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ORAL PRESENTATIONS

GENETICS

QUANTITATIVE GENETICS

Creation and use of Bacterial Artificial Chromosome (BAC), genomic resources for *Theobroma cacao* L.

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Abstract

To develop genomic resources to study the structure and evolution of *Theobroma cacao* L. genome, we constructed a BAC library for the genotype Scavina 6 (Sca6).

Nuclei were isolated from leaves with protocol to eliminate pectin contamination and reduce polyphenol oxidase activities present in the cocoa cell extracts. Digestion of the DNA was carried out with the *Hind* III restriction enzyme. A cocoa purified genomic DNA were ligated to pCC1BAC *Hind* III–Cloning Ready vector and after ligation, transformations were carried out with *Escherichia coli*

The BAC library contains 36864 clones with an average insert size of 120 Kbp which represent 10 haploid genome equivalents. Screening of the BAC library with a set of 14 genetically-anchored single copy probes identified an average of 9 BAC clones per probe, showing that all ten linkage groups are well represented in the BAC library. A set of resistance gene analogs (RGAs), previously mapped in cocoa, were used to identify RGA-containing BACs. This represent the first step to characterize identified QTLs for resistance to cocoa pathogens like *Crinipellis pernicioso* and *Phytophthora spp.*

This library represents the first available BAC resource for structural genomic studies in cocoa and a valuable resource to physically characterize mainly QTLs for disease resistance and map-based cloning of resistance genes to major cocoa pathogens like *Phytophthora spp* (*palmivora* and *megakarya*) and *Crinipellis pernicioso* . Also this library represents a valuable resource to physically characterize QTL for other agronomic traits and it is available for the cocoa researchers' community.

RESULTS AND BENEFITS OF THE CFC/ICCO/IPGRI PROJECT ON 'COCOA GERMPLASM UTILISATION AND CONSERVATION, A GLOBAL APPROACH'

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Abstract

The CFC/ICCO/IPGRI project on 'Cocoa Germplasm Utilization and Conservation, a Global Approach' (1998-2003) has significantly increased international collaboration on evaluation, selection and conservation of cocoa germplasm with the aim of producing better varieties. National research institutes in ten cocoa producing countries, CRU, CIRAD, the University of Reading and IPGRI have participated in the activities. The project has been jointly financed by the Common Fund for Commodities (CFC), supervised by the International Cocoa Organization (ICCO) and co-ordinated by IPGRI, in collaboration with CIRAD. The activities undertaken are characterization, evaluation and selection of new cocoa genotypes (clones and hybrids), improvement and enhancement of germplasm populations, exchange of information and of selected germplasm accessions between project sites, and transfer and adoption of improved cocoa selection and breeding technologies. Emphasis in the evaluation and selection process has been on disease and pest resistance.

Standardized working procedures for cocoa germplasm evaluation and selection, including disease and pest resistance screening methods, were agreed, published, and adopted by the project. Approximately 85 ha of new variety trials (clones and hybrids) have been established at the different project sites. An 'International Clone Trial' was established in nine cocoa producing countries, permitting the evaluation of stability of economically important traits world-wide of 20 selected and diverse accessions. Resistance of the 'international clones' to the three major species of *Phytophthora* proved to be generally stable in relation to more than 20 fungal isolates from the ten cocoa producing countries involved. New sources of resistance to black pod disease, to witches' broom disease and to cocoa mirids have been identified in most cocoa collections. Germplasm enhancement for resistance to *Phytophthora* pod rot has been successfully carried out in Trinidad and Tobago, using the genetic diversity present in the International Cocoa Genebank, Trinidad (ICG, T). The large efforts on germplasm evaluation and information exchange have enabled us to identify an international working collection, called 'CFC Project Collection', that contains 110 accessions possessing valuable agronomic traits and wide genetic diversity. Distribution of this collection to user-countries, through the intermediate quarantine facility at the University of Reading, UK, has been initiated.

The positive effects of the project include increased human capacity building, more effective and coordinated use of limited resources, increased collaboration between cocoa conservation and utilisation efforts, and enhanced sustainability of cocoa breeding programmes. The achievements in the current project will permit the partners to foster further international collaboration and to involve the farmers directly in the process of developing new varieties within the framework of a new project proposal on 'Cocoa Productivity and Quality Improvement, a Participatory Approach'. A short description of this new proposal is also presented.

Description of the « CFC/ICCO/IPGRI project collection »

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Abstract

One of the activities of the CFC/ICCO/IPGRI project entitled « Cocoa germplasm utilization and conservation : a global approach » was the choice of around one hundred cocoa clones to be distributed to all participating countries. The purpose was to help worldwide cocoa breeders to introduce in their research stations a cocoa germplasm enriched in sources of favourable alleles for several traits such as resistance to several diseases (Black pod and witches' broom diseases, moniliosis and *Ceratocystis fimbriata*) and bean and pod characteristics (mean weight of one bean, fat content, pod index, pod hardness). The data used to establish this choice were obtained at the Cocoa Research Unit (C.R.U), in Trinidad, for most of them, but also from other research institutes, by reading through the International Cocoa Germplasm Database (ICGD)(University of Reading), or through informal contacts with researchers.

In addition, the choice of the clones was done with the aim of including a large amount of genetic diversity. This was ensured by choosing clones from a large number of populations, and by the use of molecular markers. Most of the clones were selected in the International Cocoa Genebank, Trinidad (ICG,T). The selected clones are currently sent to the Reading International Cocoa Quarantine, from where they will be distributed to the research institutes.

A NEW INTERNATIONAL COCOA GENETIC DATABASE

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Abstract

A new cocoa database that comprises molecular genetic, genomic and phenotypic data is being developed through a collaborative project involving CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France), University of Reading (School of Plants Sciences, UK) and USDA (United States Department of Agriculture, USA). This database will combine molecular genetic information contained in TropgeneDB (a molecular database developed by CIRAD) with phenotypic data contained in ICGD (International Cocoa Germplasm Database developed by University of Reading).

TropgeneDB is organized on a crop basis with presently three modules (cocoa, sugarcane and banana). The most common data stored in TropgeneDB are genetic and physical maps, marker information, QTL (Quantitative Trait Loci), sequence data, and molecular data on genetic resources. Presently, the cocoa module comprises around 500 clones with their genotypes at various markers (RFLP, AFLP, microsatellites, isozymes, etc.), genetic maps and information on the markers themselves. The ICGD database provides phenotypic information on cocoa germplasm. It comprises around 13800 different entries with detailed information on genetic and geographic origins, history of collection expeditions, morphology, pest, disease and stress reactions, quality and agronomic characteristics, and anatomical data.

The new international Cocoa Genetic Database will integrate all these data. Phenotypic information will continue to be submitted by users to ICGD and molecular genetic data to TropgeneDB. New and updated data will be regularly transferred into the common cocoa genetic database. The common database is implemented using ACEDB (<http://www.acedb.org/>) which is a system capable of storing and retrieving complex biological information. ACEDB provides an intuitive object-oriented view of biological data, and a graphical user interface with many specialized data visualization tools (genetic map viewer, sequence annotation display, etc.).

The common cocoa database will be available through the internet. A user-friendly and intuitive web consultation interface will allow performing complex queries combining genetic and phenotypic information; examples of such queries would be "What are the clones resistant to *Phytophthora* which have specific alleles at a range of loci?" or "Are two clones identical for the whole range of their phenotypic and genetic data?". Clear recognition of the data source will be obtained through a color code: data from ICGD in one color, data from TropgeneDB in another.

This new database is specifically designed to allow end-users (breeders or molecular geneticists) to best exploit genetic information available on cocoa germplasm.

Cacao somatic embryogenesis technology transfer to Côte D'Ivoire and Ghana

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Abstract

Clonal propagation of elite germplasm has the potential to greatly improve cocoa cultivation in West Africa. Unlike some systems of clonal propagation in cocoa, somatic embryogenesis produces plants with seedlings like dimorphic growth and a tap root system. It can be used to produce stock plants for an orthotropic budwood production. This association of the somatic embryogenesis and budding can yield a low vegetative propagation system that can produce elite clonal material with the typical cocoa dimorphic growth suitable to farmers in West Africa. The embryogenesis potential of cocoa clones from farmer's plots and research stations was investigated in Cote d'Ivoire and Ghana through a technological transfer from the Pennsylvania State University. Out of 38 clones (from farmers and CNRA research station) tested in Cote d'Ivoire, 27 produced in somatic embryos. In Ghana 33 clones (from farmers and CRIG research station) out of the 50 tested, produced embryos. Close to 10,000 somatic embryos were produced in Cote d'Ivoire and 3000 in Ghana. A strong genotypic effect on the embryogenesis was observed and genotypes with higher percentage of embryogenic explants also exhibits higher number of embryos per explants. Primary embryogenesis efficiency varies between 5% and 20 with an average of 2 to 4 embryos produced per responsive explant. The efficiency of a secondary embryogenesis system that uses primary embryogenesis somatic embryos as explants was 4 to 13 higher than that of the primary embryogenesis that utilizes flower explants. Up to 225 secondary somatic embryos could be observed from 30 explants in one petri plate of a highly embryogenic clone. In both countries the subsequent maturation of the embryos and their conversion into plantlets was successfully achieved. At the moment over 200 somatic embryo derived plants have been produced and successfully acclimated to ex vitro conditions in the two countries. The first plants produced using somatic embryogenesis in the two countries have just been transferred to the field in both countries for further evaluation.

**PERFORMANCE OF COCOA GENOTYPES IMPORTED FROM OTHER COUNTRIES AT
ALMIRANTE, BAHIA, BRAZIL.**

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Abstract

The cacao disease, Witches' Broom (WB), was able to spread very quickly and severely damage the cocoa production of the farms in Bahia due to the narrow genetic basis of the existing susceptible tree stock. An enlargement of this genetic basis became an imperative requirement to establish an effective breeding program. Aimed at acquiring resistance to this disease Almirante started a program of systematic importation of clones from different origins in cooperation with the Brazilian Federal Cocoa Agency, CEPLAC. In addition to creating a collection for conservation it is important to evaluate rigorously to select for promising genotypes. Each of the imported clones is grown in plots of 16 trees and carefully characterised for resistance to WB, Black Pod (BP) and for traits of agronomic interest eg, number of beans per pod, dry bean weight, husk/bean ratio, pod index and dry bean weight per tree. WB incidence for shoots and flowers is assessed at three monthly intervals. For pods a Disease Index (DI) for each harvest is developed by calculating the ratio of diseased pods and total number of healthy pods harvested. The same procedure is used for Black Pod. In this way it has been possible to segregate the clones into groupings of low to high DI. Currently over 300 clones are being actively evaluated although in this paper we only report on those with more than 4 years of continuous data collection (> 30 harvests). Several clones have demonstrated an excellent level of resistance to WB in association with high yield. Of special merit are clones in the COCA, LCTEEN, AMAZ, EET and GNV series with a very high frequency of harvests (90-100%) with losses of less than 5% and 10% for WB and BP respectively. Conversely other clones in the series UF, POUND, Ucayali and Huallaga have a higher frequency of DIs above 40% for WB and above 20% for BP. A good pod index of below 20 (number of pods required to produce 1 kg dry beans) has also been recorded in the COCA, EET, GNV and LCTEEN series. While other series have also recorded a low pod index they have accompanied by high DI for WB eg. UF, POUND, and IMC. Analysis for DNA has demonstrated that the COCA, AMAZ and LCTEEN groups are distinct from the traditional source of WB resistance Scavina. It would appear that now different sources for resistance to WB is now available in Bahia. While still preliminary in nature these promising genotypes constitute an important genetic resource for the breeding programs of cocoa in Brazil.

Perfecting a simplified method of assessing cocoa tree productivity in Côte d'Ivoire

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Abstract

For most producing countries, productivity is a primordial selection criterion. Assessing it by usual methods at a research station is restrictive and virtually non-existent in a farming environment. This work aims to seek a simple and reliable evaluation method for this characteristic which can be applied to peasant plantations. Following a brief description of the frequently used production assessment method based on counting the fruit, we present a new method based on visual ratings which quantify roughly the number of fruit on the trees. With highly significant correlations established with the previous method, using this method has considerably reduced personnel and working time requirements in "selective" plots and produced quality information which can be used in cocoa selection.

Germplasm enhancement for resistant to black pod disease: strategy and prospects

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Abstract

Black pod disease (BP) of cacao causes an estimated 44% of global crop losses due diseases. The ultimate solution to this problem rests on the development of resistant varieties. However, limited progress has been achieved in breeding resistant varieties due to lack of sufficient genetic diversity in most cacao breeding programmes. To reinforce the existing breeding strategy, a germplasm enhancement programme (GEP) was initiated to accumulate resistance genes (BP) from different sources by exploiting the rich genetic diversity in the International Cocoa Genebank, Trinidad (ICG,T). The GEP was initiated in 1998 and since then, over 500 genotypes have been screened in ICG,T for resistance to BP. From the data accumulated on BP and Witches' broom (WB) resistance, and pod index, 136 genotypes were selected and used as base parents in the GEP. Over a period of four years, ninety-six progenies were established and 3,486 seedlings were screened for resistance to *P. palmivora* using a leaf disc method. Among the 3,486 seedlings, 0.2% (6) were found highly resistant, 11.2% (389) resistant, 52.5% (1829) moderately resistant, 35.3 % (1231) susceptible and 0.9% (31) highly susceptible. No immune genotype was observed. The resistant and moderately resistant seedlings form 63.7% of the population evaluated. This may be considered unusual in a crop that is highly vulnerable to *Phytophthora* infection, but it demonstrates the effectiveness of the selection criteria for the base parents and confirms that resistance to BP is heritable. The progeny distributions of most families showed the occurrence of useful transgressive segregants with higher levels of resistance than the parental genotypes. Narrow sense heritability was estimated at 0.64 for a sub-population of 16 progenies. Using the same population, expected genetic gain was estimated at 0.72. This implies that the selected seedlings population would have an average genetic resistance level of 2.63 on a 0-5 resistance rating scale, which is more resistant than SCA 6 (2.79), a resistant control clone in the experiment. Among the 3,486 seedlings screened, 856 consisting of the resistant and moderately resistant seedlings have been established in the field. These seedlings will be evaluated for vigour, precocity, BP and WB resistance. Further selection will be guided by the expression of these traits in the individual plants. A second cycle of crosses is proposed among the promising resistant genotypes arising from the first cycle. The various selection criteria adopted in this programme should facilitate effective selection of promising genotypes / populations with enhanced levels of resistance to BP. This material will be made available to national cacao breeding programmes and should create the opportunity to combine resistance with good yield potential in new cacao varieties.

Evaluation by leaf inoculation test of resistance levels of thirty clones to various species of *Phytophthora*.

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Abstract

The aim of the project on *Cocoa Germplasm Utilisation and Conservation*, financed predominantly by CFC and coordinated by IPGRI, is to strengthen the evaluation of genetic resources by using traditional selection methods. Since 1997 this project has produced an evaluation network for cocoa genotypes in ten producing countries in America, Africa and Asia. One of its principal objectives is to find varieties with increased resistance to cocoa diseases and insects.

Multi-site clone and hybrid tests were planted by all the project partners. To achieve this, 42 clones, including 20 common ones, were delivered to all countries during the first two years by the quarantine centres at CIRAD, Montpellier, and the University of Reading. CIRAD in Montpellier evaluated the resistance levels of thirty or so of these clones to black pod by a leaf inoculation test using 28 different isolates of *P. palmivora*, *P. megakarya* and *P. capsici* originating from the ten cocoa-producing countries taking part in the project. Each clone/strain combination has four repeated inoculations, with 10 leaf-discs per repetition. The article firstly characterises the different isolates and species used. The aggression level of the isolates is influenced by inoculum culturing and preparation conditions. The results of the inoculations subsequently presented show significant effects on isolates and clones. Details are given of the classification of the isolates and clones according to their respective aggression and resistance levels. The most resistant clones are IMC47, PA120, GU255/V, GU307/V, NA33, SCA6, AMAZ15-15, MAN15-2 and P7. The most sensitive clones are LAF1, N38, EET59, VENC22, BE10, LCT-EEN46, MXC67 and EQX33603. For the species *P. palmivora*, the Trinidad isolates and one from Côte d'Ivoire are more aggressive than the isolates from Papua New Guinea, Ecuador, Ghana or Malaysia. For the species *P. megakarya*, the Nigerian isolates are more aggressive than those from Cameroon or Ghana. For the species *P. capsici*, the isolates from Trinidad are more aggressive than the Malaysian ones. The effects of the host-parasite interaction are generally less significant than the effects of the clones and strains. This relative resistance stability towards different *Phytophthora* species and strains could facilitate the selection of varieties with a wide spectrum of resistance.

COMPARISON OF VARIETIES USED BY IVORIAN COCOA FARMERS

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Abstract

Ivorian cocoa is made up partly of local Amelonado introduced many years ago into Côte d'Ivoire and considered the unimproved traditional material and partly by various hybrids, including F1 hybrids created under the improvement programme and distributed from 1975 onwards via seed fields using natural pollination, their F2 progeny from open pollination of the F1 hybrids and FI hybrids obtained from controlled pollination.

A test containing the plant materials cultivated in Côte d'Ivoire has been set up to assess the gain in productivity and the knowledge acquired from the cocoa tree improvement programme. This involves those mentioned above as well as new hybrids created but not yet widely distributed.

Data collected between 1995 and 2001 concerns healthy pod production, in total number of pods and in the weight of fresh beans. The different varieties have also been assessed for their resistance in the field to *Phytophthora palmivora*.

The results indicate that the traditional cocoa made up of the Amelonado is far less productive than the other varieties. A ratio of 1 to 4.5 is noted between the productivity of the Amelonado and of the improved varieties. In addition, Amelonado is the least resistant to *Phytophthora palmivora* as defined by the number of pods rotting in the field. For the improved varieties, the hybrids produced by natural pollination in seed fields are less interesting than those pollinated manually. The results are discussed in the light of their impact on overall cocoa production in Côte d'Ivoire, the distribution of plant material and also the research knowledge acquired.

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OR: FRENCH

Parameters and genetic structuring of wild cocoa trees in French Guyana, studied by isoenzyme electrophoresis

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Abstract

Wild cocoa trees (*Theobroma cacao* L.) sought and collected in the south-eastern area of French Guyana between 1985 and 1995 constitutes an original group and one of the poles of genetic structuring of the species. Nineteen populations (demes) originating from five rivers (Oyapok, Euleupousing, Yaloupi, Camopi and Tanpok) of the two main river basins in French Guyana are represented in collections in Guyana, many producing countries and quarantine stations. This study concerns the genetic structuring of this group and the genetic parameters of the populations for all of them (a total of 138 clones) and for the eight most substantially represented. The method used is isoenzyme electrophoresis, using six enzymatic systems (9 loci). The results show a high average number of alleles per locus (1.9), a high percentage of polymorphous loci (77.7) and a weak average heterozygosity observed (0.085). The various populations show very variable gene frequencies. Wright's fixation index of around 20% indicates tremendous genetic differentiation between populations. The group's genetic structure shows that the River Camopi populations are separated into two groups, confirming previous results from other descriptors, and that the populations from the other rivers are different, the Kérindioutou population being the farthest from the others. Controls from other morpho-geographical groups (high Forastero and low-Amazonia, Trinitario) confirm the originality of the Guyana cocoas. The overall wealth of alleles, the originality and the low heterozygosity of the clones show the potential interest in genetically improving this genetic group. The strong differentiation between populations is an argument for pursuing surveys throughout the Guyana Plateaus.

**FIELD EVALUATION OF AGRONOMIC CHARACTERS OF SOME SELECTED COCOA HYBRIDS IN
A MARGINAL AREA OF GHANA**

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Abstract

A trial was set up in Akomadan, a marginal and *Phytophthora megakarya* endemic area in Ghana to evaluate the yield, black pod infection and bean characters of eleven selected hybrids with the view to developing suitable planting materials for denuded and black pod endemic areas. Six inter-Amazon hybrids, three Amazon x Amelonado hybrids and two Amazon x Local Trinitario hybrids were evaluated. The inter-Amazon hybrids were PA 150 x T85/799, T60/887 x Pound 15, T60/887 x Pound 7, NA 227 x T85/799, EET 399 x T85/799 and T60//887 x Pound 10. The Amazon x Amelonado hybrids were NA 79 x P30, PA 150 x P30 and EET 399 x P30 whilst the two Amazon x local Trinitario hybrids were PA 150 x Y44 and EET 399 x Y44. Two hybrids, D26 x PA7/808 and T85/799 x P30 were used as controls. For net yield and black pod infection, there were significant differences ($p = 0.05$) between the hybrids and most of them performing better than the controls. PA 150 hybrids generally combined high yields with low black pod losses with PA 150 x Y44 giving the highest yield of 908.4 kg/ha and lowest black pod loss of 19.6%. Hybrids with better cropping efficiency index were also generally better for net yield and low black pod infection. There were no significant differences ($p = 0.01$) were found in their pod values, bean number and conversion ratios. There was a no significant positive correlation between yield and black pod infection. However, there was no significant correlation between the number of surviving plants and net yield. Cropping efficiency index based on the first three years significantly correlated positively with yield and appears to be a better selection criterion than stem circumference at the three years. Hybrids of T60/887 x Pound 7, 10 and 15 originally selected for their apparent drought tolerance /resistance both in the greenhouse and in the field were among the hybrids with lowest net yields, suggesting that drought tolerance may be at the expense of overall yield. These findings are discussed in relation to replanting of cocoa in drought-prone and *P. megakarya* endemic areas.

Cocoa breeding –an experience at CPCRI

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Abstract

Research on cocoa (*Theobroma cacao* L.) was initiated at CPCRI Regional Station Vittal in the late 1960's with the introduction of this species as an intercrop in arecanut (*Areca catechu* L.) gardens with main objective of crop diversification. The majority of the introductions were from Malaysian & Nigerian Estates while the rest were from other sources. The pedigree of collections have been traced to be Pa, Na, ICS, IMC, EET, C, RIM, UF, Amelonados, etc. The basic strategies involved in cocoa crop improvement were-introduction (germplasm enrichment)- selection- evaluation- hybridization- seed/ scion bank establishment- mass multiplication-distribution for commercial cultivation. There are 137 cocoa collections majority of which have been evaluated for their canopy, dry bean yield, bean quality, tolerance to drought and disease.

Parallel to systematic field evaluation of collections over the years there had been five Progeny row trials involving 40 hybrid progenies (I set – 5, II set –17, III set-9, IV set-9) with four CRIN Synthetics and eight Nigerian elite clones in RBD layouts. Conclusions are derived on analyzing data on their five years stable bean yield along with their data on growth and quality traits employing M-STAT program. ANOVA values have been provided separately for each set of the experiment. Observations on their quality parameters and drought tolerance also have been done and analyzed for supplementary assessment of the progenies. *In vitro* screening for resistance to Black Pod disease has yielded certain line with a mild field tolerance to the prevailing isolates of *Phytophthora palmivora*, *P. capsicii* and *P. citrophthora*.

Screening for drought tolerance (water relation components and stomatal behavior) is paid due attention since cocoa passes through 6-7 months of drought every year while protective irrigation is provided. This has led to the selection of NC 23, NC 29, NC 31, NC 39 & NC 42 (tolerant clones) utilized in crossing programmes. The following clones have been selected as the best performers in terms of their annual dry bean yield (- all with an average of > 1.0 kg/ plant-), pod index & stability indices (CV=20-30%) from the entire five sets of the experiments: Na 33 x ICS 89(I-set); I-56 x II-67, I-14 x II-67, I-56 x III-105 & I-14 x NC 42/94 (II- set); ICS 6 x SCA 6 (III-set); II-67 x NC 29/66 & II-67 x NC 42/94 (IV- set) & NC 45/53 (Nigerian clonal trial). All have been with a moderate canopy indicating their feasibility in the cropping design of arecanut. Wide range (20.57 to 58.98 %) has been observed for their fat content. Many precocious & pre-potent clones have been selected. The over all improvement programs have yielded good clones that have been used in developing six bi-and poly-clonal orchards and two scion banks with sufficient number of clones to produce nearly 50, 000 soft wood grafts annually for commercial cultivation.

Induction of gene expression in cacao (*Theobroma cacao*) by elicitors of the pathogen response.

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Abstract

Pathogenic diseases are a major constraint to cocoa (*Theobroma cacao*) production, causing annual crop losses of 30-40%. Although disease resistance pathways have been intensely studied in a number of plant species, studies in cocoa are limited. In many plants, activation of defense responses is controlled, in part, by signal transduction pathways mediated by endogenous signaling molecules such as salicylic acid (SA), jasmonic acid (JA) and ethylene. In an effort to gain a better understanding of how cocoa plants respond to pathogens, we have employed suppressive subtractive hybridization (SSH) cDNA libraries, macroarray hybridization technology, high throughput DNA sequencing and bioinformatics in an effort to identify cacao genes induced by these signaling molecules. In addition, we also investigated gene activation by nep1, a defense elicitor protein produced by some plant pathogens. We have screened over 4000 SSH cDNA clones and identified a set of genes induced by these elicitors. Their potential roles in the defense response and the regulatory interactions between these pathways in cacao will be discussed.

MOLECULAR BIOLOGY

Update on the molecular diversity of *Theobroma cacao* L. populations

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Abstract

Genetic diversity of traditional cacao cultivars: Criollo, Amelonado and Trinitario was studied as well as that of all the available Upper Amazon clones from the Pound, Allen and Chalmers collections using 13 microsatellite markers. The genetic structure of these cacao populations was also studied by analyzing the polymorphism of Resistant Gene Homologues using the Single Strand Conformational Polymorphisms (SSCP) technique. This extensive study of more than 500 individuals allowed for a clear understanding of the genetic relationships among and between the traditional cultivars and the Upper Amazon wilds. The genetic structure of the species suggests a pattern of differentiation according to geographical regions. The limited genetic diversity exploited in the breeding programs, compared to the genetic diversity available in the species is discussed. Application of results in a recurrent selection breeding program is shown. The genetic relationships among disease resistant clones are also shown. The problem of mislabeling clones (caught by means of molecular markers) is also addressed.

A Preliminary Genomic Map Based on SSR Markers and Resistance Gene Homologues, and Stress-related WRKY Genes in an F₂ Population of *T. cacao* L.

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and R. J. Schnell¹

Abstract

A genetic linkage map has been created with 151 trees from an F₂ population of a cross between SCA6 and ICS1. Simple sequence repeat markers were used principally for this map with a total of 131 SSR markers. In addition, seven markers developed from Resistance Gene Homologues (RGH) and four markers developed from WRKY (stress related) genes were included. Joinmap® software from Plant Breeding International was used to create the map from the scored data, and 10 linkage groups were obtained in the preliminary map. The 10 linkage groups were found to correspond well to the map of Lanaud *et al.*, and cover approximately 715 centimorgans, as opposed to 759 in the map of Lanaud *et al.* Approximately 30% of the markers showed serious segregation distortion, however, and this distortion tended to map towards six areas of the genome. Three different factors were identified which could explain this segregation distortion, namely population size, factors involved in circumventing the self-incompatibility mechanism of the F₁, and possibly genetically based segregation distortion. Nonetheless, a basic map exists which could be used to locate the inheritance of resistance to *Crinipellis*, as phenotypic data does exist from both field scores for one-half of the population, and for seedling inoculation response for the entire population. Our future plans involve the creation and addition of additional SSR markers in house, and the addition of all possible publicly available SSR markers. We also plan to saturate the map additionally with AFLP markers, thereby also facilitating comparisons with other maps also containing AFLP.

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**DEVELOPMENT AND INTEGRATION OF NEW MICROSATELLITE MARKERS INTO COCOA
LINKAGE MAP
A NEW COCOA REFERENCE MAP**

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Abstract

Two hundred and twenty newly developed microsatellites were produced from a genomic library enriched in simple sequence repeat (SSR) and integrated into an existing high density molecular linkage cocoa map. Microsatellites (SSR) markers have been widely used in many crop species due to their abundance, high degree of polymorphism, locus specificity, reproductibility, low amount of DNA required and codominance heredity. The microsatellites were mapped on 135 individuals from the same mapping population used to establish the reference map. This progeny resulted from a cross between two heterozygous cocoa clones: an Upper amazon Forastero (UPA 402) and a Trinitario (UF 676).The new microsatellite markers were assigned to the ten different linkage groups that correspond to the ten gametic chromosomes of cocoa. Mapping of the new markers as well as previously reported microsatellites provided genome-wide coverage of the 10 chromosomes, with an average distance of 1 SSR per 3 cM. Only codominant marker were conserved to establish the new cocoa reference map. This new map includes 176 RFLP, 270 microsatellites markers and 5 isoenzymes. It will be a useful tool to identify and compare between several progenies the most important regions involved in the variation of the traits of interest and to develop Marker Assisted Selection strategies.

GENETIC DIVERSITY OF WILD CACAO (*THEOBROMA CACAO* L.) FROM THE BRAZILIAN AMAZON DETECTED BY MICROSATELLITE MARKERS.

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Abstract

The cacao germplasm repository at the “Estação de Recursos Genéticos do Cacau José Haroldo” ESJOH, CEPLAC, located at Marituba-PA, results from the systematic collection of representative samples of the genetic variability of the Brazilian Amazon populations. The objectives of this work were to analyze the available genetic diversity and to determine the genetic structure of populations using CAB (“Cacao da Amazônia Brasileira”) genotypes originally collected in Amazon river basins using microsatellites. Ninety-four CAB clonal genotypes, representing 19 river basins, grouped according to geographic origin into four distinct populations (Acre, Rondônia, Lower Amazon and Upper Amazon) were analyzed. From the 19 pair of microsatellite primers tested, 11 amplified a total of 49 alleles with good resolution and consistency. The data was analyzed using the softwares TFGA (Tools For Populations Genetics Analyses) version 1.3 and GENETIX 4.02. The results indicated a strong intra-population structure and an elevated total genetic diversity, despite the little genetic differentiation between populations, confirmed by the Nei’s Index of Diversity and the Fixation Index of Wright. The dendrogram derived from cluster analysis based on UPGMA using Nei’s original distance (1972), demonstrated a trend of genotypes to group according to geographic origin, with the largest identity found between the populations from Lower and Upper Amazon. The population from Acre grouped as the most genetically distant from all the others. The dendrogram derived from an identity matrix to analyze the sub-populations (river basins) presented a similar pattern, with a trend to group sub-populations according to original population. It was noteworthy that groups were formed by the sub-populations from the Lower Amazon and the one from Xeriuíni (Roraima state) from Upper Amazon region, geographically distant, and by the sub-populations from Acre and the one from Purus from the Upper Amazon region, close geographically, but from distinct populations. The use of microsatellite markers was highly informative for cacao populations, presenting a high average number of alleles per locus, allowing a clear differentiation between them. An additional advantage for this method was that the primers designed to be used with radioactive labeling presented a good resolution and consistency when analyzed using polyacrylamide gels stained with ethidium bromide.

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Identification of resistance to Witches' Broom disease in the International Cocoa Genebank, Trinidad.

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Abstract

Witches' Broom disease (*C.A. Crinipellis pernicioso*), is recognized as one of the major diseases faced by cocoa producers in the Americas. The selection and development of Witches' Broom resistant cocoa types appears to be the most likely long-term solution for the control of Witches Broom disease (WBD), since phytosanitation and chemical control, which have been used in the management of the disease so far, have achieved only limited success. As part of a germplasm enhancement project supported by the CFC, a pre-breeding programme was undertaken with a mandate to identify cocoa clones, with resistance to major diseases and with superior characteristics, for use in breeding programmes. Since almost half of the accessions held in the ICG, T, were collected based on their apparent resistance to WBD in the area where they were found, it was thought therefore that there is a greater than normal chance of finding accessions with resistance to Witches' Broom disease within this diverse cacao collection.

Between 1998 and 2003, over 800 accessions of *Theobroma cacao* representing 50 populations were screened for resistance to Witches' Broom disease in green house experiments (in 24 series) using a modified spray inoculation technique. Four accessions were used as control in each series to allow for comparison between series. Each accession was represented by five clonally propagated plants obtained through top-grafting each accession on to TSH root stocks. Data were collected on the proportion of plants showing symptoms, broom base diameter, broom length, broom weight and time to symptom development. There was considerable variation in the degree of symptoms shown by the resistant clones, with very promising resistant clones identified in the majority of populations screened. Approximately 65% of the accessions were classified as susceptible or highly susceptible. The resistant accessions are being verified using an agar drip inoculation technique.

Exploring the plant-pathogen interaction *Theobroma cacao*-*Crinipellis pernicios*a using random sequencing and SAGE analysis.

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Abstract

Since 1989, the Witches' broom disease due to the pathogenic fungi *Crinipellis pernicios*a increased in Brazil, destroying the cultivation of cocoa trees and leading to important economical and social changes in the concerned areas such as in the Bahia state. The aim of the research developed in the laboratory is to acquire a good knowledge of the determinism of the interaction between the cocoa tree and the pathogen *Crinipellis* based on functional genomic studies, and to contribute to the development of effective methods for the genetic improvement of resistance to this disease, so as to create new varieties obtained through a Marker-Assisted Selection (MAS) strategy.

We report here studies related to gene expression in meristems of resistant and susceptible cocoa trees inoculated or not with *Crinipellis pernicios*a spores. Plants were harvested from time zero up to 70 days after inoculation (necrosis symptoms), then used to generate Expressed Sequence Tags (ESTs) by random sequencing or by the Serial Analysis of Gene Expression (SAGE) method. First, we developed a successful and reliable procedure for the isolation of RNA from any cocoa tissues particularly those containing a high content of polyphenols and polysaccharides such as meristems and fruits. Then, we generated two cDNA libraries corresponding to inoculated resistant and susceptible plants. The cDNA libraries were cloned in an expression vector that can also be induced to produce proteins. We randomly sequenced 10 000 ESTs of both libraries. Finally, we compared, using bioinformatics tools, these results with those obtained using the SAGE method.

The collection of ESTs obtained by the way of these two methods provide new data about the physiology of the interaction between *Theobroma cacao* and *Crinipellis pernicios*a. In a short term we plan to do a functional analyze of some candidate genes which can also be used for mapping, to facilitate introgression strategies and to create new varieties resistant to *Crinipellis pernicios*a. These data may also be related to research on gene expression of cocoa challenged with other pathogens, such as *Phytophthora ssp*.

Comparison of levels of homozygosity and heterozygosity between cocoas (*Thebroma cacao* L.) of the National genotype and others which exist in Ecuador, using what is molecular marker termed microsatellites (SSRs).

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Abstract

Since the beginning of cocoa research in Ecuador, the theory has been promoted that the original genotype of Ecuadorian “National” coffee had among its genetic characteristics high levels of homozygosity, but this hypothesis has not been proved in any way to date on a scientific basis, due to various factors which have limited this type of study. This work tries to amplify and establish the bases for strengthening this hypothesis, proof of which will help in the design of future genetic improvement programmes. Among the samples selected are: two farms which produce National cocoa, Las Brisas and La Gloria, with 30 and 25 trees over 70 years of age respectively, 8 clones with the EET abbreviations from the INIAP, 10 clones from the Colección Cacao Aroma Tenguel (CCAT) [Tenguel Aroma Cocoa Collection], 4 clones with the EB abbreviations, and the ICS-95, IMC-67 and CCN-51 clones. This completes a sample of 80 materials analysed. For this molecular technique (SSRs) 15 different primers were used; the DNA obtained was amplified by means of the polymerase chain reaction (PCR), and one or two fluorescent peaks (fragments) of DNA were analysed by means of capillary electrophoresis, which were interpreted as homozygosity or heterozygosity respectively.

The levels of homozygosity found in the materials from the two farms selected were high; this did not occur in the other materials (clones) used, since high levels of heterozygosity were found and in some cases such as CCAT-18-58, CCAT-21-43, CCAT-33-45, CCAT-46-75, CCAT-49-98, EET-19 the proportion of heterozygosity was 100%. The trees from Las Brisas and Gloria are genetically closer, they form more compact groups, and grouping analysis shows that there is sufficient variability between them, which may well be used for improvement programmes. On the other hand, well-defined groups were not found in the other clones used and in the majority they were remote from the National cocoas from the farms, particularly CCN-51 and ICS-95. These results strengthen the hypothesis that National cocoa from Ecuador was originally, and before the introduction of foreign cocoas, highly homozygotic; in addition good variability among the National materials used was observed.

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AGRONOMY

SOILS / NUTRITION / PHYSIOLOGY

Gas transfer measurements on young cocoa trees in the field and modelling of photosynthetic activity.

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Abstract

Very varied situations and behaviours characterise the domestication and distribution of the cocoa tree, depending on combinations of radiative, edaphic and climatic conditions and cultivation methods.

A great deal of research has revolved around factors limiting yield, including physiological factors. Given the diversity of environments, the principal difficulty for such a study on cocoa is controlling the factors linked to its environment. Gas transfers measured in the field on young, one-year old plants were therefore studied in Vanuatu in order to understand better and model the behaviours observed.

Gas transfer capabilities of both the traditional and improved hybrid varieties were compared during this study. The average maximum intensity of the photosynthesis, in the order of $4 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, is low compared with numerous other tropical species. It is characteristic of ombriphilous plants and is reached under relatively weak rays, from $500 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The relationship between illumination and photosynthetic activity can be adjusted by two curve models, a negative exponential form and a hyperbolic form approaching a Michaelian equation.

The results indicate that no difference can be made in the photosynthetic activity, conductance and transpiration of the two varieties. However, a study of the ratio of internal CO_2 on external CO_2 suggests a better use of CO_2 for the more vigorous hybrid variety. In addition, a gradient of photosynthetic activity was revealed within a unit of foliage growth, maximum values being obtained for medium leaves.

The rapid saturation of the photosynthetic activity seems to indicate that it is not the major factor in limiting production. The strength of the hybrids results in a "spring-well" connection which encourages vegetal development to the detriment of fruit production. This preliminary work opens the way to new avenues for research in better management of the production/vigour ratio, through genetic improvement or agronomic techniques such as pruning or shade.

Effects of different thermal growing environments on the growth and chlorophyll fluorescence of contrasting cocoa genotypes

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Abstract

The thermal environments of three cocoa-growing regions (Bahia, Brazil; Tafo, Ghana; Lower Perak, Malaysia) were simulated in within a triple-span glasshouse located at the University of Reading, UK, which was purpose-built to study effects of the photo-thermal environment on cocoa whilst maintaining other parameters constant. Three clonally propagated genotypes (SCA 6, AMAZ 15/15, SPEC 54/1) were introduced into each glasshouse compartment along with seed-grown Amelonado. Early vegetative growth was studied over a year in terms of main stem height and girth. Additionally, data on chlorophyll fluorescence were collected at monthly intervals over the same time period.

The increase in main stem height of seed-grown Amelonado was a positive linear function of thermal time. The increase in main stem cross-sectional area differed significantly between genotypes ranging from 2.6 cm² yr⁻¹ for SCA 6 to 8.3 cm² yr⁻¹ for Amelonado. Whilst growth was always highest in the 'Malaysia' glasshouse, the response to differing thermal environments was not the same between clones.

Chlorophyll fluorescence differed significantly between genotypes, the average ratio of variable to maximum fluorescence (Fv/Fm) ranged from 0.677 for SPEC 54/1 to 0.713 for SCA 6. Moreover, the effect of genotype interacted both with growing environment and time of the year such that the ratio of Fv/Fm could be modelled in terms of temporal variability in temperature for two genotypes (SCA 6 and SPEC 54/1) but was independent of temperature (over the range measured) for the other two.

It was concluded from both the growth analyses and chlorophyll fluorescence data that there was considerable genotypic variation in the response of cocoa to the thermal environment.

SOLUBLE CARBOHYDRATE AND PHENOL LEVELS OF CACAO GENOTYPES WITH DIFFERENTIAL TOLERANCE TO WITCHES' BROOM DISEASE UNDER DIFFERENT LEVELS OF MANGANESE

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Abstract

This work studied the effects of Mn on the total soluble sugar levels, total soluble starch and phenols in two cocoa (*Theobroma cacao* L.) genotypes with differential resistance to witches' broom, inoculated or not with the fungus *Crinipellis pernicioso*. Genotypes Catongo, susceptible, and the hybrid Theobahia, tolerant to witches' broom, were cultivated in nutritive solution with three levels of Mn (0, 2,5 and 20 μ M). Evaluations and analytical determinations were carried out at 1, 5, and 60 days after inoculation. Generally, in the presence of 2,5 and, or, 20 μ M of Mn, the tolerant genotype presented higher levels of total soluble sugar, starch and soluble phenols. For the susceptible genotype, in the presence of different Mn levels, only higher levels of starch were observed. For inoculated plants of the susceptible genotype, at the first, and, or fifth day, levels of soluble sugar and phenols were lower at higher Mn level. In inoculated plants, the tolerant genotype showed higher sugar levels than the susceptible in young leaves, especially at the higher Mn level. In the presence of Mn, the tolerant genotype showed higher starch levels than the susceptible. In the absence of Mn these levels were higher in the susceptible genotype. Inoculation with the fungus modified the distribution patterns of soluble sugars and significantly reduced starch levels in both genotypes. Levels of soluble phenols increased in inoculated plants from the tolerant genotype for the higher Mn level at the first day after inoculation, except for young leaves. Only at 2,5 μ M Mn level the tolerant genotype showed lower percentage of infected plants. The infection percentage was similar in every Mn level for the susceptible genotype, which showed a higher percentage of plants with symptoms than the tolerant. At a level of 2,5 μ M Mn, the tolerant genotype showed higher levels of total soluble sugars, starch and phenols, which probably contributed to its higher tolerance to witches' broom disease.

Clonal variability of polyphenols in cocoa (*Theobroma cacao* L.) seeds from Cameroon

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Abstract

Polyphenols were analysed at 280 nm by HPLC device using a Photodiode Array Detector (PDA). Total phenols were estimated with folin ciocalteu reagents. Anthocyanines were separated with the SEP-PAK Vac 6cc 1000 mg (Waters) column and estimated at 520 nm with a PDA. Ten cacao clones from Cameroon genebank were used. Fresh and germinated seeds were used. Our results showed that two main polyphenols are present in cocoa seeds : catechin and epicatechin. Substance A, substance B and substance C were also found in cocoa seeds. Epicatechin represent 2 % to 4 % of the dry mass of defatted powder of cocoa seeds. Substance A (retention time 6,967 min) is discussed to be a derivative of caffeic acid and an ester bound-compound. Substance B (retention time 12,652 min) and C (retention time 20,432 min) are oligomeres of proanthocyanidins. Two anthocyanines were found in cocoa seeds: cyanidin-3-galactoside and cyanidin-3-arabinoside. They represent 0.5 % to 1 % of the dry mass of defatted powder of cocoa seeds. Amount of total phenols, catechin, epicatechin and anthocyanidines in the fresh and germinated seeds was genotype-dependent. Protocatechiuc acid and quercetin were not detected in cocoa seeds from Cameroon. Protocatechiuc acid is generally found in cocoa seeds in stress conditions. When polyphenols from seeds of two different pods of the same clone were compared, no qualitative difference was observed but quantitative significant differences could be recorded. The results suggest that the metabolism of polyphenols in cocoa pods is probably linked to the expression of only a few number of genes. The accumulation of these storage molecules which was different from one clone to another may be at least in part due to a differential expression of their genes and/or the influence of various factors such as climate, pod and bean size.

Key words: Cameroon, HPLC, polyphenols, seeds, *Theobroma cacao*

Use of ^{32}P and N^{15} to determine the efficiency of utilization of phosphorus and nitrogen fertilizers in cocoa seedlings.

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Abstract

Isotope dilution technique was used in a greenhouse study to evaluate the differences among seven cocoa varieties namely; PBC 112, PBC 130, PBC 140, UIT 5, PA7, NA 32 and UA 13 in their uptake of nitrogen and phosphorus in Typic Paleudult soil amended with fertilizer. Nitrogen was applied as ammonium sulphate enriched at 3% atom ^{15}N at the rate of 100mg N/pot while Phosphorus was applied as Triple superphosphate with an activity of 100 Uci/ pot at a rate of 100 mg P/pot. Nitrogen and phosphorus fertilizers were solely applied per pot and top dressed with muriate of potash at 100g K/pot. The experiment was designed as a randomized complete block with fourteen treatments and three replicates. Total dry matter yield (TDMY), total N, fertilizer N, Percent Fertilizer Utilization Efficiency FUE (P) were all significantly different ($p = 0.05$) among the cocoa varieties. Percentage FUE (N) ranged from 18.9% (PA 7) to 46.5% (PBC 112). Total P, fertilizer P and % FUE were found to be significantly different among cocoa varieties ($P < 0.05$). The correlation coefficient between TDMY and fertilizer N was higher than that of TDMY and fertilizer P. PBC 112 in this screening exercise was found to be the best in most parameters examined and offers a higher potential for higher productivity. Differences among the cocoa varieties in their nitrogen and phosphorus requirement as well as utilization emphasise the need to evaluate newly introduced cocoa varieties for their responses to the nutrients.

EFFECTS OF NITROGEN RATES AND FREQUENCY OF APPLICATION ON COCOA YIELD, SOIL AND COCOA LEAF NUTRIENT COMPOSITIONS

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Abstract

An investigation was carried out at the Cocoa research Institute of Ghana, Tafo to assess the effects of N rates and frequency of application on mixed hybrid cocoa yield, soil and leaf nutrient composition over a 3 year period. The experimental design was a 6 x 3 factorial with six nitrogen rate including the control and three different times of N application in a year with four replicates. The N fertilizers rates were 0, 30, 60, 90, 120 and 180 kg N/ha applied as sulphate of ammonia either all at once in April (1T), or split into two equal parts and applied in April and November (2T) or split into three equal parts and applied in April, August and November (3T). All the plots received a basal dressing of P and K at the rate of 115 kgP₂O₅/ha and 76.5 kgK₂O/ha. The results indicated non-significant time effect for the yield for both the individual and combined three years but with generally consistent 2T > 3T > 1T trend. The differences in yield among the N fertilizer treatments were also non-significant but the effects of the highest N applications (120 – 180 kgN/ha) were generally negative thus causing yield depression. The highest mean yield of 2414.0kg/ha representing an increase of 25% over the control was achieved with the 90 kg N/ha split applied in April and November (2T) with the combined three years data. N fertilizer significantly different among the N treatments with the highest value of April and November (2T) application plots. There were significant differences in nitrogen and phosphorus concentrations in the leaves for the N treatments with significant time effect of 1T = 2T > 3T. As an insurance against mining of the N reserves in the soil, it is suggested that sulphate of ammonia fertilizer at the rate of 60 –90 kg N/ha split applied in April and November may be incorporated into fertilizer application package on mature cocoa farms in Ghana.

**USE OF ORGANIC AND INORGANIC FERTILIZERS TO IMPROVE THE RATE OF COCOA
SEEDLING ESTABLISHMENT IN THE FIELD**

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Abstract

This investigation studied the effect of poultry manure, green manure, urea and single super phosphate on the field establishment of F3 Amazon cocoa seedlings in the Ago-Iwoye area, North-East of Ijebu-Ode in the South-West of Nigeria. The organic fertilizers used were applied at the rate of 500g/seedling (i.e. 0.56 tonnes/ha). There was decrease in the leaf number and leaf area of the seedlings in the first few weeks after transplanting due to leaf abscission. Later, poultry manure gave the highest rate of leaf emergence in the seedlings, followed by green manures, urea and single super phosphate in that order. No mortality was recorded in the experiment. The stem height, stem diameter, leaf number and leaf area of the seedlings increased with the application of all the fertilizers. By 32 weeks after planting, the poultry manure and the green manure treatment plants were significantly larger (height, stem diameter, leaf emergence and leaf area) than those in the urea and single super phosphate ($P \leq 0.05$) treatment.

**EVALUATION OF THE FREQUENCY OF WATERING AND LEVELS OF FERTILISATION IN THE
DEVELOPMENT OF COCOA SEEDLINGS**
(Theobroma cacao L.)

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Abstract

The production of seedlings of cocoa (*Theobroma cacao L.*) is an activity which lacks technological innovation in the production process, since they stay between 5 and 6 months in the nursery and in many cases do not achieve the vigour needed for sowing. The work was carried out at the Mata Larga Experimental Station, San Francisco de Macorís, during the months of May-November 2002, with the objective of determining the most appropriate frequency of watering and level of fertilisation for the production of cocoa seedlings. A divided parcels design was used with watering daily, more than once daily, every three and every four days; 6 levels of fertilisers: 0, 2, 4, 6, 8 and 10 grams with four repeats. The best height data were found with daily and more than once daily frequencies, with 56.31 and 53.33 cm. and the addition of 4, 6 and 8 grams of fertilisers, with 53.87, 54.70 and 55.05 cm. For the diameter of the stem daily and more than once daily watering showed the highest values with 6.89 and 6.68 mm and incorporation of 4 grams of fertilisers, with 6.67 mm. For the number of leaves daily and more than once daily watering, with 23 and 22 leaves; 4, 6 and 8 grams of fertiliser, with 23 leaves on each. On the biomass, daily and more than once daily watering, with 20.07 and 18.62 grams and incorporation of 4 and 2 grams of fertilisers, with data of 19.30 and 18.75 grams. Seedlings with watering every three and four days concentrated more nitrogen, with 1.99 and 1.95 %. For phosphorus they were equal, while as for potassium daily watering recorded the highest data, 0.17 %. The highest cost of production was recorded when water was applied every day, with a cost of RD\$ 1,511.86 per 1,000 seedlings.

Effect on cocoa production of variations in rainfall in South-West Côte d'Ivoire

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Abstract

As many other regions of Africa, Côte d'Ivoire remains dependent on agricultural resources. This agricultural economy is principally based on cocoa culture which accounts for over 40% of world production.

The heavy rains required for cocoa culture (between 1,200 mm and 2,000 mm) are found in the southern half of the country due to the indefinite equatorial weather pattern. However, for over thirty years, and in common with the rest of the country, the cocoa-producing zones have known significant variations in rainfall patterns. This change is marked by increasing water shortages and a reduction in the length of the growing seasons. In the main growing zone of South-East Côte d'Ivoire, the long rainy season has changed from 4 months 10 days to 3 months 10 days between 1950 and 1999. Some crops, particularly cocoa, are very vulnerable to these new climatic constraints, resulting in a significant drop in agricultural yields.

The ultimate objective of this study is the relationship between the variation in rainfall and agricultural productivity taking cocoa as an example. For the period 1982-1994, we have revealed a strong relationship between the accumulated water deficit and the weight of the fresh bean, an important element in cocoa yield, on several experimental plots in South-West Côte d'Ivoire. For several hybrids studied, the variations in bean weight are over 50% explained by the inter-annual fluctuation of the water deficit. This relationship (linear regression) suggests that, in this region, a 200 mm reduction in water from the cumulated rains between April and June the previous year causes an average reduction of 13 g in bean weight.

The significant fluctuations in rainfall over the past few decades ultimately present a considerable risk to cocoa production, above all when they affect the South-West, the principal production zone.

Keywords: Cocoa, South-West Côte d'Ivoire, water deficits, drop in agricultural yields, bean weight.

EFFECTS OF NITROGEN SOURCES ON THE PRODUCTION OF COCOA TREES

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Abstract

With a view to studying the effects of different sources of nitrogenous composts in the production do cocoa tree, a test was conducted on a dystrophic Red-Yellow Lato solo, in the Guandu municipality, Bahia, Brazil. The treatments used were: Sulphonitrate Nitromag (40% of N), calcium nitrate (15,5% of N), ammonium nitrate (33,5% of N), urea (45% of N), nitromag (26% of N) e ammonium sulphate (21% of N) in 60 kg of N/ha doses. A reaction curve was generated by the application of urea in the doses 0, 30, 60, 90, and 120 kg of N/ha, complemented by phosphorus, potassium and zinc in the of 90, 60 and 5 kg/ha of P₂O₅, K₂O e Zn, respectively. The experimental design was in randomized blocks with four replicates and 16 cocoa trees per plot. The results of the production, expressed in kg of dry cocoa/ha, showed that there was a significant effect on the assessed parameter. The most significant reactions, relating to the 1996-1999 period, were found with the application of 60 kg of N/ha in the calcium nitrate form and in the presence of 90, 60 and 5 kg/ha of P₂O₅, K₂O and Zn, respectively, but not differing statistically from the sulphonitrate, ammonium nitrate, ammonium sulphate and the urea.

It was noted that among the tested sources, the one, which presented the highest index of agronomical efficiency was the calcium nitrate, without, nevertheless, showing significant differences in relation to the ammonium nitrate and the urea (90 and 120 kg N/ha). The nitromag presented significantly lower effects than the other sources under study, but did not differ statistically from the ammonium sulphate, from the do sulphonitrate, from the urea (60, 90 and 120 kg N/ha) and from the ammonium nitrate. The regression analysis of the reaction curve indicated that the maximum production of cocoa should be obtained with the application of 102,3 kg of N. The best suitable model was: Production (kg cocoa/ha)= 1219 + 12,02 (dose N) – 0,05(dose N)²; r²= 0,99.

Key Words: Cocoa tree, Sources of Nitrogen, Lato solo Red Yellow,

CULTURAL SYSTEM

Agronomic evaluation of different weed management options in young cocoa

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Abstract

Studies on the effect of different weed management techniques on the ease of establishment, growth and yield of young cocoa, soil fertility as well as changes in the composition of the weed seed bank were carried out at the Cocoa Research Institute of Ghana, Bunso sub-station from 1993 to 2001. The treatments evaluated were: clean manual weeding, two, three or four times per annum; high slashing four times per annum; clean weeding of cocoa lines (1.0m diameter) three times per annum with *Mucana pruriens* cover crop in the inter-rows; clean weeding of cocoa lines (1.0m diameter) three times per annum with double rows of *Flemingia macrophylla* in the inter-rows to provide mulch and chemical weed control with paraquat applied four times or glyphosate applied three times per annum. The trial was designed as randomized complete block with five replicates.

Significantly higher ($P \leq 0.05$) girth increments of cocoa seedlings were recorded twenty-two months after transplanting in the line weeding + *F. macrophylla* treatment than in all the other treatments with the exception of those clean-weeded thrice per annum or sprayed with glyphosate. The height increments followed the same trend. Significantly lower seedling mortalities ($P \leq 0.05$) were recorded after two dry seasons in treatments clean weeded four times/annum, sprayed with glyphosate or paraquat as well as the line weeding + *F. macrophylla* treatment. Percentage flowering in the line weeding *F. macrophylla* treatment was significantly ($P \leq 0.05$) higher than all the other treatments, except the paraquat-treated plots twenty-two months after transplanting. Higher percentage jorquette formation was recorded in the line weeding + *F. macrophylla* and the chemical weed control treatments than the other treatments at twenty-two and thirty four months after transplanting. Percentage canopy formation was also higher in the line weeding + *F. macrophylla* and the chemical weed control treatments than the other treatments, four years after transplanting. Cumulative yield of dry cocoa beans during the initial five years of bearing was significantly higher ($P \leq 0.05$) in the line weeding + *F. macrophylla* and the paraquat and glyphosate treatments than in treatments clean-weeded 2 or 3 times per annum or line-weeded thrice per annum with *M. pruriens* in the inter-rows. The implications of the various weed management options on the ease of cocoa establishment and initial yield as well as their effects on the levels of some soil nutrients, soil moisture during the dry season and the composition of the weed seed bank are discussed.

**COCOA/COCONUT INTERCROPPING TRIAL IN GHANA: EFFECT OF THE CROPPING SYSTEMS
ON SOIL NUTRIENT DYNAMICS.**

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Abstract

The effects of some cropping systems on soil properties at three soil depths of tropical ferric lixisol were investigated at the Cocoa Research Institute of Ghana as part of efforts to search for an evergreen permanent shade for cocoa in Ghana. The cropping systems were cocoa planted at 2.5m triangular with coconut at 8.8m triangular; cocoa planted at 3m x 3m with *Glyricidia sepium* planted at 12.1m x 12.1m; cocoa planted at 2.5m triangular with coconut at 9.8m triangular; cocoa planted at 3.0m x 3.0m with coconut planted at 9.1m triangular; cocoa planted at 3.0m x 3.0m with coconut planted at 12.2m x 11.0m. The experimental design was a randomised block with four replicates. The soils were sampled from the cropping systems and an uncultivated bush in an adjacent plot used as the standard. Land clearing and the cultivation of the crops / crop combinations generally influenced the soil chemical properties determined at the end of fifteen years of cropping. Soil pH and organic matter content increased with cultivation.

Irrespective of the intercrop system, total N, available P and exchangeable K contents of the soils declined.

The cocoa / *G. sepium* plot recorded the highest level of nitrogen among the intercrop systems but cultivation significantly decreased the nitrogen content of the soils especially in the cocoa spaced at 3.0 x 3m and coconut spaced at 12.2m x 11.0m plot. Cocoa spaced at 3 x 3.0m with *G. sepium* or with coconut spaced at 12.2m x 11.0m treatments had significantly (P=0.05) lower available P contents. The exchangeable K contents were however similar among the intercrop systems. The results are discussed in relation to the nutrient requirements of mature cocoa as well as cocoa fertilization programme in Ghana.

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OR: FRENCH

CHANGING DENSITY IN COCOA CULTURE: THE NEED FOR THINNING

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Abstract

A frequently excessive density of cocoa trees could be one of the causes of their drop in production and degradation. This can quickly trigger (sometimes long before 10 years) significant competition which weakens the physiology of the trees and changes their architecture, whilst reducing their production and increasing their sensitivity to various hazards. The economic lifetime of the plots, in other words their sustainability, is seriously affected. However, in current situations high densities are necessary, even essential, to the plantation to ensure rapid ground coverage and to fight weeds and some insects efficiently, economically and ecologically.

One solution to this dilemma could be to thin the trees, a practice well-known to foresters, thereby applying the concept of “changing density” to cocoa culture.

Tests carried out in French Guyana and Côte d’Ivoire on initial densities of 1,333 to 1,666 cocoa trees per hectare (shared values) have shown that systematic thinning at 10 years and at a rate (“relative weight”) of 50% (one row thinned out of two, or two rows thinned out of four) have a favourable influence on the yield by many hybrids and also on the size of the cocoa beans. Thinning could therefore become a major research theme focusing on clarifying optimal application conditions (planting material, age, rate, systems, frequency, associated agronomic practices, etc.) with, alternatively, research into dwarf cultivars and their high-density cultivation, inspired by temperate arboriculture and coffee cultivation. The problems of sustainability of cocoa culture should therefore include “changing density”: planting at a relatively high density and changing this density by controlled thinning.

EFFORTS TO RE-ESTABLISH COCOA IN DENUDED COCOA GROWING AREAS OF GHANA

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Abstract

Ghana`s cocoa crop is produced under rain fed conditions in forest areas with rainfall of at least 1000mm per year. Protracted and severe drought has recently become a major limiting factor mitigating efforts aimed at increasing cocoa production. The wide spread deterioration of shade and soil conditions over much of the old and denuded cocoa growing region make the re-establishment of cocoa very difficult for farmers who in certain years report of 100% seedling mortality. This situation taunts farmers desire to rehabilitate old farms and or establish new ones. Field trials were set up at three locations (Afosu, Bechem and Akumadan) in the affected areas to evaluate the prospects of some agronomic techniques in reducing cocoa seedling mortality in the target areas. These involved the use of mulch, anti-transpirant, growth regulators, soil-moisture conditioner and temporary shade provision. A split plot design with three replications was used for trials on mulches, anti-transpirant and shade treatments while a randomized block design was used for those involving soil-moisture conditions and growth regulators. The plantation pseudo-stem mulch significantly improved seedling survival rate by 31.7 percent over the control treatment, whilst the black polythene sheet mulch improved survival by 25.5% over the control., Folicote `20` the anti-transpirant, reduced seedling mortality by 8.4% compared to the control. A growth retardant, Paclobutrazol (Cultar), increased cocoa seedling survival by 11.2% whilst the growth promoter, Ergostin decreased seedling survival by 14.4%. The soil-moisture conditioner, Grow-soak 400, achieved 8.7% success over the control. Using Cassava as temporary shade gave a survival improvement of 14.7% over the control treatment. Amongst the agronomic techniques evaluated in these trials plantain pseudo stem as mulch offers the most effective means of improving seedling survival during re-establishment of cocoa in degraded areas.

**USE OF GRAFTING TECHNIQUES IN THE APPLICATION OF THE CANOPY
OF PRODUCTIVE COCOA TREES WITH NEW VARIETIES TOLERANT TO WITCHES' BROOM**

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Abstract

The main focus of this test is aimed at improving the productivity of cocoa trees in Southern Bahia, applying different means, such as : replacement of susceptible canopies by other varieties more tolerant to witches' broom; implementation of different grafting procedures in order to replace the cocoa tree canopy; and an increase in cocoa tree yield by the adequate management of new plants. The experimental line-up was carried out in randomized blocks with 4 repetitions, 36 useful plants per plot and five 5 treatments: 1) standard, no grafting; 2) side forking, on the main orthotropous axis (GL-stem), 1 to 2 grafts carried out at a height of 40cm from the ground; 3) side forking on the plagiotropic branches (GL-canopy) carried out 60cm above the fork, 2 to 4 grafts per plant; 4) bubbling on the plagiotropic branches (B-canopy), 60 cm above the fork, 2 to 4 grafts per plant; and 5) bubbling on the basal chupon (B-basal), carried out at 40cm from the ground. Grafting was carried out by means of a clonal mix, consisting of 36 genotypes, WB tolerant (Sca-6 or 12 versus selections of Maranhão variety), each plant receiving one genotype only. The recovery process for the recuperation of the deteriorating cocoa trees by means of a side forking procedure, above the fork (GL-canopy), has been faster than the other techniques applied during the present research study: GL-stem and B-canopy or B-basal chupon. The total number of fruit harvested in the GL-canopy plants would suggest that the GL-canopy treatment, side canopy forking, has stood out against the standard and the remainder. This was evident in every harvest, between 1999 and 2002.

YIELD EVALUATION OF COCOA REHABILITATION THROUGH COPPICING

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Abstract

Monthly coppicing trial was carried out in 1989 at the Cocoa Research Institute of Nigeria, Ibadan headquarters to rehabilitate old and unproductive cacao trees. 120 F3 Amazon cacao trees were coppiced at about 30 cm from the ground for 3 chupon densities. Coppiced trees during each of the 12 months of the year and the control constituted the 13 treatments which were each assigned 10 replicates stumps at random in a completely randomized design.

The yield data from 1996 to 2000 was collated and analysed for assessment of the rehabilitation exercise on the economic returns over the period. The result showed that coppicing in the month of November performed best on the average. All the treatments except the control had significant increase in pod yield during the period under review. Differences between average yield of all coppiced treatments were found to be statistically significant from the control ($P \leq 0.05$) with T11 recording the highest (2.38 tons/ha) while T10 recorded the lowest (1.08 tons / ha) amongst the coppiced treatments.

THE EFFECTS OF SHADE ON POD SET, CHERELLE WILT, FREE SUGAR AND MOISTURE CONTENT OF COCOA PLANTS

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Abstract

As part of the long-term study of factors affecting the vegetative and reproductive characteristic of cocoa, the total free sugar level of the pulp and bean were determined for Amazon hybrids and Amalonado cocoa trees growing under two shade regimes (Heavy shade – 30% of full sunlight and No shade – 100% of full sunlight) provided by *Gliricidia sepium* staud. The levels of pod set and physiological cherelle wilt were also studied. The experiments were conducted in a field of cocoa located at Cocoa Research Institute of Ghana (CRIG), Tafo . The genotypes included Amalonado and Amazon hybrids. Plants were spaced at 3m x 3m . A split – plot design with three blocks were used with shade regimes as main plots and cultivars as sub-plot. Pod set and cherelle wilt were recorded every other week. Mature – ripe pods were harvested every other week for the determination of pulp and bean sugar and moisture content. In the Amazons where flowers were produced through the year, two distinct peaks of pods set were recorded in April and September for the minor and major seasons respectively. The shaded trees showed a wilt increase of 57.14% over the unshaded trees. The average quantity of sugars in the pulp was 310.2 mg for the non-shaded trees and 224.5 mg for the shaded trees. Thus heavily shaded cocoa trees produced pods with lower pulp sugar content, which may impact on the fermentation process and thus affect quality of fermented beans. Values for pulp sugar content were 238.8 mg and 2567.8 mg. for Amazon hybrids. And Amalonado cultivars respectively but the difference was not significant. Notably, the greatest variations in sugar content of the pulp were encountered in the Amazon hybrids. Shaded trees produced beans with significantly lower content of fermentable sugar/beans (78.9mg/beans compare to the non-shaded trees (155.2 mg/bean). Differences amongst the cultivars were however not significant. Shading of the trees showed no significant effect on the moisture content of the bean. The findings help to partially explain the high acidity often associated with large scale fermentation particularly of Amazon cultivars thus suggesting the beneficial effects of shade removal (adjustment) as a major management practice affecting cocoa yield and bean quality.

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Cocoa replanting on improved fallow land: a solution for sustainable cocoa culture

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Abstract

Cocoa, cultivated today on two million hectares, remains the driving force of the Ivorian economy. and today provides over 20% of the Gross Domestic Product and over 70% of the country's domestic savings. It is also a major source of income for many farmers and their families. However, these performances are not synonymous with the modernisation of Ivorian cocoa culture - its continuing shifting and extensive nature has resulted in the production zones moving from the East to the South-West of the country, thereby clearing several thousand hectares of forest. The fallow lands thus created are covered with *Chromolaena odorata* which prevents forest regrowth and planting of cocoa, which usually requires a forest environment. Work has started to plant the fallow lands with fast-growing legumes for two to three years. Planting of cocoa trees on these fallow lands has been compared with the cocoa tree-banana palm association. Using fast-growing legumes to improve the fallow lands has produced some very convincing results, which give hope to the sustainable replanting of cocoa.

Keywords: Replanting, legumes, improved fallow land, *Chromolaena odorata*, sustainable cocoa culture, Côte d'Ivoire.

The influence of paclobutrazol and ringing, applied at different phenological stages of cacao for flowering and leaf flux control

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Abstract

The application of ringing and paclobutrazol has allowed for the control of the cacao vegetative and reproductive process. The identification of the plant's phenological stage which best responds to these strategies, promoting a better asynchrony between the times of *Crinipellis pernicios*a spore liberation and the emission of susceptible tissues, could result in lower infection levels and a greater capability to control witches' broom. An alteration in the times of emission of leaves, flowers and the consequent displacement of production for a date less prone to the disease, would allow for a possibility to elude the disease in Recôncavo da Bahia. This essay was carried out at the CEPLAC's Experimental Station, situated at Recôncavo da Bahia, coordinates, 12° 30' South and 38° 29' West. Made up of twelve tests, repeated monthly, from May 2001 to April 2002, set out in cultivar "Catongo" cacao plants, 12 years old, 3x3m spacing, showing a good vegetative growth and an annual average yield of 675 kg/ha. Experimental units for each essay were set out in five blocks with four plots. Each unit, made up of one plant, was subjected to the following randomized treatments: paclobutrazol injected on the stems at 0,4 g.i.a./plant (PBZ-I); paclobutrazol applied directly on the ground at 1,5 g.i.a./plant (PBZ-S); ringing of the stem at 1,5 mm; and the standard. Regardless of the application periods, all treatments reduced the vegetative growth, PBZ-S being the most efficient, followed by ringing and PBZ-I. Results have shown that September and May are the best months for proceeding with ringing, with 40% and 32% reductions against the standard. August, October, May and June were the best times for the application of PBZ-S, showing a reduction of 51%, 45%, 28% and 22% in the total number of emitted leaves. Considering the total annual flowering, PBZ-I excepted, all the treatments have produced increases at every application times, ringing being the most efficient, followed by PBZ-S. Both for ringing and PBZ-S, its application at the end of the plant's vegetative repose period (February/March and August/September) has proved to be more efficient regarding a flowering increase, with values 150% to 850% higher in relation to the standard plants. Treatments have induced the anticipation and/or the amplification of the flowering periods, in up to seven weeks.

Indexing terms: Vegetative growth, flowering, paclobutrazol, ringing.

**MANAGEMENT OF EPIPHYTES AND MISTLETOES INFESTING
COCOA PLANTATION IN NIGERIA**

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Abstract

Three experiments were conducted at Cocoa Research Institute of Nigeria, Ibadan headquarters between 1999 and 2002 to quantify the effects as well as evaluating both cultural and chemical control measures of Bryophytes, Lichens and Mistletoes infesting Cacao trees. Experiment 1 and 2 involves four treatments each laid out in completely randomized design with five replicate while experiment 3 was laid out in randomized complete block design with ten treatments and three replicate both for Bryophytes and lichens respectively.

The results showed that Lichens at 60% and above stem coverage was responsible for depression in the flowering and fruit setting while Bryophytes infested trees recorded high incidence of cherelle wilt and diseased pods. Reduction in fruit setting, fruit size and weight was recorded for Mistletoes affected trees.

Cultural method of scrapping off the Bryophyte did not show any significant effect on pod yield whereas cutting off of Mistletoes significantly improve the yield ($P \leq 0.05$) Folar at 1.21 / ha followed by Glyphosate at 1.5 L / ha and paraquat at 1.51 /ha respectively significantly reduce the effect of Bryophyte infection ($P \leq 0.05$).

CROP PROTECTION

ENTOMOLOGY/IPM/RODENT

**STUDY ON THE RESISTANCE OF COCOA TREES TO MIRIDS IN COTE D'IVOIRE:
CLASSIFICATION OF MAJOR COCOA TREE GROUPS BY THEIR LEVEL OF
RESISTANCE/SENSITIVITY**

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Abstract

Classifying the major cocoa tree groups by their level of resistance/sensibility to mirids and identifying the resistant genotypes has been studied. Various cocoa genotypes introduced from different Latin American countries were planted in a collection plot at the Research Station of the National Centre for Agronomic Research at Divo, Côte d'Ivoire. These genotypes belong to the major groups of cocoa trees such as the High-Amazonian, Amelonado, Criollo, Trinitario, Catongo and non-cultivated species. Other genotypes belong to specific groups such as the Guyanais, the Venezuelan, the Ecuadorian, the double haploids from the High-Amazonian and those of unknown origin. All the genotypes were assessed on the basis of mirid damage in the field. Each genotype is marked according to leaf scorch and dieback. The results show that the cocoa tree groups differ significantly in their reaction to mirid attacks. The High-Amazonians, the cloned hybrids and the Guyanais turned out to be the groups least attacked by the mirids. The most sensitive groups were the Catongo, the Trinitario and the Amelonado. The different genotypes also recorded significant differences.

MASS PRODUCTION AND UTILIZATION OF EGG PARASITOID *Trichogrammatoidea bacteriae fumata* Nagaraja FOR THE CONTROL OF COCOA POD BORER *Conopomorpha cramerella* Snellen, IN MALAYSIA

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Abstract

The past research have made considerable advancement in the technology for mass rearing and field release of an indigenous egg parasitoid *Trichogrammatoidea bacteriae fumata* Nagaraja or Tbf for the control of cocoa pod borer *Conopomorpha cramerella* Snellen or CPB in Malaysia. However, the technology attracted little attention for commercial application because of high cost and inconsistency. In taking stock of the available technology and experience, studies were conducted to assess the system for mass production of rice moths and Tbf sufficient for release in 100 ha of cocoa fields and to evaluate the efficacy of field release of Tbf in commercial cocoa fields. The results show that Tbf were successfully multiplied in large quantity using rice moth eggs (*Corcyra cephalonica* Stainton) or RME as alternate host. The rice moths were reared on a feed formulation consisting of rice bran, rice bindled and milk powder. With the inclusion of a daily batch of 20kg inoculated feed, the mass rearing system was able to produce an average of 100g to 120g RME/day or at 5 to 6g RME/kg feed/day. This represents a production factor of 25 to 30 for RME. The field release study confirmed that Tbf is an effective bio-agent for the control of CPB. Crop loss due to CPB was substantially reduced from the initial levels of 28 – 43% to 4 – 9% at 6 months after field release of Tbf. Tbf released at higher density of 75,000 or 150,000 females/ha at 3 days release intervals have had a greater effect on CPB as compared to the lower density of 37,500 or 12,500 females/ha. But the effect was less apparent at the later stage. Rainfall, density of CPB eggs and density of pods on the trees and the quality of Tbf were found to influence the effectiveness of Tbf. The constraints and cost effectiveness of mass rearing and use of Tbf for the control of CPB are discussed.

TEMPORAL AND SPATIAL DISTRIBUTION OF THE COCOA STEM BORER, *EULOPHONOTUS MYRMELEON* FLDR. (LEPIDOPTERA: COSSIDAE)

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Abstract

The cocoa stem borer, *Eulophonotus myrmeleon* Fldr. (Lepidoptera: Cossidae) is increasingly becoming an important pest of cocoa in Ghana and other West African cocoa growing countries. Intensive studies on the development of an Integrated Pest Management (IPM) strategy for its control at the Cocoa Research Institute of Ghana (CRIG) are near completion. To determine the appropriate timing of the application of IPM, a study on the temporal and spatial distribution of the pest was conducted in years 2000-2003 in four cocoa farms located in three regions where the pest is prevalent. The experimental design was a Randomized Complete Block Design (RCBD) with three replications of 0.4 ha plots. The numbers of fresh borer holes on all cocoa trees in each plot were recorded at monthly intervals using the whole plant sampling method. The values for a and b (aggregation parameters) were obtained by linear regression of $\log S^2$ against $\log m$. There were primarily two periods of severe borer infestations. Larval populations started to build up in March reaching a first peak in May, dropped slightly in August and rose again reaching a second peak in October/November. The spatial distribution of borer infestation followed a contagious (aggregated) distribution pattern ($S^2 > m$) due possibly to oviposition behaviour of the female, which results in high densities of immatures resulting from egg masses. The study provides useful information for embarking on timely control measures, which should start in March when populations start to build up. Larger populations can be controlled using Gastoxin (aluminium phosphide) paste plus cultural methods of removal and destruction of infested branches.

**BARN OWL DEPLOYMENT FOR RODENT MANAGEMENT IN SOME MALAYSIAN COCOA
SMALLHOLDINGS**

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Abstract

Sixteen barn owl nest boxes were set-up in June 1999 in ten cocoa smallholdings in Muar, Johore at distances of 500-1,000 m apart. The ten cocoa smallholdings were about 1 ha each, spread out over 4 villages, intercropped with durian and the cocoa trees were being rehabilitated with matured budding. Crop loss and damage were assessed with barn owl occupancy and activity recorded at monthly interval over a three year study period. Initial high cocoa pod damage 30% (180-350 fresh damaged pods/plot) were noted to decline within 3 months to less than 2% (0-5 pods/plot) and fluctuating within that level. Similarly high durian fruit damage (averagely 25%, 50 out of 200 fruits noted) in the durian fruiting season declined to 0-5 fruits damaged noted for subsequent seasons. Owl occupancy was noted within 2 weeks of nest set-up and followed by breeding and establishment of a colony. The total number of new owls recorded present was 36 with 15 being recaptured, suggesting the latter being residential and 21 as emigrating/dispersal. The longest detected owl was 39 months. Nest box occupancy could be single as well as sharing over time between two or more owls, suggesting maximum nest exploitation for breeding. There was one prolonged breeding season per year coinciding with the on-set of rain and cooler weather. *Rattus tiomanicus* carcasses noted in the nests during the young owls development showed that the rat pest of cocoa was prey upon. The absence of squirrels culminated with low/no cocoa and durian fruit damage and a good crop showed that barn owls can be improvised for rodent management in cocoa, which is environmentally friendly and sustainable economically.

EVALUATION OF ORGANIC MATERIALS FOR THE CONTROL OF RATS (*Rattus rattus*) IN THE SYSTEM OF PRODUCTION OF ORGANIC COCOA (*Theobroma cacao* L.).

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Abstract

The production of organic cocoa is an opportunity for the Dominican Republic to sell on the international market and thus earn currency which will help to improve the level of entry and the producers' quality of life, since it is sold at a higher price which causes losses of up to 20% of production. An investigation was carried out to evaluate five organic materials, Cuban pine seed roots (*Gliricidia sepium*), avocado seed (*Persea americana* L.), roots of rice (*Oriza sativa*), stalks of cane (*Diffembachia sequium* L.), and coconut copra (*Cocus nucifera*) boiled with salt, with the objective of selecting the most effective material and thus reducing the percentage of cocoa pods attacked in the system of production of organic cocoa. The different materials were evaluated in the laboratory in separate experiments; for each material different doses were used and a final trial with the materials which presented toxicity at the most lethal doses was carried out. A completely randomised experimental design was used with five treatments and ten repeats in the final study. The materials which were toxic for the rats were edible pine seed roots, avocado seed, and coconut boiled with salt. Coconut boiled with salt and avocado seed had a 100% controlling effect.

PHYTOPATHOLOGY

**Containing the Spread of Witches' broom of Cocoa into Central America:
12 years later**

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Abstract

Between 1990 and 1994, FAO, with the support of OIRSA and the Ministries of Agriculture and Animal Husbandry of the Region, various projects were conducted aimed at securing the spread of witches' broom to the other countries of Central America, after the disease was registered in the southern part of Panama, bordering Columbia where the disease is endemic. Of all the measures taken, designed to stop the spread of the disease in uninfected areas of Panama, Costa Rica, Nicaragua, Honduras, El Salvador and Guatemala, monitoring its development in its new habitat and securing its spread within, and to other countries, took priority. A disease free buffer zone was determined, situated alongside the eastern length of the Panama Canal, approximately 40 km in width. Within this buffer zone, mismanaged and abandoned cocoa trees were removed and a search made, for then, known alternative host to the pathogen *Crinipellis pernicioso*. Quarantine measures were strengthened at inspection post at ports, airports and roads, and others posts installed at points of strategic importance. Mass public awareness campaigns were conducted at all levels of the community and technical personnel received training in the biology of the pathogen and management of the disease in the region. A further selected group, from each of the C. American countries, received specialized training to execute the role of key persons in the eventuality of witches' broom outbreaks.

The strategy that contemplated eradication and exclusion measures were directed specifically at witches' broom disease of cocoa. However, as an outcome of this project, the technical training received provided a standard methodology in management strategy of a disease that has been since applied for other diseases and in other crops in the area. Now, 12 years later after the projects were first implemented, other than its entry point in Panama, there has been no record of its presence in the other countries of C. America. Considering the lapse of over a decade in time since its exclusion into the countries under risk, validates the actions undertaken to be classified as successful, and outstanding for a tree crop. The region, estimated to have had 120,000 ha of cocoa, benefited socio-economically. A further benefit is that over the period when the disease remained stationary, considerable advances have been made in the management of witches' broom after it first appeared in 1989 in Bahia, Brazil. Thus, allowing for technology, more recently generated, to be applied in the case of an outbreak in the C. American countries, presently free of the disease.

THE EFFECT OF ECOLIFE-40 ON THE CONTROL OF WITCH'S BROOM DISEASE (*Crinipellis perniciosa*) IN THE COCOA TREE

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Abstract

The witch's broom disease caused by the fungus *Crinipellis perniciosa* (Stahel) Singer, is one of the most important diseases of the cacao tree (*Theobroma cacao* L.) in Brazil, in the Amazon region and in Southern Bahia, and is considered a restricting factor for its cultivation. This pathogenic affects seedling growth, flower cushions and developing fruit, causing a decrease in productivity of more than 70%, and severe uncontrolled epidemics. The removal of all affected tissues has been one of the main control procedures adopted, aiming at reducing inoculum sources and the damage caused by *C. perniciosa*. However, the efficiency of these methods, in isolation, depends on various factors, mainly on the infection level of the plants. The application of protective conventional fungicides, with copper and systemic components, is often un-economic, as it requires frequent applications in a short period of time. This is due to the continuous and fast growth of the affected tissues, linked with a regime of abundant rains. Thus, it has become necessary to test new products, mainly those with systemic action, with a capacity to induce fungus resistance and which do not pollute the environment. This research focuses on the assessment *in vitro* and *in vivo* of Ecolife-40's efficiency in the control of *C. perniciosa*. Ecolife-40 is a natural product, with invigorating and anti-stress properties for the plants, with a polysynegetic action, capable of improving the vigour and resistance of infected plants. In laboratory conditions, the inhibition of basidiospores germination and nuclear growth were determined, using different concentrations of the product. In nursery conditions, both the protector and systemic effects on the pathogenes were evaluated, using 2-month old cocoa seedlings. To evaluate its protective capabilities, Ecolife was applied at dosages of 3000 and 5000 of the p.c., 6 and 2 days after inoculation. To evaluate the systemic action, the product was applied at a concentration of 5000 ppm of p. c. and after 15 days, the apical shoots of the plants were removed and the new budding were inoculated 35 days after. Inoculations were carried out by the application of 30 μ l of a basidiospore suspension of *C. perniciosa* (1×10^6 basidiospores/ml) in the apical gemmas. The results of *in vitro* experiments have shown that Ecolife produced a 100% growth inhibition in germination and micelial growth, at 100 and 500 ppm concentrations, respectively. As far as the protection effect is concerned, the plants treated with Ecolife have shown a decrease in the incidence of the disease as compared to the standard, varying between 30 and 93%. Systemic action was observed, as the plants treated and, later, pruned, and after inoculation on the new apical shoots, have shown a lower percentage (30%) of infected plants, when compared with the standard (60%). Fungitoxic substances produced or induced by Ecolife-40 have shown a capacity to inhibit the growth *in vitro*, in tissues of treated leaves, of *C. perniciosa* up to 18 days after application.

Understanding the relationship between host and pathogen in developing an optimised screening method for identifying resistance in cacao to Witches' broom disease.

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Abstract

The main components of the disease triangle namely host-(leaf number, clone/seedling), pathogen-(spore-concentration) and environmental (incubation time) factors on inoculation success to Witches' Broom Disease were investigated in greenhouse experiments using susceptible cacao genotypes. Comparisons of three methods of inoculation (agar-drip, water-drip and spray) were done to determine inoculation success against the pathogen. The optimised inoculation method was tested for its repeatability as well as its ability to discriminate between various levels of resistance to *C. pernicioso* in cacao. The optimised method (350,000 viable basidiospores/ml; 60h incubation; agar-drip technique) was able to produce 100% infection on both clonal and seedling plants of a susceptible genotype, on a repeatable basis. Leaf number did not significantly affect percentage symptomatic plants or broom characteristics. This method effectively discriminated between the various levels of resistance in 14 cacao genotypes and is recommended as a screening method to identify levels of resistance in germplasm collections. Symptom severity has been shown to be a better measure of resistance than infection success.

Keywords: *Crinipellis pernicioso*, inoculation method, resistance screening, *Theobroma cacao*, witches' broom disease.

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GENETIC DIVERSITY OF THE CACAO PATHOGEN *Moniliophthora roreri* (Cif.) Evans *et al.* IN TROPICAL AMERICA.

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Abstract

Moniliophthora roreri causes moniliasis or frosty pod disease, an extremely destructive pod rot of cacao in South and Central America and a permanent threat to cacao cultivation world-wide. In spite of the economic importance of this pathogen, several aspects of its basic biology remain unresolved, in particular those related to the level and distribution of its genetic diversity.

Ninety-four isolates of *M. roreri*, collected throughout the natural and invasive range of the fungus, were studied to determine the level of molecular diversity of the species and to relate the information to the possible origin and biogeography of the pathogen. The study was carried out using two molecular techniques: AFLP and ISSR, and the results were compared. A significant genetic diversity of *M. roreri* was found. The highest level of genetic diversity was found in Colombia, where the fungus probably also originated. Five distinctive genetic groups were identified which show a particular geographic distribution. Origin and dispersal of these groups is discussed. Human activities appear to be the most important factors for the historical dispersal of the pathogen over long distances.

**GENETIC RELATIONSHIP BETWEEN THE FUNGUS *Moniliophthora roreri* (Cif.) Evans *et al.* AND
BASIDIOMYCETES DETERMINED THROUGH MOLECULAR TECHNIQUES**

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Abstract

Morphological evidence analysed in the 1970s demonstrated that the fungus *Moniliophthora roreri*, the causal agent of moniliasis or frosty pod rot disease of cacao, belongs to the Basidiomycetes rather than to the Ascomycetes. However, further classification of the fungus was not possible until recently, with the use of modern molecular techniques.

In order to determine the taxonomic affinities of *M. roreri* within the Basidiomycetes, different portions of its genome were amplified, sequenced and compared with the sequences obtained from other basidiomycetes and in particular from the witches' broom pathogen of cacao *Crinipellis pernicioso* (*Agaricales*). Two segments of the ribosomal RNA genes in nuclear DNA were amplified using specific primers: the internal transcribed spacer (ITS) region (partial 18S, ITS1, 5.8S, ITS2, partial 25S) and the 25S ribosomal RNA gene. The mitochondrial small ribosomal subunit RNA gene was also targeted for PCR amplification. Sequences from *M. roreri* and *C. pernicioso* were compared with those available in the NCBI (National Centre for Biotechnology Information) database using a BLAST search and taxonomic affinities were determined. The results of the study confirmed at a molecular level that *M. roreri* is a Basidiomycete and strongly suggest that it belongs to the order Agaricales. Sequences analysis also demonstrated that this fungus is closely related to *Crinipellis pernicioso*, although molecular differences were detected between them. This evidence indicates that the frosty pod rot pathogen belongs in the genus *Crinipellis*, and can be separated from *C. pernicioso* at the species level.

CANCER AND *CERATOCYSTIS*-WILT CONTROL IN COCOA TREES IN BAHIA, BRAZIL

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Abstract

Cancer or *Ceratocystis*-wilt in cocoa trees is caused by the fungus *Ceratocystis fimbriata*, which is essentially a secondary and opportunist parasite, usually associated with lesions. Presently, the illness is spread throughout all plantations of the variety Theobahia seminal (Sca-6 x ICS-1), and is the cause of major scale death amongst the cocoa tree population, and is now affecting grafting in clonal gardens. Consequently, a study has been designed, focusing on the developing of new strategies to control the illness.

Research was conducted in three experimental layouts, located in two different target- areas, with a variable degree of illness incidence. Commercial plantations of cocoa, of the Theobahia seminal variety, were used. 100 useful plants for treatment were earmarked in each layout. Each plant was used as a repetition. The treatments tested were: 1) integrated management, 2) relative standard and 3) absolute standard. In integrated management the following testing procedures were carried out: debudding, clearing of undergrowth, eradication of dead plants, scrapping of damaged areas, painting of the damaged areas with a solution of sodium hypochloride (1:1), application of a paste of copper oxide at 10% in addition to benomyl at 1%, as well as methmydophs at 0.5%. The damaged areas in the relative standard were subjected to scrapping, clearing of undergrowth, debudding and eradication of dead plants, whilst the absolute standard was subjected to the clearing of the undergrowth, debudding and eradication of dead plants only. All the data obtained, such as number of healthy sick, dead, recovered plants, cicatrix lesions and pathogenic sporulation on the surface of cuttings in sick plants, was registered on a monthly basis, for all the treatments involved.

Symptoms of cancer- *Ceratocystis* only were noticed on affected stalks, but no infection was found on the roots of the cocoa tree. Three species of *Xyleborus* (*X. affinis*, *X. ferrugineus*, *X. gracilis*) were associated to the cancer. In the integrated management layout, all the plants have survived, while in the relative and absolute standards, 38 and 46% of the plants, respectively, have died. In the integrated management layout, 67% of the affected plants were recovered against the standards. The association of the fungus *Gliocladium* sp. in the surface of the lesions was noticed, but no effect of this fungus was observed in lesion inhibition. In the new cocoa plantations, no infections or contaminations of new plants by means of materials remaining on the ground, was found.

C. fimbriata wilt on the cocoa tree is just a "cancer" associated to lesions found in the aerial part of the plant. The treatment of integrated management was more efficient both as far as illness control and the recovery of infected plants was concerned, as well as in the healing of pathogenic lesions.

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DEVELOPMENT AND STRATEGY IN THE FIGHT AGAINST SWOLLEN SHOOT DISEASE IN COCOA IN TOGO

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Abstract

Cocoa is one of the agricultural products exported from Togo. Depending on whether it is a good year or a bad year, domestic production varies between 6,000 and 10,000 tonnes. The traditional cocoa-growing areas are Litimé and Kloto, which are very far apart from each other.

Swollen shoot, one of the scourges of Togolese cocoa, was discovered for the first time in 1955 in the region of Agou (Kloto), which led to over 3.5 million trees being uprooted. Today, the swollen shoot problem is even more intense. The disease which for three decades was localised in the single region of Kloto - in particular Agou, Kpélé and Nyivé - is now found in Litimé, the main production area. Several diseased plots showing characteristic symptoms of a severe form of the disease (swelling of the shoots, reddening of the veins, mosaic) have been reported by planters. Researchers have also confirmed the existence of pockets in full expansion.

Apart from studying the epidemiology of the disease, the IRCC pathology laboratory in Togo, which was founded in 1977, has focused its work of fighting the Cocoa Swollen Shoot Virus (CSSV) on selecting tolerant hybrids and perfecting suitable replanting methods in the virus-affected areas.

As early as 1978 comparative tests on 14 hybrids brought in from Ghana for their productivity and tolerance to CSSV and 6 hybrids from Togolese seed fields were introduced into the virus-affected area of Tové in total randomisation tree by tree. Early selection tests on seedlings by bean inoculation were also carried out. This allowed the sorting of hybrids which were subsequently tested in the fields under high inoculum levels for over 10 years. Agronomic practices of uprooting host plants and isolating plots (physical barrier) were associated with the field evaluation of these hybrids. Satisfactory results were obtained.

Since 1995 the parents of the best combinations have been sown in seed fields to produce eight tolerant hybrids for replanting in the diseased areas. Armed with this knowledge, the Coffee and Cocoa Sectors Coordination Committee (CCFCC) in Togo is currently promoting evaluation of the magnitude of the disease (identification of the Litimé strains, districts with affected areas) with a view to successful replanting.

SOURCES OF RESISTANCE TO THE COCOA SWOLLEN SHOOT VIRUS DISEASE

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Abstract

The cocoa swollen shoot virus disease (CSSVD) continues to seriously affect cocoa production in Ghana especially in the Area of Mass Infection (AMI). In attempts to develop resistant cocoa varieties, several cocoa germplasm materials have been assembled in Ghana as a working collection to be used, in part, for breeding and selection of resistant cultivars. Four cocoa groups, namely, the Nanays (Na), the Parinaries (Pa), the Iquitos mixed calabacillo (IMC) and the Trinidad introductions (T) which have been utilized elsewhere successfully were investigated as sources of resistance to the disease. Clones (buddings) were created from 19 Nanays, 18 parinaries, 21 IMCs and 13 Tinidad introductions. The following F1 progenies were also created; within group crosses, between group crosses and selfed progenies. The clones/parents and the various seedlings progenies were screened in the gauzehouse by inoculating the individual seedlings using viruliferous nymphs of the mealybug vectors of the virus and observing the plants for 12 months in the greenhouse for virus symptoms. Randomized complete block design was used. Variability was low between and within the clones as well as the progenies of the selfed seedlings and the intragroup crosses but significant variability was observed in the intergroup crosses. Relatively large numbers (20-30%) of resistant progenies in the intergroup crosses were recorded especially those involving the Trinidad introductions (T) as female parent whilst the other progenies revealed zero or negligible numbers of resistant individuals. The results indicate that resistant genes are not confined to any one group but scattered among them. However, there was ample demonstration that intergroup crosses can generate some level of variability to aid selection for CSSV resistant genotypes. Some resistant clones (8) and progenies (12) have so far been selected and planted in the AMI for further field evaluation.

Variability of the cocoa swollen shoot virus (CSSV): cloning and analysis of four new complete sequences

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Abstract

The cocoa swollen shoot virus (Caulimoviridae family, badnavirus genus) is transmitted by scale insects and is found throughout the West African cocoa-producing regions. The characteristic symptoms of the disease are reddening of the veins on young leaves and swelling of stems and roots. The first full sequence of a CSSV isolate was obtained in 1993 (Hagen *et al.*, 1993), but very little information is available on the molecular variability of this virus. A new Togolese isolate (Nyongbo 2) and three from Ghana (N1A, New Juaben and Peki) have been cloned after expanding the total sequence by PCR. One clone per isolate was chosen for the sequencing.

The size of the total genome varies between 7024 pb for isolate N1A to 7242 pb for isolate Nyongbo 2. The ORFs are organised overall in the same way for all the isolates and differ basically in the partial disappearance of ORFX for the three Ghanaian isolates and the existence of an additional open reading frame for the Peki isolate, which encodes for a potential protein of 11 kDa. The five isolates can be clearly separated according to geographic location (Togo or Ghana) rather than their aggression (N1A and Peki cause reduced symptoms, without swelling) by aligning the nucleotide and protein sequences. Four phylogenetic trees were built using amino acid sequences encoded by open reading frames 1, 2, 3 and Y of the five CSSV isolates.

The ORFX of the three Ghanaian isolates is 20% less than for the Togolese isolates and there is far greater distance between the encoded proteins than between those encoded using other ORFs. The amino acid substitution rate is very important for this ORF compared with other ORFs, which implies a non-functional role of open reading frame X as of open reading frame 4 of the Peki isolate (no similar protein to the one potentially encoded by ORF4 of the Peki isolate was revealed by BlastP investigation and this open reading frame is not found in the other isolates). At the same time, ORF Y which encodes for a protein with an unknown function is well preserved. The overall variability between the five isolates (around 80% of nucleotide identity) is not very high compared with the variability found in the corresponding field of the ORF3 reverse transcriptase, between the isolates from two different regions in Togo (60% of nucleotide identity). It would be particularly interesting now to obtain full sequences of these very diverging isolates. Transmission tests involving bombarding cocoa trees with these clones are in progress to confirm these various conclusions.

***Trichoderma* : its potential for control of diseases of cacao**

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Abstract

Chemical-based control strategies to combat diseases of cacao are financially and ecologically expensive, and are not very effective. Biological control offers the possibility of a less expensive, more environmentally sensitive and effective alternative to chemical control. Species of *Trichoderma* are being used or evaluated for use in control of cacao diseases in Brazil, Cameroon, Costa Rica Ecuador, and Peru, and in one instance a *Trichoderma* species is the active ingredient in a patented product, TRICHOVAB, that is effective against witch's broome. Additional species or more effective strains are required.

Time, effort and expense in finding novel biological control species and strains can be saved by directing the search either by exploration for new strains in native areas of the host and/or its pathogen (classical biological control technique) or by studying species that are known to be phylogenetically related to species that are effective in biological control (molecular phylogenetics).

Species of *Trichoderma*, and other fungi, have been isolated from cacao trunks in the upper Amazon region. These comprise species that are already being used in biological control applications but from non endophytic sources (*T. stromaticum* in Brazil, *T. cf. koningii* in Ecuador). The endophytic strains of *T. stromaticum* are more effective in control of witch's broom than the original non endophytic strains. In addition, several new species of *Trichoderma* have been found among the endophytes of cacao. These are being evaluated for their biological control possibilities.

A phylogenetic framework of *Trichoderma* is being established through the use of DNA sequence analysis. Morphologically-based names used for *Trichoderma* species are often incorrect or refer to more than one species. DNA sequence analysis helps to define species limits and reveals previously unrecognized species. DNA sequence analysis also permits the development of a phylogenetic tree. Such a tree reveals the relationships of *Trichoderma* species and – significant to the search for biological control species – enables the prediction of biological properties of an unknown species based on its relationship to species, the properties of which are known.

Biological control of the cacao black pod disease causative agent *Phytophthora megakarya* with *Trichoderma asperellum*.

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Abstract

As part of efforts for developing alternatives for cacao black pod disease management in Cameroon, 200 strains of *Trichoderma spp*, isolated from a forest natural reserve and from farmers' fields were screened in the laboratory for their potential for biocontrol towards *Phytophthora megakarya*. Four isolates PR10, PR11, PR12, 659-7 identified as *Trichoderma asperellum*, were selected for effective mycoparasitism, and could completely suppress *P.megakarya* on precolonized plates. These isolates were further evaluated in a small scale field test conducted in a farmer's field at Esse (60km south of Yaounde); treatments included isolates of *Trichoderma asperellum* (PR10, PR11, PR12, 657-9), a chemical fungicide (Ridomil plus 72 WP) and the control. For each treatment, plots were made of a set of four cacao trees replicated five times in a completely randomized design. Data collection was made every week. Results of this preliminary field test reveal effective reduction of diseased pods for some isolates of *T.asperellum* (PR10 , PR11) below 5% whereas the disease level of the non treated control was around 20%, three weeks after spraying.

Key words: Biocontrol, *Phytophthora megakarya*, *Trichoderma asperellum* , on-farm experiment.

Biological Control of *Phytophthora* Pod Rots of cacao: Strong fungicidal activity shown by a *Myrothecium verrucaria* strain against several pathogenic *Phytophthora* species.

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Abstract

Black pod caused by several species of *Phytophthora* is the most important yield-suppressing disease on cacao especially in Africa. This plant disease presents a persistent challenge to cacao producers and plant pathologists alike. The major control methods used such as plant resistance and chemical applications are not entirely effective. This suggests that alternative or complementary methods are required for management of the disease. One such option could be a biological control approach using antagonistic microorganisms. A renewed interest in biological control of plant diseases in agriculture and horticulture has evolved over the last two decades partly as a response to public concern about the use of hazardous agrochemicals. Despite the recent advances made more progress is still needed in this area of research. In this context, the ability of several antagonistic strains of *Myrothecium verrucaria* and *Myrothecium roridum* to inhibit a broad range of pathogenic *Phytophthora* isolates (*P. megakarya*, *P. palmivora*, *P. capsici* and *P. citrophthora*) was evaluated. One *Myrothecium* strain was found to exhibit a particularly strong *in vitro* antagonistic effect against the causal agents of pod rot. The characterization and properties of the fungicidal compounds produced by this strain are now being investigated in the laboratory.

**BIOLOGICAL CONTROL OF WITCHES' BROOM DISEASE,
(CRINPELLIS PERNICIOSA), WITH THE CO-EVOLVED FUNGUS, TRICHODERMA
STROMATICUM: TESTING DIFFERENT DELIVERY REGIMES**

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Abstract

Witches' Broom disease, caused by *Crinipellis perniciosa* (Stahel) Singer, is the most serious disease of cacao (*Theobroma cacao* L.) in Brazil. While recommended control measurements are the use of host resistance and cultural- and chemical control, biological control with antagonistic microorganisms, e.g. *Trichoderma stromaticum* Samuels & Pardo-Schultheiss, sp. nov., could become an important part of a future IPM control strategy. *T. stromaticum*, which is a mycoparasite that avoids the basidiocarp formation, has shown good potential in the laboratory and when applied to infected material on the ground. When applied in the canopy, however, the results have been less promising. The standard application practice is to mix *T. stromaticum* with water only. A field experiment was therefore carried out to evaluate the efficacy of adding adjuvants and/or nutrients to inoculum of the antagonist applied to infected tissue in the canopy. The antagonist was applied to hanging brooms as recommended or separately mixed with four adjuvants (three surfactants (Agril-320, Extravon and TacTic) and one mineral oil (OleoNatural)) and/or nutrients (sucrose). The treatments were compared with a non-treated control. Applications were repeated on half of the brooms after 15 days.

The success of delivering the antagonist was determined by field observations of visual *T. stromaticum* sporulation on the brooms and colonisation of isolated broom fragments, incubated in a humid chamber in the laboratory. Field observations and laboratory results showed that 56.7 - 78.3% and 83.3 - 96.7% of the brooms were sporulating in the field and showed colonisation in the laboratory, respectively. Although not statistically significant, all adjuvant and/or sucrose added suspensions showed a higher degree of field sporulation (14.7 - 38.2% increase) and a higher laboratory colonisation percentage (6.0 - 16.0% increase) compared to recommended treatment. On a long-term scale the anti-sporulating effect of the antagonist on the pathogen was also evaluated. Results indicated that antagonist-treated brooms produced significantly less pathogen basidiocarps, compared to non-treated control brooms. This was due to a significantly lower basidiocarp production per broom as well as significantly less brooms initiating sporulation by the pathogen. Although not statistically significant, adding an adjuvant and/or sucrose to the *T. stromaticum* suspension further reduced basidiocarp production with 32.3 - 75.6%, compared to the recommended treatment. Field and laboratory results showed that applying the antagonist twice instead of once did not significantly affect *T. stromaticum* sporulation or colonisation by the antagonist or the basidiocarp production of *C. perniciosa*.

INOCULATION OF BENEFICIAL ENDOPHYTIC FUNGI INTO *THEOBROMA CACAO* TISSUES

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Abstract

Endophytic fungi appear to be ubiquitous in healthy plant tissues and much evidence suggests that endophytes of at least some plants help hosts tolerate adverse abiotic and biotic factors including pathogens. This suggests the potential use of endophytes as biocontrol agents. Nevertheless, few studies have focused on (1) determining the ecological relationships of endophytes associated with plants of agricultural significance or (2) using this information to guide the cultivation and inoculation of beneficial endophytic fungi into host plant tissues. Here, we describe a series of studies regarding endophytes associated with *Theobroma cacao*, with the dual goals of understanding the abundance and diversity of endophytes associated with this host, and of assessing those endophytes for use as biocontrol agents. First, we made a systematic collection of endophytic fungi of *T. cacao* from different agro-ecosystems in the Republic of Panama. We then conducted preliminary *in vitro* trials to assess the antagonistic activity of 50 morphotypes of endophytes against three major pathogens of cacao: *Phytophthora sp.*, *Moniliophthora roreri* and *Crinipellis pernicioso*. Forty percent of tested morphotypes showed antagonistic activity against at least one of the three pathogens. A subset of these active morphotypes was used to evaluate a method to introduce endophytic fungi into cacao tissues. Under greenhouse conditions, we sprayed spore suspensions in water gelatine (0,5%) to successfully introduce endophytic fungi into leaves of *T. cacao*. Next, we assessed the *in vivo* activity of endophytic fungi by exposing both treated (+endophytes) and untreated (-endophytes) leaves of *T. cacao* to a *Phytophthora* strain. We observed that leaves with a mix of endophytes showed nearly 50 % less damage due to the pathogen when compared with leaves without endophytes. Finally we conducted a field trial in the Bocas del Toro region of Panama to evaluate the effect of endophytes in the control of Moniliasis and Black Pod disease. Effects of three different endophytic species were assessed in four different farms. Once a month for 7 months, we used hand-held compression sprayers to apply spore suspensions of endophytes to developing and mature cocoa pods. Combined data for all the farms showed significant reduction in the proportion of cocoa pods lost to *Phytophthora sp.* in one of the endophyte treatments compared with the control during the last 4 months of the study. Together, these results suggest that endophytes may be used as biocontrol agents to benefit cacao plants, and that they are worthy of further exploration.

BIOLOGICAL CONTROL OF BLACK POD DISEASE IN COTE D'IVOIRE: IDENTIFICATION AND USE OF NATURAL ANTAGONISTS TO *PHYTOPHTHORA SP.*

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Abstract

The appearance of *Phytophthora megakarya* in the eastern cocoa areas has resulted in a substantial increase in losses caused by black pod disease. Losses in this region have gone from 10 to 15% to 30-45% with *P. palmivora*. Controlling black pod disease has now become a national priority. The strategy adopted is to develop inexpensive integrated pest management compatible with environmental concerns. One of the strategy's favoured approaches is to use natural antagonists to *Phytophthora* sp. Biodiversity has therefore been investigated in the cocoa tree ecosystem. A collection of micro-organisms comprising fungus, bacteria, actinomycetes and yeasts has been established. The antagonistic action of these micro-organisms against *Phytophthora* sp. was assessed *in vitro*. The best antagonists were selected and fermentation and formulation techniques perfected. The programme has currently reached the field experimentation phase.

Effects of Resistant Genotypes of *Theobroma cacao* L. on the genetic diversity and pathogenicity of *Phytophthora megakarya* , Causal Agent of Black Pod Rot in Cameroon

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Abstract

A study on the effect in the field of resistant clones of cacao (*Theobroma cacao* L.) on the epidemiology and evolution of populations of *Phytophthora megakarya* was carried out in collaboration between CIRAD and IRAD . In this presentation, only results of the characterization of strains and their impacts on the epidemiology of the disease , are developed.

A totally randomised design was employed using the clones SNK10 (susceptible) , SNK413 (resistant), ICS84 (medium resistant) and UPA134 (medium resistant), planted in 1982 in Barombi-Kang, Cameroon. A maximum of 50 fruits per tree were observed on 20 trees per clone during 3 growing seasons from 1999 to 2001. The isolation of the pathogen was carried out on each black pod and all the strains were sent to Cirad Montpellier for genetic and physiologic characterisation. The pathogenicity of the strains was assessed on the four clones planted in Barombi-Kang and on 12 other clones with known level of resistance to black pod by leaf disc inoculations in Montpellier and by detached pod infections in Cameroon.

Only 2 genotypes of *P. megakarya* and 1 genotype of *P. palmivora* were genetically characterized on this clonal field (on the 432 strains that were obtained at Montpellier). The spatial and temporal population structure of these 3 genotypes were identical for the 3 years of the study, suggesting that the stability of the population structure is mainly due to asexual reproduction of the two pathogens present in this field (all the *P. megakarya* strains were the A1 mating type and all the strains of *P. palmivora* were the A2 mating type). Whereas the genotype 1 of *P. megakarya* was isolated at similar levels from pods of the four different clones, the genotype 2 was mostly found on pods of the susceptible clone SNK10. The 5 isolates of *P. palmivora* were only obtained from pods of SNK10 and UPA134. It was found that there was a strong specialization *P. megakarya* genotypes on the cacao clones in this field, particularly between ICS84 and the genotype 1.

The pathogenicity tests and the genetic characterization allowed us to improve both the leaf disc and the detached pod inoculation tests. These results, as well as those obtained in the epidemiologic study were discussed and gave us good information on the impact of environmental conditions on cacao resistance and on the pathogenicity of the strains of *Phytophthora* in Cameroon.

The discovery of *Phytophthora megakarya* on a forest host in Korup National Park (Cameroon)

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Abstract

Phytophthora megakarya is having an increasing impact on cocoa production in Central and West Africa and poses a direct threat to the Ivory Coast following recent reports of its occurrence near the border with Ghana. Current cultural and chemical control strategies are either ineffective or too expensive for the resource – poor farmers to implement. Alternative control measures are urgently required, preferably sustainable, cheap and easily integrated into existing management practices. Classical biological control, which has been successfully used to control invasive alien weeds, is one such option but is dependent on identifying the centre of origin or diversity of the target weed, pathogen or pest. The humid forest zone around the Nigerian/Cameroon border has been highlighted as a possible centre of origin of *Phytophthora megakarya*, and thus a potential source of highly adapted or coevolved natural enemies.

During 2001, an exploratory survey was undertaken in the primary forests of Korup National Park in south-west Cameroon, concentrating particularly on potential Sterculiaceae hosts such as *Cola* spp., in which this ancient forest ecosystem is particularly rich. There was no evidence of *Phytophthora* disease on *Cola* pods but black pod lesions were observed on fallen but still green fruit of a member of the *Irvingiaceae*, probably *Irvingia gabonensis* (elephant mango). Isolations were made from these lesions onto selective media.

Following molecular characterisation in the UK, using ITS fingerprinting, these isolates have been positively identified as belonging to *Phytophthora megakarya*. However, AFLP comparison with other isolates of *P. megakarya*, derived from cocoa agro-ecosystems in West Africa, revealed that the forest strain is distinct, suggesting that it probably represents an ancient progenitor of the cocoa pathogen. In addition, a range of fungal antagonists were consistently-associated with *P. megakarya* on the isolation plates, often severely restricting or completely inhibiting its subsequent growth and development. It is concluded that this forest habitat and host represent a potential source of novel, coevolved natural enemies which could be exploited as classical biocontrol agents for either inoculative release or inundative application (mycofungicide) in the cocoa plantations of Central and West Africa.

058E

OR: FRENCH

Effectiveness of a *P. palmivora* leaf inoculation test on cocoa to assess genetic parameters of the fruit's resistance in the field

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Abstract

This is a genetic analysis of resistance to *P. palmivora* examined in a factorial test 4 x 2 established in the field in 1988. The two resistance traits tested are resistance on leaf disks measured according to notations on disks and resistance of the fruit in the field, assessed by the percentage of rotting pods. The results reveal a distinction between the eight progeny tested by each of these two resistance evaluation methods. The general traits from this combination are the main source of variation and indicate that the additive effects predominate for these 2 traits. Heritability of resistance in the strictest sense is 0.55 for notations on leaf disks and 0.42 for the percentage of pods rotting in the field. The existence of positive and significant genetic and phenotypical correlations between these 2 resistance traits shows that the leaf disk test can be used effectively to predict the reaction of the pods in natural infection conditions in the field.

EVALUATION ON THE EFFICACY OF EPIPHYTIC BACTERIA AS BIO-AGENT FOR THE CONTROL OF BLACK POD DISEASE

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Abstract

A study on the use of epiphytic bacteria as bio-agent to control black pod disease of cocoa was conducted at Tawau, Sabah in Malaysia. Screening for potential biocontrol agent through dual culture method demonstrated that eight isolates of potential epiphytic bacteria from cocoa pod surface were identified as *Pseudomonas spinosa* (LKM/B/2), *Pseudomonas aeruginosa* (LKM/B/5; LKM/B/62b), *Pseudomonas putida* Biotype A (LKM/B/1; LKM/B/35; LKM/B/76c), *Burkholderia gladioli* (LKM/B/4) and *Burkholderia* spp. (LKM/B/6) had a significant antagonistic effect that substantially inhibited the growth of *P. palmivora*. *Pseudomonas putida* Biotype A (LKM/B/76c) was also found to significantly inhibit the growth of *P. palmivora* through the production of antifungal substances and *Burkholderia gladioli* (LKM/B/4), *Pseudomonas aeruginosa* (LKM/B/5) and *Pseudomonas putida* Biotype A (LKM/B/76c) also had the capability to produce volatile antifungal substances that inhibited the growth of *P. palmivora*. It was observed that most of the epiphytic bacteria(s) disintegrated the cell wall of *P. palmivora*. It was proven that the hyphae was observed to have the stages of hyphae lysis which consequently had stunted the growth of *P. palmivora*.

The eight epiphytic bacteria isolates were mixed together in a liquid formulation and mass produced in order to give intense synergistic effect for used in field evaluation. The preliminary results demonstrated that, the liquid formulation containing the eight isolates of epiphytic bacteria had reduced significantly the infection of black pod disease as compared to the check plot without the microbial treatment and it was comparable to the copper treatment in effectiveness. The potential of these epiphytic bacteria as bio-agent for the control of cocoa black pod disease is clearly indicated.

Keywords : Epiphytic bacteria, antagonist , black pod disease, bio-control agents.

**INDEPENDENCE OF GENETIC DISEASE REACTION TOWARDS PHYTOPHTHORA
PALMIVORA AND MONILIOPHTHORA RORERI, AND POPULATIONS OF EPIPHYTIC,
ANTAGONISTIC FUNGI IN COCOA (*THEOBROMA CACAO*)**

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Abstract

Genetic disease resistance and biocontrol are two important control options in the management of black pod, caused by *Phytophthora* spp., and moniliasis, caused by *Moniliophthora roreri*, of cocoa (*Theobroma cacao*). Their simultaneous employment in integrated disease management requires knowledge of the interaction between cocoa genotypes and potential antagonists of these pathogens. The objective of this study was to compare the populations of mycoparasitic fungi naturally found on cocoa hybrids with a differential reaction to *P. palmivora* or *M. roreri* and relate their abundance to the disease reaction of the germplasm.

The cocoa hybrids used in this study form part of CATIE's on-going breeding programme. Disease reaction categories were defined according to information provided in progress reports. Mycoparasites were isolated quantitatively from cocoa flowers using agar plates precolonised with the respective pathogen as baits. Their counts were compared using a general linear model for Poisson-distributed data with a hierarchical (nested) arrangement, where the disease reaction classes represented the upper level and individual trees of the segregating F1 population within each reaction class, the lower level.

The F1 generation of the hybrid Catongo × Pound 12 segregated into trees with a susceptible, intermediate or resistant reaction to *Phytophthora palmivora*. The three categories supported similar numbers of total mycoparasites ($0.134 \leq P \leq 0.788$) and of the predominant mycoparasite genera (*Clonostachys*, *Fusarium*, *Penicillium* and *Trichoderma*) ($0.057 \leq P \leq 0.870$). One susceptible tree supported significantly ($P=0.042$) more total mycoparasites than one intermediate and one resistant tree with low mycoparasite populations. No other differences between individual trees were found ($0.057 \leq P \leq 0.992$). The F1 progeny of various crosses between CATIE-1000, CC137, CCN-51, ICS-95, UF-273 and UF-712 were categorised as susceptible, moderately susceptible, moderately resistant and resistant reaction towards *M. roreri*. Again, these classes supported similar populations of total mycoparasites ($0.575 \leq P \leq 0.891$) and the principal taxa (*Clonostachys*, *Fusarium* and *Trichoderma*) ($0.446 \leq P \leq 0.992$). No differences between individual trees were found ($0.416 \leq P \leq 0.992$).

We conclude that genetic disease resistance to black pod and moniliasis, and natural mycoparasite abundance are independent disease control mechanisms. Therefore, their simultaneous use in integrated disease management should lead to an additive effect.

CHEMISTRY/TECHNOLOGY/ QUALITY

COCOA PROCYANIDINS AND THEIR LOSSES DURING FERMENTATION AND DRYING

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Abstract

Polyphenols are known to provide the basis of colour, flavour and astringency to cocoa, but in recent years scientific evidence about the health benefits of these compounds has also been revealed. Polyphenols in cocoa are classified as flavonoids and their content in cocoa beans is between 12% and 18% of dry weight. Flavanols and more complex procyanidins represent 90 % of cocoa flavonoids. The rest are mainly purple anthocyanins that are responsible for the colour of fresh cocoa beans. After the cocoa bean fermentation and drying only small proportions of cocoa flavonoids remain in original, unoxidised form. The scope of this work was to determine how fermentation and drying affect levels of monomeric and oligomeric procyanidins in different cocoa genotypes.

Flavanols in cocoa are mainly monomeric (-)-epicatechin and (+)-catechin, the former being much more abundant. Procyanidins are complex molecules consisting of two or more flavanol monomers. Recently our laboratory has developed a normal phase HPLC method that measures the quantity of individual cocoa procyanidins with greater sensitivity and selectivity. This method uses a mixture of purified procyanidin oligomers as a quantitative external standard, permitting a detectable limit of 0.01 – 0.02 mg/g and a quantifiable limit of 0.03 – 0.06 mg/g for (-)-epicatechin monomer to decamer, respectively.

Large variations in procyanidin content and differences in proportions of each oligomer were revealed in HPLC analysis of cocoa beans from diverse cocoa origins and of diverse genotypes. The main differences are based on the genetic origin of cocoa planting materials and the degree of fermentation. Measurements of genetically defined procyanidin content showed a large range of values from 20.8mg/g to 80.5mg/g of dry nibs. To define how procyanidin content in different cocoa genotypes is affected by fermentation and drying, samples of beans from 15 cocoa genotypes were prepared for analysis. Four samples were prepared from beans of each genotype: unfermented/freeze-dried, unfermented/sundried and fermented/freeze-dried, fermented/sundried. All samples were analysed by the reference HPLC method. These experiments allowed us to determine the genetically defined content of procyanidins in each cocoa genotype and also helped us to understand how these quantities were affected by fermentation and drying. Results showed significant variation in procyanidin losses during fermentation and drying for each tested cocoa genotype.

DEFINED COCKTAIL OF MICROORGANISMS FOR COCOA FERMENTATION

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Abstract

A cocktail of microorganisms was inoculated onto cocoa beans immediately after the pod was broken open and left to ferment in a newly designed stainless steel vessel that can be sterilised, and capable of turning a 50kg load of cocoa beans. Inoculum, aeration and turn rate was controlled, the temperature was monitored and samples were taken at regular intervals for microbial and sensorial analysis. The defined microbial cocktail was prepared for use as an inoculum consisting of one good fermenting yeast, one pectinolytic yeast species, two lactic acid bacterial species and two acetic acid bacterial species. All the strains used were isolated from natural cocoa fermentations. The yeast *Saccharomyces cerevisiae* is a well-known good ethanol producer and the yeast *Kluyveromyces marxianus* selected due to the ability of producing pectinase. Both yeasts can ferment all pulp sugars at pH 3.5-4.2, were ethanol tolerant and were present at the beginning of natural fermentations. The lactic-acid bacteria *Lactobacillus lactis* and *L. plantarum* were selected because they are able produce lactic acid in the particular conditions in cocoa pulp: pH of 3.5, low oxygen levels and temperatures between 33°C. and 39°C. The best producers of acetic acid that are also tolerant to temperatures of 45 °C were isolates of *Acetobacter aceti*. *Gluconobacter oxydans* has been added as well to oxidise the ethanol to acetic acid, to CO₂ and to water. Each group of microorganism was left to ferment separately. From the results obtained the fermentation was not complete when only one or two groups were used as inoculum. This proved that it is necessary the interactions between yeasts and bacteria to perform the chocolate flavour. When the microbial cocktail was used as inoculum the three key metabolites in the pulp, namely ethanol, lactic acid and acetic acid showed similar sequential rises and falls to that found in spontaneous fermentations. Contamination with extraneous microorganisms was kept to a minimum. The beans were then dried and roasted and chocolate was produced by the usual means. A taste panel found the product as good as that formed by a natural fermentation, thus suggesting that the fermentation occurred normally. Defined cocktails of microorganisms are more reliable, because of the lack of spoilage organisms. These results showed that the use of fermenter mimics the natural conditions of fermentation boxes and produces fermented beans in five days. Combined with the use of defined inocula there is the prospect of producing the best quality chocolate reliably and in less time.

EFFECT OF BEAN LOADING ON THE QUALITY CHARACTERISTICS OF COCOA BEANS DRIED UNDER A DIRECT SOLAR DRYER

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Abstract

Direct solar dryers were developed to assist the smallholders in areas where the harvesting season coincides with the rainy season. The drying concept was such that the product is placed inside a transparent enclosure on a perforated wooden platform and dry primarily using direct sunlight. The objective of the study was to examine the effect of loading (20 kg, 30 kg and 60 kg wet fermented cocoa) on the quality characteristics of cocoa beans dried under the direct solar dryer. Surface mouldiness was light on the 20 kg treatment but moderate on the 30 kg treatment. In contrast, dried beans from the 60 kg treatment appeared blackish with heavy surface mould. Odour assessment showed that all the dried beans were reasonably acceptable in terms of vinegary odour ranging from weak to strong. All the dried beans were found weak in terms of alcohol type odour. However, unclear trend was observed for the fecal, rancid and cheesy odour rating. The 60 kg treatment was rated at the stronger level of wet sock odour due to poor drying condition. In terms of pH and titratable acidity, significant different ($P \leq 0.05$) was found between the 60 kg treatment and the lower loading treatments (20 kg and 30 kg). Prolonged drying period has allowed the 60 kg treatment to achieve lower acidity which could be due to the neutralization effect by the alkaline compounds derived from the putrefactive activities. Cut test assessment showed that in general the cocoa beans of lower loading treatment have the lowest percentage of purple beans but highest in percent brown beans. The 20 kg treatment showed the highest cut test score which is significantly different ($P \leq 0.05$) from the 60 kg treatment. Degree of browning was better at thinner bed layer due to better aeration. However, fermentation index showed no significant difference ($P \leq 0.05$) among the treatments but the lower loading treatment showed the tendency for higher index. No significant difference ($P \leq 0.05$) was found among the treatments in terms of cocoa, astringency, bitterness and sourness flavour notes in liquor sensory evaluation. However, the 20 kg treatment showed the tendency for better score in terms of cocoa, astringency and bitterness flavour notes as compared to the other treatments. No mouldy off-flavour was found in all the dried beans. Bean loading at 20 kg of wet fermented cocoa was hence recommended. At this loading, the beans did not suffer from putrefactive activities, produced beans with good appearance, extremely light in surface mould, high in cut test score and fermentation index and acceptable in terms of odour and liquor sensory evaluation.

092E
OR: FRENCH

**DETERMINING THE GEOGRAPHIC ORIGIN OF COCOA BY NEAR-INFRARED SPECTROMETRY:
PRELIMINARY STUDY**

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Abstract

The traceability of food products (including cocoa) is a major preoccupation among consumers. Simple, fast methods for the inductive control of raw material origins are therefore necessary.

Cocoa is a good example of the complexity of the problem for the variability in quality depends on numerous factors, including, in particular, the variety, the land and the post-harvest practices. This quality is linked to the biochemical composition; however, although biochemical variety markers do exist, albeit rarely, to date there are none for geographical origin.

Global fingerprinting methods are currently a simple way of addressing this problem.

Near-infrared spectrometry has been tested as a means of identifying three geographical origins. For this study, the spectrums (400-2500 nm) of 149 hulled and ground samples, 71 from Côte d'Ivoire (Amelonado, Forastero hybrids, free progeny), 33 from Trinidad (Forastero and Trinitario) and 45 from Venezuela (modern Criollo), were acquired through diffuse luminous reflectance using a Foss 6500 monochromator. These samples allow for variability due to post-harvest practices and three years of production.

Different classification methods based on the spectral data (such as partial least square regression, discriminant analysis on principal components and a stepped discriminant analysis) were tested.

The discriminant model developed from these principal components extracted from the spectral data, having eliminated the water absorption bands, results in 100% success in classifying the samples according to their country of origin.

The model's prediction performances were tested as follows: 20 samples taken from the 149 at random were correctly classified (100%) by applying a discriminant model built from the remaining samples.

These results are promising for setting up a rapid and non-destructive control method for the geographical origins of cocoa. This tool requires a wider database incorporating the main producing countries, the principal varieties grown and covering several harvest years. Developing such a tool also requires assessing the capability of the models to predict mixes of geographical origins.

Keywords: Cocoa, geographical origin, near-infrared spectrometry, classification

094E
OR: FRENCH

CHANGES IN COCOA QUALITY DURING CONTROLLED-ATMOSPHERE STORAGE SIMULATING PORT CONDITIONS

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Abstract

Cocoa is generally embarked in the month following post-harvest processing. However, part of the mid-crop cocoa in West Africa and the stocks are held for several months before being exported.

To assess the effects of storage on their quality, the following cocoas:

- 2 healthy lots (November 2000 and January 2001 harvests) pods opened respectively 0.5 and 9 days after harvesting, fermented for 6 days and dried in the sun,
 - 2 healthy lots (December 2000 and January 2001 harvests) fermented respectively 0.3 and 6 days (pods opened immediately) and dried in the sun,
- were stored for 9 months in a temperature-controlled unit (27°C, RH 75%), with timed withdrawals at 0, 2, 3, 5, 7 and 9 months.

The content of free fatty acids, purines (HPLC), procyanidins (HPLC), sugars (HPLC) and volatile compounds (GC) were determined.

Over the total 9 months, the water content increased from 1 to 2% and the free fatty acids by 0.4%, which therefore remained well below 2% at the end of the storage period. Purine and procyanidin content remained fairly steady whereas the sugar content dropped dramatically:

- regardless of the delay in opening the pods, the content of reducing sugars of the cocoa fermented for 6 days dropped from 80% to 100% for glucose and 20% to 50% for fructose respectively
- the same applied to the cocoa (immediate pod opening) fermented 3 or 6 days, whilst non-fermented cocoa barely changed.

Regardless of the delay in pod opening or the harvest period, the volatile fractions were decreased overall from 40 to 50% due, partly, to a significant reduction in the acid fraction.

The drop in the volatile fraction developed during post-harvest processing whilst being stored, together with a reduction in the reducing sugars, essential precursors of the thermal-origin aroma, demonstrates that the aromatic quality of the cocoa deteriorates over time. In our temperature and humidity conditions, storage up to 3 months seems to remain acceptable.

**IDENTIFICATION OF QTLs RELATED TO FAT CONTENT, BEAN SIZE AND SENSORIAL TRAITS
IN THEOBROMA CACAO L. THROUGH A JOINT MASTERFOODS/CNRA/CIRAD RESEARCH
PROJECT.**

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Abstract

A genome mapping approach was developed to gain a better knowledge of the genetic basis of the following cocoa quality traits: flavour, fat content and seed weight.

The study was carried out on a progeny located on the CNRA research station of Divo (Cote d'Ivoire), corresponding to the cross UPA402 x UF676. UPA402 is a Forastero from Upper Amazonia, and UF 676 is a Trinitario clone. Pods were harvested on those trees in two different seasons: in 1997, on 97 trees pollinated by Catongo, and in 2001, on 112 trees pollinated by Catongo and open pollinated. Fresh beans extracted from those pods were fermented on site in a homogenous fashion, using the microfermentation technique.

The sensorial traits evaluated by a panel of 10 tasters in the Masterfoods facilities in Slough were: intensity of cocoa, floral, winey and fruity flavours, intensity of astringency, bitterness and viscosity. Fat content and seed weight were also measured.

The map was established with 193 markers, including 82 microsatellites, and using JoinMap V 3.0 software. QTLs analyses were carried out using MapQTL V 3.0; interval mapping, simple marker analyses and Kruskal and Wallis rank test were used to assess the significance of the QTLs.

Several QTLs were identified for each trait. Three QTLs, explaining in total more than 50% of the phenotypic variations of fat content (evaluated by NIRS) were identified in chromosome 1, 4 and 9.

For seed weight, three major QTLs were also identified on chromosome 6, 8 and 9 explaining also more than 50% of the variations of this trait.

QTLs were also identified for sensorial traits. A region of about 20cM located on chromosome I seems particularly involved in the aromatic flavours. It contains one QTL for cocoa flavour, explaining 15% of the variation of this trait, one QTL for floral flavour, one QTL for Winey flavour and one QTL for fruity flavour explaining 10 to 12% of the variation of each one of these traits. Two other QTLs related to floral flavour were identified on chromosomes 4 and 9, and one QTL for fruity flavour was identified on chromosome 7. QTLs for astringency were identified on chromosome 2, 7 and 9. One QTL for bitterness was identified on chromosome 2, co locating with the QTL related to astringency.

These results show clearly that sensorial traits, fat content and seed weight depend on genetic origins and don't depend only on post harvest processes. Thus, it will be possible to improve varieties for these traits by cumulating favorable QTLs alleles using a Marker Assisted Selection.

A preliminary investigation into possible processing location and growing environment influences on the flavour attributes of cocoa (*Theobroma cacao* L.)

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Abstract

The possible influence of processing locations and growing environments on the flavour attributes of cocoa were investigated by two experiments both conducted over two growing seasons. One experiment examined the possible influence of three different processing locations with uniform sun drying on the flavour of four cocoa accessions each harvested from the same field. A second experiment looked at the possible influence of four different growing environments on seven cocoa accessions growing in at least two of the four locations. The results from the first experiment showed significant processing location effects in several flavour attributes as well as significant cocoa accession and processing location interactions. Results from the second experiment also showed significant growing location effects in different flavour attributes. The results from these two experiments provide preliminary evidence that both processing location and growing environment may have significant effects on the flavour attributes of cocoa. This study also highlights the effective application of optimized sensory evaluation techniques to address research needs in the area of flavour in cocoa.

**TRANSFER OF
TECHNOLOGIES / OTHERS
ASPECTS OF RESEARCH**

Farmer techniques for cocoa rehabilitation in Côte d'Ivoire

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Abstract

Created by the dynamics of extending the pioneer fronts by felling and burning forests, the Ivorian cocoa tree stocks have progressed historically and geographically from the East to the West of the country. Today only vestiges remain of the virgin forest which has been denuded by this phenomenon for over a century. In this framework, a research-action project led by the National Centre for Agronomic Research (CNRA) and the Centre for the Development of International Cooperation in Agronomic Research (CIRAD) is attempting to perfect alternative planting techniques on newly-broken forest land.

Amongst techniques already known, rehabilitation and "rejuvenation" of cocoa trees are studied under this research programme. A definition of the term "rehabilitation" is proposed firstly. The method employed relies on knowledge sharing. This involves identifying cocoa rehabilitation techniques practised in rural environments and comparing them with those already perfected by research, the final objective being to validate some of them jointly by researchers and producers.

Over 1,000 farmers were therefore surveyed over 4 months in 7 representative sectors of the main cocoa producing areas in the country. These surveys showed that rehabilitation methods in the widest sense, from soil regeneration to different techniques of cutting back and pruning, are employed by less than 15% of the farmers questioned. A detailed agro-economic description of existing farmer rehabilitation techniques could also be produced using these surveys. The decision rules applied by producers in the choice of rehabilitation method are analysed based on these elements. It appears that rehabilitation initiatives most frequently involve plantations that have been temporarily abandoned rather than plantations reaching the end of their life, which are usually maintained until their spontaneous transformation into bush fallow or plantations for other perennial crops, particularly rubber, oil palm or coffee depending on the region.

Keywords: Côte d'Ivoire, cocoa, rehabilitation, farmer innovations, technical itineraries.

Cocoa production in Côte d'Ivoire: current state of the tree stock and farmer practices

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Abstract

It is generally agreed that the Ivorian cocoa tree stocks are currently entering an ageing phase following significant waves of extension during the 1970s and 1980s. What little work exists on this phenomenon deals with frequently restrictive geographical areas. In the framework of the Research-Action Project to regenerate Ivorian tree stocks, led jointly by the National Centre for Agronomic Research (CNRA) and the Centre for the Development of International Cooperation in Agronomic Research (CIRAD), it was agreed to update and widen existing knowledge to have available a reliable and recent description of all production zones.

A survey network was therefore set up by the project in seven representative zones of all the country's producing regions. Information on 900 cocoa farmers was collected by semi-structured surveys for six months. Four analytical levels can be presented using the results of these surveys:

- an updated age pyramid by region of cocoa plantations
- a description by region of yields and factors explaining its variations (geographic location, population densities, technical itineraries applied, etc.);
- a detailed presentation of peasant technical itineraries and innovations adopted;
- a status report on peasant holdings incorporating cocoa culture (surface areas, cropping plan and planned changes)

This presentation will end with an analysis of farmer technical itineraries, with particular emphasis on the existing relationship between farmer maintenance practices and the current health conditions of the plantations.

In conclusion the interest of such a diagnosis will be discussed. This work has identified accurately the farmer production constraints and has thus resulted in better understanding of the determining factors and cocoa replanting and rehabilitation dynamics in Côte d'Ivoire.

Keywords: Côte d'Ivoire, cocoa, production system, technical itineraries

The evolution of areas planted with cocoa in Côte d'Ivoire between 1995 and 2002

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Abstract

This paper aims to present the lessons learned from a comparison of two surveys of production systems in the cocoa-growing areas of Côte d'Ivoire which were carried out at almost 10 year intervals (1993/4 - 2001/02). This comparison offers a current vision of the sector and how it might evolve in the medium term. Uncertainties weigh on the future of Ivorian cocoa culture with the saturation of forest areas, increased losses caused by black pod disease, the effects of liberalisation on the sector and social changes in the rural environment. This presentation will attempt to describe the evolution of the tree-stocks over the past eight years, highlighting its technical aspects.

The most recent survey has been carried out in Côte d'Ivoire by ENSEA in 2002 under the Sustainable Tree Crop Programme and involved 1,500 rural households in cocoa-growing areas. The results of this survey were compared with those from a previous survey led by CIRAD and the Petit-Bassam IRD Centre in 1993/94, which involved 500 cocoa farmers. The presentation reveals the lessons from this comparison on the technical aspects linked to changes in cultivation systems, such as the general development of smallholdings, the use of areas which can be cultivated and the place held by cocoa in cultivation systems, together with the state of the tree-stocks, the technical itineraries and performances obtained in the different regional situations of the pioneer fronts of the South-West, the post-pioneer areas of the Centre-West and the original areas in the East. The analysis of the variations observed between 1995 and 2002 reveal relative stability in the age pyramid of the tree-stocks, but a slight reduction in the average surface area of each holding. The trend towards diversification is confirmed, but the technical itineraries have barely intensified with yields still in the region of 400 kg/ha. Use of ordinary seeds has become generalised. Strong regional disparity continues to exist. The paper concludes by suggesting that this approach is useful for identifying breakdowns and continuity in production systems and for a future vision of the sector. It also emphasises the need to pursue this approach with more in-depth studies on socio-economic factors, to provide information necessary to formulate research and extension programmes better suited to farmers' current and future needs.

**FARMER FIELD SCHOOL AS EXTENSION STRATEGY IN THE GHANAIAN COCOA INDUSTRY:
PROSPECTS AND CHALLENGES**

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Abstract

A number of farmer-led approaches to technology transfer have emerged in recent times to replace the largely discredited Transfer of Technology (ToT) and Training and Visit (T&V) models of agricultural extension. One such initiative is the Farmer field School (FFS), an experimental learning approach originally developed in Southeast Asia for the integrated management of rice pests but adapted to other crops and even to livestock and fisheries. The experiences in the use of FFS in other crops are being replicated for cocoa in an initiative being developed in four cocoa farming communities surrounding the Kakum National Park in the Central Region. This initiative is a collaborative effort between Conservation International, Ghana, an International NGO, the Ministry of Food and Agriculture, Cocoa Research Institute of Ghana and Kuapa Kookoo Limited, a licensed cocoa buying company. A socio-economic survey to provide background information was carried out in four communities surrounding the Kakum National Park in April 2001. The results were discussed with all the stakeholders at a Workshop. Sites were selected in all the communities for a number of validation trials. The studies so far indicates that there was a dearth of technological information on cocoa production to farmers, a consequence of poor extension service provision. A Training of Trainers programme is in progress to train farmers who will be the master trainers in new FFS in the adjoining communities. The prospects for the FFS as an extension approach in cocoa are bright, as the communities embrace the training enthusiastically at the monthly meeting by taking them as part of their usual social gatherings. The long gestation period for cocoa as a tree crop compared to other annuals where this model has achieved considerable success coupled with the issue of scaling up the process pose special challenges, which are being addressed.

The SUCCESS Project: A Model for the Transfer and Efficient Utilization of Results from Cocoa Research

The Sustainable Cocoa Extension Services for Smallholders (SUCCESS) Project is a model for the transfer of cocoa research results to smallholders on a large scale. By the end of June 2003, the SUCCESS Project will have trained over 35,000 smallholder cocoa farmers in Indonesia on the identification and control of the cocoa pod borer moth (*Conopomorpha cramerella*). The objective of the SUCCESS Project is to increase incomes to smallholders through the control of cocoa pod borer (CPB) utilizing cultural control methods developed by the Cocoa Pod Borer Management Project, during the years of 1996-1998.

Research showed that smallholders could greatly reduce CPB infestation by slightly changing their crop husbandry practices. The recommended, cultural practices were proven in field trials. However, the challenge was how to disseminate information concerning CPB and these pesticide free, cultural control methods to hundreds of thousands of smallholders in a way that would result in them changing their practice and behavior towards cultivating cocoa.

To do this, the SUCCESS Project adapted the Farmer Field School approach, from the rice IPM work done in Indonesia, to cocoa, and more specifically to controlling the cocoa pod borer. After the initial training approach was agreed upon, and training modules adopted, the remaining challenge was to implement trainings across a large geographic area and among different ethnic groups while also maintaining a high level of consistency and quality control. The SUCCESS Project was to attain this through extensive training of trainers, building a network of farmer trainers and leaders, and empowering farmers to organize and carryout trainings themselves.

Analysis to date show a cumulative increase in income of US\$19.97million for all the farmers who have participated in the farmer field schools and continued the recommended methods over the last two years. This means that farmers should be receiving an extra US\$500 per year. While 25-40% of the increase can be attributed to a decrease in CPB loss, 10-20% is a result of an increase in pod size and 40-60% is an increase in the number of pods produced by the trees resulting from good crop husbandry practices. The former two figures make a very good argument for the universality of the program as it is not necessarily pest or problem specific - it increases the overall health and productivity of the cocoa system.

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Regional research project “Perfecting competitive and sustainable cocoa culture systems in Africa” - SCCS Project

J-L Battini

Abstract

In the humid tropical zone of Africa, production systems based on perennial crops offer substantial advantages in the fight against poverty, the economic integration and stability of the rural world, the balance of national economies as well as the sustainable management of natural resources.

Increasing cocoa production has until now resulted in increasing cultivated areas in forest lands through breaking new ground and burning (pioneer fronts). In the main producing countries, the accessible forest reserves are now almost non-existent, to the point where it appears impossible to maintain production levels and market shares by again extending cocoa farms into virgin lands. Maintaining production levels therefore implies transforming development systems based on pioneer fronts into stable systems.

The aim of the current *scientific regional cooperation* project is to perfect renewal methods for cocoa plantations resulting in competitive and sustainable cocoa culture systems in West (Côte d’Ivoire, Ghana, Togo and Nigeria) and Central (Cameroon) Africa through studies and *research-action operations carried out in peasant environments*.

The project will seek factors in all the countries involved restricting the “sedentation” of cocoa culture and the sustainability of the plantations, perfect suitable innovations and ensure that these innovations are promoted on a local and regional scale.

To this end, a call for study and research proposals on precise themes has been launched to national agronomic research bodies in the region and all upstream operators in the cocoa chain. These projects are monitored and run by a Coordination Management Cell based at the University of Ghana-Legon in Accra (Ghana).

The studies and research operations are dealt with in a multi-disciplinary fashion, although they come under one of five major disciplines:

- *Crop science*: study of the vegetative state of cocoa trees and how they are managed by the farmers;
- *Varietal selection*: identifying, assessing and adapting selected varieties to peasant cultivation conditions;
- *Physiology and nutrition*: characterising soils and their fertility; maintaining and restoring this fertility;
- *Crop protection*: perfecting inspection and integrated management techniques against weeds, insects and cryptogamic cocoa diseases;
- *Socio-economic environment*: characterising the technical, economic and social environment of the farmer and the resulting strategies.

Seven research operations have been implemented since the beginning of January 2003:

- *Cote d’Ivoire*: Perfecting replanting and rehabilitation techniques
- *Ghana*: Improving replanting techniques and competitiveness in cocoa culture in Ashanti country
- *Togo*: Characterising cocoa culture systems and participative selection in Litimé
- *Nigeria*: Assessing the sustainability of rehabilitation options for small producers
- *Cameroon*: Characterising and well-considered intensification of production systems and recommendations for integrated management methods against diseases and pests.

AN INSIGHT INTO GENDER ISSUES OF COCOA PRODUCTION IN GHANA

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Abstract

Gender issues imply the roles, appearances, behaviour, qualities and relationships that are expected from males and females as members of society. The study of gender issues in Agricultural production has assumed prominence in recent times. This is because the gender approach focuses attention on the different social roles and duties of men and women as it relates to productive, reproductive and community-related functions. Gender analysis provides the basic key to understanding structures and actions, including production relationships within and across households, the setting of goals and priorities and the mobilization of resources. To address the problem of the dearth of information on gender issues in cocoa production in Ghana, a study of three cocoa farming communities, Nyinahin, Ayomso and Kwabeng in Ashanti, Brong Ahafo and Eastern regions respectively commenced in 2001. A total of 100 farmers, fifty males and fifty females, were interviewed individually using a checklist as a guide. Information sought included socio-economic features of the respondents such as age, level of education, marital status, number of children, and number and yield of cocoa farms. An insight was also made into households division of labour, decision-making processes, access to and control of resources and the distribution of benefits. The average age of all respondents irrespective of sex was 57 years (with a range of 34-75). There was a clear division of labour in all households visited. Cultural values and norms decide who does what. Household decision-making processes involve men and women but men set the agenda and influence the outcome. The study found that men have the most secure rights and access to productive resources such as land, labour and capital. There were also elements of discrimination or unfairness with respect to inheritance. The implications of these findings are discussed. It is concluded that providing researchers with a body of information from the outcome of the gender analysis will make them much more gender sensitive when developing cocoa production technologies.

BY-PRODUCTS/NEW AND TRADITIONAL USES OF COCOA

ELABORATION OF ALCOHOLIC BEVERAGE FROM COCOA (*Theobroma cacao*) PULP

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Abstract

Cocoa pulp is the raw material on which the fermentation proceeds. Not only is the quantity of pulp crucial in affecting the efficiency and nature of the fermentation, but excess pulp can also be sold as a high value commodity. Processing of post-harvest residues and by-products of cacao (eg. cacao juice, cocoa pulp fermented beverage, cocoa jam, vinegar and liquor of cacao juice) may offer opportunities for diversification on farms, especially where cocoa production is the major enterprise. Revenue generated by these products exceeds that obtained from selling cocoa beans to processors. The aim of this work was to define the methodology to produce and evaluate the acceptance of alcoholic beverage made from cocoa (*Theobroma cacao*) fruit pulp. The fruit pulp used was chemically characterised (sugars, acidity, pectin, vitamin C, pectinases, starch and phenols). The cocoa fruit pulp had its sugar content adjusted to 22 °Brix with a sucrose solution. The must was deacidified using CaCO₃ until it reached pH value of 3.8 and then enzymatically treated with Ultrazym AFP-L (Novo DK). Sulphur dioxide, as potassium metabisulfite, was used as an inhibitor of bacterial growth and as an antioxidant. Bentonite was also added to aid the must clarification. After these adjustments the must was inoculated with 10⁷ cell/ml of *Saccharomyces cerevisiae* wild type strain. The fermentation was carried out at 22 °C for 10 days, with daily monitoring of Brix and fermentation activity by the liberation of CO₂. At the end of the fermentation, the fermented must was stored at 10 °C for 10 days and a first separation of the yeasts and solids particles was done. The second separation was done 30 days later, before the filtration. Ethanol, glycerol, organic acids, higher alcohols, methanol, esters and acetaldehyde were analysed in the final product. There was a high concentration of higher alcohols, which are usually responsible for the flavour found in alcoholic beverages. The acceptance of the drink was tested with 45 non-experienced panellists using the hedonic scale (1-9). The beverage was well accepted and might be a good investment for small or medium companies.

**POTENTIAL OF COCOA HUSK PULP AS A SOLE FEED INGREDIENT FOR AFRICAN GIANT LAND
SNAILS (*Archachatina marginata*)**

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Abstract

The advocate for large-scale afforestation programme has led to the production of vast quantities of tree crops' by-products, which are becoming a nuisance in our plantations. One of such by-products is cocoa husk pulp (CHP). CHP is the succulent part of the cocoa husk (CH). It constitutes about one-third of the cocoa husk. Snails have been known and highly appreciated as a valuable source of nutrition in many countries. Snails are herbivorous in nature and hence could convert inedible farm wastes into edible item in form of snail meat. This premier investigation aimed at evaluating the potential of cocoa husk pulp(CHP) as alternative feed resource to snails.

A total of 144 snails with weight ranging from 20g-45g were used for this study, which lasted for 112days. The experimental snails were grouped into 4 treatments and 3 replicates. The treatments include 100% PPF (control), 100% CTP, 100% KNT and 100% CHP. The experiment was designed to be completely randomized. Results of dietary nutrient composition showed the crude protein, crude fibre, ether extract and ash of snails fed PPF as 1.320%, 0.570%, 0.130%, and 0.710%, CTP as 0.234%, 12.520%, 0.002% and 7.55% KNT as 1.585%, 1.720%, 0.164% and 1.460, and CHP as 1.480%, 14.880%, 0.061% and 6.450% respectively. Result from the performance evaluation of snails allotted to the difference treatment showed significant difference ($P \leq 0.05$) in average total weight gain average total shell length increment, average total shell width increment, and average total aperture radius increment. The values of average total weight gain for snails fed PPF was 181.11g, CTP (154.14g) KNT (159.27g) and CHP (175.78g) total average shell length increment for PPF (8.585cm/snail) CTP (6.549cm/snail) KNT (5.858cm/snail) and CHP (6.61cm/snail) total average shell width increment PPF (3.504cm/snail). CTP (3.413cm/snail), KNT (3.23cm/snail) and CHP (4.203cm/snail), total average aperture radius increment for PPF (6.825cm/snail), CTP (5.986cm/snail), KNT (5.334cm/snail) and CHP (5.951cm/snail). It could therefore be inferred that the use cocoa husk pulp in feeding African giant land snails enhanced performance.

**CONSUMER ACCEPTIBILITY OF JUICES PREPARED FROM COCOA (Theobroma cacao) AND
CUPUASSU (Theobroma grandflora) PULPS**

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Abstract

In order to cope with the demand and preferences of the consuming market, the cocoa pulp industry in Bahia had to diversify its activities by processing other tropical fruits. One of the uses of these pulps is for the preparation of juices for consumption at home, in snack bars, restaurants and hotels. Acceptability tests of juices prepared from cocoa and cupuassu pulps were carried out aiming at assessing the consumer preference towards these products. Test samples were prepared maintained their Brix constant throughout the whole experiment and the tasting tests followed the pertinent recommendations for this kind of evaluation. Each test was replicated three times for each group of 50 people and in each tasting test it was assessed the reaction of the consumer to the following sample inherent characteristics: flavor, appearance and aroma. The data obtained from the experiment were analyzed using multivariate statistical techniques. The results showed that the consumer had distinct levels of preferences in relation to the two kinds of juice preparations and their characteristics. Tasters tended to give higher scores for the flavor of cocoa juice and lower scores for the appearance and aroma of this juice. Conversely, cupuassu juice preparations were preferred with respect to their appearance and aroma, but not in relation to their flavor. In a scale ranging from 1 to 9, the overall score attributed to the two types of juices, the cocoa juice received a score of 8 from about 60% of the people surveyed, while cupuassu had the same score from about 50% of the tasters for the appearance of the samples. For the other two characteristics, only 40% of the surveyed persons gave the score 8 to aroma and flavor.

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Development of Cocoa Powder based confectioneries as a method of increasing the local utilization and consumption of cocoa in Nigeria

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Abstract

Nigeria is one of the largest producers of cocoa in the world. Most of the cocoa is exported in the raw form and a small percentage of the processed cocoa in form of chocolate and cocoa beverages are imported back into the country. These imported products are only consumed by the high socio-economic class of the society that have acquired the foreign food habits and that can also afford the price. Recently, local utilization of cocoa powder has been limited to cocoa beverage production only.

This paper has attempted to increase the consumption of cocoa products in Nigeria by incorporating cocoa powder into local and every home confectioneries. This will help in reducing the effects of the glut in the supply and unstable price of cocoa in the world market, since more of the cocoa beans will be processed into cocoa based confectioneries like cocoa-cake, cocoa-pie, cocoa-biscuit and cocoa-sweet.

The development of these products are as described and discussed in this paper.

OPTIMISING LOCAL CONSUMPTION OF COCOA THROUGH COCOA POWDER SUPPLEMENTATION IN BREAD

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Abstract

As a means of optimising consumption of cocoa in the producing countries of West Africa, the inclusion of cocoa powder in acceptable bread recipes was thought worthwhile. Bread is widely consumed in Cameroon, Nigeria, Togo, Ghana and Cote D' Ivoire. In Nigeria, bread has become the number one staple food of the masses. An acceptable cocoa bread recipe in Nigeria with its big market of over 120 million people, will no doubt improve the cocoa economy of the nation.

A study was conducted at the Cocoa Research Institute of Nigeria (CRIN) as a starting point of this objective. In preliminary trials, bread recipes supplemented with graded levels (1 - 10%) cocoa powder as percentage of wheat flour, were produced. Repeated trials indicated the 2% cocoa powder supplementation was best in terms of colour, taste, puffness and flavour.

The selected recipe was mass-produced in collaboration with a neighbouring bakery. Sales were announced and made to CRIN staffers for several weeks. The demand rose so sharply that the Institute decided to move to the next stage, that is, building an own bakery. This is presently in the pipeline.

The control (CO) and cocoa bread (CB) recipes were subjected to organoleptic, chemical and economic analyses. CB was rated better for colour, flavour and overall acceptability by a group of trained panelists. It also showed better keeping quality, retaining its freshness till the 4th day after production at ambient temperature without turning hard as CO. Calculated proximate components (crude protein, fat, crude fibre and ash) were not significantly increased by cocoa powder supplementation. CB was slightly (~5%) more expensive than CO. This extra cost is expected to be buffered by the higher demand.

SUSTAINABILITY STUDY OF COCOA BUTTER USED AS METHYL SALICYLATE OINTMENT BASE

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Abstract

Universal ointment base has not yet been found for pharmaceutical use. Its selection in the formulation of an ointment depends on the release rate of the particular drug substance, absorption of the drug, occlusion of moisture from the skin, stability of the drug in the ointment base and consistency of the ointment base. All of these factors must be considered in finding the most suitable ointment base. The release of medicament from cocoa butter base preparation was studied *in-vitro* and *in-vivo* experiments. Results showed that cocoa butter was able to enhance the release rate and percutaneous absorption of medicament from cocoa butter based pharmaceutical products compared to mineral oil based pharmaceutical products. However, report on the stability of the drug in the cocoa butter ointment base and its consistency is limited. This paper reports the sustainability of cocoa butter used as methyl salicylate ointment base in term of stability of the drug in the ointment base and the consistency of the ointment base. Accelerated storage study at 40 °C/ 75% RH for a period of 6 months showed no significant difference with the original freshly prepared sample in term of methyl salicylate degradation. However, the color of the products was lighter, greener and less chromatic at the end of the study. Bleaching of the color may be due to the oxidation of vitamin E in cocoa butter. Flow consistency index of the product reduced to 32,389 cps from 33,674 cps at the end of the study.

POSTER PRESENTATIONS

GENETICS

Sources of resistance to *Phytophthora* pod rot at the International Cocoa Genebank, Trinidad

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Abstract

The International Cocoa Genebank, Trinidad (ICG,T) contains about 2000 accessions and is a potential source of genes for the genetic improvement of cacao resistance to *Phytophthora* pod rot (black pod disease). Black pod disease is a global problem and the aim of this study is to identify resistant genotypes in ICG,T that could be used in cacao breeding programmes. Based on the availability of pods, a subset of the germplasm at the ICG,T (528 accessions) was evaluated for resistance to *Phytophthora* pod rot using a spray inoculation method. Detached, fully-grown, unripe pods were inoculated with a moderately aggressive isolate of *P. palmivora* (100,000 zoospores mL⁻¹) using a Chromist atomiser and incubated at 25 °C and high relative humidity for four days. The levels of resistance of the incubated pods were assessed on a 1-8 disease rating scale. Two to four pods were tested per accession in each of two experiments conducted to confirm the reaction of each genotype to *P. palmivora*. SCA 6 (resistant) and IMC 67 (susceptible) were included in each experiment as standard genotypes. Analyses were conducted to understand the pattern of variation among genotypes, populations (families) and major groups in ICG,T. Subsequently, major sources of resistance were identified within the subset evaluated. The distribution of scores for resistance to *P. palmivora* showed skewness towards the susceptible end of the disease rating scale indicating that a large proportion of the sample evaluated was susceptible. Significant differences were observed between wild and cultivated accessions and among the Forastero, Trinitario and Refractario groups. Marked differences were also observed among 10 populations represented with a minimum of 15 accessions. A higher proportion of resistant and moderately resistant genotypes were observed among the wild accessions than in the cultivated varieties. The Forastero group, consisting of many wild types, was found to possess more resistant and moderately resistant genotypes than either the Trinitario or Refractario. Among the populations assessed, higher percentages of resistant and moderately resistant genotypes were found in the PA and NA populations (Forasteros) than in the other populations. This study identifies 78 promising resistant accessions among the 528 genotypes evaluated. These accessions are potential sources of resistance genes for the development of high-yielding resistant varieties.

Genetic parameters assessed from a diallel crossing programme in Togo

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Abstract

The strategy for the genetic improvement of cocoa in Togo is based on setting up a network of crossing programmes to evaluate as clearly as possible the genetic parameters of characteristics to be selected whilst allowing a varietal output in the short term. Amongst other things, this network includes a 12-seed stock triangular diallel planted in the Litimé and Kloto regions in 1987. These trials are set up using total randomisation systems on single-tree plots which gives access to individual heritability and a selection of high-performance trees within the crosses carried out. An initial assessment of these tests is presented. The characteristics taken into account are production and the resistance of the pods to rotting caused by *Phytophthora* sp. The data recorded over 3 consecutive years were analysed using the Diogene quantitative genetic software. The general traits from this combination are always highly significant whereas the specific traits are weaker and sometimes without significance. Heritabilities in the strictest sense are very close therefore to heritabilities in the wider sense, indicating that there is additional preferred transmission of the traits studied. The seed stock classification shows the superiority of the Scavina 6 parent for the different production variables, whereas T85/799 and SNK 64 parents pass on less sensitivity to pod rotting to their progeny. Heritabilities nevertheless remain quite weak and additional observations are necessary to improve the reliability of the estimations and suggest new combinations to be introduced to the seed fields. Some seed stocks, such as T86/45 and SNK 64, although little used in the genetic improvement of the species *Theobroma cacao* L., have proved very interesting

**Spatial factors in the epidemiology of pod rot due to *Phytophthora* sp.
How should they be taken into account in selection tests?**

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Abstract

In selection tests, the principal objective of experimental systems is generally to control the environmental effects. This control reduces the error terms in the variance analysis and thus improves accuracy when comparing the different variants being tested. Resistance to rotting of cocoa pods caused by the various species of the *Phytophthora* genus is an important trait to be considered. It is measured in the field by the number of rotted pods per basic plot. In tests in Cameroon and Vanuatu, analysed here, basic plots are made up of one tree, with trees from different crosses being introduced under a total randomisation system. Trees tested come either from diallel crossing programmes or from hybrid comparative tests corresponding to “single pair” crossing programmes. The rotting rates were evaluated over several consecutive years with the aim of selecting productive families or individuals with the least sensitivity to disease. Once details of all trees in the test fields had been noted, the impact of the disease in the plots could be seen and the spatial correlations estimated. Apart from vertical gradients indicating that the pods close to the ground were more likely to be infected, gradients or pockets of disease appeared, suggesting that the disease was regionalised. This is confirmed by the form of the semi-variograms which reveal the spatial correlations. Taking the spatial effects into account should be assisted by this in the selection processes for resistance to *Phytophthora* evaluated in the field. The accuracy of comparisons between the different varieties tested can in fact be improved by the use of spatial models. For the combined “individual/family” selection, these models should improve the choice of individuals for all the target characteristics and thus compensate for the inconvenience of the uniqueness of genotypes in traditional crossing programmes.

Genetic Diversity among Cocoa (*Theobroma cacao* L.) Elite Clones from Ecuador

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Abstract

The present research has the objective to quantify the genetic diversity among some elite cocoa accessions with good agronomic traits using RAPDs markers. Ten clones from INIAP's collection at EET Pichilingue (EET-clones) were used, including commercial ones, some Nacional clones and promising sources of resistance against witches' broom and frosty pod. A Forastero type clone was used as a reference. DNA extraction of each clone and RAPDs were performed. The amplification products were visually scored after an electrophoresis and Nei and Li (1979) genetic distances were analyzed by the Multidimensional Scale method (MDS). According with the results, the Nacional clones are genetically diverse and dissimilar of SCA 6, an important source of witches' broom resistance, evidencing the potencial of the existence of different genes of resistance. Among the clones with resistance against frosty pod, there is a high diversity, indicating that possibly they have different genes for this character. The clones recommended for planting by INIAP were not highly diverse, this emphasizes the need of including new sources of resistance in INIAP's breeding program. Finally, Nacional clones were genetically dissimilar of Sca 6 clone confirming that Nacional clones should not be classified as forastero. This research was executed as part of the BIOMOL Project, a cooperative research between Ecuador, Brasil and Perú to obtaining disease resistant cocoa varieties.

Behaviour of diverse varieties of cocoa on industrial plantations in Sao Tomé.

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Severino Neto Espirito Santo, CIAT, CP 375, Sao Tomé, Sao Tomé and Principe
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Abstract

Sao Tomé and Principe is a small country with cocoa plantations covering a third of its surface area. In 1999 exports were around 4,000 tonnes, half of which came from large agricultural companies under private management. One of these companies, Socomé, supported by CIAT, decided from 1995 onwards to undertake a study to identify the main factors responsible for the weak yields obtained. Several varieties of cocoa from seed fields in different countries (Brazil, Côte d'Ivoire, Cameroon, Gabon, Nigeria and Sao Tomé) plus original amelonados were planted on 2,500 ha in varied environments.

The study used a test sample of 28 sites representative of the diversity of the planting material and environment. Two types of plots are compared:

- The **Production Blocks**, with surface areas between 2 and 27 hectares.
- The **Control plots**: small plots (700 to 1,100 m²) comprising 100 trees chosen from the **Production Blocks**.

Standard maintenance (cleaning, suckering, plant health treatments) was identical in both cases. Additional, systematic surveys of the main damage were carried out on the **Control** plots.

The results show good homogeneity from one year to the next and good representativeness of the **Control** plots. The varieties show very variable behaviour but the loss percentages remain high, between 31 and 42%.

There are two main groups. The best one is made up of varieties from Gabon, Cameroon and CIAT, which adapted without significant disturbance to the growing and flowering cycles. The second group mainly comprises the varieties from Côte d'Ivoire and the amelonados, with weak flowering, regardless of the period and sometimes advanced vegetal development, clearly shown through regular observation. Other indicators studied, such as the number of pods per tree and the weight of cocoa per pod, confirm this behaviour. These variations therefore reveal that the varieties differ in their ability to adapt to the Sao Tomé environment.

An increase in productivity seems possible by altering the work timetables and the plant health treatments in order to reduce losses. Based on the results obtained, new technical itineraries are currently being evaluated.

061E
OR: FRENCH

Plant production by somatic embryogenesis using genotypes selected for their agronomic value in Côte d'Ivoire and Ecuador

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Abstract

The objective of the Firelone project financed by the cocoa research incentive fund allocated to Cirad by the Ministry of National Education and Research is the technical evaluation and comparison of different forms of clonal reproduction. This project is being carried out on a private plantation in Côte d'Ivoire (SAO) and at the National Agronomic Institute of Ecuador (INIAP). A dozen genotypes were selected for their agronomic interest in both countries from collection clones or cultivated hybrid material. The study involves firstly the feasibility of multiplying these genotypes by somatic embryogenesis carried out by Cirad or at INIAP, by attempting to link the embryogenic capacity to physiological factors. Separation and hardening off conditions are subsequently described and compared for both places. Lastly, the project plans to set up a field trial in each country to compare the agronomic value of these *in vitro* seedlings with cuttings, grafted plants and sowings (free progeny) for the selected genotypes. The *in vitro* multiplying capabilities can be compared with the horticultural multiplying capabilities using the first results from cuttings and grafting. Information on the establishment capability, vegetal development and marketable cocoa production for these three clonal forms and for seed tubers (from seeds taken from cloned trees) planted in 2004 will be supplied by field behaviour trials.

Relatedness and Inbreeding in a Cacao Breeding Population

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Abstract

Heterosis in cacao was recognized since the first crosses aiming to combine resistance and productivity were made. Also, inbreeding depression has often been observed, particularly when certain clones are selfed. In these cases the selfed progenies are below average for many traits. On the other hand, inbreeding can be a good strategy for purging deleterious genes from breeding populations. The concerns on the relatedness and inbreeding arise from the fact that many genetically related cacao clones, regarded as potential sources of resistance to witches' broom disease, are involved in the crossing programs to form the populations of breeding. Further, more complex crosses are being produced for pyramiding resistance genes and associating resistance with other traits of agronomic importance. Therefore, aiming to plan better the new generations of breeding it is important to examine some important features such as the level of relatedness, the effective population size and the inbreeding in that population. In this study we report the level of inbreeding and effective population size for the first and second generations of breeding. Overall, the level of inbreeding is still very low, despite the frequent involvement of some clones.

**Genetic and physical mapping of resistance gene analogs colocalised with QTL of resistance for
Phytophthora in cocoa**

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Abstract

Resistance gene analogs (RGA) have been isolated in cocoa using a PCR-based approach. After genetic mapping, three clusters of RGA have been identified in the cocoa genome. Two clusters corresponded to genes encoding cytoplasmic receptor-like protein containing a nucleotide-binding site (NBS). Another cluster gathers four analogs to the tomato *Pto* gene and one analog to the *LRK10* wheat rust resistance gene, corresponding to genes which contain a serine threonine kinase domain (STK). The last cluster is colocalised with 4 QTLs of resistance to *Phytophthora* identified in previous QTL mapping studies. A BAC library constituted using nuclei isolated from the Scavina 6 resistant genotype was used to begin physical mapping of the chromosome regions containing these RGA clusters.

SELECTION OF HYBRID COCOA TREES (*Theobroma cacao* L.) FOR CLONES OF HIGH YIELD AND QUALITY

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Abstract

In the Dominican Republic, yields from the cocoa crop (*Theobroma cacao* L.) are relatively low. This is a consequence of the advanced age of the plantations (80 to 100 years), poor varietal selection, and mixing of genetic material, in addition to inadequate management of the plantations. The selection of genetic material with good yield and good quality is important, since they could be used as progenitors in programmes of hybridisation with imported materials. In addition they could be used in the development of clonal plantations. A localised exploratory investigation was carried out, to identify, evaluate and select cocoa trees within a hybrid plantation with potentially high yield and quality in the North East region of the Department of Agriculture on an estate owned by Mr. Héctor Rizek, located in the Los Ancones, San Francisco de Macorís section. A non-experimental design was used where the trees which showed desirable phenotypic characteristics for the study were examined in their place of origin. The work started in 1999 and ended in 2002. The results obtained show that yields fluctuate between 0.61 and 13.36 kg/tree; cocoa pod index between 11.23 and 64.95 pods/kg; seed index between 0.71 and 1.95 g/seed. According to the results obtained it is concluded that of the 100 trees evaluated, 10 were selected. These selections are called “Rizek-1”, “Rizek-12”, “Rizek-16”, “Rizek-36”, “Rizek-43”, “Rizek-44”, “Rizek-61”, “Rizek-83”, “Rizek-84” and “Rizek-100”.

PROPAGATION OF COCOA TREES (*Theobroma cacao*, L.) VIA SOMATIC EMBRYOGENESIS.

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Abstract

An efficient vegetative propagation system for selected trees, the identification and preservation of genetic resources and the creation of homozygous parents are some of the major limitations in cocoa breeding. Somatic Embryogenesis (SE) can overcome some of these limitations especially in the conservation and exchange of disease free Germplasm. Penn State University, USA has developed a successful protocol for inducing plant regeneration using staminoids from cocoa flowers. However, this protocol did not work well at the Almirante Center for Cocoa Studies, Bahia, Brasil when the staminoids were taken from trees growing in field conditions. Although some improvement was found by prior covering of the flower button with plastic bags the level of embryo production was still unsatisfactory. An adaptation was made to the protocol by substituting staminoids by the flower petals. Even with field collections the contamination level was very low (5%). Seasonal variations in response were found for some of the clones but overall a 75% rate for embryo production was achieved with a high level of regenerated plants. Successful transfer to a soil substrate was obtained in the greenhouse. Currently trees from several clones are now growing in the field and are already yielding pods. SE is a promising technology for propagating desirable genotypes, facilitating Germplasm exchange and where space is at a premium a small area can be used for maintaining a Germplasm collection. As plants from SE show orthotropic growth are already being used in Almirante nursery as a source of single buds and/or bud sticks for budding and grafting on rootstocks to generate cocoa trees with dimorphic growth.

DEVELOPMENTAL ANATOMY AND ULTRASTRUCTURE OF SOMATIC EMBRYOS IN *Theobroma cacao* L.

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Abstract

Somatic embryogenesis procedures for *Theobroma cacao* L. have recently been developed. However, very few anatomical and ultrastructural studies were carried out. The study here aims to elucidate some of the processes of somatic embryogenesis of *Theobroma cacao* L. at the cellular level.

Staminode explants were dissected from the immature flower buds and cultured. Staminode calli (10-12 weeks old) of clone KKM19 with somatic embryos proliferating were cut into 2 mm³ pieces and fixed overnight in fresh 3% (v:v) glutaraldehyde in 0.1M phosphate buffer, pH 7. Histological sectioning was then carried out. Tissues were stained with 0.5% (w:v) toluidine blue in aqueous 0.1% (w:v) sodium tetraborate for 5 min or with a 1.0% (w/v) aqueous solution of Safranin for 24 hours and counterstained with 0.1% (w/v) solution of Fast Green, prior to light microscopic examination. Somatic embryos were frozen in liquid nitrogen for 5 minutes and mounted on a scanning electron microscope (SEM) support and kept at -194 °C in a vacuumed chamber. The samples were covered with a thin layer of gold (250-300 Å) using a sputter coater and examined with a JEOL 25-S-II SEM at 10 Kv.

Histological sectioning of the callus revealed that it was comprised of embryogenic and non-embryogenic cells. Embryogenic cells were denser than non-embryogenic ones. Somatic embryos also exhibited a closed vascular system. A meristematic outer surface area, the protoderm, which will form the future epidermis, was observed. The pith cells at the center were more vacuolated than other cells. Heart-shaped embryos were divided into two distinct zones, the hypocotyl and cotyledon, and showed the emergence of a shoot apex.

Somatic embryos were observed to differentiate from callus tissue. Fusion of somatic embryos was often seen, as well as somatic embryos with more than two cotyledons. Somatic embryos usually differentiated in a cluster, from globular to heart. Secondary embryos were also seen to differentiate from the surface of primary embryos. Trichomes were observed on the surfaces of these embryos. Somatic embryos followed the normal developmental sequence: globular, heart, torpedo, and cotyledonary stages. In heart-shaped embryos, polarity was established by the development of cotyledons and procambial strands. Torpedo- and cotyledonary-stage embryos had a continuous protoderm, well-defined cotyledons, and connecting procambial strands. Histological sections showed globular embryos, in some cases with a central mitotically active region. Protoderm was present throughout. SEM revealed globular, early heart, torpedo stage, and adult embryos with well-developed cotyledons.

Biotechnology Research by the Malaysian Cocoa Board

DOUGLAS FURTEK, Rosmin Kasran, Tan Chia Lock, Lea Johnsiul,
Azhar Mohammed, Meriam Mohd. Yusof, Khairul Bariah, Kelvin Lamin, and M. T. Lee

Abstract

Somatic Embryogenesis and Plant Regeneration

Somatic embryogenesis and plant regeneration procedures are being refined in order to propagate cocoa clones commonly used by Malaysia breeders and farmers. Stamnodes are placed on induction media containing 2,4-D and either 2iP or TDZ. After several weeks, calli are transferred to expression media without growth regulators. Primary somatic embryos begin to appear after three to six weeks. Embryos are then placed on induction and expression media again to stimulate the formation of secondary somatic embryos. Normal-appearing embryos are then maintained on media without growth regulators until at least 1 cm long and with primary and sometimes secondary roots. They are then transferred to media with reduced minerals and placed in the light room. To date, 13 normal-appearing plants from 4 different clones have been removed from tissue culture and placed in pots in Malaysia.

Molecular Marker Assisted Breeding

An integrated molecular and agronomic map of cocoa is being constructed using amplified fragment length polymorphic (AFLP) markers and several agronomic traits, such as resistance to VSD and *Phytophthora* pod rot, yield, and precocity. The 100 plants in the segregating population are 13 years old and were derived from a cross between UIT2 and NA33. DNA has been extracted from all plants and AFLP markers are being generated using an Amersham MegaBACE DNA sequencing and fragment analysis machine. This map will allow breeders to select for trees with superior adult traits while plants are still seedlings.

Pod Abscission

Harvesting is a major cocoa production expense that could be greatly reduced if pods dropped when mature. Pod abscission would also ensure that only mature pods were collected. Two strategies are being studied to incorporate an abscission zone into the pedicels of cocoa fruit. The first is to make interspecific hybrids between *T. cacao* and *T. grandiflorum*. Five cocoa clones have been pollinated by *T. grandiflorum*. The second strategy is to engineer an abscission zone into cocoa. As a first step, we are investigating a gene in cocoa that is homologous to the tomato *jointless* regulatory gene that controls fruit abscission.

Artificial Diet for Cocoa Pod Borer

An artificial diet for cocoa pod borer (CPB; *Conopomorpha cramerella* Snellen) is being developed in order to study the toxicity of compounds such as *Bacillus thuringiensis* (B.t.) delta-endotoxins and protease inhibitors to larvae. Our best diet consists of cocoa pulp juice, sucrose, glucose, soya protein, flax seed oil, vitamins, and agar. CPB larvae exit media blocks after 15 days – about the time larvae in the field exit pods for pupation – and crawl onto the plastic petri dish. But they fail to pupate after 5 additional days. This work is being conducted in collaboration with Dr. Monique Royer and Dr. Laurence Alemanno of CIRAD in France who have generously provided purified toxins for testing.

Storage Protein Alleles

The importance of the vicilin-like storage proteins for cocoa flavor development has been well established. And differences in chocolate flavor among clones is also well known. But nothing is known about how differences in storage protein composition among cultivars contribute to differences in flavor. Thus we are cloning and sequencing the storage protein alleles from a variety of cocoa clones and hope to correlate cocoa flavor with allele.

**GENETIC IMPROVEMENT IN RESISTANCE OF COCOA TO BLACK POD DISEASE IN COTE
D'IVOIRE**

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Abstract

During the first phase of the genetic improvement of cocoa programme in Côte d'Ivoire, resistance to black pod disease caused by *Phytophthora palmivora* was considered a secondary criterion to be taken into account at the final selection stage only. With the recurrent, reciprocal cocoa selection programme starting in 1990 and particularly with the appearance of *P. megakarya* in the cocoa tree stock, selecting for resistance to *Phytophthora* has become a priority. The selection scheme is based on two extended populations made up of High Amazonian and Low Amazonian. This increased-performance strategy should lead to an accumulation of favourable alleles. Resistance evaluation tests on planting material in collections has shown the judicious choice of first seed stocks and is directing choices towards a second wave. The analysis of test results on intra- and inter-group hybrids suggests the possibility of improved cultivar creation.

AGRONOMY

DIAGNOSIS OF THE PRODUCTION OF SHOOTS AT THE CACAO BIOPANT INSTITUTE

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Abstract

The Cacao Biopant Institute (IBC) is a unit geared to large scale production and multiplication of high yielding cocoa plant genotypes tolerant to the witch's broom disease, caused by the *Crinipellis pernicioso* (Stahel) Singer fungus, which, during the last 10 years, has decreased cocoa production in the State of Bahia, Brazil, by more than 60%. Production of shoots by cuttings was started in 1999 by the IBC. The Cocoa Research Centre (CEPEC), of the Executive Commission of the Cacao Agricultural Project (CEPLAC), has been supporting the IBC with technical and scientific means since its inception. Analyses were carried out aimed at checking the quality of the shoot production process, at defining the nutritional status of the plants and at setting out a research program to improve the process. These analyses included an assessment of the total number of nutrient contents in root understorey and shoot growth, as well as foliar analyses of TSH 1188, TSH 516 and EET 397 clones, according to the weather. Understorey analysis has shown a relatively high concentration of the iron element and a concentration of sodium in the coconut fibre component, sodium in excess can contribute to an increase in electrical C.E. conductivity in the solution, thus affecting water and nutrient absorption. The values of N, P, K, Ca and Mg observed in the foliar tissues of the various clones may be considered normal; however, those of micro-nutrient contents may not. New leaves have shown chlorosis, suggesting toxicity, probably caused by an excess of the Fe element, in consequence of its presence in the understorey, of the reduced environment and low pH, factors which contribute to an increase in its availability. A striking reduction of foliar content of macro-nutrients during the first 90 days in the nursery, mainly N, P and K, was observed. The magnitude of the differences in macro-nutrient contents observed amongst the clones, suggest that there is a differentiated absorption of elements, which can result in different programmes of fertilizing for clonal gardens as well as in the use of fertirrigation.

015E
OR: PORTUGUESE

Evaluation of Heavy Metals in Agricultural Sub-Products in the Area of Cocoa Production in Bahia, Brazil.

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Abstract

The presence of heavy metals in post-harvest husk of the cocoa bean (pod), in the bean testa - a by-product of the chocolate industry, and in pork fertilizer, has been evaluated. The latter is a by-product of the pig breeding industry, an activity presenting a high level of polluting capacities, because of the excessive number of pollutants present in their effluents, which represent a source of air, water resources and soil pollution. These materials were combined in various ratios and then submitted to composting and vermicomposting procedures. This study was conducted at the Cocoa Research Centre - CEPLAC / CEPEC, at Ilhéus, State of Bahia, Brazil. Based on the heavy metal contents detected, it becomes evident that the possibility exists to use these materials as a substratum for seedlings in nurseries as well as an organic fertilizer, the contents found being in accordance with those required by law. The agronomic use of these by-products can be an added value; these are products which, discarded in the environment *in natura* may cause damage both to the environment and to public health.

Comparison in performance between two technical itineraries in a large cocoa holding in Sao Tomé.

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Abstract

The State of Sao Tomé and Principe acquires a large proportion of its resources from cocoa exports. Production is currently less than 4,000 tonnes. In 1999, following a land redistribution programme, cocoa was produced by small farmers, medium-sized companies and six large agricultural companies under private management. One of these companies, Socatomé, covers a total area of 2,500 hectares. In collaboration with CIAT, it decided to compare 2 types of technical yield itineraries between 1997 and 1999 to compensate for the low yields being experienced. The hybrid planting material came from different geographical origins: Côte d'Ivoire, Cameroon, Gabon, Nigeria and Sao Tomé.

28 sites or plots were selected within the plantation, itself divided into 2 operational sub-units, to take account of the variability of these geographical origins. Two types of plot are compared at each site:

- **Control plots** where standard maintenance (cleaning, suckering, plant health treatments) are identical to the maintenance specified for all the company plots under production.
- **Garden plots** where a specific, intensive technical itinerary is applied. A complete set of agronomic knowledge and cultural control is implemented.

In both cases the fruit is harvested on a monthly basis and weighed and all losses are identified: these concern in particular damage due to *Phytophthora spp*, *Selenothrips rubrocinctus*, *Bathycoelia thalassina* and pests.

The results reveal a significant improvement in yields of marketable cocoa per hectare when the **Garden** type technical itinerary is used. The differences also come from the origin of the planting material and the pedoclimatic environment. In addition, the high number of fungicidal and insecticide treatments on the **Garden** plots considerably reduce the average loss rate, from 40% to under 15%.

The influence of both the technical itineraries on the weight and number of fruits per tree and the bean weight per pod is also studied.

Finally, annual maintenance costs of the garden plots are €600 per hectare for gains in production which are significant, but nevertheless limited and unprofitable given the price of cocoa in 1999. Simplified itinerary techniques allowing for economic reality should lead to a compromise being found between improved yields and profitability.

Variability in leaf parameters in cocoa foliage in Vanuatu.

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Abstract

Photosynthetic ability, based on the quantity of intercepted light, is one influential factor in developing the yield from the cocoa tree. Tremendous variability is often observed during measurements of gas transfers on the leaves. It is linked to their sampling on the trees and the instability of the measurements, regardless of the experimental conditions. With a view to characterising the factors responsible for this variability, samples of adult leaves were taken from foliage in the different areas accessible, corresponding to areas where gas transfer measurements are taken. Several factors were considered: the orientation of the tree (according to the four cardinal points), the height and position in the tree of the terminal leaves, all taken from the same foliage shoot the length of a plagiotropic axis. The measured parameters are the length, width and weight of each leaf. The calculated parameters are the leaf density and surface area, this area being determined using two different methods. The study was carried out on the island of Espiritu Santo, Vanuatu (15° latitude South) on 10 year old Amelonado trees.

The influence of the orientation and height was shown overall by an increase in size and surface area of leaves on the lower, south-facing part of the tree, in other words leaves less exposed to visible radiation. The position of the leaf on the plagiotropic axis also influences its weight and size: the leaves are heavier and larger the further they are away from the apex. However, leaf density remains stable, which shows that the increased weight of the leaf is therefore linked to the increased surface area.

The results obtained could lead to comparative physiological studies between trees within a cocoa population taking into account their leaf characteristics linked to the positioning on the plant.

EFFECTS OF SPACING IN THE PRODUCTION AND FITOSANITARY CONDITIONS OF COCOA UNDER A CANOPY OF PEACH PALM IN THE BAHIAN COCOA GROWING REGION

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Abstract

The arrival of cloning has prompted a need to reassess the questions dealing with the management of cocoa plantations in Bahia, as the spacing used presently is connected to information provided by hybrids and common cocoa plants. As the clones are high yielding material, it is not really known whether the spacing recommended by CEPLAC (3x3m) fully satisfies its development and production. This research focuses on the evaluation of the cocoa plant behaviour, whenever an agro forestry system using groves of peach palm is adopted. The peach palms were planted using a spacing system of 6x6 m and the cocoa plants were planted using different spacing: 2x3; 2x2; 2x1,5; 2x1 and 3x3 meters. The outline used was in randomised blocks with 5 treatments and 4 repetitions. The cuttings were planted in the field, applying the system of double and diverging ranks, and grafted with clone propagum TSH565 and TSH1188. Preliminary results have indicated that, for the time being, the highest yields, were obtained for the 2x2 and 2x1 m spacing. As far as the witches' broom disease is concerned, generally the smaller spacing has shown a higher incidence of the illness. As far as the two clones used, it was confirmed that clone TSH1188 is more tolerant, both in relation to witches' broom and black rot. According to the preliminary results, higher yields were obtained in the smaller spacing, i.e. 2x2 and 2x1 meters. Clone TSH1188 continues to prove a higher tolerance to witches' broom.

Assessment of an adequate time for pruning and number of tubes per tray for the development system at the Instituto Biofábrica de Cacau (IBC).

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Abstract

The Instituto Biofábrica de Cacau (IBC) is an unit geared for the multiplication of cacao clones tolerant to the witches' broom disease, caused by the fungus *Crinipellis pernicioso* (Stahel) (Singer), which, in the last few years, has caused a decrease of more than 60% in the yield of *Theobroma cacao* L. in the State of Bahia, Brazil. The development of cacao cuttings by means of grafting was first tested in Brazil during the 30s; however, in major scale, it is a new technique, begun in 1999, by the application of a protocol of development of cuttings from forestry companies, adapted to the morphological and physiological conditions of cacao. It has been noticed that the small area reserved for each cutting and the fierce competition for light on the trays, were promoting unequal growth amongst the seedlings, and therefore, an appropriate time for pruning and number of tubes should exist, which inter-act to promote a better growth of the plants' aerial part and roots. This research study has assessed the ideal period of time for pruning and number of tubes per tray, and its influence on the growth of seedlings. Initially, on each tray, with a capacity for 54 tubes, cuttings of clone (TSH 1188) were planted; each tube contained a total volume of 288 cm³ and was filled in accordance with the IBC routine. Every 40, 50, 60, 80 and 100 days, pruning was carried out for occupation percentages of 17; 33; 50; 67; 83 e 100 % respectively. After 150 days, plants were assessed for height, stem diameter and survival percentage. The factorial system was applied, with a 6% rate of tray occupation and 4 periods of pruning, in randomised blocks, with three repetitions. To compare the treatment effects, the Student Newman - Keuls test, at 5% probability was applied. Differences were observed in the treatments as far as the plant height was concerned; this varied between 26,2 cm and 14,6cm, for occupation percentages of 33 and 100% respectively. Plant survival varied between 74,1 and 100%. Results observed indicate that 27 tubes per tray and pruning at 60 days are ideal for the development of grafts with a better height and percentage of plant survival.

EFFECT OF BEAN CONDITION AND SOWING MEDIA ON GERMINATION AND NURSERY PERFORMANCE OF SOME SELECTED *THEOBROMA CACAO* L. GENOTYPES.

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Abstract

This investigation was carried out to test the influence of mucilage and weathered sawdust on germination and early growth of some double crosses involving selected single hybrids of *Theobroma cacao* L. and also some F3 Amazon cocoa beans. Germination capacity varied significantly from one genotype to another. The cleaned beans (without mucilage) sprouted faster and recorded higher percentage germination than uncleaned beans (with mucilage). The sawdust medium gave faster germination than the corresponding topsoil medium regardless of seed condition. There was a significant difference in the number of days to the cotyledon drop. The sawdust medium also contributed to earlier cotyledon drop in the seedlings.

The stem diameter and stem height showed significant differences in the first two months of sowing ($P = 0,05$). The number of the leaves produced by the seedlings also differed significantly ($P = 0,05$) in the first three months between the two sowing media

**BARE-ROOT TRANSPLANTING OF COCOA SEEDLINGS: I.
EVALUATION OF DIFFERENT STORAGE METHODS TO ENSURE GOOD FIELD ESTABLISHMENT**

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Abstract

Different storage techniques were tried to determine the most suitable methods of keeping bare-root cocoa seedlings prior to transplanting to the field. Storage periods were also taken into consideration to determine the number of days the seedlings could keep after removal from the potting medium without drying. Erosion of the soil around the root system ensured high root volume because just a few of the roots were damaged. This favoured early establishment of the seedlings on the field while the uprooted seedlings recorded lesser values of stem height, stem diameter, leaf number and leaf area from eight weeks of transplanting. However, there were significant differences ($P = 0,05$) in the leaf number and leaf area of the seedlings from eight weeks of transplanting. The height of the seedlings also differed significantly ($P = 0,05$) at 32 weeks after transplanting. The rate of leaf abscission (leaf fall) was significantly higher ($P = 0,05$) in uprooted seedlings than seedlings with washed roots especially from fourth week of transplanting. Wrapping the roots of the seedlings in wet mud also recorded higher rates of abscission than those soaked in water. The seedlings whose roots were washed and soaked in water for twenty four hours before transplanting recorded the higher rate of leaf appearance after one year of transplanting (31.36). There was very low mean percentage mortality (below 6%) for all the treatments.

APPLICATION OF A "LYSIMETRIC" DEVICE TO MEASURE THE HYDRIC BALANCE IN COCOA CLONAL SEEDLINGS GROWN IN GLASS TUBES

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Abstract

Substrata are used for the growth of plants in tubes, the so-called soil-less culture. It is considered that its main function is to provide support to plants in this particular media and to adjust its availability in water and nutrients. Water dynamics in substrata are very different from ground dynamics, due to the specific outline conditions that the relationship between its mass and volume inside the container require. Close monitoring of the hydric balance in glass tubes is a condition sine qua non to quantify the relationship water/air and to understand the complex system substrata-plant-atmosphere, which is the main objective of this study. This project was carried out at the nurseries where clonal cuttings are developed in glass tubes (5cm inside diameter and 19cm long) at the Instituto Biofábrica de Cacau (IBC), Banco do Pedro, Ilhéus, Bahia, Brazil, latitude 14°45'S and longitude 39°16'W. In order to quantify the water output in the glass tube, a rubber preservative (condom) was inserted, which has a modus operandi similar to a bleeding lysimeter. The amount of water applied in automatic irrigation was collected, in four pluviometers (plastic cups), at regular intervals; in the centre a glass tube coupled with a preservative and six repetitions were set. The in and out blades were counted and by difference, what was left in the substrata: plantmax (PLT), cocoa bean testa ITAC), coconut fibre (FC) and the mix plantmax+coconut fibre (PFC). Preliminary results have shown that, FC excepted, all substrata drained more than 60% of the water applied, during a period of approximately 10 hours. It should be stressed, however, that coconut fibre retains approximately 80% of the water applied, whereas TAC drains that same percentage. Present substrata are, for the most part, mixes of two or more components and those two factors combined should be maintained in order to keep the adequate supply of water and air to the rooted grafts. The condom applied as a "lysimetric" device has proved to be a perfect method for the assessment of the hydric balance, as well as an excellent collector for water, fertilizer and other residues drained by the substrata.

**MATURITY OF THE COCOA POD AND POSITION OF THE SEED IN THE DEVELOPMENT OF
COCOA SEEDLINGS (*Theobroma cacao* L.).**

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Abstract

The development of cocoa seedlings is a decisive factor when encouraging the renewal and restoration of a cocoa plantation. Proper selection of the seed is important to ensure proper development of the seedlings in the nursery, with effective vigour and stability in the field. In many cases, seedlings which do not meet the minimum requirements for being raised in the field are produced in nurseries, and are affected by the nutritional deficiency of the substrate and inadequate selection of the seed. The objective of this study was to evaluate the effect of the state of maturity of the cocoa pod and the position of the seeds in the development of cocoa seedlings. The parameters used for maturity were: semi-ripe, ripe and very ripe. For the position, the bottom, base third of the pod (bottom); the middle third (middle) and the apical third (top) of the fruit were considered. It was carried out in the nurseries of the Mata Larga Experimental Station, San Francisco de Macorís. A completely randomised design was used, with factor adjustment (three degrees of ripeness and three positions of the seed), which consisted of 9 treatments and 4 repeats. The results showed that the best heights were obtained in seedlings from ripe cocoa pods, with a value of 44.49 cm and diameter of 6.45 mm. The seeds with the highest - 100% - germination were from semi-ripe cocoa pods. It is concluded that seedlings from ripe cocoa pods and top and middle position had the best survival index with 94% and 98% respectively. As for the number of leaves, there was no statistical difference between the treatments. Seedlings which came from seed from middle and ripe cocoa pods presented the highest values for diameter of the stem and height.

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OR: SPANISH

EVALUATION OF SUBSTRATES IN THE ROOTING OF CUTTINGS OF COCOA (*Theobroma cacao* L.).

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Abstract

Cocoa plantations in the Dominican Republic have low yields and are of poor quality. The advanced age of the plantations, poor varietal selection and the mixing of genetic material are factors which influence the situation. One of the alternatives for resolving this problem is asexual reproduction, since it ensures new material with little genetic variability and of high quality. A study was carried out with the objective of evaluating 7 substrates in the rooting of cocoa cuttings. It was carried out at the Mata Larga Experimental Station, San Francisco de Macorís, in the period March - November 2002. A completely random design was used, with 7 treatments and 5 repeats. The treatments were coffee pulp, sawdust, decomposed poppy, carbonised rice husks, soil, sand and soil, soil +sawdust. Phytohormone (alphanaphthalene acetic acid, 0.4% concentration) was applied to the cuttings. The substrates in which rooting was best were soil + sawdust and soil. The lowest values were recorded in the coffee pulp and poppy substrates. It is concluded that under conditions of 75% screen, damp chamber of transparent plastic, the soil + sawdust, soil, sawdust and sand substrates are adequate for rooting of cuttings in a polyethylene case.

SPACING TRIALS IN COCOA/ KOLA/CITRUS INTERCROP

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Abstract

Spacing experiment on cocoa/kola/citrus intercrop was established in 1995 at Ajassor substation of Cocoa Research Institute of Nigeria to determine the optimum spacing and plant population for cocoa/kola/citrus intercrop. The experiment was laid-out in Randomized Complete Block Design with seven treatments of kola and citrus each at 17,34,54 and 69 plants/ha and cocoa, kola and citrus at 1,110, 173 and 173 plants/ha respectively under three replications.

Data was collected on growth parameters such as number of leaves, leaf area, plant height, stem girth and canopy scores during the first 4 years of establishment. The results showed that stem girth and canopy scores of cocoa, kola and citrus in cocoa/kola/citrus were better at larger spacing and lower plant population densities of 17 plants/ha each of kola and citrus than at smaller spacing and higher plant population of 69 plants/ha, although the differences were not significant ($P = 0,05$). Similarly, the growth performance of poly-cultured cocoa, kola and citrus was comparable to that of their corresponding pure stands indicating that there were no deleterious or allelopathy effects on any of the component crops.

Field performance of cocoa hybrids treated with Paclobutrazol

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Abstract

The Cocoa Research Institute of Ghana (CRIG), in the early 1960s developed the Series II cocoa hybrids to replace the old West African Amelonado (Amel) as a result of the former's high seedling vigour, precocity and yield. However, recent observations appear to indicate that the early seedling vigour is not always reflected in the yield during the cropping years. Growth retardants usually reduce vegetative growth of most crops to improve their agronomic performances and which may promote a shift towards improving the reproductive growth of the crop. This study was therefore aimed at improving the agronomic performance of the vigorous Series II cocoa hybrids through the application of Paclobutrazol, a growth retardant (with commercial name "Cultar" – ICI, England) and to look for the most effective application rate. Results from a field trial at CRIG, Tafo using four levels (0, 250, 500 and 1000 ppm) of Paclobutrazol as foliar sprays on young cocoa seedlings of four varieties (viz: Amel x Amel, T85/799 x T79/501, T85/799 x Amel and T60/887 x Pd15), in a split plot design, showed that the growth retardant caused a significant decrease of 16.5% ($P \leq 0.05$) in plant girth and a non-significant decrease of 18.0% in plant height. The hormone reduced the rate of leaf production by 30.2% ($P \leq 0.05$) thus slowing down the rhythmic flushing of cocoa, which is believed to be the main vegetative trait of cocoa plants during cropping. The initiation of flower production was, however, delayed in the plants that received the 1000ppm concentration with a corresponding decrease of 87.8% in the intensity of flowering at first flowering. The application of the hormone at 500 and 1000ppm reduced seedling mortality rate by 35.4% and 32.6% below the control and 250ppm respectively. The prospects of improving the agronomic performance of the vigorous varieties by the growth retardant are discussed.

TRADITIONAL COCOA AGROFORESTRY. 2. INDIGENOUS SELECTION CRITERIA FOR SHADE TREES ON COCOA FARMS IN A TYPICAL COCOA GROWING DISTRICT IN GHANA.

C.Ameyaw Oduro¹, K. Osei-Bonsu², and J.P. Tetteh³

Abstract

Sixty cocoa farmers were interviewed in the Osino District of the eastern region of Ghana to determine the selection criteria used to manage forest tree shade on their farms.

The major reason for eliminating certain tree species during land preparation appeared to be to create a congenial environment for the establishment of food crops for the farmer. Sixty three percent of farmers interviewed gave this as the main reason for grubbing some forest shade trees. Indigenous farmer knowledge of tree species however identified tree species which are not compatible with food crops and others which serve as alternate hosts for pests and diseases of cocoa. In established cocoa farms, the main reason (55% of respondents) for eliminating forest tree species was concerned with shade reduction and improving the microclimate for the cocoa. Only a few farmers (9%) were able to identify trees with disease or pest associations (9%) whilst another 9 percent identified trees which were incompatible with cocoa on account of making the soil too dry or causing yield reduction in cocoa. Up to nine percent of the trees felled were used for household construction/repairs, for timber or for canoe construction. Other reasons for eliminating some tree species was to reduce the incidence of rodent attack (4%) or reduce damage to cocoa trees from falling branches (1.8%). A tree species like *Cola Gigantia* was noted to create a nuisance by causing a lot of seedling sprouts and producing litter which takes too long to rot.

Farmers' knowledge on the morphology of desirable tree species was impressive. Various tree species were retained in the cocoa farms for providing good adequate shade throughout the year (40% of respondents). Contrary to expectations, a high proportion of the trees on cocoa farms which can be exploited for timber are consciously left by the farmers. Indigenous knowledge also protects species with multipurpose uses for firewood, gun butt construction, fruit for income generation, soil improvement or even to attract snails for farmers' use. Undesirable trees perceived by farmers as having other beneficial effects are inadvertently conserved in the cocoa ecosystem. Further dialogue with farmers should be undertaken on which tree species should be eliminated or retained to make the cocoa ecosystem sustainable for cocoa production.

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Biological and economic evaluation of some cocoa food crop intercropping packages at establishment

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Abstract

An experiment to investigate effect of intercropping cocoa with some food crops during the first three years of establishment without the use of fertilizers was carried out at the Cocoa Research Institute of Ghana substation at Bunso. Plantain, cassava and maize were planted alone or as mixtures with cocoa to give the following intercrop combinations: cocoa/plantain, cocoa/cassava, cocoa/maize, cocoa/cassava/plantain, cocoa/cassava/maize, cocoa/plantain/maize and cocoa/plantain/cassava/maize. Sole cocoa served as the control in a randomised complete block design with four replicates. The effect of the treatments on the growth of cocoa seedlings, some soil chemical properties and profitability during establishment were evaluated. Intercropping significantly ($P=0,05$) reduced the girth of cocoa seedlings in the cocoa/cassava and cocoa/cassava/plantain only during the first year. Plant height was not significantly influenced by the treatments. The cocoa/plantain/cassava/maize package had the lowest but non-significant levels of N, P and K at the end of three years of food crop intercropping. The effect of this observation on cocoa yield is yet to be determined. Economic analysis of the treatments showed that intercropping was generally economically beneficial. Higher net returns were achieved especially where the mixtures contained cassava.

BEHAVIOUR OF THE COCOA CLONES FROM CUTTINGS IN A *DYSTROPHIC RED-YELLOW LATO SOLO UNIT* UNA UNDER DIFFERENT SIZES OF HOLES

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Abstract

The aerial and radicular growth of two cocoa clones (TSH-516 and TSH-1188) from cuttings were evaluated, under different sizes of holes, planted in a dystrophic Red-Yellow Lato solo *unit* UNA, in the south of Bahia, Brazil. The treatments were: holes of 0,20x0,20x0,20, 0,20x0,20x0,30, 0,30x0,30x0,30, 0,30x0,30x0,40, 0,40x0,40x0,40 and 0,40x0,40x0,50 m. All the treatments received 240 kg.ha⁻¹ of the 30-90-30 formula; fractioned 25%, 50%, 75% and 100%, at two months, 12 months, 24 months and 36 months after the transplanting of the shoots, respectively. At 18 and 30 months a complementary fertilization based on nitrogen (50% of the N doses) was administered. The experimental design was in randomized blocks with three replicates and twelve useful plants by plot. In the young phase (24 months of age): height, diameter and the number of shootings, the length of the latest shooting and gas exchanges were evaluated. In the plants radicular system: the weight of the dry material, the number, the diameter, the length and the density of the roots were evaluated. It was noted that holes with a depth of 0,40 m and/or bigger holes with 0,40x0,40x0,40 m provided a bigger growth of the aerial parts (height, diameter and length of the shootings) ($P \leq 0.05$) in both the clones. The TSH-516 showed a better efficiency in the use of water when planted in holes with sizes of 0,30x0,30x0,40 m. On the other hand, the TSH-1188 showed higher photosynthetic rates and efficiency in the use of water in holes with sizes of 0,40x0,40x0,40 m. In the two clones, 75% of the radicular system (second and third order roots) was placed at a depth of 0-0,20 m. The clone TSH-1188 showed a bigger average number of the second order roots (2.47 m) when compared to the clone TSH-516 (1.99 m) both planted in holes of 0,40x0,40x0,40 m. The radicular system (second and third order roots) of these clones did not exceed a depth of 0,80 m.

TRADITIONAL COCOA AGROFORESTRY: 1. SPECIES ENCOUNTERED IN THE COCOA ECOSYSTEM OF A TYPICAL COCOA GROWING DISTRICT IN GHANA

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Abstract

A survey was undertaken in the Osino District of the Eastern Region of Ghana between 1997 and 1998 to identify tree species retained by farmers on their cocoa farms. The exercise covered four different farm classes denoted as newly established (0 to 7 years old), juvenile (8 to 15 years old), mature (16 to 23 years old) and old (above 24 years old) farms from four large extension units each covering about one thousand hectares in the erstwhile Cocoa Services Division. The sample size chosen from each age class in each extension unit was based on probability proportional sampling and the unit of farm size taken for the survey was one hectare. The data collected on each farm included the number of different tree species found on the farm, the frequency of occurrence of different tree species on the farm, the frequency of occurrence of each tree species and the presence of non-tree species.

One hundred and sixteen different tree species were recorded from the sixty farms surveyed. Newly established farms contained three times as many trees per hectare than the other age classes to provide nursing shade for the young cocoa. An average of nine different tree species were encountered per hectare on newly established cocoa farms. Compared to six on the mature farms and about four on the juvenile and old farms. About twenty-one tree species were the commonest on all the farm classes with *Rauvolfia vomitoria* occurring on 95% of newly established and juvenile farms whilst *Milicia excelsa* was found on 82% of the mature and old farms. An undesirable tree species, *Sterculia tragacantha* was found in a large number of farms and in very high frequencies indicating possible ignorance by farmers of the types of tree species required for managing the cocoa ecosystem.

Fruit trees like *Citrus sinensis*, *Cola nitida*, *Persea americana*, *Mangifera indica*, and *Elaeis guineensis* and non-tree food/fruit species like plantain, banana, pawpaw and cassava were frequently encountered in the farms providing shade as well as food / fruit for the farmers. The study reveals that the cocoa ecosystem in this district has a rich tree species diversity which needs to be managed properly for cocoa cultivation.

Crop Protection

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OR: FRENCH

Variation in glucanase activity in the cross cells of nine *Theobroma cacao* clones after being inoculated with *Phytophthora megakarya*.

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Abstract

Black pod disease in cocoa (*Theobroma cacao* L.) caused by *Phytophthora megakarya* is the main constraint to production in Cameroon. The β -1.3 glucanases (EC 3.2.1.6) and β -1.4 glucanases (EC 3.2.1.4) are identified as playing a role in the defence of plants against fungicidal diseases, due to the nature of their substrates, glucans β -1.3 and β -1.4, which are major components of the cell wall in the majority of pathogenic fungus. In this study, the activity of β -1.3 and β -1.4 glucanases have been measured in the cross cells of nine *Theobroma cacao* clones varying in sensitivity towards the pathogenic agent. These are: SNK10, UPA134, SNK13, SNK213, SNK64, ICS95, SNK416, ICS84 and SNK413. These proteins are extracted from the acetate buffer pH 5.2 and the enzymatic activity measured using laminarin and carboxymethyl cellulose as substrates for β -1.3 and β -1.4 glucanases respectively. In soluble and ionically-linked fractions, the β -1.3 glucanase activity increases on average by 106% and 50% respectively, after the pods of the least sensitive clones, ICS84 and SNK413, have been inoculated. In the same way, the β -1.4 glucanase activity of the soluble fraction increases in inoculated pods of clones ICS84 (56%) and SNK413 (50%). However, the activity of these enzymes decreases or remains constant in the other clones. The β -1.3 et β -1.4 glucanase activity could therefore be used as sensitivity level indicators in cocoa clones.

Keywords: β -1.3 glucanase, β -1.4 glucanase, *T. cacao/P. megakarya*

Analysis of amino acids in the cortex of *Theobroma cacao* L. when inoculated with *Phytophthora megakarya* Bras. and Grif.

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Abstract

Amino acids and proline were analysed in the cortex of 9 clones of *Theobroma cacao* L. with varying degrees of sensitivity: SNK10, UPA134, SNK13, SNK213, SNK64, ICS95, SNK416, ICS84 and SNK413 with increasing sensitivity to *Phytophthora megakarya*. The strain of fungus L₂C₂ used is regularly maintained on a Potatoes Dextrose Agar (PDA) medium. Total amino acids and proline were measured using ninhydrin five days after the pods were inoculated. From a constituent point of view, the amino acid content is 745% higher in SNK413, a clone with the least sensitivity, than the very sensitive clone SNK10. When the pods are inoculated, the amino acid content increases significantly in clones SNK13, SNK213, ICS95, ICS84 AND SNK413, but decreases in clones SNK10, SNK213 and SNK416. The highest amino acid content (604 µg/g of PF) is measured in inoculated pods of the clone SNK413 whereas the lowest value (71 µg/g of PF) is measured in clones SNK10 and SNK64. A negative correlation ($r_p = -0.646$; $P = 0,05$) is noted between the amino acid content and the area of necrosis. The proline level in sound pods depends on the genotype. After inoculation, the level of this amino acid in the cortex increases significantly in clones SNK213, ICS95, ICS84 and SNK413 whilst decreasing in the other clones. When inoculated, the highest proline content is measured in clone SNK413 (0.9 mg/g of PF) and the lowest value is measured in clone SNK10 (0.12 mg/g of PF). The increased accumulation of amino acid content in general and proline in particular in the cortex after the fungus has been inoculated does not appear to offer resistance to the pathogen, but can serve as a resistance level indicator for a clone.

Keywords: amino acids, proline, *T. cacao*, *P. megakarya*

Evidence of resistance of National cocoa of Ecuador to the principal diseases which affect it

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Abstract

In Ecuador, *Crinipellis perniciosa*, causal agent of witches' broom, and *Crinipellis roreri* (previously *Monilia/Moniliophthora roreri*) act simultaneously on the crop causing between 40% and 100% of losses with a national average of 60%. Historical records of these diseases began at the beginning of the 20th century, not many years after the crop had become an important economic activity in the country, which led to the introduction of cocoa from Venezuela, Trinidad and other parts of the continent. Less spectacularly but also marking important milestones in the history of Ecuadorian cocoa is the incidence of *Ceratocystis fimbriata*, the agent of cocoa wilt. However, the first two organisms are known to be native to the cocoa region and they have also lived together with the "National" variety, which is also a native of the same region, without epidemics of these diseases being recorded. This behaviour suggests that there is an inherent level of resistance in the National germplasm but there is very little information or related experimental evidence, because all the information generated on this topic comes from material introduced on the Ecuadorian coast (Trinidadian and Venezuelan types) or collected directly from the High Amazon. The present study presents the results obtained in the EET Pichilingue (Autonomous National Institute of Agricultural research) when groups of plants from a population of National origin were subjected to artificial inoculation with the three organisms to determine their reaction to the three diseases. With *C. perniciosa* and *C. roreri*, a tendency for the behaviour of the population analysed to be normal was observed, individual plants with a good level of resistance and some very susceptible ones at the extremes being found. For *C. fimbriata* the tendency was not so clear; however, some plants with levels of resistance similar to or even better than the resistant control, IMC-67, were found. The methodologies used are described and the sources of resistance encountered in this population are identified.

**Effects of Resistant Genotypes of *Theobroma cacao* L. on the Epidemiology of *Phytophthora megakarya* ,
Causal Agent of Black Pod Rot in Cameroon**

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Abstract

A study on the effect in the field of resistant clones of cacao (*Theobroma cacao* L.) on epidemiology and evolution of genetic diversity of *Phytophthora megakarya* was carried out in collaboration between CIRAD and IRAD. In this presentation, only results of the epidemiologic study are developed.

A totally randomised design was employed using the clones SNK10 (susceptible) , SNK413 (resistant), ICS84 (medium resistant) and UPA134,(medium resistant) planted in 1982 in Barombi-Kang, Cameroon. A maximum of 50 fruits per tree were observed on 20 trees per clone during 3 growing seasons from 1999 to 2001. The response variables were: instantaneous severity of disease, expressed as a percentage of rotten fruits per tree and per week, number of days before disease was observed on each fruit, and yield loss due to black pod rot, wilt and feeding damage. The isolation of the pathogen was also carried out on each black pod and all the strains were sent to Cirad Montpellier for genetic characterization. The disease-progress curves for each clone showed a clear difference in severity of disease among the three years of observation. In 2000, the first severity peaks appeared 15 weeks later than in 1999 for all the clones. Between 2000 and 2001, disease severity was increased 6-fold for the clone UPA134 and doubled for the resistant clone SNK413. However, the population structure and the pathogenicity of the strains of *P. megakarya* between 2000 and 2001 were the same on UPA134 and SNK413. This suggests that the increase of pod losses in 2001 on UPA134 and SNK413 was more due to the increase of rain fall for that year than to a change in pathogen populations. Logistic regressions for the age of diseased pods per clone, showed that black pod of SNK10 were significantly younger than for all the other clones. This was connected to the fructification cycle that differs between the different genotypes. Yield loss due to *P.megakarya* was not significantly increased between 2000 and 2001, in spite of the important outbreak in severity. However, wilt rate was increased by 20% for all clones, suggesting that a secondary effect of high disease pressure could also be an augmented cherelle wilt. Finally, an estimation of total harvest considering tree survival after 20 years of plantation showed that resistant and moderately resistant clones performed better, than the susceptible SNK10. The effects of environmental and disease stresses on the physiology of the cocoa tree, their importance on the resistance mechanisms in the field and its implications for the grower will be discussed.

Characterisation of different *Phytophthora* pathogen species of the cocoa tree on the American continent and in the Caribbean.

Hérial C., Vezian-Bonnemayre K. and Ducamp M.

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Abstract

Several species of *Phytophthora* cause pod rot disease in cocoa (*Theobroma cacao* L.) on the American continent. Molecular characterisation studies have been able to separate certain species such as *P. palmivora*, *P. capsici* and *P. citrophthora* taxonomically, but they have not been enough for intra-species characterisation. The study of ITS (Internal transcribed spacer region of ribosomal DNA) sequences has confirmed the specific classification of some strains which in addition have been characterised for their sexual identity and their ability to produce spores.

The species *P. palmivora* is found throughout the American continent and the Caribbean. All the strains have the sexual identity A2, except those isolated in Jamaica which are A1 or A2. The cocoa pathogen from species *P. capsici* has been separated into three entities: *P. capsici* sensus stricto, *P. capsici* cocoa and a new species, *P. tropicalis*. *P. capsici* cocoa is the dominant species in Mexico. The strains with complementary sexual identities between *P. capsici* sensus stricto and *P. capsici* cocoa are fertile (production of oospores), which suggests adapting this species of Solanaceae to cocoa. However, the *P. tropicalis* strains are not fertile with the *P. capsici* strains, which allows these two species to be separated. A1 and A2 strains can be isolated for both species in all the countries where they are found.

In Brazil, all strains are very close to *P. citrophthora* (citrus pathogen) and *P. colocasiae* (cocoyam pathogen) and are sterile, in addition, which means that it is impossible to tell if they are fertile specifically with one or other of the two species. Their name of *P. citrophthora* cocoa should therefore be discussed.

Strains of *P. parasticia* (tobacco pathogen) have been isolated from rotting pods in Cuba alone.

The characterisation of the species responsible for the disease in a given place is primordial, for each species has a different epidemiological cycle with an impact on the prevention methods to be set up in the field for each species.

Development of microsatellite markers for the genetic analysis of *Crinipellis pernicioso*.

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Abstract

Witches' broom disease of cacao (*Theobroma cacao* L.), caused by *Crinipellis pernicioso* (Stahel) Singer, is the main important disease of cacao in the cacao growing areas of South America and Caribbean Islands. Very little is known about the genetic biology of the pathogen population, and this information is very important to the host improvement programs and deployment of resistant planting material. A collaborative international program was initiated to identify and describe the genetic diversity of *Crinipellis pernicioso* in South America. Microsatellites (SSR) constitute highly informative genetic markers for population genetic studies due to their co-dominant and multiallelic nature and distribution in the genome. SSR primers were searched in the *C. pernicioso* genome data base were designed as potential candidates to define an efficient, standardized, molecular fingerprinting protocol for this pathogen. These primers have been evaluated for reliability, widespread distribution across *C. pernicioso* genome and their ability to discriminate isolates of *C. pernicioso*. The final objective is to study the diversity structure of the *C. pernicioso* population from the main cacao growing area in Brazil. Preliminary results are reported.

Cocoa plant phyloplan hosts as possible agents for the bio-control of witches' broom in cocoa plants

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Abstract

This research study focuses on the possibility of biological control of the witches' broom in cocoa plants, by means of procaryote phyloplan hosts. Samples of healthy phyloplan cocoa plants, such as: new budding, totally expanded canopy, young beans and flower cushions, were collected, from two different collections, in commercial plantations in the Southern region of Bahia. Samples were submitted to the extraction of their epyphitotic flora by phyloplan washing and planting of wash in culture means. Thus, 377 procaryote host cultures were obtained. Mass selection was carried out by the combination of the data obtained in tests *in vitro* where the capability of isolates to inhibit basidiospore germination and the inhibition of the fungus saprophytic mycelic growth were evaluated. By combining the test results obtained by mass selection, 20 isolate were selected, which were also assessed in regard to their antagonistic activity against the mycelic growth of three isolates of *Trichoderma* spp. tested for the bio-control of the disease. For this investigation, various tests of culture pairing were developed, both in BDA and King B media, tests for the development of volatile anti-fungal composts, as well as extra-cellular anti-fungal composts. The culture filtrate activity was also identified. A greater activity antagonist to the mycele in the disease producing fungus as compared to the isolates of *Trichoderma* spp. was observed. The results obtained allow for the possibility of the implementation of phyloplan hosts and micro-parasites in association. In antagonist testing, isolates B42, B142, B143, B145, B146 e B147, have proved to be the most efficient for the development of anti-fungal composts against *C. pernicioso*.

Potential role of endophytic fungi in the management of cocoa pests and diseases

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Abstract

Endophytes, principally represented by fungi, are organisms which live asymptotically within healthy plants in a purportedly symbiotic or mutualistic association. It has been proposed that such fungi may confer resistance to pests and diseases and this potential has already been realised for certain associations, predominantly those involving graminaceous crops. However, there is a paucity of information pertaining to tree crops.

The aim of this study was determine if endophytic fungi commonly occur within cocoa tissues and, if so, whether they can reduce the impact of pests and diseases and thus be exploited as systemic biocontrol agents. Endophytes are known to exhibit antagonism through multiple mechanisms such as the production of inhibitory metabolites or toxins, competitive exclusion and activation of the defence mechanisms of the host (induced resistance).

We opine that wild populations of cocoa and its relatives in the forests of South America have evolved in association with a specialised endophytic mycobiota which helps to improve their fitness and competitive ability by increasing their resistance to pest and disease attack. As cocoa has been moved away from these natural forest ecosystems, we suggest that this has resulted in a loss of coevolved endophytes over time leading to an increased susceptibility to pests and diseases in exotic plantations, especially in Africa and Asia.

With this hypothesis in mind, surveys were undertaken within the native ranges of *Theobroma* spp., concentrating on the Upper Amazon region which is considered to be a centre of origin or diversity of *T. cacao*. Endophytic fungi were isolated directly in the field from both healthy pods and stems onto selective media. In addition, isolations were made from cocoa in exotic plantations (Bahia; Brazil; Cameroon; Costa Rica; Ghana; India) for comparison. Selected endophytes are also being assessed for their ability to colonise cocoa seedlings and pods. Almost 2000 fungal isolates have been obtained thus far from healthy wild cocoa populations representing a broad range of genera (*Acremonium*, *Clonostachys*, *Stilbella*, *Trichoderma*, *Verticillium*) and comprising a high percentage of new species. In contrast, exotic cocoa plantations are significantly depauperate, both quantitatively and qualitatively, in endophytic fungi.

A high proportion of the selected endophytes which have been tested so far have proven to be able to colonise germinating cocoa beans and to grow systemically within the tissues. A number of these endophyte strains have been successfully re-isolated from asymptomless cocoa shoots several months after inoculation. In addition, the results of preliminary greenhouse trials are also showing that some isolates appear to increase resistance to witches' broom disease (*Crinipellis pernicios*).

Field screening in Costa Rica of Ecuadorian mycoparasites of the cocoa frosty pod rot pathogen *Crinipellis (Moniliophthora) roreri*

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Abstract

Frosty pod rot disease, caused by the basidiomycete fungus *Crinipellis roreri* (formerly known as *Moniliophthora roreri*), is now becoming a major invasive pathogen of cocoa in Latin America. The failure of conventional control measures to halt its progress and reduce losses, has necessitated evaluating alternative methods of managing this dangerous and damaging pod pathogen. Biological control, and in particular the so-called classical strategy, is currently being investigated in Costa Rica. This classical approach centres around the premise that the most efficient, and hence exploitable natural enemies will be found in the native range of the target pest (arthropod, pathogen or weed). In the case of the frosty pod rot pathogen, the area of origin is purported to lie in the foothills of the western Andes, in northern Ecuador and Colombia. The search for coevolved natural enemies, specifically mycoparasitic fungi colonising the spores and pseudostroma which cover the pod surface, concentrated on submontane forests in north-west Ecuador where wild populations of *Theobroma gileri* infected by the frosty pod pathogen were discovered.

Mycoparasites were isolated, either directly *in situ* on selective media, or in the laboratory from diseased pods transported back to the UK. Fungal isolates were characterised and screened for mycoparasitic activity using the pre-colonised plate technique. Those isolates which exhibited high levels of mycoparasitism against *C. roreri* culture, including *Clonostachys rosea* and *Trichoderma stromaticum*, were selected for field trials in Costa Rica, after a pest risk analysis and following permission from the Costa Rican Plant Health Authorities to import the exotic isolates.

Two-month-old, hand-pollinated pods were inoculated with spore suspensions (1×10^6 spores/ml) of the selected mycoparasites and bi-weekly samples were taken over a 10-week period in order to assess their ability to colonise and persist on pods in the field. The results of this first phase of the project are presented and discussed. The methodology of the second phase, which will concentrate on determining the efficacy of the mycoparasites to reduce the sporulation of *C. roreri* is also discussed.

Characterisation and evolution in populations of *Phytophthora megakarya* pathogens of cocoa in Cameroon (1989-2002).

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Abstract

Phytophthora megakarya is the main species responsible for rotting pods in cocoa (*Theobroma cacao* L.) in Cameroon. This pathogen destroys on average 50% of the Cameroon pod production. To fight this disease effectively, it seems primordial to improve the characterisation and monitor the genetic and physiological evolution of the *P. megakarya* populations found in this country. Improved knowledge can be acquired from monitoring this evolution of the various field parameters (edaphic, climatic, growing practices, genetic control, biological control, etc.) which encourage the different genotypes (optimising their propagation cycle and increase their aggression). It can also support explanations during traditional epidemiological studies where the knowledge of planting material and environmental conditions alone is not enough to explain the extent of rotting which is sometimes noted.

Strains obtained from rotten pods during the periods 1989-1990, 1994-1996 and 1999-2002 were characterised molecularly by RAPD, and some by their ITS (internal transcribed spacer regions) and microsatellites. Their genotypes were compared. For the 1994-1996 period, all the genotypes characterised were different from those studied for the 1989-1990 period (where all the strains were "Central African"), but it showed good stability of the genotypes between the last two periods studied. Note that since 1994 asexual or clonal reproduction seems to dominate in Cameroon as it has been impossible to isolate a strain with the sexual identity A2. However, since 1994-1996, from the border zone between Nigeria and Cameroon, strains from the West Africa population seem to be propagating thanks to their powerful aggression. Strict control of planting material transfers from this zone to other cocoa areas in Cameroon should be planned to prevent the propagation of these highly-aggressive strains.

Production levels of sporocysts, zoospores and chlamydospores in the representative strains of the different periods were also studied under different climatic conditions, to attempt to explain differences in aggression between certain strains and the predominance of certain genotypes in some regions of Cameroon.

Using molecular markers and reliable evaluation tests for aggression, the aim is to monitor the evolution of populations of the pathogen according to control methods introduced in the cocoa farms and on other Sterculiaceae plants which could be infected by this parasite (cola, for example).

Geographical distribution of genotypes of *Phytophthora* from cacao by RAPD markers in Papua New Guinea.

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Abstract

In Papua New Guinea, losses caused by 'black pod' are close to 40 % of the annual cacao production. The identification and characteristics of the *Phytophthora* strains from the different producing provinces in PNG is important for the development of proper integrated disease control measures, particularly in breeding for resistance to the disease.

Objectives of the work were, firstly, to collect pathogen samples in local smallholdings. A large number of isolates were collected (474) around seven mainly cacao producing provinces visited: East New Britain (45), Madang (190), East Sepik (52), Manus (37), New Ireland (85), Oro (42) and Morobe (23). Secondly, the physiology and the isozyme pattern of each isolate were characterized in CCRI. And finally, PCR technique on a range of some representatives samples of *Phytophthora* strains (55) was used in Cirad Montpellier, with nine primers, four OPM (4, 6, 7, 17) and five OPR (4, 5, 6, 11 and 14).

Two important results were obtained:

1. The subsequent geographical distribution of the genotypes (G1 to G7) in PNG pointed out a particular genotype discrimination concerning Madang province (G5, G6 and G7).
2. Clustering with other strains of *Phytophthora* from cacao as well as from other hosts (coconut, rubber, durian, bamboo and orchids) showed the parentage with *P. palmivora* but with a clear proper branching.

Keyword: RAPD markers, Isozymes patterns, *Phytophthora*, black pod cacao disease.

COMPARISON OF CLONE RESPONSE AND DIFFERENT CONTROL STRATEGIES OF WITCHES' BROOM OF CACAO IN CLONAL PLANTATIONS, ON THE SAME CONDITIONS AS IN BAHIA, BRAZIL

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Abstract

Witches' broom is the major pest affecting cacao culture in Brazil. In 1989 the presence of its pathogen was noticed for the first time in the main Brazilian production area. The lack of adequate control measures has resulted, amongst others, in a decrease in yield. The present research study was developed as very little is known about the various strategies available for the genetic, cultural and chemical control of the disease, in the conditions existing in Southeastern Bahia, its main objective being the comparison between 10 genotypes and three treatments, for the control of the disease. The essay was carried out in Uruçuca, BA, in an area where cacao plants grafted with ten different genotypes and approximately 3 years old after grafting, are present. The experimental area was submitted to the following treatments: phytosanitary pruning every six months, monthly phytosanitary pruning and phytosanitary pruning combined with a monthly application of a fungicide. Copper oxide was applied as a fungicide, at a dosage of 3 g of the i.a./plant/application. The number of branches and the percentage of fruit showing disease symptoms were quantified every month. The experimental outline was totally randomised, with 10 genotypes, three treatments and a different number of repetitions. The theory of generalised lineal models was applied, for the assessment of the data obtained. Results achieved indicate that the treatment combining pruning and spraying with fungicide presents a significant difference in the reduction of the percentage of fruit affected by witches' broom. Genotypes NO-34, NO-17 and NO-02 presented lower percentages of infected fruit with broom, being significantly different from genotypes NO-24 and NO-13. Levels of genetic resistance to witches' broom on branches and fruit were not co-related inter alia. Thus, testing on fruit should be carried out as a matter of routine for all improvement programmes.

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The endophytic community of cocoa (*Theobroma cacao* L.) and the biological control of *Crinipellis pernicioso*, causal agent of Witches' Broom Disease .

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Abstract

The basidiomycete fungus *Crinipellis pernicioso* (Stahel) Singer is the causal agent of witches' broom disease of cacao (*Theobroma cacao* L.) which is the main factor limiting cocoa production in the Americas. Pod losses of up to 90% are experienced in affected areas as evidenced by the 70% drop in production in the State of Bahia, Brazil following the arrival of the *C. pernicioso* in the area in 1989. The disease has proven particularly difficult to control and many farmers in affected areas have given up cacao cultivation. In order to evaluate the potential of endophytes as a biological control agent this phytopathogen, the endophytic fungi and bacterial communities of resistant and susceptible cacao plants as well as affected branches were studied in 2001 and 2002. Dominant bacterial isolates were characterized by 16S rDNA sequencing as *Micrococcus* sp., *Streptomyces* spp., *Bacillus subtilis*, *Bacillus* spp., *Bacillus pumilus*, *Kocuria* sp., *Acinetobacter* sp., *Methylobacterium* spp., *Pseudomonas* spp. The fungal community was identified by microscopy observation and ITS sequencing as belonging to the genera *Verticillium* sp., *Gliocladium* sp., *Monilochaetes* sp., *Trichoderma* sp., *Geotrichum* sp., *Syncephalastrum* sp., *Fusarium* sp., *Acremonium* sp., *Xylaria* sp., *Cladosporium* sp., *Blastomyces* sp., *Pleurotus* sp., *Botryosphaeria* sp. These fungi and bacteria were evaluated *in vitro* and *in vivo* as the ability to inhibit *Crinipellis pernicioso*. Among these, 68 microorganismos were identified as potential antagonists, but only 1 fungus (*Gliocladium catenulatum*) and 2 unidentified bacteria reduced till 80% the incidence of witches' broom disease in cocoa plants.

Genetic variability of endophytic and pathogenic strains of *Crinipellis pernicioso*.

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Abstract

The basidiomycete fungus *Crinipellis pernicioso* (Stahel) Singer is the causal agent of witches' broom disease of cacao (*Theobroma cacao* L.) which is the main factor limiting cocoa production in the Americas. Pod losses of up to 90% are experienced in affected areas as evidenced by the 70% drop in production in Bahia province, Brazil following the arrival of the *C. pernicioso* in the area in 1989. The disease has proven particularly difficult to control and many farmers in affected areas have given up cacao cultivation. Any useful control strategy for witches' broom disease must be effective against a range of strains of the pathogen. It is already known that pathogenic variation exists among isolates of *Crinipellis pernicioso* obtained from different areas and host plants. However, any study was developed about the variability between endophytic and pathogenic population. In order to evaluate the genetic variability of 35 isolates of endophytic and pathogenic populations of *Crinipellis pernicioso*, the RAPD technique, ITS sequencing and fungicide susceptibility were performed. Genetic variability between 35 isolates of *C. pernicioso* was analysed by the random amplified polymorphic DNA (RAPD) technique, which indicated that isolates from other host plants were more diverse than isolates obtained from cacao plants. Among cacao isolates was observed at least two groups, one formed mainly by endophytic isolates and other by pathogenic ones. Analysis by ITS sequence grouped isolates independently of endophytic or pathogenic status. Fungicide susceptibility showed that cupric oxide inhibits statistically more endophytic isolates than pathogenic ones, showing that could have physiological differences between these populations. The present study highlighted the possible genetic and physiological differences between endophytic and pathogenic population of *C. pernicioso*.

INCIDENCE AND GEOGRAPHIC DISTRIBUTION OF CACAO DISEASES IN BAHIA IN RECENT YEARS.

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Abstract

Diseased plant materials from cacao (*Theobroma cacao* L.) collected by growers are usually sent to the Plant Pathological Clinic of the Phytopathological Section of the Cacao Research Center (CEPEC/CEPLAC) for disease identification and classification of the pathological agents. These records are kept in registration books and fed to a computer program (Sgrillo/CEPEC). Data can be assessed by host, disease, pathogen and locality. Analysis of these data from January 2001 to December 2002 shows the prevalence, geographic and season distribution of cacao pathogens and respective diseases. The most common pathogens and diseases caused by them were: *Ceratocystis fimbriata* (wilt); *Lasiodiplodia theobromae* (dieback, canker, decline); *Crinipellis pernicioso* (witches' broom); *Rosellinia pepo* (root rot); *Fusarium solani* (root rot, damping off, canker, fruit rot, dieback); *Fusarium* spp. (root and fruit rots, damping-off); *Xylaria* spp. (trunk rots); *Phytophthora* spp. (pod rots: few materials collected due to easy diagnostic); *Phomopsis* spp. (dieback); *Pestalotiopsis* spp. (leaf spots); *F. decemcellulare* (flower cushions galls); *Ganoderma philippii* (root rot); *Colletotrichum* spp. (anthracnosis); *Corticium salmonicolor* (pink disease); *Nectria* spp. (fruit rot, canker). Ilhéus, Ubaíra, Itajuípe, Canavieiras and Itacaré were the localities with higher disease records, specially *Ceratocystis* wilt. Diseases occur mostly during the rainy season (April to September), except diebacks and wilts which prevail during the dry season (October to March). Several minor pathogens and diseases were also recorded. *Verticillium* wilt was serious in the past, but has not been recorded in the last three years; while *Ceratocystis* wilt was registered in Bahia only in 1998. *Witches' broom*, *Ceratocystis* wilt and *Phytophthora* pod rot are, presently, the most important diseases in Bahia, followed by root diseases.

**GENETIC DIVERSITY OF THE ISOLATES OF *TRICHODERMA* SPP. AND *HYPOCREA STROMATICA*
IN THE CACAO PRODUCING REGION OF BRAZIL.**

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Abstract

The isolates of *Trichoderma* spp. and *Hypocrea stromatica* present a high antagonistic potential to *Crinipellis pernicioso*, the causal agent of witches' broom in cacao plants (*Theobroma cacao* L.). *Trichoderma stromaticum* has been used experimentally with good results in the biological control of witches' broom, since 1995. This antagonist presents high re-combinatory capabilities, which could generate segregates with a variable efficiency. The objective of this research study was to check: **(i)** the variability (both genetic and morpho-physiologic) of isolates of *Trichoderma* spp. in the cacao producing areas of Bahia and Brazilian Amazon and **(ii)** the viability of the application of a mutant isolate of *T. stromaticum* (resistant to benomyl, TVC 5.15) for epidemiological studies of the antagonist (Sardagna & Melo, 2001). Nine decamer primers generated 202 marks (RAPD) with an average of 22,4 marks per primer. A high genetic variability rate was observed amongst *Trichoderma* spp. isolates of cacao (1 to 85%). There was no genetic differentiation between the wild and mutant type isolates (=96%). Analyses of the morpho-bio-metric characteristics comprised shapes of conids, types of phyalids, presence/absence of clamidospores, sporulation and types of colouring in culture means. The characteristic type of phyalids was the one offering a higher contribution towards the divergence of isolates. It was concluded that the mutant type isolate may be used for epidemiological studies, as it keeps the species' genetic characteristics and that the use of molecular markers show a potential for the indication of the antagonistic capabilities of the species *Trichoderma*.

Mycoparasitism on the cacao black pod disease pathogen *Phytophthora megakarya* by fungal endophytes from cacao leaves in Cameroon .

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Abstract

The presence of mycoparasites of *P.megakarya* within fungal endophytes from cacao leaves was investigated. 735 endophytic fungi isolated from cacao (*Theobroma cacao*) leaves were screened using the pre-colonized plate method. Nine of them (BC177, BC108, BC252, BC134, BC150, BC181, BC118, BC13, BCX) expressed mycoparasitism against *P.megakarya*. However eight (BC108, BC252, BC134, BC150, BC181, BC118, BC13, BCX) expressed biotrophic mycoparasitism. Some necrotrophic mycoparasitism was observed with [BC177], a *Geniculosporium species* isolate. Necrotrophic mycoparasitism has much potential for biocontrol of black pod disease but was found to be very scarce within the investigated population of endophytic fungi. Necrotrophic mycoparasites can be used as any commercial fungicide, but could also be exploited as natural regulators of the disease by reducing the level of primary inoculum of the pathogen in the farm. .

Key words: Biocontrol, *Phytophthora megakarya*, mycoparasitism, *Geniculosporium sp*, endophytes.

Pathogenicity genes and pathogen-host interactions – a comparative study in relation to *Crinipellis pernicioso*, causal agent of cacao witches' broom.

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Abstract

The knowledge of pathogenic genes and their expression mode may turn possible the control of a given disease. The purpose of this work was to describe the *Crinipellis pernicioso* fungus and its importance in the cacao areas of Brazil, to discuss briefly the concepts of avirulence (Avr) genes and host-specific toxins (HST), and to relate and compare some pathogenicity genes described in the literature, which could be homologous to ones in the genome of this fungus. To determine sequence similarities among pathogenicity genes in the genome of *Crinipellis pernicioso*, a search was firstly made using the key-words 'pathogenicity', 'avr genes' and 'resistance genes' in the GeneBank (NCBI homepage). The sequences of genes obtained were compared with sequences stored in the database of the *Crinipellis* Genome Project. Afterwards, specific sequences of genes already described as related to pathogenesis were obtained from the GeneBank and also compared with sequences from the *Crinipellis* Genome Project. In both methods, which used either key-words or established pathogenic genes sequences from other fungi, mainly those that express the formation of infection structures, degrade the cuticle or the cellular wall, respond to the host environment, produce fungic toxins or that were not yet classified, sufficient sequence similarities between the GeneBank and the *Crinipellis* Project entries were not found. High levels of similarity were only found with components of signal transduction cascades, mainly genes of mitogen-activated protein (MAP) kinases of *Ustilago maydis* and *Magnaporthe grisea*. Most of these genes are similar with MAP-kinases found and studied in *Saccharomyces cerevisiae*.

Population structure of the witches' broom pathogen of cacao in Trinidad and Tobago

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Abstract

A study on pathogenic variability of *Crinipellis pernicioso* in Trinidad, conducted in 1987, reported one biotype, B, of the pathogen. Variation in disease reaction response has been frequently reported by researchers at CRU, in cacao accessions used as controls in different witches' broom greenhouse screening trials. In this study amplified fragment length polymorphisms (AFLP) were used to discern the genetic diversity of the witches' broom cacao pathogen in Trinidad and Tobago. Isolates of *C. pernicioso* were collected from farmers' estates on both islands from both Trinidad Selected Hybrid (TSH) and the original Trinitario cacao. At the 0.9 similarity level, two clusters were revealed from the polymorphisms of 173 fragments in 74 isolates using UPGMA cluster analysis with Dice's coefficient. The majority or 90% of the isolates were genetically similar while the other cluster was comprised of two closely related groups with 99.8% and 90.2% *P* values, respectively. The relatively rare groups were not localized to any particular region or estate, but were only present in Trinidad on the Trinitario genotypes. These results support previous findings in that the majority of isolates were genetically similar. The results also indicate that the *Crinipellis* pathogen population in Trinidad may consist of at least two distinct clonal lineages.

Rational fungicide use in cocoa: improving agents and application techniques

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Abstract

We provide an overview of a number of USDA funded trials on cocoa pod disease control, carried out at La Lola, Costa Rica, together with supporting laboratory research. The objective of this work was to assess the interactions between application techniques and fungicides (both biological and chemical) for the control of frosty pod rot *Crinipellis (Moniliophthora) roreri* and other pod pathogens.

Copper fungicides have long been amongst the most important cocoa fungicides, but concern is growing about the toxicity of these agents and their build-up in cocoa crop soils. Although copper is still permitted for organic production, this is increasingly being seen as an undesirable and incompatible control measure, with restrictions placed on its application. We have examined two groups alternative agents: systemic chemicals (for non-organic production) and hyper-parasitic fungi; copper hydroxide was used as a standard (contact) control chemical. The patents of a number of systemic fungicides, among the triazoles and oxathiins, have recently expired and products (that might previously have been dismissed on cost grounds) have become cheaper. The oxathiin, flutolanil, appears to show significant and cost-effective activity against basidiomycete pathogens such as *C. roreri* and evaluation is continuing as a short-term measure. Fungal control agents constitute medium-term control tactics, and trials have included treatments of *Clonostachys* and *Trichoderma* spp. However, isolate selection and formulation of these agents are still in their infancy; this trial evaluation is linked to other research described in these proceedings.

Most cocoa growers apply pesticides with simple manual, hydraulic equipment (if at all), although motorised mistblowers (which were originally developed for this crop) are used often, being more cost effective on larger holdings. In both cases, little research has been done to optimise pesticide dose transfer to the biological target in spray operations, and knowledge at the distributor / farmer level is extremely limited. We describe progress made in optimising mistblower machinery settings, and dose transfer to pods with hydraulic sprayers. In the latter case, substantial (>2x) improvements in efficiency may be achieved using narrow-angle cone nozzle combinations and pressure regulating valves.

Research to date has focused on selecting agents and improving application techniques and is ongoing. There is further scope for reducing disease control costs by more targeted timing of application, reducing the number of applications per season.

PROGRESSION OF MONILIOPHTHORA ROT IN CACAO PODS IN PERU

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Abstract

Cacao is one of the main cultures in Peruvian Amazon. Diseases are the main restrictive factor as far as production is concerned, particularly after the 90s, when *Moniliophthora Rot* (PM), caused by *Moniliophthora roreri*, was first sighted and started its fast spread. In all Peruvian cacao-producing regions, losses can reach 100% in plantations where disease control is not implemented. On the other hand, losses of between 40 and 60% are quite common in commercial plantations. It is essential to reduce these high values of losses, and bearing this in mind, measures to be implemented should be based on research studies under Peruvian conditions. Therefore, the objective of this research study was to evaluate PM's progression, in cacao pods, correlating this with climacteric and phenological factors.

Studies were carried out in the Tingo Maria area, from 1996 to 1999. Disease progression was studied, in relation to the phenological stages of the beans, as well as inoculum production (number of collected conides), influenced by meteorological factors (pluvial precipitation, relative humidity and temperature).

The occurrence of this disease during every month of the year was observed, which would suggest that in Peru, the pathogene has, or finds, favourable conditions to infect cacao plants throughout the year. However, infection peaks were identified, mainly from January to July, coinciding with the predominance of optimal conditions for infection.

These conditions are related to the existence of a rainy season, from October to April, the presence of a high level of inoculum and bean formation in susceptible conditions from December to May. In some years, lower peaks of infection were observed in different months, in accordance with climatic variations. Assuming that susceptible beans are always available, *M. roreri* may infect pods throughout the year, and the fungus seems to be less dependent on climatic variables than some other pathogens of cacao pods.

This research project has demonstrated the importance of beans with *M. roreri* sporulation, for the development of PM epidemics, and consequently, the phytosanitary control of the disease should focus on these fruits. Frequent removal should reduce inoculum sources and, consequently avoid future infections during the rainy season.

**STUDIES ON THE FEEDING AND DAMAGE CHARACTERISTICS OF THE COCOA POD BORER
CHARACOMA STICTIGRATA HMPS (LEPIDOPTERA: NOCTUIDAE)**

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Abstract

The feeding and burrowing and damage characteristics of *Characoma stictigrapta* HMPS was studied. Many of the larvae (61.7%) were observed to burrow into the pod through the underside of the peduncular end. Larvae found protective cover at the point of contact of the pod and other parts of the tree or in furrows on cherelles and pods. Larvae did not burrow into the pod until the third instar stage. Cherelles were more susceptible to damage than mature pods as 76% of cherelles infested with third instar larvae were totally damaged while none of the mature pods so infested was totally damaged. Multiple infestation of pods was found to be common, ranging from 1-12 larvae/pupae per pod. About 27% of all pods sampled had a larvae/pupae density of 3. No cannibalism was observed in multiple infestations.

Considering the observed tendency of *Characoma* larvae to hide and cover the entrance to its burrow with mass of silk-bound frass, spot spraying of pods is recommended as against general spraying of cocoa trees. Spot spraying will deliver more spray chemicals that will penetrate the protective wall of frass and silk.

Towards biological control of pod rot in cocoa

Carmen Suárez-Capello & Karina Solis Hidalgo

Abstract

In Ecuador, two fungus diseases, witches' broom disease, caused by *Crinipellis perniciosa*, and “moniliasis”, “cold” or “fruit rot”, the agent of which is *Crinipellis roreri* (previously *Monilia/Moniliophthora roreri*), continue to depress cocoa production. The damage due to these two organisms can reach 80 and 100% of any harvest and the national average fruit loss is 60%. The pruning of infected fruit before *C. roreri* sporulates leads to their rapid decomposition on the ground; however, the frequency with which this work has to be done does not make it attractive to producers and the INIAP (National Institute of Agricultural Research) through the National Department of Plant Protection of the Pichilingue Tropical Experimental Station has continued to search for sustainable strategies to control these diseases, especially *C. roreri*. Since 1999 studies aimed at discovering Biocontroller organisms in the pods have been going on; the starting point was the organisms which speed up the initial decomposition of the pods which fall to the ground in the plantations. Using the technique of maceration of tissues and dilution in agar plate, seven collections were carried out one, three, seven, fifteen, thirty, sixty and ninety days after the pods with an initial infection were left on the ground in a plantation. Subsequently, by means of successive replatings, 94 isolates of filamentary fungi from the epicarp and mesocarp of the reference pods were obtained. From these, fifteen were selected which came from the first three collections and which, in comparison with the isolates, had a greater speed of growth on the agar plate and therefore greater competitive capacity. With these fungi, competitive bio-assays were carried out both with a colony of *C. roreri* already established in the medium and with simultaneous sowing. Seven isolates of *Trichoderma* spp (5) and *Xilaria* sp (2) types were found which inhibited the development of the fungus, two others (*Trichoderma* sp) which overlapped the colony of *C. roreri* preventing it from sporulating and eventually macerating its mycelium, and another (*Trichoderma* or *Gliocladium* (?)) which completely inhibited the advance of the fungus in the medium. Later studies until now have been aimed at identifying the organisms involved specifically, deciding on media for the production of inoculum and carrying out *in vitro* bio-assays to confirm their Biocontroller capacity.

**SELECTION OF COCOA GENOTYPES AS PARENTS FOR RESISTANCE TO *PHYTOPHTHORA*
MEGAKARYA AND *PHYTOPHTHORA PALMIVORA***

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Abstract

The application of chemicals to control *Phytophthora Palmivora* and *Phytophthora megakarya* pod rot (the two types of black pod disease in Ghana) in fraught with many problems and the use of genetically resistant materials has been advocated. *Phytophthora palmivora* was for several years the only causal agent accounting for most infections in Ghana. Following the detection of *P. megakarya* in Ghana, a programme was started in two endemic districts to determine the level of resistance of cocoa trees to the new strain. The first evaluation was by field observation at the end of which twenty-five trees were selected as promising black pod resistant parents. In the subsequent evaluations, the selected trees were hand pollinated and attached pods inoculated with zoospores of *P. megakarya* and *P. palmivora* isolates. Two methods of inoculations (point and spray) were tested on attached pods. For the point inoculation, the level of resistance was assessed by counting the number of infected pods, measuring the size of lesions on and in the cocoa pod husk and the amount of sporangia production on the pod husk. The number, size and type of lesion on the pod was used to score the trees using a scale of 1 to 8 in the spray inoculation. The test inoculations showed that most of the trees classified as resistant to black pod based on field observations were mere escapes. The pod infections from the field observations ranged from 0.0% to 14.4%, the point inoculation from 3.6% to 90.5% and the spray inoculation had a mean score of 1.00 to 4.67. There were wide variations in the level of tolerance of the best trees and five distinct categories were identified; trees with less than 5% black pod infections or a score of 1.00-1.10, trees with 20%-29% pod infections or a score of 1.59-2.00, trees with 30%-40% infections or a score of 2.35-2.57, trees with 50%-60% infections or a score of 3.07-3.87 and trees with over 70% infections or a score of 4.60-4.67. There was a positive correlation between the point and the spray inoculation methods. The results showed that the pods from trees classified as tolerant in the test inoculations had less sporangia than pods from trees classified as susceptible. However, *P. megakarya* was found to be more prolific and produced about six times more sporangia than *P. palmivora*. *Phytophthora megakarya* was more virulent and infected pods at a faster rate than *P. palmivora*. Tolerant trees have been selected and planted as part of a clonal collection at Bechem.

Field trials on thiamethoxam (Actara 240 SC) and fenobucard (Bassa 500 EC) for the control of cocoa mirids (Heteroptera: Miridae) in Ghana.

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Abstract

Large-scale field trials were conducted during 1999-2002 to evaluate the efficacy of thiamethoxam (Actara 240 SC) and fenobucarb (Bassa 500 EC) for control of the cocoa mirids [*Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.)]. Two applications of Actara 240 SC at 20g a.i./ha in October and November and four applications of Bassa 500 EC at 280g a.i./ha in September, October, November and December showed that both chemicals were equally effective against mirids. Each gave mean mortality value of more than the acceptable level of 95%. Studies on population recovery (cumulative numbers) and fresh damage during the entire season after insecticide application indicated that significantly more mirids and damage were found on the Bassa plots than on the Actara plots ($P= 0.05$). The results of the study support the potential of using the two insecticides for the control of capsids on cocoa in Ghana.

STUDIES ON TERMITE PREFERENCES FOR COCOA PROGENIES .

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Abstract

Termites (Isoptera) have assumed a major pest status on cocoa in Ghana. The situation poses a serious hindrance to establishment of the crop in the country. Synthetic chemicals have been the major means of their control, and there is increasing need to find alternative control measures, in view of the hazards associated with such chemicals. One such option is the use of cocoa varieties that are less attractive to pest termites. From March 2000 to December 2002, monthly data were taken on plot M3 at Afoosu to study the incidents of termite pests on nine cocoa progenies with either T85/799, T60/887 or 10p as one of the parents. Twelve randomly selected seedlings per plot and three replications. Twelve randomly selected seedlings were examined in each plot for incidence of the termites and samples were preserved for identification. The dominant termite pest was *Ancistrotermes* sp. And it showed preference for crosses involving T85/799, followed by those involving T60/887 and involving T85/799, followed by those involving T60/887 and 10p.

**PRELIMINARY STUDIES OF THE CONTROL OF COCOA SWOLLEN SHOOT DISEASE BY THE
USE OF IMMUNE CROPS AS BARRIER**

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Abstract

A field trial was established at the Cocoa Research Institute of Ghana in 1993 to investigate effectiveness of using crops such as citrus, oil palm and kola which are immune to cocoa swollen badnavirus (CSSV) to separate new cocoa planting from old after, monthly inspections were carried out in the outer cocoa for spread of CSSV. Cocoa yields as well as yields from the establishments to reduce the risk of CSSV reinfection. The trial was planted out in a randomised block design replicated three times. The crops used for the barriers formed the treatments. These were citrus, oil palm, kola and cocoa. The treatments were laid out in such a way that healthy core plants of 25 cocoa trees planted at 3m x 3m were surrounded by two rows of barrier crops. Four years after establishment, the inner core of 25 plants were inoculated with CSSV severe strain IA. There after monthly inspections were carried out in the outer cocoa for spread of CSSV. Cocoa yield as well as yield from the barrier crops were recorded. Five years after CSSV severe IA inoculations, 30 trees across the barriers have been infected by the CSSV. Eighteen of the infections were across the cocoa barrier, while eleven were across the kola and one across the oil palm. These results indicates that the citrus and oil palm barriers are effective in isolating the test cocoa trees from CSSV infection. The kola barrier has not been effective due to poor establishment. Economic analysis of the produce from the three barrier crops suggests that the citrus and oil palm adequately compensated for the cocoa space they occupied. The results are discussed in relation to swollen shoot disease control in West African sub-region with particular reference to Ghana.

How do *Phytophthora* species infect cocoa plants?

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Abstract

Phytophthora species belong to the Oomycetes (water moulds), a group of fungal-like organisms, which contain some of the world's most destructive plant pathogens. Collectively, they account for multibillion-dollar losses in the world's cash crops. The mechanisms that make *Phytophthora* species successful pathogens on major economic crops such as cocoa has been the subject of our investigations for a considerable number of years. Our research focuses on understanding the molecular triggers underlying the different growth phases of *Phytophthora* prior to infection of host tissues and the mechanisms of host-pathogen interaction itself. We have examined the nature of the signals that allow the motile zoospores to locate and home in on plant tissues, the pattern of accumulation of competing zoospore populations from different species at well-defined regions around roots and the role of certain ions prior to the establishment of successful disease infection (van West *et al.* 2002 *Molecular Plant-Microbe Interactions* **15**: 790-98).

We are currently dissecting, at the molecular level, the specific role of genes encoding for the channelling of potassium and calcium ions, which have been found to play key roles in the pre-infection stages. We are also analysing the transcript profiles of genes and proteins expressed in the stages prior to infection using proteomics, gene silencing and functional genomics approaches. We believe these studies hold great promise in identifying novel targets for effective control of *Phytophthora* diseases including black pod disease of cocoa, as many of the currently available control measures are ineffective and of great cost to the environment.

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ORIGINAL

**EVALUATION OF FOUR SYSTEMIC FUNGICIDES FOR THE CONTROL OF *PHYTOPHTORA* POD
ROT OF COCOA IN NIGERIA**

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Abstract

Black pod disease caused by *Phytophthora megakarya* is the most important and major disease of cocoa in Nigeria. Four systemic fungicides were evaluated for their effectiveness against black pod diseases at Ibadan, Owena and Ikom during 2000, 2001 and 2002. Ridomil gold, Funguran, Champ D. P were compared with Ridomil plus in a field trial. Compared with unsprayed plots, Ridomil gold and Champ D. P. significantly reduced black pod infection from an average of 72% for the year to 21.7% and 27.2% respectively.

The other fungicides tested also reduced infection but to a less extent. Ridomil gold and Champ D. P were more effective than the Ridomil plus. Surveys for fungicides resistance isolates of *P. megakarya* are proposed. Future trial work and control recommendations will depend on the outcome of such survey.

**A SURVEY ON THE ROOT DISEASES IN DIFFERENT COCOA CROPPING SYSTEMS IN
PENINSULAR MALAYSIA**

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Abstract

A study was conducted on cocoa root diseases in cocoa fields of 3 different cropping system in Peninsular Malaysia, namely monoculture of cocoa in Baling, Kedah; cocoa intercropping with coconut in Hilir Perak, Perak and cocoa intercropping with fruit trees (durian) in Muar, Johor. The results show that the incidence of root diseases in the cocoa fields under study was low (0.3-0.5%). White root disease (*Rigidoporus* sp) and brown root disease (*Phellius* sp) were found in all three cocoa systems, while red root disease (*Ganoderma* sp) was detected in monoculture cocoa and cocoa coconut intercropping system. *Rigidoporus* sp was the predominant root disease representing 57.9% of the root disease found in monoculture system, 68.2% and 71.4% in cocoa/coconut and cocoa/fruit tree system. It was followed by the *Phellius* sp at 36.8%, 18.2% and 28.6% respectively. *Ganoderma* sp represented 5.3% and 13.6% of the root disease found in monoculture cocoa and cocoa/coconut intercropping system.

CHEMISTRY/TECHNOLOGY/ QUALITY

A NOVEL PROTOTYPE OF A FERMENTER FOR CONTROLLED COCOA FERMENTATIONS

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Abstract

A small-scale drum type fermenter with forced ventilation and mechanical turning was designed for experimental work on controlled fermentation of 50 kg cocoa batches. Process control is done through monitoring and control of the aeration rate and inoculation of the cocoa batch with yeast cocktails. The prototype was made of stainless steel and it has a supporting frame on which two concentric cylinders are mounted horizontally. These cylinders have an axle connected directly to a gearbox, which is driven by a 3 HP electric motor. The rotation speed of the fermenter is controlled by an electronic motor drive, which acts on the frequency of the current fed to the motor. The inner cylinder has perforated walls allowing free drainage of sweating and air diffusion. The sweating from the fermenting cocoa is collected in the bottom of the external cylinder and drained out, from time to time, through three outlets with threaded lids. Artificial ventilation is provided by an air compressor connected to the fermenter through a hose distributing the air by means of a stainless steel manifold. The airflow rate to be used in the tests is controlled by means of constriction valves and a rotameter fitted in the line. Temperatures of the fermenting mass and the ambient were monitored using an eight-channel datalogger. To date the fermenter was tested with both natural inoculation and natural aeration as yet, using cocoa beans from ripe pods harvested and open on the same day, either in the field or in the laboratory. The temperature profiles varied considerably with the conditions with which the beans were processed prior to being loaded into the fermenter. Fermentation runs without inoculation showed temperature profiles with maximum temperatures of about 33 ° C. The curing process observed was abnormal due to lack of natural inoculation and aeration, since the fermenter was kept closed during the whole test. Fermentations of cocoa beans inoculated naturally, the fermenter closed, showed higher temperatures (40 to 42° C). Fermentations carried out with the fermenter open gave the best results in terms of temperature rising. Temperature peaks of about 48 ° C were observed. The curing process finished before the fifth day of fermentation.

DETERMINING THE PURINE CONTENT OF COCOA BY NEAR-INFRARED SPECTROMETRY

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Abstract

Methods normally used for purine dosing (caffeine and theobromine) in cocoa are fairly long and ill-suited to routine controls. A simple, quick dosing method is necessary, given that these compounds play a significant role in developing the cocoa aroma and that their content in chocolate is also used to determine the cocoa content in the product.

The feasibility of measuring caffeine dose by near-infrared spectrometry, a method already used in coffee, has been tested for cocoa.

The theobromine and caffeine content of 148 samples (at different stages of fermentation and from different geographical and varietal origins) tested in the laboratory (water extraction, HPLC-UV) were found to be between 0.58% and 1.48% for theobromine and 0.08% and 0.74% for caffeine.

At the same time, the spectrums (2400-2500 nm) of the hulled and ground samples were acquired by diffuse luminous reflectance using a Foss 6500 monochromator.

The Partial Least Square (PLS) regression by using a cross validation was applied to develop a prediction model for purine contents using spectral data. This model adjusts the data perfectly, the coefficient of determination (R^2) is 0.96 for caffeine and 0.80 for theobromine. Standard errors of cross validation (SECV) are 0.041 for caffeine and 0.08 for theobromine.

To test the models' prediction performances, 118 samples representative of the spectral variability were used to develop equations. The prediction errors (SEP), estimated by applying these equations to 31 remaining samples, are 0.036 for caffeine and 0.085 for theobromine.

The coefficients of determination of the regressions between the reference values and the forecast values are 0.95 (caffeine) and 0.77 (theobromine).

These results are promising for setting up a rapid and non-destructive control method for cocoa purines. The prediction model should be refined, particularly for theobromine, by completing the database with samples from the principal varieties grown covering several harvest years. The accuracy of theobromine dosing should also be improved using physico-chemical methods.

In addition, this study has demonstrated that it is perfectly possible to envisage characterising the genetic origins of cocoas by studying the theobromine/caffeine ration.

Keywords: cocoa, theobromine, caffeine, near-infrared spectrometry

097E
OR: FRENCH

DETERMINING THE ORIGIN OF THE ENZYMATIC COMPLEX INVOLVED IN THE FORMATION OF FREE FATTY ACIDS IN COCOA

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Abstract

The physico-chemical and rheological characteristics of cocoa butter depend greatly on its free fatty acid (FFA) content. A significant increase in cocoa butter acidity from Côte d'Ivoire has been noted periodically for several years. This results in financial loss for the producer and extra processing costs for the butter manufacturers.

Although this has never really been proved, lipases are suggested as causing FFAs to form. The aim of the study is to identify and characterise the enzymatic complex in question.

A lipase was revealed in de-fatted cocoa powder. It has optimum activity at two pH (5.2 and 7.4), implying the presence of two lipases or of an isoenzymatic system. It shows obvious preference for cocoa butter compared with other fats such as olive oil or soybean oil. It is expressed 3 to 4 times higher in cocoa powder than in the proteinaceous extract.

None of the factors such as genotype, pod ripening, pod-opening delays, length of fermentation and extended storing of cocoa (temperature-controlled tank - 27°C, HR 75%) produced samples with high FFA levels or significant lipasic activity in healthy cocoas.

Conversely, defective beans (black beans, mouldy beans) and broken bits show high FFA content, which increases during storage.

The comparison of the microflora content and particularly the mouldiness between healthy beans and defective and broken beans, before or during storage, shows that a relationship exists between the contamination level and FFA content.

The following microflora have been identified in order of abundance and frequency: *Absidia corymbifera*, *Rhizopus oryzae*, *Penicillium chrysogenum*, *Aspergillus flavus*, *A. tubingensis*, *A. tamaritii* and *Monilia sp*

FFA formation in cocoa butter is unlikely to come from an enzymatic autolysis, but (exclusively?) from a microbial source combined with other factors such as cocoa bean quality, their physical condition and how they are stored.

KEYWORDS: Cocoa, free fatty acids, technology, microflora.

**EVALUATION OF THE IMPACT OF MICRONUTRIENTS AND BACTERIA ON THE
FERMENTATION AND DRYING OF COCOA (*Theobroma cacao* L.)**

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Abstract

Fermentation is one of the most important processes in the improvement of the quality of cocoa (*Theobroma cacao* L.), where the physical, chemical and organoleptic qualities of the bean change. This quality is affected when the cocoa is not fermented or if inadequate methods of fermentation are used: this is reflected in use of a single market, low prices on the market and less access to more demanding markets. The investigation was carried out at the Mata Larga Experimental Station, San Francisco de Macorís, during the months of May and June 2002, with the objective of evaluating the impact of micronutrients, bacteria and environmental conditions on the fermentation and drying of cocoa. A totally random design was used, with 4 treatments: Sánchez cocoa, fermentation in boxes, fermentation in heaps, and fermentation in sacks and 5 repeats. The pH of the triturated cocoa beans rose as the process of fermentation advanced, for Sánchez cocoa from 4.89 to 5.49, boxes from 4.79 to 5.57, heaps from 4.74 to 5.41 and sacks from 4.91 to 5.31. The best temperature was achieved in fermentation in boxes with 46 °C, followed by fermentation in heaps with 44.2 °C and in sacks with 39.4 °C. The temperature of fermentation was inversely proportional to the environmental temperature. The most appropriate values of humidity for dry cocoa were supplied by fermentation in sacks with 7.65 %. Micronutrients - iron, manganese, copper and zinc - are not involved in the process of fermentation of cocoa, although iron is present in large quantities in all the treatments, except in heaps. The genera of bacteria encountered in the process of fermentation of cocoa were: *Proteus sp.*, *Mucos sp.*, *Bacillus sp.*, *Corynebacterium sp.*, *Candida sp.* and *Bacillus sp.*

EVALUATION AND COMPARISON OF THREE METHODS OF FERMENTATION OF COCOA
(*Theobroma cacao* L.)

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Abstract

Cocoa from the Dominican Republic is of poor quality, due to inadequate post-harvest management, the culture of no fermentation, and genetic aspects. 85% of the cocoa which is exported is not fermented or Sánchez type. The producers who ferment use wooden boxes, heaps and plastic bags. The latter method has not been duly evaluated. The objective of this work was to evaluate the methods of fermentation used by the producers, to determine which is the most efficient, and with them to help to improve the quality of fermented cocoa. The study was carried out at the Mata Large Experimental Station, San Francisco de Macorís. A completely random design was used with 4 treatments and 5 repeats. The treatments were wooden boxes, heaps, plastic bags and a control with no fermentation. For each treatment, 100 kg of hybrid cocoa were used. The fermentation process developed over 6 days with two periods of sampling at 48 and 96 hours. Physical analyses were carried out, such as pH, ammoniacal nitrogen, ashes, fat and protein. In relation to temperature, the maximum values were reached in the boxes and heaps, with 48 °C each, while those in the sacks were lower with 40 °C. In the cutting test, the highest values for completely brown, were recorded in the boxes and heaps 70.34 and 64.33%, while for partial brown, sacks and the heaps were lower: 28.07 and 27.53%. For purple coloured beans, Sánchez cocoa and the method in sacks reached higher values with 40.53 and 30.46%, likewise for the yields of 41.70 and 40.06 kg. In relation to flat and uneven beans, there were no differences in the treatments. In the chemical tests, the highest values of ammoniacal nitrogen were obtained in the boxes 431.7%, the lowest in the heaps. In relation to fats, ash and proteins there were no significant statistical differences. As for pH, boxes and heaps were statistically equal, while pH was more acid in sacks - 5.260. In conclusion, fermentation in boxes is the most appropriate, for good quality cocoa, followed by fermentation in heaps.

DEVELOPMENT OF NON-CONVENTIONAL THERMO-RESISTANCE CHOCOLATE FOR THE TROPICS

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Abstract

Chocolates are confectioneries consumed primarily for ectasy/enjoyment. Unfortunately most of the chocolate imported into the country are expensive and are out of the reach of the general populace. Apart from this, they also melt at room temperature (25-33°C) causing bloom formation in storage. This situation had led to low consumption of chocolate in the tropics.

This experiment had focused on the production of thermoresistant chocolate that are heat labile at ambient temperature. Studies were carried out in two stages. The first stage was the incorporation of corn starch at different percentage of 2.5, 5, 7.5 & 10% and the second stage was the use of gelatine at 2.5, 5, 7.5 & 10%.

At 10% inclusion of corn starch and 10% of gelatine, the two non-conventional chocolates melts at temperature between 40°C-50°C compared to that of conventional chocolate (25°C -33°C).

The production of these thermoresistant chocolates will allow the wide distribution and display of chocolate in the tropics and this will therefore increase the consumption of chocolate in the tropics.

The two non-conventional chocolate were compared with the conventional (control) sample using their sensory attributes. A 10-man panelistic approach was used with 9-point hedonic scale.

All the sample showed no significant difference at 5% probability level except for texture of the non-conventional chocolate that was stable at ambient temperature.

EFFECT OF HACCP ON THE MICROFLORA PROFILE OF CRIN MILK CHOCOLATE

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Abstract

The hazard analysis critical control point (HACCP) is a system directed to identify microbiological hazards existing in an industrial operation or process in order to identify critical control points (CCP) in which such hazards can be controlled and tests systems established to be able to monitor or supervise control effectiveness. This system was applied in the processing procedure of CRIN milk chocolate. Critical control points (CCP) were identified and microbiological examinations were carried out. The identified CCPs were: raw material, roasting, conching, tempering and storage. At each of the identified CCPs, Total viable count was taken on nutrient agar and fungal counts on potato dextrose agar. Biochemical Analysis were carried out to identify organism. The result indicated the presence of lactic acid bacteria in the raw/fermented cocoa bean. These organisms are responsible for the production of volatile characteristics of cocoa bean flavour. Mean counts of 90 cfu/g was found in the raw, unfermented beans, 77cfu/g for fermented beans, no count was obtained at conching stage. This is because of the high conching temperature 60-75 °C which may be unfavourable to the survival of the organism that are not thermophilic in nature. Minimum Yeast counts (10⁶ propagule/g) was prominent on samples stored under ambient condition. Counts of microorganisms increased with storage time in the refrigerator, then constant and later reduced. This erratic changes in the counts was necessitated as a result of unstable electric supply. Quality of chocolate products produced at CRIN was assured. This is the result of Good Manufacturing Practice (GMP) and Clean in Place (CIP) adopted during processing.

BIOCHEMICAL INDICES OF THE QUALITY OF BEANS OF THEOBROMA CACAO CULTIVARS .

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Abstract

A comparative study was conducted to develop simple and accurate methods for assessing and monitoring bean quality of some new cocoa varieties in the CRIG breeding programme. Unfermented and fermented dried cocoa beans were used for this work. The result indicate that the mean fermented nib pH for selected CRIG Inter-Amazon Hybrids and normal mixed hybrids was 5.34 ± 0.15 . Mean total acidity of these hybrids was 0.98 ± 0.15 mEq of acetic acid. Fermentation index (colour index for anthocyanins content) of the fermented beans of the hybrids was 1.044 ± 0.14 compared to 0.658-0.978 for the unfermented. The hybrids had $51.85 \pm 2.0\%$ to $55.61 \pm 2.4\%$ fat by weight and the protein content was $51.32 \pm 4.44 \mu\text{g/ml}$ (unfermented) and $46.37 \pm 4.43 \mu\text{g/ml}$ (fermented). Cut test on the fermented hybrids gave Grades 1 and 11 cocoa beans. Unfermented beans had higher carbohydrate content ($5.0 \pm 1.0 \text{ng/ml}$) than the fermented ($3.0 \pm 1.0 \text{ng.ml}$). SDS-PAGE, in the absence of protease inhibitors, revealed four bands of low molecular weight proteins of 29.1, 22.3, 16.0 and 12.6 kDa in all the hybrids.

These biochemical parameters conform to observed values of pH, low acidity and fat content generally associated with Ghanaian cocoa. A matrix, which could be used to fingerprint cocoa for quality has been constructed using the biochemical parameters determined in this study.

**THE EFFECT OF ROASTING TEMPERATURES ON THE FLAVOUR QUALITY OF CHOCOLATE
COUVERTURE MADE FROM MALAYSIAN COCOA BEANS**

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Abstract

The choice of sources of cocoa beans for chocolate production is mainly related to the concern of the industry about the cocoa flavour quality. Roasting process that magnifies the complex interaction between the flavour precursors in the beans is known to have an effect in the improvement of the flavour quality of cocoa. The objective of this study is to determine the flavour profile of cocoa liquor and the chocolate couvertures made from Malaysian cocoa beans subjected to different roasting temperature treatments. Cocoa beans of SMCIIA from a cocoa plantation in Raub, Pahang, peninsular Malaysia were selected for use in this study. The beans were processed into nibs and roasted at three different temperatures which are considered as low roasting (125 °C), medium roasting (135 °C) and high roasting (145 °C), using a pilot plant scale roasting facility. The roasted cocoa beans were ground into cocoa liquor. The flavour quality of cocoa liquor was measured using descriptive analyses of 0–10 scale by 10 expert panels for 3 replications. The five main flavour attributes namely the cocoa flavour, bitterness, sourness, astringency and mouthfeel/thickness were tested. The cocoa liquor was further processed into milk and dark chocolate couvertures. The sensory evaluation of these couvertures, using descriptive analyses, was determined from the means of 60 judgments by the expert panels. The flavour quality in terms of cocoa/chocolate flavour, sweetness, milky taste, bitterness/astringency, sourness and panels' preference were measured for the couvertures. The sensory evaluation result of cocoa liquor shows that there is no significant difference in terms of cocoa flavour, bitterness, sourness and mouthfeel between cocoa liquor roasted at different temperatures. However, difference was observed for astringency where cocoa roasted at 135 °C and 145°C are significantly more astringent than cocoa roasted at 125 °C. However it can be seen to influence the flavour profile of dark couverture more than it does to milk couverture where it is observed that at higher roasting temperatures (135 °C and 145°C), the dark couverture gave a higher bitter/astringent taste when compared to couverture made from cocoa nibs roasted at lower temperature (125 °C). In the case of milk couverture, it seems that the composition of other ingredients such as milk and sugar tend to influence the final flavour quality where the astringent taste has diminished. Other attributes tested, even though showed some effect from the roasting process, the differences were however statistically not significant.

**BY-PRODUCTS/NEW &
TRADITIONAL USES OF
COCOA**

FREEZING OF COCOA PULP IN COMMERCIAL CHAMBERS

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Abstract

The cocoa pulp produced in Bahia is commercialized as frozen product so as to supply the consuming market with a natural and healthy product. Therefore the pulp should be extracted soon after the pod breaking operation, then it should be packed in plastic bags or containers of variable size and frozen as quick as possible to minimize quality losses. Freezing and storage is usually carried out in industrial freezing chambers at temperatures in the range of -20 to -25° C. A study of the freezing process of fresh cocoa pulp was carried out in a commercial chamber maintained at -20° C. The product was stored in cylindrical plastic containers of 20 L and stacked in piles of three units in the chamber. Air and product temperatures were monitored using 24 AWG type K thermocouples connected to a data acquisition system. The ambient air temperature was measured at several locations inside the chamber and at locations at about 5 cm of the container walls. The pulp temperature was measured at the center position of the containers. Temperature profiles for cocoa pulp were analyzed and compared with profiles obtained for other fruit pulps. The temperature profiles obtained for pulp of cocoa and other fruits showed a similar behavior and were characterized by presenting a initial period of faster temperature decay, which lasted for about 20 hours, followed by a shorter period, about 10 hours, at which the product temperature remained nearly constant, and a final period of slower temperature decrease rate which ended in about 55 hours, when the product temperature approximated that of the cold air. The average time required for the temperature at the center of the containers to be lowered to the safe value of 0° C was around 12 hours. The temperature of -2° C was reached after an elapsed time of about 20 hours.

**CARCASS EVALUATION AND NUTRITIONAL QUALITIES OF AFRICAN GIANT LAND SNAILS
(*Archachatina marginata*) FED COCOA HUSK PULP AS A SOLE INGRIDIENT.**

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Abstract

The problem of protein malnutrition is real in most developing countries of the world. These countries are mostly located in the warm-humid tropics where the level of animal protein intake represents about one-tenth of the animal protein intake in the advanced countries. Snail meat constitutes an accepted source of animal protein, containing 18% crude protein, which makes it complete favourably with the expensive conventional animal protein sources such as beef with 18% crude protein, mutton, 18% and poultry 20%. Snails are herbivorous by nature and could feed on various farm wastes. One of such farm wastes is cocoa husk pulp (CHP). This premier study assesses the carcass value and nutritional qualities of Africa giant land snails fed cocoa husk pulp.

A total of 144 snails with weight ranging from 20g-45g were used for this study. The experimental snails were grouped into 4 treatments and 3 replicates. Treatments include 100% PPF (control), 100% CTP, 100% KNT and 100% CHP. The experiment was designed to be completely randomized. Results of carcass analysis showed the dressing percentage of snails fed PPF as 38.46%, CTP 36.9%, KNT 27.16% and CHP 33.68%. Statistical analysis revealed significant differences ($P = 0,05$) in values such that $PPF > CTP > CHP > KNT$. Proximate analysis results indicated no significant difference ($P > \leq 0.05$) in crude protein values for meat samples. Result from sensory evaluation of the meat samples showed significant differences ($P \leq 0.05$) only in scores for flavour, tenderness and overall acceptability. Average scores for overall acceptability were 7.4 for PPF, 6.9 for CTP, 5.5 for KNT and 6.1 for CHP. Values can therefore be ranked as $PPF > CTP > CHP > KNT$. Although the cocoa-pulp had lower yield in terms of carcass than PPF and CTP, the study revealed no detrimental effect on nutrient composition of the meat samples.

SEPARATE INCLUSION OF TEMPEH AND COCOA POWDER IN BISCUIT PRODUCTION - A COMPARATIVE ANALYSIS

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Abstract

Different additives included in biscuit production have been known to have some desirable or undesirable processing effect on physical, nutritional and organoleptic qualities of the final product. This allusion necessitated the inclusion of 'Tempeh', a known protein rich, fermented soyabean powder and cocoa powder separately in biscuit formulation. Various wheat flour -tempeh mixes and wheat flour - cocoa powder mixes were prepared by varying the proportions of both the tempeh and cocoa powder in each mix of a total of 500g, the percentage inclusions were (a) 0% (b) 5% (c) 10%, (d) 25% and (e) 40% respectively. The different wheat flour - tempeh and cocoa powder mixes were evaluated for pH, titratable acidity (TTA) and water absorption capacity (WAC). The product so produced was analysed for biscuit strength, moisture content, protein, fat and carbohydrate. Sensory evaluation was carried out to assess the biscuit by 12 regular tasters of biscuit in terms of taste, colour, texture and overall acceptability. The results obtained in the study indicated an increase in the pH of wheat flour - tempeh as the inclusion of tempeh increases. Same trend was observed for cocoa powder mixed with wheat flour. Other parameters such as: titratable acidity (TTA), water absorption capacity (WAC), protein, moisture content follows the same increasing trend with increase in the addition of tempeh and cocoa powder. The sensory evaluation carried out indicated a very high preference for tempeh/cocoa free biscuit. A(0%) in terms of all the appraised sensory attributes. Samples containing (10%) cocoa powder had a very hard texture and very chocolatey flavour. For tempeh powders, the most acceptable limit to which it can be added without impairing its quality organoleptically was found to be at 10%, above which the taste becomes unacceptable. Same is applicable to cocoa powder inclusion. Above the 10% level of addition, the biscuit become too dark and unattractive. The two powders shared the same physicochemical properties in biscuit production. The addition of the two powders in biscuit formulation beyond the 10% level of unfavourable.

THE CHARACTERISTICS OF ACTIVATED CARBON FROM COCOA POD HUSKS

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Abstract

Cocoa pod husk, which is abundantly found as waste, is a good source of carbon for the production of activated carbon. The objectives of this study is to explore the possibility of producing and to understand the characteristics of activated carbon prepared from cocoa pod husk. In this study, the cocoa pod husks were used as the starting material. The pod husk samples were shredded to chips and then dried in an oven at 80 °C. The pod husk chips were ground, sieved and then simultaneously underwent carbonization and activation process in a flow of inert gas (N₂) and CO₂, respectively. The processed pod husks were heated at different levels of preset temperatures (500, 600 and 700°C), phosphoric acid (0, 20, 40%) and activation period (1, 2, 3 hours) using response surface methodology (RSM) experimental design. The results show that the pod husk samples treated with 40% phosphoric acid at 700°C exhibited a higher yield of activated carbon (53.9%) than other treatments. The characteristics of the activated carbon such as surface functional groups, iodine adsorption, pH, ash content and phenol adsorption were presented for discussion.

**IMPROVEMENT OF COCOA
CONSUMPTION THROUGH
OPTIMAL UTILISATION OF
THE COCOA BEANS**

CHALLENGES OF INCREASING COCOA CONSUMPTION

Lawrence K. OPEKE

Abstract

Cacao (*Theobroma cacao*), food of the gods, first used as food by the Aztecs and Mayans, native of the forests of the Upper Amazon, was introduced to Africa for cultivation by Spain. The primary objective of Spain for the introduction was to OBTAIN ON REGULAR BASIS CHEAP SUPPLIES OF CACAO BEANS from its colonies in Africa. Since the introduction, every effort has been made to adhere to that objective.

The main product of cacao is the beans which constituted about 48 percent of the pod-wet weight. The major by-products are the cacao pod husk (CPH), the bean testa and the mucilage. For over a century of cultivation of cacao in Africa, no serious effort was made to find economic usage for the by-products.

Current research efforts at CEPLAC, Cocoa Research Institute of Ghana (GRIG) and Cocoa Research Institute of Nigeria (CRIN) have shown that the by-products could be processed into livestock feeds, organic fertilizers, alcoholic and non-alcoholic beverages. CPH is a good source of Potash for soap making.

Consumption of cocoa has centred solely on the production of quality temperate-type chocolates. The temperate-type chocolates are thermo-unstable; this defect has limited its consumption in the cocoa bean producing tropical countries. No serious effort has been made to develop cacao based recipes which are thermo-stable and acceptable to the palates of the primary cacao producers. To increase cocoa consumption worldwide, all these omissions must be corrected. This will involve huge investments in:

1. Research and Development of:
 - (a) Thermo-stable novel cocoa based recipes which are acceptable to the palates of the target consumers.
 - (b) Design and fabrication of machinery for industrial production of the novel recipes.
2. Publicity and Aggressive Promotion of such novel recipes e.g.
 - (a) Supply of the recipes, at subsidized prices or FREE to
 - (i) educational institutions
 - (ii) Public establishments – hospitals, etc.
 - (iii) Merchantile houses for use in products promotions.
3. Follow-up Research to document effects of the novel recipes on consumers.
4. Increase publicity and promotion of cocoa products in countries which are currently consuming very little. E.g. Russia, China, etc.

Sustainable cocoa production will be enhanced and remunerative producer prices achieved when fruitful and appropriate investments are made to ensure consumption worldwide.

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**TRANSFER OF
TECHNOLOGIES/OTHERS
ASPECTS OF RESEARCH**

Mathematical model to simulate the cocoa production recovery in Bahia, Brazil

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Abstract

The cocoa production in Bahia, Brazil decreased from an average of 350.000 T (1989-1990) to 100.000 (1999-2001) after the introduction and spread of the witches' broom disease. In 1996 a program was initiated to recover the cocoa production through the distribution, by CEPLAC, of genetically improved material with high productivity capacity and highly resistant/tolerant this disease. With the participation of the State government and other organizations started the mass production of seedlings and scion-wood for grafting. In the last three years more than 60.000 ha were recovered through grafting of the new material on the old cocoa trees. At the same time, many producers also started to increase the plant density from an average of 650 plant/ha to 1000-1100 plant/ha. It is expected a field productivity of more than 1000 kg/ha in the renewed areas. Many farmers leave the old trees in production for 1-3 years before pruning, while the grafted plant grow, avoiding this way a drastic drop in the farm production, after the grafting. The cocoa production of Bahia, in the next 10 years should depend essentially on the increase in recovered area. The plan is to recover 300.000 ha in the next few years, but the financial resources expected from the federal government to attain this goal are more or less erratically released. The development of a mathematical model to simulate the individual farm, the regional production and the cost/benefit relations of the recovery process as a function of different recover strategies could help the decision making process of the farmers. Also the model could support strategies for needed adjustments in the overall program.

With these objectives a deterministic compartmental model to forecast the Bahia cocoa production was developed. The model input are: grafting rate and starting year, seedling planting rate and starting year, total farm/region area, target area, average productivity of old areas, average expected productivity of the recovered areas, plant density in the old areas and target plant density. Optionally, for the cost/benefit analysis it is necessary to input the expected cocoa prices and the recovery/production costs. The outputs of the model are: yearly expected production (in each category area), yearly costs, expected yearly income and cost/benefit index.

In the better scenario preliminary simulation shows that if 300.000 ha are recovered at the end of 2004 then the Bahia production could increase to 300.000 T up to the year 2011. However other scenarios shows that production can remain below 200.000 T for the next 10 years.

Absorption of heavy metals on the pod husk of *Theobroma cacao*

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Abstract

Due to increasing industrialization especially in Nigeria and other countries, most water bodies have been polluted arising from the textile, mining, plastic, dyes and pigment industries. This wide spread contamination has caused great concern about its potential hazards on plants, human and aquatic lives. Removal of heavy metals ions from waste water still remains an important problem.

Conventional method of removing heavy metal ions employs the use of chemicals and other synthetic resins which are often too high in price and thus uneconomical. In the continuous search for alternate systems for waste water/effluent treatment, the pod husk of *Theobroma cacao*, an agricultural waste, was investigated for its sorptive behaviour for heavy metals such as cadmium, lead and zinc ions. In this study, the binding capacity of aqueous solutions of lead, cadmium and zinc ions on cocoa pod husk was investigated by equilibrium sorption studies at $30^{\circ}\text{C}\pm 1$. The maximum metal ion binding capacity was determined from the sorption isotherm for the metal ions and were found to be 0.03 mmol g^{-1} , $0.039 \text{ mmol g}^{-1}$ and $0.016 \text{ mmol g}^{-1}$ for pb (II), cd (II) and zn (II) ions respectively. It was also found that the rate of sorption followed the particle diffusion controlled process for pb (II) ions and the particle diffusion control rate coefficient was determined to be of the order $6.4 \times 10^{-3} \text{ mm}^{-1}$. There is therefore an indication that cocoa pod husk can be used to scavenge waters of effluent containing these heavy metal ions.

International donors and sustainable cocoa economy

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Abstract

It is a fact that many of the world poorest people depend on one or more of the agricultural commodities. These commodities include cocoa, rubber, palm oil etc. for their livelihood. Many least developed countries derive most of their export earnings from them as well.

With time, the State owned structures and methods of commodity administration (production, marketing, processing etc.) did not meet the yearnings of the countries production and their economy.

The International Communities came up with different antidotes including globalization of production and liberalization of trade. These new reforms had their toll on the commodities, the practitioners i.e. exporters and importers alike as many of the countries were ill prepared.

There had since been various efforts to improve on the situation of the entire industry by notable International/Multi-national bodies such as Food and Agricultural Organization (FAO), International Cocoa Organization (ICCO), Sustainable Tree Crop Project (STCP), Common Fund for Commodities (CFC) and Cocoa Producers' Alliance (CPA) amongst others who had raised fund, materials and men in attempt to redeem the worsening situations.

This paper attempts to look at the objectives and programs of some of these bodies, their successes and failures and had suggested, some way forward from an observers wiew point.

**SPECULATIVE STRATEGIES OF THE HEDGE FUND AND THE COMMODITIES FUND IN THE
FUTURES CACAO MARKET**

Mario M. Amin

Abstract

Prospects about demand and offer of commodities play an important role in price definition. Social, political and economic situation in the various cocoa-producing countries also exerts an indirect influence. There are, however, some daily activities in the New York market, which strongly affect the determination of international prices for commodities.

The cacao international market is the perfect example of a market whose main characteristic is a speculative process, carried out by the Hedge Funds and by the Commodities Fund; its participation in the futures market, geared to short term gains, will significantly influence the development of international prices.

As a consequence of the speculative process, inherent to the functioning of the futures market itself, cacao is considered, amongst all the commodities transacted, as the item with a higher index of instability in the formation of international prices.

By making use of the information made available by the Commodities Futures Trading Commission – CFTC, as well as by the reports published by the Coffee, Sugar and Cocoa Exchange Market – CSCE, about the daily trade in the cacao futures New York market, this research project aims to identify the participation, position and level of influence of the Hedge Fund and the Commodities Fund, in the formation of the international cacao prices, for the period 1992 to 2001.

Weekly statistics presented at the Commitments of Traders in Futures, at the CFTC, allow for the identification of the positions assumed by the speculators (non-commercial and small traders) and by the hedgers (commercial traders), for a certain period.

As soon as long and short positions are identified, the key point of this essay will be to estimate net long position and net short position values both for speculators and hedgers. This process estimated three series estimated: one for the non-commercial (representing the activities of the Hedge Fund and of the Commodities Fund), another for the small traders, and the last one, for the commercial traders or hedgers. The separation of the various positions within the futures cacao market indicates, very rapidly, the weekly position taken by non-commercial traders, commercial traders and small traders.

Analysis results of cacao international prices have demonstrated that most of the oscillations occurring in prices, for the period 1992 to 2001, are a consequence of a significative activity of the Hedge Funds and the Commodities Fund in the New York futures market. Prices varied between US\$ 700 and US\$ 1.800 per ton.

**COPING WITH AGRICULTURAL MARKET REFORMS IN THE '90S:
Winners and Losers among Ghanaian Farmers.**

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Abstract

The Ghanaian government has been put under strong pressure by the international organisations to complete the liberalisation of the cocoa sector. These institutions visualise the full liberalisation of the market as the optimal policy to enable farmers to directly manage production and marketing activities. The repercussions that this step would have on farmers have to be put in the context of both the institutional framework of the country, and of the disadvantaged position of farmers who would become potential vulnerable "losers" in the liberalisation process. Is it really the case that a full liberalisation of the marketing system will create a win-win scenario from which all cocoa farmers in Ghana will benefit? The equity-impact of a full liberalisation is being questioned.

This study analyses the impact of agricultural reforms in Ghana mainly from an empirical angle by exploring the following research hypotheses: 1) Have cocoa farmers gained better market access of their produce as a result of liberalisation? 2) If so, what factors determined a greater level of production? 3) Are there observable discriminations across different group of farmers responding to the new incentives created by the partial marketing reforms?

These questions are addressed using a unique dataset of 500 farmers collected by the author in 2002 and purposely designed to identify *winners* and *losers* from cocoa marketing reforms in Ghana. These data are partly linked to the Ghana Living Standard Survey (GLSS) as the author revisited 180 farmers from the 1998/99 round of the GLSS. The purpose of this exercise was three-fold. Firstly, to follow up the changes occurred over a period of four years for a set of households surveyed in the last Ghana Living Standards survey in 1998/1999. Secondly, to research the marketing conditions of cocoa farmers in the most important areas of Ghana's cocoa production. Finally, to identify in some greater detail the current labour inputs going into cocoa production. The supply of labour on cocoa farms with its differentiation in its family and hired component is considered one of the major constraints in the farmers' ability to expand their level of cocoa production.

Two major areas emerged as presently hampering a sound and efficient growth of the sector:

i) *Inputs use*: Traditionally cocoa has been grown in primary forest zones with negligible use of fertilisers and insecticides, causing land degradation and declining soil fertility. In addition the wide range of pests and diseases have caused major output losses. In this respect only moderate signs of improved crop management techniques were found.

ii) *Farm and off-farm labour allocation*: A combination of imperfections in the credit market and of better non-farm earnings for rural household has contributed to the inefficiency of resource allocation among Ghanaian farm household labour. Better investment incentives are needed to attract a new generation of farmers and signal better farm labour allocation opportunities.

Participatory Cacao Clones Evaluation for Resistance to Witches' Broom in Agrossystems from Bahia, Brazil

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Abstract

Participatory approaches of research have been more efficient in transferring technologies as well as in reducing costs to the research institutes. This is particularly important when a large number of varieties has to be tested, to increase the chances of finding disease resistant ones. In 1999, the Cacao Research Center (CEPEC/CEPLAC) launched a large program of testing clonal varieties with farmers' participation. Now, around 150 clones are under evaluation in different agrossystems of the main cocoa region in Brazil. In this paper it will be reported the preliminary results of six of those clonal tests installed in six farms: Massaranduba farm (Itajuípe, BA), Nossa Senhora da Conceição farm (Ubatã, BA), Santa Úrsula farm (Camacã, BA), Novo Horizonte (Belmonte, BA), São Francisco farm (Itabuna, BA) and Corcovado farm (Ipiáu, BA). In all farms, the same 30 clones (2 controls for resistance to witches' broom + 2 controls for susceptibility + 26 new clones) were used, under a complete randomized design, with single-tree plots and 20 replicates. Grafting on basal chupons of adult plants was used to propagate the clones. Farmers supervised by CEPEC's personnel made all activities, since the installation of the experiments to the evaluations. One year after grafting, it was started the measurements of the number of vegetative brooms in the canopy and the number of pods in the tree. Overall the 26 clones being tested test presented statistically less brooms per tree than the susceptible controls (SIC-23, SIAL-169) and similar number of brooms as the resistant controls (TSH-1188, Sca-6). Although not statistically different of the other resistant clones, VB-1145 and PH-16, both selected on farms, presented the smallest number of brooms across environments. Genotype-by-environment interactions was highly significant for both resistance and production. The clonal heritability ranged from 0.26 to 0.62 (average $h^2 = 0.44$) for number of brooms per tree and from 0.09 to 0.46 (average $h^2 = 0.22$) for number of pods. A positive relationship was observed between heritability for number of pods and for number of brooms, indicating that environments which allow good discrimination for production is also good discriminator for resistance to witches' broom.

**On Farm Selection for Witches' Broom Resistance in Bahia Brazil –
A Historical Retrospective**

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Abstract

Witches' broom, caused by *Crinipellis perniciosa*, was introduced in Bahia in 1989, causing severe economical, social and ecological losses. Since the beginning, resistance was chosen as one of the key points of the integrated pest management adopted by the Cacao Research Center (CEPEC). Unfortunately, by the time WB was arrived in Bahia, around 600 thousands hectares were planted as a continuous monoculture, mostly with susceptible varieties. Fortunately, among the varieties deployed to farms in the past, some resistant parents were included and chances of finding resistant segregant plants on a large screening program was expected. Today, 10 years after the beginning of the program, more than one thousand trees were pre-selected by CEPEC and more than five thousands pre-selected by farmers. Among these, highly productive and resistant clones have been found. In this paper, we report the strategies adopted, the results and some drawbacks on that program.

Sustainability of Cocoa Production within the Context of the Generation, Transfer and Adoption of Improved Cocoa Technology in Nigeria.

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Abstract

One of the problems associated with low cocoa output and sustainability of cocoa agriculture in Nigeria is low level of adoption of improved cocoa technologies. Linkages between researchers, extension staff and farmers, if any at all are usually supply driven rather than based on actual needs of farmers. Results from research-extension-farmer linkages in Nigeria show that the influence and participation of farmers in the generation of improved cocoa technologies have been minimal. Transfer of technologies have been “top-down” via extension staff of the State Ministries of Agriculture which did not provide the opportunity for feedback of information from farmers to researchers.

It is concluded that program designed to facilitate the adoption of improved cocoa technologies at the farm level in Nigeria must take cognisance of farmers real need. Specific strategies for effective research extension farmer linkages are suggested.

Farmer techniques for cocoa planting and replanting in Côte d'Ivoire

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Abstract

Since its introduction into Côte d'Ivoire at the end of the 19th century, cocoa production has increased in the main through planting areas being extended through forest clearance. These pioneer front dynamics have made the country the leading world producer for the last twenty-five years. But today, Ivorian cocoa culture viability is compromised by the depletion of forest reserves and therefore of the "forest rent" provided by clearing and burning them.

A research-action project has been initiated by the National Centre for Agronomic Research (CNRA) and the Centre for the Development of International Cooperation in Agronomic Research (CIRAD) to attempt to find answers to this pressure. One of the project's avenues of research involves knowledge of farmer techniques for replanting on land not previously covered by forest.

Farmer techniques for cocoa planting on forest land and replanting on land previously given over to different crops have been surveyed, therefore, involving 650 farmers questioned over 5 months in seven representative sectors of the main cocoa producing areas in the country. Following a suggested definition for the terms "planting" and "replanting", existing farmer techniques are described in agro-economic detail in the first instance. The decision rules applied by producers on the choice of land, planting material, propagation and installation technique methods are analysed based on these elements. Lastly, technical planting itineraries are compared with those used for replanting to demonstrate the adaptations by producers when planting cocoa trees on land not previously covered by forest. The most suitable of these innovative techniques will be adopted and validated at a research station and in a farmer environment. This is a knowledge-sharing approach adopted between researchers, farmers and developers.

Keywords: Côte d'Ivoire, cocoa, planting, replanting, farmer innovations, technical itineraries.

**GENETIC DIVERSITY OF CUPUASSU (*THEOBROMA GRANDIFLORUM*) POPULATIONS USING
MICROSATELLITE MARKERS**

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Abstract

The objective of this work was to characterize the genetic structure of seven populations of cupuassu (*Theobroma grandiflorum*), an important tropical fruit tree of the Brazilian Amazonia using microsatellite markers to support the breeding and genetic conservation program. Three natural populations collected at the putative center of maximum diversity; three populations from the germplasm collection; and another one collected from commercial plantings were analyzed using 21 microsatellite loci, disclosing an average of 74.1 alleles per population. A large genetic diversity of the species was detected based on percentage of polymorphic loci ($\bar{P} = 0,839$), average number of alleles per locus ($\bar{A} = 3.5$ alleles) and expected heterozygosity ($\bar{H}_e = 0,428$). The mean allelic fixation index ($\bar{f} = 0,161$) within natural populations detected no inbreeding, but it was significant in populations of the germplasm collection. The divergence among natural populations ($\bar{\theta}_p = 0.307$) may represent a preliminary process of differentiation. This divergence was more pronounced between the natural populations from Tucuruí and Nova Ipixuna, Para state, corroborating the hypothesis that this region is the center of maximum diversity of *T. grandiflorum*. The structure of the wild populations suggests for *in situ* conservation, the definition of a reasonable number of genetic reserves, enough to maintain rare alleles at medium to long term, as well as, for collections, that sampling of various populations should be emphasized. A low genetic divergence was observed among the populations kept at the germplasm collection, and that most of the genetic diversity was within populations. The characterization of these accessions allowed the first insight for the future establishment of a core collection. The high genetic diversity observed in commercial plantings indicate the possibility to use them as alternative source of genes and genotypes for the breeding program of cupuassu.

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Farmers Organization and Sustainability of Cocoa Agriculture in West African Sub-region.

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Abstract

In the wake of the liberalization regime across the West African Cocoa Community, the vast majority of small holder producers remain in the state of flux. The once structured cooperatives gave way to a highly fragmented cocoa chain that constitutes severe implication for production capacity, quality pricing and income generation potential. Whereas, the severity of the impact continuous varies from country to country, the foundation to re-inventing the robust cooperative frame work that ensure discipline and a coherent trade chains are still very much in place. Without strong farmer driven organization, it is difficult to see how the majority of Cocoa farmers in West Africa will have sufficient leverage as a credible and effective partner for sustainability not only in trade relations that the overall future of cocoa agriculture.

It is however concluded that farmers owned methodology present a paradigm that is worth exploring within the private sector context. We are of the view that a robust coalition of private, public NGOs and other relevant stakeholders integrating various farmer-friendly perspectives in a holistic manner to strengthen farmer organizations to meet shared values and long term sustainability of the cocoa sector is indeed the way forward in the post market- led regime

Selection for disease and pest resistance in cocoa in Sulawesi, Indonesia

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Abstract

The aim of this project, sponsored by the Australian Centre for International Agricultural Research (ACIAR), is to demonstrate that it is possible to select for resistance to a number of disease and pest problems confronting cocoa production in Sulawesi using farm-based and farmer-assisted selection from among the great genetic diversity of cocoa evident on the farms in the region. The model for this project has been the selection for resistance to vascular-streak dieback (VSD) of cocoa that occurred in Papua New Guinea during the initial destructive epidemic of this disease in the 1960s and 1970s - in this case, extremely susceptible genotypes of cocoa among the great variability of cocoa types in Papua New Guinea were killed and more resistant types survived and were propagated, enabling the survival of a productive industry in the region. It is evident that such a selection has probably occurred also in Sulawesi, where VSD is common but not destructive.

The expansion of cocoa production in Sulawesi since the 1980s has involved the spontaneous planting of a productive crop by small holders with access to land, to the extent that about 400,000 smallholders have made Sulawesi now the third largest exporter of cocoa in the world. However, after an initial period of trouble-free production, the crop is now seriously threatened by two main problems - pod rot (PPR) and canker caused by *Phytophthora palmivora* and cocoa pod borer (CPB) (*Conopomorpha cramerella*). There is great genetic diversity of cocoa in Sulawesi, based upon the planting of old Trinitario types from Java, Amelonado types and hybrids including Upper Amazon types introduced from Malaysia and elsewhere in Indonesia, and the hybridisation that has occurred within and between these types in a crop which is still largely outbreeding and largely propagated by seed in this region. Among this diversity, types with apparent resistance to PPR and CPB and high yield are occasionally observed on the farms. These genotypes, selected initially by farmers and extension staff, have been collected as budwood and sidegrafted onto mature trees in a replicated trial at two sites in Sulawesi, where, along with a range of well known clones, they will be exposed to natural infection by PPR, CPB and VSD and assessed for their performance. They will also be assessed for cocoa quality.

The essence of this project, that started in 2001, is field selection in the face of natural epidemics from among the great genetic diversity evident over many thousands of hectares of this crop, representing a vast natural experiment with the people most closely in contact with the experimental material (the farmers) being pivotal in the observation and selection process. It is a lesson in the benefits of genetic diversity in a world where the opportunity for such farm-based crop improvement is being increasingly closed down by inbreeding, clonal propagation and monopolisation of genetic material.

**Diversification and sustainability of cocoa farms
The case of Côte d'Ivoire**

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Abstract

Surveys conducted in Côte d'Ivoire between 1999 and 2002 under two successive major research projects covered almost 1,000 farms in the country's major cocoa-producing regions. The most significant results tally. Independently from crop diversification, the first determining factor for diversification of cocoa farms is neither price nor the market, but the key problem of cocoa sustainability - the difficulties of cocoa replanting. Farmers actively seek new sources of revenue when the cocoa trees age and they face or re-face difficulties in replanting. Investments should therefore be made in information, access to new planting material and new plantations. Some crops, rubber for example, involve a relatively sophisticated planting material, clonal material and a wait of 5 to 6 years before it starts producing. The "information/capital" binomial is therefore the second major determining factor in diversification. Farmers do not have available a credit market and information to enable them to make the most of their production systems individually. Producer and sector organisations are still in their infancy. Indeed, in many cases, particularly those studied under these research projects, the "information/capital" binomial is provided by a development project. In Côte d'Ivoire, the rubber and oil palm plantations and the fishing sector, all of which require both information and capital inputs, have been developed historically through development plans and public funds. Once this type of crop or activity is firmly established, the farmers themselves are frequently sufficiently dynamic to boost diversification without a project or outside capital. Nevertheless the rate is far lower than if the country received some financing. When less capital is required for diversification, cashew, for example, with its easily accessible seed, the crop can develop spontaneously, but a certain minimum of information was necessary, firstly provided by the forestry projects of the 50s and 60s. Raising small ruminants is another possibility which many farmers wish to develop, but find themselves faced with technical (disease) and social (theft) problems. They need external aid. This does not involve reproducing full-scale projects as for the first oil palm or rubber programmes, but to draw lessons from them to encourage diversification at reduced cost. This cocoa diversification offers two advantages. It expands the country's agricultural and economic base and in return consolidates the chances of cocoa replanting. Diversification is a key factor in cocoa sustainability.

**THE STATUS OF COCOA IN THE AGRARIAN SYSTEM OF SOME RURAL COMMUNITIES
SURROUNDING THE KAKUM FOREST RESERVE, GHANA**

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Abstract

The status of cocoa farming in four rural communities around the Kakum Forest Reserve in the Central region of Ghana was studied in a survey carried out in April 2001 to provide background socio-economic data to guide possible interventions. Four groups of twenty farmers within each community were interviewed using Participatory Rural Appraisal (PRA) approach. Further focused interviews were carried out on four farms in each community. The interviews focused on issues such as social structures of the communities, family and livelihood systems, constraints, aspirations and perceptions to intervention strategies. The communities are inhabited by indigenous and migrant smallholder farmers who cultivate cocoa, oil palm, citrus, maize, cocoyam, yams, plantain and vegetables. Cocoa farm size ranged from 0.4 to 4ha and aged between 3 and 50 years. Average cocoa yields were 570 kg/ha. Main constraints included perceived high cost of inputs especially agro-chemicals, absence of land for new plantings and the dearth of technological information. The potential for higher productivity was evident as the population is relatively young and has high aspirations. The perceptions of the farmers with respect to intervention strategies, their relationship with the forest reserve and other details are discussed. It is concluded that given adequate extension support, farmers in these communities can raise their productivity and live in harmony with the forest reserve.

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**AN EVALUATION OF THE DYNAMICS OF COCOA MANAGEMENT TECHNOLOGIES AND THEIR
IMPLICATIONS FOR SUSTAINABLE PRODUCTION IN GHANA.**

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Abstract

Ghanaian cocoa farmers have shown growing interest in applying various levels of recommended improved cocoa production technologies to increase output and incomes from their farms in recent times. Five levels of cocoa production technologies are described. These are subjected to economic analysis, and compared with the demonstrated potentials of a package of cocoa production technologies developed at the Cocoa Research Institute of Ghana (CRIG). The analysis indicates that given 2002/2003 cocoa inputs and producer prices, the CRIG-developed technologies are most profitable from both the static (average year), and dynamic (over time) points of view, investment cost are, however, high and are likely to continue to rise as most of the key inputs are imported. Nevertheless, opportunities exists for farmers to increase and sustain cocoa production indefinitely by organizing themselves into viable farmer-based associations to enhance access to credit and to enable them to organize their production and marketing activities at reduced cost. Future research directed at finding substitute technologies with lower per unit production costs will also be an added advantage.

Note 1: The title has been modified to reflect suggestions by the Editorial Board.

Note 2: The information as contained in the paper has not been communicated to the GSA. It has existed as a technical report since 1992 when it was first presented at a CRIG Seminar at the request of the Executive Director and the Research Committee. People who know of its existence have been calling for its publication and at the recent STCP workshop in Accra I was called upon by more international researchers who came to CRIG for copies to have it published called me upon. The paper has been modified to reflect current trends in sustainable cocoa production.

**Strengthening the smallholder cocoa sector in Sulawesi through
PRIMA Cocoa (Pest Reduction and Integrated Management for Cocoa)**

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Abstract

The cocoa sector is the largest agricultural activity on Sulawesi involving over 300,000 smallholders and contributing strongly to Indonesian export earnings. Over the last two years the smallholders have experienced a dramatic decline in saleable cocoa with losses now averaging 40% due to damage caused mainly by the cocoa pod borer (CPB). Incidence of this insect pest is on the increase, its rapid progression posing a serious threat to the sustainability of the cocoa industry on Sulawesi.

Presently the farmers on Sulawesi are battling with the cocoa pod borer and other pest and disease issues, with insufficient knowledge about the problem they are dealing with. Programmes like the USDA-funded SUCCESS initiative are trying to train farmers to deal with the pest.

Masterfoods and the worldwide chocolate industry are committed to promoting sustainable cocoa production systems. With this in mind PT Effem together with its European sister unit Masterfoods Europe, Veghel, approached Senter International, (part of the Netherlands government international development agency). A project was proposed which would introduce on a pilot scale an integrated management system to control CPB while also raising bean quality and production by improved cultural practices and post-harvest treatment. A number of promising biocontrol methods would be further tested and developed in cooperation with the University of Hassanuddin, Makassar and a local entrepreneur to see if they can have wide-scale application.

The total pipeline approach will further include cocoa collectors in the pilot area, who will be trained with regard to proper drying and grading of beans purchased by PT Effem for processing. The proposed improvements will substantially increase the income of smallholders and benefit the environment through the promotion of sustainable cultural and biological control methods. Given the importance of the cocoa sector, its relevance to development on Sulawesi is potentially huge and it is intended that all stakeholders (smallholders, cocoa collectors, cocoa processors) will greatly benefit.

“PRIMA Cacao” The integrated management and chain approach to be piloted in this project will be known and promoted as “**PRIMA Cocoa**” (Pest Reduction and Integrated Management for Cocoa).

The role of cocoa agroforest management in view of poverty alleviation in rural forest area of southern Cameroon

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Abstract

In southern Cameroon, the forest has supplied timber, fuelwood, Non Timber Forest Products (NTFP like food, medicine, etc...), animal resources (meat and fish) and land for food and cash crops such as cocoa, to the population on a regular basis. The practice of cultivating cocoa in the forest, for several decades, has contributed not only to shape a new forest landscape in the area, but also to sustain the rural economy (by being responsible for meeting food, health needs, etc. ...) of the villages.

The poverty period, Cameroon went through in the middle of the 80's, was fuelled in the cocoa agroforest sector by a drop in the price of cocoa. This occurred when food and health needs required more money than before following the liberalisation of many sectors (economy, health, agriculture, etc...). This thus led to an increased dependence on natural resources for survival. In this context, local dynamics forced people to (i) create farmer associations dealing with the management and commercialisation of outputs from the cocoa agroforest and to (ii)- search for alternatives by increasing food crops and/or diversifying forest resources in plantations. This makes these systems open for (a) providing the same services as the forest in meeting farmers' needs, but mostly (b) for conserving biodiversity thus in keeping with a clearly expressed will of the international community i.e. that of better conserving forest resources while using these resources as rationally as possible to meet the needs of local people.

This paper highlights (i) the place of cocoa agroforest in the mosaic of various land use modes, (ii) the influence of the international context on the management of cocoa agroforest, (iii) the dependence on cocoa agroforest within the context of rural poverty, (iv) the sustainability of cocoa agroforestry system within the perspective of poverty alleviation and (v) concludes by giving some recommendations for the better utilisation of cocoa agroforest as one of the driving forces in poverty alleviation in forest areas.

Key Words: Southern Cameroon, rural area, cocoa agroforest, poverty alleviation, sustainability.

The Sustainable Tree Crop Program – Realizing a new development paradigm

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Abstract

The **Sustainable Tree Crops Program (STCP)** constitutes a coordinated and innovative effort made by farmers and producer organizations, industry and trade, national governments, research institutes, the public sector, policymakers, donors and development agencies to facilitate the improvement of smallholder agricultural systems based on tree crops in West and Central Africa. The key constraints in tree crop production have been identified and include: inadequate production technologies and practices, unresponsive and expensive input supply systems, insufficient organisational and management skills amongst farmer groups, inefficient marketing channels, limited access to information and finances, inconsistent quality standards, low farmgate prices, and an inadequate incentive environment.

Collectively, the above-mentioned coalition of partners, which typically bring different perspectives to the table, have shaped consensus around four common interests and concerns. They include: 1) promoting the production and marketing of quality tree crop products, 2) improving market access and income for small-scale producers, 3) creating systems that are environmentally friendly, socially responsible, and economically sustainable, and 4) developing an integrated Action Plan initially targeting cocoa, cashew nuts, and associated tree crops, in Cameroon, Cote d'Ivoire, Ghana, Guinea and Nigeria.

The goal of STCP is to improve the economic and social well-being of smallholders and the environmental sustainability of tree crop farms of West and Central Africa.

Following the Regional Workshop held in Accra in November 2002, the STCP Action Plan moved into the implementation of its 3-year pilot phase. The members of the Sustainable Tree Crop Development Alliance ensured their support for this Action Plan. The Action Plan includes pilot projects in each of the member countries focusing on developing the technical, financial and institutional capacity of farmer organizations, and regional projects that provide technical support to the pilots in addressing issues on trade and information systems, technology dissemination, and participatory research. At the same time, a new component on child labour was added to the STCP fabric by developing new alliances and responding to a set of studies on the use of child labour in tree crop systems. Existing linkages with complementary programs are being formalized (e.g. with SOCODEVI, CABI Biosciences, USAID Guinea) and additional ones are being pursued (e.g. Creative Associates, Winrock International).

A results framework with five strategic areas has been adopted: 1) strengthening farmer and community-focused organizations, 2) technology dissemination and research, 3) policy change and implementation, 4) market system and information system development and, and 5) labor and social systems. The general approach being taken within each strategic area is to build on the existing efforts and activities of relevant stakeholder groups, to add value to them, and to co-ordinate future collaborations.

If successful, the outcome of these efforts will be a more sustainable global economy for the focal tree crop systems, characterized by: increased rural incomes, reduced risk and greater stability in the supply of quality products to end users, increased demand for and use of tree crop products, better working conditions on farms, and an improved status of environmental resources for current and future generations of West and Central Africans to pursue their interests. Please see www.treecrops.org for more information.