

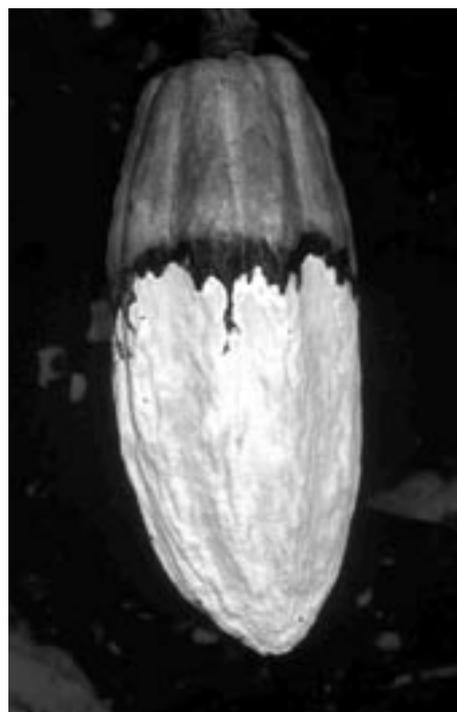
Global Research On Cocoa - *working with and for farmers*

Strength in Diversity

We were delighted with the positive feedback on our first issue. Thanks must go to the contributors and funder, who kindly gave of their time and money, respectively, without any real idea of what would come out of it. The first issue has helped to redress this, but many in the cocoa sector may have slipped through our distribution net. Please tell us of anyone else who would like to receive *GRO-Cocoa*.

This issue looks at diverse work on two emerging diseases: frosty pod (*Moniliophthora roreri*) in Latin America and *Phytophthora megakarya* black pod in Africa. Articles on the development of a tool to enhance decision making in frosty pod management in Costa Rica, and a wide range of research on strategies for black pod control in Cameroon contrast with ones about research to clarify the taxonomic status of *M. roreri*, and on *Phytophthora* infective stages. While solution-based research focuses on generating control measures that can be adopted and adapted by farmers, basic research feeds into this process by identifying new approaches.

The emphasis on diseases does not detract from the importance of other pests. Cameroonian work on insects is also



Frosty pod: telltale symptoms of *Moniliophthora roreri* infection (H.C.Evans)

covered, while an article from Malaysia discusses vertebrate pests from a perspective farmers are familiar with - the signs they leave.

Modelling the Future of Frosty Pod Management

Simulation models are powerful tools for answering 'what if...?' questions. A model of the field dynamics, management and economics of frosty pod rot (moniliasis) caused by *Moniliophthora roreri* allows smallholder farmers in Costa Rica to test various harvesting and phytosanitary measures, and assess best management options. It can also answer and/or anticipate a range of scientific, strategic or policy questions. Although still at the developmental stage, the model has been received very favourably in Costa Rica, and it has the potential to be adapted for other countries and/or diseases.

Moniliophthora roreri covers the pod with a white mycelial mat of spores, giving it a frosted appearance. The disease inflicts losses ranging from less than 25% to total crop failure. First identified as a cocoa disease in Ecuador in 1919, it spread rapidly, causing millions of trees to be abandoned and an 80% reduction in cocoa yield by 1933. Similar levels of damage have been reported in other countries, including Costa Rica, as it spread further. Its current range covers large parts of northwestern South America and Central America.

Cocoa is grown in Talamanca in south-eastern Costa Rica, predominantly on small farms. Once the most important commercial crop, production fell by two-thirds in the last quarter of 20th century through yield loss, declining cocoa prices and (consequent) cocoa abandonment, particularly because of frosty pod rot.

Cultural management is the main practical means of frosty pod management for smallholders, involving frequent removal of pods. Infected pods have to be removed at the first sign of external symptoms and before spores have been produced. Frosty pod is characterised by conspicuous bumpy swellings on the pod surfaces. Sporulation (and infectivity) starts within 12 days of these appearing. Two interventions are practised:

- **Harvesting:** picking and removing mature ripe 'clean' pods, which provide a return to the farmer.
- **Pod stripping:** picking visibly infected pods of any age during harvesting. Stripped pods provide no financial return. They are placed on the ground, not removed, because of the cost and the fear that infection rates would increase if sporulating pods were carried through the plantation. Normally infected pods rapidly degrade on the plantation floor.

Recently biocontrol has been shown to have an added impact, especially where the timing of application is closely linked to the cocoa production cycle.

Building the Model

The model was written in an Excel 97™ spreadsheet and runs on any Windows-based PC. Spreadsheets are useful tools for modelling: they are user-friendly and transparent in their workings, and can be designed as separate worksheets to help the user understand how sub-models interact. Proof of this was the speed with which scientists and managers and trainers in growers' cooperatives learned to use this model and recognised its usefulness as a decision-making tool. It consists of

In This Issue

- *Managing frosty pod rot*
- *Frosty pod's secret life*
- *Focus on Cameroon*
- *Phytophthora infection mechanisms*
- *Recognising vertebrate signs*
- *Conference update*



Model at a Glance

The main page is the first worksheet, shown here. Users can input various strategies to see how they affect disease incidence, yields or returns. Tree density, fruiting pattern, pod counts and different management and economic criteria can be added. The results, also on this page, may include tables of economic outputs, or graphs of cocoa weight, number of pods harvested and percentage of infected pods in the field.



five interacting sub-models:

- 1. Pods:** The main engine of the model. It calculates numbers of pods in weekly age classes for each week of a 2-year period. Users can enter average pod count (number of pods/kilo dry beans) for each year, and age (from flowering) at which pods become harvestable.
- 2. Epidemiology:** This contains interactive functions describing the number of sporulating pods and the proportion of pods that become infected. The more sporulating pods in the field, the greater the chance of a pod becoming infected, but pods are less likely to become infected as they get older.
- 3. Rodents:** This allows farmers to see how different harvesting strategies may affect losses to rodents (mainly rats and squirrels). Farmers in Talamanca estimated rodents inflicted losses of up to 3% of mature pods/day.
- 4. Management:** Removal of infected pods is currently the most effective measure for reducing disease impact. This sub-model allows farmers to test strategies, either singly or in combination, and to evaluate them for control efficacy and financial return. At present, variables are the frequency of (a) harvesting and (b) pod stripping. Farmers can perform either at chosen intervals that they can vary over time and see how this affects both yields and returns. Efficiency of harvesting (proportion of ripe pods picked at each harvest) can also be altered, which allows a farmer to calculate how often he should strip his pods and how efficient

he should be to optimize returns, and whether early intervention against the disease reduces its impact.

- 5. Economics:** This factors in changing labour costs and returns from cocoa sales. Labour costs include number of man-days/ha/week for harvesting and stripping, and cost of labour/day.

Model Answers

The model helps predict optimal management strategies and practices by showing how disease incidence, crop yields and financial returns will be affected by different choices, and how returns will be affected by variables such as labour costs and cocoa prices. Although still in the developmental stage, confidence in the model has been increased by qualitative verification by a major buyer and exporter of Costa Rican cocoa, and by scientists at CATIE (Centro Agronómico Tropical de Investigación y Enseñanza).

For farmers, it gives interesting results.

- In the absence of pod stripping, harvesting more often than current practice (every 4-5 weeks) does not offer relative economic benefits. At prevailing labour and cocoa prices, a marginal reduction in number of infected pods is outweighed by the extra harvesting costs.
- Adding in infected pod stripping as a management tool, however, allows more frequent harvesting/stripping to reap considerable benefits, with striking impact where rodent damage is high.

The model also has great potential as a training tool and to inform policymak-

ing. For example, it enables the user to change the age at which infected pods can be recognised, and thus to estimate the potential impact of an extension programme aimed at their early detection and removal, and how this could affect the farmers' incomes. The model suggests that early (pod age 10 weeks rather than 15) recognition of symptoms, combined with harvesting every 4 weeks, gives an increase in net return of about 50% over 2 years. However, relative benefits are greatly reduced as harvesting frequency is increased to weekly. Such information would help an extensionist to devise a strategy that both improves productivity and is acceptable to farmers. Farmers may be keener to learn to recognise the disease at an earlier stage if they can continue harvesting/stripping at current intervals rather than more often.

The model can be used to explore any management permutations, so that policy makers, extensionists, buyers and farmers can test changes in interventions and returns on employment before implementation. It is currently being evaluated in Costa Rica by the Asociación de Pequeños Prodoctores de Talamanca (APPTA) and the Organic Commodity Project.

Source: Leach, A.W.; Mumford, J.D.; Krauss, U. (2002) Modelling *Moniliophthora roreri* in Costa Rica. *Crop Protection* 21, 317-326.

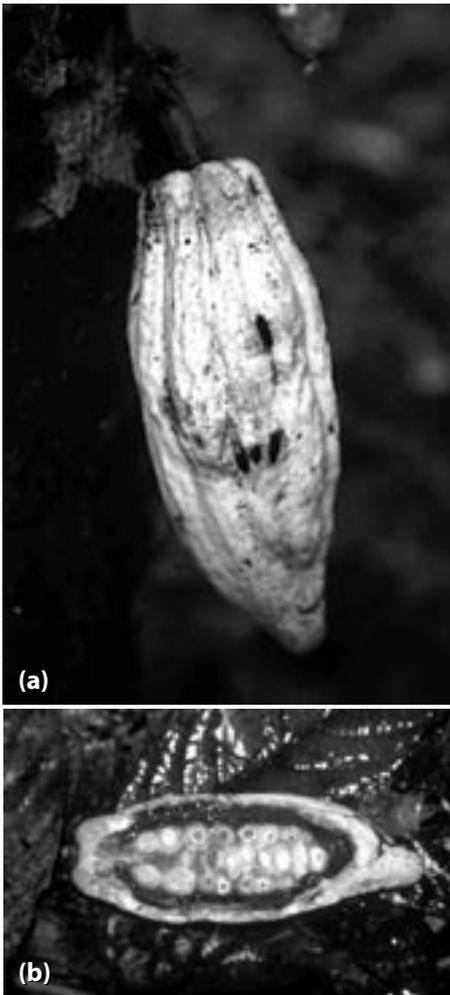
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Frosty Pod Taxonomy in Meltdown

A recent paper¹ has presented new evidence supporting a long-running claim that *Crinipellis pernicioso* and *Moniliophthora roreri*, the species responsible for the New World's worst cocoa diseases (witches' broom and frosty pod rot, respectively) are very closely related.

To normal mortals, and even entomologists, fungal taxonomy is a bit of a mystery, so it is gratifying if slightly alarming to discover that it can be a puzzle for pathologists too. The Box, 'Unravelling Fungal Taxonomy' explains some of the terminology and sources of confusion.

Frosty pod rot (moniliasis) was first recognised as a significant disease of cocoa at the beginning of the 20th century in Ecuador, then the world's largest producer. Anecdotal disease history illustrates one major obstacle to identifying fungi: their life forms vary. In 1914, C.J.J. Van Hall described 'mancha' (rot) which rotted the



Moniliophthora roreri (a) early external symptoms
(b) internal necrosis (H.C.Evans)

whole pod and 'helada' (frost) with abnormal growth of pod and beans, but we now know that both can be induced by *M. roreri*. Apparently different diseases may be symptoms of different life stages of a fungus. Symptoms may also vary between cocoa varieties.

The causal agent of the disease was identified by R.E. Smith (University of California), from specimens sent by J.B. Rorer, as a deuteromycete *Monilia* sp. closely related to a serious brown rot disease of stone fruit in the USA. It was described as *Monilia roreri* by R. Ciferri in 1933. However, in 1981 Harry Evans controversially suggested that *M. roreri* and *C. perniciosa*, a basidiomycete, were closely related, based on similarities in biology and symptomatology and that they shared a common ancestor, possibly a forest saprophyte. Based on TEM (transmission electron microscopy) studies, it was then proven that *M. roreri* is a basidiomycete rather than an ascomycete, and the new genus *Moniliophthora* was proposed to accommodate what was assumed to be the asexual stage. Further surveys, however, failed to find the sexual ('mushroom') stage.

Now, from research funded by the USDA Agricultural Research Service, Evans and

Unravelling Fungal Taxonomy

Advances in microscopic and molecular techniques have caused a number of taxonomic surprises and upsets to supposed phylogeny, particularly in the Fungi. To illustrate the complexity of fungal taxonomy and terminology, we consider two phyla of the True Fungi containing the species causing frosty pod (*Moniliophthora roreri*) and witches' broom (*Crinipellis perniciosa*).

Ascomycota: Most ascomycetes have a teleomorph or sexual stage that produces sexual spores (meiospores) called ascospores usually in an ascus, and an anamorph or asexual stage that produces asexual spores (mitospores) called conidia on free hyphae (mycelial threads) or in asexual fruiting structures (which have many different names). Here we meet a first source of confusion: because they look different, the sexual and asexual stages of an organism may have been described as different species. For example, *Clonostachys* and *Trichoderma* spp. are showing promise as cocoa disease biocontrol agents, but these are the anamorphs of fungi whose sexual stages have been described as *Nectria* and *Hypocrea* spp., respectively. Mycologists are familiar with such arrangements, but they can come as a bit of a shock to others.

The Ascomycota includes the class *Deuteromycetes* (Imperfect fungi or asexual fungi), in which *M. roreri* was placed. Sexual forms are rare or absent. This class is something of a catch-all for asexual stages, and sexual stages have been described for some, e.g. *Trichoderma*. More confusing are cases where the sexual stage of a deuteromycete has been placed in a different phylum, for example the Basidiomycota.

Fungal taxonomy is complicated partly because the most significant identifica-

tion characteristics are the spores and spore-bearing structures, yet the asexual and sexual stages may look completely different, and thus be described as separate species in different genera, classes, even phyla. Add this to the plethora of common names assigned to diseases on the basis of symptoms which may vary with disease stage, host, or environmental conditions (e.g. *M. roreri*), and confusion seems inevitable.

Basidiomycota: Commonly interpreted as the 'mushroom' fungi, basidiomycetes have a sexual stage that produces spores called basidiospores on a basidium. Asexual stages have never been found for many. Importantly, for us, the phylum includes *Crinipellis perniciosa* (class *Agaricales* - the true mushrooms). The paper on which this article is based argues that *M. roreri* should be re-assigned to this class.

New molecular methods which allow us to compare genetic material and draw conclusions about the relatedness of taxa, and in some cases that two species are the same, do not signal an end to confusion. Another complication lies in the highly adaptable life cycles of fungi. Either the sexual or the asexual phase may be suppressed, meaning that one or other may be found rarely, if at all. Sexual forms of some deuteromycetes have never been recorded, and neither have the asexual forms of some basidiomycetes. Fungi that are predominantly asexual, and thus placed in the *Deuteromycetes*, may be cytologically and genetically closer to the Basidiomycota, although their sexual form may never have been recorded. In the case of *M. roreri*, the 'mushroom' has evolved into a multifunctional spore-bearing structure combining asexual and sexual characteristics.

colleagues have reported findings that not only support the close relationship between the two cocoa pathogens, but also provide evidence that the observed reproductive phase of *M. roreri* is sexual, rather than asexual, which confounds its current placement in the asexual *Deuteromycetes*.

- Molecular studies showed considerably more genetic variability than expected for a preponderantly asexual fungus. Reasoning that genetic recombination must therefore be common in the life cycle, they looked at the reproductive structures more closely.
- Cytological studies of the supposed conidia and conidiophores showed that meiosis occurs during sporogenesis and germination (see Figure, 'Frosty Pod Life Cycle'). The haploid parasitic phase lives only in the host tissue, but produces few external symptoms of

disease. An unidentified signal stimulates the transition to the necrotrophic diploid phase, which produces the characteristic frosty pod symptoms.

- Further molecular studies, which compared specific regions of *M. roreri* ribosomal DNA with these regions in other fungi, found significant similarities with fungi in the basidiomycete class *Agaricales*; in particular, there was an extremely close match with *C. perniciosa*.

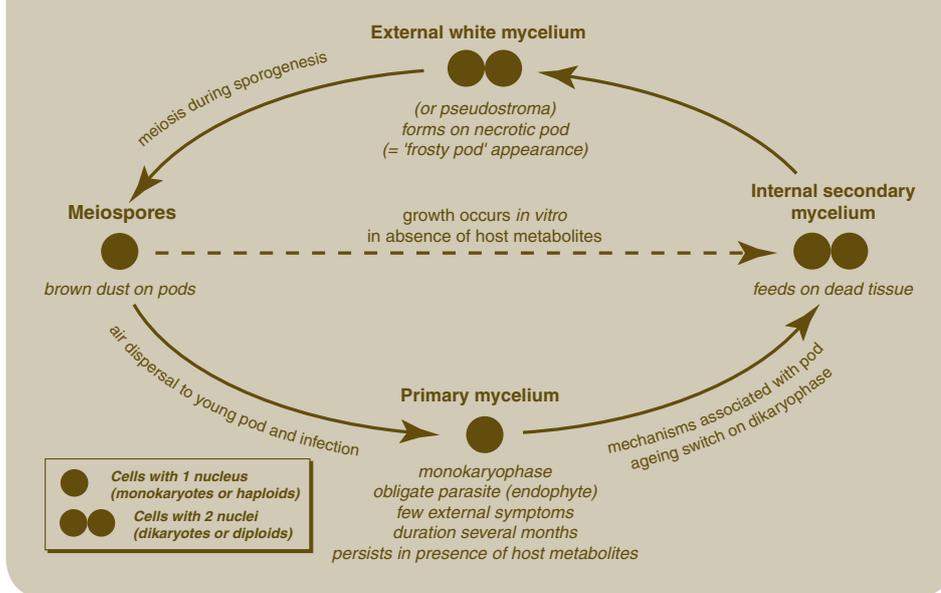
The authors argue that the current status of the frosty pod pathogen cannot be maintained, and have formally proposed the name *Crinipellis roreri* n. comb.

What's in a Name?

One criticism levelled at taxonomists is that their work has little relevance to applied science. But is this true? Not in this case, certainly, for the recent phylogenetic



Frosty Pod Life Cycle



clarification has practical implications.

- Elucidation of the life cycle means we now know why resistance to *M. royeri* has been so hard to find: the enormous numbers of spores (~44 million/cm²) ensure a high rate of variability in a sexually reproducing organism.
- For resistance breeding purposes, more needs to be known about the breeding strategy of the fungus, and particularly the mechanisms for switching from parasitic to necrotrophic phases.

The authors of this paper turn the tables on detractors of taxonomy, noting that "crop scientists in general and plant pathologists in particular are slow to accept mycological name changes..." and argue that this perpetuates confusion. In the case of *M. royeri*, frosty pod has continued to be compared to soft rot diseases of orchard fruits caused by ascomycete fungi, when it is something quite different, which calls for radically different research and management priorities.

¹Evans, H.C.; Holmes, K.A.; Phillips, W.; Wilkinson, M.J. (2002) What's in a name: *Crinipellis*, the final resting place for the frosty pod rot pathogen of cocoa? *Mycologist* 16, 1-4.

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IRAD: Leading Cocoa Research in Cameroon

Cocoa is cultivated on some 370,000 ha in the forested zones of Central, Southwest, Coastal and parts of Southeast Cameroon. The shaded cocoa agroecosystem is not only a source of revenue to smallholders,

but also a significant reservoir of biodiversity and a good sink for carbon sequestration (compared to annual cropping systems). In recent years, as cocoa prices have fallen, yield and production have declined and farmers no longer able to make a living from cocoa have begun to abandon it. The Government of Cameroon is striving to solve their plight, seeing sustainable agroforestry with cocoa at its heart as fundamental to improving the livelihoods of the 400,000 farmer households who grow cocoa as their main cash crop. Foremost amongst their pest constraints are black pod and mirids.

IRAD (Institut de Recherche Agricole pour le Développement) is the Government of Cameroon's main agricultural research institute with some 30 stations spread over the country. IRAD has established a worldwide network of cocoa collaborators around the world, including the USAID chocolate-industry sponsored STCP (Sustainable Tree Crops Programme), the USDA Agricultural Research Service (ARS), ACRI (American Cocoa Research Institute), Masterfoods (formerly Mars), IITA (International Institute of Tropical Agriculture), CABI Bioscience, CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), universities in Cameroon (Dschang and Yaoundé) and UK (Reading), and the cocoa research institutes of Côte d'Ivoire (CNRA), Ghana (CRIG) and Nigeria (CRIN).

Black Pod Disease

IRAD is taking a broad-based approach against the virulent new black pod disease caused by *Phytophthora megakarya*, which currently threatens cocoa production across West Africa. Untreated, it causes more than 50% of potential production

to be lost and reduces cocoa quality. It is thought to have originated in the forest zone of Cameroon/Nigeria and, as cocoa is not indigenous to Africa, to have transferred to cocoa from its original host.

Chemicals provide the only efficient control currently, but are too expensive for smallholders. They are also potentially damaging to the environment and non-target organisms, and may ultimately lose potency if the pathogen develops resistance. Farmers rely largely on diverse cultural practices. As high humidity and pod wetness are key elements in rapid disease spread, farmers reduce humidity and facilitate air circulation by:

- Regular weeding at the beginning of and during the wet season
- Pruning trees during the dry season in Central Province
- Shade management in cocoa farms established under shade in forested land in Central and Southern provinces

Sanitation (removal of infected pods) was only 25% effective when regularly practised as the sole control measure, probably owing to unidentified infection sources and the difficulty of removing infected pods from tall trees.

IRAD is conducting research in crop breeding, biocontrol, botanicals and agroforests to develop technologies to be integrated with cultural measures for sustainable management of *P. megakarya*.

Crop Breeding

Despite the relatively narrow genetic basis of cocoa in Cameroon, there has been progress over the last decade, towards



Phytophthora megakarya infected cocoa (A.Appiah)



developing cultivars with increased resistance to or tolerance of *P. megakarya*. Clones and hybrids were evaluated in different agro-ecological zones; e.g. 30 promising genotypes were selected in a diallelic mating design, using indices of production potential and resistance to pod rot based on several years' observational data for natural disease development and tree yields. Field trials to confirm their resistance were planted. Other cocoa materials exhibiting low incidence of black pod were identified through field evaluation. Based on attributes of precocity, vigour and level of resistance, several local and introduced progenies have been selected as part of an ongoing breeding programme.

A method for early screening for cocoa resistance to pod rot (based on inoculation of leaf discs during early plant development) developed using clones and progenies from the IRAD collections is providing insights into its genetic basis. Current evaluation of the 'Old German Cocoa' population and local progenies indicates the presence of a suitable source of resistance.

Significant correlations were obtained between level of field attack (pod rot rate) and assessment of resistance by artificial inoculation tests (in leaves and pods). Genetic correlations between leaf and pod tests appeared positive, although a slight negative environmental correlation was observed at individual tree level.

Based on resistance components including an escape mechanism (duration of pod maturation) and true resistance, genotypes such as Amelonado and some Upper Amazonian clones present in Cameroon should contribute to the creation of more resistant varieties. The level of resistance of several introduced clones has been quite stable across different environments and in relation to different *Phytophthora* spp.

IRAD's breeding and selection strategy aims to accumulate resistance genes within progenies obtained from the most resistant local (farmers' and IRAD selections) and introduced clones. Early selection (leaf disc inoculations, possibly in association with marker selection) of progenies and the comparison of individual seedlings with parental clones will allow new hybrid varieties to be created with higher levels of resistance than found in existing breeding trials.

Biocontrol

The Biocontrol and Applied Microbiology Unit is conducting key research on *P. megakarya*. As Cameroon may be the species' area of origin, it is an ideal place to look for

co-evolved natural enemies that may offer best hope for its control.

The initial step of screening microbes for potential for disease control is a major bottleneck. Therefore, the team at IRAD concentrated initially on setting up a large, well-characterized collection:

- Some 1000 fungal and 274 bacterial endophytes, epiphytes and saprophytes collected from cocoa farms in Central and Southern Provinces, and from forest reserves (Dja and Korup) where fungicides have not been used, have been isolated and stored
- New leaf disc and pod screening methods developed by IRAD and USDA-ARS (Beltsville, Maryland) have led to promising candidates being isolated from cocoa leaves and pods, and field tests have begun. Interest is focusing on endophytic *Trichoderma*, wood-decaying *Geniculosporium*, *Actinomycetes* with potential for antibiotic production, a saprobic *Cunninghamella* sp. and spore-producing *Bacillus* spp.
- A new formulation based on a local low cost product developed by the IRAD/Masterfoods/USDA team has shown promise in the field.

Next steps will progress research on promising biocontrol agents, through: expansion of field-testing in collaboration with farmers and extension services; further isolation and screening; completion of identification and risk analysis assessments; determination of mechanisms of action; fermentation and formulation trials using local low cost materials; and development of appropriate application techniques for biopesticides in farmers' fields.

Knowledge dissemination is an important part of IRAD's work. It has developed links with universities and cocoa farmers' organizations in Cameroon. Under the STCP, a regional training workshop was held at IRAD, Yaoundé, in collaboration with IITA, on microbial methods for biocontrol of plant disease, which was attended by scientists from Nigeria, Côte d'Ivoire and Cameroon. IRAD is also taking the lead in a Central and West African working group on biocontrol of plant diseases.

Botanicals

In countries such as Cameroon with active IPM programmes, botanicals can offer a low-cost, environmentally safe alternative to synthetic pesticides. After the Government removed pesticide subsidies in 1993, farmers found fungicides for black pod control prohibitively expensive, and began to apply botanical preparations, selected



Training on disease biocontrol agents (H.C.Evans)

and prepared using indigenous knowledge. At IRAD, collaborative research is being conducted with farmers to quantify the efficacy of these local solutions, although financial constraints have so far limited this to just one species.

A survey in two areas of Central Province, funded by PNVRA (Programme National de Vulgarisation et de Recherche Agricole), led to hemp being selected for a field trial. Results showed that farmers' extract gave a promising level of control compared to a copper fungicide and an untreated control. Just over one-quarter of hemp-treated pods were infected by black pod, compared to one-sixth of those treated with the fungicide and more than one-half in the untreated control. Funds will be needed for extending surveys to all cocoa-growing regions and for testing more plants.

Crop Agroforests

Cocoa agroforests in southern Cameroon are rich in tree species and are an important reservoir for conserving biodiversity, notably in degraded forest areas. Their diversity is also the basis for important ecological functions that drive and maintain productivity of the system. The Cocoa Agroforestry Unit is conducting investigations on farmers' local knowledge and management of cocoa multistrata systems in southern Cameroon. Findings are expected to shape both the design and delivery of extension messages and the scope of national research programmes. The research will also deliver sustainable capacity in participatory research methods.

Farmers' knowledge of plants pertained to their usefulness in terms of system management. Functions of plant species were classified into four groups:

1. Planted species: cocoa, fruit trees, oil palm
2. Very useful spontaneous species: high timber, fruit, fertility, medicinal and/or spiritual value
3. Spontaneous species of minor use, providing shade and/or fuel wood
4. Spontaneous species with negative effects on cocoa: host plant for pests,



incompatible rooting habits, above ground competition, or allelopathy

Farmers had considerable knowledge of fruit trees in their cocoa agroforests in locations with reasonable market access. Some 5-10 spp. are commercially exploited, and are perceived as more important to their livelihoods than cocoa.

Farmers articulated sophisticated understanding of pests and diseases in terms of occurrence, control and impact on cocoa yield. The loss caused by cocoa black pod was estimated at 80% of potential production if control was not applied. Farmers have specific knowledge of cocoa cultivars. They consider local landraces to be more resistant to drought, pests and diseases than hybrid varieties, while the hybrid varieties are early producing, with large bean size and higher bean index. Farmers often preferred the hybrids but, confronted by seedling scarcity and difficulty of affording chemicals for pest and disease control, generally used local landraces.

Farmers had detailed understanding of shade management and its impact on cocoa yield, and pest and disease control. Pruning cocoa trees and control of shade through maintenance and lopping of shade trees were important for pest and disease management because they regulate the humidity level in the plantation.

Farmers' knowledge of site selection is based on soil types and biological indicators, such as plant species. Soil types are distinguished by texture, colour and position in the landscape. Seven classes of soil have been identified and their effects on cocoa growth and production described. Trees, shrubs and herbaceous species are used as key indicators for site selection. Farmers have unambiguously identified over 65 tree species and their effects on cocoa farming systems.

The investigations revealed that 99% of the knowledge was held by older men, 67% by younger men and 39% by women. The spread of knowledge between age groups and genders highlights the risk of local knowledge depletion.

The potential for diversifying and enhancing productivity and environmental resilience of tree-based cropping systems, including cocoa agroforests, has been highlighted before. Many indigenous species which farmers have adapted to these systems have not been researched. There is a need to consider farmers' knowledge of non-cocoa tree species, particularly fruit trees. In many cases economic returns from these can contribute significantly to incomes and if judiciously incorporated

make sustainable cocoa agroforests more attractive to farmers.

Mirids and Other Insect Pests

Two species of mirids (or capsids) (*Sahlbergella singularis* and *Distantiella theobroma*) are by far the most damaging insect pests of cocoa in Cameroon. Mirids occur in cocoa at low densities, but tend to aggregate in breaks in the shade canopy. Extensive feeding leads to further canopy degradation and badly affected trees may eventually die. However, Cameroon's other insect pests should not be overlooked. *Helopeltis bergrothi* (cocoa mosquito) is very common but economically less important than mirids. Thrips (*Selenothrips rubrocinctus*) cause leaf yellowing and premature shedding, and infestations are severe in plantations with little shade. Attacks of scolytid beetles such as *Xyleborus morstatti* (now renamed *Xylosandrus compactus*) are also observed. Various *Bathycoelia* and *Atelocera* (pentatomid) and *Pseudothraupis* (coreid) species cause significant damage in Southwest Province. Sporadic but severe attacks on leaves are inflicted by the noctuids *Achaea catocaloides*, *Earias biplaga* and *E. insulana* (spiny bollworm). A noctuid pod borer, *Charcoma stictigrapta*, is also found. In nurseries and young plantations, cocoa suffers from attacks of psyllids, most commonly *Tyora tessmanni* (now re-named *Mesohomotoma tessmanni*).

Chemical control of mirids is expensive. Cultural control, including chupon (central stem) removal, shade management and elimination of alternative host plants reduces attacks. Ants (*Oecophylla*, *Tetramorium* and *Wasmannia*) may act as beneficial predators but this has not been exploited.

Research on mirid control at IRAD is focusing on botanical compounds (from hemp and the leguminous timber tree *Erythrophleum ivorense*) and on plant resistance. Experiments with 23 cocoa cultivars investigated attractiveness of cocoa to mirids, response of cocoa to attack, and mirid development and survival. One cultivar, SNK 413 was less vulnerable to attack than Catongo varieties. Analysing results of all three test types identified five cultivars that are fairly tolerant to attack, and a further seven with some resistance. Together these could form a basis for breeding cocoa with substantial mirid resistance.

In another initiative, studies are being conducted on the contribution of termites and ants to black pod epidemiology.

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How Does *Phytophthora* Infect Cocoa?

A research group at Aberdeen University in Scotland, led by Professor Neil Gow, is using cutting-edge science to research *Phytophthora* infection. As understanding of the molecular biology of the *Phytophthora*-host interaction and the mechanisms of infection is poor, improving knowledge may open up new possibilities for disease control.

Phytophthora spp. are oomycetes (water moulds) in the kingdom Chromista, whose members have fungal-like characteristics but are not true fungi. *Phytophthora palmivora* and *P. megakarya* are amongst the most serious constraints to cocoa worldwide. In the late 1980s when 10-30% of global production was estimated to be lost to *Phytophthora* diseases, financial losses were put at US\$2.5 billions/year. Global losses have risen to some 44% owing to widespread devastation of cocoa by *P. megakarya* in West and Central Africa. Chemical control has been at enormous cost to the environment and farmers, and in many cases has failed due to the presence of diverse genotypes within populations, early development of resistance to active ingredients, and rapid evolution of higher resistance to the selection pressures of fungicides. Clearly, new approaches are needed.

Zoospore Navigation

The lifecycle of oomycetes is shown on the next page. We are particularly interested

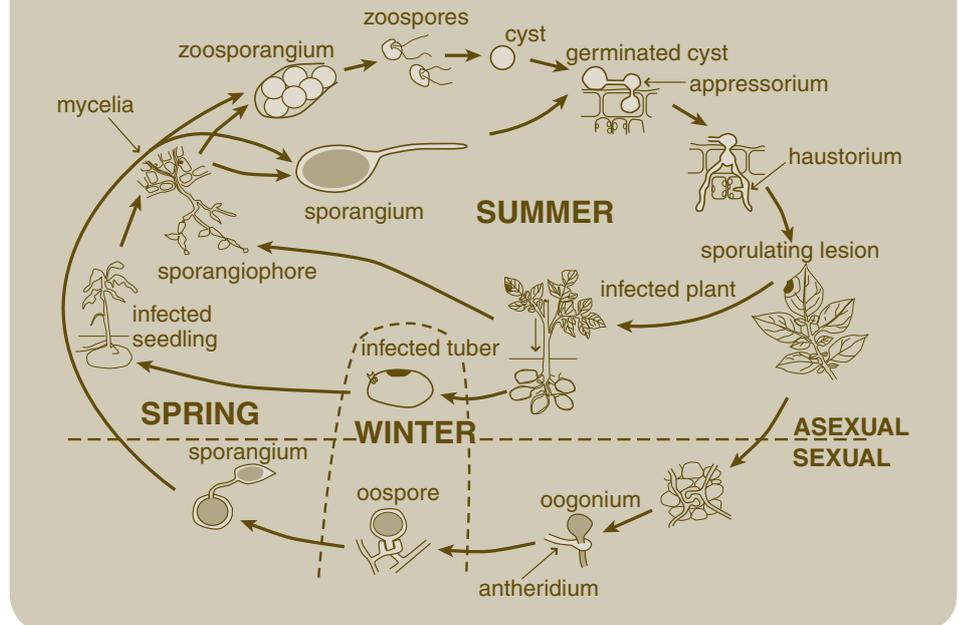


in the stages from which the infection process begins. The zoospore is motile in water and swims in search of host tissue. Some 10 years' research at Aberdeen on the nature of the signals that allow zoospores to locate plant roots, and on patterns of accumulation of competing zoospore populations at well-defined regions around roots, has led to progress in understanding these processes. In particular, calcium is liberated upon encystment and taken up again at germ tube growth in *P. palmivora*, suggesting it has a key regulatory role in pre-infection processes. We have also developed reporter gene systems in *P. palmivora* that allow the monitoring of gene expressing *in vivo*, *in situ* and in real time without harming the infection structures, a useful tool for studying host-pathogen interactions.

Zoospores may respond to a chemical gradient associated with root exudates to find their host, but directional swimming (taxis) has not been demonstrated unequivocally. However, plant roots act as batteries in the soil, and endogenous circulating ionic currents and associated electrical fields in the rhizosphere have been measured with voltage-sensitive vibrating electrodes. Importantly, these electrical fields have a reproducible profile along the root surface and the total inward and outward ionic current remains balanced as a whole. Zoospores move towards electric fields (electrotaxis) generated by ionic circulation from root cells, and research at Aberdeen has confirmed that zoospores are genuinely electrostatic in fields smaller than those capable of being generated in the root environment. Zoospores of *P. palmivora* migrated towards the anode (positive electrode) while those of another oomycete, *Pythium aphanidermatum*, migrated towards the cathode (negative electrode). The direction of movement in an electrical field was governed by differences in the electrical charges of anterior and posterior flagellae.

Recently, by extending these *in vitro* experiments to real roots of plants including cocoa, we have shown that there is a close correlation between *in vitro* electrostatic behaviour and sites of zoospore accumulation at natural root anodes and cathodes. Zoospores of *Phytophthora palmivora* swam towards and encysted preferentially on anodic regions of plant roots but were repelled from cathodic wound sites. In contrast, zoospores of *Pythium aphanidermatum* were attracted to wounds and to cathodic regions behind the root apex, but were actively repelled from the root apex. Mixtures of zoospores of the two species added

Life Cycle of *Phytophthora* (from the potato pathogen, *P. infestans*)



together became spatially segregated at the root surface. It seems that electrotaxis is an important and dominant short-range guidance cue that determines zoospore localisation patterns around roots.

Seeking Targets

We are currently analysing the specific role of candidate genes including those encoding calcium and ion transport into cells selected from a very large expressed sequenced tagged (EST) library database in the zoospore-root interaction. We have screened a set of promising genes for their specific regulation in the different stages of the *Phytophthora* lifecycle and are conducting experiments to obtain and analyse mutants for gene function.

Members of our group have pioneering expertise in the field of gene silencing of *Phytophthora*, functional genomics and proteomics techniques. The proteome is the expressed protein complement of a genome, and accordingly, proteomics is essentially functional genomics at the protein level. With technical support from the Aberdeen Proteomics Facility, we are using this approach to identify proteins specific and important to key stages of infection. We have demonstrated differential expression of proteins isolated from zoospores and germinated cysts of *P. palmivora*. Currently, we are analysing secreted and stage-specific proteins from specific phases of the *Phytophthora* lifecycle. This provides the basis for constructing peptide fingerprint databases of the protein profiles, and enables us to identify interesting proteins. Combining the gene silencing and proteomics approach, we hope to find useful targets for control of *Phytophthora* diseases.

We are hoping to develop research projects in collaboration with other cocoa research centres to investigate specifically the *P. megakarya*-host interaction and what makes it a more aggressive pathogen of cocoa than other species, using some of the techniques and approaches outlined here, and also hope to tackle other major diseases of cocoa in the near future. We believe these approaches can improve understanding of the molecular basis of host-pathogen interactions and offer effective ways to identify targets for disease control and enhance crop improvement programmes. Please, contact us for further information on our research and a reference list, or with any enquiries on the issues discussed in this article.

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Recognising Clues to Vertebrate Thieves

Cocoa crop losses attributed to vertebrates vary from place to place, from less than 15% in Ghana and Nigeria and 20-50% in Sierra Leone to 80-100% in some cases in Fiji and Malaysia. In Malaysia, vertebrates are a problem in a number of crops, with recorded losses in cocoa as high as RM470/ha/week [US\$1 = RM3.8].

Worldwide, more than 60 vertebrate species have been recorded as potential cocoa depredators. Parakeets and woodpeckers



The ripe pod damage (A) suggests squirrel attack. The hole is big and devoid of beans. Squirrels prefer ripe pods, but will attack matured unripe pods in their absence. The unripe pod damage (B) is characteristic of a rat. The hole is smaller with bean remnants in the damaged pod. The pectin layer of the husk is still quite extended and the gradient of the damage is gradual from the outer green layer to the edge of the hole. Closer examination of pod and husk reveals more: only 2-3 teeth chips were made to reach the beans in (A), consistent with the larger teeth of a squirrel, but more chips were needed to reach the beans in (B), suggesting a smaller animal such as a rat (A: S. Soetikno; B: J.D. Mumford)

are serious pests in Brazil, Jamaica and New Hebrides. Cattle, elephants and buffaloes are the menace in Africa. Monkeys, wild pigs, civet cats, deer, rats and squirrels inflict severe losses in Malaysia. This diverse array highlights the importance of being able to identify the animal species responsible for observed damage, as well as knowing the serious vertebrate pest(s) in a particular area. Systematic procedures and strategies for management and control are needed, as the approach for each species is different. Here we look at how vertebrates can be identified from the trademark clues they leave behind them.

Pods may be damaged at any age, and are attacked irrespective of their position on the main stem or within the canopy. The type of damage can help to identify the culprit.

- Monkeys move in groups of 8-12, tramping through cocoa fields, nipping off young tender shoots, ripping pods from trees and scattering husks all over the place.
- Wild pigs are indicated by disturbed soil, footprints, detached budded scions of seedlings, injured flower cushions, damaged pods with only the upper half of the cocoa pod husk dangling from the tree and pilfered beans with chunks

of husks scattered on the ground.

- Civet cat damage will leave the upper half of the pod still dangling from the tree, chunks of pod husks scattered on the ground, and no pilfered beans - but piles of cocoa beans will be found in faeces in the area.

Cocoa pods with holes bored in them and perhaps remnants of beans in the pods are symptomatic of damage from squirrels, rats and woodpeckers. A discerning eye can tell the difference (also see photos).

- Squirrel damage is recognised by the relatively larger hole, bigger husk chippings and steeper hole gradient, and there are usually beans devoid of mucilage scattered over the ground.
- Rats, in most cases, will leave bean remnants in the damaged pods, and there will also be damaged and bitten beans on the ground.
- Woodpeckers leave the smallest hole of the three, bored more-or-less horizontally straight into the pod. Beans will have been pulled out and the mucilage fed upon. In most cases >60% of the beans are left in the pod and are predisposed to secondary fungal infection.

It is important to be able to differentiate the damage between pests such as civets and wild pigs, or rats and woodpeckers, as the strategies and approaches towards their management and control are totally different. Similar measures are sometimes be used for different pests, e.g. physical and mechanical approaches to rat and squirrel control (trapping and shooting), but chemical strategies differ, and rodenticides deployed against rats would be ineffective against squirrels. Thus a pest problem erroneously identified can lead to inappropriate strategies of control, and wasted effort and resource utilization. Clearly, the need for correct identification of vertebrate pest damage is an essential step towards an understanding of the problem and applying the right management tools and strategies to ensure maximum output and minimum costs in addition to harmonizing with the environment.

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Conference in Prep

The 14th International Cocoa Research Conference will be held on 13-17 October 2003 at the International Confer-

ence Centre, Accra, Ghana. Deadlines for submission of summaries and full papers are 31 January and 31 May, 2003, respectively. Send a copy of each document to both officials below, who can also supply further details and a registration form. Intending participants should register and pay by 1 September 2003.

Secretary General (Re: 14th International Cocoa Research Conference), Cocoa Producers' Alliance, Tafawa Balewa Square, National Assembly Complex, PO Box 1718, Lagos, Nigeria
Fax: +234 1 263 5684
Email: copal@alpha.linkserv.com

Chairman, National Organizing Committee, 14th International Cocoa Research Conference, Ghana Cocoa Board, PO Box 933, Accra, Ghana
Fax: +233 21 667104/674302/227291
Email: cocobod@africaonline.com.gh

The INCOPED 4th International Seminar and INGENIC Workshop will follow the conference. Contact Joao Luis Pereira (pereira@cepec.gov.br) about INCOPED and Michelle End (michelle.end@bccca.org.uk) about INGENIC.

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