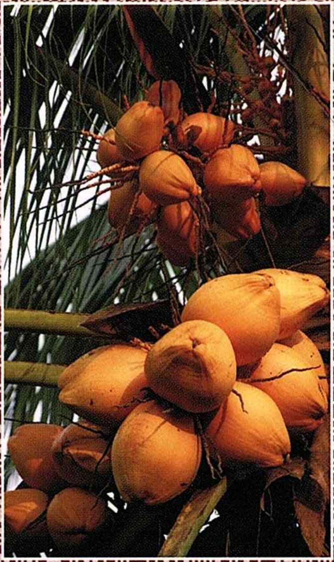
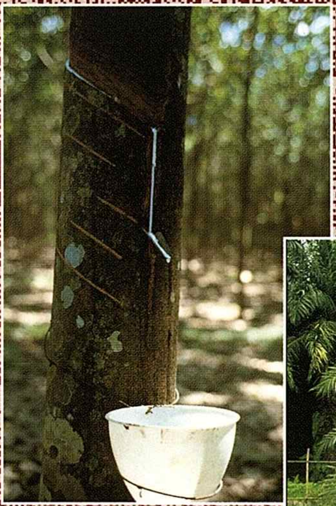
Spotlight on tree crops





Plantations, recherche, developpement

ISSN 1254-7670

The Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) is a French scientific organization specializing in agricultural research for development for the tropics and subtropics. It is a State-owned body, which was established in 1984 following the consolidation of French agricultural, veterinary, forestry, and food technology research organizations for the tropics and subtropics.

CIRAD's mission is to contribute to the economic development of these regions through research, experiments, training, and dissemination of scientific and technical information.

The Centre employs 1800 people, including 900 senior staff, who work in more than 50 countries. Its budget amounts to approximately French francs 1 billion (€ 152 million), more than half of which is derived from public funds.

CIRAD is organized into seven departments: CIRAD-CA (annual crops), CIRAD-CP (tree crops), CIRAD-FLHOR (fruit and horticultural crops), CIRAD-EMVT (animal production and veterinary medicine), CIRAD-FORÊT (forestry), CIRAD-TERA (territories, environment and people), and CIRAD-AMIS (advanced methods for innovation in science). CIRAD operates through its own research centres, national agricultural research systems, or development projects.

Cover photos
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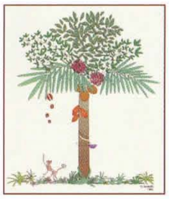


In recent decades, there has been a boom in tree crops in the humid Tropics. Although such development has sometimes been spontaneous, it has usually been induced by determined agricultural policies, resulting in an immense expansion of cultivated areas, to the detriment of forests.

In these early years of the new century, sustainable management of land areas and natural resources are new dimensions to be reckoned with, and strictly productivist models are being called into question. Moreover, the new context of liberalized economies, and increasing demand from consumers for food quality and safety add further constraints that development-oriented agricultural research has to take fully into consideration if it is to make a significant contribution to poverty alleviation in developing countries.

In order to take up these challenges, the Tree Crops Department is organized in five programmes based on the problem issues arising in five commodity chains that are often essential for the economy of countries in the humid Tropics: cocoa, coffee, coconut, rubber and oil palm. Scientific action is structured in multidisciplinary projects taking an integrated approach combining research operations along three interdependent lines:

- a socio-economic line, covering the stakeholders involved, their organization, their decision-making procedures, from farmer to consumer, and including the entire chain of operators involved in trading, transport, processing and policy making,
- a technological line, which studies products, the determinism of their characteristics and qualities, and changes induced by processing methods,
- an agronomic line, which identifies appropriate crop management sequences, from seed production to harvests (see also storage and transport), depending on the farming systems, expected results, edapho-climatic conditions and spatial and environmental constraints.

This document, which takes the same editorial form as the journal *Plantations, Recherche et Développement*, is intended to shed light on the scientific activities of the department, and highlights some of the main results obtained over the last two years. The reader will find articles covering the diversity of the department's activities: research in the fields of molecular biology, varietal improvement, integrated control and ecophysiology, along with an analysis of perennial crop-based farming systems in Côte d'Ivoire, Indonesia and Guinea.

Denis Despréaux

Cocoa commodity chain: economic context and research overview

Weak prices despite buoyant demand

With almost three million tonnes harvested annually, world cocoa production has increased sharply in recent years, primarily through three countries: Côte d'Ivoire—1,400,000 t in 1999-2000, i.e. 45% of world supplies—,Indonesia—which has exceeded 400,000 t with steady growth since the end of the 1980s—and Ghana—where the revival of the cocoa sector was confirmed with 435,000 t in 1999-2000.

This increase can barely keep pace with clearly growing consumption—worldwide grindings increased by more than 8% in 1999-2000—through strong demand for chocolate products in traditional major consuming countries, such as Western Europe, and in new emerging markets in Eastern Europe and Asia.

World production in seven of the last ten years was below consumption estimated from grindings, and production shortfalls are being predicted for the coming years. However, the volume of cocoa stocks cumulated over the previous years, which exceeded 1,200,000 t (i.e. over 40% of annual grindings), the accelerated liberalization of the Ivorian cocoa commodity chain, and changes in European legislation on use of the term "chocolate" have had a considerable effect on world prices. For instance, cocoa prices on the London exchange reached a record low in 2000. World cocoa prices are expected to improve in the short term, under the effect of strong demand but also through the low prices in 2000, which led to an upward revision of world consumption and a decrease in production, as plantings were abandoned and more extensive cultivation was adopted.

Cocoa Programme research priorities

The research priorities of the Cocoa Programme for the 1998-2002 period were defined from a foresight exercise carried out in the sector in 1997-1998. The current five priorities are periodically reviewed to take into account any changes in economic circumstances, scientific progress and changes in the programme's operational arrangements.

African cocoa cultivation: worrying prospects!

Cocoa is a cash crop found throughout the humid intertropical zone. However, Africa holds a major position with 70% of world supplies. The attention of the different stakeholders in the cocoa commodity chain is therefore focused on the future of African cocoa cultivation, particularly as the cocoa butter and chocolate industry, notably in Europe, has adapted its means of production to processing West African beans.

Plantations have been traditionally set up on cleared forest land in a pioneer front process, with low input farming systems. Yields per hectare are often low and the transfer of technical innovations is not always satisfactory. In these traditional systems, plantation productivity slumps after 25 to 30 years—this is the senescence phase—and farmers can only maintain their income by continually setting up new plantings on cleared forest land.

However, the reproducibility of this system is currently threatened by the exhaustion of available forest reserves and through the deterioration of production conditions: drier climate, the threat of *Phytophthora megakarya,* degraded soils, invasion of fallow land by very aggressive weeds, serious damage by insect pests, etc.

The development of competitive and sustainable cocoa farming systems in West Africa, and integrated control of *Phytophthora* pod rot are the Programme's top two research priorities.

In terms of sustainable farming systems, CIRAD is working with producing countries such as Côte d'Ivoire, Cameroon and São Tomé and Príncipe, and is drawing up projects with Ghana, Togo, the Dominican Republic and Nigeria. This research effort has been boosted by the recruitment of a farming systems agronomist and an entomologist.

For the control of pod diseases (*Phytophthora* sp.), research is being conducted in Côte d'Ivoire, Cameroon, Trinidad and Tobago and Papua New Guinea, notably in connection with an international project coordinated by the Programme, and backed by European cocoa industry (CAOBISCO). The importance and complexity of this research theme led to the recruitment of an epidemiologist to strengthen the Programme.

Which cocoas for which chocolates?

For several years, cocoa consumption trends worldwide have been characterized by two phenomena: vitality in terms of volume (increase in grinding volumes) and segmentation in terms of quality. The increasing consumer demand for diversified products (organic products, products from specific areas, guaranteed origins with particular flavours, etc.) is a notable development in the market for agricultural produce from the temperate zone, but also from the tropical zone. This is particularly the case for cocoa. Greater production of such cocoas with specific qualities faces technical obstacles—for instance, fine cocoa varieties are often low yielding, susceptible to diseases and little is known of their genetic make-up—but also organizational problems: lack of market transparency, a marketing system offering no incentive for quality efforts; insufficient differentials, contractual relations between operators that need to be redefined, etc.

It is in order to take up this challenge that improved production, marketing and promotion of fine or flavour cocoas has been included in the Programme's research priorities, and that an agricultural economist specialized in marketing products with specific qualities has been recruited.

However, the quality concept for a food product such as cocoa is a complex set of characteristics that it would be wrong to limit merely to the taste aspect. These characteristics are technological, health-related, organoleptic and nutritional. Their consistency from one batch of cocoa to the next, or from one shipment to another is also very important. Processing yields, related to the size and fat content of the beans, or to the rheological properties of the cocoa butter, which depends particularly on the rate of free fatty acids, illustrate the technological aspects. The interest recently shown by the European Union in the existence of ochratoxins [1] in numerous foodstuffs also shows how important the health-related quality of chocolate is.

Thus, in order to fine-tune our understanding of the numerous factors governing the different quality aspects of cocoa, CIRAD carries out multidisciplinary research and development support operations in Montpellier, France, and in the main producing countries, in partnership with public or private organizations in the countries of the South and North: Côte d'Ivoire, Ecuador, Venezuela, Cameroon, São Tomé, Dominican Republic, Vanuatu, Madagascar, etc.

The Cocoa Programme at a glance

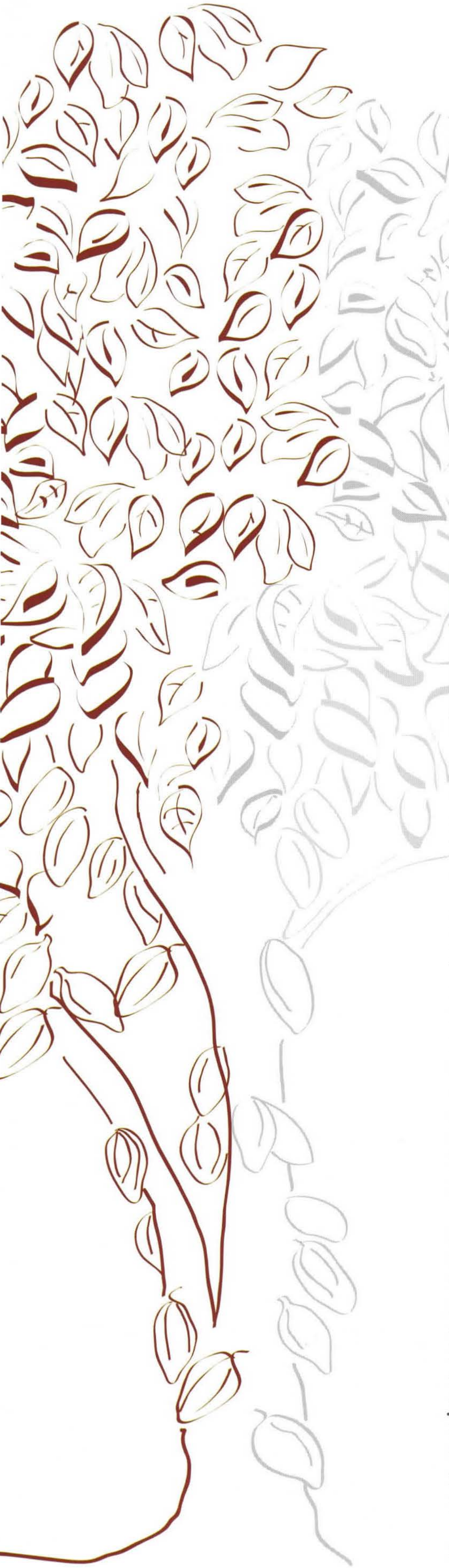
21 staff, including 15 researchers
Partner countries: France (Montpellier and French Guiana), Cameroon, Côte d'Ivoire, Ecuador, Papua New Guinea, São Tomé and Príncipe, Trinidad and Tobago, Vanuatu
Institutes:
France: INRA, IRD, University of Montpellier II
International: CABI, USDA, ICCO, IPGRI
Industries: Mars M&M's, FCC, CAOBISCO
Research themes:
- Sustainaibility of cocoa-based farming systems in Africa
- Integrated control of *Phytophthora* black pod rot
- Revival of fine cocoa cultivation in America
- Understanding of cocoa quality chains
- Utilization and preservation of cocoa germplasm

Germplasm characterization and exchanges

The different lines of research described above (productivity, sustainability, resistance, quality) call for cocoa germplasm. In order to be of use, the germplasm has to be collected, preserved, characterized and made available: this is the spirit of the Programme's fifth priority.

It is to that end that the Cocoa Programme, which now has its own germplasm collected from the wild in French Guiana and which has developed tools for genetic diversity assessment and accession characterization, has decided to take an active role in setting up an international cocoa germplasm network. This network is primarily based on the operations of the project entitled *Utilization and preservation of cocoa germplasm: a global approach* under the aegis of the CFC, ICCO and IPGRI, a project in which the programme is taking an active part alongside 13 other research organizations from 11 countries. ■

(1) Ochratoxins: toxic metabolites produced by fungi, found primarily in cereal-based products, but also in dried fruits, coffee and, to a lesser degree, in cocoa products.



Agricultural diagnosis of a region of former pioneer fronts in Côte d'Ivoire

Historically, cocoa cultivation worldwide has virtually always developed on cleared tropical forest land, in a pioneer front process, with low intensification farming systems. Production levels are low and the transfer of technical innovations is rarely satisfactory. After twenty-five to thirty years, the plantings enter a senescence phase and productivity declines. When this happens, farmers open up new plots on freshly cleared forest land, which explains the historic phenomenon of shifting poles of production.

In West and central Africa, it is precisely along these lines that cocoa cultivation has developed, and nowadays provides 70% of world cocoa supplies. However, the reproducibility of this system is now jeopardized by the exhaustion of available forest reserves, which can sometimes reach land saturation. It is for this reason that developing and identifying conditions for the adoption of more sustainable cocoa growing systems is one of the main challenges for the future of the cocoa commodity chain, particularly in West Africa.

To that end, the Cocoa Programme embarked upon a farming systems diagnosis in an ageing Ivorian cocoa growing zone, in the Abengourou region (East of the country). This operation was conducted in partnership with BNEDT in Côte d'Ivoire (*Direction de l'agriculture et de l'aménagement rural et Direction du plan foncier rural*) and with the French national agronomy institute (INRA), Paris-Grignon. In the Abengourou zone, it received support from ANADER regional management.

The purpose of such diagnosis operations is to understand how agrosystems function and identify innovations taking place, be they spontaneous or not.

Main results

This agrarian diagnosis was undertaken in 1999 in the Abengourou region of Côte d'Ivoire, in the Affalikro-Kouakou Ndramanekro sector. It is a zone of former pioneer fronts, and typical of the senescent phase of cocoa cultivation.

The study made it possible to trace the changes that have occurred in the local agrarian situation and identify how producers take up the challenge of post-forestry cocoa cultivation, by applying appropriate technical and organizational innovations.

Changes in the agrarian situation (1900-1999)

At the turn of the century, the farming system was based on slashing and burning, with two years of crops (yam-taro-banana, then groundnut-maize-banana), combined with hunting and forest gathering.

Cocoa cultivation was introduced into the region between 1910 and 1950, and was first adopted by chiefs and eminent members of society. The dominant agrarian system combined food crops and cocoa trees, with cocoa seedlings planted among the food crops. The food crop-tree fallow system was replaced by a succession of forest clearance-food crops-cocoa plantings, a system which meant continuing to extend on cleared forest land.

Up to World War II, plantations were under 10 ha in size and were reserved for eminent members of society. The main limiting factor at the time was the work force. The manpower was primarily family labour, completed with a few, gradually emancipated slaves. In the 1930s, coffee cultivation developed to the detriment of cocoa, due to its early production and the low price fetched by cocoa.

From the 1950s and up to the 1980s, the system was characterized by the major development of plantations on pioneer fronts with the massive influx of migrant workers: it was a land rush. The system was largely dominated by plantations and food crops played a secondary role. The forest (interfluvial zone) rapidly diminished and farmers began clearing lowlands to plant cocoa, but with mediocre results. At the end of the 1980s, the forest had virtually disappeared, and cocoa replantings were set up in coffee plots and on fallow land left after old plantings had been abandoned. In order to cope with a food shortage, food crops were grown in lowlands, notably by share-croppers (*aboussan*).

Since the 1980s, owners have been replanting cocoa trees in coffee plots after pulling up every other coffee tree. This technique makes it possible to continue planting cocoa, though there is no longer any forest available, and to benefit from labour in a semi-productive young plantation. Indeed, labour is becoming increasingly rare: migrants are now moving to the West and Southwest. At the end of the 1980s, replanting was carried out on fallow left by old plantations, as young people returned to the village, having been unable to find work in town. Food crops have regained a dominant position in the farming systems. Lastly, since 1993, there has been a clear tendency towards farming system diversification, with the development of landless activities, market garden crops, irrigated rice and oil palm.

Current situation

Four types of farming system were identified in the study region (East of Abengourou) :

- type 1, which comprises "large farmers". They farm areas of more than 15 ha in the interfluvial areas, mostly occupied by plantations, but also by reserve fallow,
- type 2, which refers to farming systems practised by share-croppers (*aboussan*), and which primarily involves food crops in lowlands,
- type 3, which comprises farming systems practised by the young sons of large farmers, who possess small interfluvial areas (under 6 ha), either planted or left fallow, with free access to lowlands,
- type 4, which comprises farming systems practised by the other farmers who became established later. These growers have smaller areas (interfluvial areas of under 15 ha), with no reserve fallow, and also farm hired land.

The distribution of types within the populations and in space, reveals high inequality within smallholder society (table). Today, 20% of farmers possess 100% of the land, whereas 80% have none (*aboussan*). These share-croppers farm for themselves 10% of the area held by the owners.

The large farmers (type 1) arrived earliest in the zone: they are Agnis descending directly from the founder of the village, on the one hand, and the other Agnis, Barbo-Koulangos, Baoules and the first Burkinese to arrive, on the other hand. The direct descendants of the founder of the village easily had access to the forest and cleared 4 ha per year as soon as the pioneer front period began. They now have farms with cultivated areas of more than 45 ha and sometimes over 100 ha in interfluvial zones. The farming systems comprise fallow areas (around 25% of the area cultivated), cocoa mono-cultures (35%), intercropped coffee and cocoa (13%), immature unproductive plantings (8%) and food crops (2%).

Each year, they use a limited area of fallow for food crops (own consumption) and to plant a mix of cocoa and coffee trees. The cocoa trees are grown either from seedlings reared in the nursery, or beans sown directly in the field (two beans every metre), from pods harvested on site. The coffee seedlings are mostly selected plants distributed by ANADER under the coffee revival project.

The productive plots mainly consist of cocoa monocultures, more rarely a mix of cocoa and coffee. Upkeep in these plots is systematically entrusted to share-croppers. Treatments are regularly carried out against mirids (capsids). Cocoa is harvested in three rounds; with yields from treated plots reaching 500 kg/ha. In the mixed plots, yields are 150 kg/ha for cocoa and 50 kg/ha for coffee.

For the last few years, rubber and oil palm plantings have been developing in the interfluvial zones and lowlands. They currently account for 4% of the total area.

The other Agnis who are not direct descendants of the founder of the village (and the first outsiders) also have large farms, with interfluvial areas of ranging from under 15 ha to 50 ha, notably among the Baoules. They also have access to bottomlands. However, their cocoa cultivation system is less intensive. Their plantings are more regularly treated against mirids.

The sons of large farmers (type 3) farm small interfluvial areas (under 6 ha) and have access to fallow.

The most diversified system consists of young plantings (40%), food crops (10 to 15%), fallow (15 to 40%) and cash crops in the bottomlands, such as market garden crops (under 25%).

Lastly, the farmers who arrived later (type 4) no longer had any fallow available for them, nor access to lowlands. The areas planted to cocoa and coffee amount to under 15 ha. Their farms comprise untreated cocoa plantings (30%), a mix of coffee and cocoa (55%), coffee plantings (10%) in the interfluvial areas and tenant-farmed food crops in bottomlands.

Conclusion: a few lessons from this case study

Three main points are revealed by this study:

- the decisive nature of access to land in the diffentiation of the agrosystems,
- a strong tendency towards farming system diversification, especially by the most fortunate farmers in terms of land and capital,
- the existence of numerous attempts to replant old cocoa plantings.

In the study region, the main farming system differentiation factor was and remains access to land, a factor which in turn governs access to labour. Access to land and labour occurred differently depending on when the farmers arrived. For the last ten

Table. Distribution of cultivated areas by type of land owner.

Farming systems	Farms (%)	Area covered (%)
Type 1		
Direct descendants of the village founder	1	25
Large farmers	8	40
Type 3		
Young sons of large farmers	3	10
Type 4		
Other farmers	8	25

years or so, young farmers have been turning to production diversification, obliged to make more use of lowlands, due to land saturation. They are developing crops that are more lucrative per unit area. Diversification through tree crops (oil palm, rubber, cashew) or food crops (irrigated rice) and market garden crops (tomato, okra, aubergine) seems to be a strategy for maximizing income per unit area and minimizing risks by limiting financial dependence on cocoa.

This phenomenon of "no cocoa" diversification may nonetheless be a factor leading to the intensification of cocoa cultivation (pesticide purchases, replanting), particularly among large farmers who reinvest part of their cash resources in input purchases for cocoa. For instance, vegetable sales from May to July provide cash-flow to buy anti-mirid products, and in turn cocoa sales provide funds for fertilizers, seeds and pesticides for market gardening.

Lastly, numerous attempts at cocoa replanting have been seen in the last few years in the region, mainly on fallow or former coffee plantings, much more rarely in old cocoa plantings. However, the study carried out did not reveal how successful these replantings have been, nor did it identify the problems encountered, apart from the observed absence of technical supervision to accompany these replanting or rehabilitation initiatives. ■

List of publications

Brayer J., 1999. Diagnostic agraire d'une petite région d'anciens fronts pionniers en Côte d'Ivoire. Quelles évolutions des systèmes de production ? Mémoire de Dea Géographie et pratique du développement dans le tiers-monde, INA PG, 76 p.

Satellite remote sensing: mapping of coffee and cocoa plantations

For a producing country, knowledge of the areas planted to coffee and cocoa, along with their location and their vegetative condition is essential for defining an appropriate agricultural policy: management of land resources and regional planning, gaining an idea of future supply, introduction of supervisory infrastructures for production and collection, etc. In Côte d'Ivoire, these two crops are grown over vast expanses in often spontaneous initiatives by smallholders to develop forest zones, the dynamics of which are not completely known and are unsupervised. Consequently, although cocoa and coffee are very important export products in Côte d'Ivoire, the areas planted are not precisely known, with estimations fluctuating between 2 and 4 million hectares in all.

Such knowledge can be acquired by conventional inventories of the stands, with identification of plantations and area measurements in the field. In order to provide an alternative to these laborious and expensive conventional techniques, BNETD-CCT (Côte d'Ivoire) and CIRAD carried out a joint study to test the feasibility of statistical inventory mapping based on Spot images and field surveys.

The methodology was developed in the department of Daloa, in central-western Côte d'Ivoire, over an area of 29,842 ha within the pilot zone of the Rural Cadastral Plan of Daloa. The map of cadastral plots, which was still incomplete, included indications of land occupation, based on 15 themes, including coffee and cocoa. Similar work was launched in the department of Soubré.

Methodology

Various operations were carried out for this project, from a preliminary survey to validation of the classifications obtained. The sequence of these different operations is shown in figure 1.

Preliminary agronomic survey and biometric analysis

The agronomic survey (January-February 1997) involved 358 plots, including 159 cocoa plantings and 197 coffee plantings. Only plots more than 5 years old were taken into account, thus excluding young plantings that were not agronomically stabilized. The 36 variables noted were identifiers and descriptors from agronomic, topographical, pedological and socio-economic viewpoints. A multiple correspondence analysis was carried out on all these data and revealed that, for both crops, the first axis was always an intensification axis. For instance, in the case of cocoa, it contrasted high-yielding plots in good agronomic condition with low-yielding plots, or virtually abandoned plots, often set up using the old cultivation system based on non-improved planting material (Amelonado). The level of intensification, characterized simply from the yield estimated by surveyors, was therefore an indicator that summed up both the integrity of the plots and the vegetative conditions of the trees within them, two parameters that were likely to be revealed by satellite remote sensing.

Preliminary processing of geographical data

Different operations made it possible to match the image obtained with the satellite

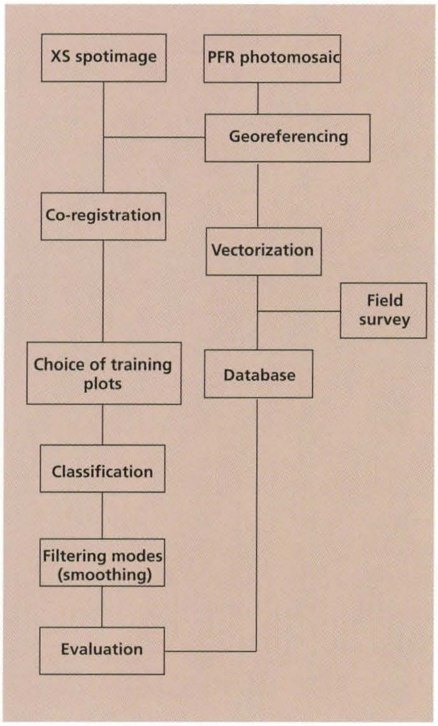


Figure 1. Diagram of operational sequence.

scene (Spot-XS 46-336 scene dated 29th March 1998) with a map comprising a 9-plate PFR photomosaic, in order to select training and check plots.

Image classification

The classification method used was "supervised maximum likelihood classification" and was carried out in several stages:

- identification of themes (classes),
- choice of training plots,
- checking of the classification rule in the test zone,
- validation on the entire scene.

Identification of themes and choice of training plots

An initial eight themes were defined: coffee, cocoa, forest, fallow (including food crops and cotton), wasteland (tall grasses, *Chromolœna odorata,* forest regrowth), habitat-denuded zone, cleared forest and bottomland. For these eight themes, 63 training "plots" (representative sample) were chosen, covering a total of 302 ha, i.e. 1% of the study area.

The training plots for the coffee and cocoa themes came naturally from the agronomy surveys. For the other themes, they were chosen by photo-interpretation based on the indications on the PFR photomosaic, which thus constituted the "field truth". Classification was then carried out using three channels (XS1, XS2, XS3). A study of spectral signature separability (Jefferies-Matusita distances) showed that, generally speaking, the themes adopted were clearly separable.

Validation in the test zone

Plot checking for the coffee and cocoa themes involved all the survey plots, except those that were abandoned and those already used for training, i.e. 174 coffee plantings and 143 cocoa plantings. The training and check plots for the other themes were chosen using the PFR photomosaic. Overall plot checking consisted of 389 elements, for 2,589 ha.

The classification was evaluated from a confusion matrix in accordance with so-called "majority" recognition (classification of the plot according to its majority theme). This corresponded to the purpose and to the results of the agronomic survey, which identified the plots according to their dominant crop.

Validation of the whole scene

In order to validate the classification results on the whole scene, i.e. 3,600 km^2, a sampling plan was drawn up in two stages: firstly stratified random sampling (based on the representativeness of the themes) of 320 uniform units of at least 5 ha, then, in order to account for access difficulties, a selection of units whose centre was located less than 1,200 m from a practicable track. 180 units were chosen in this way, 133 of which were effectively investigated, i.e. an investigation rate of 0.2%. During the validation survey, the centres of the units were located by a GPS (Global positioning system).

Results

In the test zone (table 1 and figure 2) the theme recognition rates varied from 66% to 100%. Coffee and cocoa were recognized at 69 and 66%, respectively. The overall percentage of correctly classified pixels was 72%.

On the entire scene (table 2), coffee and cocoa were recognized at 86 and 94%. 80% of pixels were correctly classified. For these two crops, statistical precision was satisfactory: 0.86 and 0.90.

Conclusions and prospects

Despite the numerous constraints, some of which were specific to the agriculture and tropical climatology (e.g. cloud cover in the rainy season prevents scenes from being taken), the results obtained in terms of theme recognition and statistical precision are acceptable for statistical mapping purposes. It seems they could be improved by using the mid infrared (MIR) channel.

Extension of the method to the entire cocoa and coffee growing zone of Côte d'Ivoire can thus be envisaged.

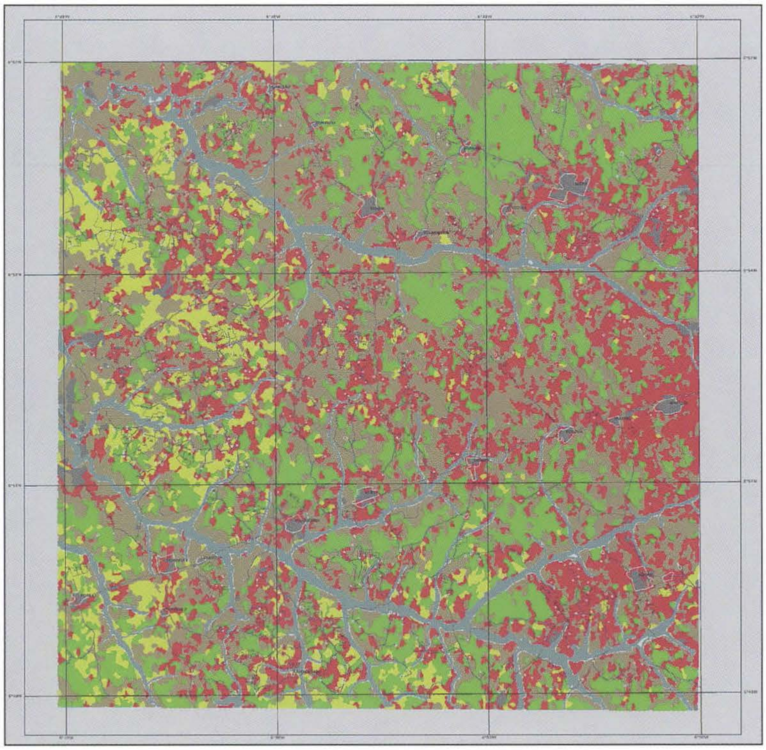
The next step of the work will be an analytical approach intended to characterize the condition of the cocoa and coffee plots and, consequently, their suitability for rehabilitation. ■

Table 1. Confusion matrix, as a percentage, omission errors on the test zone.

Theme	Classification							Omission* (%)
	1	2	3	4	5	NC	Total	
Coffee	69	22	2	2	1	3	99	31
Cocoa	20	66	7	1	4	1	99	34
Forest	3	9	87	0	0	0	99	12
FWBL	5	0	0	95	0	0	100	5
HDZCF	0	0	0	0	100	0	100	0

NC : unclassified; FWBL: recomposed theme (fallow-wasteland-bottomland); HDZCF: recomposed theme (habitat-denuded zone-cleared forest).

*Omission for a theme A: error by which a plot belonging to the land in theme A is classified in another theme.



Legend:

Coffee
Cocoa
Forest
Fallow-Cleared forest
Habitat-Cleared forest
Bottomland

Crop check plots:

Coffee cf
Cocoa cc

Figure 2. Classified image of the test zone.
True scale 1:30,000

Table 2. Confusion matrix, as a percentage, and parameters: omission and commission errors (as a %) and statistical precision (SP = 1 – com / 1-om) on the scene.

Theme	Classification						Omission (%)	Commission* (%)	PS
	1	2	3	4	5	Total			
Coffee	86	7	3	3	0	99	14	22	0.91
Cocoa	0	94	4	2	0	100	6	20	0.86
Forest	4	33	54	4	4	100	46	19	1.50
FWBL	30	5	0	55	10	100	45	21	1.43
HDZCF	0	0	0	0	100	100	0	20	0.80

NC : unclassified ; FWBL: recomposed theme (fallow-wasteland-bottomland); HDZCF: recomposed theme (habitat-denuded zone-cleared forest).

*Commission for a theme, A = error by which a plot belonging in the field to theme B is classified and mapped in theme A.

List of publications

N'Doume C., Lachenaud P., Hussard A., Nguyen H., Flori A., 2000. Etude de faisabilité pour l'élaboration d'une cartographie statistique d'inventaire des vergers café et cacao en Côte d'Ivoire par télédétection satellitale. Bulletin de la Sfpt, 157, 3-10.

Nguyen H., Lachenaud P., Flori A., 1997. Cartographie analytique et statistique des vergers cacao et café de deux zones pilotes (Daloa et Soubré) de Côte d'Ivoire. Montpellier, France, Cirad, CP-SIC n° 859, 60 p., cartes (internal document)

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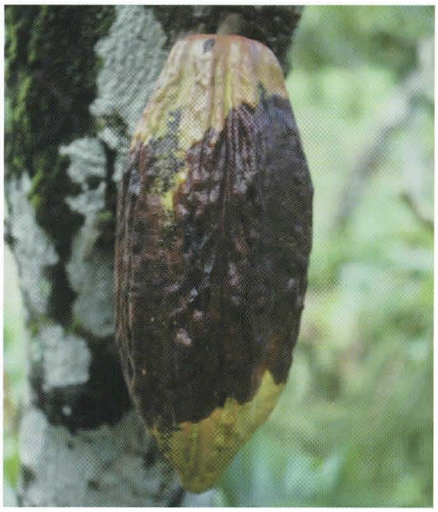
Working for better resistance to cocoa pod rot

Production losses caused by *Phytophthora* rot diseases (photo) are estimated at 15-20% worldwide; the selection of cocoa trees that are less susceptible to pod rot has therefore become a top priority. Despite a great deal of work, the search for cocoa trees with total resistance to this disease has drawn a blank. However, studies based on observing attacks in field trials have revealed the existence of genetically transmissible partial resistance. Transmission of this trait was primarily additive. An international project entitled Genetic bases of cocoa tree resistance to *Phytophthora* diseases was implemented between 1995 and 2000, with a view to proposing techniques for the sustainable control of *Phytophthora* diseases causing pod rot. The project received financial backing from European chocolate makers through CAOBISCO and involved several research centres: CRU, CNRA, IRAD and CIRAD. Its main objectives were to:

- improve knowledge of the structure and distribution of pathogen populations,
- identify the different factors involved in resistance,
- develop and validate resistance tests,
- locate areas of the genome involved in the resistance trait, by searching for QTLs,
- launch a process of genetic improvement for this trait.

The results obtained were released in publications and conference papers, and are to be compiled in a final book currently being drafted. This article sets out to highlight a few salient facts in our knowledge of resistance mechanisms and their genetic determinism.



c. Cilas

Photo. *Phytophthora* pod rot symptoms.

Resistance measured by the rotten pod rate in the field

The results shown here came from several mating designs and a clonal trial set up in zones affected by the disease in Cameroon and Côte d'Ivoire. They are compared with data obtained in Togo by ITRA's forest zone agricultural research station.

Losses caused by pod rot were estimated from potential production (minus rodent damaged pods) using a formula giving the rot rate per tree:

$$\text{Rotr} = \frac{\sum \text{rotten pods}}{\sum \text{rotten pods} + \sum \text{ripe pods} + \sum \text{healthy pods at last count}}$$

Analyses of variance carried out on the mating designs indicated that general combining abilities (GCA) were preponderant, confirming that transmission of the resistance trait was primarily additive.

The parents were classed according to the rot rate observed in their progenies. This involved a multiple comparison of GCAs estimated per parent (table 1). All in all, the parent classifications tallied well between the three countries despite different pathogen species. For instance, the selection carried out in Côte d'Ivoire for resistance to *P. palmivora* will be useful should the species *P. megakarya* invade that country.

Genetic and environmental correlations were calculated between the rot rate and potential production, estimated from the total number of pods produced per tree. Genetic correlations between potential production and rotten pod rates were favourable (negative). It was therefore possible to proceed with combined selection for these two traits. Combined "individual-family" selection based on an index combining high yields and good resistance to black pod was proposed for Cameroon and Côte d'Ivoire, in order to select individuals within worthwhile families, which were suitable for use as clones or as parents for new crosses. On the other hand, environmental correlations were systematically positive between rot rate and potential production. This correlation might have been due to secondary infections, from pod to pod, which would increase in line with the density of fruits on the trees.

Narrow sense and broad sense heritabilities were estimated for rot rate (table 2). The larger the number of years taken into account, the greater were the heritability values. Indeed, genetic values increased in accuracy in line with the number of years' observations. For instance, the data for the design in Côte d'Ivoire should be considered the most reliable, whilst additional observations are required in Togo.

Trinitario parents were generally the most susceptible to the disease. The length of the fruiting cycle in this material may have contributed to its poor performance in the field. Amelonado type Lower-Amazon parents and some Upper-Amazons, such as Sca 6, P 7, Pa 150 or T85/799 should help in creating less susceptible varieties, notably in Cameroon, where these parents are not used.

This classification, obtained with GCAs, only had one inversion compared to that based on the actual clone values estimated in a clonal trial located at the same experimental station in Cameroon.

Tests on leaves and pods

Methods

The leaf test is a method of artificial inoculation for early assessment of resistance in genotypes. This test was first developed on whole leaves, then it was applied to leaf discs with a diameter of 15 mm, with a view to reducing the space necessary for comparing genotypes.

Table 1. Classification of the different parents for their susceptibility to black pod rot. Newman and Keuls test (5%).

	Cameroon *P. megakarya* (data over 3 years)		Togo *P. megakarya* (data over 1 year)		Côte d'Ivoire *P. palmivora* (data over 9 years)	
- susceptible						
			IFC 5	a	Pa 150	a
			SNK 64	ab	Sca 6	a
			T86/45	ab	P 7	a
			T85/799	ab	T85/799	b
			T60/887	ab	T60/887	bc
			Sca 6	ab	T79/416	bc
			ICS 100	ab	T79/501	bc
	UPA 134	a	UPA 134	abc	Pa 7	bcd
			ICS 40	abc	T63/967	bcde
	SNK 413	b	Na 32	abc	Na 32	bcde
	ICS 84	bc	UF 676	bc		
	IMC 67	bc	IMC 67	c	IMC 67	bcde
	ICS 95	bc			T63/971	bcde
	SNK 10	c			T79/467	bcde
					Na 79	cde
					IMC 78	de
					Pa 35	e
+ susceptible						

Table 2. Narrow sense heritability (h^2) and broad sense heritability (h^2,L) of the rotten pod rate.

Country	Pathogen species	Number of years' observations	h^2	h^2_L
Cameroon	*P. megakarya*	3	0.109	0.133
Togo	*P. megakarya*	1	0.061	0.061
Côte d'Ivoire	*P. palmivora*	9	0.681	0.681

Leaf discs were placed in trays, then inoculated with 10 µl drops of a *Phytophthora* sp. zoospore suspension. Observations were carried out after 5 and 7 days of incubation at 26°C.

The pod test consisted in measuring the diameter of a rot patch on fruits inoculated artificially with a calibrated zoospore suspension. Such inoculations can be carried out on fruits still on the tree, or on loose fruits, with or without wounding of the epidermis.

Correlations between leaf tests and pod tests

Data relative to leaf and pod tests carried out in a clone comparative trial were examined using a multivariate analysis of variance, in order to determine the different correlations.

"Clone" effects were significant for each of the traits studied. Phenotypic correlations between leaf tests and pod tests were positive and significant.

Based on the multivariate analysis of variance, it was possible to calculate genetic and environmental correlations between the different variables. Genetic correlations corresponded to correlations between the means per clone. They were systematically positive between the leaf tests and pod tests. However, they were not significant, as they were calculated on too small a number of clones (5 clones).

On the other hand, environmental correlations were negative for most of the variables considered. Test expression was therefore subject to environmental effects, and those effects differed depending on the organ tested.

Heritability of the resistance trait estimated with the leaf test

The heritability of the scores obtained by the leaf test was estimated in different trials in Côte d'Ivoire. The first trial was a diallel planted in the nursery at CNRA's Bingerville station. It was a 5 x 5 design including selfs (without the reciprocal crosses). The parents used were: P 7, PA 150, T60/887, IFC 1 and a hybrid tree UPA 402 x UF 676, identified for its resistance to *P. palmivora*, assessed on leaves (table 3). The assessment involved thirty plants of each progeny in two series of inoculations. Each series comprised two batches of 250 plants, and fifteen plants per family were tested in each batch.

The second trial was a 4 x 2 factorial mating design (table 4). Eight progenies, all made up of adult trees, were planted in an experimental plot at CNRA's Bingerville station in a randomized single tree plot design. The female parents were Upper-Amazons and Trinitarios, whilst the male parents were two Upper-Amazons. The assessment protocol used in the diallel trial was also used here.

In both the mating designs studied, the heritability of the scores obtained in the leaf test increased with the number of series taken into account, as the precision of the genetic values was better when the plants were assessed several times.

Correlations between resistance tests and rot rates in the field

As regards relations between the results of the leaf test and rot rates observed in the field, it was found that no strong phenotypic correlations (tree by tree) existed, be it in the clonal trials or in the hybrid trials. On the other hand, a significantly positive genetic correlation was often detected between the rot rates and the pod tests, and especially with the leaf tests. This indicates that the means per clone or per hybrid were correlated and that it is therefore possible to select a clone or cross on the basis of leaf tests carried out in an appropriate experimental design.

However, the selection of genotypes within a progeny is not reliable yet with leaf tests, and further trials need to be carried out before considering this method for early selection of individuals.

The correlations between leaf tests, pod tests and rot rates in the field were also studied on clones in Trindidad and Tobago (table 5).

Resistance tests on leaves or pods were significantly correlated to the rot rates observed in the study plots. They were correlations established on a clonal level, hence determined from the means of several trees for each of the clones. Unlike the leaf tests, the pod tests were better correlated to the rot rates in the field when they were carried out without wounding the epidermis.

In Côte d'Ivoire, fifteen clones compared in a trial set up in a totally randomized "single tree" plot design were assessed for their reaction to *Phytophthora palmivora*. These clones, planted in a trial at the Bingerville station (BL7), were assessed for several reasons:

- compare leaf tests and pod tests (attached pods and loose pods),
- assess tree effects within clones (environment),
- compare inoculation tests and resistance in the field,
- assess the influence of morphological traits (cortex thickness, pod size) and environmental factors on pod resistance.

The clonal values estimated by the different tests were then correlated to each other. It appeared that the rot rates were quite well correlated to the pod inoculation tests, and to a lesser degree with the leaf tests.

Mapping areas of the genome involved in resistance

In work to map areas of the genome involved in resistance to *P. palmivora*, several significant QTLs of resistance, assessed from the percentages of rotten pods, were identified. One QTL was located on chromosome 1 of clone UF 676, another on chromosome 10 of T 60/887, and another on chromosome 4 of IMC 78. Other putative QTLs, with lower *lodscore* values were also identified. An improvement in the experimental design, adapted to QTL analyses, notably with a larger number of individuals, would make it possible to increase the power of QTL detection. In progenies UPA 402 x UF 676 and T 60/887 x Amelonado, QTLs of resistance assessed by leaf tests on adult trees were identified on

Table 3. Individual heritability values for resistance to *Phytophthora palmivora* measured on leaves in two series of inoculations.

Heritability	Series 1	Series 2	Series 1 + Series2
Broad sense	0.490	0.427	0.662
Narrow sense	0.294	0.203	0.320

Table 4. Individual heritability values for resistance to *Phytophthora palmivora* measured on cocoa tree leaves in the field in three series of inoculations.

Heritability	S1	S2	S3	S1 + S2	S1 + S3	S2 + S3	S1 + S2 + S3
Broad sense	0.103	0.325	0.581	0.248	0.451	0.666	0.685
Narrow sense	0.103	0.292	0.565	0.248	0.451	0.657	0.662

Table 5. Correlations between resistance tests and rot rates in the field (Trinidad and Tobago).

	Leaf test		Pod test		Field observations	
	WW	NW	WW	NW	% BPTP	% BPRP
Leaf test						
WW		0,469 **	0,410 **	0,862 **	0,496 **	0,471 **
NW			0,106 ns	0,438 **	0,3734 *	0,415 *
Pod test						
WW				0,543 **	0,536 **	0,586 **
NW					0,660 **	0,642 **

WW: with wounding; NW: no wounding
% BPTP: percentage of black pods / total pods
% BPRP: percentage of black pods / ripe pods
* significant at 5%; ** significant at 1%; ns: not significant

several chromosomes, but the reproducibility of the results between experiments was found to be poor. Nevertheless, a few QTLs were detected on the trial mean. These trials were highly sensitive to environmental effects, which might explain the few significant effects of the QTLs and the weak correlations between the different resistance assessment methods applied to old trees. Possible interaction with other agronomic traits was also studied in two progenies in Côte d'Ivoire. In IMC 78, co-location was found between the QTL linked to resistance in the field, that linked to vigour (trunk circumference) and pod weight. On the other hand, in the progeny derived from T 60/887, the most important QTL for resistance in the field, found on chromosome 10, was not co-located with the QTLs associated with the vigour and yield traits. The regions identified in resistance to *P. palmivora* were located in different parts of the genome; a cumulation of different resistance genes therefore seems possible, with a view to increasing levels of resistance to this disease. Similar results were obtained for resistance to *P. megakarya*.

Discussion and conclusion

Resistance tests on leaves and pods, carried out in an appropriate experimental design, can be used to select less susceptible clones or hybrid families. However, these assessment techniques are not reliable, at this stage, for selecting resistant individuals within hybrid families. In this case, the unicity of the genotypes tested prevented these tests being carried out with robust experimental designs. Likewise, rot rates estimated individually on trees in a trial cannot be used to precisely determine the level of susceptibility in those trees. Indeed, the observed rot rate may depend on the immediate environment of the tree, its pod load, the layout of the pods, etc. In fact, field observations depend on many environmental factors that are difficult to control by conventional experimental designs.

In order to estimate the reliability of leaf tests, it is suggested that they be applied in the nursery and validated by observing the performance in the field of material tested in that way at an early stage. In fact, it is now a matter of validating these assessment techniques on a true scale, under actual breeding conditions, and of estimating the genetic gains obtained in rot rate for different selection rates determined from leaf tests.

Leaf testing was also assessed using molecular tools, notably with a view to identifying QTLs for this trait. Several resistance QTLs have been identified, and it is planned to cumulate resistance genes with a view to increasing levels of cocoa tree resistance to *Phytophthora* diseases. Some of these QTLs need to be confirmed by appropriate experimental designs and could be used for marker-assisted selection. ■

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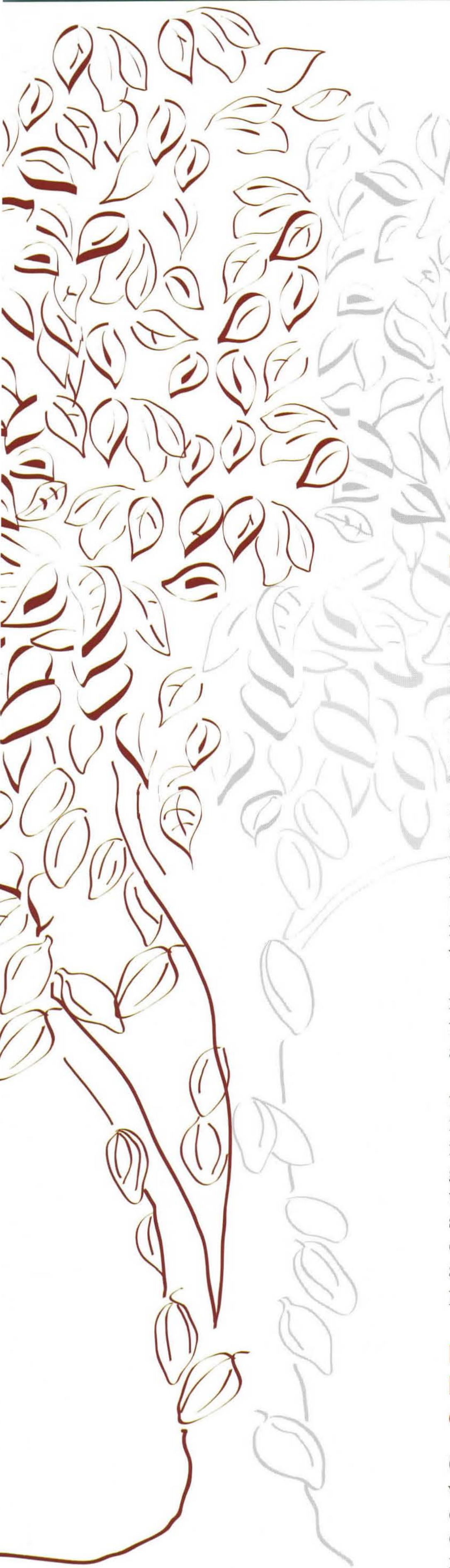


The fine cocoa revival in Ecuador

The project to revive production and improve the quality of Nacional cocoa in Ecuador was implemented in connection with European Union programmes to alleviate poverty, in cooperation with Ecuador and France, and with the collaboration of CIRAD and INIAP. It was intended to improve the standard of living of cocoa smallholders by raising the price of traditional Ecuadorian cocoa on the international market. The aims were to help small family farmers to maintain or increase production by renewing or rehabilitating their plantings, and to restore quality by regaining the specific flavour of cocoa from Ecuador, by ensuring good post-harvest processing of their product.

The project was set up in 1995 and ran for five years up to October 2000. It covered the provinces of Guayas, Manabi, Los Rios and El Oro.

Despite difficult environmental conditions (*El Niño,* the economy and social policy in the country), the project conducted its operations in three closely linked programmes: action-research, technology transfer, smallholder organization and associative marketing. Producers, traders and cocoa industrialists actively participated in all phases of the project cycle: diagnosis, programming, operations.

Background of the Ecuadorian cocoa commodity chain

Cocoa cultivation in Ecuador goes back a very long way and underwent its strongest development in the second half of the 19th century, up to World War I. Then, for numerous reasons, the importance of this crop greatly declined: natural causes such as the occurrence of new diseases (witches' broom, frosty pod rot), but also for socio-economic reasons (sharing out of the large *haciendas*, overcapacity in cocoa processing in the country, reconversion of agro-industry to banana exports, etc.). Since 1980, production has fluctuated around 80,000 t, and Ecuador is the world's eighth largest cocoa producer.

Ecuadorian cocoa has established itself a reputation on the original floral flavour, the *Arriba* flavour, produced by Nacional type cocoa trees. This genetic group falls between the Forasteros and Criollos. However, at the beginning of the 20th century, higher yielding Trinitario type cocoa trees, which were also assumed to be more resistant to new diseases, were introduced into Ecuador from Venezuela. Through a series of natural hybridizations, genetic erosion of Nacional cocoa occurred. Despite this genetic contamination, the *Arriba* flavour of traditional Nacional cocoa batches was maintained, though it decreased in intensity.

At the same time, due to an inappropriate marketing system that did not involve a quality premium, and did not sanction redhibitory defects, most small-scale producers stopped marketing well-prepared cocoa. In 1994, the decline in quality led the ICCO to downgrade Ecuadorian cocoa from 100% to 75% fine or flavour cocoa.

Nacional cocoa, which is considerably hybridized, currently accounts for around 95% of the country's production, but it is gradually being replaced by a clone of Trinitario origin, CCN-51, which is a very high yielder but does not have the *Arriba* taste. Gradual substitution of Nacional cocoa by cocoa from CCN-51 could lead to a loss in the specificity of Ecuadorian cocoa, and would set the country in direct competition with countries producing bulk cocoa, such as Côte d'Ivoire, with production volumes that are more than ten times larger.

Evaluating and characterizing the *Arriba* flavour

A complete study was launched in 1996 with the assistance of INIAP; it involved the physical, chemical and sensorial characterization of different types of cocoa produced in Ecuador: Nacional, Trinitario, and natural Nacional x Trinitario hybrids. The study revealed the existence of the floral *Arriba* flavour in the beans of pure Nacional and in certain natural hybrids, which were also characterized by their mildness, absence of bitterness and astringency. All these traits seemed to be linked to genetic factors provided by Nacional cocoa; environmental factors promoted the development of these original flavours. A reference *Arriba* liquor was created from cocoa at the collecting centres supported by the project. It was made available to any producers, traders or industrialists who wished to check the organoleptic quality of their product.

Safeguarding the *Arriba* flavour

Two operations were conducted at the same time by the project, one intended to rapidly offer producers Nacional type cocoa seedlings with the *Arriba* flavour to renovate their plantings, and the other, with a more long-term perspective, intended to build up a collection of flavour material for use in cocoa genetic improvement programmes in Ecuador.

INIAP recommended the use of six Nacional type clones from the Pichilingue research station. The project assessed the adaptability of this material to the different agro-ecological conditions in the cocoa growing zone of the coastal plain. The results from 30 plots spread throughout that zone revealed quite high production potential for the material as a whole, apart from clone EET 19 which did not do well; the organoleptic quality of the cocoa was maintained (mildness, absence of bitterness and astringency), and the *Arriba* flavour was only found in clone EET 62.

Action was undertaken to safeguard Nacional material rich in the *Arriba* flavour. An original pre-breeding method with farmer participation, based on fresh bean tasting, led to the collection of Nacional flavour cultivars from old cocoa plantings (photo 1). The proportion of Nacional cocoa trees with a strong floral flavour was around 1% due to genetic erosion resulting from natural Nacional hybridization with introduced Trinitarios. The project selected 115 cocoa trees and grafted buds onto rootstocks of IMC 6, which is resistant to *Ceratosystis* wilt caused by *Ceratocystis fimbriata*. This material was planted out in two study collections called SNA *(Selección Nacional Aromatica)* in which the agronomic characteristics of the material were studied. One collection was at INIAP's Pichilingue station, the other on a smallholding in the Balao region.

Ensuring the quality of post-harvest preparation

The project helped small farmers to organize themselves in smallholder associations, in order to transfer simple post-harvest preparation techniques to them that were adapted to their needs, and make available to them equipment and infrastructures for optimizing preparation (photo 2). More than 25 associations were either restructured or created in the project zone, around twenty of which are operational. The elected representatives were trained and acted as technical managers.
Within the associations, the project installed fermentation centres, concreted or mobile tray sun drying areas and warehouses. At the INIAP research station, a modern and didactic post-harvest processing centre was set up for better cocoa preparation, but also for demonstration purposes during technician and farmer training.

Renovating and rehabilitating old plantings

Maintaining or increasing Nacional cocoa production involved increasing plantation productivity, achievable either by renovating the planting material, when the plantation had been excessively weakened, or rehabilitating the planting material, when the trees showed a positive and rapid response to the cultural practices used. In this context, the project set out to validate Nacional type planting material and the cutting back techniques recommended by INIAP.

Despite their susceptibility to diseases, the Nacional type cocoa clones recommended by INIAP showed quite high production potential: around one tonne per hectare, which was around 30% lower than clone CCN 51. A regionalized analysis of the results led to selection of the two or three most appropriate clones for each agroclimatic zone.

On the other hand, the cutting back techniques were difficult to implement and were not cost-effective.

The project therefore set up nurseries of Nacional type cocoa seedlings in the smallholder associations and at the INIAP research station, with an annual production capacity exceeding 280,000 seedlings.

At the same time, the project implemented a technology transfer programme intended for the smallholders grouped in the associations. Through actions such as demonstration plots, "open days", and introductory training courses, it invited farmers to renovate or rehabilitate their plantings.

Guaranteeing a better sale price through direct associative marketing

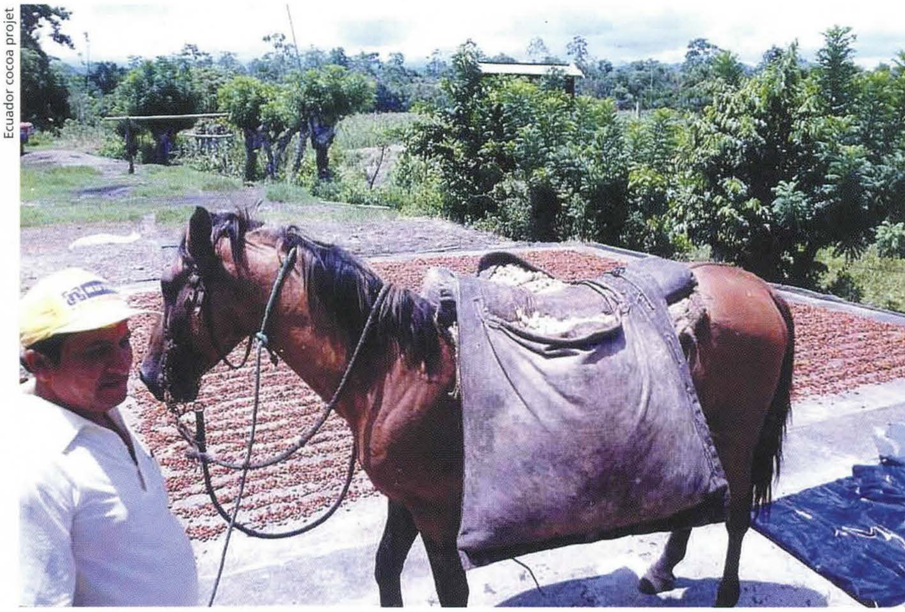
Another purpose of the smallholder associations was to ensure direct marketing of the cocoa produced by their members, and strengthen the unity of action with respect to the authorities and cocoa buyers in order to ensure that their rights were respected. The project was responsible for training members and their managers in marketing matters.

Among the operations conducted for that purpose, it is worth mentioning:

- restructuring or gaining legal status for more than 25 associations, around twenty of which were operational by the end of the project,
- setting up a body, UNOCACE, covering these organizations and responsible for managing direct exports of quality cocoa, and taking on a representative role in cocoa sector decision-making processes,
- supervisor training in company management, marketing techniques, accountancy, etc. A high-profile information campaign was instigated. For instance, producers and the associations received a quarterly information bulletin from the

Ecuador cocoa projet

Photo 1. Nacional variety cocoa tree.



Ecuador cocoa projet

Photo 2. Cocoa collection within a smallholder association.

project concerning the national and international cocoa markets,

- introduction of quality standards applied within the associations for cocoa purchases from members, with price differentials for selling fermented, properly dried cocoa to the association,
- construction of a cocoa packing centre for export batches, for successful associative marketing of quality cocoa,
- granting of small, short-term credit facilities to facilitate the purchase and sale of cocoa produced by members,
- introduction of a specific technical and commercial scheme enabling the collection and direct associative marketing of Nacional cocoa batches,
- marketing of batches of fine and flavour cocoas that are much sough-after by chocolate makers, especially in Europe. For this operation, the project received support from CIRAD, which was involved in developing and marketing batches of cocoa prepared by the project on the international gourmet markets.

These operations resulted in an increase in prices paid to cocoa producers, through:

- premiums received directly at the time of the sale,
- a rebate granted by UNOCACE at the end of the year depending on sales (volumes and export prices),
- competition from independent small-scale buyers, who offer higher prices to producers than the association when they have to acquire the volumes essential for their trade.

In addition, the project helped in drawing up a programme intended to improve fine and flavour cocoa marketing, by determining objective differentiation criteria that could be measured between fine cocoas and bulk cocoas. This programme, which was submitted by INIAP to the CFC, via the ICCO, in partnership with Venezuela, Trinidad and Tobago and Papua New Guinea, was approved and was scheduled to be introduced in the first quarter of 2001.

Conclusions and prospects for the sustainability of cocoa project operations in Ecuador

After operating for five years, the project is in a position to propose technological packages which, when applied immediately, can improve the economic situation for smallholders, but can also help in promoting Nacional cocoa from Ecuador.

Several achievements will ensure the sustainability of the project. For instance, INIAP has acquired international recognition and is already involved in numerous international research projects, funded by donors such as the CFC, ACRI, FIRC, and multinationals. These include molecular characterization of the new Nacional material (CIRAD-BIOTROP), comparative assessment of international clones (CFC-IPGRI), determination of objectively measurable flavour quality parameters (CFC-ICCO), etc.

In addition, the producer associations in UNOCACE have acquired independence and sufficient capital to continue with direct marketing and export of quality cocoa, to the benefit of small-scale producers.

Lastly, several NGOs backing the Ecuadorian Ministry of Agriculture are ready to participate in technology transfer and coordinate technical assistance programmes for associated smallholders.

The cocoa project is a pilot project in which development of the smallholder sector is ensured by direct associative marketing of a specific quality product, backed up by technology transfer and action-research programmes. ■

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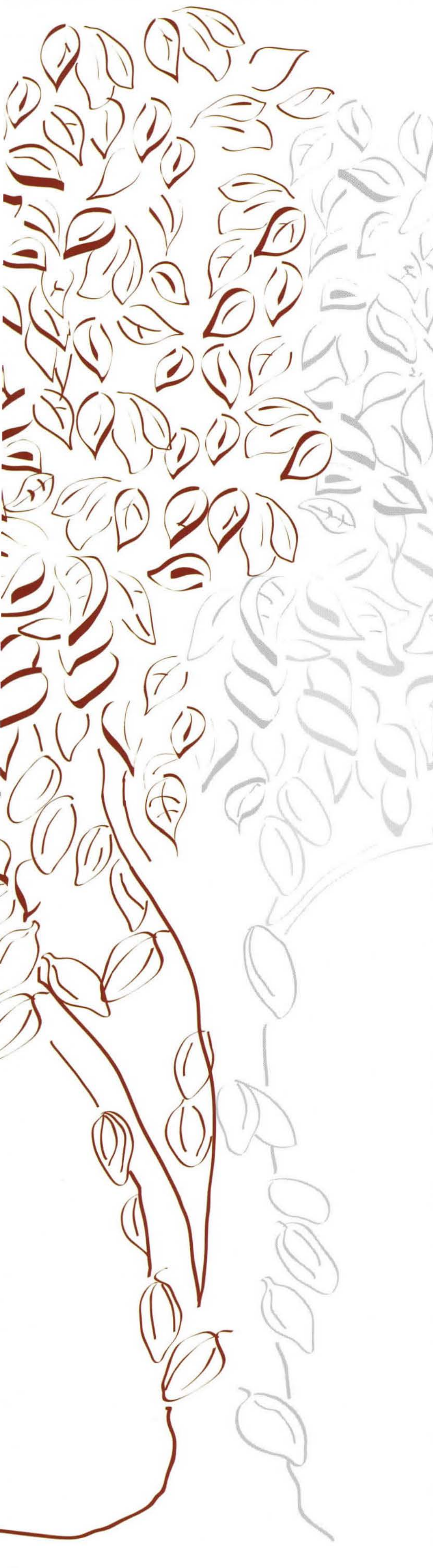
Cocoa genetic resources: assessment and use



Conservation, evaluation and utilization of germplasm are the three basic stages in the cocoa breeding process, which is a fundamental aspect in developing a sustainable high-yielding cropping system. Since April 1997, fourteen national or international research centres, including CIRAD and those of the main 10 producing countries in Latin America, Africa and Asia providing 80% of world cocoa supplies, have been focusing their efforts on that objective. It is the first time in the history of cocoa research worldwide that such a wide-ranging international project has been implemented.

The project

The five-year CFC/ICCO/IPGRI project on the use and conservation of cocoa germplasm is funded by the partner research institutions and by the Common Fund for Commodities (CFC), and is coordinated by IPGRI. Its aim is to enhance the assessment of cocoa germplasm using traditional selection methods to obtain improved clone and hybrid varieties with improved resistance to diseases.

The operations involve:

- setting up international and local clone trials in the ten countries taking part in the project,
- hybrid trials in five of those countries,
- germplasm enhancement and population improvement programmes in five of the countries.

Improved populations and selected genotypes will be distributed to user countries.

After actively taking part in the preparatory phase, CIRAD-CP is now involved in international coordination of the project and in conducting certain research activities, in Montpellier,Trinidad and Côte d'Ivoire. The working methods used for joint activities under the project were defined at an initial workshop in 1998.

Germplasm evaluation, identity checking and exchange

Identity

Twenty-five clones were selected for the international clone trial. The clones were multiplied in 1997 and distributed to all the project partners in 1998 and 1999 from quarantine centres at CIRAD in Montpellier and the University of Reading in the UK. Over the last two years, CIRAD and the University of Reading have analysed 200 and 100 samples respectively, to check genetic identity using microsatellite markers. In around 20% of these clones in the collections of different countries, the sample did not correspond to the reference, indicating the existence of off-types in the collections.

The same clones were used to study their resistance to 25 strains of *Phytophthora* spp. from countries taking part in the project ring test. Three sets of trials were conducted at CIRAD in Montpellier using inoculations on leaf discs. An analysis of the first results revealed highly significant effects for the *P. palmivora* strains, and for the cocoa clones, but there was no pathogen-host interaction. The aggressiveness and identity of the *Phytophthora* spp. strains were also investigated. This work is continuing with inoculation tests on nine international clones recently introduced from the University of Reading.

Genetic diversity

All the information available on the agronomic value of accessions of the international collection in Trinidad (ICGT) were used to select the most promising clones, in order to build up a "CFC/ICCO/IPGRI Project Collection". This collection, containing around a hundred clones, is available to all those taking part in the project. Apart from their agronomic value, the clones were also selected for the genetic diversity they represent. High genetic diversity is maintained through representation of a large number of populations (stratification), and through a choice based on the results of genetic diversity analyses using morphological traits, biochemical markers (isozyme electrophoresis) and molecular markers (RAPD). The degree of diversity observed within the cocoa populations in the ICGT made it possible to determine the number of clones to represent each of them (N strategy). A study of how genetic diversity was organized, using multivariate analyses, then made it possible to choose clones that ensured maximum maintenance of that diversity. The detection of off-types in the ICGT collection is also under way, using RAPD techniques, giving priority to those clones that are candidates for inclusion in the CFC project collection.

Resistance to diseases

Monthly field observations of resistance to witches' broom disease *(Crinipellis perniciosa)* and black pod rot *(Phytophthora palmivora)* began in November 1998 on a batch of 228 clones. After a 12-month observation period, the results were analysed in terms of the percentage of pods affected by *Phytophthora* and *Crinipellis,* and the percentage of branches affected by *Crinipellis*. Resistance categories were defined according to the levels of attack on control clones. The results showed average productivity of 14.1 pods per tree, a mean of 15.3% of pods affected by *Phytophthora* (from 0 to 87.5% for clones producing more than 10 pods per tree), 10.8% of pods affected by *Crinipellis* (from 0 to 49.5% for clones producing more than 10 pods per tree) and 0.72% of branches affected by *Crinipellis* (from 0 to 13.9%). In November 1999, observations continued on 226 clones, this time including 50 control clones from the first batch (variables for their resistance), 45 clones from the first batch that revealed a good level of resistance to black pod rot and 131 new clones included in the list of clones to be studied as a priority.

Assessment of clones in the international collection for resistance to witches' broom, by artificial inoculation, began in July 1999. The method adopted by ACRI was the spray inoculation system developed by the University of Florida. At ACRI's request, the clone grafting and inoculation programme was launched directly on a large scale. In all, 221 clones were grafted up to the end of December 1999. Four series of 40 clones were inoculated at the standard dose of 350,000 basidiospores/ml at a rate of 1 ml/plant. No symptoms were seen in the four months following inoculation. This result was attributed to unsuitable inoculation and incubation conditions. The method was improved, primarily by modifying the conditions for incubation and symptom development, and by carrying out preliminary trials to ascertain the optimum inoculation stage. Screening was resumed in February 2000 using a dose of 250,000 basidiospores/ml and 2 ml/plant. Research is also under way, outside the CFC project, to develop another early laboratory test to assess resistance to witches' broom.

Clone trials

Budwood from some 25 international clones was introduced in each of the countries, and clone trials (international and local clones) were set up in Ecuador, Malaysia and Papua New Guinea. Selection of new local clones has begun in all the countries and observation plots have already been set up in Brazil, Ghana and Papua New Guinea. Trials on the interaction between rootstocks and scions are being prepared in Ecuador and Ghana. Assessment of the level of resistance to black pod rot in clones and hybrid progenies has begun in Trinidad, Brazil, Ghana, Nigeria, Papua New Guinea, and France. The genetic diversity and pathogenicity of isolates of *Phytophthora palmivora*, *P. megakarya* and *P. capsici* collected in the different countries are being studied in Montpellier. An assessment of resistance to mirids is under way in Côte d'Ivoire, Ghana and Cameroon, where significant differences have already been found between clones. A ring test to measure resistance to witches' broom is being carried out in Brazil, Trinidad and at the University of Reading.

Hybrid trials

Around 250 crosses have been created and planted in Cameroon, Ecuador, Nigeria and Papua New Guinea. Variations have been found between hybrids for black pod resistance in Nigeria and in Papua New Guinea.

Field resistance data for 230 genotypes in the international collection in Trinidad were used to choose the most resistant genotypes to be crossed with each other, and over 1,000 plants derived from 30 of these crosses have already been evaluated by leaf inoculation tests. The 150 most resistant plants are then to be planted out for further observations.

Improvement of populations

In Brazil, the main objectives are to recombine genes of resistance to witches' broom, using two mating designs, selection in the field of trees identified in the trials, and screening of the progenies for resistance.

Under the recurrent selection programme in Côte d'Ivoire, involving a technique developed by CIRAD in 1991, observations of the two base populations were completed in 1999 and a second selection cycle has begun. The selection of second cycle parents is based on the performance of first cycle parental clones, on the progenies of these clones, and on the characteristics of individuals within the best progenies. Forty parents from each population were used in 1999 to carry out 160 crosses in incomplete factorial designs. Analyses of the data from the first cycle were also used to identify between-group crosses (Upper-Amazon x Criollo or Lower-Amazon Amelonado x Guianese crosses) and within-group crosses of potential interest for variety creation. Crosses were carried out for a preliminary between-group combination trial (16 families plus controls). A trial including within-group and between-group crosses for selection based on resistance to *Phytophthora* spp. is to be set up in 2001 at Abengourou.

In Ghana, around 500 within-population and between-population crosses have been carried out and an assessment of resistance to Swollen Shoot virus and to black pod rot has begun. Almost 200 progenies had already been planted out in 1999.

In Malaysia, 56 progenies were obtained to combine traits of resistance to Vascular Streak Dieback and black pod rot with the fat content trait. More than 70 clones have been grafted for resistance tests and field trials have been set up.

Conclusion

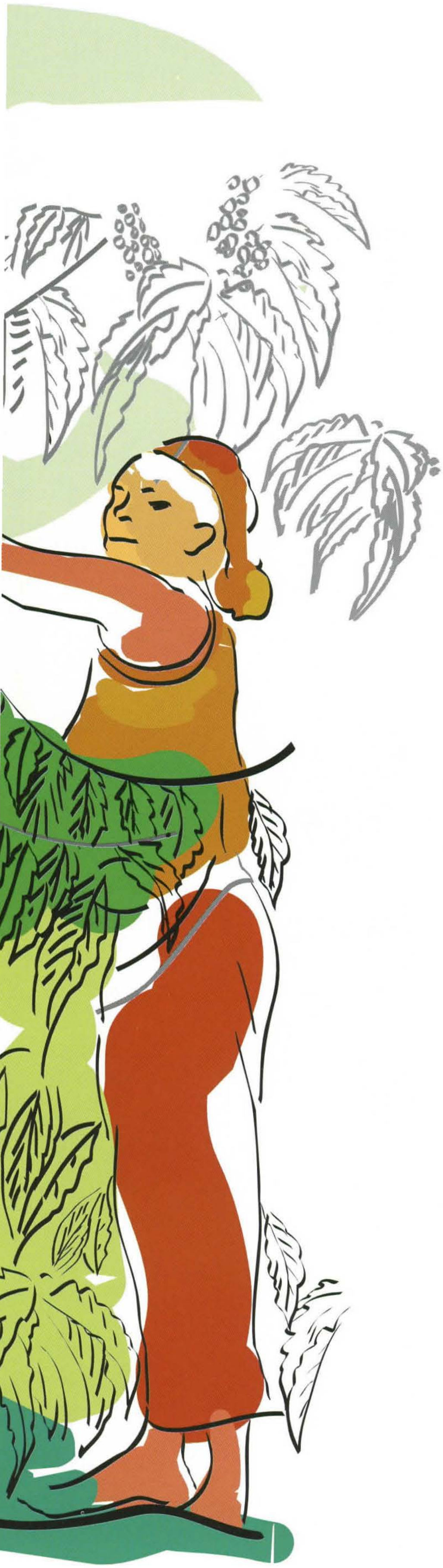
After operating for only two years, the achievements of the CFC/ICCO/IPGRI project are already impressive: more than 20 hectares of trials have been set up in a concerted manner at research centres in producing countries, notably using clones of certified origin; the breeding programmes in Côte d'Ivoire, Ghana, Brazil and Malaysia have been relaunched; and common procedures have been defined for the characterization and evaluation of genotypes. At the same time, good headway has been made in defining a "working collection" of around a hundred clones to be made available to project partners. ■

List of publications

ESKES A.B., ENGELS J., LASS R.A., 1998. The CFC/IICO/IPGRI project: a new initiative on cocoa germplasm utilization and conservation. Plant. Rech. Dév. 5 (6) : 412-417.

ESKES A.B., ENGELS J., LASS R.A., 2000. Working procedures for cocoa germplasm evaluation and selection. Proceedings of the CFC/IICO/IPGRI project workshop, 1-6 Feb. 1998, Montpellier, France. Rome, Italy, International Plant Genetic Resources Institute, 176 p.

Coffee commodity chain: economic context and research overview



Stagnating consumption and rising stocks: the crisis is deepening

The some six million tonnes of coffee (70% Arabica) produced worldwide bring in earnings of 9 to 15 billion dollars for producing countries. Farmers receive 40 to 60% of that some directly, with the rest going to commercial operators in the national commodity chain and to various State taxes.

The speculative nature of the coffee market gives rise to substantial and sudden price variations. However, four years of relatively stable prices (1994 to 1997) were followed by a steady decline. Between January 1998 and November 2000, the average Arabica indicator price fell from 4.01 to 2.01 $/kg (3.64 € to 1.70 €) and from 1.82 to 0.79 $/kg (1.65 € to 0.67 €) for Robusta. This trend has been worsened by stagnating consumption, particularly for standard coffees, to the benefit of other beverages. Coffee is primarily produced by smallholders practising mixed cropping on one to five hectares, and remains their main cash source. Maintaining crop profitability, and risk management are made difficult by this situation, and producers are driven to reducing upkeep and halting renewals.

In order to cope with this volatile market, which is virtually 70% controlled by four multinationals, the Association of Coffee Producing Countries (ACPC) attempts to stabilize prices by holding back or releasing stocks. Despite apparent unanimity, the dominant role played by Brazil (Arabica), and more recently Vietnam (Robusta) makes it difficult to implement such measures. One channel being considered involves reviving consumption in producing countries and promoting consumption on potentially strong markets such as Eastern Europe, China and Russia.

Various initiatives are envisaged, such as strengthening farmer organizations to limit price fluctuations. Special "quality" coffees —gourmet coffees, single origin coffees, organic coffees and low-caffeine coffees— ought to provide more lucrative opportunities for producers: it is mostly the South American countries that are turning to this outlet. This drive for quality generally goes hand in hand with sustainable, environment-friendly production. Likewise, coffee's fair trade status is confirmed. However, the commodity chain is now concerned by the risk of coffee contamination by mycotoxins, especially ochratoxin A (OTA).

Coffee Programme priorities

Based on an analysis of the major constraints in coffee cultivation, and taking into account the needs of the different stakeholders in the commodity chain, the Coffee Programme is developing research designed to:

- improve smallholder farming systems,
- successfully achieve sustainable *C. arabica* cultivation in intensified farming systems in Central America,
- control major parasites,
- control quality.

Sustainable farming systems: an essential target

For producers, this target entails risk management, in order to maintain crop profitability, and taking environmental protection into account.

In many Robusta producing countries, recent liberalization of the commodity chain and weak prices have led to a slump in plot productivity and a deterioration in quality due to a reduction in plantation upkeep and a halt to renewals. The Coffee Programme has therefore stepped up studies on smallholder practices and of the way producers take on board technical innovations. Farm types and their operating methods have been characterized during joint projects with partners, in West and Central Africa in particular.

In an attempt to remedy the limited use made of selected planting material, a reciprocal recurrent selection programme undertaken in partnership with Côte d'Ivoire led to the identification of new between-group (Guinean x Congolese) type *Coffea canephora* hybrids that are more productive and more uniform than the old clones. This work, which was covered by a PhD thesis, led to a revolution in Robusta variety creation, by opening up the way for the dissemination of selected hybrid varieties in the form of seeds produced in a seed garden.

In Central America, a coffee-based farming system agroforestry programme is being conducted in partnership with CATIE and CIRAD-Forêt, with a view to proposing alternatives to intensive farming systems and increasing plantation sustainability. This research, which is initially geared towards understanding environmental effects, is being carried out in close connection with studies being conducted with INRA in Avignon on yield components and quality.

Crosses between commercial varieties and wild Ethiopian genotypes have resulted in the creation of hybrid *Coffea arabica* varieties possessing production, resistance and cup quality characteristics.

Under cooperation with countries in Central America, varietal outputs are planned with these hybrids, propagated notably by somatic embryogenesis, though this innovative procedure needs to be validated before considering it for mass production on an industrial scale.

Disease and pest management is a priority for all producers. The Coffee Programme is focusing its research on developing integrated control methods. In Honduras, results obtained on the effects of soils and crop management sequences on Coffee Leaf Rust, have shed original and innovative scientific light on how the type of farming system and the physico-chemical factors of the environment can affect disease development. In Guatemala, results on the characterization and bioecology of the complex of nematode species in the genus *Pratylenchus* have provided new information, in terms of both biosystematics and host-parasite relations. The importance of numerous edaphic, climatic and varietal factors has been elucidated, laying the foundations for a strategy to search for varietal resistance.

In Africa, studies being conducted in partnership (CRF in Kenya, IRAD in Cameroon and CIFC in Portugal) on Arabica Coffee Berry Disease caused by the fungus *Colletotrichum kahawae* have been developed under a project funded by the European Union. This research has led to the identification of genotypes that are sources of sustainable resistance to the disease, and has furthered knowledge of genetic variability in the pathogen, which is a prerequisite for rational management of resistance sources. With the recurrence of tracheomycosis, which is restricted to East Africa for the time being, fresh research is under way in joint projects with CABI-ARC (African Regional Centre), CORI (Uganda), Facagro (Kinshasa, DRC) and UCL (Belgium).

The Coffee Programme at a glance

34 staff, including 23 researchers
Partner countries: France (Montpellier), Côte d'Ivoire, Guinea, Cameroon, Kenya, Tanzania, Costa Rica, Guatemala, El Salvador, Dominican Republic, Laos, Uganda
Institutions:
France: IRD, INRA, University of Montpellier II
International: CABI, CATIE, FAO, ICO, CIFC
Industries: Europe (ISIC), major distributors, SNICC
Networks: ACRN/RECA, IICA-PROMECAFE
Research themes:
- Improvement of smallholder farming systems
- Sustainable use of *C. arabica* in intensified farming systems in Central America
- Management of major phytosanitary constraints in East Africa
- Genetic control and trapping against the coffee berry borer
- Quality control

The development of ways to control the Coffee Berry Borer has provided the opportunity to develop innovative research on genetic modification and trapping. An effective coffee modification methodology has been developed and has resulted in a model for resistance to the leaf miner, using *Bacillus thuringiensis* genes. A trial has been set up in Guiana to validate this model in the field. In addition to testing the efficacy and utility of the modification methodology in the field, this trial provides an opportunity to study the impact of transgenes on local populations of target and non-target insects, along with pollen dissemination. A Coffee Berry Borer trapping technique has been developed in partnership with El Salvador (PROCAFE). In order to confirm the promising results obtained under experimental conditions, it is necessary to assess the agronomic impact of trapping on yield improvements, in both quantity and quality terms.

Quality characterization and control

Although remunerated to varying degrees, quality remains a priority for producers. Faced with market segmentation after the appearance of "special" coffees, the Programme has continued its research on biochemical characterization of coffees and stepped up its efforts on two essential fronts: how to produce and market these different types of coffee, how to define and certify origins and flavours. Providing training in sensorial analysis for quality control has been one of the main activities of the Programme, with a view to creating networks of experts in producing countries.

In order to preempt the imminent limitation in coffee mycotoxin contents, a project to prevent coffee contamination by OTAs has been drawn up with European industry (ISIC), several producing countries and the FAO. The Programme is coordinating research which focuses on the detection of toxins and the identification of conditions for OTA contamination and development, and which takes into account producer practices according to their socio-economic constraints. ■

Forest Guinea: coffee cultivation and smallholder constraints

Agricultural development operations in Guinea, especially in the forest region, were launched at the beginning of the Second Republic in the form of projects, which were based on intensive technical models or "packages", which were agriculturally coherent when the conditions for their application were assembled. In terms of smallholder economics, these intensive technical models called for high investments (setting up plantations, hydro-agricultural work in bottomlands) concentrated on a single crop. They also presupposed strong commitment of the work force to this crop at times of the year when it might enter into competition with other crops that were equally as important for family economics. Given this concentration of the means of investment and of labour on a single crop, these models, when applied to many family farms, could weaken them and expose them to increased technical and economic risks.

This document shows that the improvement of coffee-based farming systems needs to be rational, through a search for technical/economic solutions that are adapted to the specificities of the zone and to the multiple activities of producers. It uses proposals from studies and results from programmes funded by French cooperation (SCAC, AFD) and implemented by Guinean agricultural research (IRAG), along with the coffee revival project (RC'2), with CIRAD collaboration and the participation of the centre for support to agricultural professional organizations (CAOPA) and the national service for rural promotion and extension (SNPRV).

The region

Most Guinean coffee production is concentrated in seven *prefectures* of the Forest Guinea region, covering almost a fifth of the total area of the country. Pedoclimatic conditions are suitable for the cultivation of a wide range of perennial, semi-perennial and annual crops. The height above sea level, which varies from 400 to 1,000 m, enables staggered sowing and several harvests per year. This situation opens up outlets for produce on the national markets of the other natural regions, but also on the subregional markets of neighbouring countries such as Mali, Senegal or the Gambia.

According to estimations, the population of the region, not counting refugees, was around 1,324,000 inhabitants in 1998. The number of farms is estimated at around 120,000 with agricultural densities of 24 to 70/inhab/km^2 depending on the *prefectures*.

Farms

In this region, farmland seems to be fixed and agricultural growth, which is booming, is on limited and relatively few areas of newly cleared forest land. The farming systems are exclusively manual and based on backyard crops, hillside crops and marshland or lowland crops. Perennial crops are located in the strip of trees around the village, usually near dwellings where wandering domestic animals limit food crops.

However, in recent years, the increase in population pressure and strong expansion of coffee plantings has encouraged planting in the fallow areas between villages previously devoted to food crops. The increase in land occupation pressure has led to a substantial increase in the length of time food crop plots are cultivated, rising on average from 0.8 to 1.5 and 2 to 3.5 years of cropping over the last ten years, depending on the zones and the families.

Rice—around 50% of the areas occupied by food crops—and coffee—around 90% of the areas occupied by perennial crops—are the linchpin crops of the main farming systems. The food crop systems involve the cultivation of one or more plots, on a shifting cultivation basis, usually for two years. These plots are then left fallow for varying lengths of time depending on population pressure, the areas attributed by the village or the extent of the family domain.

The main characteristic of farms in the forest region is the multiple activities of rural families. Apart from work in the field for food crop and cash crop production, rural families devote a large share of their work time to gathering (oil palm bunches, mushrooms, wild pepper and medicinal plants) and the harvesting of palm wine (*Elaeis guineensis* or raphia depending on the zones), whose sale brings in regular income spread out over the year. In addition, women, who account for around two thirds of family agricultural workers, carry out more than half of the work in the field and are responsible for harvest management and processing of products intended for family consumption and for sale.

There is a marked tendency towards reducing fallow to the benefit of perennial and food crops. However, the main constraints vary depending on the type of farm. For small farms, a land occupation constraint means limited diversification possibilities. For medium and large farms, there is expansion in the areas devoted to food crops and perennial crops, but also diversification through large plots planted with cassava or groundnut, to the detriment of rice. Labour management is one of the major constraints for both large farms and small family farms. Figure 1 shows, for an average family with 7 members, including 3.6 active workers, that family labour availability is insufficient for the work to be done for six out of twelve months.

The labour shortage period corresponds to the first half of the year, when food crops are planted, when plantation upkeep is carried out, and when large quantities of fruits, such as oil palm bunches, are gathered. Whilst mutual assistance groups alleviate the laboriousness of some tasks, such as land clearance and felling, they are not an additional source of manpower, since any day's work acquired must be paid back in return. Thus, unless they reduce the areas cultivated, families have to call in outside labour for some of the work. The cost of such manpower, 760 to 910 GNF per day of mutual assistance or 1,000 to1,500 GNF per day's wages, leads a large number of rural families to sell their product stocks and first harvests at a very cheap price. These sales, which severely reduce cash availability at the end of the first half of the year, are the main factors behind the tiding over period from July to September. For the least fortunate families, it begins as early as June.

Area planted to coffee

The area planted to coffee is not precisely known and is estimated, depending on the sources, at between 100,000 and 200,000 ha, but was probably between 160,000 and 165,000 ha in 1999. It is also difficult to specify yields, for which estimations vary, depending on the sources, between 15,000 t and 20,000 t. Although export volumes remain modest when compared to the major African producing countries, coffee is nevertheless the country's main export crop.

So-called "wild coffee plantings"

Wild coffee plantings are often considered as a separate entity, since they are intercropped with other perennial species with unselected materials. They are in fact highly diversified. Recent studies showed that they are mostly young, since 75% to 80% of the plantings are apparently under 20 to 25 years old. Although they are always planted under forest tree or palm cover, the degree of shading between and within plots is highly heterogeneous, ranging from very dense shading under patches of forest trees, to slight shading under Albizzia or sparse palms. According to some studies, 43 to 51% of coffee plantings only benefit from slight shade, or none at all, 39% to 42% have moderate shade, and 7% to 18% have dense shade, depending on the zones.

In shaded plantings, weeds mostly consist of regrowth of shade-loving dicotyledons and creepers, which do not particularly compete with coffee for minerals. However, when creepers cover the coffee trees, competition for light severely reduces coffee yields. Cultural practices are limited to weeding and sanitary pruning to remove exhausted lower branches and parts of branches broken by harvesters. Coffee sale prices, which rose from 1994 to 1999, led most coffee growers to carry out two weeding rounds per year, in June-July and October-November. In that way, competition with creepers was limited. What will happen if coffee prices, after the substantial drop in 2000, remain at that level in the long term?

Apart from a few very limited operations, producers hardly have the resources necessary to reduce pressure from the numerous pests that attack coffee plantings. Pesticides on sale at the coffee producers' federation are only enough to treat no more than 2% of plantings. Producers report the existence of numerous pests: variegated grasshoppers, twig and berry borers, etc. Stem borers, which kill a large number of coffee trees, and tailed caterpillars (*Epicampoptera*), which can cause defoliation over thousands of hectares some years, cause the most serious damage.

In most plantings, coffee trees are interplanted with kola trees (30 to 60 trees/hectare) and oil palms (20 to 40 palms/ha). In some cases, they may be intercropped with raphia palms, cocoa trees, banana and citrus fruits. This combination of species makes it possible to con-

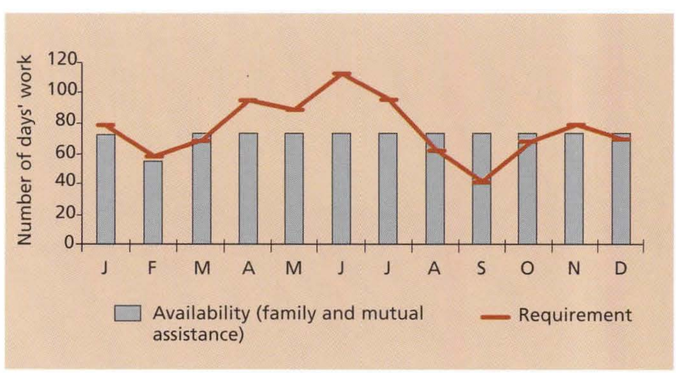


Figure 1.
Labour availability and requirements for an average family.

siderably reduce upkeep work, and to multiply and spread crops over the year. In that way, producers benefit from staggered potential income, limiting the effects of price fluctuations for one product or another.

Improved coffee plantings

Improved coffee plantings occupy around 5,800 ha, i.e. around 3% of the regional area planted to coffee. This has resulted from coffee revival programmes launched in 1986 by the Guékédou agricultural project and extended in 1988 to the whole of Forest Guinea by the RC'2 project. The proposed crop management sequence involved selected clones or hybrids planted without shade, with free growth and regular cutting back, which involved 4 to 5 weeding rounds per year, sucker removal every two months in the rainy season, and fertilizer applications. Due to low sale prices or low productivity, income from coffee was often insufficient to hire part-time labour for food crops. Families stepped up oil palm harvesting and oil production, which was rapidly sold and used to pay the hired labour. Consequently, in the first half of the year, a minimum amount of time was devoted to cash crops, and upkeep in the coffee plantings was often put off until August-September. This work constraint explains why intensive models proposed by the coffee revival project failed. For instance, apart from the distribution of new varieties, the only innovation really adopted by producers, the expansion of coffee cultivation, was largely achieved by increasing areas without any true intensification. Under these agricultural management conditions, selected plantings cannot express their potential and, after the first production cycle, they rapidly give yields equivalent to those in traditional plantings (figure 2).

Discussion

Population growth—approximately 3% per year—incites families to increase the area given over to food crops and coffee trees, and to multiply their activities to limit risks and secure income. Food crop fallow areas are therefore declining significantly and their duration has been reduced overall by half in the last ten years. Yet, with traditional slash and burn practices, without mineral fertilization, it is the duration of fallow that determines the fertility of the soil, hence food crop yields. A drop in soil fertility encourages an increase in the areas given over to food crops, leading to extra work to cultivate them. Due to the extension and diversification strategy, family farms do not devote the amount of work, or the financial resources required for any intensification, even when reduced to a single field. Projects that have attempted to develop a single crop, such as coffee or irrigated rice, have to acknowledge that the results do not match the investments made.

In addition, crop management sequences based on a monoculture, developed by research, no longer meet the expectations of producers, who wish to limit investments in a context of less available production factors, such as land or labour, and fluctuating sale prices. Indeed, the models used as a reference to date, appear to be inadequate in view of the diversification strategy adopted by producers. This results from economic liberalization and improved access to the region through the construction of asphalted roads, which offer true opportunities of outlets on the national and subregional markets, which are less risky and easier than the world market. Strong price fluctuations are undermining the cost-effectiveness of intensifying farming systems based on *Coffea canephora*.

Descriptions of coffee-based farming systems bring out the great diversity of the coffee plantings, and of growing conditions, meaning that general technical messages are meaningless. On family farms, an improvement in coffee production can only come about through the application of flexible techniques making it possible to stagger work in line with producer constraints, leading to a gradual and sustainable improvement in rural family incomes. This approach involves targeted actions, requiring a prior assessment of the resources and constraints of family farms. That is why it is impossible to consider intensifying a given crop without taking all the other components of the farm into account. It is therefore important to develop tools for a rapid diagnosis of farms and of the plot in which action is to be taken. These tools should make it possible to evaluate with the producer which actions, out of several possibilities, will have the greatest likelihood of leading to improvements in the long term, without adversely affecting production potential. Thus, based on on-site observations, producers will be able to make a rational choice of the actions they are in a position to go through with.

Since its creation in 1988, the Guinean agricultural research institute (IRAG) has developed crop management sequences and has bred varieties of rice and coffee adapted to them. Since 1998, with CIRAD support, IRAG has developed an overall approach. It has set up teams of crop specialists, systems agronomists, and socio-economists, with a view to developing innovations that are more suited to the resources and concerns of producers. For instance, in collaboration with smallholders, operations to conserve soil fertility have been launched in villages and at system study sites:

- development of intercropping systems with coffee and food crops, or coffee and other perennial crops, such as oil palm and banana, which are of nutritional, economic and technical interest (complementarity of the farming calendars, staggering of income to cover monthly cash requirements),
- selection of the best varieties for intercropping,
- study of planting designs and crop management sequences making it possible to balance out labour requirements and availability,
- study of the physical and organoleptic characteristics of selected and wild planting material,
- improvement of harvesting and post-harvest processing,
- identification of zones with pedoclimatic conditions suitable for the production of high altitude Robusta coffee, which is sought after by the profession and fetches a more lucrative price than standard Robusta, which depends entirely on world prices. ■

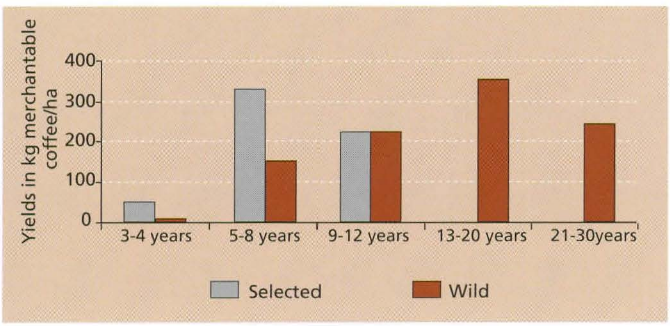


Figure 2. Average coffee yields depending on the age and origin of the planting material.

List of publications

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Excellent *Coffea canephora* hybrid varieties

The cultivation of *Coffea canephora* trees, which produce coffee known commercially as Robusta, is extensive in most cases on the African continent. Yields from this crop reach 600 kilograms of merchantable coffee per hectare per year in Asia and Latin America, as opposed to 300 kilograms in Africa. Under optimum conditions, yields can exceed 2 tonnes of merchantable coffee per hectare per year. Fluctuating coffee prices, ageing plantations and limited use of selected *C. canephora* planting material (from 10 to 20% of plantings), are generally the reasons given to explain the gap between optimum yields and those actually achieved.

In Côte d'Ivoire, the *Centre National de Recherche Agronomique* (CNRA) and CIRAD have been working together for more than three decades on the genetic improvement of *C. canephora*. The setting for this collaboration has evolved over the years and now takes the form of a strong partnership based on clearly defined joint research objectives; these include the identification of high-yielding varieties that are easy to distribute to smallholders.

Coffea canephora clones and hybrids

A clone is a set of trees corresponding to the same genotype. A hybrid is a set of progenies derived from a hybrid combination between two parents or clones.

Production of hybrids and clones

The biology of the species *C. canephora* makes it possible to create either hybrid varieties in seed form, or clonal varieties in cutting form.

In Côte d'Ivoire, hybrid seeds are produced in biclonal seed gardens. The strict cross-fertilization of *C. canephora*, along with geographical isolation of the seed gardens, guarantee that all the seeds harvested are derived from the cross between the two parents of which they are composed. One hectare of seed garden produces 500 kg of seeds per year, on average, for approximately 1,000 ha of new plantings. The viability of the seeds lasts barely more than two months under uncontrolled conditions, but it can be maintained for up to a year with relatively simple methods.

Clonal varieties are propagated from cuttings of green orthotropic branches, with a rooting phase in trays, then transfer to bags. Direct cutting in a propagating tunnel improves the efficiency of the technique. A one-hectare budwood garden can produce up to two million cuttings per year, for approximately 1,000 ha of plantings.

Recent developments in *in vitro* microcutting techniques and somatic embryogenesis suggest that increasingly high multiplication rates can be achieved. Little use is yet made of these techniques, except in Uganda, where somatic embryogenesis by temporary immersion (RITA) is used.

Coffee growers very often use seeds collected from selected varieties (hybrid or clonal) to set up new plantings. Unfortunately, seedlings obtained in that way are of a limited agronomic level; only seeds from seed gardens guarantee strong agronomic potential.

The key to choosing between clones and clone hybrids

The biology of *C. canephora* enables the selection either of hybrid varieties distributed in seed form, or of clonal varieties distributed in cutting form.

The strict self-incompatibility of *C. canephora* means that there is a certain degree of heterozygosity in hybrid parents. In theory, the progeny of a hybrid combination thus possesses a degree of genetic variability that can be exploited through clonal selection. The genetic progress provided by the best trees can rapidly be fixed through cuttings.

Seedling production is ten times cheaper than plant production from cuttings. Moreover, growers have a clear preference for seeds, as they offer many practical advantages over cuttings. Clones are distributed in rooted cutting or budstick form (for direct planting in a propagating tunnel). In both cases, the material is fresh and has a limited storage life: a few days. The conditions and duration of transport between the cutting production centre and village nurseries sometimes result in fatal deterioration of a large number of cuttings and budsticks.

Seeds are less fragile, easier to transport and can be kept longer: a few weeks. Coffee growers are perfectly familiar with seedling rearing techniques.

Hybrid varieties distributed in seed form would therefore seem to be more interesting from a socio-economic point of view. In Côte d'Ivoire, as in many countries, clones have nonetheless been chosen. In fact, the yields of the best clones were 30 to 40% better than those of the best hybrids. Of course, one of the aims of *C. canephora* varietal improvement is to identify hybrid varieties that are equivalent to the best clonal varieties.

Rational use of *Coffea canephora* genetic diversity

Up to the end of the 70s, *C. canephora* variety creation involved carrying out a large number of controlled or uncontrolled crosses, with a view to detecting clones with a strong general combining ability. However, these crosses were carried out at random and did not amount to exploitation of known genetic diversity in the species.

At the beginning of the 80s, two major genetic groups were identified within the species *C. canephora* : the Guineans, which originated from West Africa (Guinea and Côte d'Ivoire) and the Congolese, which originated from central Africa (Congo, Gabon, Democratic Republic of Congo, Cameroon, Central African Republic, Uganda, Angola). In addition, natural progenies derived from parents between these two groups, and the clones from such progenies, revealed vigour likely to have resulted from a heterosis effect. A reciprocal recurrent selection scheme (RRS) using Guinean and Congolese materials as the base populations was subsequently proposed.

CNRA and CIRAD then set out resolutely down that path in Côte d'Ivoire. The RRS programme was launched in the mid-80s (figure). The first genetic gains expected for yield through the selection of between-group hybrids from RRS were estimated at 60% compared to the mean of the hybrids under test.

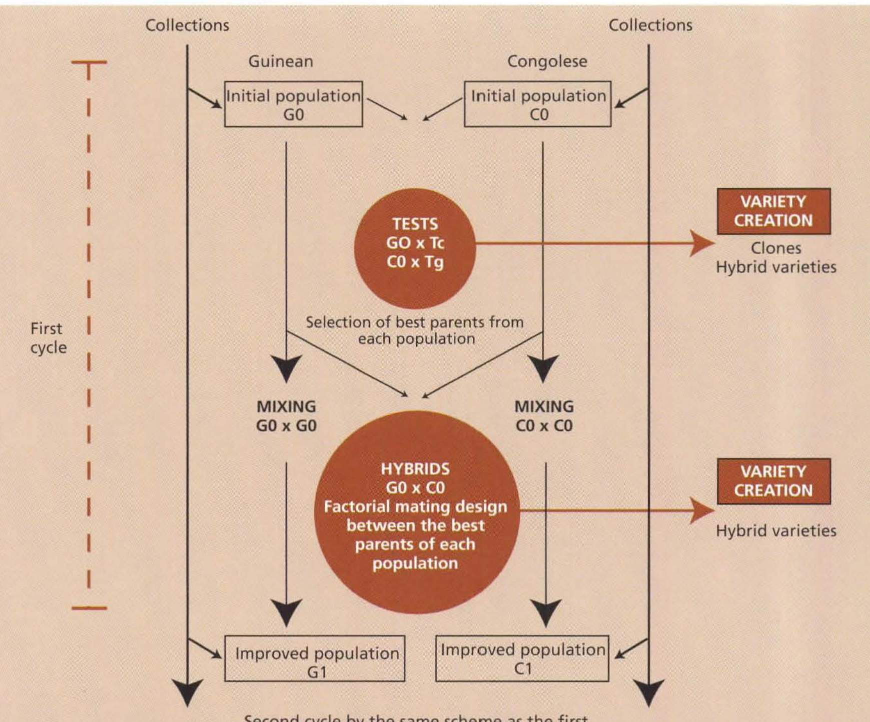
Hybrids as high-yielding and as uniform as clones

The first RRS cycle is now finished. The second cycle began in 1998. The highlights of the first cycle were presented in a thesis.

Following a comparison of the best parents from each population in a complete factorial design, it turned out that the production of the best between-group hybrids from the first selection cycle (RRS hybrids) was equivalent, on average, to 85% of that of the best clonal control currently disseminated (clone 461). Among this set of elite hybrids, the highest yielders exceeded the clonal control, reaching 120% of its production. The potential of hybrids has therefore been virtually doubled in less than three decades (table).

These hybrids give very satisfactory average yields, at around 2.5 to 3 tonnes of merchantable coffee per hectare per year in absolute values—on-station potential—and reveal surprising genetic homogeneity. Their coefficients of variation for yield vary between 25 and 40%, corresponding to the coefficients of variation observed for clones. Moreover, in view of such remarkable homogeneity any attempt to select exceptional trees from these hybrids in order to identify clones that are better than the hybrid mean is difficult.

The good performance of the hybrids does not involve yield alone. Some of the



Each base population consists of around a hundred parents. Each parent in each population is crossed with one or two constant testers from the reciprocal population. Thus, with two testers 400 progenies are obtained (200 per population). The progenies are compared with each other in a field trial. The best progenies reveal the identity of the best parents tested, i.e. ten or so per population. These are first crossed with each other (within-population mixing) in order to create improved populations which will be used as the base for the following cycle. They are then compared in a between-population factorial mating design, making it possible to identify hybrid combinations suitable for distribution to growers.

Figure. Reciprocal recurrent selection programme applied to *Coffea canephora* in Côte d'Ivoire (according to Leroy, 1993).

most spectacular genetic progress obtained for these new varieties has been for their compact and bushy architecture (photo). This architecture offers several advantages: easier harvesting, better ground cover limiting weed development, and increase in the harvest index (ratio between production and vegetative development), possible suitability for modernization of cultural techniques (increase in density combined or not with topping).

Bean size is acceptable (between 13 and 14 g for 100 beans with a 12% moisture content) and these varieties have leaf rust tolerance. Chemical and organoleptic analyses under way reveal quality that satisfies Robusta market expectations.

Conclusion

C. canephora varietal improvement has made a remarkable quality leap with the creation of hybrids that perform just as well as clones. This progress is down to active collaboration between CNRA and CIRAD.

Seed gardens are already being set up in Côte d'Ivoire and seed production for this new generation of hybrids can begin in the near future.

Proposing these hybrids to growers in seed form could "reconcile" them with improved varieties, from which many had turned away, discouraged by the problems encountered with obtaining and rearing clone cuttings in the nursery. ■

Photo. Four-year-old *Coffea canephora* hybrid variety.

C. Montagnon

Table. Production potential of clonal and hybrid varieties before and after introduction of the reciprocal recurrent selection scheme (RRS).

	Clonal varieties	Hybrid varieties
Before RRS (1970s)	100	65
After RRS (2000 and beyond)	120	120

Production potential, base 100, for clonal varieties before reciprocal recurrent selection.

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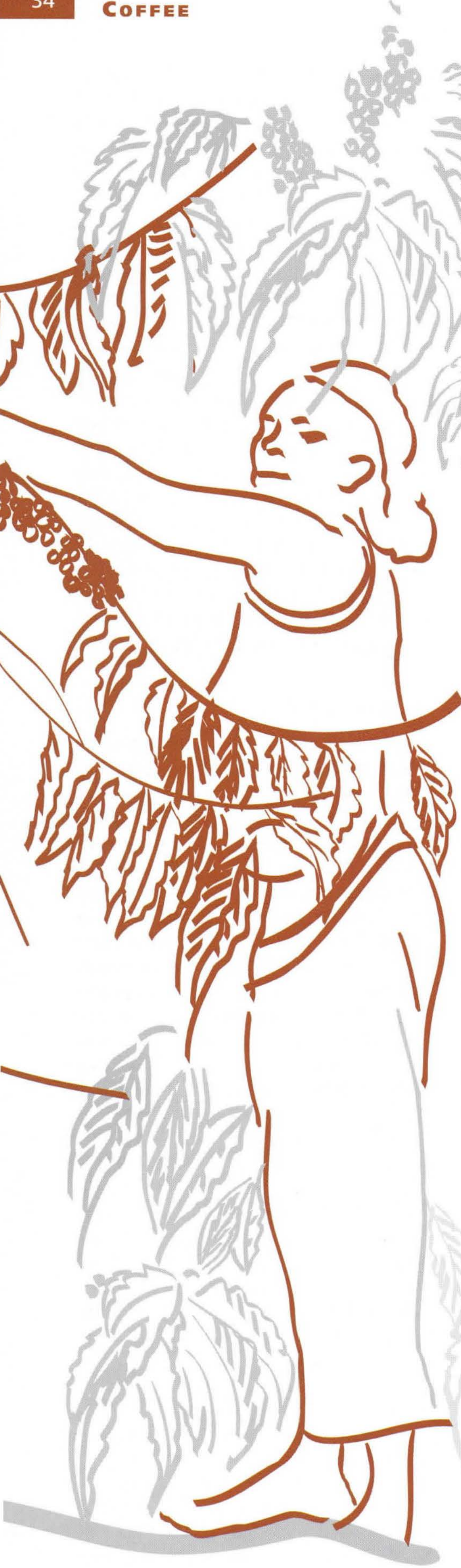

Rational and optimized control of coffee leaf rust in Honduras

The research described below was conducted under cooperation between CIRAD and IICA-PROMECAFE in Honduras, with the assistance of the Honduran coffee institute (IHCAFE).

A decline can currently be seen in the economic viability and ecology of Central American coffee cultivation. In such a context, it is essential to reduce inputs and production costs. In crop protection terms, a reduction in production costs means more effective management of the main fungal disease on Arabica coffee in the region, leaf rust caused by *Hemileia vastatrix*, against which control methods are still highly stereotyped. However, rational and optimized control of leaf rust requires better knowledge of the disease and of factors propitious to its development.

Coffee leaf rust survey-diagnosis

We conducted an integrated study, based on a survey, of all the physical (climate and soil), phytotechnical (crop management sequences and plantation structures) and biological (production variables and leaf mass) factors propitious to the disease in six coffee growing regions of Honduras. The survey covered 73 plots. Observations were carried out over three consecutive years in 25 of the plots, over two years in 10 of the plots and over just one year in the remaining 38 plots. Different types of multivariate analyses revealed that local characteristics, specific to each plantation, mainly determined the development of coffee leaf rust epidemics. Regional factors such as rainfall ranked second. The local factors linked to epidemic development were primarily the production and leaf mass characteristics of the coffee trees, nutritional characteristics, such as soil acidity and fertilization, along with the height above sea level and percentage of shading. The production and leaf mass of the coffee trees were positively linked to epidemic development. Conversely, soil pH and fertilization were negatively associated to epidemic development. Fertilization was a variable that had never been included so far in prediction models for this disease. The same applied for shading which, whilst not limiting production, probably affects the microclimate in such a way that leaf rust incidence increases. Lastly, height above sea level played a substantial role in restricting epidemic development.

A decision support tool

A segmentation analysis of all the variables mentioned above resulted in a leaf rust management tool adapted to conditions in Honduras. It is the first management tool for this disease to be proposed in the Central American region. It can be used to measure the predisposition of a given plot to the development of a leaf rust epidemic, depending on certain plantation characteristics (table) and to suggest to producers what action to take in line with the epidemic risks incurred. In other words, this tool defines recommendation domains. The control measures to be recommended will be all the more stringent, the higher the risk probability. This thereby helps to reduce production costs and protect the environment in certain cases with a low epidemic risk.

Table. The risks of coffee leaf rust epidemics in Honduras.

Plantation characteristics	Epidemic risk
Fewer than 230 fruiting nodes per coffee tree	Low
At least 230 fruiting nodes per coffee tree Fertilized plot Height above sea level over 1,100 m	Low
At least 230 fruiting nodes per coffee tree Fertilized plot Height above sea level under 1,100 m Soil pH over 6	Low
At least 230 fruiting nodes per coffee tree Unfertilized plot Under 56% shade	Average
At least 230 fruiting nodes per coffee tree Fertilized plot Height above sea level under 1,100 m Soil pH under 6 Mean leaf mass under 7.6 young leaves per branch	Average
At least 230 fruiting nodes per coffee tree Fertilized plot Height above sea level under 1,100 m Soil pH under 6 Mean leaf mass over 7.6 young leaves per branch Mean fruit crop under 1.6 fruits per young leaf	Average
At least 230 fruiting nodes per coffee tree Unfertilized plot Under 56% shade	High
At least 230 fruiting nodes per coffee tree Fertilized plot Height above sea level under 1,100 m Soil pH under 6 Mean leaf mass over 7.6 young leaves per branch Mean fruit crop over 1.6 fruits per young leaf	High

Effects of fertilization and of soil acidity on coffee leaf rust

An additional factorial experiment confirmed the effects of fertilization and soil acidity on leaf rust levels; these two factors figure in the definition of recommendation domains (table). In the short term, and this is a new fact, nitrogen fertilization only, based on urea, causes early disappearance of leaves affected by coffee leaf rust, which may explain the negative effect of fertilization on epidemic development. This type of fertilization is probably inappropriate, as no effect has been seen on yields, but it is widely practised. In addition, soil acidity is clearly and positively associated with disease development, so long as the life span of the leaves is sufficient. However, in the long term, extreme soil acidity becomes detrimental to coffee tree development and is antagonistic to epidemic development. In addition, adequate compound fertilization (NPKMgS), leading to an increase in the life span of leaves and higher yields, also leads to an increase in disease levels.

Effects of intensifying coffee cultivation on leaf rust

The results of the survey, and of this experimental work, provide an understanding of how intensifying coffee cultivation affects leaf rust epidemics. The intensification of coffee cultivation primarily involves reducing shading and resorting to fertilization. In the short term, i.e. probably on the scale of a production year, fertilization and shade reduction have negative effects on leaf rust development. However, in the medium term (probably less than five years), these practices cause an increase in leaf mass and yields, which are factors that increase the risks of leaf rust epidemics. The effects of increasing yields and leaf mass on leaf rust seem to increase in line with soil acidity. Indeed, carrying out fertilization and shade reduction contributes to soil acidification, which consequently favours disease development. In the long term (more than five years), it is to be feared that the cumulated effects of soil acidification and leaf rust attacks will lead to irreversible low yields. Leaf rust would therefore play a part in the decline of intensified coffee cultivation. To our knowledge, this study is one of the rare illustrations of the link between agricultural intensification and an increase in epidemic risks for a perennial plant.

A control method

After defining the recommendation domains making it possible to decide on whether or not to take control measures against leaf rust, emphasis was placed on the control method to be applied. Leaf rust control in Central America relies on the use of copper-based fungicides, which have recognized long-term pollutant effects. This sole means of control is also intended to protect leaves from an exogenous or secondary inoculum. The existence of an endogenous inoculum at the beginning of the rainy season on old coffee tree leaves, a residue from the previous year's epidemic, is therefore not taken into account in the current control strategy.

A factorial trial, in which old leaves were removed by hand before the beginning of the rainy season, showed that this residual inoculum was responsible for initial development of the epidemic. It also showed that using a curative fungicide on the first spraying reached primary infections and therefore slowed down the development of the epidemic. The number of cuprous applications required to control the epidemic could thereby be reduced. This strategy seems all the more important in that spraying fungicides increases the quantity of residual inoculum, subsequently leading to early epidemics in the case of an inappropriate control programme. Sometimes, such programmes lead to disease levels at the end of the campaign that are no better than those obtained if no control measures are taken.

Conclusion

The outcome of the research conducted in Honduras was a decision support tool for coffee leaf rust control, and a new, less polluting control strategy based on the control of primary infections. It should therefore help in improving the profitability and sustainability of Central American coffee plantations. ■

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Coffee berry borer trapping

A project to study trapping of the coffee berry borer *Hypothenemus hampei* Ferr. was launched in 1997, after an examination of the preliminary results obtained at the start of that year. For 1998, the CIRAD Tree Crops Department gave priority to trapping studies in its research on the coffee berry borer (CBB). At the same time, the Salvadorean foundation PROCAFE included this topic in the research programme of its crop protection department. Collaboration with INRA and CALLIOPE-NPP led to scientific and technical exchanges, though without resulting in a true partnership.

In 1999, Salvadorean coffee growers exerted considerable pressure for a trapping method to be developed. The scientific contribution was insufficient to enable studies to be made of olfactory attraction. It was thus decided to examine the parameters required for creating a specific highly efficient trap for CBB control. The attractant to be used in the trap was a mixture of allelochemicals that had been amply tested in earlier studies.

Objectives

The main aim of the project was to develop a mass trapping method that captured the majority of CBB populations migrating from fruits left behind after the harvest, i.e. fruits that had fallen to the ground and those not harvested. The idea was therefore to significantly reduce these populations that were destined to infest the new crop of fruits.

Of the secondary objectives, studies of the actual trap are worth mentioning, particularly the physical parameters that determined catch levels, and the study of active elements in the trap, forming the attractant mixture, the dispenser and the diffusion rate. Also of importance is the testing of mass trapping efficiency under natural conditions, along with studies on the different aspects of CBB migration-capture during the post-harvest period, along with the effect of trapping on the ecological balance of coffee plantations and on biodiversity.

Results

The trap

The initial principle of the trap, which consisted of a capture recipient, the dispenser and the capture liquid was kept (photos 1 et 2). The only changes made were to the shape, volume and colour, in order to emphasize the determinant parameters. Firstly, a comparison of five models, three of which were experimental and two commercially available, revealed the importance of size, and the positioning of the trap openings. For instance, CBB enter the trap more quickly if the openings are large and easily accessible. The commercial traps tested, such as Multipher® A and B, which were of a different design, proved to be totally unsuitable. Secondly, the size of the openings was confirmed using new experimental single-aperture models, in the shape of a large-diameter, inverted cone, prototypes A and B. These models also ensured better attractant diffusion and showed that the position of the aperture, in a horizontal plane, made it easier for the insect to land on the trap.

Visual attraction of the CBB, which has been studied by several authors, had always been carried out in the laboratory. In El Salvador, the tests were carried out in coffee plantings, with coloured traps, which revealed that CCB are very strongly attracted to red, with a wavelength of $\lambda = 750$ nm.

B. Dufour

Photo 1.
Initial trap model (1B).

B. Dufour

Photo 2.
Prototype B of the trap, precursor of the Brocap® trap.

Another factor was involved in the size of catches: trap height. The results obtained with traps diffusing at a height of 1.20 m from the ground were three times better than with those diffusing at 0.40 m.

Active trap elements

The attractants comprised an alcohol mixture with different terpenes identified by Mathieu (1998) during chemical analyses on coffee cherries. In a binary or compound mixture, and at low concentrations, terpenes in fact gave results that were similar to or not as good as those obtained with the alcohol mixture alone. The synergy effect of the terpenes seen in earlier trials was not reproduced, despite setting up ten or more comparative trials, at different times and at different sites. Several hypotheses can be put forward to explain the results obtained with terpenes: the attractiveness depends on the particular physiological state of the insect, exceptional ecological conditions, or the effect of chance. The allelochemical attraction of CBB needs to be studied more closely to prove whether terpenes are worthwhile.

Evaporation is the simplest way of diffusing alcohol mixtures. The diffusion rates for the mixture, from 0.12 to 0.35 g/day, in no way changed the capture levels. The Picodrop® system for diffusion of more complex mixtures proved unusable due to trap design. However, although a ceramic wick dispenser did not notably improve diffusion of the alcohol mixture, it could be used for complex mixtures.

Evaluation of mass trapping efficacy under natural conditions

Large catches obtained during migrations of colonizing CBB females did not necessarily mean that trapping significantly reduced infestation on new fruit crops. It was therefore important to carry out an initial study of trapping efficiency and thus assess the potential of the method. Trials were launched in March 1998, just before the first significant migration, and continued up to mid-June, when flights became insignificant. The experimental design was a systematic layout of 16 type 2A traps per manzana (0.7 hectares), i.e. the optimum number defined in the very first trapping tests. It consisted of two treatments with different attractant mixtures and a control.

During trapping, catches occurred in peaks reflecting flight intensity. On the whole, the number of CBB captured only amounted to a small proportion of the initial residual populations. However, efficacy measured in terms of reduced infestation reached 34.8% with the alcohol mixture alone, and 50.7% with the alcohol mixture enriched with terpenes. It needs to be said that the sharp increase in catches obtained with the second mixture did not last.

More recently, a replicate of the efficacy study was carried out with the Brocap® trap and the basic alcohol mixture; it improved the previous results, increasing efficacy to 57%. A trapping effect therefore does exist. It needs to be confirmed by validations currently being carried out.

Study of different aspects of CBB migration-capture during the post-harvest period

This trial was set up in the same plots as those used for mass trapping efficacy. It showed that rainfall played a decisive role in triggering migrations, and therefore in the occurrence of capture peaks. An increase in temperature within coffee plantings also contributes towards CBB activity, encouraging flight. The most intense activity during the day was seen in the afternoon, when warming of the coffee planting reached maximum.

Capture peaks systematically corresponded to the quantities of colonizing females found in the residual fruits, mainly those lying on the ground. Captures of CBB from residual fruits still on the trees did not follow the same rule: migrations were incessantly masked by the comings and goings of CBB of various origins. These fruits were more or less a refuge for migrating CBB that had yet to find a suitable host.

Hence, in order to achieve maximum catches, trapping needs to begin before the first rainfall. Flight intensity can be estimated beforehand from data obtained on the CBB populations, primarily those in residual fruits on the ground.

Examination of the merits of CBB trapping in terms of biodiversity preservation

The attractant used for CBB trapping attracts few other insects apart from the coffee berry borer. Of 68 species identified during the capture period, fewer than ten or so were attracted by the trap, and primarily by the existence of water (capture liquid). *Chrysopa* sp. was the only useful species captured, but in insubstantial numbers.

Trapping is therefore a selective capture method with a good degree of specificity.

Conclusion

Given the results obtained on trapping procedures, the physical characteristics of the trap, its efficacy and its relative neutrality as far as other insects are concerned, studies can now be continued, primarily to develop a trap that is perfectly adapted to CBB capture.

The prototypes that revealed their trapping efficacy have led on to the first specific CBB trap, registered under the Brocap® brand. A preliminary series was manufactured industrially, in order to have a sufficient number of units for tests. The trap was then validated under natural conditions in 15 coffee plantations in El Salvador, to confirm its efficacy. With the results of these trials it should now be possible to launch the trap on the market.

At the same time as this validation operation, other tests have been set up to gain a clearer idea of how the Brocap® trap performs under true operating conditions: number of units per hectare, CBB capture rates, prevention of accidental plugging of the traps, etc.

Other improvements will follow, primarily the development of a more specific and more powerful attractant. ■

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Coconut commmodity chain: economic context and research overview

Recent trends in the coconut commodity chain context

The coconut palm, which is cultivated on eleven million hectares in more than 60 countries, is a smallholder crop. Whilst it is still geared towards copra, which continues to be its main commercial product, coconut cultivation also involves a marked food crop aspect: many nuts are consumed in production zones. Although it has become a minority element in world trade, copra has maintained its market through its lauric specificity. As for all tropical products, world prices fluctuate considerably, but this trend is even greater for copra due to highly variable yields.

A minor, but rapidly increasing share of production is processed fresh. Indeed, national commodity chains are diversifying and devoting part of their processing capacity to new higher value products, such as desiccated coconut or different coconut-based products. In importing countries, the agrifood industry is increasingly seeking new flavours, creating demand for these fresh coconut-based products.

In 1998-1999, production in the coconut commodity chain was completely disrupted, with considerable repercussions for world prices. Production was severely affected by drought due to El Niño, reaching a record low at the beginning of 1998 and keeping copra prices high. With the resumption of normal rainfall in 1998, production returned to a good level in 1999, triggering an immediate drop in prices. Prices fell to half their value at the beginning of 1999 and the trend continued in 2000, fuelled by record non-lauric oilseed production. In other respects, the recent arrival of Lethal Yellowing in Mozambique and Central America, spreading at lightning speed in some zones, have made this disease a major constraint in Africa and Latin America.

Highlights

The last three years have been very important for the Coconut Programme, in scientific terms and as regards partnership developments.

Efforts were concentrated on strengthening Coconut Programme ecophysiology skills, incorporating numerous results acquired over the years in a functional model, and developing innovative methodological tools such as sap flow measurement. This was made possible through the excellent collaboration established between Coconut Programme ecophysiologists and those from the CIRAD AMAP Programme, but also through the "model plant" nature of the coconut palm due to its very simple architecture. There is strong demand from our partners for these scientific skills in two fields: the development of yield forecasting models that can be broken down into prediction tools (production management, local or national production forecasts, etc), and the estimation of carbon flows around a coconut planting (development of tools to estimate carbon sequestration by a coconut stand).

A "farming systems" approach has been adopted in Vanuatu, where the Optimization of Coconut-based Farming Systems project is taking a participatory approach with a view to developing high-yielding coconut-based farming systems that truly meet the expectations of producers.

Research on the olfactory trapping of coconut insect pests (INCO project being implemented in collaboration with INRA) has made very significant progress, notably with the identification of synergistic substances of plant origin, and dissemination of the results obtained in a very large number of international publications and conference papers.

Collaboration with the COGENT-IPGRI network (Coconut Genetic Resources Network, under the authority of the International Plant Genetic Resources Institute), which was launched 8 years ago, has been stepped up and extended. This partnership has resulted in: the development of dedicated software packages for defining and monitoring experimental protocols, and for processing and managing the data acquired; definition of a germplasm collection strategy in zones yet to be explored; contribution to the participatory breeding programme in the Pacific—definition and description by smallholders themselves of the types of worthwhile coconut palms they are seeking to preserve in their villages; development of molecular markers for the characterization of coconut genetic resources.

Lastly, the launch of the Coconut Products Diversification initiative led to implementation of the "Technical Support to Women's Processing Activities" component of the AFD project in Ghana, and the drafting of numerous tenders for technology transfer projects involving small and medium-sized processing enterprises.

The Coconut Programme is therefore renewing its scientific expertise, through simultaneous development of new skills in promising fields (biotechnologies, ecophysiology and agrifood sciences) and by taking development requirements more into account (family agriculture, strengthening of commodity chains through the diversification of uses, small-scale processing). This trend, which should lead on to a renewal of the Programme's partnerships, is beginning to bear fruit. For instance, this repositioning has already attracted the interest of the AFD, which has called upon CIRAD's coconut skills to provide scientific and technical support to coconut rehabilitation projects in Africa (a project under way in Ghana, involving 8,000 ha of the 40,000 ha in existence, and a second project being prepared in Mozambique). ■

The Coconut Programme at a glance

20 staff, including 15 researchers
Partner countries : France (Montpellier), Côte d'Ivoire, Papua New Guinea, Vanuatu
Institutions:
France: IRD, INRA
International: IPGRI, EMBRAPA, PCA, VARTC
Networks: COGENT, APCC, BUROTROP
Industries: CIIF Oil Milling Group (Philippines), Ducoco (Brazil), Nagua Agroindustrial (Dominican Republic), RSUP (Indonesia), Danone (France), Madal (Mozambique)
Research topics:
- Improvement of crop competitiveness and appropriate farming systems
- Control of lethal decay diseases
- Support for the diversification of uses
- Genetic diversity

Model for the simulation of coconut growth and production

In order to understand and model the performance of palms under unfavourable trophic conditions, studies were carried out under a thematic research project entitled *Development of a physiological model integrating the architectural dimension of plants to simulate coconut growth and production*, in partnership with the Vanuatu Agricultural Research and Training Centre and the Philippine Coconut Authority's Davao Centre.

Methods

Various ablation-pruning treatments were applied (figure 1):

- ablation of bunches to limit carbon sinks and simulate optimum trophic conditions,
- frond pruning to limit carbon sources and simulate unfavourable trophic conditions,
- removal of part of the root system to assess the effect of edaphic limitation or trauma on root development.

These experiments were carried out at the same time over three years on 10-year-old VRD x VTT hybrids in Vanuatu and on 25-year-old local Talls (Laguna Tall) in the Philippines.

Observations were carried out on the organogenesis and morphogenesis of the palms in response to treatments, and on photosynthesis and hydric functioning on the scale of a frond (measurement of leaf gas exchanges) and of the whole palm (sap flow measurements) depending on climatic conditions.

Three-dimensional mock-ups of coconut palms were created as a support for simulating frond lighting and carbon assimilation.

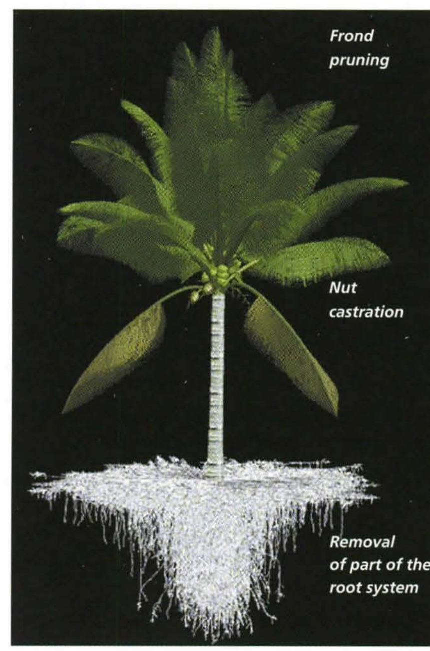


Figure 1. Treatments applied to the coconut palms.

All the results obtained served as a database making it possible to validate a simulated carbon balance based on observed growth results, and to analyse the allocation of resources for growth and production under different conditions.

Coconut palm architecture and light interception

The architecture of the palms was described in detail at the two trial sites, and was used to construct realistic three-dimensional digital mock-ups of the palms.

The digital mock-ups were positioned to reconstitute a plot in a coconut plantation and were used to simulate radiative transfers according to a MIR-MUSC model developed in earlier projects, particularly the STD3 project on *Functioning and evaluation of coconut-based intercropping systems*. The radiative simulations were validated using *in situ* radiation measurements carried out under coconut.

Changes made to the MIR-MUSC model during this project made it possible to integrate simulation of the photosynthetically active radiation (PAR) balance on a frond scale (figure 2a) and use of that balance for photosynthesis (figure 2b) and transpiration simulation, in the same software chain.

Photosynthesis and transpiration

An extensive campaign of gas exchange measurements was undertaken on fronds in Vanuatu to determine the response of leaf carbon assimilation to PAR, ambient CO_2 and temperature. These data were used to parameterize a Farquhar type model. The experimental results did not reveal any seasonal effect or frond rank effect on assimilation. Single model parameterization was adopted.

At the same time, stomatal conductance data (g_s) were used to establish a Jarvis type stomatal model integrating the effects of PAR, VPD (vapour pressure deficit) and temperature. Despite the low predictive value of the model, its integration within the assimilation model did not induce any major bias (figure 3).

Carbon assimilation was calculated on digital mock-ups (figure 2b) from simulations of frond lighting and the combined assimilation and stomatal conductance models (figure 2a). These assimilation calculations were carried out over a short time lapse of an hour or less, then integrated on a daily scale, in order to avoid bias linked to use of the mean lighting value.

Removing part of the root system did not induce any measurable drop in stomatal conductance. However, pruning lower fronds led to maintenance of stomatal conductance, and a higher level of photosynthesis than in the control during the dry season. This effect, which could be explained by the reduction in leaf areas consuming water resources, needs to be elucidated. The so-called Granier sap flow measurement method that enables overall measurement of tree transpiration, was adapted to coconut. The main difficulty consisted in evaluating the radial variability of sap conduction in the stem. Long sensors to take measurements at different levels in the stem were specially made for the study. The sap flow rate seemed to be largely constant over the entire radius. This result made it possible to precisely calculate the flow per palm and per day. The measurements taken were used to make an overall analysis of the response of palm transpiration to climatic conditions. They also made it possible to calculate the conductance of the cover, integrating overall variability in stomatal functioning on that scale. Cover conductance could be used directly in a model of plot transpiration depending on climatic conditions.

Palm growth in response to ablation-pruning treatments

The trophic regimes induced by the treatments affected both organogenesis (fronds, bunches, etc.) and growth. For example, it was necessary to take into account a period of 12 months between the initiation and emergence of fronds, then another period of 7 months for ripening. The ablation-pruning trials were therefore continued for three years, to allow the time for the coconut palms to stabilize their reserve compartments and the allocation of reserves per organ.

Aerial growth

For the nut compartment, exponential curves were obtained for the relation between dry matter and circumference in the controls, from rank 9 to rank 15 (young nuts). For the following ranks, the circumference no longer varied, unlike dry matter, which increased steadily, stabilizing from rank 20 onwards. The increase in nut dry matter could therefore be simply estimated from their circumference, their rank, the dry weight of ripe nuts, and the bunch emission rate.

Nut growth potential was attained when the sinks were reduced—limitation of the number of nuts—while leaving sources intact. The maximum potential nut weight observed was higher than normal: 55 cm circumference as opposed to 45 cm for the controls. The daily increase in the nut compart-

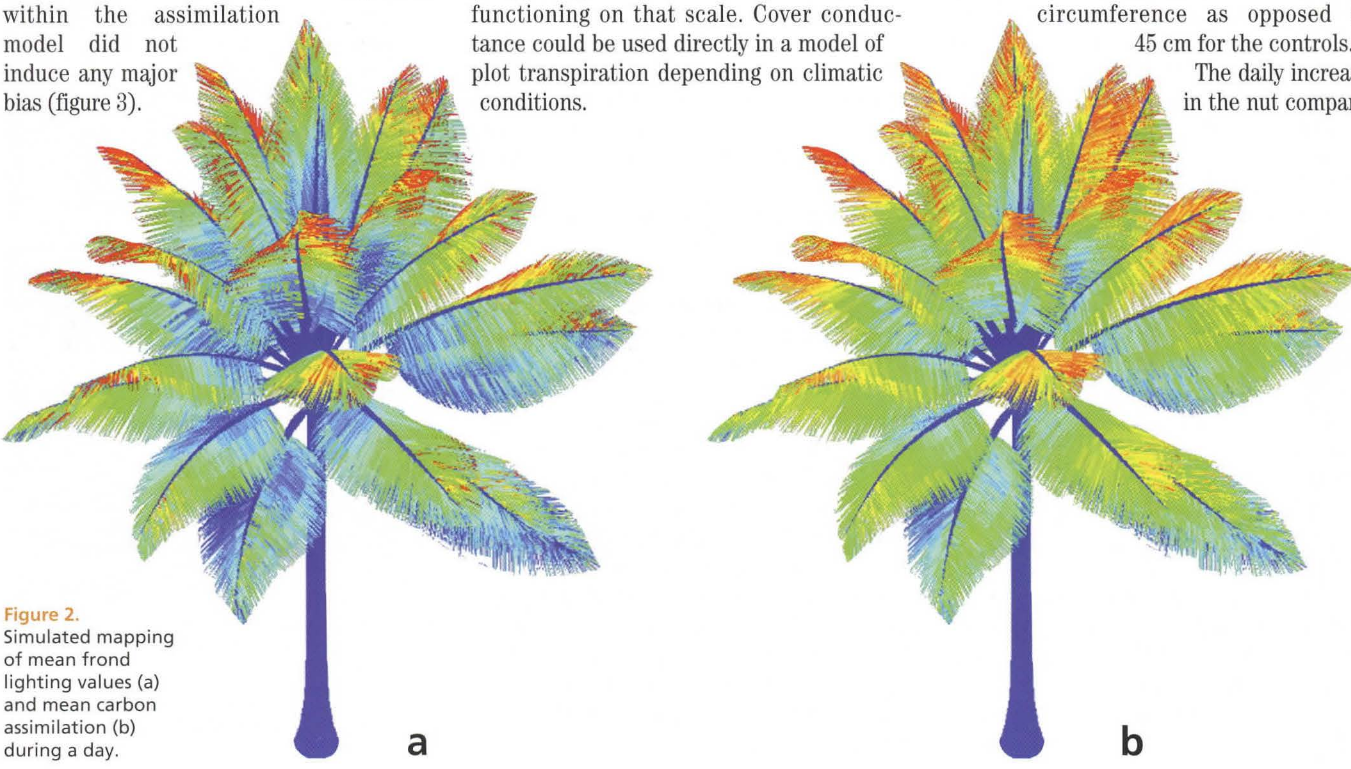


Figure 2.
Simulated mapping of mean frond lighting values (a) and mean carbon assimilation (b) during a day.

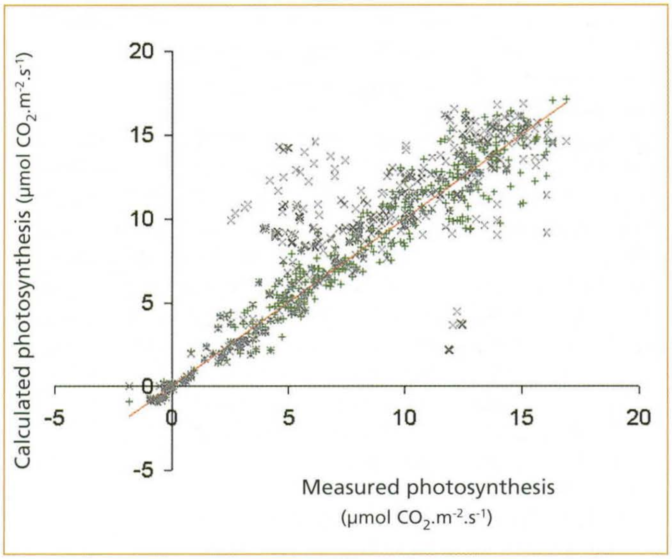


Figure 3. Comparison of measured leaf photosynthesis with that simulated by a Farquhar model. The simulations were carried out with measured (+) or calculated (x) stomatal conductance.

ment was relatively constant in Vanuatu (300 $g.d^{-1}$ of dry matter on average), with moderate fluctuations and two seasonal peaks. On the other hand, in the Philippines, the coconut palms were affected by a major drought caused by El Niño and bunch growth declined substantially (170 $g.d^{-1}$ of dry matter on average).

In terms of male or female flowers, sink suppression led to a substantial and rapid increase—only 4 to 6 months after castration—in the number of flowers. The number of flowers per bunch was proportional to castration intensity, with the largest number of flowers occurring after severe castration.

In additioning, root removal and frond pruning had a depressive effect on the number of flowers.

The frond emission rhythm did not vary much from one treatment to the next, though there was a slight increase in the castrated palms (15.5 $fronds.year^{-1}$ as opposed to 14 $fronds.year^{-1}$ in the controls).

The dry weight of fronds on coconut palms subjected to frond pruning increased steadily over time, as did the number of leaflets per frond. On the other hand, total frond length, and the length of the largest leaflet seemed to decrease somewhat.

Root system growth

The hefty techniques used for root system observations (large excavations, rhizotrons), made it possible to estimate coconut root biomass, along with daily, seasonal and annual growth dynamics. Average total root biomass for a 25-year-old tall coconut palm was 200 kg in the Philippines, whereas it was only 75 kg for a 10-year-old Dwarf x Tall hybrid in Vanuatu. Annual root growth was 47.8 $g.d^{-1}$ and 37.6 $g.d^{-1}$respectively.

Sectioning of the root system, each year for 3 years, in a semicircle 1 m from the palm in the Philippines and 2.5 m from the palm in Vanuatu down to a depth of 1 m, had no significant effect on the growth rate of all root types, irrespective of their distance from the stem, led to a significant increase in root emission at the base of the stem, with uniform radial distribution, and an absence of reiteration at the cut site. The different castration intensities led to a significant increase in root biomass at the foot of the stem for all root types, but did not affect root biomass changes 2.5-m from the stem.

Frond pruning did not have any effect on root biomass or root emission.

Coconut palm growth results

Figure 4 shows the distribution of annual biomass increase in coconut palms in Vanuatu and the Philippines. The importance of the nut compartment (over 50% of total allocation) and the frond compartment (30%) can be seen. Dry matter allocation to the stem and roots was much lower (under 10%). The distribution of coconut standing biomass was not similar. Indeed, the stem (37% of total biomass), frond (28%) and root (17%) compartments accounted for more than two thirds of palm biomass; these compartments must therefore contribute mostly to respiration.

Growth and production modelling

Initially, the general approach consisted in simulating carbon fixation by combining the radiative, stomatal and assimilation models. This simulated carbon fixation, minus respiration costs, was then globally compared, on a palm scale, to the carbon equivalent estimated from the biomass balances obtained from biometric observations. Once the carbon balance had been established on a palm scale, an allocation model was used to generate fixed carbon distribution to the different sink organs.

Assimilate allocation

An original assimilate allocation model was used. The model considered a source compartment (photosynthesis apparatus), sink compartments (bunches, fronds, stem, roots), and a reserve compartment functioning simultaneously as a sink and a source. Allocation from the sources to the sinks was managed by demand from the sinks—depending on the degree to which their demand was met for potential growth —and by the set of resistances reflecting their ability to compete in relation to the sources. The average rate of satisfaction for all the sinks defined the trophic status of the coconut palm, a variable that was used

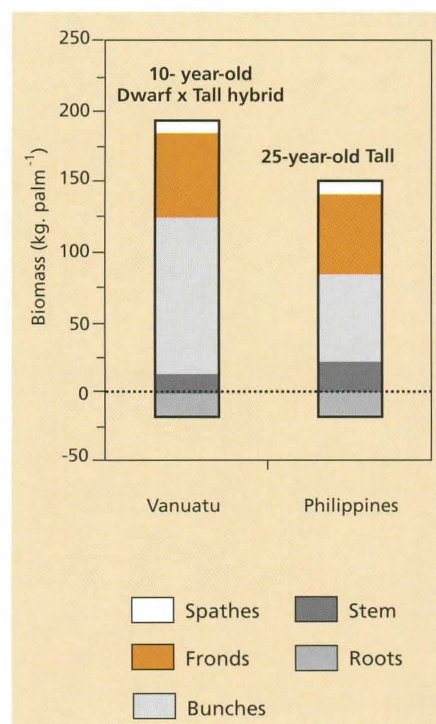


Figure 4. Mean annual allocation of dry matter per coconut palm in Vanuatu and the Philippines.

to establish an "organogenetic" model managing the formation and abortion of vegetative organs.

The allocation model was tested with Model Maker software and used to simulate biomass distribution to the different compartments depending on the treatments applied.

In addition to biomass distribution, the model was used to simulate the buffer effect of reserves when conditions changed. For example, figure 5 illustrates how the model performed when the sources were reduced, in this case by frond pruning.

The allocation model now needs to be integrated into the AMAP software chain and coupled to the organogenetic model.

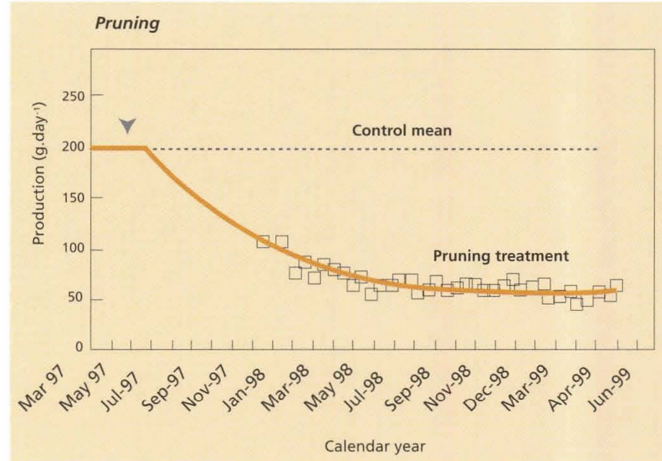
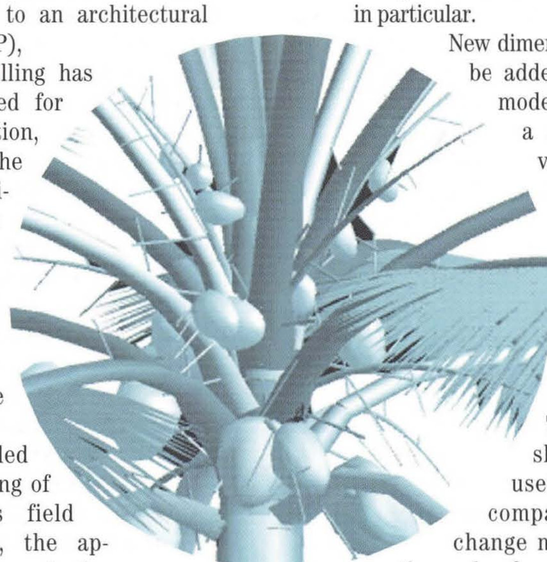


Figure 5. Coconut yields following pruning of simulated (▬) and observed (□), fronds, compared to the average yields of control palms (- - - -).

Conclusions and prospects

Our approach to coconut palm functioning is original in two ways:

- it is the first time that a functioning model including an explicit carbon balance has been coupled to an architectural mock-up (AMAP),
- original modelling has been attempted for carbon allocation, which avoids the need to attribute constant coefficients to the organs, and the rules of priority are modified depending on the organ fill rates.



This study called for the monitoring of fairly laborious field trials. However, the approach was made easier because coconut palm architecture and growth are particularly simple.

The next stage will be to validate our simulated carbon balance using measured growth results. A calibration phase may be necessary. A sensitivity analysis will be carried out.

Once this validation is complete, we shall develop this model further, by optimizing simulated production using planting density in particular.

New dimensions will have to be added to the current model, so as to give it a greater scope of validity: sensitivity to a water deficit in the soil, to fertility, etc.

This work consisted in integrating leaf gas exchanges on a plant scale. This approach should prove very useful in its future comparison with exchange measurements on the scale of a plot or the ecosystem limited to the plantation, in a carbon sequestration and water balance context. This stage could be dealt with using the turbulent fluctuations micrometeorological method currently being applied in Vanuatu.

It will then be possible to construct a new functional model from empirical relations between gas exchanges in the cover and the climate. ■

List of publications

Dauzat J., Eroy M.N., 1997. Simulating light regime and intercrop yields in coconut based farming systems. Eur. J. Agron. 7(1-3) : 63-74.

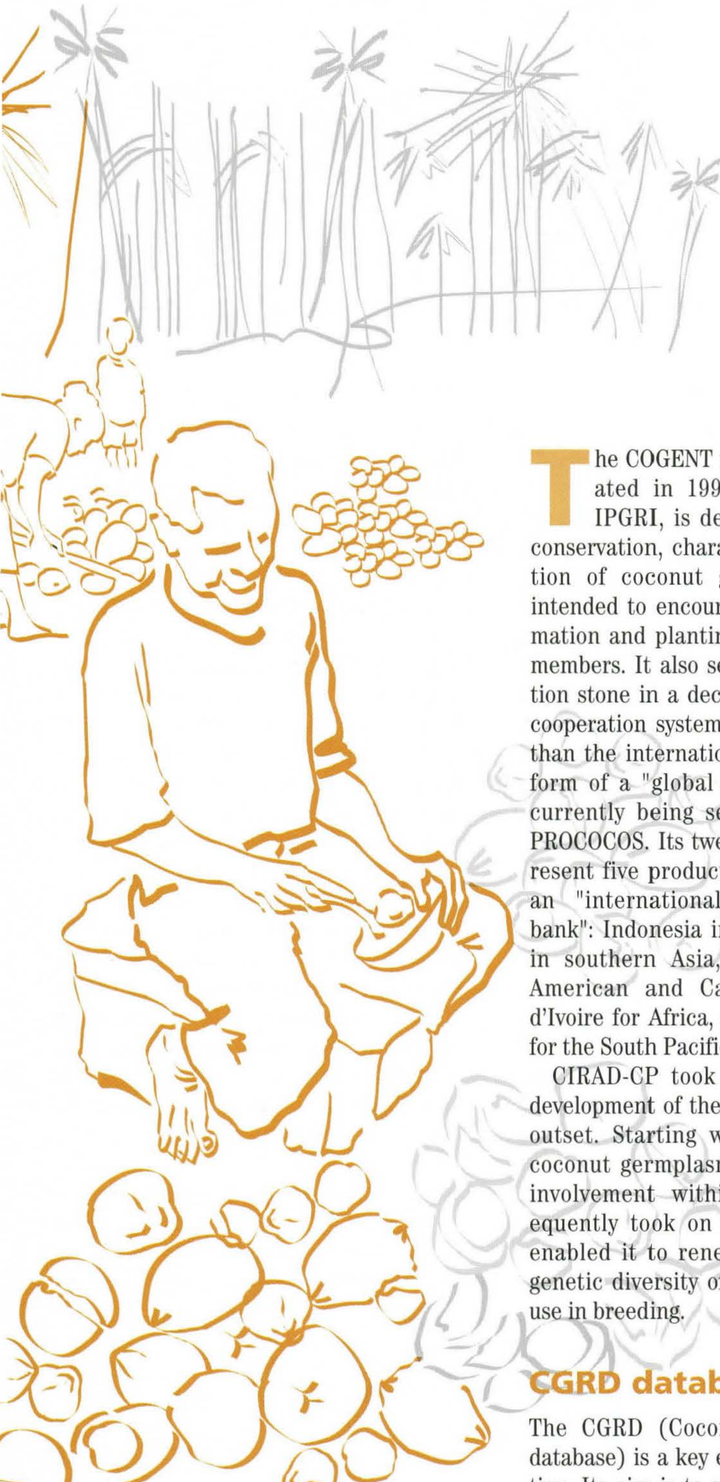
Jourdan C., 1998. Observation du système racinaire du cocotier dans le cadre de l'ATP n°10_96. Rapport de mission au CARFV, 13/03 - 03/06/98. Montpellier, France, CIRAD, document CP SIC n°990, 22 p. (internal document).

Labouisse J.P., Roupsard O., 2000. Coconut research in Vanuatu. *In* : COGENT Newsletter (3) : 7-8.

Mialet-Serra I., Dauzat J., Auclair D., 2001. The use of plant architectural models for estimating radiation transfer within coconut-based multi-strata agroforestry systems. Agr. Syst. (in press).

Quencez P., Dauzat J., Braconnier S., Jourdan C., Margate R., Eroy M., Lamade E., Mialet I., Coffinier J.F., Rey H., 1998. Développement d'un modèle physiologique intégrant la dimension architecturale des plantes pour la simulation de la croissance et de la production du palmier à huile et du cocotier. Compte rendu annuel de l'ATP n°10_96, 2e année, 68 p. (internal document).

Management of coconut genetic resources



The COGENT network, which was created in 1991 on the initiative of IPGRI, is designed to promote the conservation, characterization and utilization of coconut germplasm. It is also intended to encourage exchanges of information and planting material between its members. It also set out to be the foundation stone in a decentralized international cooperation system that was less unwieldy than the international centres, taking the form of a "global programme", which is currently being set up under the name PROCOCOS. Its twenty or so members represent five production regions, each with an "international coconut germplasm bank": Indonesia in Southeast Asia, India in southern Asia, Brazil for the Latin American and Caribbean region, Côte d'Ivoire for Africa, and Papua New Guinea for the South Pacific.

CIRAD-CP took an active role in the development of the network right from the outset. Starting with the creation of a coconut germplasm database, CIRAD-CP involvement within the network subsequently took on many forms. This has enabled it to renew its approach to the genetic diversity of the species and of its use in breeding.

CGRD database

The CGRD (Coconut genetic resources database) is a key element in this cooperation. Its aim is to store and share the data available on coconut cultivars planted at research stations in countries taking part in the COGENT network. These are of two sorts: passport data relative to the identity of the planting material, and characterization and evaluation data concerning agromorphological particularities of the conserved planting material.

This database is devoted to traditional cultivars, or ecotypes. Research material and advanced hybrids are not included. All network members can input their own data, access the data of the other members, and search for the information they need.

The software

The structure of the database is relatively simple: it consists of a directory of conservation sites and a table listing all the populations conserved in the field. Each entry in the database represents a set of palms of the same origin, planted at the same time in the same place.

The software provides all the conventional functions of a database:

- addition, deletion, modification, consultation of information on screen,
- search for accessions meeting selection criteria chosen in the lists,
- edition of various types of reports at the end of the search,
- export of passport and characterization data in DIP format (Data interface protocol, the format used by IPGRI) for insertion into other databases taking that format,
- storage on a medium chosen by the user, and data restoration, in the event of a failure, from a previous back-up.

The software initially functioned with the MS-DOS operating system, but has kept pace with developments in hardware and operating systems. Version 3, which came out at the beginning of January 2000, was entirely re-written in Visual-FoxPro language under Windows. This version offers users new data search possibilities and provides access to a large number of photographs illustrating various aspects of the cultivars studied (figure 1).

Data acquisition and management

The 195 descriptors in the database can be organized in several categories. Passport descriptors contain information relative to the type of planting material and collection conditions. Characterization and evaluation data cover germination, vegetative measurements on the stem and fronds, observations relative to the start of flowering and floral biology, inflorescence and fruit measurements, along with quantitative yield data.

From the beginning of 1998 to the end of 1999, the number of accessions increased from 940 to 1,277 (+36%), reflected also in the number of data items inputted, which increased from 41,500 to 64,800 (i.e. a 55% increase). The Solomon Islands have joined the database, bringing the number of countries to 18 (table 1).

The software allows data updates by participants. However, many of them reach us in various forms, meaning that the data have to be inputted again. In all cases, data coherence is checked. Moreover, CIRAD staff have visited numerous countries to provide training in software use, and to assist collection managers with their data inputting.

The database already provides access to a mass of information on the identity of coconut cultivars in collections worldwide. For many of them, there are also biometric data, making the database a valuable tool for breeders. However, the availability of biometric data varies considerably from one case to another. In some cases, this is normal: they may be recent collections, which have yet to undergo observations, or old collections for which some data may no longer be available. Even so, it is possible to considerably improve the quantity and quality of the data stored in this database.

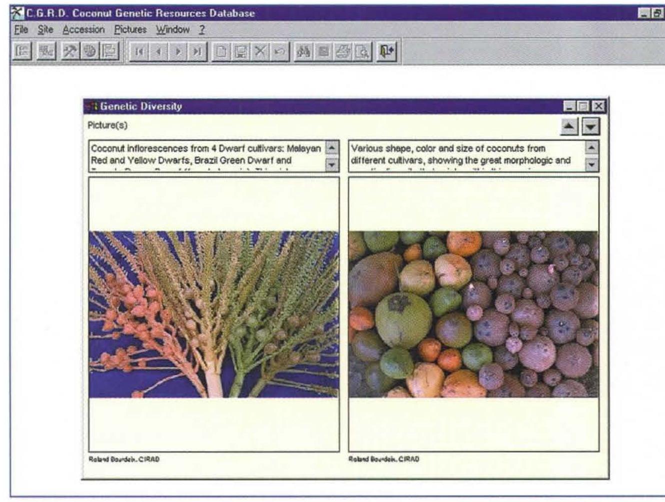


Figure 1. Numerous photograph archives can be consulted with the new version of the CGRD.

Table 1. List of countries contributing to the database. The number of entries for each country is given in brackets.

Africa	Latin America and the Caribbean	Southern Asia	Southeast Asia	South Pacific
Benin (4)	Brasil (16)	India (212)	Indonesia (156)	Papua New Guinea (57)
Côte d'Ivoire (99)	Jamaica (60)	Sri Lanka (78)	Malaysia (99)	Fidji (11)
Tanzania (72)	Mexico (20)		Philippines (224)	Solomon Islands (21)
			Thailand (52)	Samoa (9)
			Vietnam (31)	Vanuatu (66)

Management of data gathered in the field

Whilst actual observations are the responsibility of the managers of each collection, the COGENT network can help in completing this task. For instance, the Stantech manual, drawn up with the collaboration of CIRAD-CP staff, proposes standard procedures for the main operations carried out in coconut breeding. During our support missions, we found that our partners in producing countries were encountering real problems in the recording and long-term storage of field observation data.

This difficulty was linked to the perennial nature of the coconut palm: the assessment of each cultivar requires the individual observation of around ten palms, for at least eight years. The data then have to be summarized before being entered in the CGRD. CIRAD-CP has experience of such situations and in 1995 it proposed the development of a second software package called CDM (Coconut Data Management) to simplify management of these data.

The software was developed in four years, operates on PC under Windows and includes a module for palm mapping in each field, making it possible not only to display the characteristics of palms, but also to modify them interactively. In fact, this module is a geographic information system dedicated to coconut collections (figure 2).

The software manages data relative to experimental protocols and can be used to input observation data, along with their statistical analysis in accordance with specified experimental protocols. Standard statistical analysis modules, used to process the most common cases, have been incorporated into the software, but there is nothing to prevent more sophisticated analyses from being carried out by transferring the necessary information to specialized software. The final version was supplied to network members in the first quarter of 2000.

This software is not only of interest for coconut. The mapping module can be used to display spatial variations, in qualitative or quantitative terms, that are observed for different plants. There are plans to develop it for that purpose. Training national managers in its use is also one of the main priorities.

Collection and conservation of germplasm in Asia and the Pacific

In Asia and the Pacific, some of the germplasm collection and characterization operations are funded by the ADB through the CGRNAP project (Coconut genetic resources network in Asia-Pacific). CIRAD-CP was assigned to assess the progress made in the project.

This mission covered all the countries in the region. Visits were made to India, Sri Lanka, Thailand, Vietnam, the Philippines,

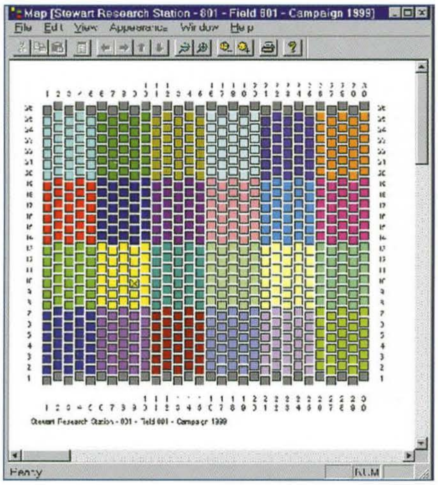


Figure 2.
Experimental factors can easily be displayed in the CDM mapping module.

the Solomon Islands, Papua New Guinea and Vanuatu. Considerable use was made of the CGRD, in particular to locate the geographical origin of the cultivars being conserved and identify the subsampled regions on the map of each country. In return, the data gathered during the mission were used to enhance the database.

In their conclusions, the CIRAD-CP experts approved the strategy developed for germplasm collection—random collection based on a geographical sampling grid—and made proposals for increasing available diversity by gearing collections towards specific regions through scientific exchanges between gene banks. In this context, *in vitro* culture seems to be a prime tool for germplasm collection and exchanges. They also highlighted the need to more effectively guarantee the longevity of certain gene banks.

These experts emphasized the fact that the value of a germplasm collection largely depends on the information gathered on the material involved. This applies as much to documentary and historical data as to on-site observation data. The latter are essential for breeders, even though taking them into account for collection management is tricky: targeted collection is sometimes justified when the traits sought are of obvious economic importance, or correspond to types that are under-represented in the region—e.g. coconut palms with a high husk percentage in the Pacific region. However, as a general rule, random collection more effectively preserves genetic diversity, in theory, even if it does involve the risk of high redundancy in the germplasm collected, and coconut collections are expensive to maintain.

Microsatellite kit for coconut genetic diversity studies

Molecular markers can be used to reduce such redundancy. In fact, they give direct access to the diversity of the genome and do not depend on the environment. They therefore make it possible to maximize the diversity conserved in a given area. CIRAD-CP, IPGRI and the COGENT network are involved in joint research to develop a set of molecular markers for the characterization of coconut cultivars, intended for collection managers. By the end of the project, over 130 populations, represented by around 600 palms, will have been characterized with a dozen highly discriminant microsatellite markers chosen from an initial set of 85 markers. It will then be possible to position populations studied during surveys in relation to those that are already known, and assess their potential usefulness for breeding. Of course, this methodology will also be usable for the management of existing collections. It will be transferred to members of the network and CIRAD-CP will be able to act as an international molecular data repository.

Other activities

CIRAD-CP also carries out numerous international visits in connection with COGENT functioning, both for defining descriptors and observation methods, and for training in software use and assistance to local researchers for data collection. It has also helped to organize several such events (table 2).

This involvement has also included new germplasm collections in the South Pacific (Kiribati, Cook, Marshall, Tuvalu) and support to national research centres in Côte d'Ivoire and Vanuatu, notably for carrying out participatory surveys in Vanuatu. This method involves asking members of a community to describe the types of coconut palms they know, then identify them in the field.

The profusion of cultivars and of their names in the database, led us to draw up a catalogue of traditional coconut cultivars, which currently has more than 300 entries.

Prospects

Further collaboration with the COGENT network

After eight years' involvement in the COGENT network, the achievements described here illustrate CIRAD-CP's contribution to its functioning. It will continue to gather and circulate data acquired within the network. Emphasis will be placed on:

- training researchers how to use CDM for management of their own data and their transfer to the CGRD,
- making customized recommendations for observations, taking planting material age into account,
- improving the CGRD software, for easier use and inputting, notably with the help of photographic documents,
- technical support to national research centres.

The development of coconut molecular markers, and the first genetic diversity studies of this species using such markers are an important step forward made in recent years. Development of the microsatellite kit will be followed by a training project for international coconut gene bank staff. This will make it possible to enter microsatellite marker data in the CGRD.

Repercussions for CIRAD-CP operations

This participation has also enabled CIRAD-CP to renew its approach to coconut genetic diversity and its use in genetic improvement.

From a methodological point of view, development of the microsatellite kit

Table 2. Missions and participation in seminars.

Year	Mission	Location
1998	ADB and IFAD seminars	Manado (Indonesia)
1999	Meeting on molecular markers	Montpellier (France)
1999	ADB appraisal	Thailand, Vietnam, Philippines
1999	ADB appraisal	India, Sri Lanka
1999	ADB appraisal	Solomon Islands
1999	ADB and IFAD seminars	Ho Chi Minh City (Vietnam)

required the development of original identification methods adapted to cross fertilizing populations. These methods will shortly be applied to other plant and animal species, in connection with a thematic research project. The practice of participatory germplasm collection in Vanuatu revealed diversity that had not been noticed in earlier surveys. In order to gain a clearer understanding of how traditional cultural and social practices affect coconut diversity, CIRAD-CP is backing a thesis that will be exploring the genetic, agronomic and sociological aspects of the phenomenon. It should also help breeders to gain a clearer picture of what farmers expect from their planting material. ■

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BAUDOUIN L., BONNOT F., BOURDEIX R., HAMELIN C., LABOUISSE J.P., ROUZIERE A., 2000. Rundown of CIRAD activities in connection with the CGRD database. Montpellier, France, CIRAD-CP, 47 p. (internal document).

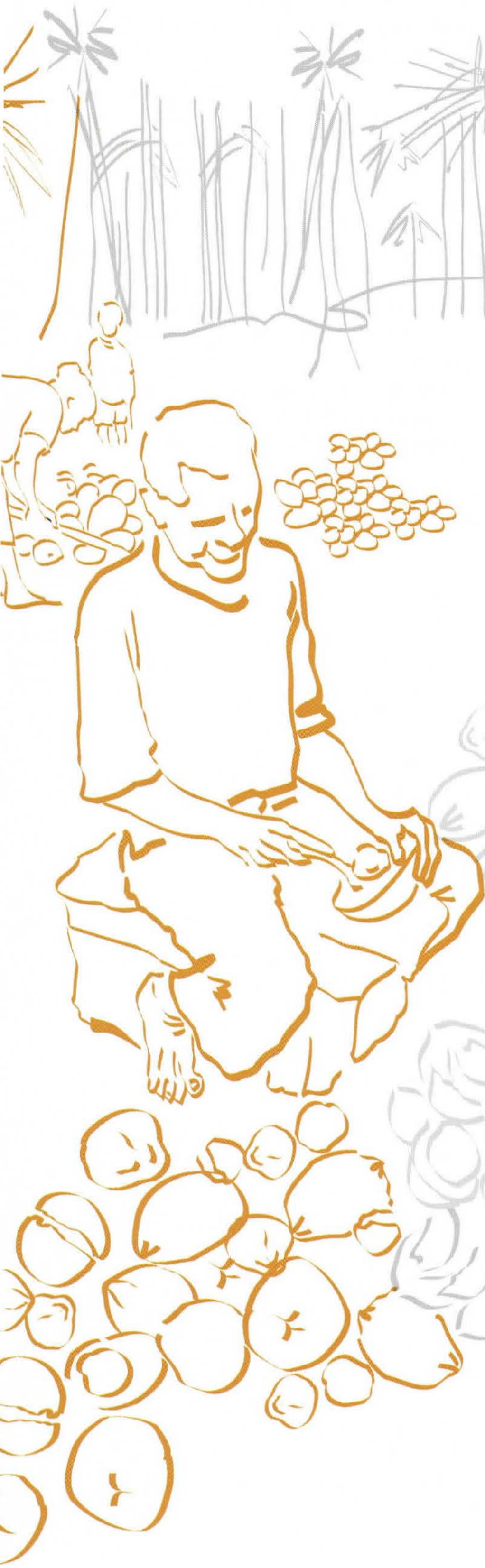
BAUDOUIN L., BOURDEIX R., BONNOT F., HAMELIN C., ROUZIERE A., 2000. COGENT establishes an international coconut genetic resources database (CGRD). COGENT Newsletter (3) : 1.

BAUDOUIN L., LEBRUN P., 2001. An operational bayesian approach for the identification of sexually reproduced cross-fertilized populations using molecular markers. *In :* Proceedings of the International symposium on molecular markers for characterizing genotypes and identifying cultivars in horticulture, Montpellier, France, 6-8 March 2000, p. 81-93.

BOURDEIX R., BAUDOUIN L., BONNOT F., HAMELIN C., 1998. The international coconut genetic resources database: rationale, progress, constraints and plans for the short and long term. *In :* ADB-COGENT meeting, 28-30 Oct. 1998, Kuala Lumpur, Malaysia, 9 p.

BOURDEIX R., BAUDOUIN L., OLLIVIER J., LABOUISSE J.P., 1999. Consultancy report on coconut collecting strategies: ADB-CGMAP report. Montpellier, France, CIRAD-CP, 110 p. (internal document).

Molecular markers: recent progress, and soon an identification kit



Since COGENT was set up in 1992, CIRAD-CP has been providing its scientific and methodological support to this network. Following the development of standard tools for characterizing the accessions collected (Stantech manual, CGRD genetic database and the CDM field data processing software), the Coconut Programme turned its attention to two aspects of coconut molecular genetics: genetic diversity and genome mapping. The genetic diversity study is currently the main aspect, with various objectives ranging from structuring a collection of coconut palms in uniform genetic groups, to cultivar identification. Then, with genome mapping, it should be possible to identify areas of the genome involved in the variation of traits such as yield, resistance to diseases or adaptation to particular climatic conditions. Such identification is the first stage in the move towards marker-assisted selection, and towards genetic modification, which should make it possible to enrich a cultivar with desirable traits.

Genetic markers used

The first molecular markers used on coconut to study diversity were RFLP, with hybridized probes usually from other plants, such as maize, rice or oil palm. This technique gave reliable and reproducible results, which made it possible to gain an initial picture of the species' diversity. Despite these qualities, the laboriousness and slowness of the operations led to preference being given to new types of markers that were easier to use in producing countries.

Although microsatellite markers are expensive to develop, they are reproducible and simple to use. They are abundant, and have numerous codominant alleles well distributed on the genome. Their only major disadvantage lies in the cost of obtaining them, though the cost of using them remains competitive. A clone-enriched library containing the motif GA (guanine-adenine) has been produced. It was obtained by the magnetic bead capture method and represents around 800 clones, 80% of which contain a microsatellite motif. After sequencing part of the 140 positive clones, then elimination of sequences unsuitable for defining primers (microsatellite motif too close to the edge of the insert, redundant clone, impossible to define a primer in the sequence flanking the microsatellite) around a hundred clones are now available. Their readability, reproducibility, and their degree of polymorphism (between 2 and 17 alleles per marker), were checked using a sample of 31 palms representing coconut diversity (figure 1).

The oil palm is another source of microsatellite markers since a third of them are usable on the coconut palm.

Although their polymorphism is relatively low in coconut, AFLP markers provide further information that is useful for mapping. The difficulty in reading them, and problematic reproducibility, led to their being ruled out for diversity studies.

Genetic mapping of the coconut palm

In order to produce the genetic map of a species, the coefficient of recombination is calculated between the different markers studied on the progeny of a cross. The lower the coefficient, the more the corresponding loci are genetically linked. This calculation can only be carried out if the parents are heterozygotes. The coconut palm is not a particularly prolific species, and crosses generally have few progenies. Appropriate populations are rare. For instance, the only genetic map of the coconut palm currently published by the Neiker team (Spain) uses a cross represented by 52 progenies, carried out especially for that purpose. The observations available on this cross are still very limited.

A new map was undertaken in collaboration with Neiker (Spain) and Long Ashton Research Station (UK) to search for quantitative trait loci (QTL). The chosen population had been planted in a genetic trial for 15 years at the M. Delorme station in Côte d'Ivoire, and therefore benefited from several years of individual observations. It was a cross between a Rennell Tall parent (PO 2664) and 12 Cameroon Red Dwarfs. This latter cultivar is self-fertilizing and can be considered as a pure line. Its uniformity was confirmed by microsatellite markers and RFLP. Only a few low intensity AFLP bands proved to be variable between the different Cameroon Red Dwarfs and were removed from the analysis. In the progeny of the cross, 2 out of 69 individuals revealing alleles that were absent from the parents were considered illegitimate and discarded. The genetic map of the Rennell Tall parent was constructed using heterozygous markers present in that individual and, for the AFLP markers, absent from the Dwarf parents.

The Rennell parent proved to be less heterozygous than expected. Of the 64 primer combinations available in the laboratory (8 Eco R1 and 8 Msel), only 21 varied in the progeny. However, 190 polymorphic markers, 78 of which were obtained at CIRAD (11 microsatellites, 6 RFLP and 61 AFLP), were positioned on 16 linkage groups. Few markers were not assigned to any group. The longest linkage group, LG3, measured 202 centimorgans and contained 22 markers; the shortest, LG 16, only contained 5 markers distributed over 34 centimorgans. Most of the markers were regularly distributed over the linkage groups, apart from a cluster on GL 3 containing 11 markers over 29 centimorgans (figure 2).

The 16 linkage groups identified most probably corresponded to the 16 chromosomes of the coconut palm. The results obtained were an initial stage in obtaining a saturated genetic map. Its construction, along with the QTL search, will be carried out under an INCO project selected by the European Union in 2001.

Coconut genetic diversity

The initial diversity analyses carried out on 26 Tall cultivars (191 individuals) and 16 Dwarf cultivars (98 individuals), using RFLP, revealed that all the coconut palms divided into three genetic groups (figure 3):

- the "Indo-Atlantic" group, which contained 5 cultivars from West Africa, India and Sri Lanka,
- the "Indian Ocean" group, which contained 3 cultivars,
- the "Pacific" group, which contained all the Dwarf cultivars, those of Southeast Asia, Papua New Guinea and the Pacific islands.

An analysis of gene flows between cultivars enabled us to define smaller, uniform units within the large Pacific group. Given the laboriousness of operations with RFLP markers, it was worth finding out whether such results could be obtained with markers that were easier to use, such as microsatellites. The speed with which they evolve is an important difference between the two types of markers. Microsatellites, which evolve much faster than the other types of markers, are very

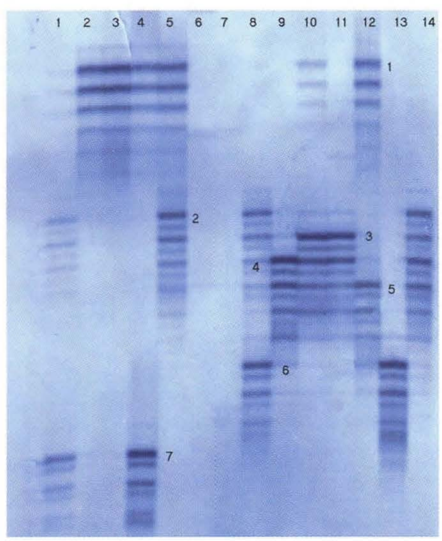


Figure 1.
Microsatellite marker CnCirC5 with 7 alleles.

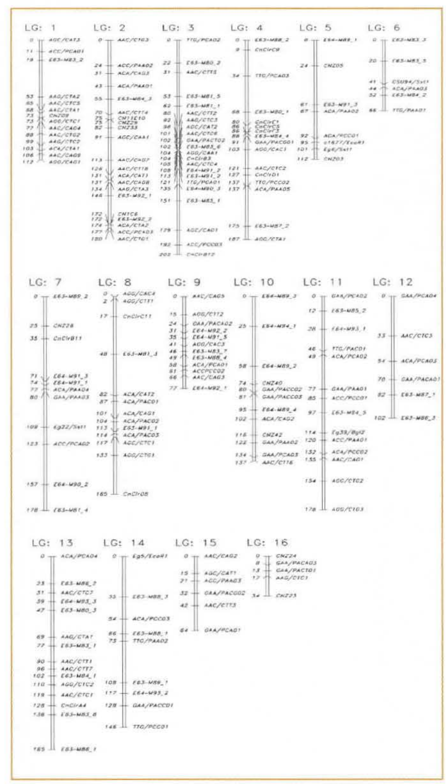


Figure 2.
Beginnings of a coconut genetic map.

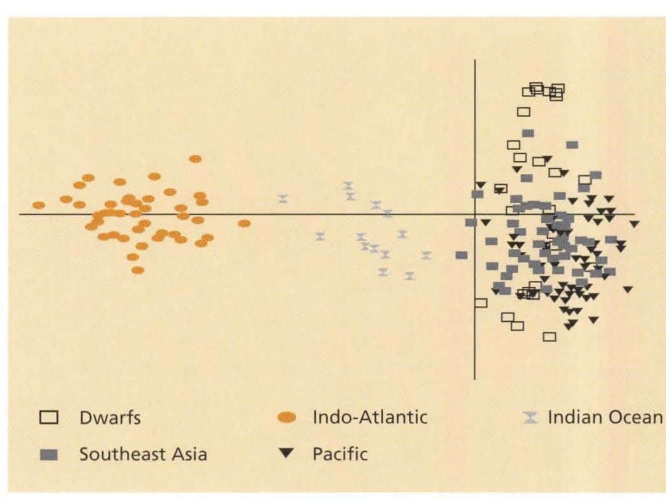


Figure 3.
Factorial analysis of correspondences carried out using RFLP data.

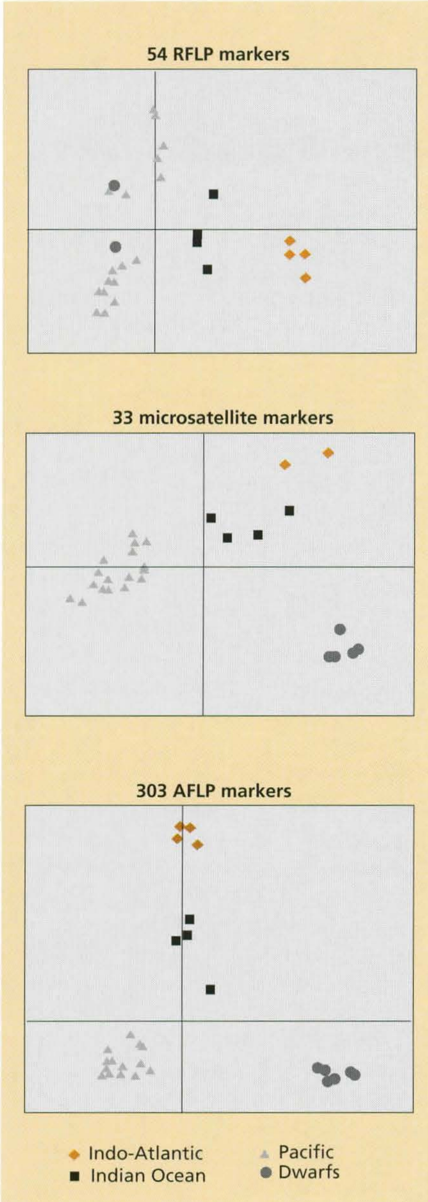


Figure 4.
Image of diversity obtained with the three types of marker.

suitable for shedding light on microevolution processes, whereas RFLP markers can be used to monitor macroevolution.

This study was carried out on a subsample of 31 individuals, chosen to represent the overall diversity of the species. Fifteen cultivars belonging to the three genetic groups were represented by 2 or 3 individuals. The AFLP and microsatellite molecular analyses were carried out at IACR's Long Ashton station, the RFLP at CIRAD. The results for the three techniques were represented by principal components analyses. The three major groups were clearly found: the Pacific, the Indian Ocean and the Indo-Atlantic group (figure 4).

Prospects and applications

The experience acquired during this research led to CIRAD-CP being chosen by IPGRI and the COGENT network to produce a microsatellites kit for the identification of coconut cultivars. This kit needs to be as discriminant as possible, and easy and cheap to use. The first requirement for success is to have a sufficient number of markers: around 130 microsatellite markers developed at CIRAD and at the Long Ashton research station are available. Only ten or so of these will be selected for their sufficient number of clearly readable alleles, and their high discriminant power, which assumes that polymorphism occurs between cultivars rather than within the cultivar.

In order to evaluate the discriminant power, it is necessary to test these microsatellites on an appropriate representation of diversity on a world scale: leaf samples from 400 individuals, representing more than 80 cultivars, have been collected. It will then be necessary to use an identification method adapted to the genetic structure of the populations in question. The Tall coconut palm is cross-fertilizing, and in traditional cultivars all individuals differ from each other: identification will therefore have to be based on the probability of belonging, and make use of allelic frequencies. A statistical identification method (Bayesian method) is currently being developed and has already given some promising results.

This kit will be used to describe coconut diversity in Papua New Guinea (on a sample of 29 Tall cultivars and 3 Dwarfs), in Vanuatu (on a dozen populations collected during participatory surveys) and in four archipelagos of the Pacific: Cook, Tuvalu, Kiribati and Marshall. In each case, the populations will be positioned in relation to worldwide diversity. The polymorphism existing between the populations and within them will be assessed. The results will make it possible to more effectively orient the strategy for the collection and conservation of diversity, and its use in breeding. ■

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Controlling palm pests: olfactory trapping

Insect chemical mediators, particularly pheromones, are an important component of integrated protection. CIRAD and INRA, in cooperation with partners from the South, have been engaged for several years in research on control methods using selective mass trapping of palm Coleoptera pests (*Dynastinae* and *Rhynchophorinae*), which are rife in Latin America, Southeast Asia and the Pacific. It is becoming increasingly difficult to control these insects as they have developed considerably with more intensive cultivation and especially with the renewal of old plantations. New rules governing pesticide use, and land preparation (zero burning), are making the choice of treatments even more complex.

The work involves indentifying the pheromones of these insects, along with allelochemical substances (plant odours) acting in synergy with pheromones to develop highly effective attractants. Work began in October 1997 and will continue up to September 2001 under an INCO project *New technology of pest management against insects pests of oil palm and coconut crops: research and development of selective trapping using synthetic attractants.*

This project, which is being implemented in collaboration with INRA, includes three oil palm and coconut producing partners from the South (CENIPALMA in Colombia, CCRI in Papua New Guinea, and IOPRI in Indonesia), along with three European organizations (CIRAD-CP and INRA in France, INIA in Spain) and two private organizations (EGNO-Chimie and Agrisense-BCS Ltd).

Methods

Characterization of a pheromone and of allelochemical attractants was the culmination of a succession of observations, and research work involving biologists and chemists.

The attraction of one of the sexes under natural conditions was discovered through trapping with living insects, along with studies on behaviour and sexual activity, and on the role played by the host plant. This initial stage was essential, since it provided information about the conditions under which the insects came together and, consequently, the conditions under which the pheromone was produced. The physiological condition of the individuals had to be taken into account, along with the rhythm of activity and whether or not the host plant had to be present.

Pheromones and allelochemical substances were isolated by collecting the effluvia of the insect on specific absorbent material (Supelpak-2 made by Supelco). In some cases, when the compounds to be analysed were very volatile, the SPME method (Solid Phase Micro-Extraction) was used. It offered the advantage of sampling odours on an adsorbent fibre directly in a chromatograph, without solvent.

The biological activity of the extracts was studied by olfactometry or by electroantennography (EAG).

A physico-chemical analysis of the extracts was carried out by gas chromatography (GC), either alone or combined with mass spectrometry. Detection of pheromone compounds was sometimes facilitated by using combined GC-EAG.

The different molecules that probably made up the pheromone were synthesized, and biological tests (EAG, olfactometry, trapping in the field), were carried out.

A formulation was developed and the effective doses were optimized in trapping trials in the field.

Results

Coleoptera *Rhynchophorinae*

Rhynchophorus are large weevils attracted by wounded palms in which their larvae develop, often after attacks by dynastid beetles. *Rynchophorus palmarun* is the most dangerous since it is a vector of the nematode responsible for red ring disease. Synthetic pheromones are now available for these insects, but trapping is only possible with a piece of plant matter (co-attractant). Work is under way to develop a synthetic mixture that acts in synergy with the pheromone, to replace the plant.

Complex mixtures that compete well with the plant have been found for *R. palmarum*. They include a major ferment fraction, marking the existence of a wound, and a fraction more particularly linked to the palm. A comparative analysis testing sub-fractions was carried out with CENIPALMA, in Colombia, to determine simplified mixtures containing 5 to 6 constituents with synergistic effects. These mixtures are also being tested on the species found in Papua New Guinea (*R. bilineatus*).

Coleoptera *Dynastinae*

Damage is caused by adults. The larvae develop in leaf mould or old rotting wood.

Scapanes australis

Studies were carried out in collaboration with CCRI in Papua New Guinea on *S. australis*, a large Coleoptera measuring 6 cm, which attacks coconut palms on several islands in the Bismarck archipelago (Papua New Guinea, Solomon Islands). The adult mines galleries in the petiole bases of fronds, and penetrates the stem. Given the size of the insect, damage is severe in young 1 to 5-year-old coconut palms. Deep *Scapanes* galleries cause fermentation of the tissues, which then very often attracts *R. bilineatus*. The latter lays its eggs and the larvae cause destruction inside the plant, with foul smelling bacterial rot diseases. These combined *Scapanes* and *Rhynchophorus* attacks inevitably kill the coconut palm and prevent any coconut planting in some regions of the country.

Studies carried out in recent years, in connection with the INCO project, have led to significant progress in our knowledge of the biology and behaviour of *Scapanes* : identification of larva sites, particular behaviour of males and flight periods. The last observation was decisive in discovering a signalling behaviour in males and in identifying the insect's pheromone.

During the day, males remain inside their galleries. At nightfall, they move to the entrance and take up a very original position, with the head in the gallery and the abdomen held erect outside the gallery; a droplet of liquid is secreted from the abdomen and spread by the hind legs (photo1). Field trials with caged insects showed that calling males were attracting both males and females of the species. The females did not show this behaviour. They were highly mobile and only remained for a short time, around one night, in the galleries.

Gas chromatography analyses, combined or not with mass spectrometry after sampling by the SPME technique (figures 1 and 2), led on to the identification of compounds in the secretion that corresponded to an aggregation pheromone. The two compounds required were 2-butanol and acetoine (3-hydroxy-2-butanone) in a ratio of 90:5 (v/v). The third compound identified in the male secretion (2,3-butanediol) did not improve captures. The synthetic mixture attracted the pest into traps. It was contained in a plastic sachet and outwardly diffused through the walls of the bag. The trap developed by CCRI is a plastic bucket with two large holes in the side. The pheromone sachet is hung from an inner box which contains the plant used as a synergist with the pheromone (photo 2). This insecticide-free trap is covered with coconut fibres to provide easier access for the insects. The insects are kept inside with a little water containing detergent. Over the last two years, experimental mass trapping with 14 traps has led to the capture of 3,000 adult insects, bringing the population down and reducing damage at the study site.

These results are very promising for the possible use of mass trapping to control *Scapanes*. Trapping trials have been set up to check the effective control of the pest in new coconut plantings with high-yielding hybrid material in zones where this crop had become impossible due to the high infestation rate.

Oryctes rhinoceros

Work undertaken with IOPRI in Indonesia showed that *O. rhinoceros* is one of the most serious pests in young oil palm and coconut plantings in Southeast Asia. The adults mine galleries at the base of young fronds. These attacks retard development and sometimes kill the palm.

The insect develops in rotting wood and in old stems left on the ground when the land is being prepared, or left standing after poisoning. Replantings carried out to replace old plantations encourage development of this pest. Protecting young palms with repeated applications of insecticide granules is expensive and involves environmental risks, notably pollution of the water table. Manual collection necessitates frequent rounds and is labour-intensive.

Identification of the aggregation pheromone ethyl 4-methyloctanoate (E 4-MO), in *O. monoceros* in 1995 and in *O. rhinoceros* in 1996 offered new prospects for controlling and reducing populations by mass trapping.

A "dose-captures" trial carried out over 163 ha near Marihat in Indonesia over a long period (15 weeks) revealed a dose effect: the greater the diffusion, the larger the size of the catches (table). The number of captures was high, at 11,482 insects, two

J. P. Morin

Photo 1. Calling *Scapanes* male.

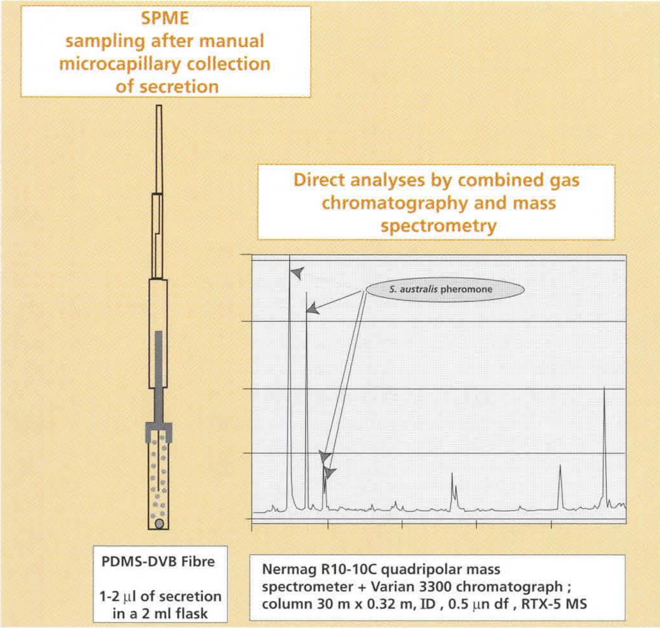

Figure 1. Sampling of the *Scapanes australis* male secretion by solid phase micro-extraction.

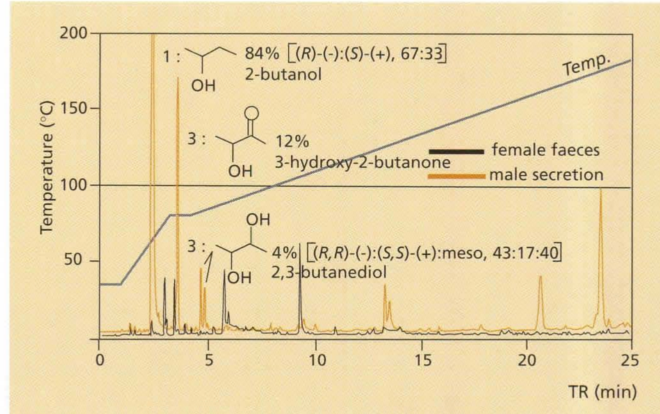

Figure 2. Gas chromatography analyses of the *Scapanes australis* male secretion.

J. P. Morin

Photo 2 . *Scapanes* trap developed by CCRI.

thirds of which were females, which enabled rapid action to be taken against the pest's multiplication potential. Given the cost of E 4-MO, it is not feasible to use the highest doses for the commercial production of dispensers. Research currently under way is attempting to find synergist compounds that will make it possible to reduce the pheromone dose, hence the cost price.

As it was not known how and when the pheromone was involved in the life of the insect, the biology and behaviour of *O. rhinoceros* were studied. A complementary study of antenna structure led to the identification of several specific sensilla, which responded to certain chemical compounds.

An examination of young attacked palms in the field showed that there was no aggregation at that level, and that the palms were colonized separately, as much by males as females. Insect grouping occurred in larva sites, such as old wood or rotting stems. Investigations carried out on those materials confirmed that insect arrivals corresponded to a stage in stem decomposition that enabled mating and egg-laying. The pheromone emitted by the males would therefore seem to play a role at that level, promoting the colonization of that medium. This hypothesis was put to the test by carrying out trapping with pheromone and rotting wood or plant compost. The synergistic effect observed led to a substantial 2 to 4-fold increase in captures. Studies are continuing with IOPRI to identify synthetic compounds that are potential synergists with the pheromone.

Table. ***Oryctes rhinoceros*** **captures depending on the doses emitted by different dispensers. Trial over 163 ha of young palms at the Laras estate, North Sumatra, Indonesia.**

Type of dispenser	Diffusion rate (mg/day)		Captures (males and females)	
	In the laboratory (INIA)	In the field	Total (60 traps for 15 periods of 4 days)	Mean/trap in 4 days
D4	54	61	3,099	22.1 a
D3	27	32	2,392	17.1 b
D2	16	17	1,962	14.0 c
D6	_	16	1,577	11.3 d
D5	_	13	1,459	10.4 d
D1	5	5	826	10.3 e
Total			11,482	

Means followed by the same letter are not different at $P < 0.05$ (Newman-Keuls test).

Conclusion

Mass trapping of Coleoptera pests of palms using synthetic attractants is now possible, but further development of dispensers—determination of mixtures and doses—and traps is still required. Strategies for their use depending on cultivation conditions also need to be tested. The method will only be required in the first 4 or 5 years after planting, when palms are highly susceptible to attacks. It will offer the advantage of being adjustable to pest population levels, and to a seasonal effect, whilst taking into account the degree of damage.

Trapping offers the advantage of having an immediate impact on damage by reducing populations of the pests responsible, whilst being environment-friendly. It complements biological control, which acts slowly and in the long term. ■

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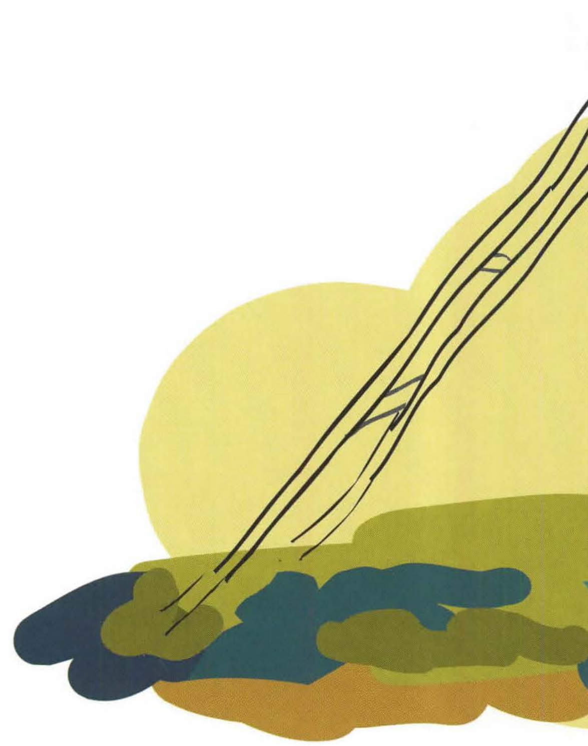
Rubber commodity chain: economic context and research overview



Recent developments in the commodity

The 1998-2000 period was marked by the disappearance of INRO, which managed the natural rubber buffer stock, which was the last such stock to be operating in the commodities field. Its disappearance was due to the profound changes in the rubber commodity chain, fuelled by the currency crisis in Southeast Asia. In fact INRO was unable to play its role, intervening on the markets, at a time when exchange rates, particularly those of the Malaysian ringgit and the Singapore dollar, which were used to quote natural rubber prices, were in a process of devaluation against the American dollar.

In addition, most sales (54%) were carried out by private contract directly between producers and consumers. That tendency is bound to increase with the appearance of e-business Internet sites where producers and consumers can make contact and easily carry out their transactions.

World natural rubber production increased from 6.4 to 6.7 million tonnes in two years. The same phenomenon was seen in Thailand and Indonesia, where production increased from 2.0 to 2.1 million tonnes and from 1.5 to 1.7 million tonnes respectively. Production in Malaysia fell from 0.97 to 0.85 million tonnes, due to competition with oil palm as a cash crop and to industrialization of the country, which is draining off manpower from the countryside to the factories. However, the crisis slowed down the movement. Production in China and India has continued to rise, increasing from 0.44 to 0.46 million tonnes and from 0.58 to 0.60 million tonnes respectively.

Another notable event was the desynchronization of growth rates for elastomer consumption between the different regions of the globe. In 1998, consumption in the USA, Canada and the countries of the European Union rose by 5 to 10%. In the Pacific countries, which were subjected to the effects of the crisis, consumption fell by 2 to 15%. In 1999, the situation was reversed.

Synthetic rubber consumption reached approximately 10 million tonnes in 1999 and the natural rubber: synthetic rubber consumption ratio remained stable: 39:61.

The current level of natural rubber prices, which have been falling since 1998, is hardly an incentive for growers, and international organizations have stopped funding rubber development projects. The replanting rate is falling each year, and the growth in production is likely to slow down, at a time when the last countries in the Pacific zone will be coming out of the crisis. IRSG expects consumption to increase by 4% in the coming years. The tyre industry has succeeded in developing chemical formulas that would make it possible to replace natural rubber by synthetic rubber in some tyre parts (around 50% in weight). The decisive factor would therefore seem to be the price, or for the same price, quality.

Main activities

In order to meet the challenges facing the rubber commodity chain, the Programme has adopted a strategy based on:

- mastering the transfer of crop management sequences to smallholders in traditional zones, but also in sub-optimum production zones,
- controlling leaf diseases,
- improving the productivity of the areas planted, work ouptut and investment,
- mass propagation by somatic embryogenesis,
- mastering and controlling quality.

Support to the development of rubber smallholdings has resulted, among other things, in the continuation of the Smallholder Rubber Agroforestery Project, in collaboration with GAPKINDO and ICRAF in Indonesia, which has revealed the good performance of selected planting material under traditional agroforestry management conditions, with regrowth in the interrows. Two smallholder adaptive research projects have been launched, one in Vietnam in connection with the Agriculture Diversification Project, in collaboration with RRIV, the other in Cambodia with the Agricultural Productivity Improvement Project, in collaboration with IRCC. Lastly, surveys have begun on smallholdings in Cameroon, under an FAC research project for support to smallholder development, in collaboration with IRAD.

The improvement of rubber cultivation productivity through fundamental genetics and physiology research has continued in Côte d'Ivoire, Thailand and Montpellier. A physiological study has been launched to estimate the impact of different types of stress on productivity and identify simple criteria for measuring the severity of such stress. The importance of carbon supplies to the laticifers and development of rubber cultivation in sub-optimum climatic zones subjected to water stress, led us to study the functioning of the whole plant, and its response to variations in the natural environment. We are seeking to estimate biomass formation using simple criteria and functions for different clones, and to establish a model for carbon allocation between the different sinks in the plant—including the artificial sink created by tapping—depending on different water supply situations.

Hevea selection based on quantitative genetics could be speeded up through rapid progress in biotechnologies. The Rubber Programme is developing various applications (genetic diversity, clonal identification, open pollinated reproduction) for microsatellite molecular genetic markers. It is aiming to study the genetic determinism of the factors involved in the metabolic typology of clones, using genetic mapping. Genetic modification of *Hevea* is currently being developed, and mastery of somatic embryogenesis is also being acquired.

As regards leaf diseases, it has been shown that leaf fall caused by *Corynespora cassicola* is caused by the action of a toxin specific to *Hevea*: cassiicoline. An early test for clone susceptibility to cassiicoline has been developed. It can be used in a breeding programme taking into account phytosanitary constraints in Southeast Asia.

Development of somatic embryogenesis procedures has continued in partnership with Michelin. New regenerating callus lines have been obtained for clone PB 260, which enable continuous experimental production of *in vitro* plantlets. Around 15,000 PB 260 *in vitro* plantlets obtained by somatic embryogenesis were sent to five rubber growing sites in 1998-1999 for acclimatization and planting out in field trials (25 ha) under research agreements with CNRA in Côte d'Ivoire, Michelin in France, Africa and South America, and with RRIT in Thailand. The first results from the trials have provided information on planting conditions for this new material and confirmed that *in vitro* plantlets have a well-balanced root system and vigorous growth and reveal juvenility characteristics. At the end of 1999, this work was considered by an external scientific review, and the conclusions served as a basis for considering further programming and agreements with our partners.

Quality control primarily relies on an understanding of the mechanisms involved, and the role of agronomic factors in quality variability. Thermo-oxidation of raw natural rubber has been studied by DSC (Differential Scanning Calorimetry).

In connection with an operation to identify physico-chemical criteria linked to natural rubber processability in manufacturing, a study was made of changes in its microstructure during blending in a roller mill. Close collaboration with the PSA, Valeo and Dunlop companies, along with IFOCA, revealed relations between the physico-chemical criteria of raw rubber and vulcanizate ageing, measured in dynamic mode.

Quantification of the influence of agronomic factors and their interactions—clones, climate, soils, cultural practices and processing—has begun in close collaboration with the Hévégo station in Côte d'Ivoire.

The Rubber Programme at a glance

44 staff, including 27 researchers
Partner countries: France (French Guiana, Montpellier), Côte d'Ivoire, Cameroon, Gabon, Guatemala, Mexico, Brazil, Thailand, Cambodia, Vietnam, Indonesia
Institutions:
France: IRD, INRA, University of Montpellier II, IFOCA
International: ICRAF
Networks: ANRA, IRRDB
Industries: Michelin, Dunlop, PSA, Valeo
Research topics:
- Diagnosis, design and transfer of *Hevea*-based farming systems adapted to smallholdings
- Study of *Hevea-Microcyclus ulei* interactions, to obtain resistant planting material
- Control of rubber cultivation productivity by applying the metabolic typology concept
- Development of *Hevea* somatic embryogenesis procedures
- Controlling the consistency of natural rubber

Scientific partners

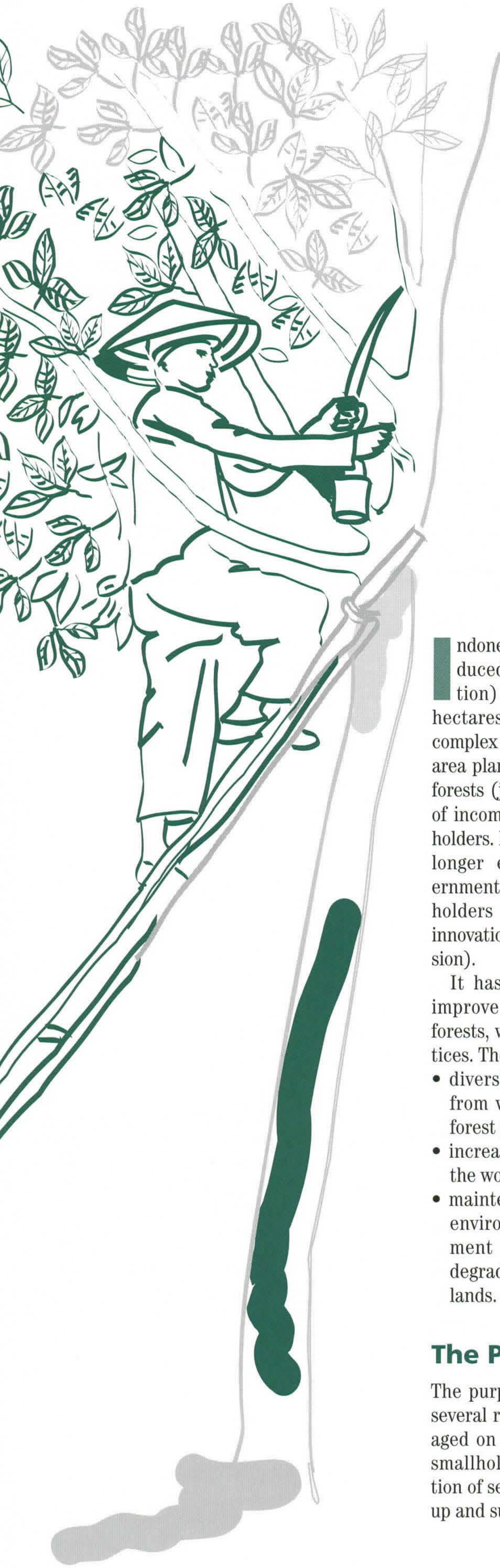
By strengthening our partnership with INRA-PIAF and the University of Kasetsart, in Thailand, in the ecophysiology field, it has been possible to organize two Thai theses, which were initiated in 2000.

Our collaboration with CIRAD's Tera Department has continued for socio-economic aspects, particularly in Indonesia (ICRAF project) and in Vietnam (ADP project).

Closer ties have been established with INRA-SAD in socio-agronomic sciences for smallholder development.

Our cooperation in Thailand has been further strengthened with the signing of a tripartite agreement with RRIT and the University of Kasetsart for molecular biology and ecophysiology. Work on the consistency of natural rubber quality has been jointly carried out with processors and natural rubber users in France, and with IFOCA. ■

Improving the productivity of rubber agroforestry systems



Indonesian rubber, which is primarily produced by smallholders (75% of production) is grown on over 2.5 million hectares in a highly extensive manner: complex rubber agroforests (84% of the total area planted to rubber). These rubber agroforests (jungle rubber) are the main source of income for more than 1.2 million smallholders. Nowadays, this farming system is no longer economically viable. Under government projects, only 15% of these smallholders have had access to technical innovations (clones, fertilization and extension).

It has therefore become necessary to improve the productivity of these agroforests, whilst preserving agroforestry practices. These practices need to enable:

- diversification of income with revenue from wood, fruits and other traditional forest products,
- increased rubber production by limiting the work time required for upkeep,
- maintenance of a sustainable system in environmental terms, or even improvement and rehabilitation of extremely degraded land, such as *Imperata* grasslands.

The Project

The purpose of the study was to compare several rubber-based farming systems managed on a more or less intensive basis on smallholdings, and to ensure the production of selected planting material by setting up and supervising budwood gardens.

The main two partners behind this SRAP project (Smallholder rubber agroforestry project) are GAPKINDO and ICRAF. Moreover, the SRAP has developed co-operation with some local projects. Particularly involved in these projects are GTZ for the SFDP (Social and forestry development project) in West Kalimantan, and the Pro-Rlk (Critical land rehabilitation project) in West Sumatra, certain private partners (Goodyear in North Sumatra, PT Finantara Intiga), research organizations such as BPPS, IRRI's Sembawa rubber research station in South Sumatra, and CRIFC in Bogor (Foodcrops Research Institute of Bogor). Lastly, CIRAD-CP is working with CIRAD-TERA on farming system characterization and technical-economic monitoring of plantations.

Smallholder trial network

The RAS (Rubber Agroforestry Systems) research programme is based on on-farm trials taking a participatory approach and involving several types of RAS at variable degrees of intensification. A network of trials was set up in three provinces, West Kalimantan, Jambi and West Sumatra (100 trials, a smallholding is equal to one replicate with several plots according to treatments). This network sought to test a certain number of technical hypotheses under natural conditions, based on three agroforestry systems (RAS 1, 2 and 3) with increasing levels of intensification in terms of inputs and work, depending on the strategies of the different communities and ethnic groups covered by the project (Dayaks and Javanese transmigrants in West Kalimantan, Minangs in West Sumatra and Malayus in Jambi).

RAS 1 type

RAS 1 is jungle rubber, where a single modification is made to the system: the *Hevea* seedlings traditionally used are replaced with clones adapted to these particular growing conditions, where *Hevea* is in competition with natural forest regrowth (photo). This agroforestry system can only be practised in non-degraded planting or replanting zones, with sufficient environmental biodiversity, such as secondary forests of the fruit and timber agroforest, or old jungle rubber type. The trees intercropped with *Hevea* are therefore derived from natural forest regrowth, some of which will subsequently be selected by the grower. In the provinces of West Kalimantan and Jambi, upkeep along the planting row is sufficient to ensure normal rubber tree growth. Slashing and hoeing frequency depends on the existing vegetation, but upkeep limited to four rounds in the first year is sufficient for zones with little *Imperata* invasion. Under such conditions, rubber tree growth is comparable to that in a monoculture, as natural regrowth encourages rubber tree growth, by helping to control grasses and maintaining soil moisture, which promotes good striking of young plants.

Likewise, the trials reveal the good growth of PB 260 and RRIC 100 (table 1); BPM 1 and RRIM 600 have significantly slower growth. PB 260 thus confirms excellent growth under agroforestry conditions. Clone growth is better than that of plants derived from seedlings.

Lastly, in Jambi, no significant difference has been found between plots with fertilization and the fertilizer-free control. This indicates the good relative fertility of the piedmont soils in that province.

RAS 2 and RAS 3 types

RAS 2 and 3 are complex agroforestry systems, where the elements of the rubber tree and intercropped tree combination are chosen from the outset. There is therefore less biodiversity, which depends on the economic interest of the intercropped trees selected by the farmer, comprising fruit and timber trees. Planting densities are 550 rubber trees/ha and 150 to 250 trees/ha for the intercropped trees. Such a structure makes it possible to stagger different productions over time: rubber between years 5 and 35, wood for paper pulp between years 6 and 10 (fast growing trees), fruits between years 10 and 50, then wood between years 40 and 50 (slow growing trees).

For RAS 2, in transmigration zones or when land is extremely limited, and in severely degraded zones, the intercrops are grown for the first three or four years of the rubber tree immature period. The major challenge here is to maintain rice production at a level that is compatible with good work output and a minimum of inputs and risks over several years running. This is the most intensive system. In West Sumatra, trials have been set up under marginal conditions for rubber cultivation (slope, height above sea level). Minang smallholders have grown successive annual crops surrounded by *Flemingia* hedges. They have succeeded in overcoming these environmental difficulties and the rubber trees have shown remarkable growth (8 cm/year), which confirms the important role of this crop for developing marginal zones. Phosphate fertilizer, or NPK compound fertilizer significantly improves rubber tree growth by 16% and 39% respectively, compared to the control (table 2).

Intercropping with annual crops, especially rice, which are fertilized, improves rubber tree growth by 9%. This result clearly shows the merits of fertilization in correcting the severe P and N deficiencies in the soils of West Sumatra. PB 260 growth is inferior to that of polyclonal seedlings, well-developed at the time of planting. Thus, seedlings can achieve good growth if strict culling is carried out in the nursery. However, for use on small-

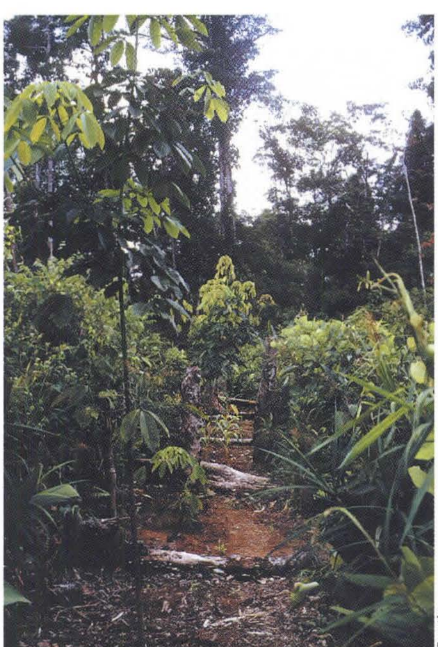

D. Boutin

Photo.
Hevea clone under forest regrowth conditions.

Table 1. Circumference at 3 years depending on environmental conditions (cm).

	Jambi	West Kalimantan (2 sites)	
PB 260	34.6 *a*	26.5 *a*	29.0 *a*
RRIC 100	31.6 *a*	25.7 *a*	
BPM1	33.9 *a*	21.2 *b*	27.0 *a*
RRIM 600	32.1 *a*	21.6 *b*	27.0 *a*
Seedlings	27.0 *b*	15.5 *c*	

Table 2. Effect of fertilization on circumference at 3 years and 2 months (cm).

	West Sumatra
No fertilization	20.6 *a*
Natural phosphate	24.0 *b*
NPK	28.6 *c*

holdings, this strict selection constraint is a handicap. Fruit trees planted between the rubber tree rows do not have any negative effect on rubber tree growth. Grasses are generally more effectively controlled by the shading effect of the intercropped trees. Durian is often the favourite choice of smallholders, but it grows very slowly. For rapid additional income, only rambutan and bread-fruit trees start to bear at three or four years. Lastly, annual crops with or without fertilization do not have an effect on rubber tree growth, contrary to observations in West Sumatra. The fertility of the soils in Sumatra is moderate, but fertilization is necessary in Kalimantan to ensure satisfactory yields.

In RAS 3, growers do not wish to grow intercrops for various reasons: lack of a market, an average level of intensification is sought, limited labour availability. In this case, the challenge is to set up a system of plants that will provide good cover and soil protection, with minimum upkeep in the first year and little or no upkeep thereafter. Such a combination calls for non-climbing, more or less self-regulating cover crops (*Flemingia congesta, Crotalaria, Setaria...*), or even cover crops that improve the low initial fertility of the soil (*Chromolaena odorata*), combined with bushy shade plants (*Leucena leucocephala, Gliricidia, Calliandra...*) or fast-growing trees (*Acacia mangium or Gmelina arborea* type). RAS 3 can also be considered in a strategy against *Imperata* to rehabilitate *Imperata* grasslands. In West Kalimantan, *Pueraria* gives satisfactory results. The other plants, such as *Crotalaria* and *Flemingia* only partially control *Imperata*. *Mucuna* disappears too quickly and needs to be combined with *Pueraria* to guarantee effective weed control. *Imperata* and *Chromolaena* significantly affect rubber tree growth (- 18% compared to *Pueraria*, table 3). The results between plots are highly variable given the flora composition and weed development. *Chromolaena* plots are often mixed with *Imperata* and the negative effect on rubber tree growth comes from the *Imperata*.

Gliricidia helps in controlling *Imperata* but requires frequent pruning. Fast-growing trees effectively control *Imperata*. *Acacia mangium* is the most efficient from two to three years onwards. However, its vertical growth means that it has to be eliminated before four years, as it starts competing with rubber. Other species, *Gmelina arborea* and *Paraseriantes falcataria* (albizzia), give variable results due to modest growth and an insufficiently developed canopy.

The network of smallholder budwood gardens

The cost of planting material amounts to approximately a third of investment expenses during the RAS immature period. It is traditionally considered that planting material production should be specialized (projects or nurserymen). Methods for planting material propagation by smallholders are being tested on smallholdings by setting up smallholder budwood gardens managed by the community, whilst nurseries remain separate. Budding courses and technical information on nursery management are provided for growers. An attempt is being made to identify constraints, which are mostly social, that are likely to prevent the development of this activity. The budwood gardens were set up between 1995 and 1996.

Caracterization of farming systems and technical-economic monitoring

At the same time as these on-farm trials taking a participatory approach, the Project carried out surveys with CIRAD-TERA to characterize farming systems in the three provinces, in order to ascertain the constraints and opportunities for adoption of the technical innovations proposed, and in order to identify an operational typology of situations and farming systems. The surveys were carried out in 1997 and 1998. Ninety-eight farms surveyed in the on-farm trial network formed the set of reference farms monitored each year.

Conclusions

RAS 1 enable the return of the natural vegetation and are particularly worthwhile, since they limit the cost of rubber plantation development.

Clone growth under agroforestry conditions is highly satisfactory and comparable to that in a monoculture (table 4). High-yielding clones are therefore suited to environmental conditions that are less favourable than in plantations, the environment in which they were selected. ■

Table 3. Effect of cover crops on circumference at 3 years (cm).

	West Kalimantan
Pueraria control	30.6 *a*
Mucuna	31.7 *a*
Crotalaria	29.0 *a*
Flemingia	29.3 *a*
Chromolaena	25.1 *b*
Imperata	26.1 *b*

Table 4. Rubber tree circumference at one metre under agroforestry conditions.

	Jambi		West Sumatra		West Kalimantan	
	Circumference (cm)	Age (months)	Circumference (cm)	Age (months)	Circumference (cm)	Age (months)
RAS 1	32.0	39	-	-	27.9	36
RAS 2	31.0	28	26.3	38	33.4	42
RAS 3	-	-	-	-	28.6	36

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For a resistant planting material *Hevea-Microcyclus ulei* interactions

The Rubber Programme is carrying out research in Brazil and French Guiana on the genetic control of *M. ulei*. The main purpose of this work, which is being carried out in partnership with the Michelin estates in Brazil, is the characterization and genetic selection of durable resistance to this parasite, which is responsible for South American leaf blight (SALB) on *Hevea*.

To that end, two main lines of research are being investigated. The first aims to acquire as clear an understanding as possible of *M. ulei* diversity, as an initial evaluation of its ability to overcome new resistances. The second seeks to exploit known sources of resistance within a programme of variety creation and genetic marking of genes involved in such resistance.

Pathogenicity diversity

The study of diversity in the pathogencity of *M. ulei* involved a sample of 88 strains from the Brazilian states of Bahia and Mato Grosso, and from the coastal strip of French Guiana. The virulence and aggressiveness of the strains were assessed under controlled conditions on a range of differential hosts comprising seven *H. brasiliensis* and *H. benthamiana* clones.

Figure 1 summarizes all the results obtained. Of the 88 strains studied, 73 distinct genotypes were observed and classed into 5 groups and 2 sub-groups. This classification did not correspond to the geographical origin of the strains. The table specifies the main characteristics of these groups. The considerable susceptibility of clone FX 3864, which is not very discriminant in the sample tested, can be seen. Group 1 is characterized by strains whose virulence seems to be directed against *H. benthamiana*, according to the differential range used. Group 3 stands out from the others as the only one containing strains that were virulent on FX 985. Groups 4 and 5 did do not have any strong specificity and group 2 shows low pathogenicity on the range of hosts in the study.

Work prior to this study revealed the existence of several races of *M. ulei*, from 4 to 10 depending on the authors. Our work, which was conducted on a larger scale, revealed the considerable diversity of the pathogen, 73/88 in the sample tested. No geographical specialization of the parasite was detected, but it is reasonable to suspect a certain degree of parasite specialization depending on the species, *H. brasiliensis* or *H. benthamiana*.

These results pinpoint the nature of the *Hevea-Microcyclus ulei* pathosystem: the substantial diversity observed largely explains the speed with which the parasite has overcome resistances set up against it to date. The current work is intended to confirm that result. The sexuality of *M. ulei* is probably the main driving force behind this diversity.

Variety creation and resistance marking

Work on the creation of *Hevea* clones resistant to *M. ulei* has made considerable progress in recent years. The definition of a clear breeding strategy along three lines with short, medium and long-term objectives enabled a start to be made to the work described below.

A large collection of original clones is available at the Michelin estate in Bahia. These clones are all derived from crosses

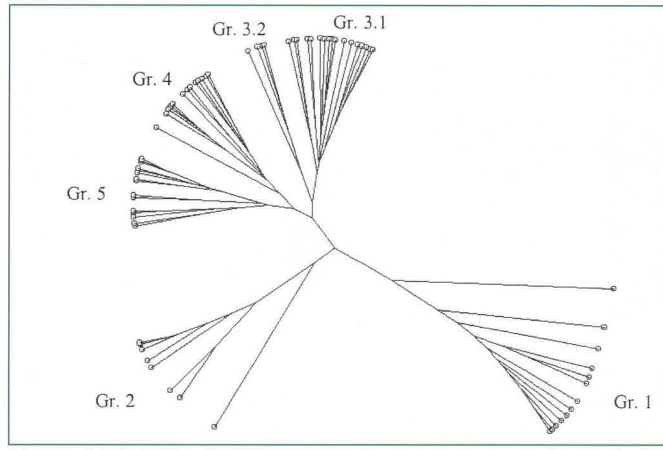

Figure 1.
Hierarchical classification of the pathogenicity of a sample of 88 strains of *M. ulei* on 7 *H. brasiliensis* and *H. benthamiana* clones.

Tableau . Distribution of the pathogenicity of 88 strains of *M. ulei* on a range of differential *H. brasiliensis* and *H. benthamiana* hosts.

Strains		Clones						
Group	Number	FX 3864	FX 985	FX 2261	FX 2804	IAN 3087	FX 3899	MDF 180
		H. bras.	*H. bras.*	*H. bras.*	*H. benth.*	*H. benth.*	*H. benth.*	*H. bras.*
Group 1	15	+ / -	-	-	+	+	+	+ / -
Group 2	20	+ / -	-	-	-	-	-	-
Group 3.1	17	+	+	+ / -	-	+	+ / -	+
Group 3.2	5	+	+	-	-	-	+	+ / -
Group 4	15	+	-	+ / -	-	-	-	+
Group 5	16	+	-	+	+ / -	+	+ / -	+

+ : susceptible; - : resistance; +/-: heterogeneous group.
H. bras.: *H. brasiliensis*; *H. benth.*: *H. benthamiana*.

with material from the Madre de Dios region, in the Peruvian Amazon, on which few studies have been carried to date, but which appears to be highly promising. Following a large-scale mass selection phase involving more than 800 clones, the best of them in terms of resistance to *M. ulei* and rubber production, are currently undergoing a large-scale test phase. In the short term, these tests should produce material that can be used to replant zones severely infested by *M. ulei*.

A programme of variety creation and selection was launched in 1993 at the Edouard Michelin estate, in Mato Grosso, Brazil. By recombining parents carrying high partial resistance and parents with a high production potential, we are seeking to assemble within the same varieties characteristics that are favourable for their cultivation in Latin America. The choice of genotypes produced under this programme was based on family selection involving quantitative genetics. Until now, selected resistant clones had only undergone mass selection. It is thus hoped to gain greater precision in the choice of best genotypes, and reduce assessment costs. The first clonal trials, planted since 1998, reveal a good correlation between the family values of resistance to *M. ulei* and those obtained in the previous phase. At that time, the trials involved seedlings, which did not offer the possibility of genotype replication. The soundness of the method used has been confirmed. It is thus possible to envisage supplying planting material in the medium term that is both high-yielding and resistant.

New prospects for the creation of *Hevea* varieties resistant to *M. ulei* have been opened up with the major work carried out on genetic mapping of *Hevea*. It culminated in the defence of a thesis in 1999. This genetic mapping work was carried out on progeny PB 260 x RO 38, whose RO 38 parent has a genetic background of resistance to *M. ulei* through its *H. benthamiana* origin. After creating a saturated consensus map using more than 700 markers—mostly AFLP and RFLP, but also isozymes and microsatellites—, a search for QTL markers of resistance was launched. Inoculation of the fungus under controlled conditions on 200 individuals of the progeny revealed 6 regions of the genome involved to varying degrees in such resistance. One of these regions, located on chromosome g13, seemed to be particularly interesting since it explained a large proportion of phenotypic variance, from 16 to 36% depending on the traits considered and the strains inoculated (figure 2). In addition, it seemed to be independent from the type of strain, since the 5 strains tested revealed a QTL of resistance in this region. This result was confirmed under natural infestation conditions: observations carried out in French Guiana on the same progeny revealed the presence of a major resistance gene in the same region of chromosome g13, for a trait that had not been studied under controlled conditions. All the resistance QTLs detected came from the *H. benthamiana* grandparent.

Conclusions and prospects

These results with genetic marking of major genes of resistance to *M. ulei* are promising, since they suggest that it will be possible to use marker assisted selection in the future, aimed at resistance to *M. ulei*. Three operations need to be carried out beforehand.

- New sources of resistance will have to be identified within the species *H. brasiliensis*, which do not have the drawbacks of RO 38 for agronomic and production traits. Some parents of Madre de Dios origin are very good candidates for this.
- Further mapping work will be carried out, and QTLs of resistance will be located on progenies derived from these parents. This phase should be simplified by the experience already acquired on PB 260 x RO 38.
- A variety creation strategy will be implemented based on early screening in

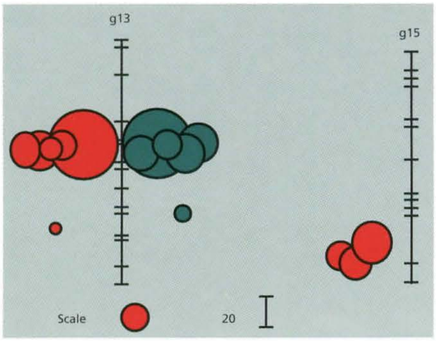

Figure 2.
Synthetic map indicating the location of the main QTLs for sporulation intensity (in red) and lesion diameter (in green) on chromosomes g13 and g15. The vertical dotted lines represent the 5 strains of *M. ulei* used under controlled conditions. (From the thesis by D. Lespinasse, 1999)

the nursery of individuals combining the largest number of favourable alleles at loci marking major genes of resistance. Those individuals will then be integrated into a conventional breeding scheme and assessed according to agronomic and production criteria. ■

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Study of open pollination in *Hevea* using microsatellites

The international survey organized in 1981 by IRRDB in the Amazon forest of Brazil has made it possible to broaden the base of germplasm available for *Hevea* breeding. Three successive projects to characterize this Amazonian germplasm were funded by the European Union over a cumulated period of twelve years. Different genetic groups were identified. Their improvement requires intense genetic mixing followed by selection. To that end, seed gardens containing samples of Amazonian genotypes from the different groups were set up in Côte d'Ivoire under close collaboration between CIRAD and CNRA. In order to validate this new approach for Hevea, it is worth specifying the conditions of the reproduction scheme resulting from open pollination in seed gardens.

The *Hevea* open pollination reproduction scheme

Hevea varieties are budded clones and seeds are usually only used for the production of rootstocks in nurseries. The seed gardens studied here did not, as for numerous forest species such as eucalyptus, have a seed production function.

The methodological study involved a planting set up in a forest environment at CNRA's Divo station, with total isolation from contamination by pollen from outside rubber trees. The planting comprised 50 Amazonian genotypes from three survey zones: Acre, Rondonia and Mato Grosso, along with the male-sterile clone GT 1, for which any selfing is excluded in principle. The seed garden therefore consisted, in equal parts (25/25), of genotypes belonging to groups I, II and III on the one hand: Acre and Rondonia except Pimenta Bueno (PB) district in Rondonia, corresponding to the western Amazon, and group IV on the other hand: Mato Grosso and Rondonia-PB corresponding to the eastern Amazon. These genetic groups were identified by Seguin *et al.*, based on molecular diversity. Each genotype was represented by 5 to 6 budded trees, randomly distributed in the seed garden, so as to maximize the number of possible recombinations. Each tree in the planting was intended to take part in reproduction as both a male pollen emitter and as a female seed producer, in accordance with its specific flowering and fruiting traits. Only clone GT1 functioned exclusively as a female. The questions raised involved the quality of genetic mixing of the rubber tree population undergoing open pollination within the planting design adopted. For a progeny harvested from a maternal genotype, what were the diversity and balance of the paternal contributions? What was the rate of selfing depending on the genotypes? Were the progenies of the different genotypes similar in their paternal make-up? Depending on the answers to those questions, what harvesting and selection strategy should be adopted to maximize variability in the harvested progenies and to optimize selection between and within the progenies?

Of the genotype characterization criteria (agro-morphological traits, isozymes, molecular markers), microsatellites appeared to be particularly efficient for varietal identification and especially for determining paternity, given the large number of different alleles found at each locus. The exploratory power of this molecular tool on *Hevea* had already been shown in a project funded by the French genetic resources bureau, involving the constitution of core collections, to which IRD and CIRAD have contributed. Using microsatellites to study seed gardens was made possible through the funding of a thematic research project programmed by CIRAD and involving CNRA in Côte d'Ivoire, for rubber and FOFIFA in Madagascar, for eucalyptus.

For the work in Côte d'Ivoire, and for the 1998 fruiting season, 468 plants derived from 14 genotypes (including GT 1) and 29 different mother-trees were analysed. For the 1999 season, 338 plants derived from 4 genotypes (including GT 1) and 8 different mother-trees were analysed. These large samples enabled a precise evaluation of the paternal participation of each of the 50 genotypes in the seed garden over two consecutive seasons.

Eight microsatellite markers were used, all independent from each other and distributed over six linkage groups of the *Hevea* genome; they comprised from 11 to 21 alleles. For each plant analysed, paternity was sought using Cervus software. Genotyping of all the parental genotypes was carried out first of all, in order to specify the possible origins of alleles observed in each progeny to be analysed. A check of clonal conformity of the parent trees in the seed garden, carried out at CNRA using isozymes, revealed the existence of 15 off-types within the seed garden; microsatellite genotyping of those 15 trees has yet to be carried out.

Paternity identification using microsatellite markers

Identification of the most likely father was completed for 463 of the 468 progenies harvested in 1998. Of the 338 progenies harvested in 1999, identification of the most likely father was completed for 334 plants. The few cases in which it was not possible to identify the probable father may have been due to the existence in the seed garden of 15 trees that were not true-to-type and not genotyped. Despite a high confidence level in paternal identification, there were sometimes mismatches between parents and progenies at certain loci. The error rate, estimated over the two years of the study, was 0.6%. These errors were probably due to inaccurate allele readings on the microsatellite migration gels. In order to take these errors into account, a probabilistic approach had to be taken to paternity testing by microsatellites. The probability that a genotype was the father of a given progeny was calculated by the Lod score method (Lod = logarithm of the odds ratio).

The sensitivity of the analysis depending on the number of microsatellite markers used was assessed. It declined as the number of markers decreased. When switching successively from 8 markers to 7, 6, 5 and 4 markers, whilst seeking to keep the most effective markers, the percentage of changes in paternal identification, compared to the best situation established with 8 markers, was 5%, 10%, 15% and 30% respectively. The small percentage of changes seen when switching from 7 to 8 markers (5%) afforded a high level of confidence to paternity identifications carried out with 8 markers.

These paternity identifications made it possible to deduce the degree of paternal participation of the different genotypes in the pollinations. A major result was that the distribution proved to be highly unequal. In the sample of seeds harvested in 1998 and 1999, 11 and 18 genotypes respectively, out of the 50 potential pollinators, did not participate in the recombination. When analyses were grouped for the two years, 8 genotypes did not make any paternal contribution. On the other hand, 4 genotypes accounted for 40% of total paternal contribution, 14 genotypes accounted for 80% of total paternal contribution and 25 genotypes accounted for 95% of total paternal contribution.

The genotypes were found to have quite a stable performance for their paternal contribution over the two harvesting years (table). The degree to which a given genotype made a paternal contribution to the seed garden progeny therefore seemed to be a genetic characteristic with high variability, which was relatively little influenced by environmental effects.

Whilst the proportion of mother-trees in the seed garden belonging to genotypes of genetic group IV was 50%, the share of those trees in the paternal contribution was 83%. The major pollinators therefore mainly belonged to group IV (East).

The distributions of paternal contributions for a given mother-tree were examined on five mother-trees for which the analysed progenies exceeded 40 seeds. It was found that the rate of participation in the total paternal contribution per mother-tree of the two predominant genotypes varied quite strongly between 0.34 and 0.71. In addition, these two predominant genotypes were not the same for a given two mother-trees. Although marked by certain important parents, the pollen pools that pollinated these trees were therefore not identical. The figure shows spatial distribution of the paternal contributions for these 5 mother-trees—and for an additional 3 mother-trees.

For the grouping of the 1998 and 1999 harvests (except progenies harvested from GT 1), the average selfing rate of the analysed genotypes (including possible crosses between trees of the same genotype) was estimated at 4.8%. As could be expected for a male-sterile clone, no selfing was seen for GT 1. This selfing rate, which was estimated less precisely for each genotype separately, due to limited numbers of individuals, varied considerably from one

Table. Contributions of paternal genotypes to formation of the progenies analysed for the 1998 and 1999 seed harvests.

Paternal population	Number and proportion of paternal genotypes	Number of fathers per genetic group*		Number and proportion of progenies		
		Groups (I, II, III) West	Group IV East	1998	1999	Cumulated 1998-1999
First 10 fathers°	10 (20 %)	0	10	300 (65%)	237 (71%)	537 (67%)
First 25 fathers°	25 (50 %)	7	18	426 (92%)	323 (97%)	749 (95%)
MT + RO/PB	25 (50 %)	0	25	385 (83%)	274 (82%)	679 (83%)
AC + RO	25 (50 %)	25	0	78 (17%)	60 (18%)	138 (17%)

° classed by decreasing number of progenies for cumulated years 1998 and 1999.

* according to Seguin et al. 1999.

AC = Acre, RO = Rondonia, MT = Mato Grosso, PB = Pimenta Bueno district of Rondonia.

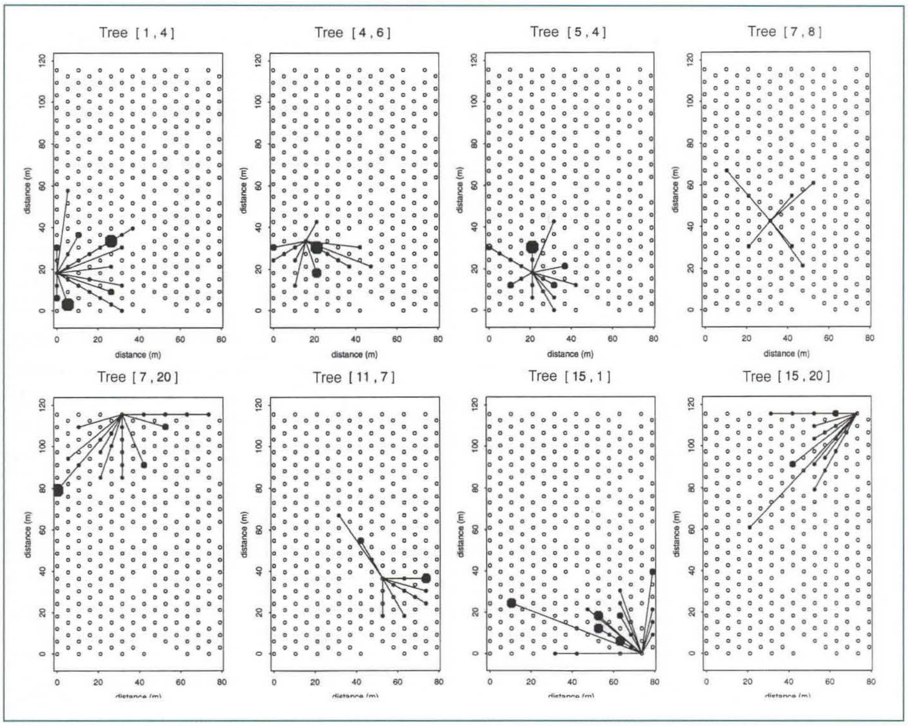

Figure. Microsatellite analysis of gene flows in a *Hevea* seed garden: paternal contributions in the progeny of 8 mother-trees in 1998. Each point represents a living tree in the seed garden. The lines link the mother-tree to the nearest trees of the parental genotypes that contributed to the progeny of the mother-tree in question. The size of the black circles is proportional to the number of progenies derived from that paternal genotype.

mother-tree to the next. The following individual results were obtained for five mother-trees: 0%, 1.8%, 6.8%, 8.6%, along with 15.9% for a mother-tree located on the edge of the seed garden and with a very large canopy. Observations on two mother-trees of the same genotype, with 56 and 44 progenies respectively, indicated selfing rates of 1.8 and 6.8%. It seemed that these variations could be partly explained by the genetic selfing ability, but also by the architecture of the mother-trees—canopy volume—the flowering and fruiting ability, along with the environment and canopy access to light—position of the tree on the edge of the garden or next to a gap.

An analysis of seeds from the same fruit revealed the possibility that two different fathers may have contributed to seed formation; this was seen in 4 of the 9 fruits studied. It is therefore not possible to envisage analysing just one seed per fruit to determine the paternal origin of the other seeds.

A further study, currently being conducted at CNRA, is intended to compare these results with architectural and phenological characteristics, along with the flowering periods and the abundance of flowering in the genotypes and individual trees in the seed garden. Indeed, considerable architectural differences are known to exist between clones from Acre and Rondonia—often marked by strong apical dominance, limited branching and flowering—and clones from Mato Grosso—often marked by strong branching and flowering. This research will provide a classification of genotypes according to their fruiting ability, and will elucidate the effect of staggered flowering on any formation of preferential crosses. The study takes into account climatic differences over the two years, and the effect of tree canopy growth from one year to the next. It could provide indications on distance effects associated with pollination.

Application to genetic variability management, and selection

The power of microsatellites, and the suitability of these markers for studying the reproduction scheme in seed gardens, are clearly shown through the abundance and precision of the information gathered.

As analyses stand at the moment, the study revealed substantial variability in the performance of parental genotypes as regards their maternal contribution to fruiting and their paternal contribution to male flowering, pollination and fertilization, and their selfing rate. The reproduction scheme in the seed garden was therefore far from a panmictic situation where all the combinations between parents would be equally probable in the progenies.

The relatively small number of genotypes (50), combined with the imbalance in paternal contributions, limited the recombinations. Since the abundance of flowering in the genotypes appeared to be the main factor limiting the diversity of paternal contributions, the propagation of each genotype by budding and randomization of trees in the field did not improve the situation much. However, it is advisable to harvest small samples of seeds from the largest possible number of mother-trees. The "East" group accounted for 83% of pollination: in order to increase the genetic diversity of the overall progeny, seeds could preferably be harvested from genotypes with a low paternal contribution, in this case clones from the "West" group, which would give a high proportion of hybrid progenies between the two groups. On the other hand, harvesting seeds from genotypes of the "East" group will give a large proportion of progenies derived from the internal recombination of that group.

The composition of the seed garden does not seem suitable for obtaining progenies of half-sib families derived from the same diversified and balanced pollen pool, which is prejudicial to effective selection.

Different arrangements need to be considered to try and diversify and improve the balance of paternal contributions in the seed garden. However, it is not sure they will be effective and would have to be systematically assessed.

Towards microsatellite marker assisted recombination

The power of paternity determination by microsatellites, used to assess the genetic recombination of the seed garden, could also be used as a tool for identifying the paternal origin of each seed harvested, after the event. The cost and time required for analysis do not necessarily rule out this possibility, notably because these analyses are set to become increasingly automated.

Sorting could be carried out in the large quantity of harvestable seeds, to make up batches according to the objectives sought:
- an overall progeny maximizing genetic diversity,
- a specialized progeny for a type of recombination, between- or within-group,
- progenies derived from the same pollen pool,
- progenies of full-sib families possibly structured in mating designs.

With this approach the particular conditions for the genetic recombination of a seed garden have very little influence—apart from isolation conditions designed to limit pollution from outside pollen—and the introduction of various constraints can be avoided when setting up seed gardens. The availability of a recombination seed garden with a large enough number of parents having undergone microsatellite genotyping could thus constitute an abundant source of naturally renewable breeding material, which is easy to dispatch in "seed" form for use by distant partners, and which is preservable in living form in the nursery at high density, sortable and usable over several years, in line with objectives which may evolve over time. This approach, which is envisaged for managing and utilizing wild germplasm, could be extended to selection within the advanced Wickham population, notably to carry out further genetic mixing involving little used parents.

Microsatellite genotyping of seed gardens is an example of marker-assisted selection applied to Hevea. The control of gene flows, which has been made possible by molecular markers, opens up new methods for two key aspects of rubber tree selection: dynamic management of genetic resources, and the acquisition of legitimate, half-sib and full-sib progenies.

In addition to seed gardens, molecular marking of gene flows in *Hevea* will also be very important when planting out genetically modified rubber trees. Microsatellite markers will make it possible to answer the question of pollen propagation distances, and the risk of GMO dispersion in this species. ■

List of publications

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Specific promoters for genetic engineering of the rubber tree

The choice of a promoter, for a genetic engineering programme, needs to take into account both the physiology of the plant and the application being sought, in order to optimize the expression of the transgene.

In the case of rubber trees (*Hevea brasiliensis)*, genetic engineering is being considered in order to improve existing varieties, but also as a physiological study tool intended to analyse or confirm the function of certain genes. In addition, several teams are interested in *Hevea* as a "green bioreactor" for production by the latex of marketable molecules other than rubber (molecular farming). In the case of an application seeking to improve rubber production or for molecular farming, it is important that the promoter used guarantee optimum expression in the latex cells (laticifers), and if possible in the latex cells only, so as to disturb the tree as a whole as little as possible. Hence our search for a promoter specific to those tissues.

We are also seeking for a promoter that can be induced by ethylene, in order to induce or strengthen transgene expression under farming practices calling for ethylene treatment (ethephon or ethylene gas) to stimulate production. The use of ethylene stimulants needs to be limited, since overstimulation, like over-tapping, can cause a physiological imbalance in the tree and penalize production. One possible strategy to limit this risk is to genetically modify *Hevea* in such a way that certain endogenous genes involved in protecting cells from oxidative stress, which is itself generated by over-exploitation, are over-expressed. By using a promoter that is inducible or over-expressed by ethylene, the activity of the transgene would be stimulated when most needed by the plant.

Chosen promoters: hevein and glutamine synthetase

A certain number of genes expressed in latex have been cloned in cDNA form. Some of them meet the expression criteria being sought.

Laticifer-specific promoter

Hevein is a small lectin-like protein involved in latex coagulation mechanisms. It is only present in the latex cells, as shown by immunolocalization tests (figure 1). The genes coding for hevein are therefore good candidates for cloning promoters that are specific to the laticifers. In addition, their level of expression in latex is very high, suggesting high promoter activity. Low expression of hevein has been detected on RNA extracts from leaves, but it probably originated from the latex cells located in the leaf ribs.

Ethylene-inducible promoter

Several genes have been identified whose expression is specifically controlled by ethylene in latex, including a gene encoding glutamine synthetase, which is highly over-expressed from 6 to 12 h after ethylene treatment (figure 2). It is the earliest response to ethylene described, in terms of gene expression.

Cloning and molecular characterization

Several genomic clones coding for hevein or glutamine synthetase have been obtained by screening a genomic library (genotype RRIM 600) with cDNA probes.

Whilst highly homologous in terms of sequence, the different genomic clones of hevein obtained could be classed in two

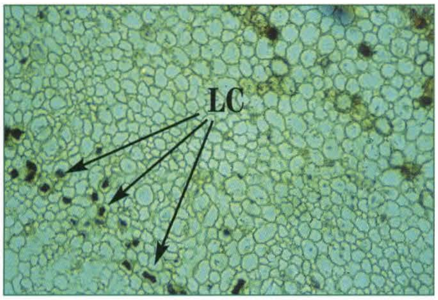

Figure 1.
Immunolocalization of hevein. Cross-section of a stem from a young plantlet incubated with polyclonal antibodies raised against hevein.
LC: latex cells

groups (I and II). A representative of each of these two groups of genes (*hev1* and *hev4*) was chosen for a detailed functionality analysis of the promoter.

Three different genomic clones coding for glutamine synthetase have been obtained (*gs1*, *gs2* and *gs3*). Clone *gs1* corresponds to the cDNA initially described and used to screen the genomic library. Unfortunately, it was truncated and could not be used to isolate the promoting region. A new cloning strategy using a flanking sequence isolation technique based on PCR (polymerase chain reaction) is currently being implemented. The other two genes are highly homologous to each other in their transcribed region, but have different upstream regions. Both differ significantly from *gs1*. No known regulatory sequence involved in regulation by ethylene was identified after sequence analysis of the upstream regions. For the four clones chosen (*gs2, gs3, hev1* and *hev4*), the size of the upstream regions isolated for functionality analysis varied from 320 pb to 1.9 kb (table).

Analysis of glutamine synthetase and hevein endogenous gene expression

An analysis of the expression of hevein and glutamine synthetase endogenous genes was carried out on samples of latex stimulated, or not, with ethylene, and on *in vitro* material at different stages of the somatic embryogenesis process (figure 3). Such an analysis was necessary to determine the choice of plant material to be used for the functionality study of the promoters. It showed that certain types of callus cells enabled expression of the GS and hevein genes.

In the case of GS, the different isoforms revealed differential expression during the somatic embryogenesis process. For instance, isoform *gs1* was preferentially expressed in proliferating friable callus, whereas isoforms *gs2/gs3* (without possible distinction) were preferentially expressed in callus undergoing embryogenesis, and in young embryos. Furthermore, the inducibility of the different GS genes by ethylene was confirmed, but with a greater range of over-expression for *gs1* than for *gs2/gs3* (without possible distinction).

In the case of hevein, the high percentage of homology between *hev1* and *hev4* made it impossible to define specific probes which would have made it possible to carry out an analysis of differential expression. The hevein genes—without possible distinction—were expressed in proliferating friable callus, to the exclusion of all other types of callus, and in the latest phases of development (mature embryo and plantlets), probably concomitantly with the development of differentiated laticifers.

Functionality analysis of the isolated promoters

The upstream region of the clones *hev1*, *hev4*, *gs2* and *gs3* was isolated by PCR and cloned in a transformation vector in phase with the *gus* reporter gene. Such a construct, after reintroduction into an appropriate cellular environment, will make it possible to study the functionality of the promoters by observing the activity of ß-glucuronidase, a product of *gus* gene expression.

Transient expression analysis

The functionality of a cloned promoter depends on the integrity of the isolated sequence, but also on the cellular environment, which generates signals authorizing or not authorizing promoter activity. For that reason, the choice of plant tissues into which the constructs will be introduced and analysed is of great importance. Based on the results of endogenous gene expression analysis (figure 3) two kinds of rubber tree callus have been choosen (proliferating friable callus and callus undergoing embryogenesis) in order to develop a transient expression system for analysing the regulation of promoter activity. The biolistic technique (particle gun bombardment) can be efficiently applied to introduce plasmid DNA into rubber tree callus. This transient expression system will be used to analyse the regulation of both GS and hevein promoter activity in response to ethylene, by treating the callus with ethylene inhibitors—as *in vitro* cultures usually produce a large amount of endogenous ethylene—and exogenous ethylene. However, other traits such as tissular specificity or response to wounding will be difficult to analyse with such a system. In particular, a study of the tissular specificity of the hevein promoter requires integration of the constructs in tissues with differentiated laticifers. Therefore, regeneration of transformed plantlets stably expressing the construct to be studied seems unavoidable.

Stable expression analysis

A process of stable genetic engineering of rubber trees, based on the *Hevea* somatic embryogenesis technique developed at CIRAD, is currently being developed. This process uses *Agrobacterium tumefaciens* as the gene transfer tool. To date, the phases of gene transfer, callus growth resumption after transformation and selection, have been optimized. It is already possible to obtain callus lines transformed in a stable manner. The final stage of regeneration has not yet been completely mastered and is currently being worked on. Nevertheless, this process is already available to analyse the activity of the promoters at intermediary stages such as callus (for studying the inducibility by ethylene) or embryos (for analysing tissue specificity), based on the knowledge that mature

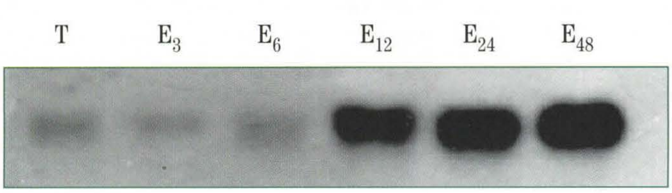

Figure 2.
Expression of glutamine synthetase genes in response to ethylene in virgin trees (first tapping).
Northern blot hybridization on latex total RNA.
T, untreated control trees; E3-E48, trees treated with ethephon 3, 6, 12, 24 and 48 h respectively before the first tapping.

Tableau. Size of the promoter region upstream of the translation initiation codon, in the initial genomic clone and after sub-cloning in a transformation vector.

	Genomic clone	Sub-clone (in transformation vector)
gs1	En cours de clonage	-
gs2	1.4 kb	1 kb
gs3	3.9 kb	0.9 kb
hev1 (group I)	320 pb	320 pb
hev4 (group II)	1.9 kb	1.9 kb

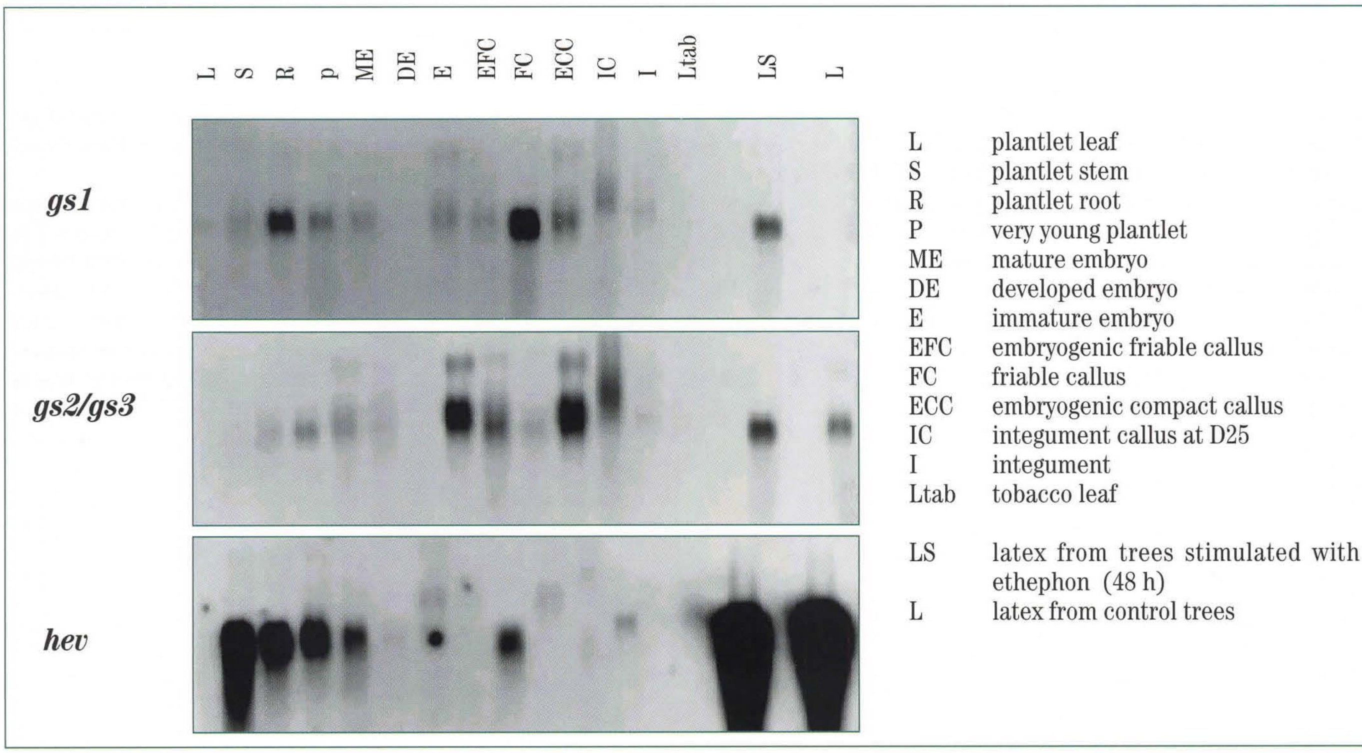

Figure 3.
Differential expression of the glutamine synthetase and hevein genes in response to ethylene and during the somatic embryogenesis process. Northern blot hybridization on total RNA; *gs1*, probe specific to isoform GS1 of glutamine synthetase; *gs2/gs3*, probe specific to isoforms GS2 and GS3 (without possible distinction); *hev*, full length cDNA probe coding for hevein (without possible distinction of the different hevein genes).

embryos already bear differentiated latex cells.

For several reasons, it seems worth studying these promoters in plant systems other than *Hevea*, especially systems for which genetic modification is faster and has been more effectively mastered. This would make it possible to demonstrate more rapidly certain regulation traits that are not specific to *Hevea*, such as the response to abiotic stress, including wounding, and to broaden the range of applications to other plants. For that reason, we have started testing our constructs in two plant systems for which genetic transformation has been effectively mastered: rice, a model plant for monocotyledons, and *Arabidopsis*, a model plant for dicotyledons.

Partnership

The hevein and glutamine synthetase genes were cloned under a Franco-Thai rubber biotechnology project involving the University of Mahidol, RRIT, IRD and CIRAD.

Molecular characterization and promoter subcloning were conducted at CIRAD-Montpellier, in the BIOTROP unit.

Work to optimize the *Hevea* genetic engineering method has been conducted in Thailand, under the DORAS (Development-oriented in agrarian systems) project, in collaboration with the University of Kasetsart and RRIT. It is now being carried on at CIRAD-BIOTROP.

Analysis of promoter functionality in both hevea and model plants will be carried out at CIRAD-BIOTROP.

This work is supported by IFC.

Conclusion and prospects

This work has made it possible to clone promoters from two glutamine synthetase and two hevein genes from rubber trees. However, the merits of these promoters will only be established after a more in-depth study of their regulation in different cellular environments.

The traits initially sought (inducibility by biotic stress, tissular specificity) with a view to *Hevea* genetic engineering programmes will be analysed either in a transient expression system, via biolistic gene transfer into rubber tree callus, or in stably transformed tissues via *Agrobacterium*. In rubber trees, analyses of promoter activity regulation will be conducted at intermediate stages of the regeneration process (callus, embryos), with ultimate demonstration in fully regenerated *Hevea* plantlets. Additionally, analysis of the *Hevea* promoters in model plants such as rice and Arabidopsis will give information about their potential use for genetic engineering of plants other than rubber trees.

Lastly, our work has revealed new traits of interest for the promoters studied. Indeed, the expression analysis of the endogenous *gs* genes revealed differential expression during the embryogenesis process. These genes therefore appear to be potential markers for the early stages of embryogenesis. The isolated *gs* promoters may be useful tools for validating this hypothesis: combined with a fluorescence reporter gene, they will make it possible to monitor changes in *gs* gene expression during development, under non-destructive conditions for the plant material. ■

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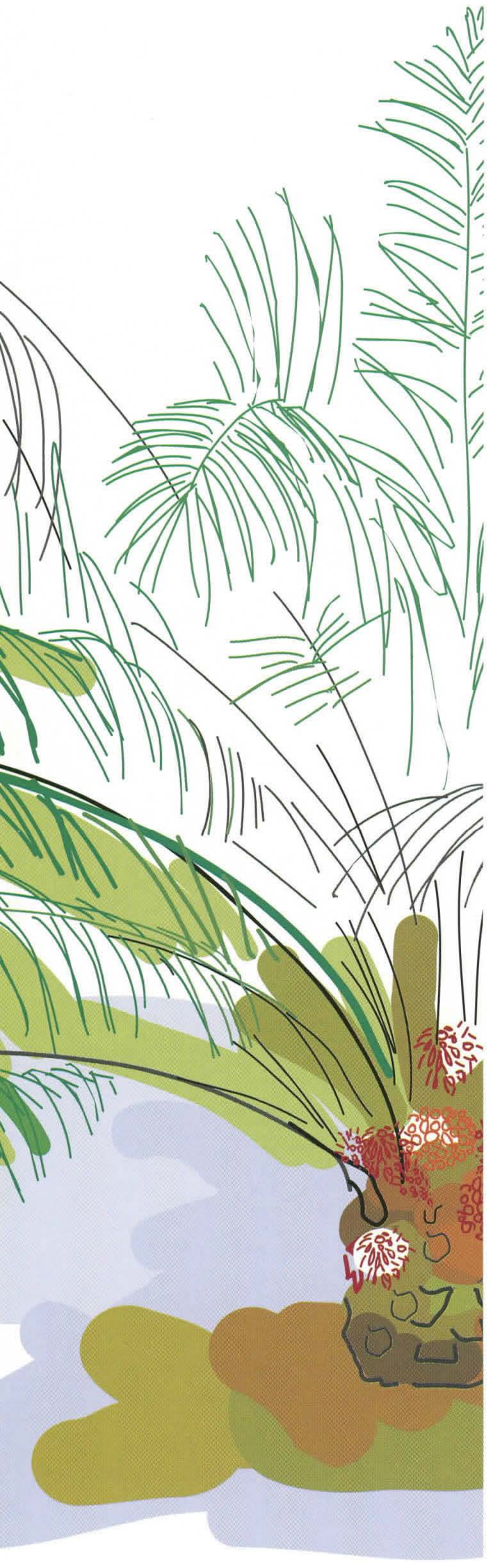
Oil palm commodity chain: economic context and research overview



Recent developments in the commodity chain

The oil palm commodity chain has recently had to deal with two distinct events. Firstly, in 1998, world palm oil prices reached their highest level for 15 years (663 USD per tonne on average) due to the economic crisis in Asia, which struck Indonesia, the world's second largest producer, head on, and the after-effect of the drought in 1997 caused by El Niño. Then, in 1999, surplus soybean production and the devaluation of the Brazilian Real led to rapid erosion of oil prices from January to July, and their subsequent stagnation (462 USD per tonne on average in 1999).

The main consequence of the Indonesian crisis was a freeze on the extension programmes of large private estates. In Africa, the price drop in 1999 threw into confusion a profession that was still insufficiently organized after the wave of privatizations, notably in Côte d'Ivoire. The cooperative sector is not yet operational and is late in compensating for State withdrawal. The privatization of State-owned companies is continuing in Cameroon (Socapalm, CDC). In Latin America, the commodity chain is becoming increasingly dynamic, especially in Colombia and Ecuador, despite the constant threat of bud rot.

Oil Palm Programme research priorities

In order to accompany and remain abreast of developments in a commodity chain in the midst of restructuring, the Programme has centred its research on four priority topics.

The first involves support to smallholders: it is important to define a "smallholder" typology and analyse organization in this sector.

The second research topic covers sustainable intensification factors: genetic improvement programmes are continuing and are now backed up by molecular biology. An overall "precision agronomy" approach, combining ecophysiological modelling and geographic information systems, is being taken to fine-tune fertility management in plantations.

The third topic is integrated control of bud rot, which is the main obstacle to the development of oil palm cultivation in Latin America.

Vegetative propagation by somatic embryogenesis is the fourth topic: research is focusing on the molecular determinism of somaclonal variations.

Support to smallholdings integrating oil palm

This research topic, which is new for the Programme, is endeavouring in the first instance to establish a typology of family farms, identify their capacity for sustainable innovations, and ascertain what they require from research. In 1999, two young researchers were recruited: an agronomist specialized in farming systems and an agricultural economist assigned to Cameroon and Côte d'Ivoire respectively.

In Cameroon, an initial study conducted in collaboration with IRAD and IITA led to the definition of a typology of farms to be studied in 2000, along with their zoning.

In Côte d'Ivoire, three lines of research will be developed under a research agreement with the University of Bouaké: institutional rules and logics in the context of commodity channel liberalization, development of the oil palm sector and land management, innovations and changes in the smallholder and small-scale production sector, and their consequences.

Oil palm sustainable intensification factors

An ambitious marker-assisted breeding programme has been launched. More than 121 microsatellite markers specific to oil palm have been developed. Reference genetic mapping of the oil palm is under way. At the same time, genetic blocks are continuing to be extended and exploited: in Indonesia with the Aek Loba Timur project (SOCFINDO); in Benin with SRPH at Pobé and in Cameroon with IRAD at La Dibamba. These trials are also intended to determine the agronomic value of planting material and study interactions between genotype and environment. They are thus helping to enrich the data in the network of breeding programmes formed by our partners.

Methods to diagnose the nutritional condition of oil palm plantings have been strengthened with the development of geographic information systems to monitor the PT SMART estates in Indonesia. An assessment of satellite remote sensing as a decision-making aid has begun. The limitations of leaf diagnosis are also being investigated, along with additional actions to be taken to improve its relevance and integration in an overall precision agronomy type approach.

In collaboration with the INRA centre in Versailles, for integrated control of *Oryctes*, the Oil Palm Programme is continuing research on volatile compounds from rotting empty bunches, which may act in synergy with pheromones to trap these Coleoptera and assess insect populations.

Integrated control of bud rot

The hypothesis that this disease is transmitted by *Scaptocoris* sp., a soil-borne piercing insect, has yet to be demonstrated. To date, genetic control remains the most promising strategy. Trials have been set up in partnership with plantation companies in Ecuador (Palmeras de los Andes, Indupalma) and in Colombia (La Cabana) in order to determine differences in susceptibility within the species *E. guineensis*. At the same time, the selection of *E. guineensis* x *E. oleifera* interspecific hybrids and introgression of *E. oleifera* genes in *E. guineensis* are continuing.

A research programme on molecular markers of tolerance has been drawn up and is now operational. Based on a backcross, embryo clones will be planted in an endemic zone with a view to associating molecular markers with the most tolerant clones.

Mastering and developing vegetative propagation by somatic embryogenesis

In order to understand and control somaclonal variations, a joint CIRAD-CP/IRD team has launched a research programme in Montpellier using molecular biology techniques. The ultimate aim of this work is to identify molecular markers of the floral abnormality, in order to eliminate off-type plants before they are planted out.

Two overall approaches have been taken: an analysis of the genome structure and of its methylation rate in relation to the floral abnormality, and a study of differential gene expression between variant and normal materials.

On a genome scale, the methylation rate has been quantified using a method based on high pressure liquid chromatography, along with another enzymatic type method, SssI-methylase accepting assay. Significant hypomethylation (0.5-2.5%) has been found in variant regenerants.

A programme of differential analysis of RNA populations from normal and abnormal regenerants was launched to identify the genes involved in the "mantled" abnormality. The technique adopted for this study was differential display reverse transcriptase-polymerase chain reaction (DDRT-PCR). This work was undertaken in conjunction with the plant biotechnology institute at the University of Paris XI, in Orsay, in connection with a project funded by the MPOB (formerly PORIM).

The Oil Palm Programme at a glance

38 staff, including 28 researchers
Partner countries : France, Côte d'Ivoire, Cameroon, Indonesia, Ecuador, Brazil
Institutions:
France: IRD, INRA, CNRS, universities of Paris VI, Paris XI, Montpellier II
Private companies:
Agro-industrial companies
Research topics:
- Support to smallholders
- Factors of sustainable intensification
- Integrated control of bud rot
- Vegetative propagation by somatic embryogenesis

Scientific partners

Research and development operations are carried out in a rich and diversified partnership with French and European research centres, national research centres in producing countries and the agro-industrial sector.

In countries of the South, these particularly involve:

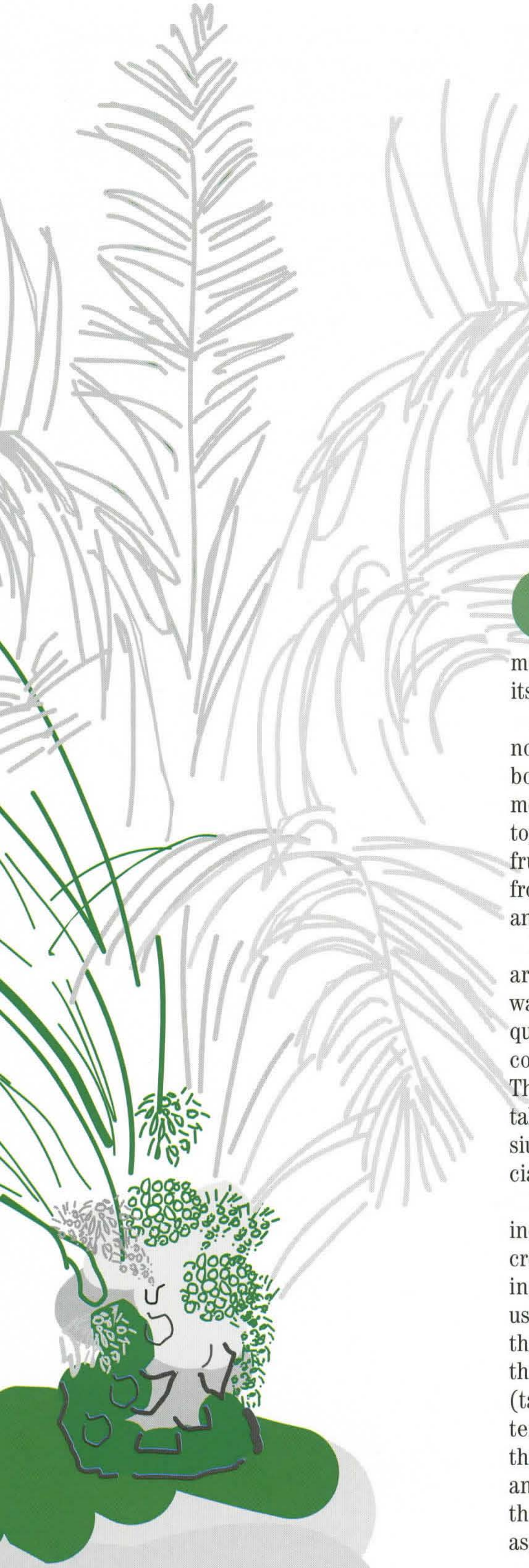
- the University of Bouaké (Côte d'Ivoire) and IRAD (Cameroon) for smallholder agricultural socio-economics,
- CNRA (Côte d'Ivoire), INRAB (Benin), IRAD (Cameroon), EMBRAPA (Brazil), SOCFINDO and IOPRI (Indonesia) for breeding activities,
- MPOB and FELDA (Malaysia), IOPRI and SOCFINDO (Indonesia), CNRA (Côte d'Ivoire) for biotechnology (*in vitro* culture and molecular markers),
- PT SMART and SOCFINDO (Indonesia) for fertilization and numerous other private and State partners, in Africa and Latin America,
- IOPRI (Indonesia), CNRA (Côte d'Ivoire), Palmeras del Ecuador (Ecuador) and Palmas del Espino (Peru) for integrated control of pests and diseases.

In industrialized countries, the scientific partners of the Programme are mainly:

- IRD for the study of somatic embryogenesis micropropagation protocols and the search for molecular markers of somaclonal variations,
- INRA in Versailles for olfactory pest trapping,
- CNRS-IBP in Orsay for molecular biology and somaclonal variations,
- Flottweg (Germany) for the Drupalm® extraction process,
- the Universities of Leicester and Exeter and IACR's Long Ashton Centre (UK) for molecular biology.

The gradual establishment of UMR (mixed research units) will enable greater integration of our research programmes in national academic structures, along with greater involvement of the Programme's researchers in teaching. ■

Oil palm empty fresh bunches: back to earth



CIRAD-CP has been working with the Indonesian group Sinar Mas since 1996 on optimizing the management of mineral and organic fertilization in its oil palm estates.

Making use of oil mill by-products, notably empty fresh bunches (EFB), for both economic, agronomic and environmental reasons, is one of the main research topics described here. These results are the fruit of cooperation involving researchers from SMARTRI (Smart Research Institute) and CIRAD-CP.

For each tonne of palm oil produced, around one tonne of EFB, considered as waste, have to be disposed of. Such large quantities of EFB were once a considerable constraint: they were usually incinerated. The ash was then spread in the estates to take advantage of the relatively high potassium content as a replacement for commercial potassium fertilizers.

Environmental concerns have led to incineration being banned in most recent crop development projects. A few composting units have been set up, but EFB are usually spread directly in the field. Farmers thereby benefit from the fertilizing value of this by-product, which is easily quantifiable (table 1). This application of organic matter offers benefits in the longer term for the physico-chemical properties of the soil, and more generally for soil fertility, even though only a few data are yet available for assessment.

Agricultural practices have been based on the results of field trials, such as the application of 60 tonnes of EFB/ha in a continuous single layer. These trials have made it possible to recommend application rates giving a level of oil palm mineral nutrition in line with production objectives.

However, optimization of EFB use requires better knowledge of the dynamics of organic matter mineralization and of mineral nutrient release depending on environmental conditions (rainfall, temperature, etc.).

Table 1. Fertilizing value of one tonne of EFB.

Fertilizers	Equivalence (kg)
Urea	6.1
Triple super phosphate	1.7
Potassium chloride	16.3
Kieserite	3.0

Methodology

An initial trial was set up under true application conditions in the field, arranged so as to enable a study of the phenomena involved. EFB were placed in the interrows of plantings, after prior characterization (individual weight, average water and mineral contents of the batch) and separated inside wide-meshed plastic nets (photos 1 and 2). At more or less regular intervals, from a few days to a few weeks depending on rainfall, the EFB were taken and analysed in the laboratory to determine the kinetics of mineral nutrition release and matter reduction. At the same time, analyses of the soil beneath the EFB were also carried out at different depths, to monitor changes in chemical properties.

[1] 1.5 kg of ash for 1.0 kg of KCl titrating 60% K_2O, sometimes increased to 2 kg of ash to take into account variability in water and potassium contents.

Infiltrometry measurements have also been programmed to try and characterize the impact on the physical properties of soils.

Dynamics of mineral nutrient release from EFB

Figures 1 to 3 show the dynamics of EFB mineralization and release of the main mineral nutrients (N, P, K, Mg). In an initial approach, simple mathematical functions were adjusted so as to facilitate interpolations for calculating the fertilizing capacity of EFB over time after their application (table 2). Rainfall recorded over open land, above the canopy, is also indicated.

Weight loss from the EFB was very rapid in the first month, with a reduction of almost 50% (figure 1). It continued thereafter at a more modest rate: eleven months after EFB application, there only remained 14% of the initial weight. Application of nitrogen to the EFB at the time they were spread (3 kg of urea per tonne of EFB) accelerated the process by almost 30% in the first ten days. This effect dropped to 25% then 20% in the following months. Application of phosphate (2.25 kg of triple super phosphate) also accelerated the mineralization process, but only in the first three months.

For mineral nutrients, the most notable result was the rapidity with which potassium, the major content of EFB, is released: one week after application in the field, 18% of the potassium was released, whilst 33 mm of rainfall were recorded in two showers. Three months after the start of the trial, there was virtually no more potassium in the EFB.

Phosphorus and magnesium dynamics were much slower and seemed to follow relatively similar kinetics. Nitrogen release was similar to that of magnesium in the first month, then it slowed down: eleven months after application, there still remained 40% of nitrogen in the EFB.

The release time for 50% of the mineral nutrients seems to be a good indicator of the different kinetics observed (table 3).

Change in the physico-chemical properties of soils

The above events occurring at the soil surface have a major impact on the physicochemical properties of the soil.

J. P. Caliman

Photos. Monitoring of changes in separate EFB in their nets.

Exchangeable potassium contents, which were initially very low—under 0.05 meq throughout the profile—were considerably increased by these ongoing supplies. These developments logically affected the surface horizons, then moved down: 0.40 m after a month, 0.80 m after 2-3 months (figure 4).

In addition, measurements taken deep down (1.40 m-1.50 m) did not seem to indicate losses through leaching.

When calculating variations in assimilable potassium over time totalized over a depth of one metre—zone extensively explored by the root system—a gradual increase was seen, reaching 20 times the initial K, three months after application, in accordance with the release kinetics for this nutrient. Eighteen months after the start of the trial, there existed eight times more exchangeable potassium compared to the outset. This potassium was then primarily between the 0.15 and 0.80 m horizons.

For exchangeable magnesium, only the 0 to 0.15 m surface horizons underwent profound changes (figure 5). Calcium seemed to be even less mobile, remaining in the first five centimetres of soil. Magnesium and calcium, which are bivalent ions, are in fact generally more effectively held than monovalent ions by organic matter, which was more abundant in these surface horizons.

Whilst the set of analyses carried out did not reveal any significant variation in nitrogen and carbon contents, the pH profile was considerably disrupted. From an acid profile at the beginning of the trial (pH ranging from 4.5 on the surface to 5.1 at a depth of 0.80 m), the pH increased very quickly on the surface, reaching neutral two months after the EFB were applied (figure 6). This effect then spread downwards with less intensity (5.4 at 0.60 m after nine months). A return to more acid pH values began after

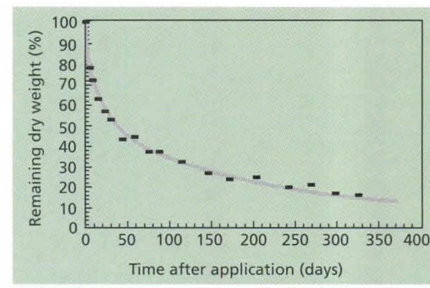


Figure 1. Kinetics of EFB mineralization after application in the field.

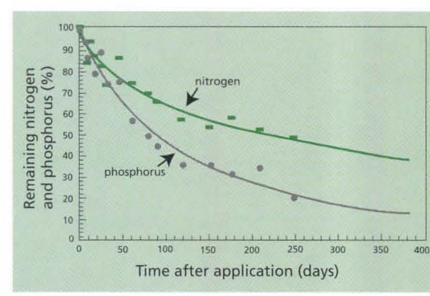


Figure 2. Dynamics of nitrogen and phosphorus release from EFB after their application in the field.

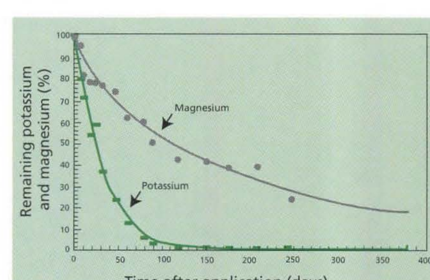


Figure 3. Dynamics of potassium and magnesium release from EFB after their application in the field.

Table 2. Fertilizing power of EFB (60 t/ha) in equivalent fertilizer released over time, without additional urea or TSP.

Time after application	Rainfall (mm)	Urea equivalent (kg/palm)	TSP equivalent (kg/palm)	KCl equivalent (kg/palm)	Kiéserite equivalent (kg/palm)
1 week	33	0.235	0.070	1.225	0.100
2 weeks	50	0.105	0.035	0.885	0.060
3 weeks	93	0.050	0.015	0.545	0.025
4 weeks	123	0.050	0.045	0.815	0.050
2 months	428	0.285	0.125	2.310	0.190
3 months	676	0.155	0.070	0.610	0.125
6 months	1 540	0.310	0.145	0.340	0.235
9 months	2 287	0.260	0.085	-	0.150
11 months	2 605	0.105	0.035	-	0.060
Total		2.600	0.725	6.800	1.125

TSP: Triple Super Phosphate

Table 3. Release time for 50% of nutrients or dry matter (in days).

N	P	K	Mg	Dry matter weight
205	85	25	115	40

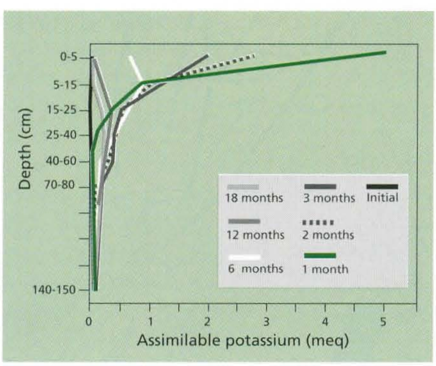


Figure 4. Variation in exchangeable potassium in the soil after EFB application in the field.

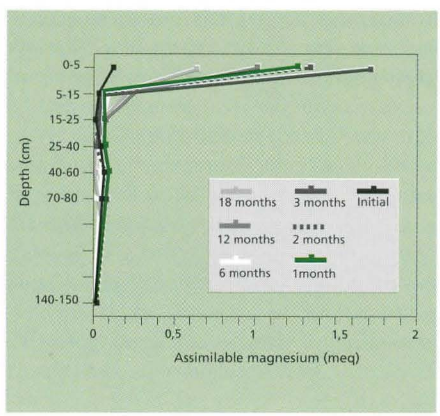


Figure 5. Variation in exchangeable magnesium in the soil after EFB application in the field.

six months for the upper horizons, later deeper down.

Opposing variations were recorded for exchangeable aluminium, which must undoubtedly have precipitated when the pH became higher than 5.5.

The first observations regarding the physical properties of the soil consisted in permeability measurements, comparing a control site that had never received any EFB and a site where applications had been carried out two years earlier. The measurements were taken using double Müntz cylinders. The initial results indicated much lower permeability at the control site, be it at the start of measurements or during stabilization of the infiltration rate (table 4 and figure 7).

All these phenomena could go some way to explaining the good performance of oil palms generally seen after EFB application, despite the apparent arithmetic imbalance in mineral nutrients (K and Mg) supplied.

Conclusions and prospects

The speed with which these nutrients are released prompts the following recommendations:

- halt mineral fertilizer applications (potash and kieserite) as soon as EFB are spread,
- apply EFB in the field as soon as they leave the mill, so as to avoid any losses through leaching, notably K, which might occur during storage, even temporary, in an open area unprotected from the climate.

The persistence of the improvement in exchangeable K and Mg contents in the soil indicates that, at the doses tested, applications every two years ensure generally satisfactory mineral nutrition for oil palms. Nevertheless, routine monitoring of the nutritional status of the palms is recommended.

Studies and analyses are continuing, with three main aims.

The first is to try and acquire a deeper understanding of the phenomena involved, so as to enable modelling for simulations

Table 4. Infiltration rate (cm/h).

Time (min)	Control	EFB
T 0	55	114
T 15	20	39

applicable to other situations. Analyses are under way using models with compartments characterized by different release speeds. Initial indications are that potassium could be totally "contained" within a single compartment, whereas the other nutrients (N, P, Mg) seem to be divided between at least two compartments.

The second consists in constructing relevant models irrespective of the application season. To that end, new observations are being carried out with different seasonal climatic conditions, primarily rainfall.

Lastly, work is continuing on a complete mineral nutrient cycle, from EFB to soil to plant. ■

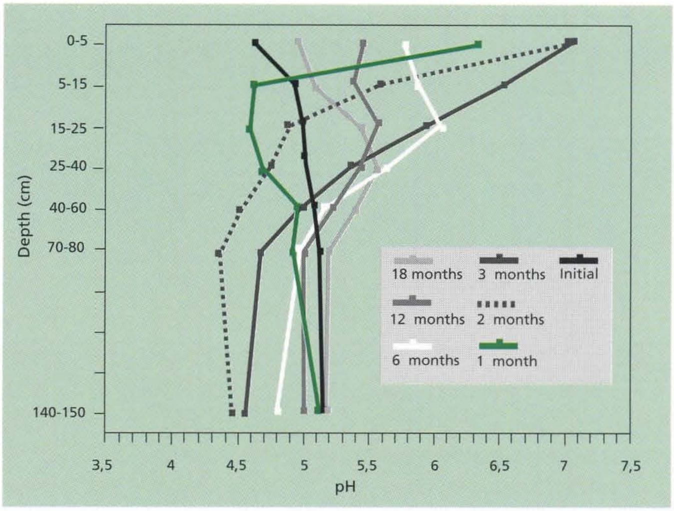


Figure 6. Variation in soil pH after EFB application in the field.

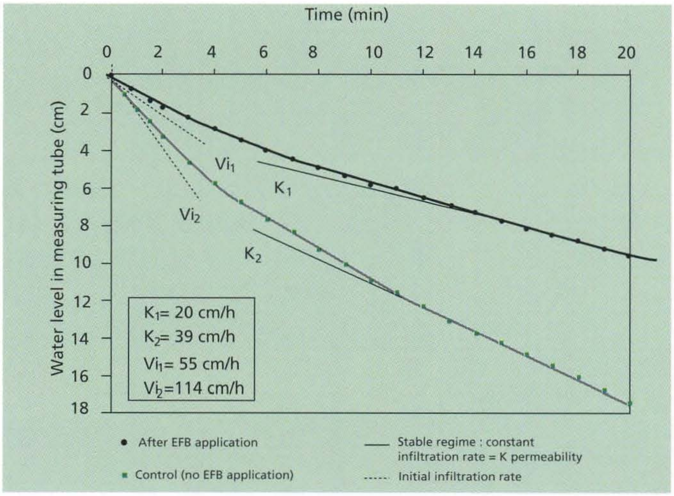


Figure 7. Impact of EFB applications on soil permeability.

List of publications

CALIMAN J.P, HARDIANTO J., NG M., 2001. Strategy for fertilizer management during low commodity prices. Proceedings of the 2001 Porim International Palm Oil Congress, 20-25 August 2001, Kuala Lumpur, Malaysia.

CALIMAN J.P., MARTHA B., SALETES S., 2001. Dynamic of nutrient release from empty fruit bunches in field conditions and soil characteristics changes. *In:* Proceedings of the 2001 Porim International Palm Oil Congress, 20-25 August 2001, Kuala Lumpur, Malaysia.

Bud rot in Latin America

The economic importance of oil palm bud rot in Latin America is considerable. The disease is particularly rife in Ecuador, Colombia, Brazil, Venezuela and Suriname. It can take several forms:

- a non-lethal form, followed by a high recovery percentage, notably in the Colombian Llanos,
- a lethal form, which is predominant in the Ecuadorian and Brazilian Amazon (photo 1).

It has already led to various estates or sectors of estates being totally abandoned: Turbo, in Colombia, in the 1960s; Victoria, in Suriname, between 1982 and 1986; Emade near Tefe, in Brazil, in 1985, along with Division I at Denpasa, near Belem, between 1985 and 1990. It has also caused considerable losses: at estates in the Ecuadorian Oriente (Huashito and Shushufindi) and on thousands of hectares in Colombia.

The bud rot research programme is the fruit of a multiple partnership, involving both the main central research centres in the commodity chain and plantation companies in Latin America.

In Latin America, CIRAD is working with two private Ecuadorian partners: Palmeras del Ecuador, the main partner, and Palmoriente, primarily for testing planting material with respect to bud rot. EMBRAPA, a State-owned Brazilian company, creates new hybrids used in part of these tests. A partnership, which is limited for the moment, is being established in Colombia with La Cabana, to assess planting material resistance to bud rot and continue the breeding programme, and with INDUPALMA, for hybrid production.

In Côte d'Ivoire, Benin and Cameroon, research is being conducted with CNRA, INRAB and IRAD respectively, to select *E. guineensis* material and test its resistance to bud rot in Latin America. Moreover, in Côte d'Ivoire, cloning of the backcross for molecular markers is being carried out with CNRA.

H. de Franqueville

Photo 1. Bud rot damage in Ecuador.

Epidemiology - Etiology

The biotic or abiotic origin of bud rot remains a controversy, and has yet to be elucidated. However, CIRAD has geared its research for many years towards biotic factors. No positive results have been obtained so far.

In1998, *Fusarium* inoculations were carried out in the soil, at the nursery stage. A few months later, in 1999, a bacterial suspension was applied to half the inoculated plants. This trial was based on the hypothesis of a soil-borne origin for bud rot, caused by hypovirulent strains of *Fusarium*, migrating to the bud systemically and subsequently encouraging bacterial development. Disease symptoms were not reproduced.

At the Shushufindi estate, in Ecuador, analysis and deduction work led to a hypothesis being put forward suggesting that bud rot type diseases of oil palm were transmitted via the roots by a sucking insect living in the soil.

The existence of a sucking insect in the soil around oil palm roots was confirmed in 1999. It was the bug *Cydnidae* of the genus *Scaptocoris*. Following these observations, transmission trials were set up in the prenursery and nursery. Results are awaited.

Detection of a neighbourhood effect

In 1983 a trial, SHGP 1, was set up in 4 Fisher blocks at the Palmeras del Ecuador estate to assess the performance of four categories derived from seed production (C1001, C2501, C6501, C7001). The experimental plots comprised 16 rows of 27 palms, i.e. a total for the trial of around forty hectares. The trial was not observed for FFB production, but mortality records have been kept on a monthly basis from planting to date.

An analysis of these records revealed the effect of certain factors involved in disease development. In this trial, the quarterly mortality rate—i.e. the proportion of palms dying in that quarter out of those present at the start of each quarter—literally exploded from 1995 onwards (figure 1). From 1983 up to the fourth quarter of 1994, the quarterly mortality rate barely deviated from the mean of 1.4 deaths for 1,000 palms. From the first quarter of 1995 onwards, that rate increased exponentially and reached 54 deaths for 1,000 palms at the end of 1999.

However, there was a substantial disparity in mortality rates depending on the cross categories (figure 2) and the replications. In order to obtain values characterizing each category of crosses, the mortality rate was modelled by logistic regression. This analysis revealed that mortality at a given date was greater in palms whose neighbours were affected by the disease. This result backed observations in the field of disease propagation from foci: palms dying at a given moment were especially those located at the edge of a focus extension (figure 3). This partly explains the suddenness with which the quarterly mortality rate increased: the more time went on, the more the palms had affected palms in their neighbourhood. The high mortality classes had an automatic increase in their number of infected palms, leading to greater overall mortality than would have been seen if all palms had remained in the same class.

The number of dead palms on each date among the nearest 24 neighbours to each palm (NdeadN) was thus included as a source of additional variation in the regression.

The importance of this factor was substantially the same as the cross category effect. Its introduction in the model considerably reduced the share of variance attributed to time, to replications and to category x replicate interaction (table 1). The assessment of a category was thus, in theory, more reproducible from one trial to the next.

Logistic regression consists in modelling the rate of probability of being affected over the probability of survival for palms in each category, in each quarter, in each replicate and for each of the 25 neighbourhood situations in which they can be found (have 0 dead neighbours, ..., 24 dead neighbours). This is known as the odds, or the relative risk of the "being affected" event for palms in a given context. The relative risk run by palms possessing any modality of a given factor varies, of course, depending on the modalities of the other factors. On the other hand, as the odds ratio run by palms only differs by a single factor, it does not depend on the level they possess for the other factors.

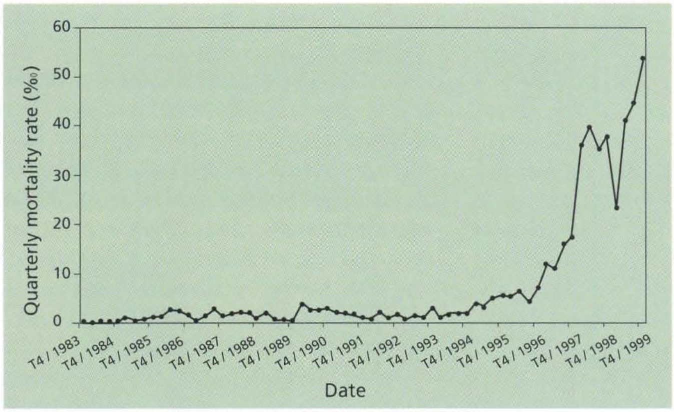


Figure 1.
Variation in quarterly mortality rate in trial SHGP 1.

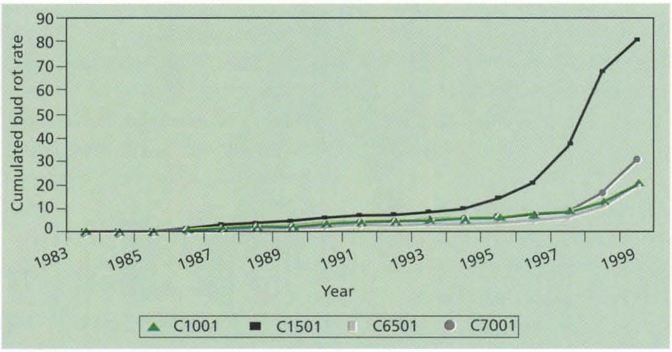


Figure 2.
Bud rot development depending on seed category.

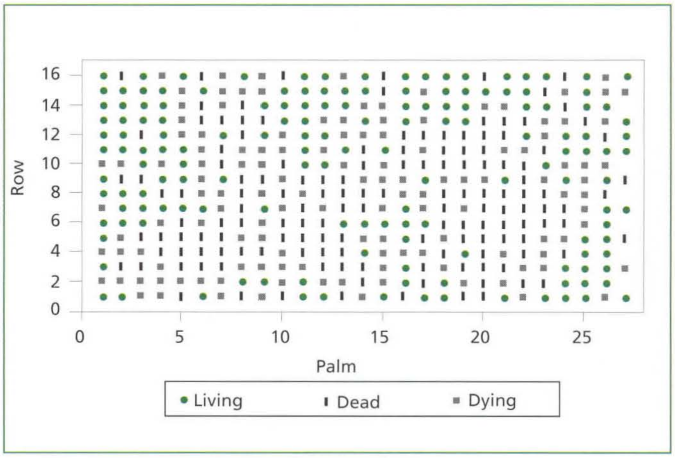


Figure 3.
Bud rot propagation from foci.

Tableau 1. Logistic regression of the quarterly mortality rate. Consequence of introducing NdeadN factor.

Source of variation	χ^2 without NdeadN	χ^2 with NdeadN
Quarter	4,911	1,433
Replicate	125	45
Category	2,100	577
Category x replicate	193	81
Number of dead neighbours	-	718

It appears that the relative risk run by palms with 4 neighbours affected out of 24 is twice as high as that for palms whose neighbours are all still alive, all other things being equal. This risk increases in line with the number of dead neighbours, becoming 10 times greater beyond 50% of losses in the neighbourhood (figure 4) than for palms surrounded by healthy neighbours.

Taking into account the neighbourhood effect, category C1501 was the only one significantly more affected than the others (table 2), the relative risk being twice as great, all other things being equal, for the palms in this category than for palms in C1001. Photo 2 illustrates differences in the field between C1501 and C7001.

This way of calculating the value of crosses, the merits of which are shown in this preliminary trial in reducing the environmental effect, will be applied as soon as the first observations in the new genetics trials become available.

The first trials were alll set up on forest and replantings began a few years ago. The effect of bud rot is particularly rapid in replantings. In a trial planted in 1997 with material of the C2501 and C1001 origins, intended to test cultural practices, the average mortality rate due to the disease was over 20%, only three years after replanting. It has not been possible to detect any effect of cultural practices.

In view of this result, it was decided to set up genetic trials with *E. guineensis* material, which could offer resistance to bud rot according to observations in the first planting generation. A honeycomb trial was set up in which 7 different crosses were assessed, each palm being surrounded by a palm of the other 6 crosses. Another trial in an elementary plot of 9 palms with 15 replicates (41 crosses, including the 7 in the honeycomb trial) was set up. An identical trial was planted on an extension (41 treatments). The data will be used employing the method already described and we shall be able to compare the efficacy of the choice of design adopted. In addition, a bud rot disease has been detected in Colombia that is similar to that in Ecuador, but with different symptoms. Various trials with the same origin as that tested in Ecuador (C07**) are planned, and the seeds have been sent to Colombia.

Search for high-yielding and resistant planting material

The provisional solution adopted in replantings is to use interspecific hybrid material between *Elaeis guineensis* and *Elaeis oleifera*. This material raises almost total resistance to the different bud rot diseases in Latin America, but oil production is 30% lower than that of *Elaeis guineensis*.

The results obtained with hybrids of the Coari x La Mé types planted without a statistical design in Ecuador are excellent for FFB production and at least equivalent to those of the best *Elaeis guineensis*. However, the oil extraction rate from FFB is lower than that of *Elaeis guineensis* due to lower fertility. The main problem encountered when using these hybrids is pollination. Indeed, this material is extremely feminine and the rare male inflorescences that appear only produce a small quantity of viable pollen. The attraction of pollinating insects to the male inflorescences is low, whereas attraction to the female inflorescences of these hybrids is good.

In order to search for hybrids with greater pollen fertility and thereby limit the practice of assisted pollination, several trials have been set up in Ecuador designed to test hybrids corresponding to *E. oleifera* materials surveyed in the Brazilian Amazon basin crossed with the La Mé *E. guineensis* origin.

Merits of *in vitro* cloning and research prospects

The programme to search for high-yielding material with resistance to bud rot requires the introgression of resistance from *E. oleifera* into high-yielding *E. guineensis*. In order to implement that programme, it is necessary, for each backcrossing cycle, to assess the resistance of each parent, which is impossible as knowledge stands at the moment. In order to remove that obstacle, each parent needs to be multiplied, so that at least 50 individuals can be planted in a trial in a zone where bud rot is rife, and an idea can be gained of the resistance of this genotype. The first step was to clone a palm derived from a first generation backcross and plant a large number of its progenies in a zone severely affected by bud rot, in both

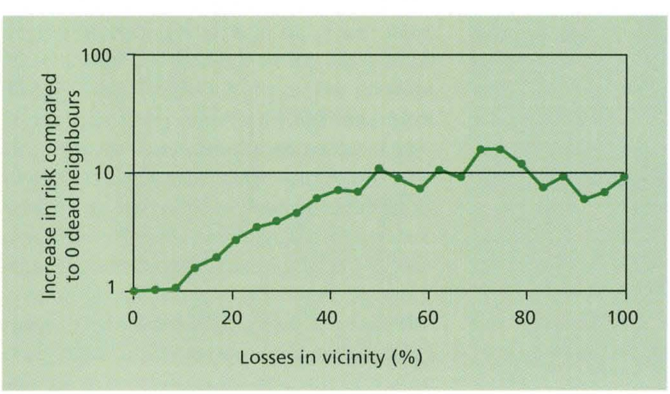


Figure 4.
Variation in risk depending on the neighbourhood situation.

Tableau 2. Risks run by the palms of each category compared to those of C1001.

Category	Oldds ratio	Group according to contrasts test
C1501	2.06	A
C6501	1.00	B
C7001	1.38	B
C1001	1.00	B

Photo 2.
Difference in mortality between seed categories.

Brazil and Ecuador. The resistance value of that clone should be known in a few years. However, it is impossible to clone all the palms of all the backcrosses created.

The search for molecular markers of resistance has been launched, in order to develop an early selection method.

To that end, embryos from a first generation backcross have been cultured *in vitro* and 200 clones, each represented by 50 palms, will be replanted in a zone severely affected by the disease. The material is currently in the laboratory, and the first plantings are to take place in 2002. The clones derived from this backcross will be mapped and markers of resistance to bud rot will be sought. This material will also be planted in a bud rot-free zone, in order to assess its other agronomic characteristics.

Development of such a tool is essential for an initial screening of all the palms of backcrosses 1 and 2 planted at various research centres and selected solely for their agronomic value.

The marker(s) of resistance to bud rot should make it possible to monitor the introgression of these traits in the best *Elaeis guineensis* palms.

At the same time, there are plans to test these clones in Africa, to search for one or more markers of resistance to vascular wilt provided by *Elaeis oleifera*. ■

List of publications

FRANQUEVILLE H. DE, 2000. Oil palm bud rot in Latin America. *In :* International Seminar: Agribusiness of palm oil: a social, economic and environmental alternative for sustainable development of the Amazon region. IICA-Procitropicos, Belem, Brazil, 16-20 October 2000.

FRANQUEVILLE H. DE, 2001. La pourriture du cœur du palmier à huile en Amérique latine. Revue préliminaire des constats et des acquis, 34 p., http://www.burotrop.org/accueil.htm

Controlling somaclonal variations induced in oil palm by *in vitro* micropropagation

Mass micropropagation of oil palm by somatic embryogenesis has been carried out by several teams worldwide since the 1970s, to multiply elite genotypes obtained in breeding programmes. This approach helps to overcome the high degree of variability within improved progenies, so that the best individuals can be exploited. The joint CIRAD-CP/IRD team in Montpellier has developed a regeneration procedure based on somatic embryogenesis, which has been transferred to several laboratories in producing countries: Malaysia, Indonesia, Côte d'Ivoire.

A network of partners has been set up, linking the team to several research laboratories in France (universities of Paris VI, Paris XI, IBP-CNRS) and to private and State sector partners in producing countries: CNRA (Côte d'Ivoire), FELDA (Malaysia), IOPRI (Indonesia), SOCFINDO (Indonesia).

Genetic fidelity of plants obtained by somatic embryogenesis

The CIRAD-IRD process has already led to the production of around 1.2 million plants regenerated *in vitro* known as "ramets", representing a thousand different genotypes. However, a proportion of ramets (5-10%) reveal a so-called "mantled" variant phenotype in terms of floral architecture (photo). In the most serious cases, the abnormality leads to sterility in the clonal palm. Clonal plantation yields can therefore be disrupted by the existence of such somaclonal variants. The abnormality rate varies from 0% to 80% depending on the line considered: this is an unacceptable risk for growers and currently limits commercial development of *in vitro* culture as a mass propagation technique for oil palm.

Several earlier observations suggested that an epigenetic phenomenon was involved, not a conventional genetic mutation. In particular, reversion towards a normal phenotype has been observed in the field for a large percentage of palms, even in the most serious cases. In addition, genetic crosses have demonstrated that the floral abnormality can be transmitted to progenies, though by a non-Mendelian process. These two observations are incompatible with the hypothesis of conventional genetic mutation and vindicate the pursual of an epigenetic hypothesis, whereby genome expression is affected rather than its structure.

The CIRAD-IRD team in Montpellier has launched a research programme using state-of-the-art molecular biology techniques, to understand and control somaclonal variations in oil palm. The purpose of this work is to identify one or more molecular markers of the mantled abnormality, in order to eliminate off-type plants before they are planted out, and perfect the propagation process so that variants are not produced.

Two overall approaches have been taken: firstly, the structure of the genome and its methylation rate were analysed in relation to the mantled abnormality (DNA approach), then differential genetic expression was investigated (RNA approach).

No difference in ploidy or in the quantity of nuclear DNA was revealed by flow cytometric analyses between normal and abnormal regenerants. A study was therefore made of genome structure, using the RAPD method. No polymorphism was detected between normal and abnormal regenerants, nor between regenerating plants and the mother-palm. AFLP was then used to carry out a more detailed analysis: as in the previous experiments, no difference was identified between normal and abnormal regenerants.

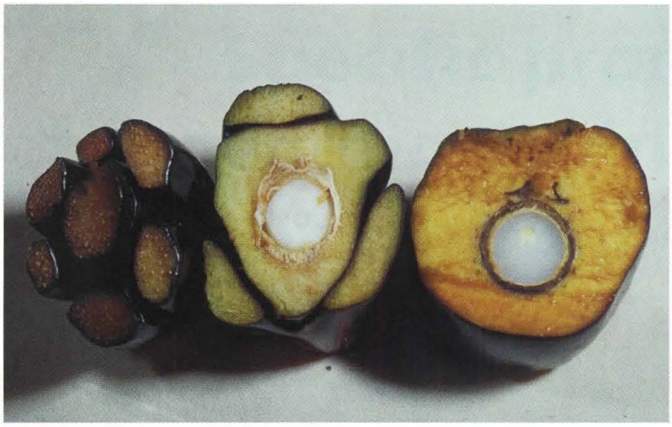

A. Rival

Photo. From left to right: fruits of oil palm regenerants with a seriously abnormal, slightly abnormal, or normal phenotype.

Study of genome structure and DNA methylation

All the results described above back the hypothesis of an epigenetic mechanism controlling the occurrence of mantled regenerants. Subsequent studies therefore focused on the potential role of DNA methylation. The DNA methylation rate was quantified on a genome-wide basis using two complementary methods: high pressure liquid chromatography (HPLC) of nucleosides, and another enzymatic type method, SssI-methylase accepting assay. A significant difference (0.5-2.5%) was detected in the methylation rate between DNA extracted from normal and abnormal regenerants of the same clonal progeny, with the variant regenerant always showing hypomethylation (table, figure 1). The difference was even more marked for *in vitro* cultures: fast-growing calli, which produced 100% variant regenerants, had a genomic methylation rate of – 4.5% compared to nodular calli, which regenerated approximately 95% normal ramets. Unfortunately, quantification of overall genomic methylation cannot be used to carry out a "blind" test of clonal conformity, as there is a genotype effect of the mother-palm that is just as powerful as the epigenetic factors arising from *in vitro* culture. Yet these results are highly encouraging, since they enable a distinction to be made at the molecular level between normal ramets and variants of the same progeny. Gene specific methylation studies, which are under way, will provide a clearer understanding of the role played by methylation and will doubtless help in developing a clonal fidelity test based on this phenomenon.

Study of differential genic expression

A differential analysis programme was launched involving RNA populations, with a view to developing genes affected by the mantled abnormality and using that information to check regenerated plants. The technique adopted for this study was differential display reverse transcriptase-polymerase chain reaction (ddRT-PCR). The work was carried out jointly with the plant biotechnology institute at the University of Paris-XI, Orsay, under a three-year project funded by MPOB (formerly PORIM, Palm Oil Research Institute of Malaysia).

Leafed shoots grown *in vitro* were chosen for the differential display experiments, in order to analyse material that was as uniform as possible. Putative cDNA markers of the abnormality identified by ddRT-PCR were cloned and sequenced, and their expression validated by the Northern hybridization technique. Each marker proving to be truly differential was then tested on other *in vitro* cultures, both true-to-type and variants, in order to assess its reliability as a marker of the "mantled" abnormality. After this second validation stage, the cDNA which still proved worthwhile were examined more closely, by analysing their tissular expression specificity. Lastly, a cDNA library was screened to obtain "full length" clones for complete sequencing. Such an approach provided information on the protein coded by the gene in question and enabled identification of its possible function.

Studies on expression markers currently stand as follows:

- total number of potential markers identified by ddRT-PCR: 46,
- number of individual cDNA cloned: 58,
- number of cDNA validated by Northern hybridization: 16
- number of normal specific cDNA: 9,
- number of abnormal specific cDNA: 7.

Several markers have been selected for more in-depth studies, including the marker illustrated in figure 2. This putative marker gave good discrimination between true-to-type and variant cultures at the cal-

Tableau . Standardized methylation index (SMI) calculated by SssI-methylase accepting assay of DNA extracted from calli.

Clone	Type	SMI ± standard deviation
LMC 458	NCC	1.74 ± 0.43[a]
	FGC	1.01 ± 0.04[b]
LMC 464	NCC	1.39 ± 0.15[a]
	FGC	0.93 ± 0.29[b]

Data followed by different letters are significantly different at 5%.

NCC: nodular compact calli.

FGC: fast-growing calli

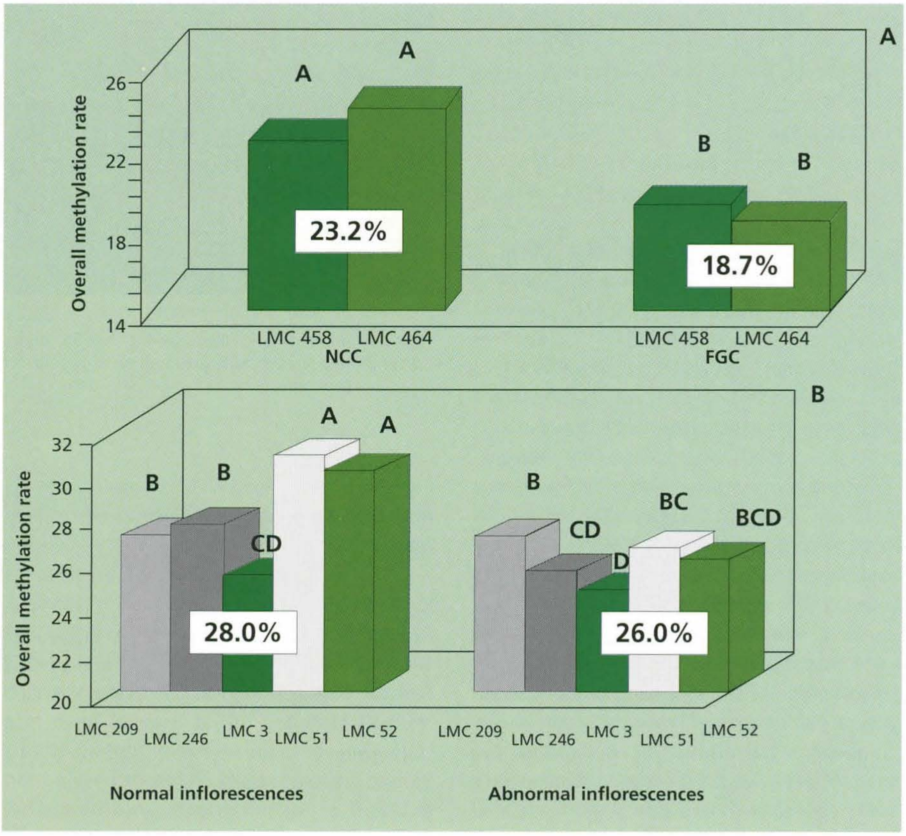


Figure 1.
Overall methylation rate in DNA from embryogenic calli (A) and immature inflorescences (B) of oil palm, calculated after HPLC analysis of nucleosides. The data associated with the different letters are significantly different at 1‰ (calli) or 5% (inflorescences).
NCC: Nodular compact calli.
FGC: fast-growing calli.

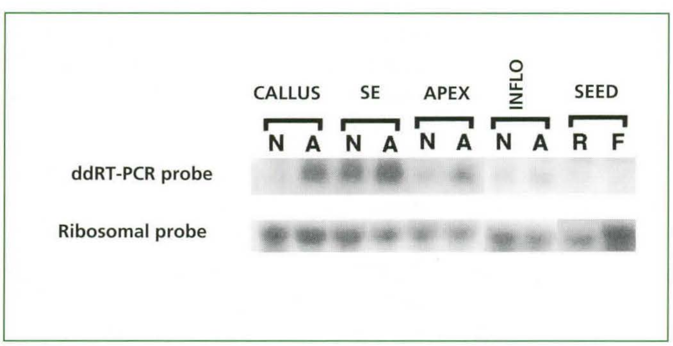


Figure 2.
Validation of a potential marker of the mantled abnormality by Northern hybridization and characterization of the tissular specificity of corresponding messenger RNA accumulation.
N: normal culture; A: culture with the mantled abnormality; SE: somatic embryo; APEX: part of leafed shoot obtained by *in vitro* culture containing stem apex; INFLO: inflorescence; SEED: seed-derived plant.

lus stage. The gene in question, which codes for a protein playing a possible role in response to stress, was expressed in *in vitro* cultures at all stages, whilst in the whole plant the transcripts were only found in inflorescences. Other "abnormal specific" genes identified in this study would also seem to code for proteins involved in the response to stress, which might be explained by the physiological effects of *in vitro* culture.

Prospects

These encouraging results will be followed up in an assessment of the markers on a wide range of genotypes. Two approaches are being considered for developing fidelity tests, bearing in mind their respective cost and ease of use. Firstly, if differential accumulation of proteins coded by the genes in question can be shown, clonal fidelity could possibly be assessed by simple quantification using an antibody, such as ELISA tests. Secondly, if one of the differential expressions identified is linked to differential gene specific methylation, another possibility will be to develop a test using methylation specific-polymerase chain reaction (MS-PCR), with PCR depending on the methylation status of the substrate DNA.

A reliable test of clonal fidelity would solve the main difficulty holding back further commercial use of clonal micropropagation of oil palm on a large scale. If somaclonal variations were controlled, growers could rapidly benefit from the genetic progress achieved in breeding programmes, and the resulting yield improvements could be substantial (10 to 30%). In addition, the regeneration programme based on liquid medium developed by the CIRAD-IRD team could then be used to obtain a considerable reduction in production costs. ■

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The Drupalm® process An exemplary research and development phase A more difficult industrialization phase

The Drupalm® palm oil extraction process originated from the idea of transferring centrifugal extraction technology, which had been widely used since the 1960s for olive oil production, to oil palm, the only other oilcrop fruit used on an industrial scale. This process, which was designed by CIRAD and jointly developed by CIRAD and the Flottweg company, with assistance from ANVAR, provides an alternative to the conventional process.

The first trials designed to determine the feasibility of the process were carried out at the oil mill of the Clermont l'Hérault oilseed cooperative (France) with sterilized fruits prepared and shipped by the Pobé research station in Benin. True scale trials were conducted in Cameroon at the Nkapa factory belonging to the SOCAPALM company.

The Drupalm® process

Unlike the traditional process, the technology does not involve separate extraction of palm oil and palm kernel oil. It extracts oil from the fruit in a single stage to obtain an oil called Drupalm® consisting of a mixture of palm oil and palm kernel oil in proportions approaching 95% and 5%. Drupalm® is a registered trademark and the process, also known as Drupalm®, has been patented.

The figure shows the overall Drupalm® process.

FFB reception

The FFB reception procedure is identical to that applied in a conventional oil mill, with FFB checking and weighing prior to storage.

Sterilization

Sterilization is carried out to enable efficient fruit stripping and to stop acidification of the oil they contain. Unlike the conventional method, no attempt is made to facilitate kernel extraction. Simple steam pressure cooking is therefore enough and the installations, for the same capacity, are therefore smaller than in the conventional process.

Stripping

Stripping is identical to that of the traditional process. Operating in two stages is recommended for large capacities, in order to achieve maximum efficiency. After initial stripping, the empty bunches are passed through a "empty bunch crusher" prior to a second stripping operation.

Washing-stone removal

Washing and stone removal are not needed in the conventional process, but are essential in this case, to protect the crushers from metal objects and stones, which could seriously damage them. This also serves to remove a large proportion of sand and soil, which occur in large quantities in FFB and are the main cause of wear seen in all the equipment used in traditional oil mills.

Crushing

The sterilized, washed and stone-free fruits are crushed (photo 1) to release the oil contained in the cells. Crushing is carried out in one or more phases, depending on the installed capacity. The supernatant phase containing most of the oil extracted during the washing-stone removal operation is added to the crusher with the fruits to facilitate the operation, reduce the energy consumed, prevent overheating, which is detrimental to the end-quality of the product, and ensure appropriate viscosity in the fruit mash.

Digestion

The purpose of digestion is to trigger and promote coalescence of the oil droplets contained in the mash, so as to form the phases that will subsequently be separated. This essential operation is carried out in a horizontal double inverted-pitch screw digester (photo 2), operating at reduced speed to avoid any risks of emulsion and to ensure efficient and uniform stirring. The digester body has a double steam jacket, to maintain the temperature by circulation of hot water or low pressure steam. Unlike the conventional process, there is no live steam injection into the mass of the product, and there are therefore no risks of quality deterioration due to excessive temperature surges.

Separation

The digested mash is transferred by a volumetric screw pump to a continuous decanter (photo 3), where it is separated into three phases:

- an oily phase containing over 99.5% oil, some dirt (0.1%) and traces of moisture (0.4%),
- an aqueous phase, known also as the heavy phase, primarily consisting of more than 96% water, traces of oil (around 0.5%) and non-oily solids,
- a solid phase containing around 50% water and 8 to 10% oil.

Oil purification

Purification is intended to reduce impurities in the oil to under 0.02%. Depending on the capacity of the installations, purification is carried out by simple static decantation in a heat-insulated tank or by passing through a centrifuge (photo 4). In the latter case, oil moisture is also reduced below 0.3%.

Oil drying

The oil moisture content is reduced to around 0.1% by dehydration carried out in a vacuum dehydrator, in order to prevent any overheating of the product. Under these

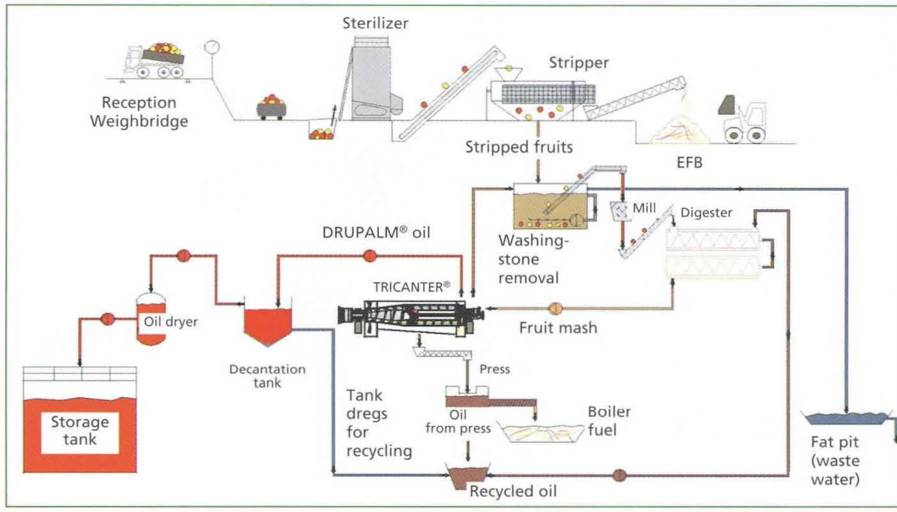

Figure. Diagram of the Drupalm® process.

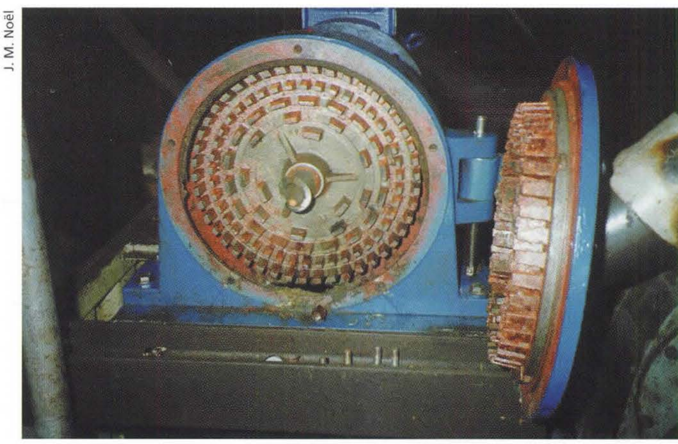

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Photo 1. Disk crusher.

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Photo 2. Digester.

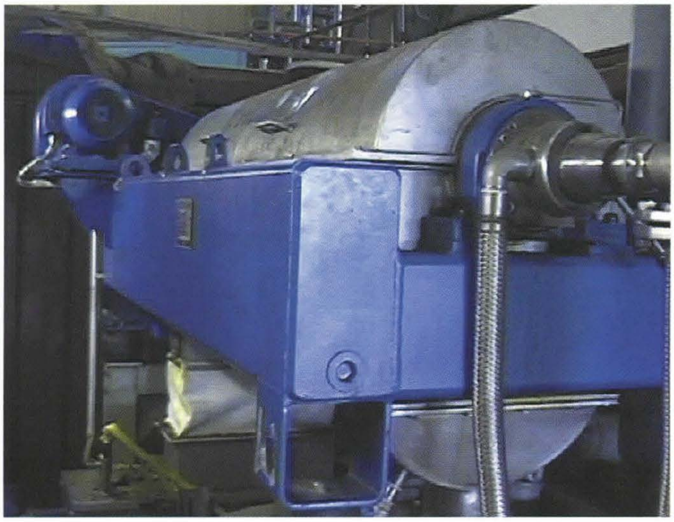

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Photo 3. Three-phase decanter.

impurity and moisture conditions, oil quality is preserved during storage, even over long periods.

Pressing

The solid phase from the decanter contains fruit fibres and pieces of shell and kernel. After drying to reduce moisture content to under 40%, it can be used as fuel in the steam boiler. However, it is preferable to press this solid phase, which still contains oil that is extractable with a simple screw press (photo 5) or even a hydraulic press, depending on the capacities installed. The operation offers the dual advantage of:

- reducing oil losses in the solid phase by about half, and thereby improving the overall extraction rate by around 1% in relation to bunch weight,
- reducing moisture in the presscake to a maximum value of 40%, which is compatible with its direct use in the steam boiler, after simple breaking up in a cake breaker-conveyor.

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Photo 4. Oil polisher.

The liquid from the press, which may contain up to 15% oil depending on the dilutions carried out before pressing, is recycled to the digester, or crusher if needed.

Drupalm® oil

Drupalm® oil (photo 6) is a new product which lends itself perfectly to all downstream processing operations, since:

- its refinability is identical to that of palm oil,
- during fractionation, olein yield is slightly better due to the eutectic effect obtained by the palm oil-kernel oil mixture,
- its possible uses as salad oil, cooking oil, soap and margarine manufacture have been verified and acceptance tests carried out with consumers have proved positive.

Its use to meet the requirements of producing countries no longer raises any problem. However, as a new product, it has yet to find its place on the international market, where it needs to be introduced as soon as production volumes are sufficient.

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Photo 5. Screw press.

Advantages of the process

The merits of the process result from a reduction in the number of operations required. The advantages are therefore multiple and include a reduction in:

- direct or induced investments, equipment and buildings (no kernel recovery station or mill for kernel oil extraction),
- the installed electrical power,
- steam consumption for sterilization and clarification,
- the quantities of effluents discharged and their BOD_5 content (biochemical oxygen demand in 5 days),
- production and maintenance costs.

In addition, the process ensures optimum performance for capacities ranging from a few tonnes of FFB per hour and its modular nature enables investment in stages, as plantation yields rise. This is all the more true in that the energy balance is always favourable, with a fuel possessing a higher thermal capacity and available in large quantities, to meet lower requirements.

Lastly, trials have shown that, unlike in the conventional process, a drift in operating conditions has little effect on the performance of the Drupalm® process.

Research and development phase

The research and development phase took place over a period of four years, from 1992 to 1996. Technical validation of the Drupalm® process was obtained in three sets of trials. During these trials, several dozen tonnes of FFB were processed by this new extraction method, producing almost 20 tonnes of oil.

Industrialization phase

The industrialization phase, which was launched in 1997, has yet to lead to the first firm equipment sale, despite major commercial efforts, such as participation in several conferences and exhibitions. In 1998, the project to construct a 12 t/h oil mill on behalf of an oil palm growers' cooperative in southwestern Côte d'Ivoire almost went ahead, but despite the major guarantees provided by the consortium of constructors and an identified operator, the main funding agency pulled out at the last minute, due to the technological risk associated with the use of a new process in an industrial context. However, that decision was primarily due to the absence of immediate outlets on the international market, as outlets at the time for this new oil were restricted to the local market.

In the Autumn of 1999, another 6 t/h unit was sold, subject to performance, to a Peruvian company. Unfortunately, there was a cumulation of very diverse problems for performance of the contract: delayed deliveries due to a ship cancellation, considerable delays in transferring the equipment from the unloading port to the site, multiple technical problems encountered with start-up of the installations, impossibility of extending the duration of temporary importation for the equipment. Flottweg was obliged to re-export the equipment before its performance could be demonstrated.

A unit with the same capacity is currently operating in Malaysia, near Kuala Lumpur. The experience acquired in Peru, which was used to optimize the equipment, and the more favourable technical environment in Malaysia suggest a more successful outcome. In addition, the duration of temporary importation was increased to one year this time, with the possibility of an extension.

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Photo 6.
Drupalm® oil leaving the decanter.

If successful, which will only become clear in 2001, it will have taken more than 5 years between the end of process validation trials in the industrial pilot unit, to operation of the commercial demonstration unit. ■

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List of acronyms

AARD	Agricultural Agency for Research and Development (Indonesia)
ACPC	Association of Coffee Producing Countries (UK)
ACRI	American Cocoa Research Institute (USA)
ADB	Asian Development Bank (Philippines)
AFD	Agence française de développement (France)
AFLP	Amplified Fragment Length Polymorphism
AMAP	Atelier de modélisation de l'architecture des plantes, CIRAD (France)
ANADER	Agence nationale d'appui au développement rural (Côte d'Ivoire)
ANRA	Professional Association of Natural Rubber in Africa (Côte d'Ivoire)
APCC	Asian and Pacific Coconut Community (Indonesia)
ATP	Interorganization thematic research project
BNETD	Bureau national d'études techniques et de développement (Côte d'Ivoire)
BPPS	Balai Penelitian Perkebunan Sembawa (Indonesia)
BUROTROP	Bureau for the Development of Research on Tropical Perennial Oil Crops (France)
CABI	Cab International (UK)
CABI-ARC	Cab International - African Regional Centre (Kenya)
CAOBISCO	Association of the Chocolate, Biscuit and Confectionery Industries of Europe (Belgium)
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza (Costa Rica)
CCRI	Cocoa and Coconut Research Institute (Papua New Guinea)
CDC	Cameroon Development Corporation (Cameroon)
CDM	Coconut Data Management
CENIPALMA	Centro de Investigación en Palma de Aceite (Colombia)
CFC	Common Fund for Commodities
CGRD	Coconut Genetic Resources Database
CIFC	Centro de Investigação das Ferrugens do Cafeeiro (Portugal)
CNRA	Centre national de recherche agronomique (Côte d'Ivoire)
CNRS	Centre national de la recherche scientifique (France)
COGENT	Coconut Genetic Resources Network (Singapore)
CRF	Coffee Research Foundation (Kenya)
CRU	Cocoa Research Unit (Trinidad and Tobago)
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuaria (Brazil)
FAO	Food and Agriculture Organisation (Italy)
FCC	Fédération du commerce des cacaos (France)
FELDA	Federal Land Development Authority (Malaysia)
GAPKINDO	Association of Indonesian natural rubber producers-exporters (Indonesia)
GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit (Germany)
HEVEGO	Société hévéicole du Go (Côte d'Ivoire)
IBP	Institut de biotechnologie des plantes (France)
IFC	Institut français du caoutchouc (France)
IACR	Integrated Approach to Crop Research (UK)
ICCO	International Cocoa Organization (UK)
ICO	International Coffee Organization (UK)
ICRAF	International Centre for Agroforestry (Indonesia)

IFOCA	Institut national de formation et d'enseignement professionnel du caoutchouc (France)
IHCAFE	Instituto Hondureño del Café (Honduras)
IICA	Instituto Interamericano de Cooperación para la Agricultura (Costa Rica)
IITA	International Institute of Tropical Agriculture (UK)
INCO	International cooperation (Belgium)
INIA	Instituto Nacional de Investigación y Tecnologia Agraria (Spain)
INIAP	Instituto Nacional de Investigaciones Agropecuarias (Ecuador)
INRA	Institut national de la recherche agronomique (France)
INRA-PIAF	Institut national de la recherche agronomique - physiologie intégrative de l'arbre (France)
INRA-SAD	Institut national de la recherche agronomique - systèmes agraires et développement (France)
INRAD	Institut national de recherche agronomique du Bénin (Benin)
INRO	International Natural Rubber Organisation
IOPRI	Indonesian Oil Palm Research Institute (Indonesia)
IPGRI	International Plant Genetic Resources Institute (Italy)
IRAD	Institut de recherches agronomiques pour le développement (Cameroon)
IRAG	Institut de recherche agronomique de Guinée (Guinea)
IRD	Institut de recherche pour le développement (France)
IRSG	International Rubber Study Group (UK)
IRCC	Institut de recherches sur le caoutchouc du Cambodge (Cambodia)
IRRDB	International Rubber Research and Development Board (UK)
IRRI	Indonesian Rubber Research Institute (Indonesia)
ISIC	Institut scientifique d'information sur le café (France)
ITRA	Institut togolais de la recherche agronomique (Togo)
MPOB	Malaysian Palm Oil Board (Malaysia)
NPP	Natural Plant Protection (France)
OTA	Ochratoxin A
PAR	Photosynthetically Active Radiation
PCA	Philippine Coconut Authority (Philippines)
PORIM	Palm Oil Research Institute of Malaysia (Malaysia)
PROCAFE	Fundación Salvadoreña para Investigaciones del Café (El Salvador)
PROMECAFE	Programa Cooperativo Regional para la Protección y Modernización de la Caficultura (Costa Rica)
PT SMART	PT Sinar Mas Agro Resources and Technology Corporation (Indonesia)
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RC'2	Relance de la caféiculture, seconde phase
RECA	Réseau de recherche caféière en Afrique
RFLP	Restricted Fragment Length Polymorphism
RRIT	Rubber Research Institute of Thailand (Thailand)
RRIV	Rubber Research Institute of Vietnam (Vietnam)
SCAC	Service de coopération et d'action culturelle (France)
SMARTRI	Smart Research Institute (Indonesia)
SOCAPALM	Société camerounaise des palmeraies (Cameroon)
SOCFINDO	Société financière d'Indonésie (Indonesia)
SRAP	Smallholder Rubber Agroforestry Project (Indonesia)
SRPH-INRAB	Station de recherche du palmier à huile - Institut national de recherche agronomique du Bénin (Benin)
UMR	Mixed research unit
UNOCACE	Union de las organizaciones campesinas cacaoteras de Ecuador (Ecuador)
USDA	United States Department of Agriculture (USA)
VARTC	Vanuatu Agricultural Research and Training Centre (Vanuatu)
VPD	Vapour Pressure Deficit

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