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GRO-Cocoa

Global Research On Cocoa - *working with and for farmers*

Research for Farmers

In this issue, we begin by looking at two projects dealing with constraints to cocoa production in Sulawesi, Indonesia's main cocoa-producing area. The first aims to improve quality and disease and insect pest resistance of cocoa through screening and selecting from local cocoa plantings, which have great genetic diversity and are adapted to local conditions. It will enhance capabilities within the country in selection techniques and diagnostic skills and improve management practices. The second project is drawing together current local research and management capacity in integrated management of cocoa pod borer (*Conopomorpha cramerella*) to develop farmer-friendly management options. It takes a commodity chain approach to cocoa production, working with farmers and collectors to improve production and quality.

Molecular research might seem far removed from cocoa farmers' fields, but a USDA-funded initiative to combine the Reading and Tropgene cocoa databases underlines just how much cocoa breeding has to gain from it. We outline what the existing databases have to offer and hopes for the new database. The increased knowledge of the cocoa genome that novel molecular methods generate may allow cocoa breeding research to be better targeted for delivering improved cocoa varieties to beleaguered cocoa farmers. Still on frontier research, we include a report from the 2nd Endophyte Working Group Meeting and look at the potential of *Trichoderma*. And now for something different: you may never worry how you could measure the surface of a cocoa pod, but it is a serious concern for spraying technologists and we include a short article on that.

From Ghana, an article on pink disease (*Corticium salmonicolor*) is intended to be the first in an occasional series on lesser-known constraints to cocoa production. We also report on a Cameroon workshop that developed a pilot curriculum for Farmer Field Schools in cocoa. Finally we update readers on the website of the International Cocoa Research Conference in Ghana this October, which we will report on in the next issue.

Improving Cocoa in Indonesia

A decade or so ago Indonesia was a relatively small producer of cocoa, the basic ingredient of chocolate and also an important component of cosmetics. However, at the same time that the introduction of witches' broom disease (caused by the fungus *Crinipellis perniciosa*) into the main cocoa-growing area of Brazil decimated that country's industry, transmigration from densely populated areas of Indonesia to Southeastern Sulawesi opened vast areas of rainforest to cocoa production. Haji Selang, a cocoa grower in Ladongi, told us that he came to Southeast Sulawesi in 1980 with 30 people from his old village in Southwest Sulawesi to farm 180 ha. Twenty years later, 600 or so people from his old village now cultivate 6,000 ha, producing about US$9 million worth of cocoa a year. There are now over 300,000 cocoa growers in Sulawesi, producing 350,000 tonnes of cocoa annually, only surpassed by the African countries of Côte d'Ivoire and Ghana.

Seventy percent of Indonesian cocoa is produced by smallholders like Haji Selang and his villagers. However, this rapid explosion of cocoa growing has created its own set of problems. Firstly, the rapid expansion has not been matched by the development of infrastructure or expertise, and this has been compounded by the rapid pace of political reform and decentralisation in Indonesia. Secondly, the expansion sometimes threatens the integrity of rainforest adjacent to the cocoa-growing regions, including the famous Lore Lindu National Park, home of a vast virgin rainforest containing many rare mammals and birds.

In 2000 the Australian Centre for International Agricultural Research (ACIAR) was asked to help the cocoa industry in Sualwesi. A team led by Dr David Guest (School of Botany, The University of Melbourne), Dr Philip Keane (Department of Botany, Latrobe University) and Dr Smilja Lambert (Masterfoods Australia/New Zealand, Ballarat, a part of Mars Inc.), developed a proposal to work with colleagues at the Agricultural Technology Assessment Institute (BPTP) in Kendari, Southeast Sulawesi, and the Indonesian Coffee and Cocoa Research Institute in Jember, East Java, to improve the quality and pest and disease resistance of cocoa. The three major pest and disease problems for cocoa in Sulawesi are:

- Cocoa pod borer (*Conopomorpha cramerella*), an insect that infests over half the cocoa plantings in Sulawesi and causes 20-30% yield loss
- Pod rot and canker caused by the oomycete, *Phytophthora palmivora*, that typically causes losses of 15%
- Vascular-streak dieback (VSD), caused by the fungus *Oncobasidium theobromae*

Both *P. palmivora* and *O. theobromae* also kill cocoa trees. Dr Keane discovered the cause of VSD during his PhD work in the 1970s, and he and Dr Guest have collaborated for over 15 years with colleagues from Papua New Guinea on Phytophthora pod rot and VSD problems. Dr Guest knew Dr Lambert from their work together in Brazil, and more recently since Dr Lambert's move to Ballarat to head up the R&D effort of Mars Inc. in Southeast Asia and the Pacific, and so this project brings together a well-established team. Dr Peter McMahon, who has had long experience of development work in Africa, was appointed to manage the project.

The ACIAR project formally commenced with a 3-day workshop in Kendari in June 2001 attended by Dinas Perkebunan (agri-

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cultural extension) staff from 10 cocoa-growing provinces of Indonesia, and cocoa researchers from Java, Papua New Guinea and Australia. The workshop summarised the current status of the cocoa industry and planned the development of straightforward methods for identifying and screening improved individual trees from existing cocoa plantings. This ensures that selections are adapted to the local environment, and it also ensures that cocoa growers and local scientists manage and control the selection process. During field trips associated with this workshop promising genotypes were identified and collections established, and trials investigating improved farm management practices were established. In June 2002 a second workshop was held at Kendari, this time focussing on technical aspects of disease diagnosis and treatment. In this way the project enhances the infrastructure and capability within Indonesia using locally available resources, especially the great genetic diversity of the cocoa already being grown in the country.

So far over 40 collections of promising genotypes have been established at two sites. Cuttings of promising genotypes are prospected with the help of farmers and extension staff, collected from the field and transported and grafted onto existing cocoa trees for evaluation under local conditions. In this way the mother trees are not damaged, and the grafts begin producing pods within 18 months. Yield, pest and disease resistance and cocoa quality attributes can then be assessed under trial conditions. The trials also represent a locally available collection of potentially useful genotypes that can be readily propagated for distribution to farmers. It is planned that elite selections will be distributed widely for propagation, use in other cocoa projects in Sulawesi, and eventual release to farmers. To date at least 10 replicates of most genotypes have been established in the trials. The majority of grafts are now flowering and setting fruit, and we are assessing their resistance to VSD, Phytophthora pod rot and cocoa pod borer, and cocoa quality attributes.

At a field site at Soppeng in Sulawesi (David Guest)

While it is expected that some useful selections of high quality pest and disease resistant planting material will be made available to growers as a result of this, and other, projects, an important aim of this project is to demonstrate the usefulness of local selection of improved planting material using straightforward methods based on locally available genetic diversity within the crop. It has been previously shown in Papua New Guinea that it is possible to select cocoa genotypes with durable resistance to VSD from among a great diversity of planting material exposed to a natural epidemic of the disease. This approach is important in a country like Indonesia that included areas with great genetic diversity of a wide range of tropical crops (e.g. citrus, banana, durian, mango, sugarcane, rice, taro, jackfruit, coconut). So far the project has taught some valuable lessons in how to proceed with this sort of research and development effort based on local resources. The method being used in trials of sidegrafting improved budwood onto existing trees also demonstrates a method of upgrading existing cocoa plantings with minimal loss of yield during the process. Several farmers are already adopting the methods following the example they have observed in the trials.

Reducing cocoa losses due to pests and diseases will also reduce the pressure to expand cocoa plantings into virgin rainforest. Previous studies have shown that integrating cocoa growing with traditional mixed farming minimises pesticide use and retains up to 70% of the original biodiversity. In a further illustration of good collaboration between universities and industry, PT Effem (a part of Mars Inc.) is assisting with the logistics of establishing the field trials and cocoa quality assessments of pest and disease-resistant selections.

ACIAR website: http://www.aciar.gov.au

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Strengthening Sulawesi's Smallholder Cocoa Sector

Pest problems in cocoa that lead to poor yields and low quality beans affect not just farmers but cascade along the cocoa chain to where the beans are processed. For this reason, PRIMA Cocoa (Pest Reduction and Integrated Management for Cocoa), a new 2-year project beginning in January 2003, is piloting both integrated management

PRIMA Cocoa will bring IPM technologies to smallholders (Martin Gilmour)

of pests and a chain approach to cocoa production in Sulawesi, the largest cocoa-producing island in Indonesia.

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 2 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, *Conopomorpha cramerella* (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 CPB and other pest and disease issues, with insufficient knowledge about the problems they are facing. Although programmes like the USDA-funded SUCCESS initiative [See *GRO-Cocoa* No 1, p 7-8] have been training farmers to deal with the pest, the situation is still serious enough that PT Effem Indonesia, based in Makassar, had to close down its cocoa processing plant temporarily in 2002 as there were simply not enough quality beans available. The situation faced by PT Effem was not unique, as evidenced by the temporary shutdown of a number of cocoa processing factories in Indonesia, and complaints of high wastes in Sulawesi beans by regional and international cocoa grinders. The situation in Sulawesi is thus of great concern to all in the cocoa industry.

Masterfoods and the worldwide cocoa

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 agricultural practices and post-harvest treatment. A number of promising biocontrol methods would be tested and validated 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. It is hoped that the proposed improvements will 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.

Senter International has approved the project with a total budget of €750,000 of which €500,000 will come from Senter, and the other €250,000 in kind from PT Effem and Masterfoods Europe.

One of the project's first activities was a Technical Brainstorming Meeting on Biocontrol Technologies for IPM in Cocoa in Makassar in June 2003. There has been a good deal of scientific and field-based research on ways of enhancing cocoa production through controlling CPB by mechanical, cultural and biocontrol methods. The majority of this knowledge is spread around different research and academic institutions and private farms in Indonesia and Malaysia. The meeting brings together the people with this knowledge to discuss and define the most promising farmer-friendly and practical options for an IPM strategy to control CPB and improve cocoa production in Sulawesi. The most promising CPB control systems will be implemented as part of the PRIMA Cocoa programme.

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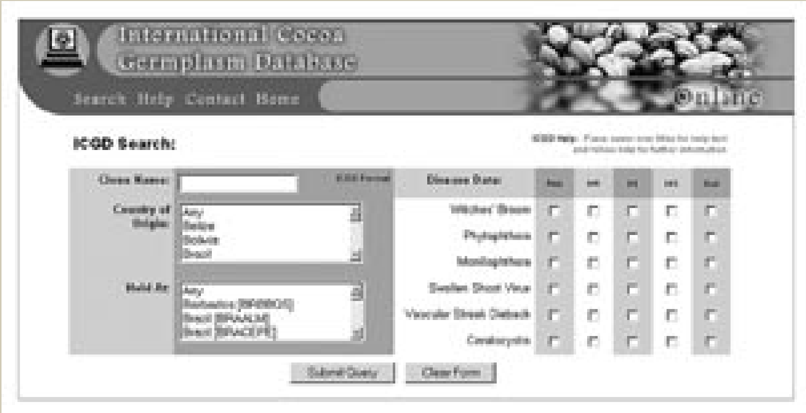
ICGD Online

The International Cocoa Germplasm Database (ICGD) collates descriptive data on a range of morphological characteristics of the tree, flowers, pods, seeds and leaves, together with results of detailed assessments of agronomic traits and quality, and reactions to stress, pests and diseases. Information is available on over 14,000 different clones/genotypes[1].

On-line[2] users can find a particular genotype and check on its availability from lists of accessions held by the 48 of the major genebanks and quarantine stations. Search facilities allow users to access all information held on individual clones, and to generate lists of clones that have resistance or susceptibility to specific diseases (or combinations of diseases), are held in specific collections, and originate from a specific region/location. The search for a clone by name allows several different formats or synonyms to be entered, but all the results are given with their standardised ICGD preferred name.



Information has been submitted to the ICGD on the disease reaction of a large number of clones. The methodology used, disease strains studied and format of the data supplied varies greatly, but the ICGD converts this information to a standard format to facilitate searching. Based on the information currently available:

- A search for clones with resistance or moderate resistance to witches' broom, *Moniliophthora* (frosty pod) and *Phytophthora* gives just one clone: ICS 95, listed as held by 29 institutions throughout the world. Clicking for further information (which can be done for any clone in the database) brings up a datasheet. This lists synonyms and related clones and provides detailed biological, agronomic and pest impact information together with further information on accessions.
- A search for clones resistant to witches' broom produces a list of 427 clones, together with details of country/region of origin, and institutions currently holding each. The search can be restricted further to specific countries of origin (e.g. Brazil: 50) or locations where the clone is held (Reading: 66). Combining these search criteria (Witches Broom resistant, from Brazil, held at Reading quarantine) produces one clone, RB 39 [BRA].

[1] The information has kindly been provided by cocoa research institutions and individuals and we gratefully acknowledge their support and collaboration. Any information on cocoa, including microsatellite data, would be gratefully received by the ICGD to be included in the database. Please contact us via the addresses in the CocoaGen.DB article

[2] ICGD website: http://www.icgd.rdg.ac.uk

The ICGD project is funded by Euronext.liffe and BCCCA.

CocoaGen.DB: a New Cocoa Genomics Database

A collaborative project has recently been set up by the University of Reading, CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), and the US Department of Agriculture to develop a new Internet-accessible database to serve the needs of cocoa molecular biologists. The new database will combine the genotypic and character data contained in the International Cocoa Germplasm Database (ICGD, School of Plant Sciences, The University of Reading), with the genomics data contained in Tropgene (CIRAD, Montpellier) [see following article], and is being funded by USDA, CIRAD, Euronext.liffe and BCCCA (UK Biscuit, Cake, Chocolate and Confectionery Alliance). Although ICGD and Tropgene are under constant development and continue to fulfil their individual roles, neither of the current databases is suited to handling the combined datasets. In the case of ICGD, the sheer quantity of genomics data currently being generated would prevent CD-ROM distribution and would also add an unacceptable level of complication for the user; the ICGD is designed to handle information on cocoa clones, whereas much of the genomics data comes from seedling populations or progenies, and the data will not be relevant for many users. The CIRAD-based database Tropgene provides genomic information on a wide range of tropical crops and not just cocoa.

The new database, named CocoaGen.DB, will be designed specifically to allow the user to carry out searches that use both datasets, for example comparing genetic markers with specific traits. Although much of the data within it will be similar to that found in the two parent databases, the user interface will be designed specifically for such complex queries, and will necessarily be very different to that of the ICGD and Tropgene.

The ICGD will continue to provide germplasm information to the cocoa community, with continued distribution of a CD-ROM version and further development of the database on the Internet*. The ICGD deals specifically with germplasm identification, origin, characterisation, current location and evaluation data, and development will continue to focus on providing this in accessible and easy to use formats. The Reading team's aim is to develop the CD-ROM and web versions of the ICGD together, so that they look similar and work in the same way, which will aid continuity in using both, as well as having practical advantages for development and maintenance. The web version will allow access to the most up to date information, and hopefully encourage more frequent update and feedback from the cocoa community as a whole. However, the CD-ROM version currently allows the user to carry out more complex searches of the data contained in the ICGD.

The three databases (ICGD, Tropgene and CocoaGen.DB) will be designed to do fundamentally different types of searches, but between them will provide wide-ranging and comprehensive information on cocoa that will be easily accessibly to the broad range of disciplines that make up the cocoa research community.

*ICGD website: http://www.icgd.rdg.ac.uk
- see Box, 'ICGD Online'

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Research behind Tropgene

How does molecular research contribute to sustainable cocoa production? At CIRAD, a major research area on cocoa is devoted to genetic improvement using molecular markers, and this is presented in the cocoa module on the Tropgene database.

Making Sense of Molecular Research

Closely related organisms (e.g. cocoa clones) share much of their genetic code, or genome, but differences arise over time through mutation. Mutations that have phenotypic (visible or functional) effects on an organism create the variation that is the basis of conventional breeding research. Historically, this has involved crossing parents with desirable attributes and rearing the progeny. For a tree crop such as cocoa this is time-consuming. However, advances in molecular techniques allow variation to be detected at the DNA level.

Even though cocoa has a relatively small genome it is too complex to study in its entirety, so researchers use markers, or tags, to focus on more manageable sections. A molecular marker is a DNA sequence or a protein whose inheritance can be monitored through polymorphisms or inherited differences between individuals. These can be genes with different alleles, or forms, but can be any part of the DNA that has detectable polymorphisms (a surprisingly large amount of DNA has no apparent function). Co-dominance, where the dominant homozygote and heterozygote are distinguishable, is a useful marker trait. There is no absolute hierarchy between markers. All have advantages and drawbacks and choice depends on the users' objectives and has to be determined on a case-by-case basis.

Isozymes are multiple forms of an enzyme that may be present at different loci [a locus is a defined and unique position on a chromosome]. They have been used widely as markers in population studies for some 30 years, although now largely superseded by DNA tools.

A significant advance was the discovery of restriction endonucleases. Each cuts DNA at unique and specific sequences of base pairs, the restriction site, creating restriction fragments of varying lengths. Whilst different cocoa populations or clones will have similar DNA and produce largely the same array of fragments, mutations at the restriction site can lead to sites being lost or created, so fragments will be longer or shorter, respectively. Electrophoretic methods allow fragments differing in length to be separated. Further techniques allow the results to be visualised, often with probes, which are labelled (e.g. radioactively or immunologically), single-stranded nucleic acid base sequences used to locate complementary base sequences.

RFLP (restriction fragment length polymorphism) uses restriction enzymes and generates mainly co-dominant markers. It has been superseded to a large extent by PCR-based techniques.

PCR (polymerase chain reaction) was a major technical breakthrough based on the discovery of a thermostable DNA polymerase, Taq, that facilitates replication of DNA at a high temperature. PCR allows specific segments of DNA to be amplified millions of time, which makes them clearly discernible, and this has led to the development of a variety of marker techniques, including:

RAPD (random amplification of polymorphic DNA) can identify a large number of polymorphisms but generates dominant markers. Although relatively undemanding technologically, problems in reproducibility mean it has been superseded by AFLPs and microsatellites.

AFLP (amplified fragment length polymorphism) detects polymorphisms at a particular locus with better reliability (and larger genome coverage) than RAPD. AFLP is useful for rapid screening for genetic diversity, although, like RAPD, it tends to generate dominant markers.

Microsatellites (or SSRs, simple sequence repeats) exploit the fact that errors during DNA replication can cause sequences in the genome to be repeated, creating polymorphisms that may have no discernible functional or phenotypic significance. Microsatellites are valuable because they are co-dominant and detect high levels of allelic diversity. They may allow differentiation below the population, indeed at the individual, level.

Genetic mapping relates specific traits with specific regions of chromosomes and/or defines how likely particular loci/markers/genes are to be inherited together. The likelihood of two markers being inherited together is described as their linkage. Linkage groups can be defined that relate to each chromosome. A linkage map shows the relative positions of the markers on a chromosome.

Family Trees

Three main groups of cultivated cocoa have long been recognised (Criollo, Forastero and Trinitario) but their origins and relationships have been far from clear. Molecular tools can be used to provide insights by comparing the DNAs of different clones to see how similar they are. Following the principle that the more similar the genomes, the more closely related the clones, conclusions can be drawn about their relatedness and area(s) of origin.

Over the last 10 years, CIRAD has analysed the genetic diversity of more than 500 genotypes from the different groups using RFLPs. A smaller sample was analysed using RAPDs and microsatellites. Their results showed that within the Forastero group a number of populations were differentiated: populations from Upper Amazon with the largest diversity, Forastero from Lower Amazon or Amelonado, and a distinct population from French Guiana. Criollo was the first domesticated cocoa but is now largely replaced in plantations by the more vigorous Trinitario, hybrid between Criollo and Forastero. However, Criollo clones from both Venezuela and Mexico were shown to be closest to Forastero populations of Colombia and Ecuador, underlining Criollo's probable South American origin. Molecular marker studies have shown that a very limited number of genotypes are at the origin of most of the modern Criollo and Trinitario varieties, both hybrid. These parents, Criollo on one side, and Forastero from Lower Amazonia on the other side, could be identified and were shown to be highly homozygous. These studies have shown the very narrow genetic base used until now in plant breeding compared to the diversity present in the species.

Mapping Genes

A population of 195 individuals derived from a Trinitario × Forastero cross was used to build the genetic map presently available in the Tropgene module. These individuals were genotyped with different marker types (isoenzymes, RFLP, RAPD, AFLP and microsatellites) that were used to ensure the best coverage possible of the genome. Detailed information on these markers is also stored in the Tropgene module. The map includes 473 loci distributed in 10 linkage groups corresponding to the 10 chromosomes. The map is available for researchers studying the genetic components of agronomic characters such as disease resistance and cocoa yield and quality.

To map genes of interest, researchers phenotype the whole population for the traits they are interested in. They look for statistical correlations between the phenotype and the markers. When such a correlation is found, it indicates the presence of a gene or a QTL (quantitative trait locus) near the significant marker. It is then possible to manipulate the allele at the gene or at the QTL through indirect selection on the alleles at the nearby markers.

For example, a genome mapping approach has been developed within the framework of a CAOBISCO (Association of the Chocolate, Biscuit and Confectionery Industries of the EU) funded collaborative project involving Côte d'Ivoire, Trinidad & Tobago, Cameroon and France, which aims to improve knowledge of the genetic basis of cocoa resistance to black pod disease caused by *Phytophthora* species. Various progenies located in Trinidad, Cameroon, Côte d'Ivoire and in France (Montpellier) have been studied for their resistance to *P. palmivora*, *P. megakarya* or *P. capsici* using leaf tests, or after field observations of pod rot. Several QTL were identified, some of which were common to a number of progenitors, particularly in the regions of chromosomes 1, 4 and 9.

Using leaf tests, a population has been studied for its resistance to various species of *Phytophthora*. A number of QTLs linked with resistance to the different species were detected, some of which were located at the same place on the chromosome. Although it is impossible with the resolution of the method to conclude whether there is just one QTL affecting all species or several QTLs clustered in the same segment, these areas are of interest for further exploration.

The accumulated information on QTL location and effect in cocoa is not yet in Tropgene but will be included soon. Tropgene graphical tools allow for easy visualisation of QTL positions on genetic maps. This CIRAD research benefits traditional breeding and has enormous potential in marker assisted selection (MAS).

Webpage: http://www.cirad.fr/presentation/programmes/biotrop/resultats/biositecirad/cocoa.htm

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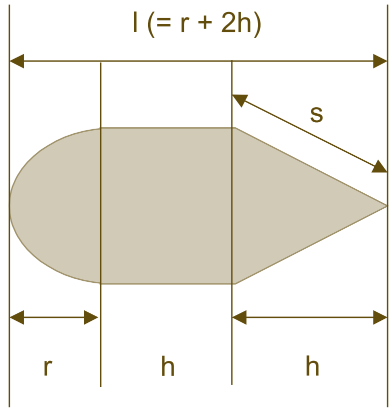
Pondering Pod Surfaces

In *GRO-Cocoa* No. 1, we introduced the concept of trying to maximise fungicide spray deposition on cocoa pods by using narrow-angle cone nozzles. In a forthcoming issue we will show that it is indeed possible to increase spray recovery, but this work has raised another interesting issue, with possible implications for other research, such as understanding disease infection processes.

In order to assess the efficiency of spray application we needed an estimate of pod surface area. The easy parameters to measure are: pod length and diameter, pod volume (by displacement in a large beaker of water) and number of furrows, with estimates of their average depth (see Fig. 1). From these measurements, pod surface area was estimated approximately using a pod model (Fig. 2) based on a hemisphere (pedicel end), cylinder and cone of equal heights (2 × height = length - radius). The trial involved the measurement of 121 pods, of various varieties, from the cocoa breeding farm at CATIE (Centro Agronómico Tropical de Investigación y Enseñanza), Turrialba, Costa Rica. Using an equivalent formula to calculate volume, the grand-mean estimate was only 1% greater than the measured mean volume (17 out of the 121 sampled pods were more than +/-10% different). A 'Zeppelin' model, using a second hemisphere instead of a cone, was 14% different, and attempts to compensate for the furrows did not improve the volume estimate.

Fig. 1. There is considerable variation in pod crinkliness and the depth of furrows (Roy Bateman)



Surface = $2\pi r^2 + 2\pi rh + \pi rs$

Fig. 2. A simple model for cocoa pod surface area.

True measurements of surface area are much more difficult to obtain, and therefore to verify estimates. Of course, the question posed is similar to a well-known fractal conundrum "What is the length of the British coastline"? The answer varies vastly, depending on the detail with which the coastline is traced. With cocoa pods, does great accuracy matter? Perhaps not for spray deposition studies, but for fungal spore adhesion, surface features of up to 10 mm might be important.

For those of us without tens of thousands of dollars to spend on techniques such as 3D-laser image analysis, have any readers come across other simple methods of estimating pod surface area? I would also greatly appreciate other views on this subject in general.

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Endophytes Report

The 2nd Cacao Endophyte Working Group Meeting, held at Rutgers University, New Jersey, USA on 14-15 April 2003, focused on exchanging information on the role played by endophytes in disease suppression. Participants included researchers from Rutgers University, Pennsylvania State University, USDA Beltsville (Maryland, USA), CABI Bioscience (UK), Almirante Cacau/Masterfoods (Brazil), Masterfoods USA and STRI (Smithsonian Tropical Research Institute, Panama).

Harry Evans, Keith Holmes and Sarah Thomas (CABI Bioscience) have been surveying endophytic fungi associated with *Theobroma gileri*, the purported original host of frosty pod (*Crinipellis roreri*), in the submontane forests of northwest Ecuador. They have found several fungal strains, including *Trichoderma stromaticum* [see Box, 'Why *Trichoderma stromaticum* is a Hot Topic']. Surveys are now complete, although there are plans being made with Allen Herre (STRI) to survey the STRI preserve in eastern Ecuador for co-evolved endophytes from *Theobroma* spp. Screening of the material collected is well underway and field trials have been established at EET, INIAP (Estacion Experimental Tropical, Instituto Nacional de Investigaciones Agropecuarias), Pichilingue in collaboration with a team led by Carmen Suarez.

Alan Pomella and Steen Hjorth (Almirante Cacau/Masterfoods) have completed work in Brazil to develop methods for isolation, screening and inoculation of bacterial endophytes for witches' broom (*Crinipellis pernisiosa*). They have some hundred isolates to screen and are planning field trials for 2004-05. Further research is needed to establish the taxonomy of the isolates, and to investigate modes of action and life cycles.

Jorge De Souza (USDA/University of Maryland) is investigating the genetic diversity of endophytic and non-endophytic *T. stromaticum*, with a view to optimizing its control of witches' broom. Studies using AFLP [see Box, 'Making Sense of Molecular Research', p. 4] on material from Bahia and Amazonas states in Brazil is complete, together with *in vitro* growth studies to characterise strains. The next step is to study broom colonization in the field.

Prakash Hebbar (Masterfoods USA) and Pierre Tondje and co-workers at IRAD (Institut de Recherche Agricole pour le Développement), Cameroon are seeking and developing microbials for control of black pod (*Phytophthora megakarya*) in Africa. Isolates have been identified and preliminary field testing has been carried out, with more planned [see *GRO-Cocoa* No. 2, p. 5].

Allan Herre, Luis Meija, Enith Rojas and Damond Kyllo (STRI) have completed preliminary field testing of endophytes isolated from cocoa material in Panama. Field testing of an intensive application regime is set for this year, with further research to investigate interactions between endophytes and mycorrhizae also planned. Looking towards an implementation phase, they will also be developing new production and application methods, and also developing and applying genetic tools for host fungal systems.

On a different tack, Tom Gianfagna and Madhu Aneja (Rutgers University) and Alan Pomella (Almirante Cacau/Masterfoods) are looking at the induction of caffeine synthesis as a possible defence response to pathogen infection. They have shown that such synthesis is a defence response in (Scavina) resistant cocoa types, and plan to investigate whether this is a widespread phenomenon in other resistant cocoa genotypes. They have also found that some endophytes produce nonanoic acid, a fungal growth inhibitor.

Bryan Bailey and Mary Streem (USDA) and Gabriela Antunez de Mayo, Joseph Verica and Mark Guiltinan (Pennsylvania State University, USA) are investigating the identity and function of genes responsible for induced resistance, with the aim of using plant defence genes to assess and monitor field tests. A series of identified genes is now being assessed in glasshouse trials, and plans are underway to evaluate endophyte/cocoa interactions for induced resistance to witches' broom.

The meeting at Rutgers gave cocoa researchers the opportunity to meet with other endophyte researchers and learn about related work in coffee (*Coffea* spp.) and turfgrass. Possible linkages with researchers working with coffee endophytes were discussed. The meeting ended with the cocoa researchers learning from staff of the university's Turfgrass Research Program and Turfgrass Farm how endophytes are now an integral and routine component in optimizing quality in turfgrass and are used to commercial advantage in the US$1 billion/year US turfgrass and sod industry, which provided both encouragement and food for thought.

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Why *Trichoderma stromaticum* is a Hot Topic

Trichoderma spp. are present in substantial numbers in soils and plant material in most ecosystems,. They are being increasingly exploited as disease control agents in a wide range of field and tree crops, and have been touted as candidates for tackling cocoa diseases. Mechanisms of action may include direct anti-fungal activity through mycoparasitism of hyphae and antibiotic production, and indirect effects such as inducing host plant resistance and competing for nutrients and space.

In 1992, Cleber N. Bastos (CEPLAC, Comissão Executiva do Plano da Lavoura Cacaueira, Brazil) found what was initially identified as a strain of *Trichoderma viride* on a witches' broom of cocoa, in Brazil's Amazon basin. This proved to be an effective mycoparasite of *Crinipellis perniciosa*, the causal agent of witches' broom. Subsequently Gary Samuels (USDA Systematic Botany and Mycology Laboratory, Beltsville, Maryland), using both morphological and molecular characteristics, described it as a new species, *T. stromaticum*.

The discovery of a new *Trichoderma* species on witches' broom in its area of origin was an exciting development. Initial small-scale field and lab trials conducted at CEPLAC showed that it could reduce formation of basidiocarps (fruiting bodies) by 99% when brooms were in contact with the soil, and by 56% in brooms remaining on trees. It also reduced pod infection by 31%. A commercial formulation, Trichovab, developed by CEPLAC is now available in Brazil for control of witches' broom.

However, results of further field trials with *T. stromaticum* from Peru and Costa Rica as well as Brazil have been inconsistent. Researchers are investigating the mechanism of action of *T. stromaticum*, particularly by assessing new endophytic strains, and how it interacts with both its host and the environment, which they hope will allow its biocontrol activity to be optimised. In addition different isolates are being assessed to improve the 'active ingredient' through strain selection. The 'teething troubles' with the promising witches' broom mycoparasite illustrate the difficulties of working with mycoparasites, and biological control agents in general. Success relies on either a great deal of luck or (more usually) considerable persistence to bring together a highly effective mycoparasite with production, formulation, delivery and application methods to optimise the biocontrol agent's activity.

Little-Known Threat: Pink Disease

A severe outbreak of pink disease on cocoa (causal agent: the basidiomycete *Corticium salmonicolor*) was reported for the first time in Ghana in 1999, although the disease has been recognised in cocoa for almost a century. It has a wide host range covering at least 141 genera and is thought to occur in most countries where cocoa is grown. It is found in a number of economically important crops but has been studied in detail only as a disease of rubber. In Papua New Guinea and Malaysia, pink disease has long been associated with the use of shade trees such as *Gliricidia* and cover crops such as pigeon pea. The disease can be severe in cocoa in parts of Malaysia and Brazil and 80% disease incidence has been reported in the Pacific. In a recent outbreak in Ghana over 60% of trees were infected in some plots.

Factfile

Signs of pink disease vary, with four distinct forms recognisable, all of which may be present at the same time. Most conspicuous and distinctive are the salmon-pink encrustations formed by hyphal fruiting bodies. These are only visible after the most vigorous phase of infection and when the silky white mycelia have spread over the surface and into the cortex of the bark. The pink encrustations consist of millions of spores and penetration of the fungus into the cortex disrupts the physiological processes of the tree leading to defoliation and death of the branches. Other forms of the disease recognised are 'cobweb' (cobweb-like white or pale-pink strands which grow out of the pink encrustation and run irregularly over the surface) and 'pustular' (old infections in which the encrustation has lost its colour and become pale pink or white, arranged in parallel lines in small cracks in the bark). The symptoms of the anamorph, *Necator decretus* consist of orange-red instead of pinkish pustules found on the upper side of attached branches exposed to sunlight. Each pustule is a mass of irregular-shaped spores which serve to propagate the disease.

The fungus survives dry periods in dormant cankers. Formation and release of basiodiospores are thought to be dependent on heavy rainfall, although more spores are released when rainfall is light and of short duration. Spore release generally begins 20-80 minutes after rain starts and can continue for up to 14 hours. High humidity, heavy shade and rainfall are important factors in pink disease spread, which is wind, rain-splash and insect (especially red ant) mediated. Spores need to settle on wet brown bark to germinate and penetrate through undamaged bark tissues. In water, germination of basiodiospores starts 60-90 minutes after release. Green pods have also been observed to be attacked in Brazil with production of pink fruiting bodies seen on them. Trees 2-6 years old are most susceptible with damage ranging from loss of individual branches to death of whole trees if the main stem is attacked.

Recorded damage symptoms in cocoa include severe die-back of young twigs and some large branches, severe defoliation and brown to pinkish encrustations of fruiting bodies and small and large cracks on affected branches. In Ghana, excessive wilting, yellowing and browning of leaves and complete defoliation of severely affected branches were observed, and later young twigs and some large branches died back. Brown to pink encrustations were seen which varied in length between about 0.3 m and 2 m. Most of the affected branches became girdled by the fungal encrustations, some of which were rough while others were rough and patchy. On some trees the affected bark was dry with several cracks, while on others it adhered tightly to the wood, and on yet others it peeled off. Microscopic examination revealed conidia oblong to irregular in shape, thin-walled, catenulate and hyaline with dimensions of 10-24 μm × 8-12 μm, and also the presence of secondary invaders such as *Colletotrichum gleosporium*.

Pink disease can be controlled by a combination of chemical and cultural control. Fungicides reported to show activity against *C. salmonicolor* include copper formulations, tridemorph paints in an ammoniated latex base, triadimefon granules, chlorothalonil paints in a latex/bitumen base, and fenpropimorph. Good drainage, shade reduction and removal of susceptible forest and shade trees have also been found effective.

Pink Disease on Trial

The outbreak in Ghana occurred in two of CRIG's experimental plots in Bunso in Eastern region, which was inconvenient for the original purposes of the trial but did permit some inferences to be drawn about the influence of different factors on disease incidence (although these should be treated with caution as none of the trials was designed for disease assessment).

The disease was more severe on a multiple-stem × spacing × pruning trial than in a shade × variety × spacing trial, with disease incidences of 50% and around 15%, respectively. There was a greater incidence of infected trees in the plots with

Dieback casued by pink disease (Cocoa and Coconut Research Institute, Papua New Guinea)

highest tree density (2.5 × 2.5 m spacing) and a greater incidence of infected trees where 2 seedlings had been planted in one hole. Also, more pink disease occurred in crosses of T85/T99 × T79/501 with over 30% of trees and 56% of their branches affected. Variety T85/799 × Pa7/808 was the least infected; 5% of the trees in general and 16% of its branches were infected. Infections were more severe in the no-shade area than the shaded area, which contradicts published reports of a higher incidence of disease in damp and shaded conditions than in the open. The apparently inconsistency could have arisen because trees in the no-shade plots may already have been weakened by excessive sunlight and thus the attack of pink disease was more marked.

The following control regime was implemented successfully in Ghana. The affected branches were removed about 5-10 cm below the apparent point of infection and burnt immediately. This was followed by spraying of affected plots with either Ridomil 72 Plus or Kocide 101 at 3-weekly intervals, using motorized sprayers to reach the canopy where the disease was prevalent.

All the infected trees survived, indicating that early action can be successful at combating this disease. However continued vigilance is necessary as the disease may be more widespread than observed. To prevent its spread, other plots on the Sub-Station as well as farmers' cocoa nearby have been thoroughly inspected and CRIG staff remains alert to this potential threat.

For references on pink disease see: Opoku, I.Y.;

Ghana Conference Website

Keep up to date with the latest news on the 14th International Cocoa Research Conference (Accra International Conference Centre, Ghana, 13-17 October 2003) by visiting the website at:

www.copal-cpa.org/conference.html

It includes information on the 4th INGENIC Workshop and the 4th INCOPED Seminar, which are being held at the same venue on 20-21 October.

Akrofi, A.Y.; Osei Bonsu, K.; Acheampong, K. (2002) An outbreak of pink disease in Ghana. *In*: Proceedings, 13th International Cocoa Research Conference, Kota Kinabalu, Malaysia, October 2000.

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Field Schools for Africa's Cocoa Farmers

A workshop held on 24-28 March 2003 in Mbalmayo in southern Cameroon saw the development of a pilot curriculum for the Sustainable Tree Crops Program's (STCP) farmer field schools (FFS). The curriculum will be further validated and implemented in STCP's four national cocoa pilot projects in the region and will involve first training of trainers (ToT) and then cocoa farmer field schools (FFS).

The workshop, which was organised by IITA (International Institute of Tropical Agriculture) with CABI Bioscience and sponsored by STCP, brought participants from Ghana, Côte d'Ivoire, Nigeria and Cameroon (who included pilot project managers, participatory training supervisors and resource people) together with IITA and CABI Bioscience staff with expertise in cocoa IPM and participatory methods. In addition, sponsorship from ACRI (American Cocoa Research Institute) brought expertise from Indonesia (in the person of Ross Jaax) where cocoa FFS have been functioning for the past 2 years (see *GRO-Cocoa* No. 1, pp. 7-8).

FFS operate on the principle that farmers need to develop fuller understanding of the constraints inhibiting their efforts if they are to manage their crops most effectively. STCP baseline surveys conducted across the region had established that farmers considered mirids and black pod to be their most important production constraints, and the curriculum focuses on integrated pest and crop management of these. However, it also covers rational and safe use of pesticides, as lack of knowledge about their use has been identified as a cause of both inefficient and hazardous practices.

The curriculum develops understanding by using simple processes of non-formal discovery learning to fill gaps in the farmers' knowledge. Exercises are designed, for example, to illustrate clearly the cause and transmission mechanisms of black pod, and to demonstrate the impact of pruning and shade management. As farmers work through the exercises they acquire better understanding of the disease and which, when and why particular interventions are most effective. They are thus better equipped to implement cultural control of black pod including sanitary harvesting, pruning and shade management.

Even with improved cultural measures, in Cameroon and Nigeria in particular, the high incidence of the virulent black pod pathogen *Phytophthora megakarya* will continue to oblige farmers to spray fungicides on a regular basis during the rainy season. Further exercises therefore help farmers to learn how to spray more efficiently. These demonstrate that smaller amounts of fungicide can be applied more effectively with low-volume spray nozzles and a change in spraying practice (aiming for full pod coverage rather than spraying to run off). By applying the combined knowledge they have acquired in the FFS to the management of their cocoa farms, farmers should see a net reduction in both the losses to this disease and the costs of inputs (fungicides) to control it.

The 24 discovery learning exercises developed during the workshop are being edited and compiled as the STCP version of the cocoa training manual, *Introduction to Cocoa Management through Discovery Learning* currently under developed by CABI Bioscience and due to be published later this year.

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Validation of agro-ecosystem analysis (J.Gockowski)

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