves (μ = 49,0 eggs/leaf). In the low infestation experiment ($F = 9,96$; $p = 0,012$) comparable results were obtained with higher rate (14,4 eggs/leaf) of oviposition on the normal as compared to mokka (8,2 eggs/leaf). These results

Table 1. Oviposition of *Leucoptera coffeella* in normal (N) and erecta (E) plants. Average number of eggs per leaf in leaf pairs of orthotropic (O) and plagiotropic (P) branches according to the first (1), second (2), third (3), fourth (4) or fifth (5) leaf pair (L) from the tip. µ denotes mean of average values. Kruskal-Wallis tests for Oµ p = 0,386 and for Pµ p = 0,043.

Block	Plant type	OL1	OL2	OL3	OL4	OL5	P1L1	P1L2	P1µ	P2L1	P2L2	P2L3	P2µ	P3L1	P3L2	P3L3	P3µ	OLµ	Pµ	OPµ
1	N	4,00	8,00	37,50	-	-	7,75	4,50	6,12	7,00	13,67	24,50	15,06	-	-	-	-	16,50	11,48	12,24
2	N	8,50	5,00	15,50	6,00	-	1,50	-	1,50	3,50	7,25	-	5,37	7,50	1,50	24,50	11,17	8,75	7,62	7,20
3	N	7,00	25,50	29,00	16,50	-	2,30	-	2,30	1,25	9,00	-	5,12	-	-	-	-	19,50	4,18	10,30
4	N	18,50	27,00	49,00	-	-	2,50	-	2,50	0,00	12,50	-	12,50	-	-	-	-	31,50	5,00	16,00
1	E	30,50	25,50	32,00	-	-	15,25	-	15,25	17,00	21,50	-	19,25	-	-	-	-	29,33	17,92	21,83
2	E	20,00	18,00	21,00	11,00	25,00	9,00	-	9,00	7,25	16,50	-	11,87	-	-	-	-	19,00	10,92	13,84
3	E	0,50	26,50	74,00	-	-	2,50	-	2,50	17,75	23,25	-	20,50	5,75	13,00	13,50	10,75	33,66	12,62	15,12
4	E	11,00	29,00	13,50	25,00	13,50	13,50	-	13,50	16,25	13,50	-	14,87	-	-	-	-	18,40	14,42	22,60

RESULTS AND DISCUSSION

Results of the oviposition in normal and erecta plants according to branch types and leaf position are shown in Table 1. A total of 867 eggs were laid in 80 leaves of normal plants (μ = 10,84 eggs/leaf) while in the erecta plants, 1486 were laid in 82 leaves (μ = 18,12) difference statistically significant ($p = 0,046$). Considering only the orthotropic branches, the normal plants presented an average of 19,06 eggs/leaf and erecta 25,10, data not significant ($p = 0,386$). Differences however were observed in the number of eggs per leaf in the plagiotropic branches with normal plants averaging 7,07 eggs/leaf and the erecta ones 13,97, data significant ($p = 0,043$). Although pairwise comparison of oviposition in branches and on correspondent leaves of normal and erecta plants showed that in most of the cases more eggs were laid in erecta plants, the differences were significant only in the second leaf pair of orthotropics and in first, second and third pairs of plagiotropics. Data on fourth and fifth pairs contributed only to overall computations provide lack of correspondence in all plants.

In the field, plants at close spacings usually show reduced levels of leaf-miner infestation as compared to fields with wider spacings. Adult erecta plants due to the upright position of plagiotropic branches have a more compact architecture and one might speculate whether it would show less preference for oviposition of the adults of *Leucoptera coffeella*. The present experiment revealed however an opposite trend, exactly as previously observed in the selection field. This could be due to the preference of the female insects for the erect architecture of the plants *per se* or due to an unknown chemical or morphological attractiveness factor, linked to or pleiotropically conditioned by the erecta genotype. In the case of existing an attractiveness factor it would be expected, as in plagiotropic, unequal oviposition also on leaves of orthotropic branches since leaves of both branches would bear the same putative attractive factor. The fact that oviposition on the leaves of orthotropic branches showed no statistical difference between normal and erecta plants would thus drive the conclusion toward the effect of architecture itself. However, a more detailed analysis of leaf pairs in the orthotropic branches showed that, despite these leaves altogether showed no significant differences, the data relative to the second leaf pair displayed significant higher oviposition rate on erecta plants. The first and third pairs although not significant displayed also higher oviposition rate therefore leaving this matter open to further experimentation. The results indicated also that in both genotypes and branch types oviposition tends to be higher in the second or third leaf pair, spatially positioned in central part of the plants.

ACKNOWLEDGEMENTS

Authors are indebt to Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café for financial support, to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for fellowship to HPMF, to José Márcio da Cruz for providing Minitab Version 14 and to Silvia L. S. Lima for technical assistance.

REFERENCES

Cook, O.F. 1911. Dimorphic branches in tropical crop plants: cotton, coffee, cacao, the Central American rubber tree and the banana. Bulletin of the Bureau of Plant Industry. Nº 198. Washington, US Dept. Agric. 64p.

Cramer, P.J.S. 1913. Gegevens over de variabiliteit van de in Nederlandschindië verbouwde koffie-soorten. G. Kolff & Co. Batavia. 696pp.

Gravena, S. 1983. Táticas de manejo integrado do bicho mineiro do cafeeiro *Perileucoptera coffeella* (Guérin-Meneville, 1842). Anais da Sociedade Entomológica do Brasil 12(1):61-73.

Gravena, S. 1984. Estratégias de manejo integrado do bicho mineiro do cafeeiro *Perileucoptera coffeella* (Guérin-Meneville, 1842). Anais da Sociedade Entomológica do Brasil 13 (1):117-129.

Guerreiro-Filho, O. 2006. Coffee leaf-miner resistance. Braz. J. Plant Physiol. 18:109-117.

Krug, C.A., Carvalho, A. 1951. The genetics of *Coffea*. Adv. Genet. 4:127-158.

Krug, C.A., Mendes, J.E.T., Carvalho, A. 1939. Taxonomia de *Coffea arabica* L. Descrição das variedades e formas encontradas no Estado de São Paulo. Boletim Técnico, 62, Campinas, Instituto Agronômico, 57p.

Medina-Filho, H.P., Bordignon, R., Fazuoli, L.C., Gallo, P.B. 2001. Possibilities of modifying height, architecture and cup quality of arabica coffee by monitoring the segregation of Caturra (*Ct*), Erecta (*Er*) and Mokka (*Mo*) alleles. 19th International Conference on Coffee Science. Trieste, Italy 14-18 May. CD-ROM. 10p.

Medina-Filho, H.P., Carvalho, A., Sondahl, M.R., Fazuoli, L.C., Costa, W.M. 1984. Coffee breeding and related evolutionary aspects. p. 157-193. In: J. Janick (ed.) Plant Breeding Reviews. Vol. 2. AVI Publish. Co. Westport.

Nantes, J.F.D, Parra, J.R.P. 1978. Influência da alimentação sobre a biologia de *Perileucoptera coffeella* (Guérin-Méneville, 1842) (Lepidoptera-Lyonetiidae). Cientifica 6:263-268.

Sokal, R.R., Rohlf, F.J. 1969. Biometry. The principles and practice of statistics in biological research. W.H Freeman and Co., San Francisco. 776pp.

The Reduced Infestation of Leaf-miner in Mokka Variety of Coffee and the Implication on its Origin and Breeding

R. BORDIGNON, O. GUERREIRO-FILHO, D. A. RAMIRO, H. P. MEDINA-FILHO

Centro de Café Alcides Carvalho, Instituto Agronômico (IAC), Av. Barão de Itapura, 1481, 13020-902, Campinas, SP, Brazil. E-mail: rita@iac.sp.gov.br

SUMMARY

Early investigations on the rate of leaf miner (*Leucoptera coffeella*) infestation in a wide collection of *Coffea arabica* of IAC under field conditions in Brazil have indicated reduced attack in the leaves of the botanical variety mokka and its derived Ibairi cultivar. Further studies in the lab with no-choice tests of leaf discs showed that the average area damaged by one caterpillar in cv. Ibairi is similar to the observed in other standard *C. arabica* cultivars. From a cross of Ibairi (*mo mo*) with Catuai (*Mo Mo*), leaves representing 10 F_3 progenies randomly chosen *mo mo* and 10 *Mo Mo* were tested for oviposition in confinement conditions in the lab. The experiment was performed in two levels of infestation. Oviposition occurred 2,2 times more intensely ($F = 11,48$; $p = 0,008$) in the normal *Mo Mo* plants under high infestation pressure (107,2 eggs/leaf) and 1,8 times ($F = 9,96$; $p = 0,012$) under low infestation pressure (14,4 eggs/leaf). The results indicate that the low rates of infestation of Ibairi cultivar is at least partially explained by differential preference for oviposition of the adult insect with reduced egg laying on its leaves. Although plants with such resistance stand out in mixed selections plots, their exploitation in breeding programs is obviously questionable in present homogenous cultivar based cropping. The uniqueness of such resistance of mokka in *C. arabica*, its fine beverage profile coupled with associated phenotypes that occasionally dissociate in segregant generations drive the speculation on mokka genotype be conditioned by a gene block introgressed from *C. eugenioides*.

INTRODUCTION

Moca, Mocha, Mokha, Moka or Mokka coffee has been known for long time in Yemen, Southern Arabia for its ancient cultivation, adaptation to local conditions and for its reputed fine beverage quality. It has been mentioned by Cramer (1913), Mc Clelland (1924), Choussy (1928), and described in detail by Krug et al. (1939) as *Coffea arabica* L. var. *mokka* Hort. ex Cramer. In the Reunion Island it has been also cultivated (Raoul, 1897) and from there, introduced in Brazil where it was subject to genetic analysis by Krug and Carvalho (1951). Accordingly, the original mokka was homozygous for two different pairs of factors: laurina (*lr lr*), recessive and conditioning low caffeine content and mokka (*mo mo*) with incomplete dominance conditioning small rounded fruits and beans (Figure 1) in addition to several associated characteristics. The two genes were separated out by segregation and *mo mo* was introduced to Bourbon originating the Ibairi cultivar (Carvalho et al., 1990). Due to its superior quality, cv. Ibairi has been used in a breeding program aiming at the association of *mo mo* genotype with caturra (*Ct*) and erecta (*Er*) (Medina-Filho et al., 2001) in order to develop lines of improved quality and compact architecture. An early investigation (Medina-Filho et al., 1977) indicated that mokka plants (*mo mo*) displayed reduced infestation of leaf-miner (*Leucoptera coffeella* Guérin-Méneville, Lepidoptera-Lyonetiidae) in field conditions.

Among several cultivars in that study and latter investigated in the whole germplasm of arabica collection at Campinas, mokka was the only *C. arabica* with reduced infestation in the field. In the course of the aforementioned breeding program, the low rates of infestation were again observed in some plants (Figure 1). However, in lab conditions with leaf discs in no-choice tests, Guerreiro-Filho (1987) has shown that the damaged area by one caterpillar in cv. Ibairi is similar to the observed in other susceptible cultivars. The present investigation had the objective of investigating the oviposition of adult insects in *mo mo* genotype comparatively to the correspondent normal *Mo Mo*.

Figure 1. From left to right: Cage used for oviposition experiments. Eggs of leaf-miner oviposited on the adaxial surface of coffee leaf. Recombinant plant *mo mo* (mokka) and caturra (*Ct Ct*) displaying reduced infestation in field condition. Fruits and beans of normal plants (above) and mokka (below).

MATERIAL AND METHODS

Ten randomly chosen individuals of genotype *mo mo* (mokka) and 10 *Mo Mo* (normal) from F_3 progenies originated from different F_2 plants of a single *Mo mo* F1 hybrid of Ibairi (*mo mo*) x Catuaí (*Mo Mo*) were selected to be tested as to oviposition of adult insects of leaf-miner. Insects were raised in confinement at 27 ± 3 °C, 80% relative humidity (Guerreiro-Filho et al., 1992) supplemented with 10% sucrose solution (Nantes and Parra, 1978). One leaf of each eighteen-month old plant was removed and exposed overnight to oviposition of insects inside a cage (Figure 1). Eggs laid on the upper surface of leaves (Figure 1) were recorded observing the leaves under a 100X magnifier. The experiment was set up in randomized block design with 10 replicates and performed twice, first with high then with low infestation intensity in the cage. Data of oviposition in leaves of *mo mo* and *Mo Mo* plants were subjected to Analysis of Variance (Little and Hills, 1978) using Minitab software version 14.

RESULTS AND DISCUSSION

Data of oviposition on leaves of normal and mokka plants are shown in Table 1. As in some blocks the number of eggs was less than ten, chiefly in the low infestation experiment including one with no eggs at all, recorded counts were transformed to $\sqrt{x + 0,5}$ before the analysis of variance. In the experiment with high level of infestation ($F = 11,48$; $p = 0,008$), oviposition (Figure 1) occurred 2,2 times more intensively on the normal (*Mo Mo*) (μ=107,2 eggs/leaf) than in mokka (*mo mo*) leaves (μ = 49,0 eggs/leaf). In the low infestation experiment ($F = 9,96$; $p = 0,012$) comparable results were obtained with higher rate (14,4 eggs/leaf) of oviposition on the normal as compared to mokka (8,2 eggs/leaf). These results

showed a clear preference of the insect to oviposite on leaves of normal genotype (*Mo Mo*) as contrasted to mokka ones (*mo mo*) and indicate that the lower infestation of mokka plants observed in the field is at least partially explained by a reduced oviposition of the insects on its leaves.

Table 1. Number of eggs/leaf in normal (*Mo Mo*) and mokka (*mo mo*) leaves in the experiment (F = 11,48; p = 0,008) with high and low (F = 9,96; p = 0,012) infestation of leaf-miner (*Leucoptera coffeella*). μ = average data.

	Block										
Experiment	1	2	3	4	5	6	7	8	9	10	μ
High											
Mo Mo	141	63	161	90	53	80	74	99	152	159	107,2
mo mo	73	36	25	83	15	21	141	34	27	35	49,0
Low											
Mo Mo	16	17	12	20	7	9	15	11	27	10	14,4
Mo mo	8	1	18	13	0	4	4	14	16	4	8,2

Brazil as the leading coffee exporter in the world has its production based on Arabicas (*Coffea arabica*) and Robustas (*C. canephora*). Both species regularly suffer the attack of leaf-miner, a main pest of the crop that must be biologically and chemically controlled (Souza et al., 1998; Scarpellini, 2004). As reviewed by Guerreiro-Filho (2006), several types of resistance occur among cultivated and wild forms of *Coffea* in addition to other genetic determined phenotypes that influence the rate of infestation of leaf-miner. Antibiosis, generally defined as a resistance mechanism that causes an adverse effect in any phase of the biology of the insect is found in many coffee species and the effect can vary from punctual lesions in the palisade parenquima of the leaves as seen in *C. stenophylla* or reduced tunneling in *C. racemosa* and its hybrids with *C. arabica* to increased larval mortality in *C. vatovavyensis* or even extended duration of the insect life cicle as seen in *C. tetragona*. In *C. resinosa* and *C. farafaganensis* larvae are unable to evolve to pupae. Varied types of antibiosis occur in *C. salvatrix*, *C. kapakata*, *C. eugenioides*, *C. dewevrei*, *C. liberica* as well as in a large group of Madagascar and Mascareine Islands native species such as *C. sessilifolia* and *C. humilis*. This type of resistance is the most promising to be exploited in breeding programs, not only because its primary type of action but also because most of such germplasm resources are assigned to the secondary gene pools of *C. arabica* and *C. canephora* (Medina-Filho et al., 2006). Antixenosis, usually defined as a host interference on the behavior of the insect can be frequently observed as a reaction of a weakened preference of the female for oviposition in certain genotypes. This is the case of *C. congensis* and Catuai as compared to Obatã, Tupi and Icatu cultivars of *C. arabica* (Gonçalves, 1986). *C. congensis* besides reduced oviposition presents also antibiosis expressed by diminished tunneling of the leaves by the caterpillars. As to breeding exploitation, antixenosis mechanisms are not attractive considering that usual cropping is conducted in no-choice conditions since regular fields are established with single cultivars or at least large plots of them. It is likely that the antixenosis expressed as reduced preference for oviposition of leaf-miner on mokka genotype of *C. arabica* has limited application in breeding programs. Therefore, leaf-miner resistance programs of *C. arabica* would continue to rely on antibiosis as the present introgression breeding of Lm_1 and Lm_2 from *C. racemosa* (Guerreiro-Filho et al., 1999). The reduced level of infestation observed in mokka is a unique occurrence among the vast *C. arabica* collection of cultivars, accessions from Ethiopia, mutants, forms and variations surveyed in the Germplasm Bank at Campinas. Interesting enough, mokka genotype is characterized by other accompanying phenotypes that are also unique among *C. arabica* such as small leaves with

large domatias, slender branched twigs with shortened internodes and a fine cup quality but those are common to *C. eugenioides*. This latter species is also resistant to leaf-miner and has a fine cup quality. On the basis of the above considerations it would be plausible to speculate whether mokka is a natural introgression of *C. eugenioides* into *C. arabica*. In the F_2 of mokka x normal plants analyzed in detail as to the several characteristics that compose the mokka phenotype, Medina-Filho et al. (2001) had observed that small pointed leaves, round fruits and beans, large domatias and short internodes among others can be occasionally dissociated by segregation, what reinforces the above speculation. Molecular analysis however did not indicate any unique marker associated with mokka (Anthony et al., 2002; Steiger et al., 2002).

ACKNOWLEDGEMENTS

Authors are indebt to Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café for financial support, to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for fellowship to HPMF, to José Márcio da Cruz for providing Minitab Version 14 and to Silvia L. S. Lima for technical assistance.

REFERENCES

Anthony, F., Combes, M.C., Astorga, C. and Bertrand, B., Graziosi, G., Lashermes, P. 2002. The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. Theor. Appl. Gen. 104:894-900.

Carvalho, A., Medina-Filho, O., Fazuoli, L.C. 1990. Recombinação genética derivada do café mokka com boa qualidade de bebida. Anais do 16th Congresso Brasileiro de Pesquisas Cafeeiras. Espírito Santo do Pinhal, Brazil 6-9 November. p. 57-58.

Choussy, F. 1928. El café. San Salvador. (2nd edition, 1935).

Cramer, P.J.S. 1913. Gegevens over de variabiliteit van de in Nederlandschindië verbouwde koffie-soorten. G. Kolff & Co. Batavia. 696pp.

Gonçalves, W. 1986. Resistência de cafeeiros (*Coffea* spp.) à raça 3 de *Meloidogyne incognita* (Tylenchida - Meloidogyne) e a *Perileucoptera coffella* (Lepidoptera - Lyonetiidae). Tese de Mestrado em Agronomia. Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Jaboticabal. 71p.

Guerreiro Filho, O. 1987. Avaliação em laboratório da resistência da variedade Mokka de *C. arabica* ao bicho mineiro. Anais do 14th Congresso Brasileiro de Pesquisas Cafeeiras, Campinas, Brazil, p.107-108

Guerreiro-Filho, O. 2006. Coffee leaf-miner resistance. Braz. J. Plant Physiol. 18:109-117.

Guerreiro-Filho, O., Medina-Filho, H.P., Carvalho, A. 1992. Método de laboratório para avaliação da resistência genética de *Coffea* spp. a *Perileucoptera coffeella*. Turrialba 42(3): 348-358.

Guerreiro-Filho, O., Silvarolla, M.B. and Eskes, A.B. 1999. Expression and mode of inheritance of resistance in coffee to leaf miner *Perileucoptera coffeella*. Euphytica 105:7-15.

Krug, C.A., Carvalho, A. 1951. The genetics of *Coffea*. Adv. Genet. 4:127-158.

Krug, C.A., Mendes, J.E.T., Carvalho, A. 1939. Taxonomia de *Coffea arabica* L. Descrição das variedades e formas encontradas no Estado de São Paulo. Boletim Técnico, 62, Campinas, Instituto Agronômico, 57p.

Little, T.M., Hills, F.J. 1978. Agricultural experimentation. Design and analysis. John Wiley & Sons, Inc. 350p.

Mc Clelland, T.B. 1924. Coffee varieties in Porto Rico. Boletim Porto Rico Agric. Exp. Station, 30.

Medina-Filho, H.P., Bordignon, R., Fazuoli, L.C., Gallo, P.B. 2001. Possibilities of modifying height, architecture and cup quality of arabica coffee by monitoring the segregation of Caturra (*Ct*), Erecta (*Er*) and Mokka (*Mo*) alleles. 19th International Conference on Coffee Science. Trieste, Italy 14-18 May. CD-ROM. 10p.

Medina-Filho, H.P., Carvalho, A., Mônaco, L.C. 1977. Melhoramento do cafeeiro XXXVII – Observações sobre a resistência do cafeeiro ao bicho-mineiro. Bragantia 36:131-137.

Medina-Filho H.P., Maluf, M.P., Bordignon, R., Guerreiro-Filho, O., Fazuoli, L.C. 2006. Traditional breeding and modern genomics: Complementary tools to exploit biodiversity for the benefit of the coffee agroindustrial chain. Acta Horticulturae (in press).

Nantes, J.F.D, Parra, J.R.P. 1978. Influência da alimentação sobre a biologia de *Perileucoptera coffeella* (Guérin-Méneville, 1842) (Lepidoptera-Lyonetiidae). Cientifica 6:263-268.

Raoul, E. 1897. Culture du caféier. 10ª ed. A. Chalamel, Paris. p. 237-238.

Scarpellini, J.R.. 2004. Controle conjunto de cigarras, broca, bicho-mineiro e ferrugem do cafeeiro. Anais da X Reunião Itinerante de Fitossanidade do Instituto Biológico. Mococa, Brazil 19 October. p. 114-128.

Souza, J.C., Reis, P.R., Rigitano, R.L. 1998. O bicho-mineiro do cafeeiro: biologia, danos e manejo integrado. Boletim Técnico, 54, EPAMIG, 48p.

Steiger, D.L., Nagai, C., Moore, P.H., Morden, C.W., Osgood, R.V. and Ming, R. 2002. ALFP analysis of genetic diversity within and among *Coffea arabica* cultivars. Theor. Appl. Genet. 105:209-215.

Life Tables of *Hypothenemus hampei* (Ferrari) on Three Coffee Accessions

J. V. ROMERO[1], G. H. CORTINA[2]

National Coffee Research Center -CENICAFE-, Chinchiná, Caldas, Colombia
[1]I.A. E-mail: JuanVicente.Romero@cafedecolombia.com
[2]I.A. M. Sc. E-mail: Hernando.Cortina@cafedecolombia.com

SUMMARY

To find sources of resistance to coffee berry borer (*Hypothenemus hampei* (Ferrari)), the life table (26 ºC ± 1; 75% ± 5 H.R) on three coffee accessions was studied. Two assays were performed: On the first one, development and survival were evaluated. The evaluated accessions were *Coffea liberica* Bull ex Hiern, *Coffea arabica* L. CCC 534 and Caturra (susceptible variety). On the second assay, the adult's survival and fecundity were evaluated. The duration of the cycle for the three accessions was similar: 20 days. The accumulated ovipositions were adjusted to quadratic functions, the confidence intervals (p = 95%), showed significant differences among the introductions and the control: CCC 534 32 ± 2; *C. liberica* 28± 2 and Caturra 42 ± 2 until 28th day. The survival until 72 days (between 58% and 63%), which did not present significant differences. For the net reproductive rate (R_o), significant differences were found among Caturra (25 ± 1 eggs), and the accessions CCC 534 (18 ± 2), and *C. liberica* (16 ± 2); in the intrinsic rate of increase (r) (0.085 ± 0.001, 0.078 ± 0.002, 0.070 ± 0.003 respectively), and the duplication time too (8 ± 0.1, 9 ± 0.3, 10 ± 0.4 days). For the generation time between Caturra and *C. liberica* significant differences were found (38±0.3, 40 ± 0.6 respectively).
Key words: coffee germplasm, resistance to insects, antibiosis

INTRODUCTION

Colombia is the largest producer of washed coffees in the world, there are 2.1 million acres planted with coffee, with 563.000 families depending totally on it. The coffee berry borer (CBB) is the most destructive insect pest of coffee crop, it damage the production of more than 1.8 million acres. A fertilized female leaves the infested berry and colonizes a new one. Its life cycle occurs totally inside the bean, making its control very difficult, causing damage and decreasing the quality of the beverage and of the production. The control methods used in an integrated pest management (IPM) program, include: chemistry and biological control and mainly cultural control (continuous harvesting of ripe berries and picking up berries that have fallen).

The development of resistant crop cultivars has been considered an important component for sustainable management of CBB, because it is economically feasible, environmentally friendly and compatible with the existing IPM program. Romero and Cortina (2004), evaluated 19 accessions (18 *C. arabica* of ethiopian origin and *C. liberica*), and found 4 of them showing significant less *H. hampei* progeny than Caturra (commercial arabica variety). In this research were characterized the effects of two of these out-standing accessions and Caturra as susceptible control. Survival curves, fecundity life tables and demographic parameters of *H. hampei* were estimated.

MATERIALS AND METHODS

On the National Coffee Research Center (Cenicafé), in a room with 26 ºC ± 1, 75 % ± 5 of relative humidity, three accessions of *Coffea* genus: *C. liberica* Bull ex Hiern, *Coffea arabica* L. CCC 534 (accession collected by 1964-1965 FAO mission; Meyer et al., 1968), and Caturra (susceptible variety) were evaluated. Two experiments were performed:

- **Juvenile's Survival and Development**: Three cohorts of 400 eggs were established, being two eggs by each bean the sampling unit. Every 4 days during 40 (10 evaluations), 20 beans by accession were dissected; CBB's survival (***lx***) and his development stages were determined.
- **Adult's Survival and Fecundity**: Three cohorts of 360 females by accession were established (rearing in the same ones). Every 4 days, 40 sampling units by accession were taken up randomly, during 9 evaluations. The CBB's survival and number of eggs by female were registered.

The function of accumulated oviposition through time and the confidence intervals for the average were estimated. In the last evaluation 36 days after infestation (DAI), the sex ratio was calculated according to the number of adults. For this analysis SAS V 8.0 statistical program was used.

With the population survival (l_X) and net fecundity (M_X), at every age (x), the fecundity life table and the following demographic parameters were calculated: Net reproductive rate (R_0), Intrinsic rate of increase (r), Generation time (T), Doubling time (DT), (Andrewartha and Birch, 1954). To estimate the parameters variation and confidence limits a program developed by Maia et al. (2000), based on the Jackknife resampling technique, was used. The significant differences of these parameters were analyzed with Student *t*-test.

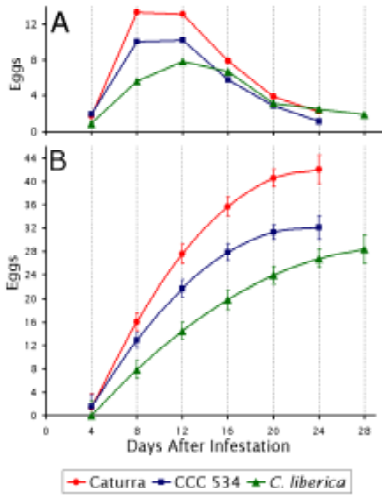
RESULTS AND DISCUSSION

Development

The CBB life cycle was 20 days, similar on CCC 534, *C. liberica* and Caturra. The development times were: egg 4 days, first larval instar 4, second instar 6, prepupae 2 and pupae 4 days approximately. These findings agree with those found by Bergamin (1943), Borbon (1989), and Montoya (1993), under similar conditions.

Oviposition

The maximum oviposition appeared between 8 and 12 DAI in CCC 534 and Caturra, whereas in *C. liberica* it was later, on day 12th (Figure 1A). The accumulated ovipositions were adjusted to quadratic functions (Figure 1B). The confidence intervals (p = 95%), showed significant differences among the accessions and the control: CCC 534 32 ± 2; *C. liberica* 28 ± 2 and Caturra 42 ± 2. The accumulated oviposition on accessions was smaller than on Caturra confirming the differences obtained in previous evaluations (Romero and Cortina, 2004).



Caturra E(t) = - 0.104 t^2 + 4.943 t - 16.825 R^2: 0.998
CCC 534 E(t) = - 0.082 t^2 + 3.849 t - 12.562 R^2: 0.998
C. liberica E(t) = - 0.039 t^2 + 2.429 t - 9.054 R^2: 0.994

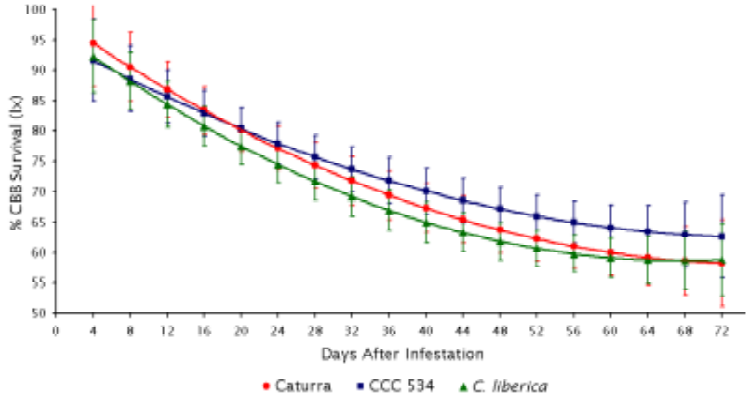
Figure 1. Female's oviposition of *H. hampei* reared in three accessions. A. Oviposition registered each 4 days. B. Accumulated oviposition, vertical lines represent confidence intervals for the average (p = 0.95).

Sex ratio

The CBB populations reared in Caturra, CCC 534 and *C. liberica* showed the following sex ratio: 9.2, 9.8 and 9.8 females by each male respectively, similar to those found by Bergamin (1943) and Montoya (1992).

Survival

For the three accessions, survival curves were adjusted to quadratic functions, with the highest mortality in immature stages. However, there were not significant differences among Caturra and the two accessions (Figure 2).



Caturra S(t)= 0,0069 t^2 – 1,0590 t + 98,604 R^2= 0.846
CCC 534 S(t)= 0,0053 t^2 – 0,8337 t + 94,901 R^2= 0.758
C. liberica S(t)= 0,0083 t^2 – 1,1259 t + 96,694 R^2= 0.799

Figure 2. Survival of *H. hampei* up to 72 days on three accessions.

Demographic parameters

Figure 3 shows the significant differences found on R_0 among Caturra's populations and the accessions CCC 534 and *C. liberica* (it was smaller in 27% and 37% respectively). ***T*** was similar between Caturra and CCC 534 (37.8, 37.5 days) and two days longer in *C. liberica*, due to the longer oviposition period. The maximum potential of increase (r_m), of CBB population was expressed on Caturra, while CCC 534 and *C. liberica* showed reduction of the expression of this potential. As consequence, the ***DT*** was different in each of the accessions.

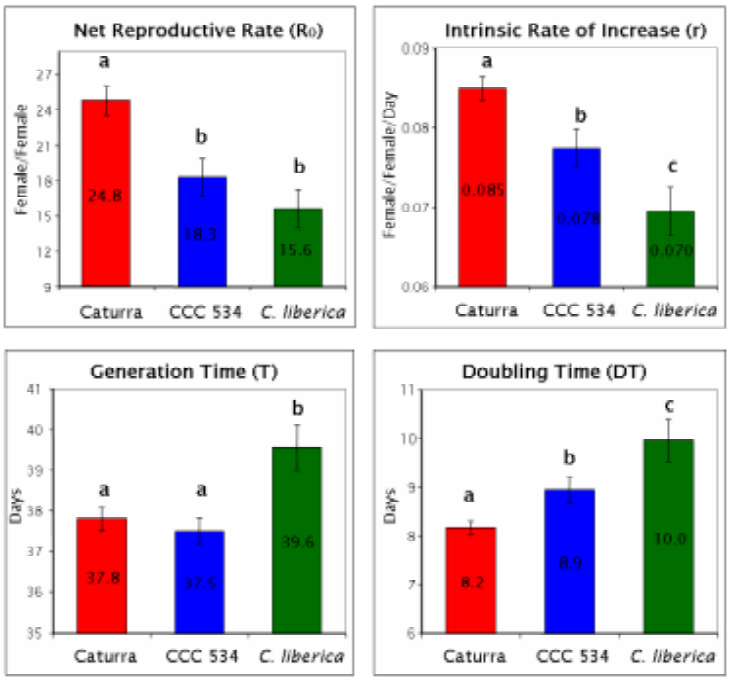


Figure 3. Life table parameters of *H. hampei* reared in three accessions. Average inside the blocks and vertical lines confidence intervals (p = 0.95). Variances estimated based on the Jackknife subsampling approach. Blocks with different letters are significantly different (Student *t*-test: P > 0.05).

CONCLUSIONS

- On CCC 534 and *C. liberica* the CBB oviposited 28% and 32% less than those reared on Caturra respectively.
- The net reproductive rate and intrinsic rate of increase of CBB were smaller on accessions CCC 534 and *C. liberica* than on Caturra, due to fewer oviposition.
- Percentage of survival and life cycle duration did not show significant differences among the three accessions.

REFERENCES

Andrewartha H. G., Birch L. C. 1954. The distribution and abundance of animals. University of Chicago press. P. 31-54.

Bergamin, J. 1943. Contribuicao para o cohnecimento da biología da broca do café *Hypothenemus hampei* (Ferrari 1867). Arquivos do Instituto Biológico, Brazil. 14: 31-72.

Borbon, M.O. 1989. Bioecologie d'un Ravageur des Baies de Cafeier, *Hypothenemus hampei* Ferr (Col:Scol) et de ses parasitoides au Togo. France. L'Universite Paul Sabatier de Toulouse. 185 p.

Maia Ade H, Luiz A J, Campanhola C. 2000. Statistical inference on associated fertility life table parameters using jackknife technique: computational aspects. Journal Economic Entomology. 93(2):511-518.

Meyer, F. 1968. FAO Coffee mission to Ethiopia 1964-1965. Italy, FAO. 200 p.

Montoya O., S.A. 1993. Ciclo de vida de la broca del café (*Hypothenemus hampei* (Ferrari)) sobre frutos en diferentes estados de desarrollo. Colombia. Universidad Nacional de Colombia, 77p.

Romero, J.V., Cortina G., H. 2004. Fecundidad y Ciclo de Vida de la broca *Hypothenemus hampei* F. (Coleoptera: Curculionidae: Scolytinae) en introducciones silvestres de café. Cenicafé 55(3): 221-231.

Population Density of *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae) and Its Effect on Coffee Berry Borer in Tachira, Venezuela

A. N. TORRES[1] AND Y. A. AMADOR[2]

[1]Instituto Nacional de Investigaciones Agrícolas
[2]Universidad del Táchira

SUMMARY

The effect of ***Cephalonomia stephanoderis*** (Hymenoptera: Bethylidae) on coffee berry borer (Coleóptera: Curculionidae, Scolitynae) was evaluated in a coffee plantation located at the INIA Experimental Farm in Bramón, Junín County, Táchira, Venezuela. Four different population densities of the parasitoid referred to proportions of the little wasp to borer infested berries were introduced into cages made of organdi which enclosed coffee branches containing ripe borer infested berries. Treatments were 1:2, 1:1, 3:2, 2:1 and a control having no parasitoids with four replicates. Twenty borer infested berries in a ripe stage were used in each cage. Thirty days after the parasitoids were released into the cages the parasitism percentage was estimated by counting the different stages of the pest and the parasitoid present inside the dissected berries. The parasitism percentage depends on the number of released wasps. Also, it was observed in this experiment that the Ivory Coast wasp caused a great impact in all the coffee berry borer stages present inside the visited berries. In addition to its parasitic activity on 2nd stadium larvae and pupae, this parasitoid also predates eggs and 1st stadium larvae, and it is capable of killing adults. It was demonstrated, in this work, that ***C. stephanoderis*** is an excellent parasitoid that can be used in a coffee berry borer integrated management program.
Key-words*:* Parasitic wasps, biological control, IPM

INTRODUCTION

Since the coffee berry borer, *Hypothenemus hampei* (Ferrari), appeared in Venezuela, the crop has had lowered its yields and quality. This insect destroys berries and allows the entrance of pathogens that accelerates the grain deterioration. To manage the pest, several methods can be used, cultural, chemical, and biological encompassed controls can be combined into an integrated pest management program. Within among the biological control alternatives, parasitoids and pathogens are the most frequently used. Among the parasitoids, ***Cephalonomia stephanoderis*** is one the most versatile. Previous experiments have shown significant mortality of eggs, larvae, pupae and adults of the coffee berry borer caused by this parasitoid, both by predation and parasitism. Because the coffee berry borer is the main pest of coffee in Venezuela, alternative control methods are needed, and this parasitoid has a good potential for controlling infested berries that remain after harvesting. The coffee berry borer stays in those berries until new berries are ready to be infested at the following harvest.

OBJECTIVES

The main objective of this work was to evaluate the effect of different population densities of the Ivory Coast little wasp for controlling the coffee berry borer under field conditions.

MATERIALS AND METHODS

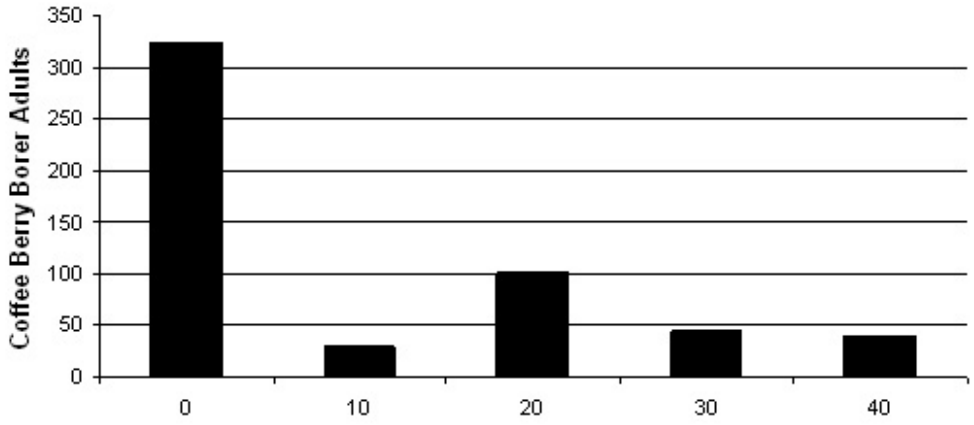
Four different parasitoid population densities and a control having no parasitoids were evaluated using a complete randomized design with four replicates. The populations evaluated were 10, 20, 30, and 40 wasps released inside cages made of organdi, wich, enclosed coffee branches containing 20 borer-infested ripe berries. The experiment was established in a coffee plantation located at the Instituto Nacional de Investigaciones Agrícolas Research farm in Bramón, Táchira, Venezuela. Berries were harvested 15 days after the parasitoids were released into the cages, peeled and stored in glass containers. Fifteen days later the parasitism percentage was estimated by counting the different stages of the pest and the parasitoid present inside the dissected berries. Polynomial regression analyses were performed to determine the model that best explains the effect of five different population densities of the parasitoid on coffee berry borer, either by parasitism or predation.

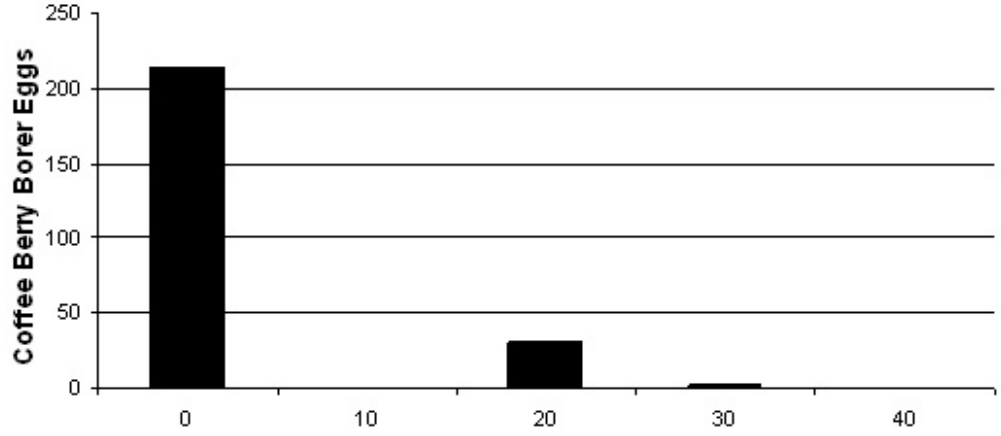
RESULTS

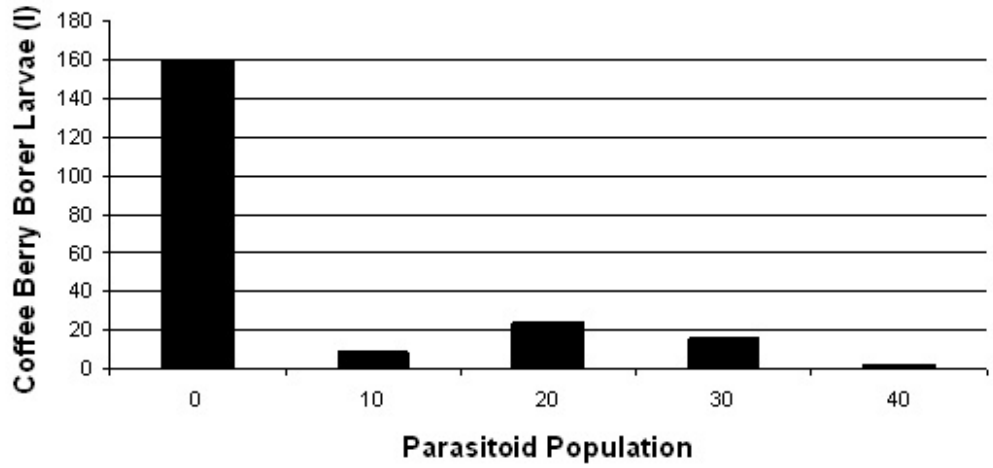
A clear pattern of predation on coffee berry borer eggs and first stadium larvae was observed as the population level of the Ivory Coast little wasp increased. However, the relationship between predation and parasitoid density was not linear (Table 1, Figure 1). Likewise, the parasitism of second stadium larvae and pupae increased as the number of wasps increased. The relationship between parasitism and the parasitoid population density also was not linear (Table 1, Figure 1). Coffee berry borer adults were killed significantly by the wasp, although the relationship between adult mortality and number of wasps was not linear (Table 1, Figure 1).

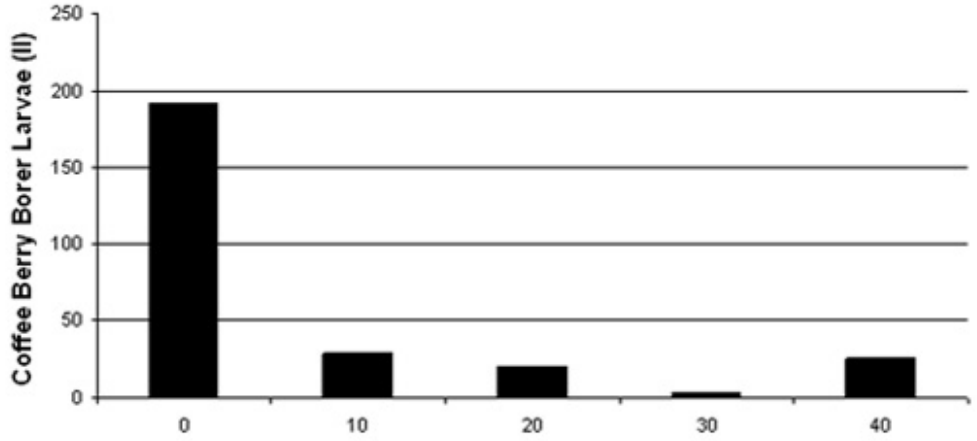
Table 1. Polynomial regression analysis to test the effect of five different population densities of the Ivory Coast little wasp on coffee berry borer adults mortality, eggs predation, first stadium larvae predation, second stadium larvae parasitism, and pupae parasitism, 39 days after releasing the parasitoids

Parasitoid Effect on	Polynomial Model	df	F	P
Coffee berry borer adults	Linear	1	52.24	<0.0001
	Cubic	3	34.47	<0.0001
Coffee berry borer eggs	Linear	1	96.91	<0.0001
	Cubic	3	70.83	<0.0001
Coffee berry borer larvae (I)	Linear	1	59.06	<0.0001
	Cubic	3	39.46	<0.0001
Coffee berry borer larvae (II)	Linear	1	66.59	<0.0001
	Cubic	3	47.27	<0.0001
Coffee berry borer pupae	Linear	1	56.58	<0.0001
	Cubic	3	34.93	<0.0001


Coffee Berry Borer Adults
350
300
250
200
150
100
50
0
0
10
20
30
40


Coffee Berry Borer Eggs
250
200
150
100
50
0
0
10
20
30
40


Coffee Berry Borer Larvae (I)
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
Parasitoid Population


Coffee Berry Borer Larvae (II)
250
200
150
100
50
0
0
10
20
30
40

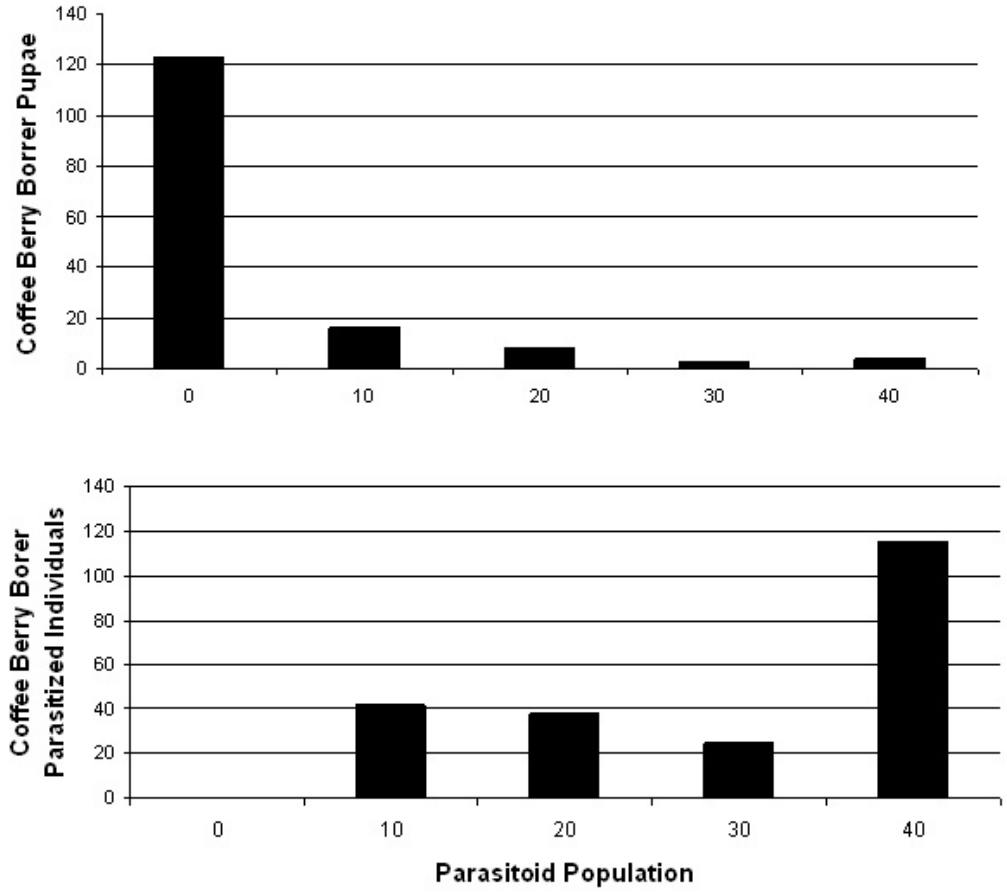


Figure 1. Total number of live coffee berry borer adults, eggs, first and second stadium larvae, pupae, and total number of parasitized individuals counted inside the berries, 39 days after the parasitoid was released into the cages.

CONCLUSIONS

It was demonstrated that Cephalonomia stephanoderis is an excellent biological control agent against coffee berry borer. This little wasp predates eggs and first stadium larvae, parasitizes second stadium larvae, prepupae and pupa and, if it not were enough, also kills coffee berry borer adults. This wasp may be used in an IPM program after the harvest to reduce the coffee berry borer population that remains inside the berries on the plants or soil, thus lowering the population of borers in the next harvest.

Screening Populations of Arabica Coffee for Molecular Markers Linked to Coffee Berry Disease Resistance

C. O. OMONDI[1], F. PINARD[2]

[1]Coffee Research Foundation (CRF), P O Box 4, Ruiru 00232, Kenya
[2]Centre de coopération Internationale en Recherche Agronomique pour le Développement, (CIRAD-CP), Nairobi, Kenya

SUMMARY

Five Arabica coffee varieties (Hibrido de Timor, Pretoria, Rume Sudan, K7 and Catimor) with known resistance to Coffee Berry Disease (CBD) and one susceptible variety (SL 28) were subjected to microsattelite (Single Sequence Repeat-SSR) analysis using 7 primers. Four primers detected polymorphism between resistant and susceptible parents. The four primers were used to analyse a segregating population of F2 plants derived from a cross between Rume Sudan x SL 28 to identify markers that co-segregate with resistance/susceptibility. A significant finding of this study was revealed by primer M24 which indicated that one of the resistance genes carried by Rume Sudan is closely linked to a 180 bp-size fragment, which can be used as a marker for resistance. This paper discusses the development of marker-assisted selection (MAS) for resistance to CBD.

INTRODUCTION

Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* is the single most important disease affecting Arabica coffee production in the high altitude coffee growing zones in Africa. Breeding and selection for resistant varieties provides a sustainable approach to the management of CBD. Efficient and reliable disease screening methods are required for successful variety development. Molecular markers linked to resistance provide the potential to screen for resistance in a large population of plants at any stage of plant development. Where several genes confer resistance, markers have the advantage over morphological assessment because plants carrying multiple resistance (broad based resistance) can easily be differentiated from those carrying single gene (narrow based) resistance. The objective of this study was to screen for molecular markers linked to CBD resistance and develop a marker assisted selection (MAS) strategy for broad- based multiple resistance.

MATERIALS AND METHODS

Five Arabica coffee varieties (Hibrido de Timor, Pretoria, Rume Sudan, K7 and Catimor) with known resistance to CBD and one susceptible variety (SL 28) were subjected to microsattelite (Single Sequence Repeat – SSR) analysis using 7 primers obtained from IRD in Montpellier, France. Table 1 indicates the primer sequence, repeat motifs and PCR product sizes as described by Combes et al. (2000).

DNA was extracted from lyophilised leaves using a modified cetyltriammoniumbromide (CTAB) protocol where CTAB was replaced by mixedalkyltriammoniumbromide (MATAB). DNA concentration in the preparation was determined by running the samples in 1% agarose gel alongside a lambda standard with known concentration of DNA fragments for comparison and quantification of samples. Polymerase Chain Reaction (PCR) was conducted in 25 ml

volume containing a final concentration of 50 ng of genomic DNA, 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 0.2 pmol of each primer, 0.2 mM of each dNTP and 1 U of Taq DNA polymerase. Reactions were performed in a programmable thermocycler (Flexigene). Amplification conditions were set with an initial denaturation step at 94 °C for 45s, 1 min primer annealing at 60 °C with decreasing temperature of 1 degree at each cycle, and 1 min 30 sec elongation at 72 °C. Then, 30 cycles of 45 sec at 90 °C, 1 min at 55 °C and 1 min 30 sec at 72 °C were performed and followed by a final 8 min elongation at 72 °C. The PCR products were electrophoresed on a 2.3% agarose gel in 1X TBE (Tris-Borate-EDTA buffer). The gel was stained in ethidium bromide, visualized and photographed under UV light 260 nm. Lambda preparation and 100 bp ladder were used as standards for estimating the size of the DNA fragments separated by electrophoresis.

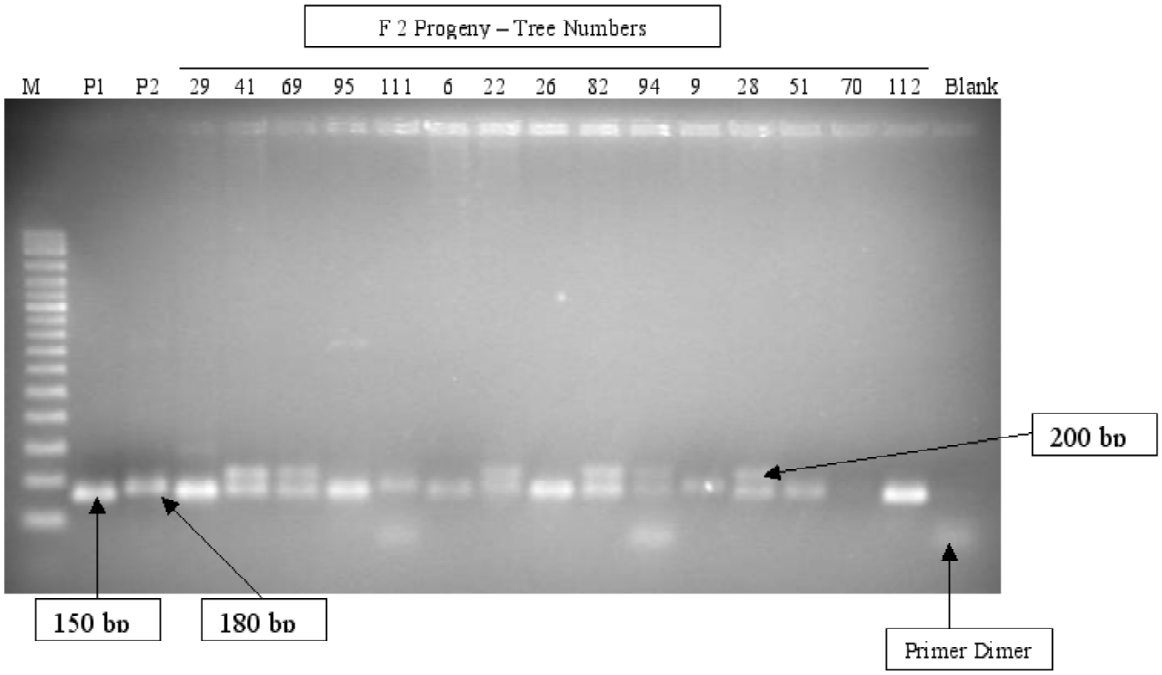
Table 1. Oligonucleotide primer sequences, repeat motifs and PCR product sizes of 7 micosatellite loci isolated from *Coffea arabica.*

Locus	Repeat Motif	PCR primer sequence (5' → 3')	Product size (bp)
M2a	$(GT)_8/(GT)_6/(GT)_7$	F: AGTGGTAAAAGCCGTTGGTG R: GCGGTTGTTGGTGAGTTGAA	205-218
M3	$(CA)_6/(CA)_3/(CA)_3/(CA)_4/$ $(CA)_3/(CA)_3/(CA)_3$	F: ATTCTCTCCCCCTCTCTGC R: TGTGTGCGCGTTTTCTTG	248-258
M24	$(CA)_{15}(CG)_4CA$	F: GGCTCGAGATATCTGTTTAG R: TTTAATGGGCATAGGGTCC	132-166
M25	$(GT)_5CT(GT)_2/(GT)_{12}$	F: CCCTCCCTGCCAGAAGAAGC R: AACCACCGTCCTTTTCCTCG	160-170
M27	$(GT)_{11}$	F: AGGAGGGAGGTGTGGGTGAAG R: AGGGGAGTGGATAAGAAGG	118-134
M29	$(CTCACA)_4/(CA)_9$	F: GACCATTACATTTCAACACAC R: GCATTTTGTTGCACACTGTA	103-122
M47	$(CT)_9(CA)_8/(CT)_4/(CA)_5$	F: TGATGGACAGGAGTTGATGG R:TGCCAATCTACCTACCCCCTT	100-132

A second stage of analysis involved the screening of an F_2 population derived from a cross between Rume Sudan (resistant parent – P1) and SL 28 (susceptible parent – P2) using primer M24 that detected polymorphison between the two parents. The F_2 screening was done alongside the parents for comparison.

RESULTS AND DISCUSSIONS

A significant finding of this study was that primer M24 was able to detect polymorphism at 3 different levels (Figure 1). The resistant parent amplified a fragment of 180 bp size. The susceptible SL 28 parent amplified another fragment of about 150 bp. These fragments were also observed in some F_2 progenies. This was evidence that the F_2 progenies segregated into parental genotypes. The third category of fragments appeared in pairs and was mainly observed in the F_2 plants. This category is believed to comprise the hetrozygotes.



M = 100 bp ladder, P1 =SL 28, P2 = Rume Sudan.

Figure 1. Amplification products revealed by primer M24.

CONCLUSION

The observed polymorphism exhibited by the F_2 progenies is an indication that one of the resistance genes carried by Rume Sudan is closely linked to the 180 bp fragment, which can be used as a marker for resistance. When visualized on an agarose gel, the fragments amplified by the resistant and susceptible parents appear to be in close proximity to each other (180 bp and 150 bp). A clear distinction could be obtained by running the products on an acrylamide gel. Further work is required to determine the sequence and the chromosome on which the fragment is located.

ACKNOWLEGEMENTS

We acknowledge with thanks the financial support obtained from the European Union to accomplish this work as part of the CBDRESIST Project No. ICA4-2001-10008. We also wish to thank the CRF staff who participated in the project. This paper is published with the permission of the Director of research, Coffee Research Foundation .

REFERENCES

Combes, M C, S Andrzejewski, Anthony F, Bertrand B, Rovelli P, Graziosi G and Lashermes P, (2000). Characterization of microsattelite loci in Coffea arabica and related coffee species. Molecular Ecology 9:1171-1193.

Assessment of Coffee Berry Disease Using Laboratory Berry Inoculation Method

F. PINARD[1] AND C. OMONDI[2]

[1]CIRAD – ICRAF, PO Box 30677, Gigiri, Nairobi Kenya. E-mil: fabrice.pinard@cirad.fr
[2]CRF (Coffee Research Foundation), PO Box 4, Ruiru 00232, Kenya
E-mail: chr_omondi@yahoo.co.uk

SUMMARY

This study was initiated to characterize the interaction between *Colletotrichum kahawae* and two coffee varieties at fruit level. Detached berries of *Coffea arabica* varieties, SL 28 (susceptible) and Ruiru 11 (resistant) were artificially inoculated and kept under controlled conditions of 100% RH and a temperature of 17 °C to 20 °C. In a first experiment, the berries were inoculated with single spore culture of *C. kahawae* at various concentrations ranging from $2 * 10^6$ sp/ml to $2 * 10^3$ sp/ml. In a second experiment, berries of various sizes were inoculated with a single spore concentration. Statistical analysis ($\bullet^2$, General Linear Model) of binary data (infected/healthy) and quantitative data (% of necrosis) revealed the existence of 2 resistance components operating against pathogen penetration and colonization of the berry tissue. The reaction was found to be controlled by at least 3 parameters: the inoculum concentration, the berry physiological stage and the host genotype.

INTRODUCTION

C. kahawae, like other *Colletotrichum spp*. (Prusky and Plumbley, 1992), is specialized on fruit tissue and produces black necrosis of the epicarpe of expanding berries. Work on interaction between *Colletotrichum kahawae* and coffee varieties is mostly conducted using artificial inoculation of young hypocotyl seedlings (Van der Graaff, 1992). Although this technique is useful for large-scale screening of genotypes for resistance/susceptibility (Van der Vossen et al., 1976), it does not provide a precise understanding of the mechanisms occurring at fruit level and little is currently known of the coffee resistance mechanisms and the pathogenicity of *C. kahawae*. Using the technique of artificial inoculation of detached berry described in early work (Nutman and Roberts 1960; Bock, 1956), and taking into consideration the appropriate experimental design (statistical analysis, berry uniformity, inoculum viability, etc.) (Van der Graaff, 1992), the work presented here focuses on the characterization of component(s) of coffee berry disease (CBD) resistance to be possibly used, later, in improving the coffee breeding programme.

MATERIAL AND METHODS

Green, expanding berries were collected from mature trees of SL 28 and Ruiru 11 varieties. The wounded stalk end was removed with a clean scalpel blade to avoid contamination. Berry surface was cleaned with liquid soap (0,01%), rinsed and dried. Berries were then aligned on a wet tissue paper supported by a metallic grid and placed in a plastic box partially filled with water. The box was then closed to provide saturated humid conditions necessary for disease development.

A spores suspension was prepared from a 7 day old culture of a single spore colony of *C. kahawae*. Inoculation was done by placing a 20 µl drop of the suspension on the berry surface. The closed boxes were then placed in a controlled temperature room at 17-20 °C. After 24 hours, the remaining inoculum drops were removed with a dry tissue paper. The necrosis extension was scored using a visual scale from 0% to 100% of the total berry surface. Qualitative observation (infected/healthy) were subjected to a χ^2 analysis while quantitative results (% of necrosis area) were analysed using the GLM procedure.

Influence of inoculum concentration on disease expression

The infection potential of 5 different inoculum concentrations: A = 2.10^6 sp/ml; B = 10^6 sp/ml; C = 2.10^5 sp/ml; D = 2.10^4 sp/ml and E = 2.10^3 sp/ml was investigated. The experiment was laid out as a two replicate randomised design with 40 expanding berries of uniform size per replication.

Berry age influence

Resistance of expanding berries at 4 different growth stages, were compared. Thirty berries were inoculated with a spore suspension of $2*10^6$ sp/ml in 3 replications. The berry being more or less spherical, it was assumed that the size of the lesion could be estimated as (% of necrosis) * (average length/2)^3. The data was subjected to statistical analysis.

RESULTS

Effect of inoculum concentration

Infection rate (% of infected berries) (Figure 1)

The disease expression was higher and faster in SL 28 reaching a maximum of 73% infection. The inoculum concentration effect was highly significant at all scoring dates (χ^2 = 1368,58; 6ddl; p < 0,000) as evidenced by the different amount of infection (χ^2 = 1033,321; 4ddl; p < 0,000). The amount of disease infection was positively correlated with inoculum concentration. The incubation period also varied with the inoculum concentration, from less than 7 day (concentration A) to 12 days (concentration E). SL 28 was susceptible enough to express symptoms at the lowest concentration used. (Figure 2). On Ruiru 11, the inoculum concentration effect was highly significant (χ^2 = 450,576; 4ddl; p < 0,000) at all dates but produced less disease than SL 28. A maximum infection level of 35% was observed at the highest inoculum concentration. No disease was observed with concentration lower than 2 * 10^5 sp/ml. The incubation period was slightly longer, varying from 8 DAI to 14 DAI .

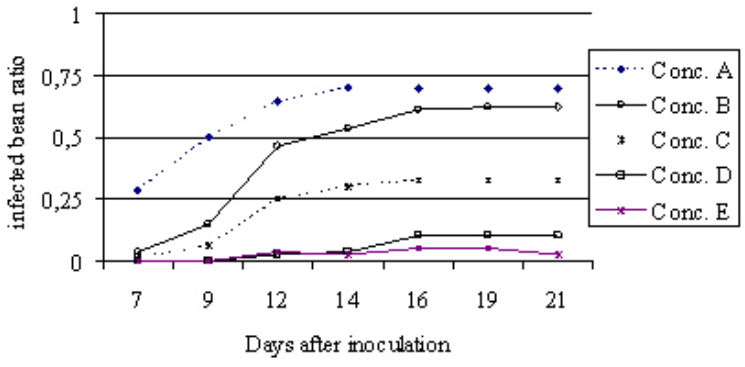


Figure 1. Infection rate of SL 28 berries infected with various inoculum concentration.

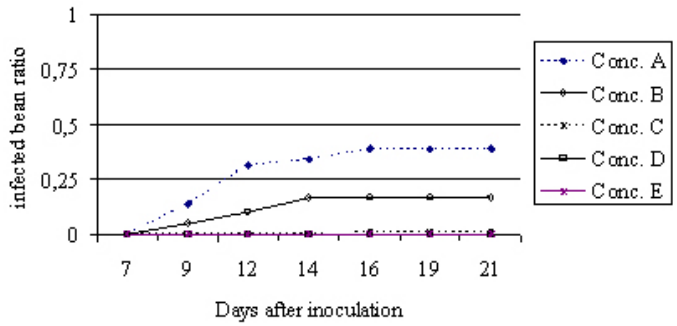


Figure 2. Infection rate of Ruiru 11 berries infected with various inoculum concentration.

Necrosis development (Figure 3)

The concentration effect was significant on SL 28, (F = 15,553; 4ddl, p < 0,000), leading to the following ranking: (A > B) > (B > C)> (C > D > E) (groups within brackets are not significantly different). At A, B and C concentration, the progression of the disease was fast, constant, generating more than 75% of necrosis on infected berries. At the lowest concentration, the disease progression was delayed by 3 days, with a significant increase in speed of 19 DAI. At the end of the observation, all treatment showed more than 50% of necrosis on infected berries. (Figure 4) On Ruiru 11, the concentration effect was not significant (F = 1,797; 4ddl, p = 0,168), mainly due to the equivalent level of disease expressed by A and B treatment, and the very low number of infected berries (3 out of 80) with C concentration. With A and B concentration, the disease progression was rather slow during the first 16 days. It then increased, leading to a maximum of 53% of disease at the end of the experiment.

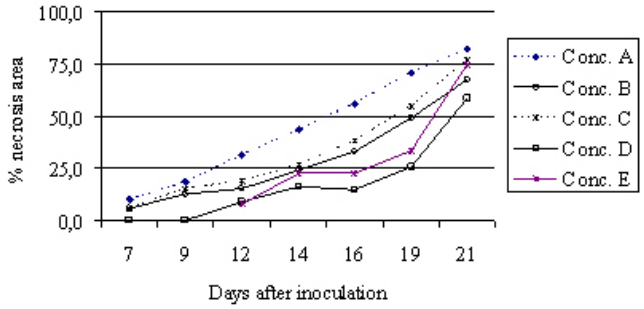


Figure 3. Necrosis development on SL 28 berries infected with various inoculum concentration.

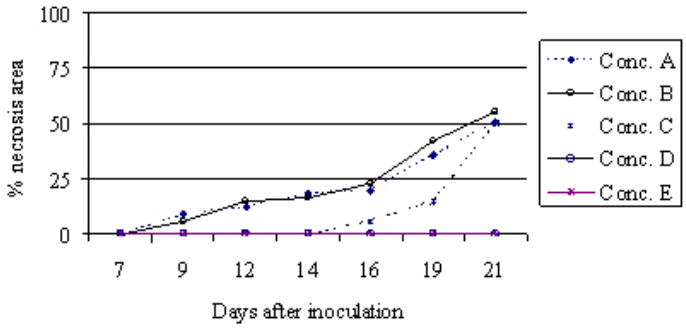


Figure 4. Necrosis development on Ruiru 11 berries infected with various inoculum concentration.

Effect of berry age

Infection rate (% of infected berries) (Figure 5)

The effect of berry-size on disease expression was significant at all dates for SL 28 ($20.031 < \bullet^2 < 142.058$; 3ddl; $p < 0.000$). The smaller berries expressed the highest level of infection (~ 100%) with an incubation period varying from 5 to 13 days and a rapid progression during the first 11 DAI. The largest berries were less infected (68%) with an increased period of incubation (7 DAI to 17 DAI) and a slower progression rate. (Figure 6) On Ruiru 11, the smallest berries were the most susceptible (88% infection). Majority of the berries were infected within 6 DAI to 14 DAI with a high progression rate. Conversely, the disease progression was slower on the larger berries, with an incubation period lasting from 7 DAI to 17 DAI, leading to a maximum infection rate varying from 29% to 47%.

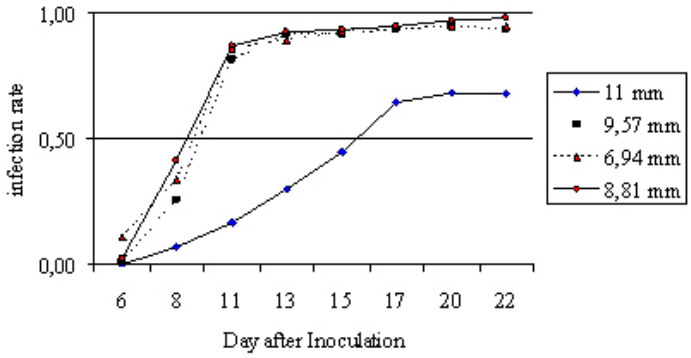


Figure 5. Infection rate of SL 28 berries of different ages.

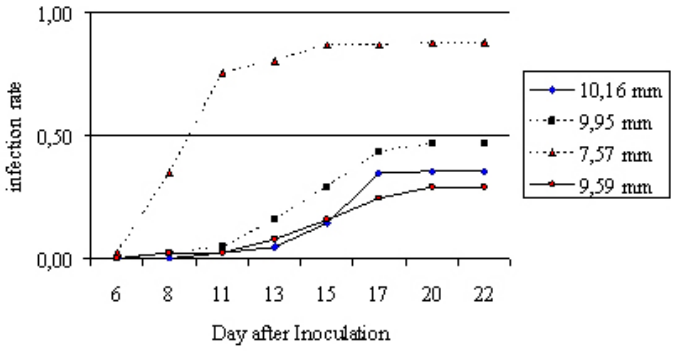


Figure 6. Infection rate of Ruiru 11 berries of different ages.

Necrosis development (Figure 7)

On the smaller berries of SL 28, the necrosis development was fast and constant. It stopped on the smallest category at 15 DAI when the necrosis reached the maximum of 100% of the berry surface (preventing any further extension, equivalent to an index of 1000 on Figure 7). Unlike this pattern, the large berries exhibited non-limited development of the necrosis. The disease progression on the largest berries was slow during the first 17 DAI, and slightly increased after this date, but still remained lower than the intermediate berries ($F = 55.856$; 3 ddl; $p < 0.000$). (Figure 8) On Ruiru 11, the necrosis developed rapidly on the smallest berries, reaching the maximum of infection (100%, equivalent to a necrosis index of 1500) 20 DAI. On bigger berries, the necrosis development was significantly slower ($26 < F < 222$; 3ddl; $p < 0.000$), especially during the first 15 DAI. After this date, the necrosis progression

increased, leading to a maximum disease expression close to the level expressed by the smallest category.

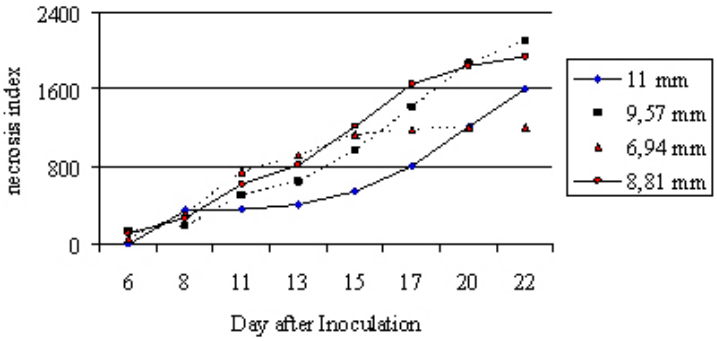


Figure 7. Necrosis development on infected SL 28 berries of different ages.

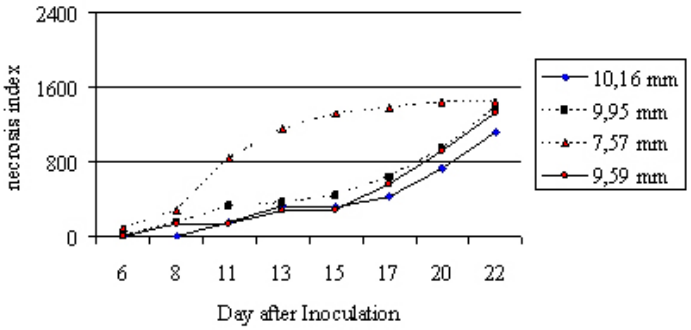


Figure 8. Necrosis development on infected Ruiru 11 berries of different ages.

CONCLUSION

Figure 1 and fig. 2 describes the effect of inoculum concentration on the pathogen ability to generate infection i.e. to penetrate the berry cuticle, to settle in the epicarpe cells and to initiate a colony growth. During this phase, the right concentration of inoculum is important to allow colony settlement as a result of increased number of successful attacks. The resistance of Ruiru11 is 2 to 3 times more efficient than the one of SL 28. During the growth phase of the colony (Figure 3 and Figure 4), the effect of the inoculum concentration is detected only with SL 28. In this case, it mainly manifests as a delay in the fungal growth, but not a reduction of the speed of growth. This could again reveal a quantitative aspect of the resistance to the colonization process. When the quantity of fungal hyphae reaches a certain threshold within the berry tissue, the expressed resistance decreases. The resistance of Ruiru 11 is higher compared to SL 28, leading to a 25% reduction of disease progress.

The effect of berry size on resistance is highly significant on both SL 28 and Ruiru 11 varieties: the older the berry, the higher the level of resistance expressed. However, the acquisition of such resistance is faster on Ruiru 11 than on SL 28: only the smaller berries of Ruiru 11 can be considered as susceptible while only the larger berries of SL 28 express some resistance.

The results obtained demonstrate that artificial inoculation of detached berry is efficient to study coffee / *C. kahawae* interaction. Using this technique, it is the first time that the disease impact is assessed both qualitatively (infected/healthy berry) and quantitatively (necrosis size). This approach was efficient in detecting 2 resistance components: resistance against the pathogen penetration and resistance to colonization of the berry tissue. These are controlled

by at least 3 parameters: the inoculum concentration, the berry physiological stage and the host genotype. This is in agreement with field observations and previous work (Bock, 1956; Mulinge, 1969; Griffiths and Furtado, 1972; Masaba and Van der Vossen, 1982). More investigation is now required for a better characterization of these resistance components.

REFERENCES

Bock K.R., 1956: Investigations on coffee berry disease – laboratory studies. The East African Agricultural Journal, October, 97-103

Firman I.D. 1963: Screening of coffee for resistance to coffee berry resistance. East African Agricultural and Forestry Journal, XXIX, n°3, 192-194.

Griffiths E. and Furtado I., 1972: A berry infection technique for assessment of the CBD strain of Colletotrichum coffeanum on coffee branchlets. Trans. Brit. Mycol. Soc. 58 (2), 313-320.

Masaba D.H. and Van der Vossen H.A.M., 1982: Evidence of cork barrier formation as a resistance mechanism to berry disease (Colletotrichum coffeanum) in Arabica coffee. Net. J. Pl. Path. 88, 19-32.

Mulinge S.K. 1969: Development of coffee berry disease in relation to the stage of berry growth. Ann. Appl. Biol., 65, 269-276.

Nutman F.J. and Roberts F.M., 1960: Investigations on disease of Coffea Arabica caused by a form of colletotrichum coffeanum Noack. II. Some factors affecting germination and infection, and their relation to disease distribution. Trans. Brit. Mycol. Soc. 43 (4), 643-659.

Prusky D. and Plumbley R.A., 1992: Quiescent infections of Colletotrichum in tropical and subtropical fruits. In Colletotrichum: Biology, Pathology and Control, edts. Bailey J.A. and Jeger M.J., C.A.B. International, pp. 388.

Van der Graaff N.A., 1992: Coffee Berry Disease, in Plant Diseases of International Importance, Vol. 4. 202-230

Van der Vossen H.A.M., Cook R.T.A. and Murakaru G.N.W. 1976: Breeding for resistance to coffee berry disease caused by Colletotrichum coffeanum Noack in Coffea Arabica L. I. Methods of preselection for resistance. Euphytica 25, 733-745.

Progress towards Searching for Durable Resistance to Fusarium Wilt (*Fusarium xylarioides*) in *Coffea canephora* Germplasm in Tanzania

D. L. KILAMBO, N. M. NG'HOMA, D. J. MTENGA, J. M. TERI, T. NZALLAWAHE, R. MIKE, L. MASUMBUKO[1]

Tanzania Coffee Research Institute, P.O. Box 3004 Moshi, Tanzania
[1]CABI Uk. E-mail: tacriced@yahoo.com – Fax no: +255 27 275 6773

SUMMARY

Since its appearance in Tanzania in 1997, *Fusarium* wilt has clearly demonstrated both its ability to spread rapidly to new areas and to cause serious losses on *C. canephora*. Commercial Robusta clones MS 1, 2, 3, 4 & 5 released to coffee growers in recent years are all vulnerable to *Fusarium* wilt. Search for durable resistance was initiated in April 2004, 175 clones each having five clonal seedlings were artificially inoculated with *F. xylarioides* at a spore concentration of 1.3 x 10^6, using stem nicking procedures. Assessment was carried out by identifying and counting free diseased seedlings every month for nine months. Out of 175 clones, 11 were found to resist *Fusarium* wilt. Survivors were re-assessed in April 2005 using root-dip procedures at the same spore concentration. After nine months, Robusta clones showed a certain level of consistency in terms of resistance with stem nicking and root dipping procedures. Although root dipping procedures showed high selection pressure than stem nicking, there is positive correlation (r = 4.75). The study indicated that there is no pathogenic variation among *F. xylarioides* isolates from Robusta coffee in Tanzania.

INTRODUCTION

Robusta coffee (*C. canephora*), has a lot of diversity with varying degree of vulnerability to leaf diseases and insect-pest attack. Coffee leaf rust (CLR) incited by *Hemileia vastatrix* (Bert & Br) on leaves and red blister on mature to ripe berries caused by *Cercospora coffeicola* had been the major diseases of Robusta coffee in the country for years. But *Fusarium* wilt commonly referred to as tracheomycosis, vascular or coffee wilt disease (CWD) is now a re-nowned coffee disease causing large-scale damage to all Robusta commercial varieties in Tanzania (Kilambo and Ng'homa, 2005). Records show presence of *Fusarium* wilt in three Robusta-growing districts that border Uganda: Bukoba, Karagwe and Muleba.

Robusta coffee growers in Tanzania are not used to applying chemicals and therefore it is difficult to adopt use of fungicides to manage CWD. The major management approach being used is eradication of diseased trees by up-rooting and burning therefore minimizing spread of the disease. However eradication has one major limitation that not all infected plant materials can be destroyed. Therefore host resistance is the only viable approach to manage CWD. This report highlights research activities to determine whether or not there is pathogenic variation within *F. xylarioides* in Tanzania, and their effects to resistance in *C. canephora*.

MATERIALS AND METHODS: PATHOGENICITY OF *FUSARIUM XYLARIOIDES*

Fourteen isolates originating from CWD-infected coffee trees in Muleba, Bukoba and Karagwe districts (Table 1) were selected basically on cultural classes; pigmentation and

growth size, and shape of conidia. The pathogenicity of the isolates was tested by inoculating 10 seedlings each, using root dip technique, a CWD-susceptible and CWD-tolerant variety, MS 1 and MS 2 respectively. Assessment on the effect of each isolate was done monthly by counting the number of diseased seedlings.

Table 1. Pathogenicity test results of Fusarium xylarioides on MS 1 and MS 2.

CWD Isolate acc. No		Location collected			Number of dead seedlings	
TaCRI	CABI UK	District	Coordinates	Altitude	MS 1	MS 2
2004/10	T 1	Muleba	S $01^0$45.901''; E $31^0$35.491''	1547 m	9	9
2004/13	T 2a	Muleba	S $01^0$46.827"; E $31^0$34.541"	1545 m	10	9
2004/07	T 3a	Muleba	S $01^0$49.702"; E $31^0$41.137"	1395 m	9	10
2004/08	T 4	Muleba	S $01^0$43.159": E $31^0$38.078"	1510 m	10	9
2004/02	T 5a	Muleba	S $01^0$41.172"; E $31^0$37.731"	1287 m	10	9
2004/06	T 8a	Bukoba	S $01^0$00.595"; E $31^0$46.582"	1189 m	10	10
2004/01		*Bukoba	S $01^0$14.836"; E $31^0$50.682"	1200 m	10	9
2004/12	T 9a	Bukoba	S $01^0$01.612"; E $31^0$32.758"	1256 m	10	9
2004/14	T 12a	Karagwe	S $01^0$18.600"; E $30^0$47.205"	1424 m	9	9
2004/03	T 13a	Karagwe	S $01^0$26.166"; E $30^0$52.801"	1317 m	10	9
2004/05	T 14a	Karagwe	S $01^0$17.308"; E $30^0$53.896"	1659 m	9	9
2004/09	T 15a	Karagwe	S $01^0$15.309"; E $30^0$57.347"	1354 m	9	9
2004/09	T 15b	Karagwe	S $01^0$15.309"; E $30^0$57.347"	1354 m	9	9
2004/09	T 15c	Karagwe	S $01^0$15.309"; E $30^0$57.347"	1354 m	10	10
Mean					9.57	9.21
SE ±					0.14	0.11
C.V					5.30	4.50
L.S.D					0.30	0.23

**Isolate 2004/1 was used for CWD resistance evaluation.*

Coffee seedlings

Studies were conducted using three months and nine months old potted seedlings of MS 1 and MS 2 for pathogenicity, and 175 clones for resistance evaluation. Five clonal seedlings were raised from a single tree (Table 2).

Inoculation of coffee seedlings

Two inoculation procedures were used; root dipping to study pathogenicity of the isolates and confirm resistance of Robusta lines, and stem nicking to study resistance of the lines. All fourteen CWD isolates were used for pathogenicity test (Table 1), but isolate 2004/1 was used for screening for CWD-resistance studies. MS1 was included as a susceptible control.

Table 2. Robusta lines survived after artificial inoculation with *Fusarium xylarioides* using stem nicking and root dipping procedures.

S/no	Coffee line/ cultivar	Mean seedlings survived		CLR resistance	Mean Clean Coffee Kg/tree (2000-2004)
		Stem nicking 2004	Root dipping 2005		
1	ML 26	4	3	R	1.86
2	NG 08	5	3	R	1.65
3	ML 35	2	1	R	2.03
4	NG 13	4	3	R	1.92
5	BK 27	4	4	MR	0.57
6	KR 21	3	3	MR	1.39
7	NG 12	4	3	MR	1.39
8	KR 11	5	3	MR	0.67
9	NG 17	3	1	MR	1.43
10	ON BK 02	3	3	MR	0.57
11	MR 10	5	4	MR	1.12
12	*MS 1	0	0	R	1.86
	Mean	3.50	2.58		1.37
	S.E ±	0.40	0.35		0.15
	C.V	40.00	35.40		38.68
	L.S.D	1.08	0.95		0.40

**Commercial cultivar, R = Resistant & MR = Moderately Resistant.*

Root dipping

Seedlings were removed from the potting soil and their roots cleaned with tap water, then immersed in the standard conidia suspension (1.3 x 10^6 spores per ml) and removed instantly (Hakiza et al., 2004). Seedlings were then carefully reported with fresh soil. The inoculated seedlings were monitored and data recorded on wilted or dead plants for nine months. The number of wilted/dead seedlings and the total number of plants per line were used to calculate the incidence of wilting. For pathogenicity test three months old seedlings were used, and resistance evaluation nine months.

Stem nicking

Potted coffee seedlings nine months old were inoculated with a viable conidia suspension of 2004/1 isolate by stem nicking procedure (Girma, 2004). A sterile scalpel was first immersed into the conidia suspension, then the stem of each seedling nicked (at about 2 cm heights from the soil level) and a drop of nearly 1 ml (spore suspension of 1.3 x 106 conidia/ml) was placed in the notch. The inoculated notch was then covered with a cotton wool and tied with a string. Means for comparison and correlations were computed.

RESULTS AND DISCUSSION

Pathogenicity studies

Results showed that there is a significant level of aggressiveness in all 14 isolates (Table 1). Mean percentage of dead plants ranged 90-100 % per isolate. Based on seedling death rates there is no significant differences in the level of pathogenicity among *F. xylarioides* isolates collected in Kagera. Pathogenic variability is reported between *Type A* of *C. arabica* and *Type*

C of *C. canephora* but very limited within-type variation has been reported (Girma, 2004; Mike et al., 2004). The results of this study are inline with these findings.

Host - Pathogen interactions

Results obtained show existence of resistance to *Fusarium* wilt among Robusta accessions within the germplasm collection at Maruku Agricultural Research Institute (Table 2). Seedlings that survived stem nicking and thereafter root dipping demonstrated their resistance to CWD. Genotypes BK 27, KR 21 and ON BK 2 are moderately resistant to rust and have yield potential of 0.57, 1.39 and 0.57 Kg per tree of clean coffee respectively. In this study they indicated highest level of resistance to CWD. Genotypes ML 26, NG 08, ML 35 and NG 13 indicated fairly good resistance to CWD, they also have potential for yield and resistance to leaf rust (Table 2). Musoli et al. (2000) also noticed varietal differences in screening Robusta coffee accessions for resistance against CWD. Booth (1971) reported association of suberization in wounds, high concentration of caffeine and chlorogenic acid, as possible source of resistance in *C. canephora*. MS 1 commercial cultivar has demonstrated its susceptibility to CWD. In this study the root dipping method showed high selection pressure. But stem nicking was positively correlated with root dipping at r = 4.75.

CONCLUSION

The study indicated that there is no pathogenic variation among *F. xylarioides* isolates from Robusta coffee in Tanzania. Also reactions of inoculated Robusta clones showed certain level of consistency in terms of resistance with stem nicking and root dipping methods. These will be multiplied in clonal mother garden to get seedlings for on-farm trials. Out of the two artificial inoculation methods, root dipping showed high selection pressure, but they are positively correlated.

REFERENCES

Booth, C. (1971) The Genus Fusarium. Commonwealth Mycological Institute: Kew, Surrey, Egham. 237 pp.

Girma, A. S. (2004) Diversity in Pathogenicity and Genetics of *Gibberella xylarioides* (*Fusarium xylarioides*). Population and Resistance of Coffea spp in Ethiopia. PhD Thesis pg 14, 16.

Hakiza, G. J., Kyetere, D. T., and Olal, S. (2004) Mode of penetration and symptom expression in Robusta coffee seedlings, inoculated with Gibberella xylarioides, the cause of coffee wilt disease in Uganda. Presented during ASIC 2004 20th International Conference on coffee science 11-15 October 2004 Bangalore, India.

Ng'homa, N. M. and Kilambo, D. L. (2005) Improvement of Coffee Production in Tanzania by the control of Coffee Wilt (Tracheomycosis). Presented during EAFCA Conference, Livingstone Zambia, February 2005.

Mike, R., Buddie, A., Crozier, J., Ineson, J., and Flood, J. (2004) Regional Coffee Wilt Programme CABI UK Centre research activities: Progress. Presented during Regional Coffee Wilt Planning Workshop Nairobi, November 2004.

Musoli, P., Olal, S., Nabaggala, A. and Kabole, C. (2000) Screening Robusta coffee germplasm for resistance against Coffee Wilt. In: CORI (Coffee Research Institute) 2001; Coffee Wilt Disease research and development in Uganda. Research progress 1997-2000 29pg.

Variability in the Insecticidal Protein Concentration within Transformed *Coffea canephora* Observed in a Field Experiment

B. PERTHUIS[1], T. LEROY[2], M. DUFOUR[2], J. M. VASSAL[2], J. L. PRADON[1], C. FENOUILLET[2], V. CONDINA[1] AND S. LAURENT[1]

[1]CIRAD-avenue de France-BP 701-97387 Kourou, French Guiana
[2]CIRAD-avenue d'Agropolis-34398 Montpellier Cedex 5, France

SUMMARY

A field trial of transformed *Coffea canephora* for the synthesis of the Bt protein cry1Ac against coffee South-American leaf miner was performed in French Guiana. Almost all the transformed clones were highly resistant against *Leucoptera coffeella*. The strategy of plant selection requires the creation of lines synthesizing large amounts of insecticidal protein. The transgenic protein in the leaves of 15 independent transformed clones was quantified once. A significant variability in the transgenic protein concentration was seen among the clones. The potential of the used genetic construct seems interesting.

INTRODUCTION

The clone 126 of *Coffea canephora* was transformed for synthesis of the *Bacillus thuringiensis* (Bt) insecticidal protein cry1Ac that is toxic for the larvae of the *Coffea* leaf miners. A multiannual field trial with 53 transformed clones and the untransformed control clone 126 was planted in French Guiana in the year 2000. It was the first field experiment with transgenic coffees. Almost 70% of the transgenic clones were highly resistant against the leaf miner *Leucoptera coffeella.* On them it was hardly seen damages (mines) of the pest (Perthuis et al., 2005). As the pest is able to infest all the leaves wherever his location on the plant, this means that the synthesis took place everywhere in the plant and that generally the dose was high enough to kill the young larvae. High insecticidal protein concentrations in the tissues well above the lethal dose for the target pest are advisable because they should delay the increase of resistance of the pest toward the Bt protein (Gould, 1998). The efficacy of the protein synthesis may be very variable between transformed lines (Perlak et al., 1991). Hence it seemed important to confirm the existence of variability in cry1Ac contents within lines. It was decided to quantify cry1Ac by Elisa immunosorbent assay in the leaves of some transformed clones at one moment.

MATERIAL AND METHOD

Transformed clones

The independently transformed clones 126 of *C. canephora* harbour a synthetic modified *cry1ac* gene (Sardana et al., 1996) under control of the constitutive promoter pEF1α from *Arabidopsis thaliana* (Van Boxtel et al., 1995) associated with an enhancer sequence.

20 plants that may be taken as independent replications were planted for each transgenic line in a field in French Guiana and their resistance was evaluated (Perthuis et al., 2005).

Sampling

It took place in May 2004 when the trees were 4 years old. Clones GM1 and GM50 were selected because they lack total resistance to the pest. 13 independent totally resistant lines were also taken. They were chosen to ensure presence of all the genetic insert copy numbers. For each transformed line, 2 groups of 6 mature leaves were collected on two sides of the tree from bottom to top on 15 out of 20 plants. They were sent frozen (-30°C) to the laboratory.

Measure of cry1Ac concentration

For each tree, 2 samples of 1 gram, one for both groups of 6 leaf blades were cut. They were crushed into uniform powder with liquid nitrogen. Extraction of soluble proteins was performed using PBST buffer. The concentration of cry1Ac was obtained with an immunosorbent assay (ELISA) from Agdia. Two test wells were filled with each extract, and colour intensity read three times on a Biotek spectrophotometer. Data for one plant is thus the mean of 12 measures: 3 measures x 2 wells x 2 extracts.

Statistical analysis

Stabilization of variance and approximation to normality was obtained by square root transformation. One way ANOVA was performed. Multiple comparisons aiming at separating homogeneous groups of concentration was then realized with Newman-Keuls test on clones displaying the same level of variance. The used software was ExelStat.

RESULTS AND DISCUSSION

Results are presented in Table 1. It can be seen that the standard deviation within a transformed line is often very high. It cannot be known whether this reflects more an environmental influence than the deviations that were induced by sampling and measurement. Influence of the specific mineral nutrition of each plant in relation to the soil is likely to occur among other agronomic factors like water or light. The influence of nitrogen fertilization on Bt protein concentration in maize has been documented (Bruns and Abel, 2003).

However, ANOVA and multiple comparison tests with transformed data may be used if the requisite of variance homogeneity is fulfilled. This leads to discard the clones displaying the highest variance (GM 10, 17, 39, 42 and 50).

One way Anova indicates significant variability in the cry1ac concentration among the 10 remaining transformed clones ($p < 0,001$). Newman-Keuls test separates 6 homogeneous groups (Table 2). Five of them are separated from all the others, hence indicating several levels of significantly different concentrations.

The admitted current rule is that a crop synthesizing a Bt insecticidal protein should exhibit a concentration 25-fold higher than the dose killing 100% of the pest (LC100) at any moment of its complete cycle on the plant where its exposition to the protein is permanent (Gould, 1998). Here, since only a few mines were counted for level as low as 40 ng/gram (fresh weight), this level is not far from the LC100 for young larvae. Therefore, LC100 after an exposition during the entire cycle has to be much lower. It appears that the inserted genetic construction is probably able to generate concentrations of cry1Ac in *C. canephora* several-fold higher than the LC100 of *L. coffeella*, and for this reason exhibits a good performance against this pest. Furthermore, it is possible to assume that a transformed line reaching a higher level than GM45 could exist in the resistant lines that have not been analyzed.

Table 1. Global results (averages).

Transformed line	Copy number of Bt gene	Relative mines numbers compared to control	Concentration of cry1ac ng/gram fresh weight	Standard deviation
GM1	1	15%	16	10
GM50	2	6,7%	40	36
GM11	2	0,20%	45	22
GM40	1	0,20%	45	29
GM8	5	1,40%	47	23
GM10	3	0,70%	53	45
GM17	3-4	0,80%	64	56
GM42	1	5,40%	66	42
GM12	3	0%	72	27
GM44	1	0%	78	19
GM27	1	0%	88	19
GM5	1	0%	103	23
GM39	4	0,10%	103	64
GM13	2	0%	138	53
GM45	2	0,10%	168	49

Ng = nanogram.

Table 2. Clones that could be classified. Means associated with the same letter are not significantly different.

Clone	Cry1Ac (ng/g)	
GM45	168	A
GM13	138	B
GM5	103	C
GM27	88	CD
GM44	78	CD
GM12	72	D
GM8	47	E
GM40	45	E
GM11	45	E
GM1	16	F

REFERENCES

Bruns H.A., Abel C.A., 2003. Nitrogen fertility effects on Bt delta-endotoxin and nitrogen concentrations of maize during early growth.Agronomy Journal 95: 207-211

Gould F., 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annual Review of Entomology 43: 701-726.Perlak FJ, Fuchs RL, Dean DA, McPherson SL, Fischhoff DA, 1991. Modification of the coding sequence enhances plant expression of insect control protein genes. Proc Natl Acad Sci USA. 88: 3324-3328.

Perthuis, B., J.L. Pradon, C. Montagnon, M. Dufour and T. Leroy, 2005. Stable resistance against the leaf miner Leucoptera coffeella expressed by genetically transformed Coffea canephora in a pluriannual field experiment in French Guiana. Euphytica 144: 321-329.

Sardana R, Dukiandjiev S, Giband M, Cheng X, Cowan K, Sauder C, Altosaar I., 1996. Construction and rapid testing of synthetic and modified toxin gene sequences cry1a (b&c) by expression in maize endosperm culture. Plant Cell Reports 15: 677-681.

Van Boxtel JM, Berthouly C., Carasco C., Eskes AB, 1995. Transient expression of béta-glucuronidase following biolistic delivery of foreign DNA into coffee tissues. Plant Cell Report 14: 748-752.

Effective Application of Chemical Strategy Against Coffee Berry Borer, *Hypothenemus hampei*, Infestation in Kenya

H. M. MUGO

Coffee Research Foundation, Entomology Department, P.O Box 4- 00232, RUIRU, Kenya
Email: mugohmu@yahoo.com or crf@kenyaweb.com

SUMMARY

The Coffee Berry Borer (CBB), *Hypothenemus hampei* Ferrari, is a major insect pest of coffee in Kenya. Its management using chemical approach has been difficult to achieve without timely spray application**.**

Three insecticides; Dursban 480EC, Marshal 25EC and Salut 500EC were sprayed against Coffee Berry Borer at various coffee berries development stages; bean formation, dry matter accumulation and ripening stages. The spraying was done twice at each stage and within an interval of three weeks as recommended. The chemicals effectively protected the developing coffee berries from CBB infestation when applied at bean formation stage than both at dry matter accumulation and ripening stages. The infestations at latter two stages were not significantly better than the control (unsprayed).

INTRODUCTION

Coffee Berry Borer (CBB), *Hypothenemus hampei* Ferr (Coleoptera: Scolytidae) is at present globally the most serious insect problem of coffee. It has become endemic worldwide causing significant damage to yield and quality of coffee in many producer countries.

The management of CBB has been difficult to achieve because of its feeding behaviour once inside the coffee berries. The adult female beetle that makes 90% of the total population of CBB is responsible for boring into a coffee berry where it lays its eggs (Le pelley, 1968). The preference is in both mature red and immature green berries though development occurs

favourably in mature yellow and red ones. The female beetle together with its brood, while inside the berry feed on the contents of the bean and affect quality. Poor control for CBB at farm level is normally extended to both secondary and tertiary processing levels. In addition, this pest is easily spread from one geographical area to the other thus increasing in number.

In the past, CBB infestation in Kenya that threatens coffee production used mainly to occur in the Western regions. At present it has spread widely and it is now found all over the coffee growing zones. There has been heavy berry borer infestation reported by coffee farmers from the Central and Eastern Provinces. The two Provinces are the main Arabica Coffee producers and CBB incidence was previously not a major problem.

The CBB management in Kenya mainly emphasizes on cultural and chemical approaches. Biocontrol using the indigenous natural enemies has not been promising. From a survey conducted in Western Kenya, only 18% parasitism occurs as compared to the borers' 80% infestation (Murphy et al., 1986; 1987).

Use of cultural and chemical approaches have a number of constraints. The recommended cultural approaches through mbuni stripping and collection of fallen infested berries are rather tedious and time consuming. Use of chemicals recommended by Coffee Research Foundation (CRF) against CBB after the main crop (Anon, 1992) are less effective once the pest enter the coffee berries since any sprays fail to get to the target.

According to Wormer (1964) coffee berries undergo various developmental stages and untimed spraying to control the CBB when the infestation has taken place become inappropriate. To improve on chemical control, studies were undertaken to establish the right stage(s) of berry development for better management of CBB.

MATERIALS AND METHODS

The studies were sited at Coffee Research Station (CRS) and Swahara Coffee Estate in Thika District, Central Province. One coffee block with mature Arabica coffee trees was selected per site. At CRS, data was collected for two years from 1999 to 2000 while at Swahara, data was availed only for 1999.

Three recommended insecticides for spraying against CBB; Salut 500EC, Dursban 480EC and Marshal 250EC at rate of 30, 15 and 5ml per 20 litres of water, respectively were applied during the study periods. Three development stages of coffee berries; bean formation (13^{th} – 20^{th} week), dry matter accumulation (21^{st} – 27^{th} week) and ripening (28^{th} – 34^{th} week) stages, identified and tagged from the date of main flowering were investigated. The actual spraying weeks, for different development stages from flowering are shown in **Appendix I**. At all the stages, each of the insecticides was applied twice at a three week interval. Unsprayed plots were used as control.

A complete randomized Block Design was used. Each block had ten (10) plots replicated four (4) times. In each plot there were 16 coffee trees and two guard rows between the blocks and plots. Sixteen (16) branches per plot, four randomly selected per tree with berries of the same age were tagged for data collection from the middle of the plot. Data was recorded on a monthly basis until berries attained maturity. Analysis of variance (Anova) was used in data analysis.

RESULTS AND DISCUSSIONS

Plots treated with the recommended insecticides during this study provided some protection to berries infestation from Coffee Berry Borer (CBB) in the field (Table1). The accumulated mean % infestations for the various berry development stages evaluated during the year 1999 at Coffee Research Station (CRS) plot 6 was below 5% (Table1). This could have been attributed by low infestation levels during this period by the CBB adults. In the year 2000 at CRS plot 6, accumulated mean percentage infestation above 15% was attained (Table 1).

The results from both CRS plot 6 and Swahara estate showed that berries timely sprayed at S1 had better protection on berries from CBB attack with mean infestation reduction of about 50.5% **(**Figures 1 and 2). The S2 and S3 had mean infestations reduction of 36.0% and 17.3%, respectively as compared with control. However, under various chemicals applied, S1 had significantly lower mean % infestation than the control (Table 1) ($p=0.05$). The S2 and S3, though the berries had some protection from the CBB attack their mean % infestations were not significantly better than the control ($p = 0.05$). This indicated that when spraying at S2 and S3 stages, some significant CBB infestations had already occurred. Thus spraying at the two stages had no significant benefits attained.

Table 1. Mean % infestation on coffee berries by Coffee Berry Borer under different spraying regimes at Coffee Research Station (CRS) and Swahara Coffee Estate.

Treatment	CRS Plot 6		Swahara Estate
	1999	2000	1999
T_1S_1	1.25C	9.99B	5.92A
T_1S_2	2.96B	15.41A	7.36A
T_1S_3	1.33C	9.92B	7.23A
T_0S_0	4.98A	14.82A	8.51A
CV (%)	47.48	30.30	54.03
T_2S_1	2.26B	8.44B	5.66B
T_2S_2	3.49AB	8.71B	6.16B
T_2S_3	3.86AB	11.79AB	8.79A
T_0S_0	4.97A	14.97A	8.51A
CV (%)	66.44	10.75	32.74
T_3S_1	2.68B	10.18B	6.68A
T_3S_2	4.29B	8.07C	6.32A
T_3S_3	3.15B	14.67A	8.93A
T_0S_0	4.97A	14.82A	8.51A
CV (%)	67.32	16.56	47.87

Means followed by the same letters down the column are not significantly different at P=0.05 T_1= Dursban 480EC; S_1= Bean formation stage. T_2= Marshal 250EC; S_2= Dry matter accumulation stage. T_3= Salut 500EC; S_3= Ripening stage. T_0S_0 = Control

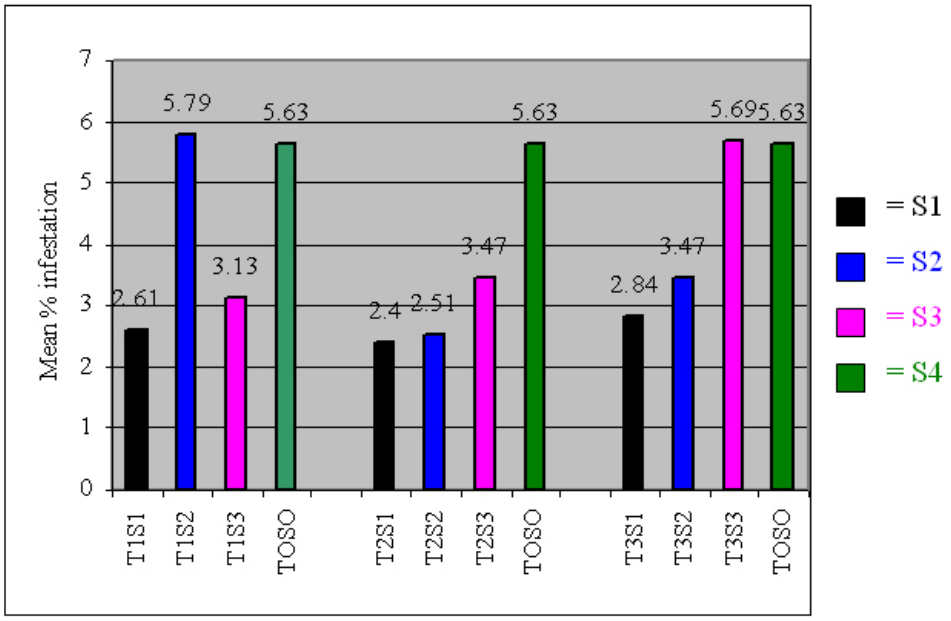


Figure 1. Infestation level s of coffee berry borer on coffee berries sprayed at various developmental stages at Coffee Research station, Plot 6.

The coffee berries picked at late stage of the season whether treated or not either at S1, S2 and S3 stages had infestations not significantly different from each other. This was attributed to fresh infestations experienced to the late maturing coffee berries from the CBB adult females emerging from earlier infested berries. The fresh infestations indicate the need for stripping of mature coffee berries after the main crop as recommended by Anon (1992). This effectively reduces the infestation of coffee berries by CBB from the previous season attack.

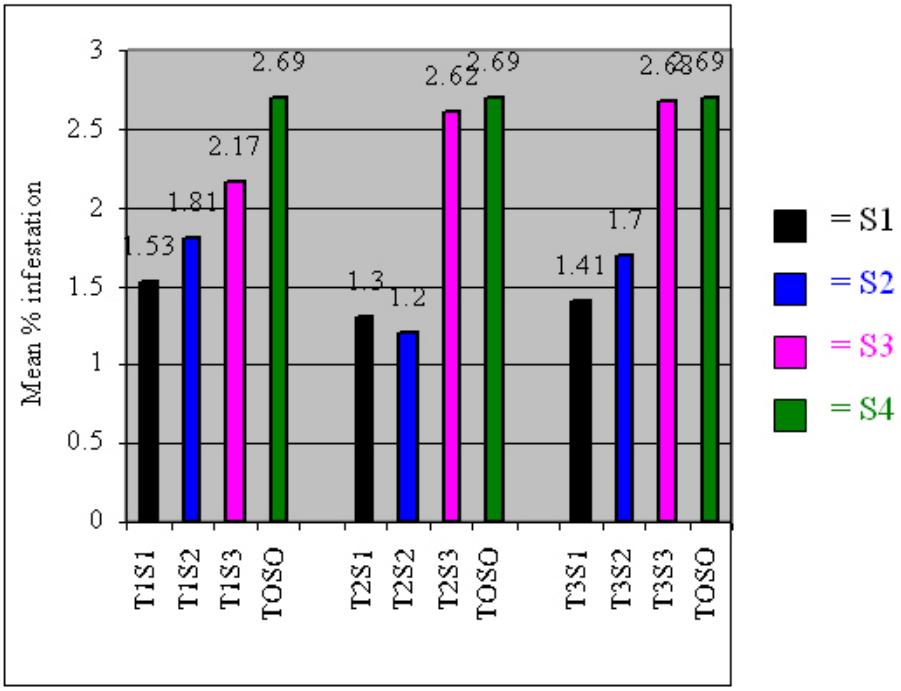


Figure 2. Infestation level s of coffee berry borer on coffee berries sprayed at various developmental stages at Swahara Coffee Estate.

CONCLUSION

Chemically treated coffee berries at bean formation stage (S1) tended to offer better control of CBB than at both dry matter accumulation (S2) and ripening (S3) stages. Therefore spraying at bean formation stage (S1) when CBB is noted occur would be recommended.

ACKNOWLEDGEMENT

The author is thankful to the Entomology Section staff for the tireless assistance rendered in the field. This paper is published with the permission of the Director of Research, Coffee Research Foundation, Ruiru Kenya.

REFERENCES

Anonymous (1992): Control of Berry Borer, *Hypothemus hamper* (Ferreri). Technical Circulars No.74: *Kenya coffee Bulletin:* Volume 57 (66): 1339-1340.

Le Pelley, R.H. (1968): Pests of Coffee 5th Edition, Longmann, London, Pg 250-295.

Murphy, S.T.; O'Donnel, D.T.; Nan'gayo, F.L.; Cross, A. and Evans, H.C. (1986): First report on the Coffee Berry Borer Project. Unpublished report, CAB International Institute of Biological Control, 23PP.

Wormer T.M. (1964): Normal and abnormal development of coffee berries. *Kenya Coffee Bulletin, March 1964.*

Appendix 1. Actual spraying weeks from main flowering date for various coffee berries development stages.

Developmental stage			
	Bean formation (S1) (13^{th}-20^{th} week)	Dry matter accumulation (S2) (21^{st}-27^{th} week)	Ripening (S3) (28^{th}-34^{th} week)
N^{th} Week for spraying after flowering	1^{st} spray = 15^{th} week 2^{nd} spray = 18^{th} week	1^{st} spray = 23^{rd} week 2^{nd} spray = 26^{th} week	1^{st} spray = 28^{th} week 2^{nd} spary = 31^{st} week

Toxicity and Ovicidal Activity of Some Botanicals against Antestia Bug, *Antestiopsis intricata* (Ghesquière & Carayon)

E. MENDESIL, C. ABDETA

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

SUMMARY

The experiment was conducted to investigate the toxicity and ovicidal activity of crude extracts of five botanicals to adult, nymph and egg of Antestia bug, *Antestiopsis intricata* (Ghesquière & Carayon) (Hemiptera: Pentatomidae) at a concentration of 5% w/v and 10% w/v, respectively. All the extracts of the botanicals tested were toxic to the adult and nymph of *A. intricata* (57.75-83.85% & 15-79.42% cumulative mortality, respectively). The extracts also inhibited egg hatching of the insect (19.29-47.43% egg mass hatch). Among the botanicals tested, extracts of *Milletia ferruginea* (Hochest) Baker and *Chrysanthemum cinerariaefolium* L. revealed promising result for the control of *A. intricata*. The potential practical use of botanicals for the control of *A. intricata* in Ethiopia is discussed.

INTRODUCTION

Antestia bug, *Antestiopsis intricata* (Ghesquière & Carayon) (Hemiptera: Pentatomidae) is the most serious pest of coffee in many African countries, which causes a considerable amount of crop loss (Le Pelley, 1968). It is a sucking insect, which causes blackening of flower buds, fall of immature berries, rotting and zebra stripping of beans and shoot damage (Le Pelley, 1968). In Ethiopia, it is the major pest of coffee, which can inflict about 9% crop loss (Mekuria et al., 1994). In addition, it contributes up to 44% darkened coffee beans, which affect coffee quality (IAR, 1996). The damage posed by this insect warrants the search for proper pest management methods.

In recent decades, intensive use of chemical insecticides to control insect pests has led to various problems such as insecticide resistance, pest resurgence, effect on non target organisms, health problem and environment pollution (Thomas and Waage, 1996). Moreover, an increase in price of chemical insecticides and fall of coffee price makes the use of chemical insecticide uneconomical (Nyambo et al., 1998). Such problems coupled with an increasing demand for organic coffee has directed research to the development of alternative pest control strategies. Use of botanical insecticides has attracted increasing attention as alternative to chemical insecticides for pest management. Several species of botanicals have shown promising result for the control of various insect pests of important crops (Stoll, 2000; Isman, 2006). However, the use of natural products to control coffee pests is the least researched and used approach (Nyambo et al., 1998). The objective of the present study is to evaluate the efficacy of crude extracts of five botanicals against egg, nymph and adult of *A. intricata*.

MATERIALS AND METHODS

Mass rearing of test insects

The initial stock of *A. intricata* was obtained from insect rearing of Entomology section, Jimma Agricultural Research Center (JARC). The insects were reared in a glass cage (20.5 cm x 26.7 cm x 7.6 cm) covered with nylon mesh. Fresh coffee twigs bearing large green coffee berries, which is the preferred food of Antestia bug (Le Pelley, 1968) were provided for the insect at 2-3 day intervals.

Plant material

The species and plant parts used for the experiment are shown in Table 1. All the plants were collected around JARC. The plants were dried under shade and then ground to a fine powder using pestle and mortar. The powder of each botanical was soaked in distilled water at the rate of 5 g 100 ml^{-1} and 10 g 100 ml^{-1} for 24 h. Then after, the powder of the different botanicals was filtered by cheese cloth.

Table 1. List of botanicals, growth form and plant parts used for the bioassay.

Species	Family	Growth form	Parts used
Milletia ferruginea (Hochest) Baker	Fabaceae	Tree	Seed
Chenopodium ambrosioides L.	Chenopodiaceae	Herb	Leaf & Inflorescence
Chrysanthemum cinerariaefolium L.	Compositae	Herb	Flower
Myrsine africana L.	Myrsinaceae	Shrub	Leaf
Ekberga sp.	Meliaceae	Tree	Leaf

Bioassay

From the rearing cage, ten newly emerged adults and nymphs were randomly selected and kept in a cage separately as described above. Insects in the cage were sprayed with 5% w/v crude extracts of each botanical to the point of run off using small hand sprayer. Control insects were sprayed with distilled water. Treatments were arranged in completely randomized design with three replications. Number of dead insects was recorded at 12 h and 24 h after treatment application. Cumulative percentage mortality was used for the analysis. To study the ovicidal activities of botanicals, egg masses (12-18 h old) were obtained from insect culture. Botanicals were applied by dipping each egg mass into 10 ml of each extract at a concentration of 10% w/v. Control eggs were dipped into sterile distilled water. There were three replicates of 12 egg masses per replicate for each botanical tested. After treatment, eggs were transferred to Petri dishes lined with filter paper. The Petri dishes were incubated at room temperature under a photo period of L:D 12:12 h. Number of hatched and un hatched were recorded and percent egg mass hatch was determined.

Data analysis

Data were analyzed using MSTAT-C computer program. Means were separated using Duncan's Multiple Range Test (DMRT) at (P= 0.05). Analysis of insect mortality was done based on square root transformation.

RESULTS AND DISCUSSION

All the extracts of the botanicals were found to be toxic to *A. intricata* compared to un treated control, but the toxicity varied among the botanicals tested. *Milletia ferruginea* (Hochest) Baker and *C. cinerariaefolium* L. induced significantly ($P < 0.05$) the highest mean percentage of 83.85 and 78.73 adult mortality, respectively at 12 h and 24 h after treatment (Table 2). Similarly, *M. ferruginea* (Hochest) Baker and *C. cinerariaefolium* L. caused significantly ($P < 0.05$) the highest respective mean percentage nymph mortality of 77.71 and 79.42 at 12 h and 24 h after treatment (Table 3). As shown in Table 4, all the extracts tested had a significant effect on egg hatching of *A. intricata*. Percentage hatch in the treated egg mass ranged from 19.29-47.43%, while it was 94% in the control treatment. Extracts of *M. ferruginea* (Hochest) Baker and *C. ambrosioides*, *Ekberga* sp. and *M. africana* L. significantly ($P < 0.05$) inhibited egg hatching of *A. intricata*, showing that these botanicals have ovicidal activity on the eggs of the insect.

Table 2. Effect of crude extracts of five botanicals on adult mortality of *A. intricata*.

Treatment	Mean percent mortality		
	12 h	24 h	Cumulative mortality
Milletia ferruginea (Hochest) Baker	83.85	0.00	83.85 a
Chenopodium ambrosioides L.	48.93	15.00	63.93 abc
Chrysanthemum cinerariaefolium L.	55.37	23.36	78.73 ab
Myrsine africana L.	30.00	36.85	66.85 abc
Ekberga sp.	48.93	8.85	57.78 bc
Control (Water)	0.00	6.15	6.15 c
CV (%)			20.07

Means followed by the same letter (s) within a column are not significantly different at P < 0.05, DMRT.

Table 3. Effect of crude extracts of five botanicals on nymph mortality of *A. intricata*.

Treatment	Mean percent mortality		
	12 H	24 H	Cumulative Mortality
Milletia ferruginea (Hochest) Baker	60.00	17.71	77.71 A
Chenopodium ambrosioides L.	27.29	28.77	56.06 B
Chrysanthemum cinerariaefolium L.	52.86	26.56	79.42 A
Myrsine africana L.	8.85	6.15	15.00 C
Ekberga Sp.	37.22	6.15	43.37 B
Control (Water)	0.00	6.15	6.15 C
Cv (%)			22. 62

Means followed by the same letter (s) within a column are not significantly different at P < 0.05, DMRT.

The result of the present study revealed that, *M. ferruginea* (Hochest) Baker and *C. cinerariaefolium* L. showed a promising result for the control of *A. intricata* under laboratory condition. According to the investigation of Bakele (1988), toxicity of *M. ferruginea* may be attributed to rotenone, which is a well known botanical insecticide with a rat oral LD_{50} = 132-1500 mg kg^{-1} through contact and stomach poisoning. The crude pyrethrum, *C. cinerariaefolium* L. contains about 30-35% pyrethrins, which is the active insecticidal component of pyrethrum. Oral LD_{50} of pyrethrins in rats range from 200 mg/ kg to greater than 2,600 mg/kg (Bennett, 2001). Many plant extracts have shown promising results for the control of insect pests of important crops. For example, fruit extracts of *Melia azedarach* L.,

Phytolacca dodecandra L. and *Schinus molle* L. for the control of maize stalk borer, *Busseola fusca* (Assefa and Ferdu, 1999), crude seed extracts of *Annona squamosa* against diamondback moth, *Plutella xylostella* L. (Leatemia and Isman, 2004), extracts of *Gmelina arborea* L. products for the control of legume flower bud thrips, *Megalurothrips sjostedti* (Oparaeke, 2006). However, there have been few efforts to use insecticidal plants for the control of insect pests of coffee. The available information include, use of neem extract for the control of green scale of coffee (Kumar et al., 1989), pyrethrum to control coffee bugs, *Lygus* spp. (Stoll, 2000), neem formulations against coffee berry borer (Sreedharan et al., 2001).

Table 4. Susceptibility of *A. intricata* eggs to crude extracts of five botanicals.

Treatment	Mean percent egg mass hatch
Milletia ferruginea (Hochest) Baker	19.29 b
Chenopodium ambrosioides L.	29.72 b
Chrysanthemum cinerariaefolium L.	47.43 ab
Myrsine africana L.	38.65 b
Ekberga sp.	31.88 b
Control (Water)	94.00 a
CV (%)	30.24

Means followed by the same letter (s) within a column are not significantly different at P < 0.05, DMRT.

The results presented in this study demonstrated the potential of using crude extracts of *M. ferruginea* (Hochest) Baker and *C. cinerariaefolium* L. for the control of adult and nymph of *A. intricata*. Moreover, the extracts of all the botanical tested showed ovicidal effect on the eggs of *A. intricata* as confirmed by a significant reduction in egg hatching. *Milletia ferruginea* (Hochest) Baker is endemic tree in Ethiopia and it is one of the important shade trees of coffee (Yacob et al., 1996).The other promising botanicals are also easily available and can be used by subsistence farmers for the control of *A. intricata* on Arabica coffee. Further investigations should be conducted on the chemical composition of the promising botanicals and method of formulation of the active plant materials for field application.

ACKNOWLEDGMENTS

The experiment was financially supported by Ethiopian Institute of Agricultural Research. The authors are grateful to Mr. Teklemariam Erge for his assistance during the experiment.

REFERENCES

Assefa G. and Ferdu A. 1999. Insecticidal activity of chinaberry, endod and pepper tree against the maize stalk borer (Lepidoptera: Noctuidae) in southern Ethiopia. International Journal of Pest Management 45: 9-13.

Bekele A. 1988. Investigation of flavonoids from Birbira. MSc thesis, Addis Ababa University.

Bennett, S. M. 2001. Pyrethrum. http://www.the-piedpiper.co.uk/th13(n).htm

IAR. 1996. Jimma Agricultural Research Center progress report for the period 1986-1991 Part I: Coffee. IAR, Jimma, 139 pp.

Isman, M.B. 2006. Botanical insecticides, detterents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 51: 45-66.

Kumar, M.G., Bhat, P.K. and Vijayalakshmi, C.K. 1989. Screening of kerosen and neem extract against coffee green scale. Journal of Coffee Research 19: 51-55.

Le Pelley, R.H. 1968. The Pests of Coffee. London, UK, Longman, 590 pp.

Leatemia, J.A. and Isman, M.B. 2004. Efficacy of crude seed extracts of *Annona squamosa* against diamondback moth, *Plutella xylostella* L. in the green house. International Journal of Pest Management 50: 129-133.

Mekuria T., Million, A, and Teklemariam E. 1994. Antestia bug as a determining factor of coffee berry fall and yield loss at Tepi state farm. In: Eshetu B., Yitbarek S., Tibebu H., Mengistu K. & Kasahun M. (Eds.). Proceedings of the first annual conference of crop protection society of Ethiopia, 14-15 March 1993, Addis Ababa, Ethiopia, P. 24.

Nyambo, B., Murphy, S.T, Baker, P., Waller, J. 1998. Biocontrol in coffee pest management. In: Sain, R.K. (Ed). Proceedings of the 3rd International conference on Tropical entomology. 3-4 November 1994. Nairobi, Kenya, pp. 27-45.

Oparaeke, A. M. 2006. Studies on insecticidal potential of extracts of *Gmelina arborea* L. products for insect pests control on cowpea. 1. The legume flower bud thrips, *Megalurothrips sjostedti* Trybom. Archives of Phytopathology and Plant Protection 39: 209-214.

Sreedharan, K., Vinod Kumar, P.K., Prakasan, C.B. (Eds.). 2001. Coffee Berry Borer in India. Central Coffee Research Institute, Codeword Process and Printers, Mangalore, India, 112 pp.

Stoll, G. 2000. Natural crop protection in the tropics, Margraf Verlag, 376 pp.

Thomas, M., Waage, J. 1996. Integration of biological control and host plant resistance breeding. A scientific and literature review. CTA, The Netherlands, 99 pp.

Yacob E., Tesfaye S., Alemseged Y., Taye K., Anteneh N. and Takele N. 1996. Advances in Coffee Agronomy Research in Ethiopia. In: J. S. Tenywa, Adipala Ekwamu & M. W. Ogengu-Latigo (Eds). Proceedings of Inter-Africa Coffee Organization (IACO) Workshop. 4-6 September, 1995, Kampala, Uganda, pp. 40-55.

Efficacy of Copper Sprays in the Management of Coffee Leaf Rust in Kenya

G. M. KAIRU

Coffee Research Foundation, P.O. Box 4, Ruiru, 00232, Kenya. Email: gmkairu@yahoo.com

SUMMARY

In a two-year field evaluation, sprays of Chemcopp 50% WP and Agcopp 75% WP formulations of cuprous oxide (American Chemet Exp. Corp.) each applied at the rate of 0.22% controlled coffee leaf rust as effectively as the standard sprays of Nordox 50% WP (0.35%); Nordox 75% WP (0.22%) and Cobox 50% WP (0.35%). The implications of the results in coffee production in Kenya are discussed.

INTRODUCTION

The occurrence of coffee leaf rust (*Hemileia vastatrix* Berk. & Br.) was first recorded in Kenya in 1913 (Rayner, 1960). It is one of the three major diseases affecting coffee (*Coffea arabica* L.) in Kenya. The disease is characterised by round, scattered, orange pustules on the lower surface of leaves (Figure 1a). Affected leaves are shed prematurely and branches, often carrying a heavy crop, are left with scanty or no leaves at all (Figure 1b). Severe leaf rust epidemics are known to have stopped the growing of Arabica coffee in parts of the world, especially, in South-East Asia. This was before the discovery and availability of the first copper fungicide: Bordeaux Mixture in 1887 (Rayner, 1960). Over the years, coffee leaf rust has been kept under control in Kenya by use of various copper fungicides (Kingori and Masaba, 1994). New copper formulations are evaluated for efficacy against coffee leaf rust following established procedures (Javed, 1979).

(a) (b) (c)

Figure 1. (a) Coffee rust on leaves; (b) Coffee tree defoliated by rust; (c) Healthy coffee trees.

MATERIALS AND METHODS

Efficacy trials with new copper formulations were conducted at Jumapili Estate during 2000/2001 and repeated again at the Coffee Research Foundation-Azania Estate during 2003/2004. The treatments are as shown in Table 1. These were laid down in randomised complete blocks of 25-tree plots replicated four times. Sprays were applied using motorised knapsack sprayers at 3-week intervals following an established protective spray programme (Griffiths, 1970). The per cent incidence of leaf rust was assessed on seventy leaves sampled

randomly from each of the nine central trees per plot. The crop was picked regularly as it ripened and yield per plot determined.

RESULTS

The incidence of coffee leaf rust at Jumapili Estate trial attained a peak of 16.47% towards the end of the long-rains season in the year 2001 (Figure 2). Sprays of Chemcopp at the rate of 0.22% resulted in a significant ($P \leq 0.05$) control of leaf rust compared to the unsprayed (control) plots (Table 1). This effect was the same as that of the standard sprays of Nordox 50% & 75% WP applied at 0.35% & 0.22% respectively and Cobox 50% WP (0.35%) (Table 1).The effect of Chemcopp and Agcopp treatments on crop yield was satisfactory (Table 1).

The incidence of coffee leaf rust at the Azania Estate trial attained a peak of 11.83% after the long rains of 2004 (Figure 3). Sprays of Chemcopp (0.22% & 0.35%) and Agcopp (0.2% & 0.22%) controlled leaf rust significantly ($P \leq 0.05$) compared to the unsprayed (control) plots (Table 1). This effect was comparable to that of the standard sprays of Nordox 50% WP (0.35%); Nordox 75% WP (0.22%) and Cobox (0.35%) (Table 1).

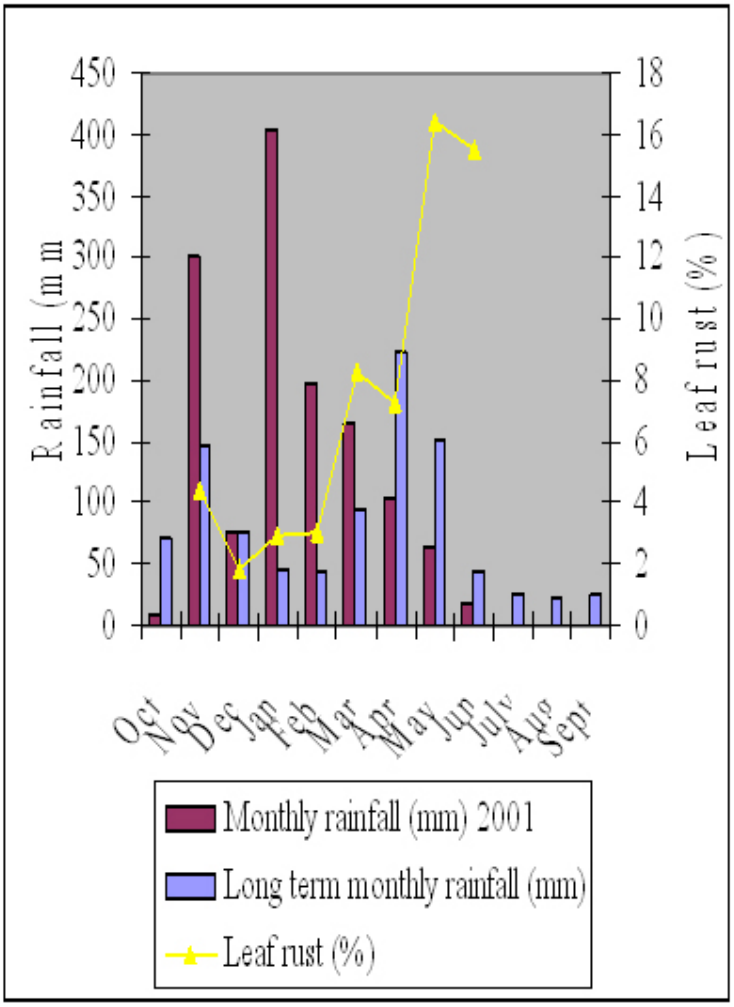


Figure 2. The incidence of leaf rust and monthly rainfall totals at the Jumapili trial during 2000/2001.

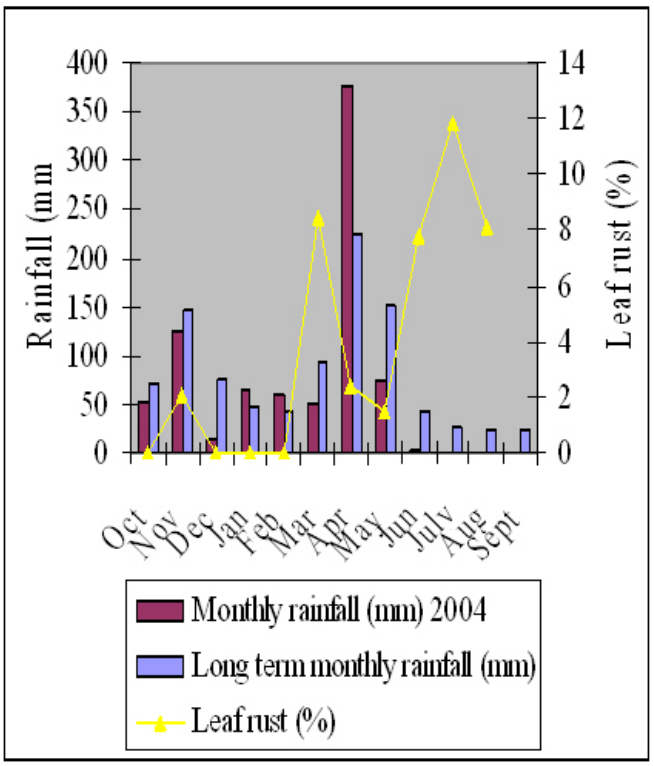


Figure 3. The incidence of leaf rust and monthly rainfall totals at the Azania trial during 2003/2004.

Table 1. Performance of Chemcopp 50% WP and Agcopp 75% WP sprays against coffee leaf rust.

	Jumapili site			Azania site
Treatment	**Rate (%)**	**Peak % Leaf Rust 31.05.2001**	**Yield (kg/ha) clean coffee**	**Peak % Leaf rust 16.07.2004**
Unsprayed (control)	-	16.47 A	585 CD	11.83A
Nordox50 %	0.35	6.83 AB	539D	0.71B
Nordox 75%	0.22	6.39 B	748 A	0.84B
Chemcopp 50%	0.35	8.50 AB	606 BC	0.40B
Chemcopp 50%	0.22	5.91B	670 B	0.60B
Agcopp 75%	0.22	5.72 B	586CD	0.48B
Agcopp 75%	0.2	7.58 AB	617 C	0.52B
Cobox 50%	0.35	5.12 B	588 C	0.63B
CV (%)		13.54	31.60	26.32

DISCUSSION AND CONCLUSION

The incidence of coffee leaf rust at Jumapili and Azania trial sites was fairly low compared to the high levels (60% to 92%) recorded in the past from various sites in the same agroecological zone (Wallis and Firman, 1962; Javed, 1981). The development of leaf rust epidemic was slowed down by the variable and inconsistent rainfall which prevailed during 2000/2001 & 2003/2004 periods as can be seen in Figures 2 and 3. Dispersal, deposition, and germination of uredospores on leaf surfaces in the field occurs entirely in rain water. Interruption of wet periods by dry spells results in inhibition of further germination of

uredospores thus curtailing the number of infections. Inspite of relatively low incidence of rust, good control was achieved by all copper treatments ranging from 48% to 69% at the Jumapili site in2001 and 93% to 97% at the Azania site in 2004. This reduction effect was of long-term strategic value in the management of coffee leaf rust. The outbreak of an epidemic has been found to depend on the amount of rust present the previous year (Rayner, 1962).

Copper formulations make effective fungicides through release of copper ions (Cu++) into the spray solution. The copper ions in spray droplets kill uredospores through contact and subsequent binding to the sulphyhydryl group of certain amino acids resulting in denaturation of proteins and enzymes (Agrios,1988).This mode of action implies that the effectiveness of a formulation would depend on how readily the copper ions are released onto the target. This is dependent on a number of factors: (a) Spray application techniques by which spray droplets are directed to the lower surface of the leaves where the rust infection takes place through the stomata; (b) Weather, particularly rainfall amounts and distribution; (c) Formulation technology which determines the physical-chemical properties of the new formulation. Commercial copper fungicides differ in the composition of their formulation. The amount of the active ingredient ; the proportions and quality of the innert material (carriers, dispersants, spreaders and stickers) determine to a large extent the degree of efficacy of a copper formulation. Against this background, Chemcopp 50% WP formulation applied at the rate of 0.22% gave the same level of rust control as the 0.22% sprays of Agcopp 75% WP formulation which contains one and a half times more copper.

The main objective in the management of coffee leaf rust, like any other disease, is to maximise on crop yield. Loss of leaves caused by rust has an indirect negative effect on the crop. This effect is usually carried forward from one season to the next as the carbohydrate budget of the tree becomes constrained with time. It is therefore, not unusual for copper treatments to give the same yields as the unsprayed treatment in one season, inspite of effective control of rust as observed at the Jumapili trial. In addition to leaf rust control, copper fungicides are also known to have a `tonic' effect characterized by better growth of coffee trees and increased yield (Griffiths,1972). This implies that coffee trees treated regularly with copper fungicides are likely to give a more enhanced yield in the longer term because of pathogen suppression and tonic effect.

On the basis of the two-year data obtained from these field trials, it was concluded that sprays of Chemcopp 50% WP and Agcopp 75% WP each applied at the rate of 0.22% were consistently effective in managing coffee leaf rust and were thus recommended for use by coffee growers in Kenya.

REFERENCES

Agrios, G. N. (1988). Mechanisms of action of chemicals used to control plant diseases. *Plant Pathology*, Third Edition, Academic Press, pp 224.

Griffiths, E. (1972). Negative effects of fungicides in coffee. *Tropical Science*,**14**:79-89

Javed, Z.U.R.(1979). Established procedures for laboratory and field screening of new fungicides for control of coffee diseases in Kenya. *Kenya Coffee*, **44**: 11-19.

Javed, Z.U.R.(1981). Field trials with new and recommended fungicides for leaf rust Control during 1979. *Kenya Coffee*, **46**: 19-24.

Kingori, P.N. & D.M. Masaba (1994). Current status of coffee rust (*Hemileia vastatrix*) in Kenya. *Kenya Coffee*, **59**: 1877-1887.

Rayner, R.W. (1960). Rust disease of coffee: Spread of the disease. *World crops,* **13**: 222-224

Rayner, R.W. (1962). The control of coffee rust in Kenya by fungicides. *Annals of applied Biology,* **50**: 245-261.

Wallis, J.A.N. & I.D. Firman (1962). Spraying arabica coffee for the control of leaf rust. *East African & Forestry journal*, **27**: 89-104.

Griffiths, E. (1970). Control of coffee berry disease and leaf rust in 1970. *Kenya coffee*, **35:** 45-46.

Biological Control of *Melodogyne incognita* on Coffee Plants using the Isolates P10 of *Pasteuria penetrans*

R. M. D. G. CARNEIRO, L. F. G. MESQUITA, P. A. S. CIROTTO, F. C. MOTA AND M. R. A. ALMEIDA

EMBRAPA Recursos Genéticos e Biotecnologia, C.P. 02372, 70849-970, Brasília, DF., Brazil

SUMMARY

Root-knot nematodes (RKN), *Meloidogyne* spp., are widely distributed in coffee plantations in Brazil, where they cause great losses to the coffee farmers and economy of the country**.** Recent surveys on coffee plantations have shown *M. exigua* in 100% of infected samples from Minas Gerais State and a substantial increase in *M. paranaensis* and *M. incognita* (races 1, 2, 3 and 4) in Paraná and São Paulo States. *M. incognita* is the most important species in São Paulo State. Public concern over the toxicity of nematicides and their impact on the environment has focused research on alternative methods to control plant-parasitic nematodes. *Pasteuria penetrans* (Thorne) Sayer & Starr, an obligate hyperparasite of root-knot nematodes (RKN) has for many years shown itself to be a very promising biological control agent. Isolates of *P penetrans* from different geographical areas were tested for the ability of the endospores to attach to second-stage juveniles and to colonize females of different populations of *Meloidogyne* spp. The bacterium isolate P10 was selected to control the four races of *M. incognita* from coffee. This work aimed to assess the potential of the isolate P10 of *P. penetrans* to control *M. incognita* race 2 on coffee over a period of two years and to improve our understanding of the factors affecting its efficacy as a biological control agent. The isolate P10 of *P. penetrans* was evaluated in greenhouse conditions, using two doses of a bionematicide powder (BP) on seedlings of 'Mundo Novo' coffee: 10^7 endospores (5.0 g BP/seedling) and 10^6 endospores (0.5 g BP/seedling). Coffee seedling substratum was treated previously with these two doses of *P. penetrans* and after 2 months the plants were cultivated in soils presenting different textures: clay-sandy-soil (38% of clay, 2% of silt and 60% of sand) and sandy-soil (17% of clay, 0% of silt and 83% of sand). When the coffee plants were 30 cm high, they were inoculated with 20,000 eggs/plant of *M. incognita* race 2. The coffee plants were evaluated 8, 16 and 24 months after nematode plant infestation. The biological control effectiveness was evaluated by the reduction of egg numbers per root system, ranging from: 60% (0.5 g in clay-sandy-soil), 70% (5.0 g in clay soil) and 80 % (0.5 g and 5.0 g in sandy soil). The suppressive mechanism caused by the bacterium was evaluated by the percentage of infected second-stage juveniles (J2), number of endospores attached /J2 and number of infected females. The high suppression rates were related to the time increasing from 8 to 24 months and to the percentage of sand in the soil. No effect of bacterium dose was observed after 24 months of experiment. Future studies in field conditions will be essential to confirm the potential of *P. penetrans* to control *M. incognita* on coffee.

INTRODUCTION

Root–knot nematodes (RKN), *Meloidogyne* spp., are widely distributed in coffee plantations in Brazil, where they cause great losses to the coffee farmers and economy of the country**.** Recent surveys on coffee plantations have shown *M. exigua* in 99 % of infected samples from Minas Gerais State and a substantial increase in *M. paranaensis* and *M. incognita* (races 1, 2,

3 and 4) in Paraná and São Paulo States (Campos and Villain, 2005). *M. incognita* is the most important species in São Paulo State (Carneiro et al., 2005). Public concern over the toxicity of nematicides and their impact on the environment has focused research on alternative methods to control plant-parasitic nematodes (Thomason, 1987). *Pasteuria penetrans* (Thorne) Sayer & Starr, an obligate hyperparasite of root-knot nematodes (RKN) has shown to be a very promising biological control agent for many years (Stirling, 1991; Chen and Dickson, 1998). The role of this bacteria in suppressing plant-parasitic nematodes has been tested on many crops, mostly in greenhouse pot test (Chen and Dickson, 1998). Recent studies proposed that in natural infection, mutual selection produces a dynamic balance between *P. penetrans* and the RKN whereby levels of infection are rarely suppressive. However, the introduction of an exotic isolate of *P. penetrans* with a different attachment profile can disturb this balance, resulting in a greatly increased proportion of infected J2 and females, increased yields of endospores and more suppression of RKN populations (Trudgill et al., 2000). *Pasteuria penetrans* isolates from different geographical areas were tested for the ability of endospores to attach to second–stage juveniles and to colonize females of different populations of *Meloidogyne* spp. Using these parameters, it was possible to select the bacterium isolate P10 for the four races of *M. incognita* from coffee (Carneiro et al., 2004). This work aimed at assessing the potential of the isolate P10 of *P. penetrans* to control *M. incognita* race 2 on coffee in greenhouse conditions over a period of two years, determining the efficacy of two bacterium endospore doses, and improving our understanding of the factors affecting its efficacy as a biological control agent.

MATERIAL AND METHODS

An isolate of *P. penetrans* designated as P10 (Carneiro et al., 2004) originating from *M. incognita* parasitizing banana plants in Imperatriz, State of Maranhão, Brazil was propagated on *M javanica* growing on tomato (*Lycopersicon esculentum* Mill. cv. Santa Cruz). The preparation of the bacterium powder was produced by grinding air-dried tomato roots infested with diseased females of *M.incognita* (Stirling and Wachtel, 1980).

Seedlings of coffee cv. Mundo Novo (highly susceptible to *M. incognita* race 3) were produced in loamy–clay soil: 31% of clay, 46% of silt and 23% of sand (Carneiro et al., 2003), previously mixed with the bacterium powder, containing two different doses of *P. penetrans*: 10^6 endospores/seedling (0.5 g) and 10^7 endospores/seedling (5 g). After two months, the seedlings of coffee were transplanted with the substratum for soils of different textures: clay-sandy soil (38% of clay, 2% of silt and 60% of sand) and sandy soil (17% of clay, 0% of silt and 83% of sand). The experiment was arranged in a randomized complete block design with a total of six blocks: two controls (without the bacteria) in clay-sandy soil and sandy soil, two treatments inoculated with 5.0 g of the bacterium in clay-sandy soil and sandy soil, two treatments inoculated with 0.5 g of the bacteria in clay-sandy soil and sandy soil. When the coffee plants reached 30 cm in length, they were inoculated with 20,000 eggs of *M. incognita* race 2 (esterase phenotype I1). Each treatment was replicated 30 times, and evaluated 8, 16 and 24 months after the plant's inoculation with the nematode (total of 180 pots). The following parameters were evaluated: height of coffee plants, fresh weight of coffee roots, infection levels caused by *M.incognita* in roots and in the soil (Hartman & Sasser, 1985; Jenkins, 1964). The suppressive mechanism caused by the bacterium was evaluated by the percentage of infected second-stage juveniles (J2) extracted from soil and counted in Peter slides, means of the number of endospores attached in 50 J2 and percentage of infected females (50) extracted from coffee roots using the technique described by Carneiro et al. (2004).

RESULTS AND DISCUSSION

The two parameters, height of the plants and fresh weight of the roots, were not related to the effect of the bacterium in the different treatments This is due to the constant manuring of the coffee during the two years of bioassay. The fertilization provided a good development of the plants included in the control inoculated with *P. penetrans* (Table 1).

Considering now the reproduction factors (RF = final population/20.000 eggs), it is possible to observe that the control presented a high number of RF when compared with the treatments with the bacteria in two doses (10^7 endospores or 5.0 g BP/seedling and 10^6 endospores or 0.5 g BP/seedling), showing that the effect of the bacteria reduced the population of *M. incognita*, after 16 and 24 months of inoculation (Table 1, Figure 1). The reduction of *Meloidogyne* population can be estimated at about 70% for the dose 5.0 g, in the clay-sandy soil and at about 80% for the same dose in the sandy soil. For the dose 0.5 g, the reduction was of about 60% in the clay-sandy soil and 80% in the sandy soil (Table 1). The best control of root-knot nematodes by *P. penetrans* on sandy soil texture has already been demonstrated by many authors mentioned by Freitas and Carneiro (2000). Although *P. penetrans* has been detected in several soil types, the suppressivity occurs especially in sandy soils due to the larger movement of J2, than in clay soils, increasing the possibility of contact between the second-stage juveniles and endospores (Mateille et al., 1995).

When the two doses of *P. penetrans* are compared, a small effect of the doses (0,5 and 5,0 g) of the bacteria can be observed in RF of *M.incognita*, after 8 months. This effect disappeared after 16 and 24 months (Table 1). Considering the number of endospores/J2, the effect of the doses of the bacterium diluted over time. After 24 months the initial dose did not interfere in the suppressivity caused by the bacteria. A small effect of the dose can be observed in the number of infected females (Table 1).

Considering now the percentage of infected J2, this parameter varied little over the 24 months. Practically, all the J2 were infected in the different treatments in the three evaluation periods. However, a significant increase in the number of endospores/J2 was observed between the first (8 months) and the last evaluation (24 months). J2 that presented 6-20 endospores in the first 8 months, presented 60-70 endospores or more after 24 months of contact with the bacteria, showing the increase of the supressivity induced by the bacteria (Table 1). The same occurred with the infected females with a significant increase of this parameter occurring over the24 months. The number of spores attached /J2 has been related to the number of endopores present in the soil, and it is the most interesting parameter to measure the degree of supressivity of a soil (Freitas et al., 2000). This parameter has also been related to the reduction of infectivity of J2 in the roots. Second-stage juveniles with 15 endospores reduced the percentage of penetration in the roots by 86% (Davies et al., 1998).

Considering now the texture of soil, the percentage of infected females increased with the percentage of sand. The results in clay-sandy soil were also satisfactory, although the supressivity indexes were slightly lower.

The obtained results were very promising, because they showed high levels of control of the nematode and increase in the suppressivity of the bacteria over time. Future studies in field conditions will be essential to confirm the potential of *P. penetrans* to control *M. incognita* on coffee.

Table 1. Effect of two doses of *Pasteuria penetrans* (Pp) isolate P10: 10^6 and 10^7 endospores (0.5 and 5.0 g/seedling) to control *Meloidogyne incognita* on coffee cv. Mundo Novo, cultivated in two different soils: clay-sand soil (clay soil) and sandy soil.

Treatments	Plant Heights	Fresh weight of roots	Gall rating index	Egg-mass rating index	Number of eggs	RF[c]	% of J2 infected	Endo-spores/ J2 rating index[b]	% of females infected
First evaluation : 8 months after nematode inoculation									
Control-Clay Soil	0.90	99.7	5	5	179.500	8.98a*	0	0	0
Control Sandy soil	0.82	106.6	5	5	160.990	8.05a	0	0	0
Pp. 5 g clay soil	0.80	126.0	5	5	97.540	4.87c	81	2.0	47
Pp. 0.5 g clay soil	0.90	145.0	5	4	124.160	6.21b	98	2.62	27
Pp. 5 g sandy soil	0.74	109.0	5	5	101.710	5.07c	91	2.12	60
Pp. 0.5 g sandy soil	0.90	159.0	5	5	127.550	6.38b	99	2.50	38
Second evaluation: 16 months after nematode inoculation									
Control-clay soil	1.29	206.2	5	5	528.083	26.4a	0	0	0
Control sandy soil	1.26	152.4	5	5	541.250	27.1a	0	0	0
Pp. 5 g clay soil	1.46	206.3	5	5	165.041	8.3b	99	3.04	70
Pp. 0.5 g clay soil	1.21	175.0	5	5	205.708	8.8b	96	2.25	66
Pp. 5 g sandy soil	1.35	182.1	5	4	64.750	4.2c	93	3.93	84
Pp.0.5 g sandy soil	1.24	157.0	5	5	99.375	5.0c	94	2.32	72
Third evaluation : 24 months after nematode inoculation									
Control-clay soil	1.59	354.1	5	5.0	527.520.6	26.4 a	0	0	0
Control sandy soil	1.70	288.6	5	5.0	572.178.9	28.6 a	0	0	0
Pp. 5 g clay soil	1.61	346.8	4.9	4.9	174.631.4	8.7 b	98	4.75	88
Pp. 0.5 g clay soil	1.56	407.6	4.9	4.9	201.120.6	10.1 b	97	4.93	69
Pp. 5 g sandy soil	1.75	304.7	4.2	3.9	84.473.0	4.2 c	100	5.03	94
Pp. 0.5 g sandy soil	1.55	308.6	4.5	3.4	114915.4	5.7 c	99	5.70	84

*[a] – Gall and egg-mass rating index number, according to the scale of Taylor & Sasser, 1978. [b]The number of endospores/ second-stage juveniles (J2)according to following scale: 0 → zero endospore/j2; 1 → 1-5 endospores/J2; 2 → 6-20 endopores/J2; 3 → 21-40 endospores/J2; 4→ 41-60 endospores/J2; 5 → 61-100 endospores/J2 e 6 → more than 101 endospores/J2. [c]FR=Reproduction Factor: final population/ initial population. *Means of different treatments of each time followed by the same letter are not different according to Scott & Knet test ($P \leq 0.05$).*

Figure 1. Roots of two years old coffee plants cv. Mundo Novo infected with *Meloidogyne incognita* and treated or not with two doses of the bacterium *Pasteuria penetrans*. A) Control presenting galls and corky root symptom; B) Plants treated with the bacterium (0.5 g/plant); C) Plants treated with the bacterium (5.0 g/plant).

REFERENCES

Campos, V.P., Villain, L. (1995). Nematode parasites of coffee, cocoa and tea. In: Luc, M., Sikora, R.A. and Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture.* CAB International, Wallingford, UK, pp. 529-579.

Carneiro, R.M.D.G., Randig, O., Almeida, M.R.A. and Gonçalves, W. (2005). Identification and Characterization of *Meloidogyne* spp. on coffee from São Paulo and Minas Gerais States using esterase phenotype and Scar multiplex. Nematologia Brasileira 29 (2):233-241.

Carneiro, R. M.D.G., Tigano, M.S., Jorge, C. L.,Teixeira, A.C.O. & Cordeiro, M.C. (2004). Selection and polymorphism of *Pasteuria penetrans* isolates in relation to *Meloidogyne* spp. from coffee. *Nematology:* 6: 37-47

Carneiro, R.M.D.G, Neves, D. I. & Mesquita, L.F.G. (2003). Influência de diferentes substratos na percolação de endósporos de *Pasteuria penetrans* em mudas de cafeeiro. *Nematologia Brasileira* 27(2): 215-218.

Chen, Z.X and Dickson, D.W. (1998). Review of *Pasteuria penetrans:* biology, ecology, and biological control potential. *Journal of Nematology* 30, 313-340.

Davies, K.G., Flynn, C.A. and Kerry, B.R. (1988). The life-cycle and pathology of the root-knot nematode parasite *Pasteuria penetrans*. In: *Brighton Crop Protection Conference-Pest and Diseases*, Brighton, UK. Proceedings Farnham: British Crop Protection Council . v.3, pp. 1221-1226..

Freitas, L.G. & Carneiro, R.M.D.G. (2000). Controle Biológico de Fitonematóides pos *Pasteuria penetrans.* In:Melo, I.S. and Azevedo, J.L. (Eds). IN: *Controle Biológico*, Embrapa Meio Ambiente, Jaguariúna, S.P., Brasil, pp.91-125.

Freitas, L.G; Dickson, D.W.; Mitchell, D.J. and Mcsorley, R.. 2000. Suppression of *Meloidogyne arenaria* by *Pasteuria penetrans* in the field. *Nematologia Brasileira*. 24:147-156.

Hartman, K.M. and Sasser, J.N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: Barker, K.R.; Carter, C.C. and Sasser, J.N., (Ed). *An advanced treatise on Meloidogyne*. Vol. II Methodology. pp. 69-77.

Jenkins, W.R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter,* 48:692.

Mateille, T., Duponnois, R., Diop, M.T. (1995). Influence of abiotic soil factors and the host plant on the infection of phytoparasitic nematodes of the genus *Meloidogyne* by the actinomycete parasitoid *Pasteuria penetrans* . *Agronomie*. 15:581-591.

Stirling, G.R. and Wachtel, M.F. (1980). Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. *Nematologica* 26: 308-312.

Stirling, G.R. (1991). Biological control of plant-parasitic nematodes: progress, problems and prospects. Wallingford, UR, CAB International, 282 pp.

Trudgill, D.L., Bala, G., Blok, V.C., Daudi, A., Davies, K.G., Gowen, S.H., Fargette, M., Madulu, J.D., Mateille, T., Mwageni, W., Netscher, C., Phillips, M.S., Sawadogo, A., Trivino, C.G. and Voyoukallou, E. (2000). The importance of tropical root-knot nematode (*Meloidogyne* spp.) and factors affecting the utility of *Pasteuria penetrans* as a biocontrol agent. *Nematology* 2, 823-845.

Evaluation of Different Alcohols in Trapping Adult Coffee Berry Borers (*Hypothenemus hampei* Ferrari)

F. L. MAGINA, D. F. MBOWE, G. S. CHIPUNGAHELO AND J. M. TERI

Tanzania Coffee Research Institute, Lyamungu, P. O. Box 3004, Moshi, Tanzania

SUMMARY

A study was carried out to evaluate the effectiveness of different alcohols in trapping adult coffee berry borers *(Hypothenemus hampei* Ferrari*)* at TaCRI Lyamungu and surrounding villages. A field experiment was conducted from July 2004 to January 2005, where five types of alcohols and three dilutions were used in a split-plot design arranged in a completely randomised block design, with three replications. Alcohol types were considered main plots whereas dilutions were considered as the sub-plots. There was a significant difference ($P \leq 0.05$) among alcohols in their ability to trap adult CBB. Methylated spirit was the most effective. The number of adult CBB trapped by different alcohol dilutions varied significantly ($P \leq 0.05$), with undiluted formulation having the highest while the control treatment trapped the least number. There was also a significant alcohols x dilutions interaction ($P \leq 0.05$) on the number of trapped adult CBB. Undiluted and diluted methylated spirit was more effective trap for adult CBB than all alcohols and their dilutions including the control. Further research to evaluate the effectiveness of more cheaply available local brews as potential traps is being undertaken.

Keywords: Alcohols, coffee berry borer, *Hypothenemus hampei*, TaCRI, trapping

INTRODUCTION

The coffee berry borer (*Hypothenemus hampei* Ferrari) is one of the most significant and widespread insect pest affecting coffee production in many countries in Africa, Asia and Central and South America (Baker, 2002). It is a major insect pest in Robusta coffee and low altitude arabica coffee in Tanzania (Le Pelley, 1968). The pest causes premature fall of cherries, reduces bean weight, reduces quality and affects flavour of coffee (Baker et al*.,* 2002). CBB was reported to cause a crop loss of up to 90 % on Robusta coffee (Le Pelley, 1968).

Control of the pest by chemical spraying has always been difficult because much of the lifecycle of the pest occurs deep inside the berry, and therefore renders the chemical control to be impractical. Moreover, inorganic chemicals are environmentally unfriendly and expensive; and in the present price crisis, very few farmers are spraying their coffee (Baker and Murphy, 1999). Cultural control method by picking and elimination of infested fruits at the end of the season leaves no food for the insect, this has shown to be a very promising methodology and hence is the standard recommendation in many countries, but is laborious and expensive (Baker and Murphy, 1999). Biological control by mass field release of *Cephalonomic stephanoderis* in El Salvador and Colombia has shown promising results; however, the cost of mass production of the natural enemies is high and hence uneconomic (Baker et al., 2002). Another shortcoming of the method is the difficult to confine the insects at the point of release.

Alternatively CIRAD has developed light traps Bracap™ which has also proved to be effective in controlling CBB in some coffee growing countries eg El Salvador (Dufour et al., 2001). However such technologies are too sophisticated in the developing world especially for small-holder farmers. In India the mixture of ethanol and methanol at a ratio of 1:1 effectively trapped many coffee berry borers in coffee plantations (Prakasan et al., 2001). Research information available in Tanzania for the control of CBB by alcohol trapping is scanty. The objective of this study was therefore to evaluate different alcohols dilutions for trapping adult coffee berry borers in Tanzania.

MATERIALS AND METHODS

The study was conducted in established mature coffee trees at Lyamungu, Moshi (at 1268 masl, 3º 14' S and 37º15' E) and surrounding villages between July, 2004 and January, 2005. Five types of alcohols and three dilutions (Table 1) were used in a split-plot design in a completely randomised block design and replicated three times. Alcohol types were considered as main plots and dilutions as sub-plots. The alcohols with different dilutions were put into transparent plastic bottles with two openings on the sides (Figure 1) and suspended on branches of coffee trees 1.5 m from the ground at an interval of 5m from one trap to another according to Dufour (2002). The assessment was done at one-week intervals by counting the number of adult CBB trapped per treatment. Regular replenishment of treatments was made to balance losses due to evaporation. The number of trapped adult CBB from the treatments was subjected to analysis of variance (ANOVA) using MSTATC statistical software (MSTATC 1993). Means were separated according to New Duncan's Multiple Range Test (NDMRT) procedure and the significant of the difference between means was assessed at alpha level of 5% ($P \leq 0.05$).

Table 1. Different types of alcohols used for trapping CBB.

Treatments(Alcohols)	%alcohol content	colour
Konyagi (Spirit)	35	Colourless
Dry red wine	15	Red
Methanol	99	Colourless
Ethanol	96.6	Colourless
Methylated spirit	70	Purple

Figure 1. Alcohol trap design for trapping CBB.

RESULTS

Efficacy of alcohols in trapping CBB is presented in Table 2. Results indicate significant variation ($P \leq 0.05$) among alcohols in their efficacy in trapping adult CBB, whereby methylated spirit and methanol trapped the largest and least number of adult insects respectively.

Table 2. Efficacy of different alcohols in trapping of adult CBB.

Treatment	Mean No. of CBB trapped
Methylated spirit	287.3a
Methanol	235b
Ethanol	219.7bc
Wine	206.7c
Konyagi(spirit)*	196.6c

*Note: Means followed by the same letter do not differ significantly (P = 0.05). *Konyagi is a local spirit distilled in Tanzania.*

The number of adult CBB trapped by different alcohol dilutions varied significantly ($P \leq 0.05$) (Figure 2) with undiluted formulation having the highest while the control treatment had the least number of the adult insect (Figure 2). There was also a significant alcohols x dilutions interaction ($P \leq 0.05$) whereby undiluted and diluted methylated spirit was the most effective trap for adult CBB.

Methylated spirit in undiluted form trapped the largest number of adult insect, followed by 50% diluted methylated spirit.

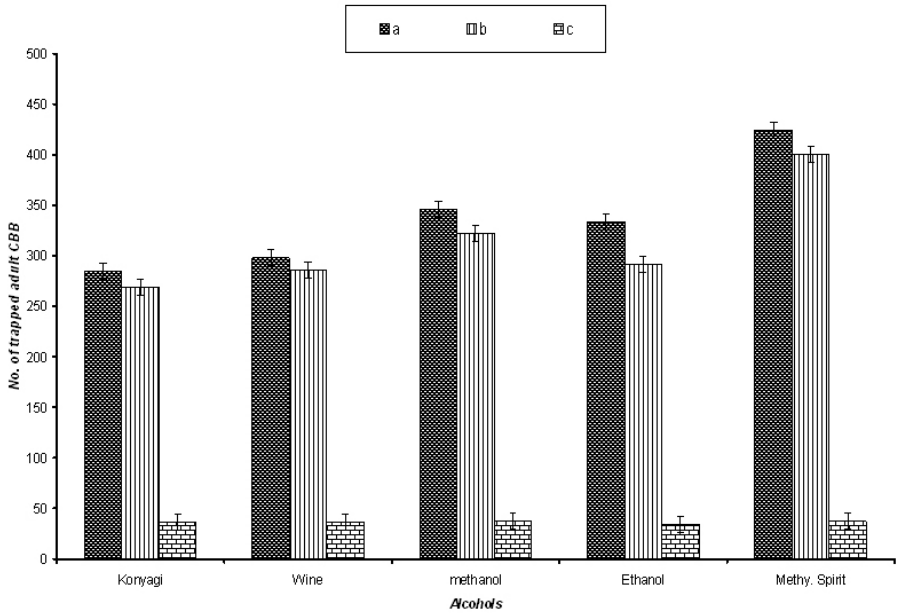


Figure 2. Effect of alcohols and dilutions on the number of trapped adult CBB. (a) = 100%, (b) = 50% and (c) = 0%. SE ± 7.951.

DISCUSSION AND CONCLUSION

The observation that Methylated spirit (a mixture of 10% methanol and 90% Ethanol) attracted a comparatively large number of CBB indicates that it is effective in trapping the

pest. This is in line with the findings reported by Prakasan et al. (2001) that a mixture of Methanol and Ethanol (in 1:1 ratio) was more effective than ethanol and methanol separately. A comparison between Methanol and Ethanol (from the results) shows clearly that methanol trapped larger number of CBB than Ethanol, suggesting that even in Methylated spirit, methanol was the key chemical, as noted by Prakasan et al. (2001).

Alcohol based traps have demonstrated a rate of efficacy of more than 50% in terms of the reduction in infestations on new fruits compared to a control without traps (Dufour et al., 1999). Therefore CBB trapping by alcohol may be termed as a special tool not only in IPM, but also in early warning systems. Efforts are under way to evaluate more cheaply available local brews and liquors as effective traps in controlling CBB.

ACKNOWLEDGEMENT

This study was supported by generous financial contribution by Tanzania coffee growers and EU/STABEX funds. We are grateful to Mr, Elisante David, for the assistance in the insectary and field data collection.

REFERENCES

Anon, (1997). Recommended Technologies in Coffee Production. Coffee Research Institute-Lyamungu 12 pp. (Unpublished).

Baker, P.S; Jackson, J.A.F and Murphy, S.T (2002). Natural enemies, natural allies-how scientists and coffee farmers forged new partnerships in the war against pests and low prices, Project Completion Report Project. CABI. Egham UK. pp129.

Baker, P.S and Murphy, R.D (1999). Biological Control of the Coffee Berry Borer, CABI commodities. CABI Bioscience, Egham, UK. In: International of coffee science, Bangalore, India), India, ASIC. pp 12.

Dufour B., Gonzalez, M. O., Frerot, B., (1999). Piegeage de masse du scolyte du café Hypothenemus hampei, Ferr. (Col. Scotytidae) en conditions reelles: premiers results. XVIII scientifique international du café, Helsinki, Finland. ASIC, p 480-491.

Dufour B. (2002). Importance of trapping for integrated management (IPM) of CBB, *Hypothenemus hampei* Ferrari In: Research and coffee growing. MONTPELLIER CEDEX France, pp 114-116.

Dufour B. P., Gonzalez, M. O., Mauricio, J. J., Chavez, B. A and Ramirez, R. (2001). Validation of coffee Berry Borer (CBB). Trapping with the Bracap® Trap, CIRAD El Salvador. In: International of coffee science (5-14 2001, Trieste, Italy), Trieste, ASIC. pp1243-1247.

Le Pelley, R. H. (1968). Pests of coffee, Longmans Green and Co.Ltd, pp590.

Prakasan, C. B, Sreedharan, K, Sambamuthy, A. G, and Gokuldas, M (2001). Mass trapping a new component in IPM of CBB. Proceedings of the 2nd National Symposium on IPM in Horticultural Crops, Bio pesticides and new molecules 17-19 October, 2001, Bangalore India pp 117-121.

The Soil Fertility Status of Coffee Growing Areas in Tanzania

G. P. MARO, H. MONYO, E. NKYA AND J. M. TERI

Tanzania Coffee Research Institute, P.O. Box 3004 Moshi, Tanzania

SUMMARY

The objective of this work was to review critically the relationship between soil fertility status and coffee productivity in the Tanzanian coffee growing areas. An inventory of soil-related information for the coffee areas in the country was made by TaCRI through consultancy from Sokoine University of Agriculture. The collected information was assessed against some established standards to give a picture of the fertility status. Total N was optimal, but its forms and dynamics make it the most limiting in the soils. Other nutrients were around optimal except P, which was on a low side. In the absence of recent soil fertility data, the low productivity (as low as 200 g/tree/year in small holder farms) was attributed to low soil fertility, as most smallholders are unable and/or reluctant to invest in soil fertility management. An overview of the causes, effects and TaCRI's efforts through integrated soil fertility management to improve coffee productivity are discussed.
Key words: coffee, fertility, ISFM, productivity, soils, TaCRI, Tanzania

BACKGROUND

In Tanzania, coffee is grown by small, medium and large scale farmers in Kilimanjaro, Arusha, Kagera, Mbeya and Ruvuma regions. It is also grown, at lesser scale, in Kigoma (Kibondo and Kasulu districts), Mara (Tarime) and Iringa (Figure 1a). The Tanzania Coffee Research Institute has suspected soil fertility problems to limit high coffee productivity and quality. A team of consultants from Sokoine University of Agriculture (Semoka et al., 2005) was contracted to review the fertility database available. This work looks critically into the findings, outlining TaCRI's effort to forge the right way forward.

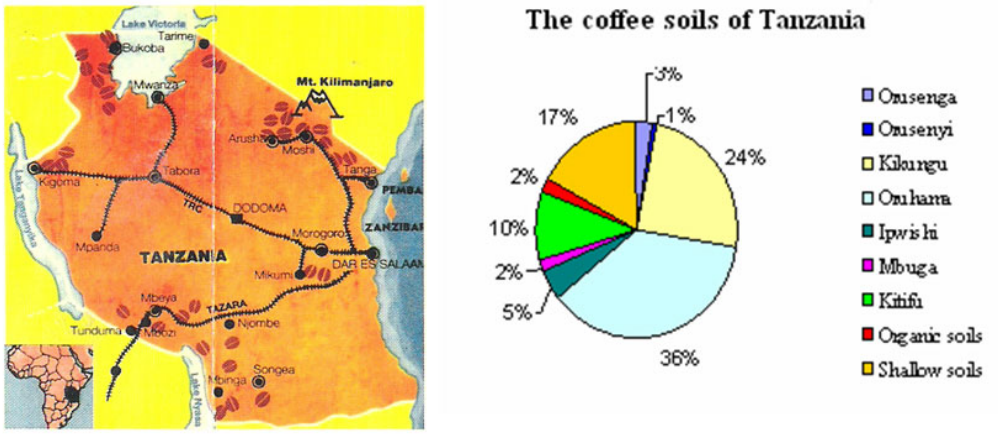


Figure 1. (a) The coffee-growing areas in Tanzania, (b) The coffee soil types.

Oosterom et al. (1998) reported coffee in Tanzania to be grown in landscapes of type C, D, E and H, ranging from 750 to over 2,000 m above sea level. Robusta (*Coffea canephora*) is grown in medium altitude, sandstone and shale areas, whereas Arabica (*Coffea arabica*) prefers higher altitude, volcanic areas of high natural fertility, and in gneiss areas of moderate

fertility. They categorized the coffee soils as shown in Figure 1(b), dominated by the *Oruhama* acid clay soils, *Kikungu* reddish loamy to clayey soils and shallow soils, by 36, 24 and 17% respectively, all of moderate to low fertility. Others include *Orusenga* reddish sandy soils; *Orusenyi* bleached sandy soils and *Ipwishi* sandy groundwater soils, all of low to very low fertility; and only 14% of high-fertility soils (*Mbuga* vertisols, *Kitifu* andosols and organic soils).

METHODOLOGY

Soil analytical data (pH-water, total N, Bray 1 P, organic carbon and the exchangeable K, Ca and Mg) collected between 1970 and 1985 from the slopes of Mt. Kilimanjaro, Bukoba, Rungwe, Arusha and Mbozi were extracted from Semoka et al. (2005) and compared to a standard range of optimal values suggested by Janssen (2005). Yield data for smallholders and estates at different locations, recorded in the mid-1990s (URT, 1998), were assessed against the standard values representing mean yields from selected single trees in experiment plots.The data were processed in Excel, and the results plotted graphically.

RESULTS

A summary of the soil results is given in Figure 2(a), with the values for total N multiplied 10 times. Soil pH and total N were optimal. Slightly higher pH was found in Arusha, which corresponds with Andosols (specifically those with sil-andic properties). Lower values were found in Bukoba, an expected situation with Ferralsols. However, N was reported as the most limiting of the macronutrients (Semoka et al., 2005) because the rate of mineralization and the fate of the available species (NH_4-N and NO_3-N) differ from place to place. Available P was generally low to very low. Exchangeable K was very high in Kilimanjaro and Mbozi, with Arusha, Bukoba and Rungwe on a low side. Ca and Mg had more or less the same trend, with Arusha and Mbozi featuring on a high side followed by Kilimanjaro. The rest featured below optimal values. All soils studied showed to have optimal OC values, with an exceptional case of 5% at Rungwe.

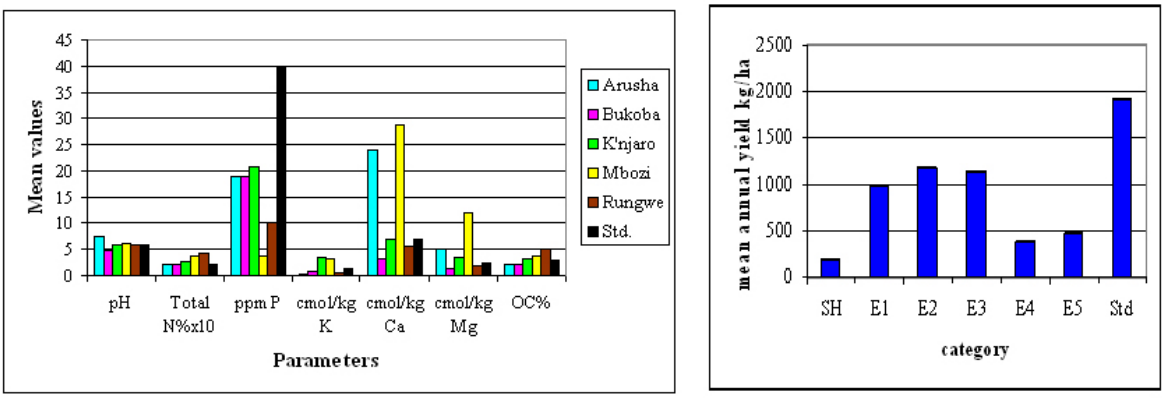


Figure 2. (a) The fertility parameters for Tanzanian coffee soils, (b) Comparative yields.

Figure 2(b) gives average yields of parchment coffee from different farming categories, where SH stands for smallholders and E1-E5 for estates in Arusha, Kilimanjaro, Oldeani, Usambara and Southern Highlands respectively.

DISCUSSION

Evidence of low fertility in coffee soils of Tanzania

In absence of up-to-date soil analytical information, productivity per tree and per ha, and the visual look of the coffee trees can serve as indicators of soil fertility. The 200kg ha^{-1} parchment coffee recorded for smallholder farmers (Figure 2b), trees showing deficiency symptoms (Figure 3a) and overbearing dieback (Figure 3b) suggest low fertility in soils. It seems that the already low natural fertility in many areas has been aggravated by improper fertility management whereby, with low and unstable world market prices, smallholder coffee farmers are unable or reluctant to invest in this area.

Figure 3. (a) Coffee tree showing N deficiency, (b) Overbearing dieback.

TaCRI efforts to improve soil fertility through ISFM

Establishment of a reliable soil database

Intensive soil survey is planned in the near future, following rehabilitation of the soil laboratory, so as to establish a reliable countrywide database. Such information will be exposed to GIS analysis and used, with spatial interpolation from nearest neighbours, to derive quick advices to farmers in situations whereby actual analysis is impractical. The laboratory facelift will also help to improve soil and plant analytical services.

Developing a quantitative fertilizer advice model

A draft fertilizer advice model called SAFERNAC (Soil Analysis for Fertility Evaluation and Recommendations on Nutrient Application to Coffee), is being developed in collaboration with experts from the Netherlands. The pilot test conducted recently indicated that it is possible to develop models applicable to Tanzania for quick fertilizer advice in coffee. Data from 25 clonal gardens around Hai and Moshi gave the following regression equations for N and P, with irregular trends in K:

$$N_{adv} = -37.568 N_{soil} + 315.65 \; ; \quad R^2 = 0.8511 \qquad [1]$$

$$P_{adv} = -99.459 \ln P_{soil} + 523.74 \; ; \quad R^2 = 0.8224 \qquad [2]$$

where: N_{adv} and N_{soil} represent the amount of N, in kg/ha to advise to a farmer, and percent total N in soil respectively. The same applies for P_{adv} and $P_{soil,}$ the latter expressed in ppm .

Proper handling and use of farmyard manure and composts

The smallholder farmer groups are advised on the proper handling and use of organic fertilizers as components of integrated soil fertility management (ISFM). Proper handling of FYM includes timely removal from the kraal, and preparation according to stack method (Ngeze et al., 1983). Dosages of about 20 kg FYM/tree are recommended. As for plant material composts, farmers are advised to go for the pit method, using 60-cm-deep pits.

Other technologies for efficient land use

TaCRI develops and disseminates a variety of tailor-made technologies for specific farming categories. These include coffee-banana intercropping (whereby farmers are encouraged to rearrange their farms, 3 coffee rows by 1 banana row, a culture whose annual income can be predicted); intercropping with leguminous crops whose residues can be returned to the soil, minimum or zero tillage, contouring with fodder plants (e.g. *Setaria* spp.), and use of leguminous shade trees like *Albizzia* spp.

CONCLUSION

The presence of TaCRI has brought new hopes to the coffee industry in Tanzania. The 10 newly released, high-yielding and disease-resistant varieties are getting adopted fast, and are expected to cut the production costs by 40-50%. This will motivate farmers to invest in fertility management. TaCRI is also encouraging farmers to utilize the analytical services available. For prompt data interpretation, fertilizer advice models will be of much use. The resultant combination of ISFM, high-yielding varieties, good crop husbandry and improved processing techniques, will result in increased productivity and quality, reduced cost of production, better incomes to growers and better quality coffee to consumers.

REFERENCE

Janssen, B.H. (2005). Report on advisory mission on crop nutrition to Tanzania Coffee Research Institute, Lyamungu, Moshi. *NMCP Project 31817 MTZ, Wageningen, The Netherlands.* 89pp.

Ngeze, P.B., Mnzava, N.A. and Ruttle, J. (1983). Methods of compost preparation. In: Brusko, M (ed). Resource-efficient farming methods for Tanzania – *Workshop proc. Faculty of Agriculture, Forestry & Veterinary Science, UDSM, Morogoro, Tanzania.* Pp 58-62.

Oosterom, A.P., Kaitaba, E.G., Schuiling, C., Onderstal, J. and Gijsbertse, H.A. (1998). Land resources of Tanzania: Use and potential for coffee production (map). *Coffee Management Unit, Min. of Agric. & Coops., Dar es Salaam, Tanzania.*

Semoka, J.M.R., Mrema, J.P. and Semu, E. (2005). A comprehensive literature review on integrated soil fertility management for coffee. *Consultancy report submitted to TaCRI. Department of Soil Science*, SUA. 142pp.

United Republic of Tanzania (URT), (1998). Kagera region: Socio-economic profile. *The Planning Commission and Kagera Regional Commissioner's Office,* DSM.

Determining the Effectiveness of Various Lures for Trapping the Black Twig Borer, *Xylosandrus compactus*, on Coffee

E. BURBANO[1], M. WRIGHT[1], V. EASTON SMITH[2]

[1]Dept. of Plant and Environmental Protection Sciences
[2]Dept. of Tropical Plant and Soil Sciences, University of Hawaii, Honolulu, HI. USA

SUMMARY

Xylosandrus compactus compactus (Eichhoff) (Coleoptera: Curculionidae) is considered the most important pest on coffee in Hawaii. The female makes an entrance tunnel into the wood and excavates a small cavity where she deposits several eggs. The immature stages feed on the fungus *Fusarium solani*, which females trasmit to their offspring. The control method used by farmers in Hawaii is to prune the infected branches. This tactic reduces the vegetative material, and many farmers leave this material inside the coffee field, inadvertently allowing the insect to enter the field again. Four lures were tested in Kona, Hawaii to attract *X. compactus*. Traps baited with vials of 75% ethyl alcohol and alcohol pouches from Phero Tech Company captured more *X. compactus* than the other lures, eugenol, alpha pinene and control (without lure). The use of traps baited with alcohol is an alternative for monitoring this beetle as part of a strategy to prevent population outbreaks.

RESÚMEN

El escarabajo *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae) es considerado una de las plagas más importantes de café en Hawaii. La hembra hace un túnel en la madera y excava un pequeño hueco donde deposita varios huevos. Los estados inmaduros se alimentan del hongo *Fusarium solani* que la hembra proporciona a ellos. El método de control usado por los agricultores en Hawaii es el corte de las ramas infectadas. Esta táctica reduce el material vegetal, y muchos agricultores dejan este material infectado dentro del cafetal, permitiéndole al insecto entrar de nuevo en la plantacion de café. Cuatro atrayentes fueron evaluados en Kona, Hawaii para determinar su eficacia en *X. compactus*. Las trampas con viales de alcohol al 75% y bolsitas de alcohol de la compania Phero Tech capturaron más *X. compactus* que los otros atrayentes eugenol, alpha pinene y el control (trampas sin atrayente). El uso de trampas cebadas con alcohol es una alternativa para el monitoreo de este escarabajo como parte de una estrategia de prevenir un brote de la población.

INTRODUCTION

The black twig borer BTB, *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae), is originally from Asia and is one of the most important coffee pests in Hawaii. This weevil attacks over 224 plant species including agricultural crops and native forest trees. There is currently no acceptable means of managing this pest, and this is a significant concern in the Kona area and potentially in other Hawaii coffee growing areas as well. Many Hawaii coffee growers are organic producers and prefer to avoid insecticide applications. The current method of control is sanitation by pruning. This tactic, however, reduces vegetative plant material and many farmers do not use adequate methods to destroy infested material, thus leaving an important infection source on the ground. This has prompted the search for pest management alternatives such as mass trapping. Traps originally designed for the Japanese

beetle, baited with a chemical attractant lure, could serve as a monitoring and research tool to assess seasonal phenology.

OBJECTIVE

To determine the effectiveness of different lures for trapping the black twig borer, *Xylosandrus compactus*.

MATERIALS AND METHODS

In March 2006, three plots were chosen in a coffee farm located in Kona, Big Island, HI, USA. In this trial we used 30 Japanese beetle traps (JB/Expando trap, TRÉCÉ® Adair, OK, USA). Traps were baited with three different lures and a control with no attractant. The attractants were dispensed as follows: ethyl alcohol in sleeves with a release rate of 7 mg/day, alpha-pinene in eppendorf tubes with a release rate of approximately 6 mg/day, and eugenol in bubble caps with a release rate of 1 mg/day. Previous research conducted in indigenous *Acacia* trees in Hawaii showed that *X. compactus* is highly attracted to ethyl alcohol (Dudley et al. 2006 in prep.)**.** To compare the effectiveness of the ethyl alcohol sleeve produced by Phero Tech Inc. (BC, Canada) and laboratory ethyl alcohol, a 15 ml plastic container with 75% ethyl alcohol was added as another treatment. The five treatments were assigned randomly to each plot. We used two traps per treatment in each plot for a total of 10 Japanese beetle traps per plot. Every 2 weeks the traps were checked and the 15 ml ethanol vials were refilled. To maintain a consistent release rate throughout the study, lures were replaced in the field every 2 months as recommended by Phero Tech Inc. To kill the captured beetles, a 2-cm strip of Hercon VaporTape II (10% 2,2 dichlorvos) (Bioquip®, CA, USA) was placed in the collection container of the trap. All the material collected from the traps was placed inside a plastic vial with alcohol and refrigerated until examination. Beetles collected from each lure were separated by species, and counted.

The five treatments were organized in a randomized complete block design. Each one of the three plots had two replicates per treatment. These data were analyzed with ANOVA. All analyses were conducted using general linear models procedure (SAS Institute, 2000).

RESULTS

Mean captures of *X. compactus*, by sampling date, trapped in different lures on coffee plants

There was no significant difference in the number of BTB trapped during the four sampling dates (Figure 1). Both ethanol 75% vial and the ethanol pouches attracted the higher number of *X. compactus* compared to eugenol, alpha pinene and the control. There was a significant difference among the treatments on all four sampling dates.

Mean captures of *X. compactus* trapped in Japanese beetle traps baited with three lures on coffee plants over four sampling dates

The number of *X. compactus* trapped in the Japanese beetle traps was significantly different among treatments (Figure 2). There was a higher number of *X. compactus* collected in traps baited with ethanol vials than traps baited with ethanol pouches. There were no significant differences in the number of insects trapped in eugenol, alpha pinene and the control.

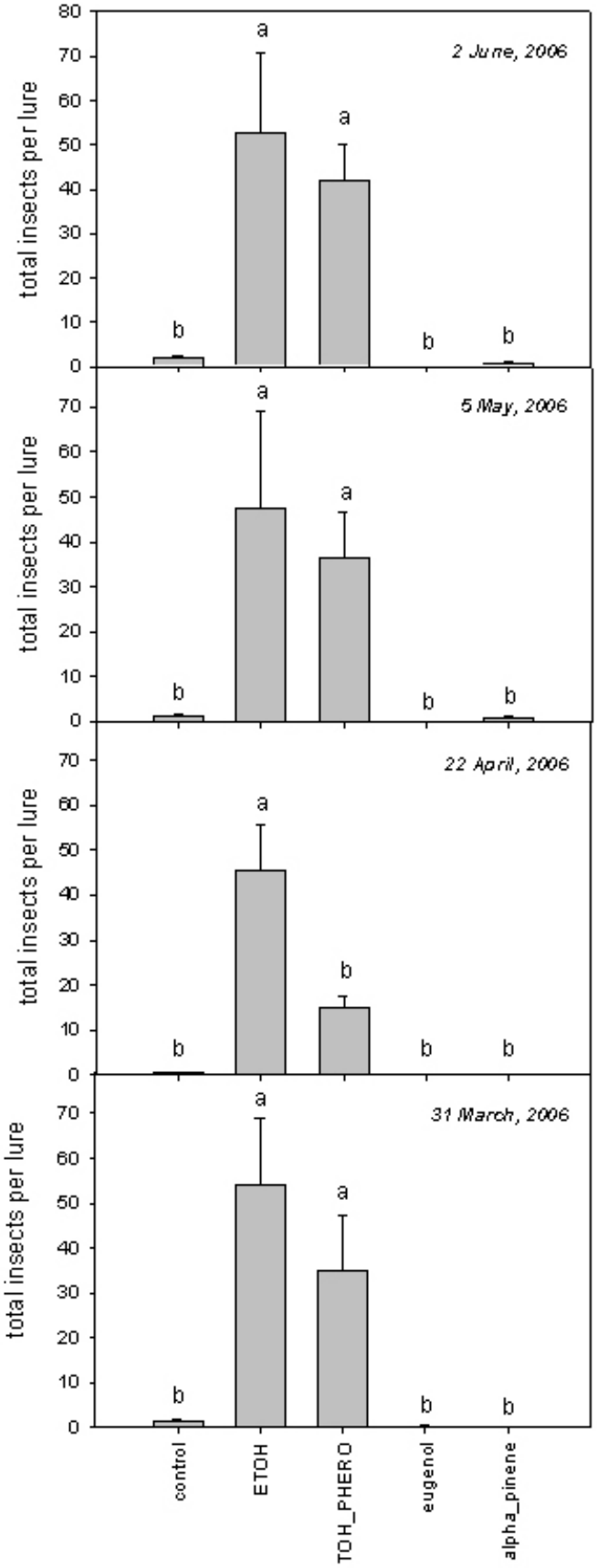


Figure 1. Average number of *X. compactus* trapped in Japanese beetle traps baited with three lures on coffee plants (mean ± SE) by sampling dates. The bars represent the means and standard error of all BTB trapped per lure.

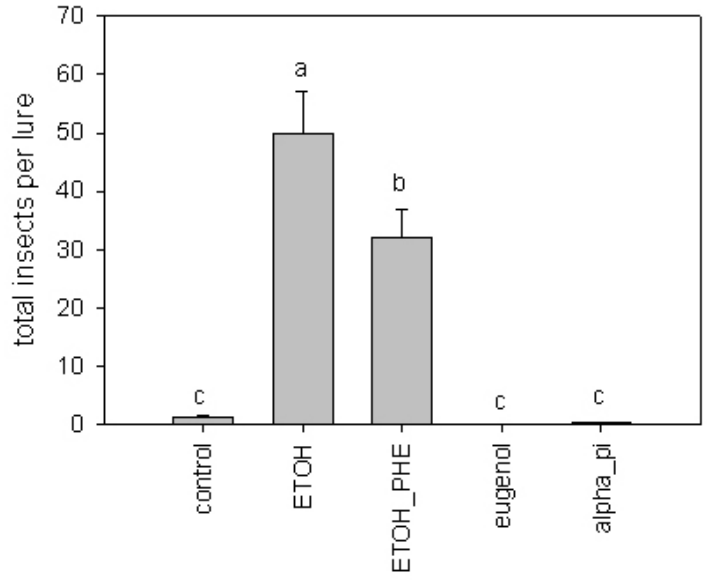


Figure 2. Average number of *X. compactus* trapped in Japanese beetle traps baited with three lures on coffee plants (mean ± SE) over four sampling dates. The bars represent the means and standard error of four trapping dates from March to June 2006.

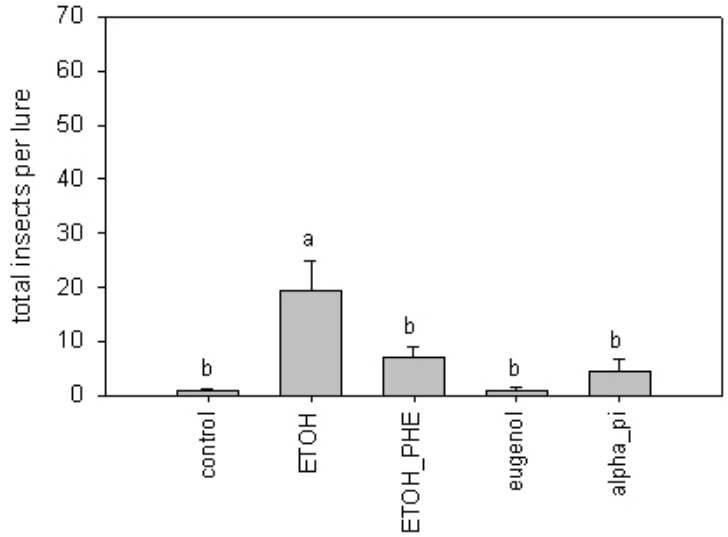


Figure 3. Average number of non-target organisms trapped in Japanese beetle traps baited with three lures on coffee plants (mean ± SE) over four sampling dates. The bars represent the means and standard error of four trapping dates from March to June 2006.

Mean captures of non-target organisms over four sampling dates

While no significant differences between eugenol, alpha pinene and control treatments were observed, there was a significant number of non-target organisms collected in traps baited with ethanol 75% (Figure 3). Other insects attracted by ethyl alcohol were *Hypothenemus obscurus, Litargus vestitus*, and other beetles of the family Corylophidae. *H. obscurus*, commonly called the macadamia shot borer, was the most abundant non-target insect collected in traps baited with ethanol because it is common to have macadamia trees close to coffee fields in Kona. This beetle is one of the major problems on macadamia in Hawaii, suggesting that the Japanese beetle trap baited with ethanol could also be an alternative for monitoring this beetle (Schultz and Dills, 2003). Low numbers of *Cryptamorpha desjardinsi*

(Coleoptera: Silvanidae) were found in ethanol baited traps. This predacious beetle has been found inside macadamia nuts feeding on *H. obscurus*, and also in coffee plantations and inside coffee berries (EGB, unpub. data). This predator can be considered a potentially useful natural enemy to control BTB.

CONCLUSIONS

Traps with ethyl alcohol captured significantly more *X. compactus* than traps baited with eugenol and alpha pinene. Attractant lures are an important tool for monitoring adult insect populations as part of an integrated pest management program. Females fly from plant to plant to lay eggs inside the twigs, causing infestations and population growth. These traps could attract and kill ovipositing females as a means to reduce the pest population at its source.

REFERENCES

Dudley N. 2006. Semiochemicals provide a deterrent to the black twig borer, *Xylosandrus compactus* (Coleoptera: Curculionidae, Scolytinae). In prep.

SAS, SAS/STAT user's guide. version 8 ed. 2000, Cary NC: SAS Institute Inc. 3884.

Schultz P. B. and M. S. Dills. 2003. Monitoring Asian Ambrosia Beetles. SNA RESEARCH CONFERENCE – VOL 48 – Virginia Tech, Hampton Roads AREC, Virginia Beach, VA 23455.

Strategies for Coffee Wilt Disease Management on Robusta Coffee in Kagera Region, Tanzania

N. M. NG'HOMA, D. L. KILAMBO, L. MASUMBUKO, J. M. TERI, T. NZALAWAHE AND M. P. L. KAIZA

Tanzania Coffee Research Institute (TaCRI) P.O.Box3004 Moshi, Tanzania
E-mail: tacriced@kicheko-mos.com – Fax: 255-27-2756773 – Tel: 255-27-2756868

SUMMARY

Coffee wilt disease (CWD), a vascular fungal disease caused by *Fusarium xylarioides,* attacks Robusta coffee (*Coffea canephora*) in Tanzania. In order to manage this disease integrated disease management approach involves eradication of infected coffee trees and improved coffee husbandry practices i.e. stumping, pruning, weed management, inorganic fertilizers application, soil conservation measures, mulching and stem/wounds coating with copper oxychloride to prevent penetration of the pathogen through wounds were used. Farmer field school (FFS) and participatory extension farmer groups (PEFG) were main mechanisms used in disseminating the information. This approach significantly reduced the disease incidences and its spread in the region. This report highlights the progress and way forward.

INTRODUCTION

Robusta coffee is the principal cash crop in Kagera region that accounts for 25% of coffee produced in Tanzania. More than 80 % of the residents of Kagera region mainly; Bukoba, Karagwe and Muleba districts depend on coffee as source of income. Since the appearance of CWD in Tanzania in 1997 with only 2 wards identified to have attacks of the disease (Kilambo and Kaiza, 1997), to date a total of 44 out of 100 wards in Kagera have reported to be affected by *F.xylarioides* causing monetary losses of $ 197,551 (Anon, 2003; Mohamed et al., 2000).

Efforts to manage the impact of CWD have been made by creating awareness in the presence and threat of CWD and initial steps to be considered to limit the its spread. This report presents progress on management strategies used towards minimizing the incidences of the disease in the country.

METHODOLOGY

Surveys

Since 2000, surveys have been carried out annually to monitor the spread of CWD and incidences. Data collected included coffee plant population, infected and eradicated coffee trees in each farm. This aims at establishing CWD incidences prevail.

Eradication programme

TaCRI in collaboration with district councils, NGOs and farmers, conducts eradication campaign of uprooting and burning of affected coffee trees in-situ, with particular emphasis on early diagnosis of the disease to reduce the risk of spread to other trees and farms.

Dissemination and training

An awareness campaign is being carried out involving CWD training for farmers and extension workers; distribution of posters and leaflets to farmers in affected areas, formation of Participatory Extension Farmer Groups(PEFG) and Farmer Field Schools (FFS). Discovery learning was the major tool used to distinguish symptoms caused by coffee wilt disease from other causes. To effect dissemination and training, TaCRI collaborated with Centre for Agriculture and Biological Science International (CABI) based in Nairobi under the financial support of Common Fund for Commodity (CFC).

Intervention packages

Trials were laid out on farmers' fields in eleven sites in CWD hot spot areas Bukoba and Karagwe districts to evaluate the effectiveness of the intervention packages on management of CWD. The trials consisted of six treatments; copper oxychloride stem paint, copper oxychloride foliar application, herbicide application, mulching, ash application and control with uprooting and burning of infected coffee trees and manure application being employed to all treatments.

RESULTS AND DISCUSSIONS

Effect of integrated disease management approach

The results from the trials conducted in eleven sites showed that use of integrated approach in CWD management has resulted into low coffee wilt disease pressure in hot spot area (Figure 1). The results showed that farmers who accepted to uproot and burn immediately after the first symptoms diagnosis their farms have less disease incidences than those who did not follow the recommendation (Figure 1).

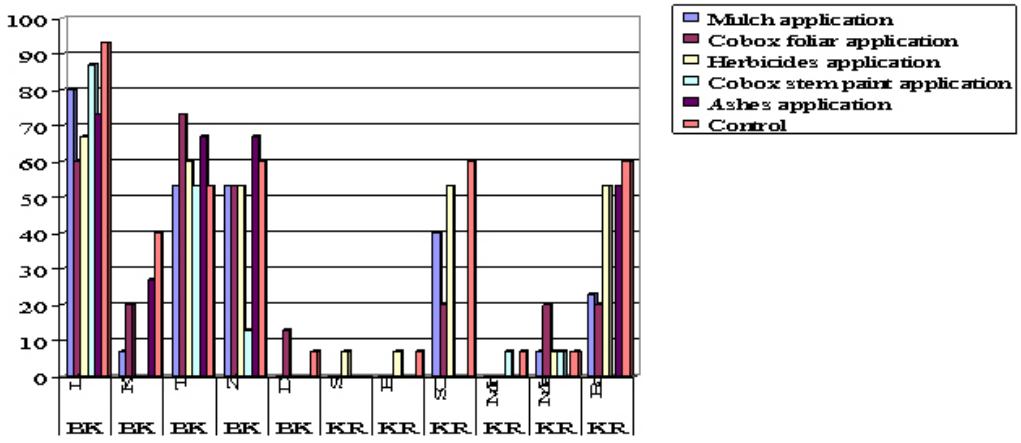


Figure 1. Reduction of CWD Incidences; 2003 to 2005.

These results are in line with the report by Booth (1971) that uprooting and burning of coffee trees affected by coffee wilt disease if done careful, assists in the control and minimizes its spread. The results from field observations and monitoring the effectiveness of immediate uprooting and burning of infected coffee trees in combination with the best crop husbandry practices in Kaisho-Murongo and Kituntu/Mabira divisions in Karagwe District showed significant reduction of the incidence and spread of the disease (Table 1). Monitoring of the disease incidences and its spread in all three districts shows that the disease is still present at high pressure in areas where farmers are reluctant in uprooting and burning of affected coffee

trees, but decreased in areas where farmers have accepted this recommendation (Table 1). Also, the results show that CWD in Tanzania is still confined to the Kagera region, in particular Bukoba, Muleba and Karagwe districts. Moreover there is no case of CWD being infected the Arabica coffee grown adjacent to robusta coffee (Kilambo et al., 2004). The results showed that coffee wilt disease incidences are lower in all coffee trees painted with copper oxychloride 50 % WP paste of 300 g per litre painted 50 cm from the ground level at interval of four months (Figure 1) and these results are similar to those of (Anon, 2005) who reported that use of copper oxychloride assists in preventing CWD pathogens to enter coffee trees through wounds. Therefore, based on these results use of copper xychloride has been scaled up on FFS and PEFG to validate its effectiveness in managing the disease.

Table 1. Sampling of CWD incidences in eight farms Karagwe district 2000 to 2006.

Village	Name of farmer	Number of coffee 1996	Coffee trees destroyed by CWD (cumulative data)		CWD % Incidence 2006
			2000	2006	
Rutunguru	Edward	463	70	363	78.4*
Nyabishenge	Christian Lutegereha	634	1	314	49.5*
Nyabishenge	David Karugendo	810	1	599	74.0*
Nyabishenge	Patrick Burengero	900	1	299	3.3**
Businde	Isa Rutenge	600	20	13	2.2**
Omukagando	Valentine Babireba	1500	50	50	3.35**
Nyakatuntu	Paulo Peter	600	65	13	2.2**
Omukagando	Christian Songamble	2000	40	40	2.00***

*Note: *Coffee trees infected with CWD remained in the farm, **Coffee trees infected with CWD immediately uprooted and burnt, ***Coffee trees infected with CWD immediately uprooted and burnt and only 308 coffee trees have been destroyed within the village.*

Dissemination and training

The significant reduction of coffee wilt disease incidences in areas where there was no any trials revealed that dissemination and training programme assisted to sensitise farmers to participate in CWD eradication campaign and therefore, minimizing the spread of the disease. Since 2000 when CWD became the serious threat of coffee production, all district authorities jointed their efforts to fight the disease. Since, then more than 15,000 farmers and 200 extension workers trained on CWD identification and its control (Table 2). Also more than 20,000 copies of leaflets and 3000 posters explaining symptoms and management of CWD have been distributed to farmers and other stakeholders.

Resistant varieties

Durable resistance to CWD is the only viable option to manage the disease. According to (Kilambo et al. 2006) there are some potential materials to be developed for commercial use. These will be planted in clonal mother garden for multiplication and on-farm evaluation.

Table 2. Number of farmers and extension workers trained on symptoms identification and control of CWD.

Forum	Number	Number of farmers & extension workers participated	
		Farmers	Extension workers
Farmer Field Schools	21	630	15
Farmer Extension Groups	23	690	23
Open days	18	8,458	44
Training for extension workers	3	3	97
Farmer training by Manyafubu Group & extension workers	1	6,134	40
Total	**61**	**15,916**	**219**

CONCLUSION

The integrated approach in CWD management is vital for significant reduction of the disease. TaCRI intends to continue implementing them for the benefits of coffee farmers in Tanzania.

REFERENCES

Anon. 2003. Surveys to assess the extent and impact of Coffee Wilt Disease in East and Central Africa. Technical report-CAB International centre. Supported by EU-CORNET

Booth, C. (1971). The Genus Fusarium. Commonwealth Mycological Institute: Kew, Surrey, Egham. 237 pp.

Kilambo, D. L.; Ng'homa, N. M.; Mtenga, D. J.; Teri, J. M.; Nzallawahe, T.; Mike, R. and Masumbuko, L (2006) Progress towards searching for durable resistance to Fusarium wilt (*Fusarium xylarioides*) in *Coffea canephora* germplasm in Tanzania. To be presented during the ASIC Conference, September 2006 Montipellier France.

Kilambo, D.; Ng'homa, N.; Mohamed, R.; Teri, J.; Poole, J.; Flori, A.; and Pinard, F. (2004) Coffee Disease Survey in Tanzania. Presented during ASIC conference Bangalore India 2004.

Kilambo, D and Kaiza, D. 1997. Investigation of Fusarium xylarioides on Robusta coffee in Misenyi division Bukoba district. Trip report, Lyamungu misc. report

Mohamed R. A., Ng'homa N.M., Sayi B. and Kabumbire A, 2002: Report on coffee wilt disease. Ministry of Agriculture and Food Security, The United Republic of Tanzania.

21st International Conference on Coffee Science
Montpellier, 10th – 15th September 2006

21 ème Colloque scientifique international sur le café
Montpellier, 10 – 15 septembre 2006

List of participants

ALGÉRIE	
Mohamed-Zine Boureghda	Université de Boumerdès / Avenue de l'Indépendance / Boumerdès / Algérie / tel. 00 213 24 81 39 87 / boureghda@yahoo.fr
Siegfried Knasmüller	Department of Environmental toxicology / Institute of Cancer Research / Medical University Vienna / Borschkegasse 8a / AU-1090 / Vienna / Austria / tel. 00 43 1 4277 65142 / siegfried.knasmueller@meduniwien.ac.at
BELGIQUE	
Piet August Stoffelen	Nat Botania Garden Belgium / Domein van Bouchout / B-1860 / Meise / Belgique / tel. 00 32 2 260 0920 / stoffelen@br.fgov.be
BRAZIL	
Aldir Alves Teixeira	Assicafé / Rva Domingos de Morais 254 / 5° andar, Confunto 502-B / 04010-100 / Sao Paulo / Brazil / tel. 00 55 11 5579 8042 / assicafe@terra.com.br
Edvaldo Amaral da Silva	UFLA-DBI-LCBM / Campus Universitario S/N / CP 3037 / 37200-000 / Lavras, MG / Brazil / tel. 00 55 35 3829 1434 / amaral@ufla.br
Alan Andrade	Embrapa / Av. W3 Norte - Final - Pq. EB / 70770-900 / Brasilia, DF / Brazil / tel. 00 55 61 3448 4795 / alan@cenargen.embrapa.br

Erika Barros	Recursos geneticos e biotecnologia / Embrapa / Parque Estagao Biologica / Avenida W5 Norte - Final - PBI / 70770-900 / Brasilia, DF / Brazil / tel. 00 55 61 3448 4670 / erikavsa@cenargen.embrapa.br
Bruna Bassoli	Universidade Estadual Londrina / Av. Nossa Senhora de Assunçao, 467 / Ap 55B - Vila Butanta / 05359-001 / Sao Paulo / Brazil / tel. 00 55 11 8551 5603 / bruestado@bol.com.br
Denisley Bassoli	Cia. Iguaçu de Café Soluvel / BR 369 - KM 88 / PO Box 199 / 86300-000 / Cornelio Procopio / Brazil / tel. 00 55 43 3524 1211 / bassoli@iguacu.com.br
Flàvia Bliska	Instituto Agronômico / Avenida Barao de Itapura, 1481 / 13001-970 / Campinas, SP / Brazil / tel. 00 55 19 3241 5188 / bliska@iac.sp.gov.br
Carlos Bloch	Embrapa / Av. WS Norte - Final / 70770-900 / Brasilia, DF / Brazil / tel. 00 55 61 3448 4636 / bloch.carlos@gmail.com
Rita Bordignon	IAC / Avenida Barão de Itapura, 1481 / 13001-970 / Campinas / Brazil / tel. 00 55 19 3241 5188 / rita@iac.sp.gov.br
Flavio Meira Borem	Departamento de Engenharia / Universidade Federal de Lavras / Campus Universitario / 37200-000 / Lavras, MG / Brazil / tel. 00 55 35 3829 1488 / flavioborem@ufla.br
Regina Carneiro	Cenargen / Embrapa / SAIN Parque Rural / Final Av. W/5 Norte / 70770-900 / Brasilia / Brazil / tel. 00 55 613 340 3660 / recar@cenargen.embrapa.br
Carlos Carvalho	Embrapa Café / Alameda do Café, 1000 / 37026-400 / Varginha / Brazil / tel. 00 55 35 32 14 14 11 / carlos.carvalho@embrapa.br
Antonio Chalfun-Junior	UFLA-DBI-LCBM / Campus Universitario S/N / CP 3037 / 37200-000 / Lavras, MG / Brazil / tel. 00 55 35 3829 1172 / chalfunjunior@ufla.br
Carlos Colombo	IAC- Campinas / Av. Barao de Itapura, 1481 / 13001-970 / Campinas, SP / Brazil / tel. 00 55 19 3241 5188 / ccolombo@iac.sp.gov.br
Miriam Eira	Embrapa / Av. W3 Norte - Final - Pq. EB / 70770-900 / Brasilia, DF / Brazil / tel. 00 55 61 3448 4010 / miriam.eira@embrapa.br
Adriana Farah	Universidade Federal do Rio de Janiero / Instituto de Quimica / Bloco A 528-A / 22550-130 / Rio de Janeiro / Brazil / tel. 00 55 21 2562 7351 / afarah@iq.ufrj.br
Luiz Carlos Fazuoli	IAC / Avenida Barão de Itapura, 1481 / 13001-970 / Campinas, SP / Brazil / tel. 00 55 19 3241 5188 / fazuoli@iac.sp.gov.br
Mariano B. Marcondes Ferraz	ITAL - Instituto de Tecnologia de Alimentos / Francisco Amendola 1817 / Jd Paineiras / 13070-241 / Campinas, SP / Brazil / tel. 00 55 19 3251 2077 / marianoferraz@gmail.com
Adriana França	DEQ/UFMG / Nucleo de Pesquisa e Desenvolvimento en Café / R. Espirito Santo / 6° andar / 30160-030 / Belo Horizonte, MG / Brazil / tel. 00 55 31 323 81 777 / franca@deq.ufmg.br
Terezinha de Jesus Garcia Salva	IAC / Avenida Barão de Itapura, 1481 / 13001-970 / Campinas, SP / Brazil / tel. 00 55 19 3241 5188 / tsalva@iac.sp.gov.br

Maria Beatriz A. Gloria	Departamento de alimentos, fafar / UFMG / Avenido Antonio Carlos 6627 / 31270-901 / Belo Horizonte, MG / Brazil / tel. 00 55 31 3498 2419 / beatriz@farmacia.ufmg.br
Maria Fatma Grossi de Sa	Embrapa / Peq EB, Final W5 Norte / 70770-900 / Brasilia, DF / Brazil / tel. 00 55 61 3448 49 02 / fatimasa@cenargen.embrapa.br
Beatriz Iamanaka	UNICAMP / R Sacramento, 481 / Apto 71 / 13010-210 / Campinas, SP / Brazil / tel. 00 55 19 3743 1814 / biathie@yahoo.com
Paulo Mazzafera	Departamento de fisiologia vegetal / Universidade de Campinas / Institut de Biologia / CP 6109 / 13083-970 / Campinas, SP / Brazil / tel. 00 55 19 3788 6213 / pmazza@unicamp.br
Herculano Medina-Filho	IAC / Avenida Barão de Itapura, 1481 / 13001-970 / Campinas / Brazil / tel. 00 55 19 3241 5188 / medina@iac.sp.gov.br
Leandro S. Oliveira	DEQ/UFMG / Nucleo de Pesquisa e Desenvolvimento em Café / R. Espirito Santo / 6° andar / 30160-030 / Belo Horizonte, MG / Brazil / tel. 00 55 31 323 81 777 / leandro@deq.ufmg.br
Marcelo Paes De Camargo	IAC / Avenida Barão de Itapura, 1481 / 13001-970 / Campinas / Brazil / tel. 00 55 19 3241 5188 / mcamargo@iac.sp.gov.br
Luciano Paiva Vilela	UFLA-DQI-LCBM / Campus Universitario s/n / CP 3037 / 37200-000 / Lavras, MG / Brazil / tel. 00 55 35 3829 1624 / luciano@ufla.br
Luiz Filipe Pereira	Lab. biotecnologia / Embrapa Café / IAPAR / Rod. Cblso Garcia Cid - KM 375 / 86001-970 / Londrina, PR / Brazil / tel. 00 55 43 3376 2399 / lpereira@iapar.br
Antonio Pereira	EPAMIG/CTZM / Vila Gianetti, casa 46 / Campus da UFV / 36570-000 / Viçosa, MG / Brazil / tel. 00 55 31 3891 2646 / pereira@epamig.ufv.br
Ludwig H. Pfenning	Departamento de fitopatologia / Universidade Federal de Lavras / Laboratorio de Sistematica e Ecologia de Fungos / CP 3037 / 37200-000 / Lavras, MG / Brazil / tel. 00 55 35 3829 1798 / ludwig@ufla.br
Ney Sakiyama	Departamento de fitotecnia / Universidade Federal de Viçosa / / 36570-000 / Viçosa, MG / Brazil / tel. 00 55 31 3899 7343 / sakiyama@ufv.br
Marta Taniwaki	Microbiology department / ITAL / Avenida Brasil, 2880 / 13070-178 / Campinas, SP / Brazil / tel. 00 55 19 3743 1819 / mtaniwak@ital.sp.gov.br
Roberto Antonio Thomaziello	IAC / Avenida Barão de Itapura, 1481 / CP 28 / 13001-970 / Campinas, SP / Brazil / tel. 00 55 19 3241 5188 / rthoma@iac.sp.gov.br
Masako Toma Braghini	IAC / Avenida Barão de Itapura, 1481 / 13001-970 / Campinas / Brazil / tel. 00 55 19 3241 5188 / mako@iac.sp.gov.br
Luiz Gonzaga Vieira	IAPAR / Rod. Celso Garcia Cid / KM 375 / 86001-970 / Londrina, PR / Brazil / tel. 00 55 43 3376 2429 / lvieira@iapar.br

CHILE	
Nicolas Franck	Universidade de Chile / / Santiago / Chile / n.franck@uchile.cl

COLOMBIA	
Ciro Bonilla	National Federacion of Coffee Growers / CRA 4 calle 16 / Feeze Dried Coffee Factory / Chinchina / Colombia / tel. 00 57 68 50 40 40 / ciro.bonilla@cafedecolombia.com
Gabriel Cadena Gomez	Cenicafe - FNC / Km 4 / Via Antigua a Manizales / Manizales, Caldas / Colombia / tel. 00 57 6850 6550 / gabriel.cadena@cafedecolombia.com
Marco Cristancho	Bioinformatics development group / Cenicafe - FNC / Km 4 via antigua a Manizales / Chinchina, Caldas / Colombia / tel. 00 57 685 06550 / marco.cristancho@cafedecolombia.com
Juan-Carlos Herrera Pinilla	Cenicafé - FNC / Km 4 - Via Antigua a Manizales / Chinchina-Caldas / Colombia / tel. 00 57 6850 6550 / juanc.herrera@cafedecolombia.com
Maria del Pilar Moncada	National Research Center - Cenicafe / / AA-2427 / Manizales / Colombia / tel. 00 57 6 850 6550 / pilar.moncada@cafedecolombia.com
Thomas Oberthür	CIAT / Recta Cali Palmira / AA 6713 / Cali / Colombia / tel. 00 57 2 445 0137 / t.oberthur@cgiar.org

CONGO DÉMOCRATIQUE	
Adrien Kalonji	Université de Kinshasa / Campus de Kinshasa / 127 / Kinshasa / Congo Démocratique /
Patrick Tshilenge	Université de kinshasa / Campus de Kinshasa / 127 / Kinshasa / Congo Démocratique /

COSTA RICA	
Jacques Avelino	CIRAD / IICA-PROMECAFEA.P. 55 / 2200 / Coronado, San Jose / Costa Rica / tel. 00 506 260 18 74 / jacques.avelino@cirad.fr
Pierre Charmetant	UMR T52 PIA / CIRAD - CP / Aptdo. 208-3011 / Barva Heredia / Barva Heredia / Costa Rica / tel. 00 506 2 60 18 74 / pcharmetant@cirad.fr
Carlos Fonseca	Instituto de Cafe de Costa Rica / San Pedro Banva de Heredia / 131 / Heredia / Costa Rica / tel. 00 506 260 18 74 / cfonseca@icafe.go.cr

CÔTE D'IVOIRE	
Louis Ban-Koffi	Centre National de Recherche Agronomique / Station de rRcherche technologique08 BP 18 / 08 / Abidjan / Côte d'Ivoire / tel. 00 225 224 428 58 / louis.bankoffi@aviso.ci

N'goran Koffi	IACO / BP V210 / 08 / Abidjan / Côte d'Ivoire / tel. 00 225 2033 2033 / maxngorank@yahoo.fr
Ronald Onzima	IACO - Interafrican Coffee Organisation / BP V210 / 225 / Abidjan / Côte d'Ivoire / tel. 00 225 20 21 6131 / rjonzima@hotmail.com

ETHIOPIA	
Girma Adugna	EIAR / Jimma Agricultural Research Center / PO Box 192 / Jimma / Ethiopia / tel. 00 251 917 803 517 / gadugna@yahoo.com
Bayetta Bellachew	Jimma Agricultural Research Center / Institute of Agricultural Research / PO Box 192 / Jimma / Ethiopia / bbellchew@yahoo.com
Taye Kufa	EARI / Ethiopian Agricultural Research Institute / Jimma Research Center / Jimma / Ethiopia / kufataye@yahoo.com
Jean-Pierre Labouisse	CIRAD / PO Box 920 / Jimma / Ethiopia / tel. 00 251 47 111 81 57 / jean-pierre.labouisse@cirad.fr
Seyoum Seifu Gebeyhu	Ethiopian agricultural Research Organization / Awada Agricultural Research Center / PO Box 205 / Yirgalem / Ethiopia / tel. 00 251 46 225 0002 / seyoum03@yahoo.com
Surendra Kotecha	BDPA-CIRAD EU / Coffee for Ethiopia CIP IV / Addis Ababa / Ethiopie / tel. 00 251 911 864 743 / sksurendra@aol.com

FINLAND	
Siamak Bidel	University of Helsinki / Pykälätie 5B / FIN-00690 / Helsinki / Finland / tel. 00 358 41 529 8909 / siamak.bidell@ktl.fi
Karla Koullias	Gustav Paulig Ltd. / Gustav Pauligin Katn 5 / FIN-00990 / Helsinki / Finland / tel. 00 358 9 31 981 / karla.koullias@paulig.fi
Atte Salomaa	Meira Ltd / PO Box 32 / FIN-00511 / Helsinki / Finland / tel. 00 358 20744 3565 / atte.salomaa@meira.fi
Jaakko Tuomilehto	Diabetes Unit / National Public Health Institute / Mannerheimintie 166 / FIN-00300 / Helsinki / Finland / tel. 00 358 9 47 44 83 16 / jaakko.tuomilehto@ktl.fi

FRANCE	
Philip Aguilar	UPR qualité produit Pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 57 45 / philippe.aguilar@cirad.fr
Edgardo Alpizar	CIRAD / IRD UMR DGPC / 911, avenue de l'Agropolis / 34314 / Montpellier / France / tel. 04 67 41 62 96 / alpizar@mpl.ird.fr
François Anthony	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 89 / anthony@mpl.ird.fr

Sylvie Avallone	ENSIA / 1101, avenue Agropolis / CS 24501 / 34093 / Montpellier / France / tel. 04 67 41 61 91 / avallone@mpl.ird.fr
Martine Barale	DIC / CIRAD / TA 383/05 / 911, avenue de l'Agropolis / 34398 / Montpellier / France / tel. 04 67 31 71 81 / martine.barale@cirad.fr
Jean-Luc Battini	UR 34 / CIRAD / TA 80/02 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 65 64 / jean-luc.battini@cirad.fr
Dominique Berry	CIRAD / TA 80/PS1 / Boulevard de la Lironde / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 58 98 / dircp@cirad.fr
Laurent Berthiot	UPR qualité produits pérennes / Cirad Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 75 49 / laurent.berthiot@cirad.fr
Benoît Bertrand	CIRAD / TA 80/IRD / Avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 91 / benoit.bertrand@cirad.fr
Carole Bessière	IRD / 911, avenue Agropolis / BP 5045 / 34032 / Montpellier / Cedex 01 / France / tel. 04 67 41 62 21 / carole.bessiere@mpl.ird.fr
Daniel Bieysse	UMR - BGPI / CIRAD - Amis / Campus International de Baillarguet / TA 41/K / 34398 / Montpellier / Cedex 05 / France / tel. 04 99 62 48 03 / daniel.bieysse@cirad.fr
Renaud Boulanger	UPR qualité produit Pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 44 28 / renaud.boulanger@cirad.fr
Arnaud-Xavier Bourasset	CIRAD / TA 178/04 / 911, avenue Agropolis / 34398 / Montpellier / France / tel. 04 67 61 71 11 / arnaud-xavier.bourasset@cirad.fr
Marty Burgaud	CIRAD / TA 178/04 / 911, avenue de l'Agropolis / 34398 / Montpellier / France / tel. 04 67 61 58 02 / marty.burgaud@cirad.fr
José Bustamante	UMR 1097 DGPC / IRD / 911, avenue Agropolis / BP 64501 / 34394 / Montpellier / France / tel. 04 37 41 62 24 / jose.bustamante@mpl.ird.fr
Claudine Campa	IRD / 911, avenue de l'Agropolis / B.P. 5045 / 34032 / Montpellier / Cedex 1 / France / tel. 04 67 41 62 43 / campa@mpl.ird.fr
Nathalie Chabrillange	UMR DGPC / IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / France / tel. 04 67 41 62 41 / nathalie.chabrillange@mpl.ird.fr
André Charrier	AGRO Montpellier / 2, place Viala / 34060 / Montpellier / France / tel. 04 99 61 22 96 / andre.charrier@ensam.inra.fr
Marie-Christine Combes	IRD / 911, avenue de l'Agropolis / BP 5045 / 34032 / Montpellier / Cedex 1 / France / tel. 04 67 41 61 00 / combes@mpl.ird.fr
Dominique Crouzillat	Centre R&D Nestlé Tours / 101, avenue Gustave Eiffel / BP 49716 / 37097 / Tours / Cedex 2 / France / tel. 02 47 62 83 83 / dominique.crouzillat@rdto.nestle.com
Philippe Cubry	CIRAD - UMR T52 PIA / TA 80/03 / Avenue Agropolis / 34130 / Montpellier / France / tel. 04 67 61 53 78 / philippe.cubry@cirad.fr
Nathalie Curiallet-Mercier	Direction / CIRAD / Boulevard de la Lironde / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 65 69 / nathalie.mercier@cirad.fr

Jean Dauzat	AMAP / AMIS / CIRAD / UMR AMAP 5120 / TA 40 / PS 2 / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 65 76 / dauzat@cirad.fr
Fabien De Bellis	UMR T52 PIA / CIRAD - CP / TA 80/03 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 56 80 / fabien.de_bellis@cirad.fr
Alexandre de Kochko	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / France / tel. 04 67 41 63 11 / alexandre.dekochko@mpl.ird.fr
Evelyne Dechamp	CIRAD / TA 80/IRD / 911, avenue de l'Agropolis / 34398 / Montpellier / France / tel. 04 67 41 62 96 / evelyne.dechamp@cirad.fr
Leandro Diniz	Résistance des plantes / IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 96 / leandrodiniz@yahoo.com.br
Pierre Dublin	/ 15, rue des Fauvettes / 21121 / Fontaine les Dijon / France / tel. 03 80 56 11 40
Magali Dufour	UMR T52 - PIA / CIRAD - CP / TA 80/03 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 59 64 / magali.dufour@cirad.fr
Barbara Dufrene	ISIC - PEC / 3, rue de Copenhague / 75008 / Paris / France / tel. 01 53 42 13 38 / b-dufrene@wanadoo.fr
Noël Durand	UPR qualité produit Pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 58 00 / noel.durand@cirad.fr
Daniel Duris	UPR qualité produit pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 71 36 / daniel.duris@cirad.fr
Stéphane Dussert	UR 141 / IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / France / tel. 04 67 41 61 85 / stephane.dussert@mpl.ird.fr
Hervé Etienne	CIRAD / TA 80/IRD / Avenue Agropolis / 34098 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 27 / herve.etienne@cirad.fr
Diana Fernandez	UR141 - UMR DGPC 1097 / IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 96 / diana.fernandez@mpl.ird.fr
Nathalie Gauthier	UR022 / IRD / Centre de biologie et de gestion des populations / Campus International de Baillarguet / 34988 / Montferrier sur Lez / France / tel. 04 99 62 33 19 / nathalie.gauthier@ensam.inra.fr
Michel Genard	INRA / Domaine de Saint paul / Agroparc / 84914 / Avignon / Cedex 9 / France / tel. 04 32 72 24 58 / michel.genard@avignon.inra.fr
Dominique Gueule	UPR qualité produit Pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 58 40 / dominique.gueule@cirad.fr
Bernard Guyot	UPR qualité produit Pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 71 44 / bernard.guyot@cirad.fr

Serge Hamon	R141 / IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / France / tel. 04 67 41 61 94 / hamon@mpl.ird.fr
Jean-Michel Harmand	CIRAD / Campus International de Baillarguet / TA 10/D / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 59 38 67 / harmand@cirad.fr
Maurice Izard	CIRAD / TA 178/04 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 58 01 / maurice.izard@cirad.fr
Thierry Joet	Pôle de protection des plantes / IRD / 15 00 00 / 7, chemin de l'IRAT - Ligne Paradis / 97410 / St Pierre de la Réunion / France / tel. 02 62 49 92 85 / joet@cirad.fr
Isabelle Jourdan	UMR T52 PIA / CIRAD - CP / TA 80/03 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 54 92 / isabelle.jourdan@cirad.fr
Boutros Kerbaje	Libios / Chemin de la Plagne / 69210 / Bully / France / info@libios.fr
Sophie Lafay	Berkem Developpement / Marais Ouest / 24680 / Gardonne / France / tel. 05 53 63 81 00 / sophie.lafay@berkem.com
Adréina Laffargue	CIRAD / TA 80/IRD / 911,avenue de l'Agropolis / 34398 / Montpellier / France / tel. 04 67 41 62 29 / adreina.laffargue@cirad.fr
Charles Lambot	Centre R&D Nestlé Tours / 101, avenue Gustave Eiffel / BP 49716 / 37097 / Tours / Cedex 2 / France / tel. 02 47 62 83 83 / charles.lambot@rdto.nestle.com
Etienne Landais	SupAgro Montpellier / 2, place Viala / 34060 / Montpellier / Cedex 1 / France / tel. 04 99 61 24 17 / landais@ensam.inra.fr
Philippe Lashermes	IRD / BP 64501 / 34394 / Montpellier / France / tel. 04 67 41 61 85 / philippe.lashermes@mpl.ird.fr
Anne-Yvonne Le Dain	Région Languedoc Roussillon / Hôtel de la Région / 201, avenue de la Pompignane / 34064 / Montpellier / France / myriamsales.regionlr@wanadoo.fr
Daniel Le Pierres	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 96 / lepierre@mpl.ird.fr
Anne-Claire Lecouls	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 96 / lecouls@mpl.ird.fr
Pascale Lepoint	CIRAD / 2477, avenue Val de Montferrand / BP 5035 / 34032 / Montpellier / France /
Thierry Leroy	UMR T52 - PIA / CIRAD - CP / TA 80/03 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 56 90 / thierry.leroy@cirad.fr
Laétitia Mahé	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 63 44 / mahel@mpl.ird.fr
Pierre Marraccini	UMR T52 PIA / CIRAD - CP / TA 80/03 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / pierre.marraccini@cirad.fr

Pascal Musoli	UMR - BGPI / CIRAD - Amis / Campus International de Baillarguet / TA 41/15 / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 44 67 / pascal.musoli@cirad.fr
Astrid Nehlig	INSERM U666 / Faculté de Médecine / 11, rue Humann / 67085 / Strasbourg / France / tel. 03 90 24 32 43 / nehlig@neurochem.u-strasbg.fr
Dominique Nicolas	Cirad CP / TA 80/P53 / Boulevard de la Lironde / 34032 / Montpellier / Cedex 5 / France / tel. 04 67 61 71 84 / dominique.nicolas@cirad.fr
Michel Nicole	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 96 / nicole@mpl.ird.fr
Hubert Omont	Direction / CIRAD / PS3 / Boulevard de la Lironde / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 55 54 / hubert.omont@cirad.fr
Jean-Jacques Perriot	UPR qualité produit Pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 58 92 / jean-jacques.perriot@cirad.fr
Anne-Sophie Petitot	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 96 / petitot@mpl.ird.fr
Sébastien Piccino	UPR qualité produit pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 5 / France / sebastien.piccino@cirad.fr
Fabrice Pinard	UPR 31 / CIRAD - CP / TA80/02 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 44 67 / fabrice.pinard@cirad.fr
Valérie Poncet	DGPC / IRD / 911, avenue l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 51 / valerie.poncet@mplird.fr
Nicole Pons	CIRAD Culture Pérennes / TA 80/PS3 / Boulevard de la Lironde / 34398 / Montpellier / Cedex 5 / France / tel. 04 67 61 69 00 / nicole.pons@cirad.fr
David Pot	UMR T52 - PIA / CIRAD - CP / TA 80/03 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France /
Francis Quetier	GenoscopeANR / 2, rue Gaston Crémieux / 91057 / Evry / France / quetier@genoscope.cns.fr
Daniel Ramiro	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 96 / ramiro@mpl.ird.fr
Fabienne Ribeyre	UPR qualité produit Pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 75 49 / fabienne.ribeyre@cirad.fr
Angela Rosso	Malongo / BP 121 / ZI 9è rue / 06513 / Carros / France / arosso@malongo.com
Olivier Roupsard	CIRAD / Campus International de Baillarguet / TA 101D / 34398 / Montpellier / Cedex / France / tel. 04 67 59 37 51 / olivier.roupsard@cirad.fr
André Rouzière	UPR qualité produit Pérennes / CIRAD Culture Pérennes / TA 80/16 / 73, avenue Jean-François Breton / 34398 / Montpellier / Cedex 05 / France / tel. 04 67 61 71 32 / andre.rouziere@cirad.fr

Didier Snoeck	UR 34 / CIRAD / TA 80/02 / 911, avenue de l'Agropolis / 34398 / Montpellier / Cedex 5 / France / tel. 00 233 21 5 31 42 / didier.snoeck@cirad.fr
John Stiles	Integrated Coffee Technologies / PO Box 1070 / 96791 / Waialua, HI / France / tel. 00 1 808 351 3502 / jstiles@integratedcoffee.com
Pascale Talamond	IRD / 911, avenue de l'Agropolis / BP 64501 / 34394 / Montpellier / Cedex 5 / France / tel. 04 67 41 62 51 / talamond@mplird.fr
Philippe Vaast	CIRAD / Campus International de Baillarguet / TA 10/D / 34398 / Montpellier / France / tel. 04 67 59 37 51 / philippe.vaast@cirad.fr
Paola Wintenberger	Malongo / BP 121 / ZI 9è rue / 06513 / Carros / France /

GERMANY	
Matthias Baum	TU Kaiserslautern / Food, chemistry and environmental / toxicology / D-67663 / Kaiserslautern / Germany / tel. 00 49 631 205 3858 / baum@chemie.uni-kl.de
Nadine Böhm	TU Kaiserslautern / Food, chemistry and environmental toxicology / Erwin-Schrödinger strasse 52 / D-67663 / Kaiserslautern / Germany / tel. 00 49 631 205 2974 / boehmnad@rhrk.uni-kl.de
Sandra Bongers	Probat - Werke / Reeser Strasse 94 / D-46446 / Emmerich / Germany / tel. 00 49 2822 912 - 0 / s.bongers@probat.com
Helmut Buchta	Nestle Deutschland AG / Lyoner Strasse 23 / D-60523 / Frankfurt/Main / Germany / tel. 00 49 69 6671 2546 / helmut.buchta@de.nestle.com
Juergen Burkhardt	INRES / University of Bonn / Karlrobert-Kreiten-Strasse 13 / D-53115 / Bonn / Germany / tel. 00 49 228 732 186 / j.burkhardt@uni-bonn.de
Andreas Degenhardt	Kraft Foods R&D Inc. / Bayerwald Strasse 8 / D-81737 / Munich / Germany / tel. 00 49 89 62738 6473 / adegenhardt@krafteurope.com
Manfred Denich	ZEF Bonn / Walter-Flex-Str. 3 / D-53113 / Bonn / Germany / tel. 00 49 228 731864 / m.denich@uni-bonn.de
Gerhard Eisenbrand	University of Kaiserlautern / Erwin-Schroedinger Strasse / D-67663 / Kaiserslautern / Germany / tel. 00 49 631 205 2973 / eisenbra@thrk.uni-kl.de
Bernd Fiebich	Psychiatry departement / University of Freiburg Medical School / Hauptstrasse 5 / D-79104 / Freiburg / Germany / tel. 00 49 761 270 6898
Anja Fischer	German Research Center for Food Chemistry / Lichtenbergstrasse 4 / D-85748 / Garching / Germany / tel. 00 49 89 289 13266 / asfischer@gmx.de
Oliver Frank	Institut für Lebensmittelchemie / University of Münster / Correnstrasse 45 / D-48149 / Münster / Germany / tel. 00 49 251 833 3899 / ofrank@uni-muenster.de
Diana Gniechwitz	Institute of Food Chemistry / University of Hamburg / Grindelalle 117 / D-20146 / Hamburg / Germany / tel. 00 49 40 42838 4349 / gniechwi@chemic.uni-hamburg.de
Helmut Günther	Research and development / Kraft Foods / Langemarckstrasse 4-20 / D-28199 / Bremen / Germany / tel. 00 49 421 599 3274 / hguenther@krafteurope.com

Thomas Hatzold	Kraft Foods R&D Inc. / Bayerwaldstrasse 8 / D-81737 / Munich / Germany / tel. 00 49 89 62738 6363 / thalzeld@krafteurope.com
Holger Hindorf	Institute of Plant desease / University of Bonn / Nussalle, 9 / D-53105 / Bonn / Germany / tel. 00 49 228 73 2444 / h.hindorf@uni-bonn.de
Christian Hinkel	Lebensmittel chemie / TU Dresden / Bergstrasse 66 / D-01062 / Dresden / Germany / tel. 00 49 351 463 37868 / christian.hinkel@chemie.tu-dresden.de
Isabelle Kölling-Speer	Lebensmittelchemie / TU Dresden / Bergstrasse 66 / D-01062 / Dresden / Germany / tel. 00 49 351 4633 3603 / isabelle.koelling-speer@chemie.tu-dresden.de
Stephan Lange	Probat-Werke / Reeserstrasse 94 / D-46446 / Emmerich / Germany / tel. 00 49 02822 912 362 / r.neuenfelot@probat.com
Gregory Mc Caffin	Coffein Compagnie / Dr. Erich Scheele GmbH & Co K 6 / Scbaldsbruecker Heerstr. 191 A / D-28309 / Bremen / Germany / tel. 00 49 421 4101 609 / iselke@coffein-compagnie.de
Christian Mendl	Kraft Foods R&D Inc. / Bayerwald Strasse 8 / D-81737 / Munich / Germany / tel. 00 49 89 6273 86270 / adegenhardt@krafteurope.com
Sigrid Oestreich-Janzen	KORD GmbH & Co. KG / Am Sandtorkai 2 / D-20457 / Hamburg / Germany / tel. 00 49 40 37 88 90 / harmgardt@kord-hh.com
Claudia Scheidig	German Research Center for Food Chemistry / Lichtenbergstrasse 4 / D-85748 / Garching / Germany / tel. 00 49 89 289 13266 / claudia.scheidig@lrz.tum.de
Bernd Schopf	Coffein Compagnie / Dr. Erich Scheele GmbH & Co K 6 / Scbaldsbruecker Heerstr. 191 A / D-28309 / Bremen / Germany / tel. 00 49 421 4101 609 / iselke@coffein-compagnie.de
Harald Schuchmann	Consultant / Schauinslandweg 1 / D-76297 / Stutensee / Germany / tel. 00 49 72 49 95 22 41 / harald.schuchmann@email.de
Dirk Selmar	Institute of Plant Biology / Technical University / Mendelsshonstrasse 4 / D-38106 / Braunschweig / Germany / tel. 00 49 391 391 5881 / d.selmar@tu-bs.de
Karl Speer	Lebensmittelchemie / TU Dresden / Bergstrasse 66 / D-01062 / Dresden / Germany / tel. 00 49 351 4633 3132 / karl.speer@chemie.tu-dresden.de
Herbert Stiebitz	Tchibo Manufacturing GmbH & Co. KG / Suederstr. 293 / D-20537 / Hamburg / Germany / tel. 00 49 40 6387 3149 / hst@tchibo.de
Kassahun Tesfaye Geletu	University of Bonn / Nees Institute / Merckenheimer Allee 170 / D-53115 / Bonn / Germany / tel. 00 49 73 21 23 / kassahuntesfaye@yahoo.com
Frank Ullrich	Kraft Foods R&D Inc. / Bayerwald Strasse 8 / D-81737 / Munich / Germany / tel. 00 49 89 62738 6473 / adegenhardt@krafteurope.com
Carola Weiss	Tchibo Manufacturing GmbH & Co. KG / Suederstr. 293 / D-20537 / Hamburg / Germany / tel. 00 49 40 6387 3253 / caw@tchibo.de
Jürgen Zapp	Life science technology department / University of Applied Science Lippe and Höxter / Liebigstrasse 87 / FB 4 / D-32657 / Lemgo / Germany / tel. 00 49 5261 702 321 / juergen.zapp@fh-luh.de

GHANA	
Frank Manu Amoah	Cocoa Research Institute of Ghana / PO Box 8 / New Tafo-Akim / Ghana / tel. 00 233 244 810 478 / fmamoah@crig.org
Esther Anim-Kwapong	Cocoa Research Institute of Ghana / PO Box 8 / New Tafo-Akim / Ghana / tel. 00 233 0243 127 380 / ekwapong06@yahoo.com

GUATEMALA	
Guillermo Canet Brenes	IICAPromecafé / Guatemala, Ciudad / Guatemala / Guatemala / tel. 00 502 2386 5907
Edwin Roldan	Promecafé / 5a Calle 0-50 Zona 14 / Guatemala / Guatemala / tel. 00 502 2337 3920 / eroldan@email.anacafe.org

INDIA	
Jayarama	Central Coffee Research Institute / Coffe Board / Coffe Research Station (Post) / 577117 / Chikmagalur Dist. / India / tel. 00 91 8265 243 029 / jayarama@myway.com
Surya Prakash Rao Nayani	Regional Coffee Research Station / Coffe Board / 1-178, PB Palli, Narsipatnam / 53116 / Visakhapatnam, Dist. / INDIA / tel. 00 91 8932 226 152 / nayanirao@yahoo.com
Giridhar Parvatam	Plant cell biotechnology department / C.F.T.R.I. / / 570 020 / Mysore, Karanataka / India / tel. 00 91 821 251 6501 / parvatamg@yahoo.com
Hosahalli Sreenath	Tissue culture & biotechnology centre / Coffee Board / Dr S. Radhakrishnan road (Bogadi road) / 570006 / Mysore, Karnataka / India / tel. 00 91 821 254 3612 / cofbiotech@vsnl.com
Mahesh Venkataramaiah	Avestha Gengraine Technologies Pvt. Ltd / Discoverer 9th Floor, ITPL / Whitefield road / 560065 / Bangalore / India / tel. 00 91 80 284 11 665 / maheshv@avesthagen.com

ISRAËL	
Yair Bezman	Strauss-Elite Ltd / PO Box 226 / 70100 / Lod / Israël / tel. 00 972 8 978 2043 / yairb@strauss-elite.co.il
Zion Cohen	Strauss-Elite Ltd / PO Box 226 / 70100 / Lod / Israël / tel. 00 972 8 978 2043 / zionc@strauss-elite.co.il
David Nini	Strauss-Elite Ltd / PO Box 226 / 70100 / Lod / Israël / tel. 00 972 8 978 2043 / davidn@strauss-elite.co.il

ITALY	
Laura Alessandrini	Department of food science / University of Bologna / P.zza Goidanich, 60 / IT-47023 / Cesena (FC) / Italy / tel. 00 39 0547 636120 / laura.alessandrini@studioi.unibo.it
Massimo Barnabà	Illycaffè SPA / Via Flavia 110 / IT-34149 / Trieste / Italy / tel. 00 39 040 3890 111 / caimil@illy.it
Bernd Bonnländer	Illycaffè SPA / Via Flavia 110 / IT-34149 / Trieste / Italy / tel. 00 39 040 3890 111 / caimil@illy.it
Enrico Braidot	Biologia ed economia agro-industriale / University of Udine / Via Cotonificio 108 / IT-33100 / Udine / Italy / tel. 00 39 0432 558 792 / enrico.braidot@dbea.unid.it
Giuseppe Buccella	Verwerkaf Spa / Via Don Minzomi 50 / IT-43015 / Noceto, PR / Italy / tel. 00 39 521 628 444 / info@werwerkaf.it
Andrea Bulbarello	University of Milan / Via Monterono 34 / IT-21013 / Gallarate, VA / Italy / tel. 00 39 2 50 31 66 31 / andrea.bulbarello@unimi.it
Giancarlo Catalano	Co. Ind S.C. / Via Saliceto 22/H / IT-40013 / Castelmaggiore, Bologna / Italy / tel. 00 39 051 632 8511 / gcatalano@coind.it
Giacomo Celi	Illycaffè SPA / Via Flavia, 110 / IT-34147 / Trieste / Italy / tel. 00 39 040 3890 111 / caimil@illy.it
Alessio Colussi	Illycaffè SPA / Via Flavia, 110 / IT-34147 / Trieste / Italy / tel. 00 39 040 3890 111 / caimil@illy.it
Ehsan Dullo	IPGRI / / IT-00187 / Rome / Italy /
Florent Engelmann	IPGRI / Via delle sette chiese 142 / IT-00145 / Rome / Italy / tel. 39 06 51 89 22 24 / f.engelmann@cgiar.org
Massimiliano Fabian	DEMUS S.p.a. / Via Caboto, 31 / IT-34147 / Trieste / Italy / tel. 00 39 348 902 8508 / mfabian@demus.it
Anna Giuseppina Fiore	Department of food science / University of Foggia / Via Napoli, 25 / IT-71100 / Foggia / Italy / tel. 00 39 881 589 245 / fioreannapina@yahoo.it
Giorgio Graziosi	Dipartemento biologia / Universita di Trieste / P.le Valmaura 9 / IT-34100 / Trieste / Italy / tel. 00 39 40 811 876 / graziosi@univ.trieste.it
Andrea Illy	Illycaffè Spa / Via Flavia, 110 / IT-34147 / Trieste / Italy / tel. 00 39 403 890 707 / andrea.illy@illy.it
Ernesto Illy	Illycaffè Spa / Via Flavia, 110 / IT-34147 / Trieste / Italy / tel. 00 39 403 890 727 / ernesto.illy@illy.it
Andreas Karanikolas	Co. Ind S.C. / Via Saliceto 22/H / IT-40013 / Castelmaggiore, Bologna / Italy / tel. 00 39 051 632 8511 / akaranikolas@coind.it
Giuditta Kravina	Department of food science / University of Udine / Via Mrangoni 97 / IT-33100 / Udine / Italy / tel. 00 39 04 32 59 07 32 / giuditta.kravina@uniud.it
Carlo La Vecchia	Mario Negri Intitute / Via Eritrea, 62 / IT-20157 / Milano / Italy / tel. 00 39 02 39 01 45 27 / lavecchia@marionegri.it

Luciano Navarini	Illycaffè SPA / Via Flavia 110 / IT-34149 / Trieste / Italy / tel. 00 39 040 3890 111 / caimil@illy.it
Maria Cristina Nicoli	Dipartimento de Scienze degli Alimenti / University of Udine / Via Marangoni 97 / IT-33100 / Udine / Italy / tel. 00 39 0432 590 744 / mariacristina.nicoli@uniud.it
Sonia Patui	University of Udine / Via Cotonificio 108 / IT-33100 / Udine / Italy / tel. 00 39 0432 558 796 / sonia.patui@univd.it
Gloria Pellegrino	Luigi Lavazza S.p.A. / Strada Settimo, 410 / IT-10156 / Torino / Italy / tel. 00 39 011 239 8452 / g.pellegrino@lavazza.it
Carlo Peresson	Biologia ed economia agro-industriale / University of Udine / Via Cotonificio 108 / IT-33100 / Udine / Italy / tel. 00 39 0432 558 796 / carlo.presson@univd.it
Marino Petracco	Illycaffè SPA / Via Flavia, 110 / IT-34147 / Trieste / Italy / tel. 00 39 040 3890 111 / caimil@illy.it
Jean-Michel Pommet	ARCHES / Via Giuseppe Leuzzi 18 / IT-70013 / Castellana Grotte / Italy / pommet@arches-centroricerca.org
Diego Rivetti	Illycaffè SPA / Via Flavia 110 / IT-34149 / Trieste / Italy / tel. 00 39 040 3890 111 / caimil@illy.it
Santina Romani	University of Bologna / Piazza Goidanich n°60 / IT-47023 / Cesena (FC) / Italy / tel. 00 39 0547 636120 / santina.romani2@unibo.it
Carla Severini	Department of food science / University of Foggia / Via Napoli, 25 / IT-71100 / Foggia / Italy / tel. 00 39 881 589 222 / c.severini@unifg.it
Furio Suggi Liverani	Illycaffè SPA / Via Flavia, 110 / IT-34147 / Trieste / Italy / tel. 00 39 040 3890 111 / caimil@illy.it
Renato Toffanin	ARCHES / Via Giuseppe Leuzzi 18 / IT-70013 / Castellana Grotte / Italy / tel. 00 39 08 04 96 85 46 / toffanin@protos-institute.org
Patrizia Tornincasa	Department of biology / University of Trieste / Piazzale Valmaura, 9 / IT-34100 / Trieste / Italy / tel. 00 39 0408 122 37 / patriziator@yahoo.it

JAPAN	
Masayuki Akiyama	Morinaga Milk Industry / 1-83, 5-Chome / Higashihara, Zama-city / JP-228 8583 / Kanagawa-Pref. / Japan / tel. 00 81 46 252 3031 / m_akiyam@morinagamilk.co.jp
Nanaka Gotoda	UCC Ueshima Coffee Co., Ltd. / 3-1-4 Zushi, Takatsuki / JP-569 0036 / Osaka / Japan / tel. 00 81 72 674 0109 / nanaka-gotouda@ucc.co.jp
Seiichi Homma	Tokyo University of Agriculture / Sakuragaoka 1-1-1 / Setagaya-ku / JP-156-8502 / Tokyo / Japan / tel. 00 81 3 5477 2317 / s3homma@nodai.ac.jp
Michio Ikeda	Morinaga Milk Industry / 1-83, 5-Chome / Higashihara, Zama-city / JP-228-8583 / Kanagawa-Pref. / Japan / tel. 00 81 46 252 3031 / mi_ikeda@morinagamilk.co.jp

Kazuya Iwai	UCC Ueshima Coffee Co., Ltd. / 3-1-4 Zushi, Takatsuki / JP-569-0036 / Osaka / Japan / tel. 00 81 72 674 0109 / kazuya-iwai@ucc.co.jp
Yoshiaki José Kawashima	UCC Ueshima Coffee Co., Ltd. / Davinci Onarimon Building / 6-1-11 Shinbashi, Minato-ku / JP-105-8577 / Tokyo / Japan / tel. 00 81 3 5400 5580 / yoshiaki-kawashima@ucc.co.jp
Aino Kazuyoshi	Japan Tobacco Inc. / 5-14, Haneda Asahicho Ota-ku / JP-1440042 / Tokyo / Japan / tel. 00 81 3 5705 7698 / kazuyoshi.aino@ims.jti.co.jp
Michio Nozaki	Takasago International Corp. / 1-4-11, Nishi-Yawata, Hiratsuka / JP-254-0073 / Kanagawa / Japan / tel. 00 81 463 25 2180 / michio_nozaki@takasago.com
Yoshiyuki Oshima	Chemi. Com. Japan Corporation / 102-6, 2, Sengen-cho Nishi-Ku / JP-220-0072 / Yokohama / Japan / tel. 00 81 45 322 3399 / y-ohshima@chemi-com.co.jp
Katsuya Tokuno	San-Ei Gen F.F.I. Inc. / Sanwa-Cho 1-1-11 / JP-561-8588 / Toyonaka, Osaka / Japan / tel. 00 81 6 6333 0521 / ktokuno@saneigenffi.co.jp
Ishiwaki Tomohiro	S. Ishimitsu & Co., Ltd / 4-40, Iwayaminami-Machi / Nada-Ku / JP-657-0856 / Kobe / Japan / tel. 00 81 78 861 7842 / t-ishiwaki@ishimitsu.co.jp
Tatsushi Ueshima	UCC Ueshima Coffee Co., Ltd. / Davinci Onarimon Building / 6-1-11 Shinbashi, Minato-ku / JP-105-8577 / Tokyo / Japan / tel. 00 81 3 5400 5580 / daisuke-tsuchikawa@ucc.co.jp
Katsuya Steve Yamagata	UCC Ueshima Coffee Co., Ltd. / Davinci Onarimon Building / 6-1-11 Shinbashi, Minato-ku / JP-105-8577 / Tokyo / Japan / tel. 00 81 3 5400 5580 / katsuya-yamagata@ucc.co.jp
Otsuka Yuuichi	Key Coffee Inc. / 22 Takase-cyo / Funabashi-shi / JP-2730014 / Chiba / Japan / tel. 00 81 47 433 1544 / yuuichi_ootsuka@keycoffee.co.jp
Yujiro Takei	Kanematsu Corporation / / J-182 / Tokyo / Japan / yujiro_takei@kanematsu.co.jp

KENYA	
Charles O. Agwanda	CABI Africa Regional Centre / ICRAF Complex / Village Market / 00621 / Nairobi / Kenya / tel. 00 254 20 722 4450 / c.agwanda@cabi.org
Anand T. Aithal	ICRAF - World Agroforesty Center / ICRAF / PO Box 30677, Gigiri / Nairobi / Kenya / tel. 00 254 20 722 4270 / a.aithal@cgiar.org
Elijah Kathurima Gichuru	Coffee Research Foundation / PO Box 4 / 00232 / Ruiru / Kenya / tel. 00 254 67 25081/2 / elijah.gichuru@mpl.ird.fr
Gilbert Kairu	Coffee Research Foundation / PO Box 4 / 00232 / Ruiru / Kenya / tel. 00 254 067 25081/2 / gmkairu@yahoo.com
Joseph K. Kimemia	Coffee Research Foundation / PO Box 4 / 00232 / Ruiru / Kenya / tel. 00 254 67 26055 / crf@kenyaweb.com
Joseph Kanjara Mburu	Entomology department / Coffee Research Foundation / PO Box 4 / 00232 / Ruiru / Kenya / tel. 00 254 067 250 81/2 / joekamke@yahoo.com

Chrispine Omondi	Coffee Research Foundation / PO Box 4 / 00232 / Ruiru / Kenya / tel. 00 254 67 25081/2 / chr_omondi@yahoo.co.uk
Noah Anthony Phiri	CABI Africa Regional Centre / ICRAF Complex / UN avenue - PO Box 633-00624 / 00621 / Nairobi / Kenya / tel. 00 254 20 722 4450 / n.phiri@cabi.org

MADAGASCAR	
Jean-Jacques Rakotomalala	FOFIFA / BP 1444 / 101 / Antananarivo / Madagascar / tel. 00 261 202 259 320 / rakotomalala.jjr@wanadoo.mg

MEXICO	
Oscar Gonzalez-Rios	Instituto Tecnologico de Veracruz / Miguel Angel de Quevedo 2779 / Colonia Formando Hogar / 91860 / Veracruz / Mexico / tel. 00 52 229 934 5701 / oscargr@itver.edu.mx
Mirna Suarez-Quiroz	Instituto Tecnologico de Veracruz / Miguel Angel de Quevedo 2779 / Colonia Formando Hogar / 91860 / Veracruz / Mexico / tel. 00 52 22 99 345701 / mirnasq@itver.edu.mx

NIGERIA	
Oletayo Fademi	CRIN - Cocoa Research Institute of Nigeria / PMB 5244 / Ibadan / Nigeria / tel. 00 234 0805 697 1046 / tayofad@yahoo.fr
Gerald O. Iremiren	CRIN - Cocoa Research Institute of Nigeria / PMB 5244 / Ibadan, Oyo State / Nigeria / tel. 00 234 08037 195 290 / geraldoiremiren@yahoo.com

PORTUGAL	
Susana Casal	Faculty of Pharmacy / Rua Anibal Cunha, 164 / P-4099 030 / Porto / Portugal / tel. 00 351 222 078 902 / sucasal@ff.up.pt
Paula Eichler	Centro ECO-BIO / Instituto de Investigaçao Cientifica Tropical / Quinta do Marquês / P-2784 505 / Oeiras / Portugal / tel. 00 351 96 522 6794 / p.eichler@clix.pt
Leonor Guerra-Guimaraes	IICTCIFC / Quinta do Marquês / P-2784-505 / Oeiras / Portugal / tel. 00 351 214 544 680 / leonrgui@iol.pt
Andreia Loureiro	IICT - CIFC / Quinta do Marquês / P-2784 0505 / Oeiras / Portugal / tel. 00 351 21494 4680 / andreiasofialoureiro@yahoo.com.br
Marco Tulio Miranda	Delta Cafés / Av. Calouste Gulbenkian, n°15 / P-7370-025 / Campo Maior / Portugal / tel. 00 351 268 699 200 / marco.miranda@delta-cafes.pt
Rui Telmo Rosa	Delta Cafés / Av. Calouste Gulbenkian, n°15 / P-7370-025 / Campo Maior / Portugal / tel. 00 351 268 699 200 / rui.rosa@delta-cafes.pt
Maria C. Silva	IICTCIFC / Quinta do Marquês / P-2784-505 / Oeiras / Portugal / tel. 00 351 214 544 680/4 / marisilva@hotmail.com

Vitor Varzea	IICTCIFC / Quinta do Marquês / P-2784-505 / Oeiras / Portugal / tel. 00 351 214 544 680 / vitorvarzea@clix.pt
RWANDA	
John Nzungize Rusagara	Programme Café / I.S.A.R. / BP 138 / 250 / Butare / Rwanda / tel. 00 250 0859 5760 / nzungizejohn@yahoo.fr
SPAIN	
Maria Concepcioñ Cid	Dept. of food science & technology / University of Navarra / Faculty of Pharmacy / Irunlarrea, 1 / ES-31008 / Pamplona / Spain / tel. 00 34 948 425 600 / ccid@unav.es
Maria-Paz De Peña	Dept. of food science & technology / University of Navarra / Faculty of Pharmacy / Irunlarrea, 1 / ES-31008 / Pamplona / Spain / tel. 00 34 948 425600 / mpdepena@unav.es
Javier Antonio Linares Pasten	Institut Quimic. de Sarria / Via Augusta, n°390 / E-08017 / Barcelona / Spain / tel. 00 34 649 372 378 / linares.pasten@gmail.com
SWEDEN	
Bertil Fredholm	Karolinska Institut / Department of physiology and / pharmacology / SE-171 77 / Stockholm / Sweden / tel. 00 46 8 5248 7939 / bertil.fredholm@ki.se
SWITZERLAND	
Jürg Baggenstoss	ETH Zürich / ILW, ETH Zentrum / Swhwelzbergstr. 9 / CH-8092 / Zürich / Switzerland / juerg.baggenstoss@ilw.agrl.ethz.ch
Daniel Baumann	HACO Ltd / Worbstrasse 262 / CH-3073 / Gümligen / Switzerland / tel. 00 41 31 950 1312 / madeleine.herzog@haco.ch
Thomas W. Baumann	Villacoffea / Dorfstrasse 17 / CH-8247 / Flurlingen / Switzerland / tel. 00 49 52 654 0817 / thomasw.baumann@mac.com
Maurice Blanc	/ Résidence de la Côte 30 / CH-1110 / Morges / Switzerland / tel. 00 41 79 607 88 25 / maurice.blanc@bluewin.ch
Imre Blank	Nestlé SA / PTC Orbe / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 7532 / imre.blank@rdor.nestle.com
Christophe Cavin	Nestlé Research Center / Vers-chez-les-Blanc / CH-1000 / Lausanne 26 / Switzerland / tel. 00 41 21 785 80 99 / christophe.cavin@rdls.nestle.com
Marc Dreyer	Nestlé PTC Orbe / Route de Charvornay / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 7320 / marc.dreyer@rdor.nestle.com
Catherine Gretsch	Nestlé Research Center / PO Box 44 / Vers-Chez-Les-Blanc / CH-1000 / Lausanne 26 / Switzerland / tel. 00 41 21 785 8836 / catherine.gretsch@rdls.nestle.com

Adrian Haiduc	Nestlé PTC Orbe / Route de Charvornay / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 7541
Marcel Hueppi	Eugster/Frismag AG / Im Hof 20 / CH-8590 / Romanshorn / Switzerland / tel. 00 41 71 466 22 45 / m.hueppi@eugster.ch
Josef Kerler	Nestlé PTC Orbe / Nestlé Product Technology Centre Orbe / Route de Chavornay / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 7381 / josef.kerler@rdor.nestle.com
Karin Kraehenbuehl	Nestec / PO Box 44 / Vers-Chez-Les-Blancs / CH-1000 / Lausanne / Switzerland / tel. 00 41 21 785 9344 / karin.kraehenbuehl@rdls.nestle.com
David Labbe	Nestlé / Chemin de Bérée, 56 / CH-1010 / Lausanne / Switzerland / tel. 00 41 76 4020 750 / david.labbe@rdls.nestle.com
Patrick Leheup	Nestlé SA / 55, avenue Nestlé / CH-1800 / Vevey / Switzerland / tel. 00 41 21 924 42 28 / patrick.leheup@nestle.com
Valérie Leloup	Nestlé PTC Orbe / Route de Charvornay / CH1350 / Orbe / Switzerland / tel. 00 41 24 442 7362 / valerie.leloup@rdor.nestle.com
Rémy Liardon	Secrétariat ASIC / Chemin de Grand Vennes 5B / CH-1010 / Lausanne / Switzerland / tel. 00 41 21 653 07 45 / coffee-science@asic-cafe.org
Christian Lindinger	Nestlé NRC / Vers-chez-les-Blancs / CH-1000 / Lausanne / Switzerland / tel. 00 41 21 785 9384 / christian.lindinger@rdls.nestle.com
Marie-Louise Mateus	Nestlé PTC Orbe / Route de Chavornay / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 7111 / marie-louise.mateus@rdor.nestle.com
Laurence Pawelczyk	Starbucks Coffee Trading Company / Rue du Grand Chêne 1-3 / CH-1003 / Lausanne / Switzerland / tel. 00 41 21 321 25 30 / lpawelcz@starbucks.com
Luigi Poisson	Nestlé PTC Orbe / Nestec SA / Nestlé Product Technology Center Orbe / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 7077 / luigi.poisson@rdor.nestle.com
Daniel Pretre	Coffee Department / Nestlé PTC Orbe / Route de Charvornay / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 72 34 / daniel.pretre@rdor.nestle.com
Alexis Rodriguez	Nestlé Nespresso SA / Route du Lac, 3 / CH-1094 / Pavdex / Switzerland / tel. 00 41 24 442 4455 / alexis.rodriguez@nespresso.com
Peter Speck	HACO Ltd / Worbstrasse 262 / CH-3073 / Gümligen / Switzerland / tel. 00 41 31 950 1311 / peter.speck@haco.ch
Heike Steiling	Nestlé Research Center / Route de Jorat 56 / CH-1000 / Lausanne 26 / Switzerland / tel. 00 41 217 858 003 / heike.steiling@rdls.nestle.com
Viviane Theurillat	Departement science / Nestlé PTC Orbe / Route de Chavornay / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 7113 / viviane.theurillat@rdor.nestle.com
Martin Von Blittersdorff	Coffee Department / Nestlé PTC Orbe / Route de Charvornay / CH-1350 / Orbe / Switzerland / tel. 00 41 24 442 73 20 / paul.eichler@rdor.nestle.com

TANZANIA	
Jeremiah Haki	TaCRI / Ministry of Agriculture / PO Box 2066 / Dar Es Salaam / Tanzania / jeremiah.haki@kilimo.go.fz
Deusdedit Lucian Kilambo	Tanzania Coffee Research Institute / PO Box 3004 / 255 / Moshi / Tanzania / tel. 00 255 27 27 56 868 / dkilambo@yahoo.com
Fredrick Magina	Tanzania Coffee Research Institute / PO Box 3004 / 255 / Moshi / Tanzania / tel. 00 255 27 275 6868 / flmagina@yahoo.com
Godsteven Maro	Tanzania Coffee Research Institute / PO Box 3004 / 255 / Moshi / Tanzania / tel. 00 255 27 27 68 868 / marogp2002@yahoo.com
Linus Masumbuko	Tanzania Coffee Research Org. Institute / PO Box 3004 / 255 / Moshi / Tanzania / tel. 00 255 27 2756 868 / linusmasumbuko@yahoo.ie
Damian Joseph Mtenga	Tanzania Coffee Research Institute / PO Box 3004 / 255 / Moshi / Tanzania / tel. 00 255 27 27 56 868 / mtenga2003@yahoo.com
Ng'homa Nyabisi	Tanzania Coffee Research Institute / PO Box 3004 / Moshi / Tanzania / tel. 00 255 27 2756 868 / nyabisin@yahoo.com
Twahi Nzallawahe	Tanzania Coffee Research Institute / PO Box 3004 / 255 / Moshi / Tanzania / tel. 00 255 27 27 56 868 / nzalla2002@yahoo.com
Msanjo Temu	Tanzania Coffee Research Institute / PO Box 3004 / 255 / Moshi / Tanzania / tel. 00 255 27 275 6868 / temumsanjo@yahoo.com
James Teri	Tanzania Coffee Research Institute / PO Box 3004 / 255 / Moshi / Tanzania / tel. 00 255 55 811 520 / tacriced@kicheko-mos.com

THAILAND	
Siripong Sanpote	Agricultural service department / Quality Coffee Products Ltd. / 999/9 Rama 1 Road / Kwaeng Pathumwan Khet Pathumwan / 10330 / Bangkok / Thailand / tel. 00 66 2 657 8214 / siripong.sanpote@th.nestle.com

THE NETHERLANDS	
E. Koen Bekedam	Wageningen University / Bomenwey 2 / Building 307 - Room 509 / NL-6703HD / Wageningen / The Netherlands / tel. 00 31 317 485 366 / koen.bekedam@wur.nl
Hans Bosma	Sara Lee Int. / Vlentensevaart 35 / NL-3532 AD / Utrecht / The Netherlands / tel. 00 31 30 297 8576 / hans.bosma@saralee.com
Ric De Vos	Plant Research International / Wageningen-UR / PO Box 16 / 6700 AA / Wageningen / The Netherlands / tel. 00 31 317 476 983 / ric.devos@wur.nl
Lex Oosterveld	Sara Lee Int. / Vlentensevaart 35 / NL-3532 AD / Utrecht / The Netherlands / tel. 00 31 30 297 8582 / lex.oosterveld@saralee.com
Henk A. Schols	Agrotechnology and food science / Wageningen University / Bomenweg 2 / Building 307 - Room 518 / NL-6703 HD / Wageningen / The Netherlands / tel. 00 31 317 482 239 / henk.schols@wur.nl

Cuno Uiterwaal	Julius Center / University medical center utrecht / Heidelbergaan 100 / NL-3508 6A / Utrecht / The Netherlands / tel. 00 31 30 250 93 69
Hetty Van der Torre	Sara Lee International / Vlentensevaart 35 / NL-3532 AD / Utrecht / The Netherlands / tel. 00 31 30 297 2342 / hettyvander.torre@saralee.com
Herbert Van der Vossen	Plant Breeding Consultant / Steenuil 18 / NL-1606 CA / Venhuizen / The Netherlands / tel. 00 31 228 54 27 65 / herbert.vandervossen@quicknet.nl
Russell Ward	Sara Lee International / PO Box 2 / NL-3500CA / Utrecht / The Netherlands / tel. 00 31 30 297 7049 / russell.ward@saralee.com

TOGO	
Komlan Wegbe	Institut Togolais de Recherche Agronomique / Centre de recherche agronomique de la zone forestière / BP 90 / Kpalimé / Togo / tel. 00 228 441 00 60 / wegbepascal@yahoo.fr

UGANDA	
Pauline Aluka	NARO-CORI / P.O. Box 185 / 185 / Mukono / Uganda / tel. 00 256 039 700 725 / cori@africaonline.co.ug
Joseph Nkandu	NUCAFE / PO Box 34967 / 256 / Kampala / Uganda / tel. 00 256 77 259 030 / nucafe@ugandacoffee.org

UNITED KINGDOM	
Peter Baker	CABI / Silwood Park / SL5 7TA / Ascot / UK / tel. 00 44 1491 829 175 / p.baker@cabi.org
Stefano Ceriali	Reasearch and development / Kraft Foods / Ruscote avenue / OX 16 2QU / Banbury / UK / tel. 00 44 12 95 223 446 / sceriali@krafteurope.com
Michaël N. Clifford	University of Surrey / / GU2 7XH / Guildford / UK / m.clifford@surrey.ac.uk
Harald Grau	Kraft Foods / Ruscote Avenue / OX16 2QU / Banbury / UK / tel. 00 44 1295 223 775 / hgrau@krafteurope.com
Fabien Guilmineau	Kraft Foods / Ruscote Avenue / OX16 2 QU / Banbury / UK / tel. 00 44 1295 22 3958 / fguilmineau@krafteurope.com
Phil Oxford	Kraft Foods / Global Coffee Technology / Ruscote avenue / OX16 2QU / Banbury / UK / tel. 00 44 1295 23778 / poxford@krafteurope.com
Euan H.M. Paul	COSIC / 12, Market Street / Chipping Norton / OX-75NQ / Oxfordshire / UK / tel. 00 44 1608 645 566 / euanpaul@hotmail.com
Simon Penson	Kraft Foods / Research and Development / Ruscote avenue / OX162 QU / Banbury / UK / tel. 00 44 1295 223 957 / spenson@krafteurope.com
Siân Plumb	Kraft Foods / Global Coffee Technology / Ruscote avenue / OX16 2QU / Banbury / UK / tel. 00 44 1295 23778 / splumb@krafteurope.com

Sylvia Robert-Sargeant	ICO / 22 Berners Street / WIT 3 DD / London / UK / tel. 00 44 20 7612 600 / positivelycoffee@ico.org
Javier Silanes Kenny	Kraft Foods / 77, Queen's Road / OX16 Q ED / Banbury / UK / tel. 00 44 1295 22 3972 / jsilaneskenny@krafteurope.com
Tim Towers	Kraft Foods / Ruscote Avenue / OX16 2QU / Banbury / UK / tel. 00 44 1295 223 402 / ttowers@krafteurope.com
Ronald Walker	University of Surrey / 44 Ash Hill Road, Ash / GU12 6AB / Aldershot / UK / tel. 00 44 1252 329 607 / profronwalker@aol.com

USA	
C. Robin Buell	The Institute for Genomic Research / 9712 Medical Center Dr / 20850 / Rockville, MD / USA / tel. 00 1 301 795 7558 / rbuell@tigr.org
Elsie Burbano	University of Hawaii / Plant and Environmental Protection Sciences / 3050 Maile Way - Room 310 / HI-96822 / Honolulu / Hawaii / tel. 00 808 956 2450 / eburbano@hawaii.edu
James R. Coughlin	Coughlin & Associates / 27881 La Paz Rd. / Suite G, PMB 213 / 92677 / Laguna Niguel, ca / USA / tel. 00 1 949 916 6217 / jrcoughlin@cox.net
Joachim Eichner	Praxis International / 15 A Melanie Lane / Unit 10 / 07936 / East Hanover / USA / joachim@roasting.biz
Loren Gautz	Bioengineering, CTAHR / University of Hawaii at Manoa / 1955 East-West Road, AgSc 218 / 96822 / Honolulu / Hawaii / tel. 00 808 956 6876 / lgautz@hawaii.edu
Susan Jackels	Chemistry department / Seattle University / Bannon Hall 516 / 901, 12th arvenue / 98122 / Seattle, WA / USA / tel. 00 1 206 296 5946 / sjackels@seattleu.edu
Charles Jackels	University of Washington / Box 358534 / Campus Way, NE / 18115 / Seattle, WA / USA / tel. 00 1 425 352 5368 / jackels@u.washington.edu
Lawrence W. Jones	Huntington Medical Research Institutes / 99 N. El Molino avenue / 91101 / Pasadena, CA / USA / tel. 00 1 626 796 8102 / joneslw@hmri.org
Geneviève Kappler	Green coffee division / Oren's Daily Roast Inc. / 177 east 87th street / Suite 409 / 10128 / New-York, NY / USA / tel. 00 1 212 348 54 00 / genevieve@orensdailyroast.com
Jackson Mel	Hawaii Agriculture Research Center / 99-193 Aiea Heights Drive, / Suite 300 / 96701-3911 / Aiea, HI / USA / tel. 00 1 808 486 5421 / mjackson@harc-hspa.com
Chifumi Nagai	Hawaii Agricultural Research Center / 99-193 Aipa Hks Dr. Aiea / 96701 / Aiea, HI / USA / tel. 00 1 808 486 5385 / cnagai@harc-hspa.com
Norm Ouellette	Starbucks Coffee Co. / 2401, Utah Avenue South / MS : S-RD/QA / 98134 / Seattle, WA / USA / tel. 00 1 206 318 4934 / nouellet@starbucks.com
Joseph Rivera	SCAA / 330 Golden Shore / Suite 50 / 90802 / Long Beach, CA / USA / tel. 00 1 562 624 4400 / jrivera@coffeechemistry.com

Henry Schwartzberg	University of Massachusetts / / / USA / schwartzberg@foodsci.umass.edu
Daniel Steffen	Kraft Foods / 10313, Long Beach Boulevard / 08008 / Beach Haven Park, NJ / USA / tel. 00 1 973 503 2562 / dsteffen@kraft.com
Shawn Steiman	University of Hawaii / 3190, Maile Way / St. John 102 / HI-96822 / Honolulu, HI / USA / tel. 00 808 956 7256 / steiman@hawaii.edu
Scott Stouffer	Starbucks Coffee / PO Box 34067 / Mail stop 5-504 / 98124-1067 / Seattle, Washington / USA / tel. 00 1 206 318 7662 / sstouffe@starbucks.com
Steve Tanksley	Cornell University / / / USA / sdt4@cornell.edu
Matt Taylor	Proter & Gamble / PO Box 538707 / 45253 / Cincinnati, OH / USA / tel. 00 1 513 627 1562 / taylor.mj.1@pg.com
Sally Vater	Procter & Gamble / 4300 Center Hill avenue / 45224 / Cincinnati, Ohio / USA / tel. 00 513 434 4240 / vater.st@pg.com
Marcela Yepes	Plant Pathology Department / Cornell University / 630 W N Street / 14456 / Geneva, New York / USA / tel. 00 315 787 2472 / my11@cornell.edu
Gerhard Zehentbauer	Procter & Gamble / 11810 East Miami River Road / 45252 / Cincinati, OH / USA / tel. 00 1 513 627 1804 / zehentbauer.g@pg.com
Bary Zeller	Kraft Foods / 801, Waukegan Road / 60025 / Glenview, IL / USA / tel. 00 847 646 3782 / bzeller@kraft.com

VENEZUELA	
Angel Torres	INIA / Instituto Nacional de Investigaciones Agricolas / Bramon, Tachira / Venezuela / atorres@inia.gob.ve

VIETNAM	
Nguyen Tu Hai	Center for Arabica Coffee R&D / Tan Linh Commune / Bayi District / Bavi / Vietnam / tel. 00 84 348 812 66 / nthaibv@yahoo.com

ZAMBIA	
Carole Hemmings	Rift Valley Coffee Research Company / PO Box 410208 / Kasama / Zambia / tel. 00 260 97 770 279 / chemmings@zamnet.zm