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

Working for the Future

The fungus *Trichoderma* has for some years been one of the brightest hopes for disease biocontrol, and has found applications in a number of crops. In cocoa its use has continued to be pursued in the laboratory and field, but we are still some way from a final product despite promising experimental results. In this issue Bryan Bailey of the US Department of Agriculture – Agricultural Research Service (USDA-ARS) indicates the key research questions which still need to be answered, in particular how the mechanisms by which endophytic *Trichoderma* impede pathogens can be exploited to facilitate its development as an effective biocontrol field agent against cocoa pathogens.

Back in June 2002 when this newsletter was launched, we ran an article detailing the global threat to cocoa from pests and diseases that were at the time regionally isolated. We pointed out the potential for them to move into new regions. Since then frosty pod rot (*Moniliophthora roreri*) has continued its progress through Latin America, arriving in Mexico in 2005, the cocoa pod borer (*Conopomorpha cramerella*) invaded Papua New Guinea in 2006, and *Phytophthora megakarya* (black pod) continues its spread through the cocoa growing countries of West Africa and was reported in western Côte d'Ivoire in 2007. The global cocoa community is responding to the invasive threat. We report on a meeting held in July this year in Côte d'Ivoire to develop a programme to identify and address these risks and develop contingency plans so as to be prepared should an invasion occur.

The Côte d'Ivoire meeting highlighted the need for rapid action, and for the knowledge and tools to facilitate this. The next article, based on a review in the journal *Biological Invasions*, describes innovations in DNA-based monitoring tools and how these are or could be used in cocoa.

We end the issue with a story of recovery from Central America. The indigenous Maya people of Belize have been growing cocoa for centuries using traditional methods. Following disasters brought about by world markets, climate and disease, links forged with the UK company Green & Black's have allowed them to rebuild an organic cocoa industry. As the organic sector booms, there are plans to expand this across national borders to include the Maya cocoa growers of neighbouring countries.

Improving Efficacy of Cocoa Disease Biocontrol: a Way Forward

There are several biocontrol programmes reaching the end of multiple year field experiments using *Trichoderma* to control cocoa diseases. These programmes use different isolates of varying species of *Trichoderma* against diverse cocoa diseases, including black pod (*Phytophthora* spp.), witches' broom (*Moniliophthora perniciosa*), and frosty pod (*M. roreri*), but have produced some strikingly similar results;

- *Trichoderma* applications enhance flowering and fruit set.
- The application of the fungus shows only limited influence on the reduction of diseased pods.
- Yield is increased because of the increase in initial pod set.
- Accumulating evidence indicates that repeated application of *Trichoderma*, over multiple years, tends to lead to continual improvements in disease control.

Several different *Trichoderma* species have been shown to colonise cocoa flower cushions. The flower cushion (Figure 1A) appears to be an ideal environment for *Trichoderma* survival since it includes available moisture for extended periods of time and nutrients originating from several sources including decaying flowers, other associated decaying plant material, and perhaps most importantly, other fungi. These other fungi may be weak pathogens that limit flowering and fruit set but may also serve as food for *Trichoderma*. It is unclear if this capability is sufficient to justify biocontrol applications by farmers, but the capability of *Trichoderma* to colonise flower cushions and enhance flowering and pod set has one obvious advantage. In some locations it may be possible to increase fruiting by a limited number of applications applied over a narrow time period: the time of flowering.

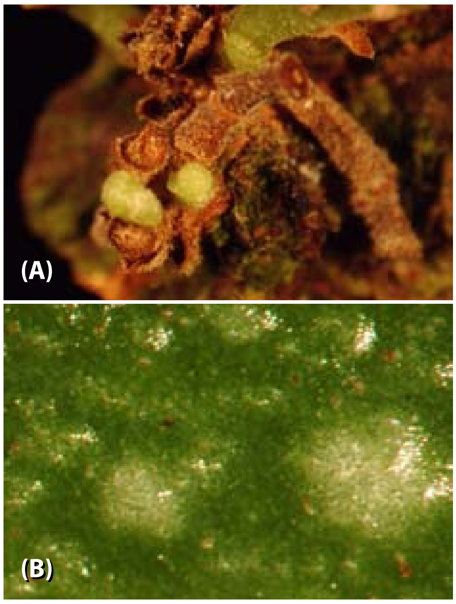

Fig. 1. Close-up of a cocoa flower cushion (A), a potentially hospitable environment, and a cocoa pod surface (B), a hostile surface to colonisation by Trichoderma. *(Bryan Bailey)*

The bigger question remains: "Why do we not get better control of disease in the developing fruit?" This could be a basic limitation of the biological system encompassing *Trichoderma* species, cocoa, and cocoa diseases. Compared to the flower cushions, cocoa pod surfaces tend to be a more hostile environment for the establishment of fungal populations because they have properties aimed at limiting disease

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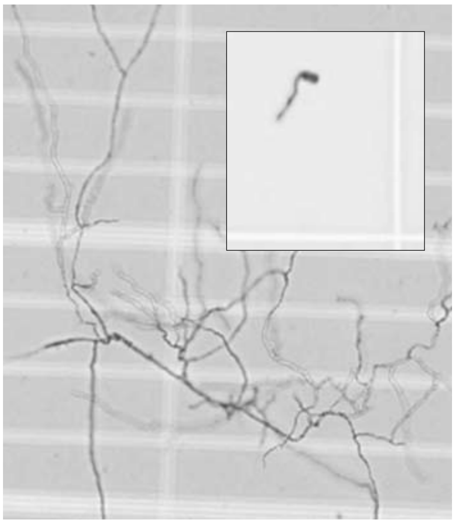

Fig. 2. Trichoderma hamatum *spore germination and growth after 24 h: growth in extract from cocoa stem (0.2% mucilage) and [inset] growth in water. (Bryan Bailey)*

and otherwise protecting the pod. The cuticle and waxy surface (Figure 1B) allow the pod to shed water, which is required for the growth and development of both pathogens and *Trichoderma*. It is unclear if the available nutrients on the pod surface are capable of supporting the growth necessary for colonisation by *Trichoderma*, especially in the time required after a biocontrol application. Yet, we know that fungi do colonise pod surfaces. Both beneficial and pathogenic fungi can be isolated from pods and the application of *Trichoderma* to cocoa pods under the appropriate conditions results in colonisation. The difficulty is that events resulting in the establishment of a pathogen can result in the loss of the entire pod, and in some cases the entire crop. In contrast, successful colonisation events by *Trichoderma* may protect only a limited area around the initial inoculation point and be insufficient to protect the pod from subsequent infection and disease.

Considering potential limitations to protecting pods from disease using biocontrol, the primary questions are: (1) Can we enhance the colonisation levels of *Trichoderma* on cocoa pods by formulation and/or isolate selection? or (2) Can we enhance the activity of the limited *Trichoderma* populations established on pods after treatment?

Current *Trichoderma* formulations, which include the matrix in which the isolate is applied to the cocoa tree, are principally water but could also include an unending list of compounds to enhance colonisation. Isolate selection could result in the identification of *Trichoderma* isolates better able to compete in the cocoa environment, in this case the pod surface, and/or better able to perform some biocontrol function. Finally, by understanding the biocontrol functions or actions, we can seek to exploit that activity by adapting methods to enhance it.

The short answer to the questions posed above is that we have every reason to expect we can improve the efficacy of biocontrol of cocoa diseases by *Trichoderma* through formulation, isolate selection, and/or exploitation of *Trichoderma*'s unique activities. However there is work to be done. We have studied only a few interactions of literally hundreds possible. The number of *Trichoderma* isolates extensively field tested numbers only in the tens and represent only a few of the many potential *Trichoderma* species available in the collections of researchers.

To date very few formulations have been adequately tested. The majority of formulations being used include surfactants as the only additives. Surfactants break down the hydrophobic barriers that result in plant surfaces repelling water. The use of a surfactant should result in the biocontrol agent (*Trichoderma*) spreading uniformly over the plant surface. Only limited testing has been carried out using formulation additives that serve as sources of nutrition for *Trichoderma*, extend the wet period after application, or otherwise enhance the biological activity of *Trichoderma*. For *Trichoderma* to grow it must have nutrition (Figure 2) and moisture/humidity. Inclusion of nutritional additives can greatly increase the growth of *Trichoderma* in a formulation. We also know that *Trichoderma* requires moisture for at least several hours after application if it is to grow and colonise cocoa tissues, yet most liquid formulations being used today would be expected to dry soon after application to exposed surfaces such as pods. Under these conditions it is likely that most of the *Trichoderma* spores never germinate, much less colonise the targeted tissues. Spores are the fungal reproduction entities included in most biocontrol formulations applied to cocoa. In the few cases where more complex formulations have been tested, the studies have been short term, with only a narrow range of isolates (sometimes a single isolate), or have had limited data gathered to quantify the resulting success of the biocontrol application. More complex formulations offer an intriguing advantage, heavily colonised cocoa pods, but also raise some new potential problems. As previously stated, extending the wet period or including nutrients that benefit *Trichoderma*, can also benefit other organisms that might be in direct competition with the *Trichoderma* and in some cases could be pathogens of cocoa. But, the possible complications should not limit experimentation with these more complex formulations since there are likely novel solutions to these potential negative impacts.

It should be clear that the goal of formulation is the establishment of large stable populations of *Trichoderma* on the cocoa tissue surfaces (epiphytes, Figure 3A) or inside the cocoa tissue (endophytes, Figure 3B). This is important since it offers the potential to limit the number of applications required to achieve the desired level of disease control. If the *Trichoderma* dies soon after application, it loses its potential to effectively control disease and may lead to the requirement for additional applications. In the case of endophytic associations, *Trichoderma* is placed within the protected environment of the plant tissues, an environment that certain isolates of *Trichoderma* are specially adapted to exploit for the mutual benefit of both the *Trichoderma* and the cocoa tree. Being a living thing, *Trichoderma* has the potential to proliferate, spread, and colonise new tissues as the tissues grow. This is an activity not possessed by chemicals and may allow a limited number of colonisation events to colonise the entire pod. This is a driving force behind the excitement concerning endophytic *Trichoderma* and to not try and take advantage of this seems short-sighted. Persistent and pervasive colonisation may not be required for biocontrol to work but it is unlikely to cause negative effects or require much additional effort.

It is in the specialised endophytic associations where the potential for *Trichoderma* as a biocontrol agent enters a new area of disease control mechanism. While on the cocoa tissue surface, *Trichoderma* can function by mechanisms such as antibio-

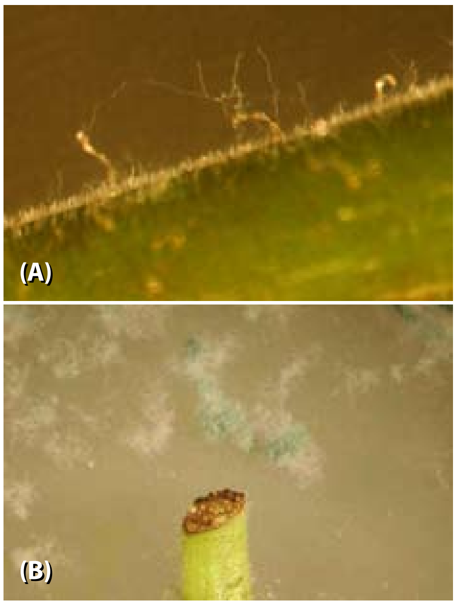

Fig. 3. Trichoderma ovalisporum *(A) growing on the cocoa stem surface (epiphyte) and (B) growing out from inside a sterilised cocoa stem (endophyte). (Bryan Bailey)*

How *Trichoderma* Controls Disease

Trichoderma species are common components of the soil mycoflora; they may also be isolated from above-ground parts of plants. There are several mechanisms by which they may confer protection from pathogens (and pests) on their host plants:

- **Niche exclusion**: outcompeting a pathogen for nutrients, or physically excluding it. In soil, *Trichoderma* may outcompete other microbes for nutritients, space and seed exudates that stimulate fungal propagule germination; *Trichoderma* species on leaves may inhibit pathogen synthesis of enzymes such as pectinases that are needed for leaf penetration[2].
- **Antibiosis**: producing metabolites (e.g. antibiotics) that hinder pathogen growth is a well known property of *Trichoderma* species. *Trichoderma theobromicola* and *T. paucisporum*, isolated from cocoa, produced a volatile/diffusible antibiotic that inhibited development of *Moniliophthora roreri* (frosty pod rot) in vitro[3].
- **Mycoparasitism** or hyperparasitism: typically by coiling round a pathogen and forming appressoria on its surface. The parasitising *Trichoderma* then produces fungitoxic enzymes and perhaps antibiotics which lyse the pathogen's cell walls and allow *Trichoderma* hyphae to enter[2]. When grown together in vitro with *M. roreri*, *T. ovalisporum* completely colonised the pseudostroma of *M. roreri* and suppressed its growth[4].

Isolates/species of *Trichoderma* frequently colonise host plant cells, notably the outer few layers of root cells, although some colonise stems, leaves – and in the case of cocoa, the pods. This endophytic colonisation provides additional opportunities for biological activity:

- **Induced resistance**: activating the plant's physical and chemical defence systems so that a subsequent pathogen challenge results in a strong and rapid response. It may be localised or systemic (i.e. act either at, or at sites remote – in space and time – from, the *Trichoderma* infection). The host plant's defence responses can be stimulated by many products originating from *Trichoderma*, including enzymes, other peptides, secondary metabolites, and cell wall fragments. Many of these products function as elicitors inducing the plant to produce phytoalexins (compounds toxic to many fungi), PR-proteins (including hydrolytic enzymes that attack fungal cell walls), and altered cell structures[2].

  Trichoderma stromaticum isolate FA 323 reduced incidence of *M. perniciosa* (witches' broom disease) by more than 70%; systemic induced resistance was implicated because the amount of total protein expressed in asymptomatic plants inoculated with both FA 323 and the pathogen was much higher than in plants inoculated with *M. perniciosa* or water alone[5].

sis, mycoparasitism, and/or niche exclusion (see Box).

Antibiosis is the production of chemicals that kill or inhibit other organisms. Depending on the isolate, these capabilities are well documented for biocontrol by *Trichoderma* and likely function in the flower cushion resulting in the enhancement of cocoa flowering and fruit set. But it is the endophytic association, the penetration of cocoa tissues by *Trichoderma* and direct interaction of the *Trichoderma* mycelium with plant cells, that has scientists working with other crops most excited.

There are numerous cases now documented in other crops where *Trichoderma* induces resistance to disease (see Box, and for review see [1]). With induced resistance the plant responds to the beneficial organism by producing a defence reaction, that, while possibly limiting the growth of the beneficial organism, also prevents disease development by subsequent infections by pathogenic organisms. For cocoa this would mean endophytic colonisation by *Trichoderma* could induce resistance to diseases such as frosty pod, witches' broom, or black pod. *Trichoderma* species are known to establish endophytic associations with cocoa. The cocoa responds to the *Trichoderma* by altering its gene expression and this includes enhanced expression of genes potentially involved in resistance to diseases and other stresses[2]. The genetic markers for carrying out evaluations of induced resistance continue to expand rapidly. It is the elucidation and exploitation of induced resistance in cocoa that may offer the greatest potential for biological disease control by *Trichoderma*.

This research also has something new to offer to the greater biocontrol research community. With annual crops *Trichoderma* is typically applied to the seed or soil. Cocoa offers to the field of biocontrol the unique aspect of having *Trichoderma* applied to a perennial tree, where interactions with the pathogens and plant can take place directly in the above ground portions of the tree. Under these conditions induced resistance can be stimulated directly in the targeted tissues. It is likely these interactions can continue over multiple years on the same tree and it is the endophytic association between cocoa and *Trichoderma* that will allow this to succeed. We know very little about the endophytic association between *Trichoderma* and cocoa but we are gaining a greater understanding with each experiment.

[1]Harman, G.E., Howell, C.R., Viterbo, A. & Chet, I. (2004) *Trichoderma* spp. – opportunistic avirulent plant symbionts. *Nature Reviews* 2, 43–56.

[2]Bailey, B.A., Bae, H., Strem, M.D., Roberts, D.P., Thomas, S.E., Samuels, G.J., Choi, I.-Y. & Holmes, K.A. (2006) Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. *Planta* 224, 1449-1464.

[3]Samuels, G.J., Suarez, C., Solis, K., Holmes, K.A., Thomas, S.E., Ismaiel, A. & Evans, H.C. (2006) *Trichoderma theobromicola* and *T. paucisporum*: two new species isolated from cacao in South America. *Mycological Research* 110(4), 381–392.

[4]Holmes, K.A., Schroers, H.-J., Thomas, S.E., Evans, H.C. & Samuels, G.J. (2004) Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. *Mycological Progress* 3(2), 199–210.

[5]Holmes, K.A., Evans, H.C. & Pomella, A. (2002) Insider knowledge: endophytes for cacao disease control. *Biocontrol News & Information* 23(4), 86N–88N.

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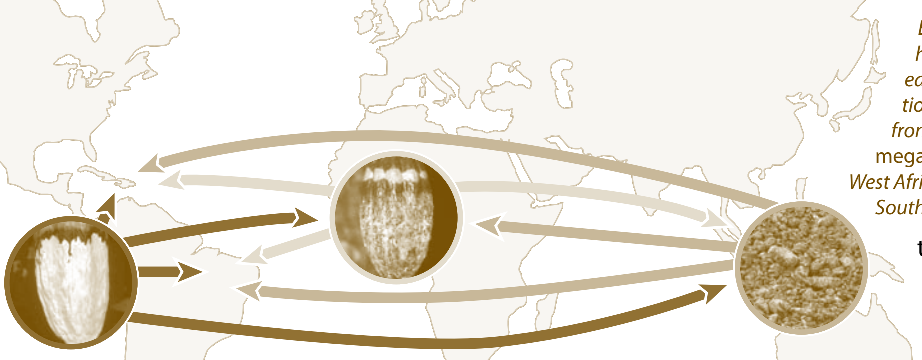
Meeting Threats of Pest & Disease Globalisation

Currently, cocoa pests and diseases have a regional distribution so frosty pod (*Moniliophthora roreri*) and witches' broom (M. perniciosa) are confined to Latin America whilst cocoa pod borer (*Conopomorpha cramerella*) remains restricted to South-east Asia and the Pacific but what would happen to cocoa production if one of these constraints were to spread to other continents? That was the question that occupied the minds of over 100 participants from 14 countries at a workshop held in Abidjan, Côte d'Ivoire on 17–20 July 2007. The workshop was organised by ICCO (the International Cocoa Organisation) and CABI with funding from CFC (Common Fund for Commodities) and local organisation by ARCC (Authorité de Regulation du Café et du Cacao) and CNRA (Centre National de Recherche Agronomique).



Each cocoa growing region harbours invasive pests and diseases that could threaten production worldwide, e.g. frosty pod rot from South America, Phytophthora megakarya *black pod disease from West Africa, and cocoa pod borer from Southeast Asia*

The aim of the workshop was to discuss the increasing threat of the globalisation of cocoa pests and diseases and to decide (a) on a strategy to minimise the risks of globalisation occurring and (b) how cocoa stakeholders and authorities should respond should an alien pest or pathogen be introduced into a new region. The participants, many of whom are experts in their chosen areas of cocoa breeding, pathology, entomology, agronomy, farmer training, etc., came from Southeast Asia and the Pacific, Latin America, Africa and Europe. They shared their collective experience in scientific presentations and in brainstorming sessions.

The workshop was held under the distinguished patronage of his Excellency, the President of Côte d'Ivoire, the patronage of the Prime Minister and the co-presidency of the Ministry of Agriculture and the Ministry of Higher Education and Scientific Research.

The workshop was formally opened by his Excellency, Mr Amadou Gon Coulibaly, Minister of Agriculture who made a keynote address in which he expressed his concern about the impact of cocoa pests and diseases on the social and economic development of the country.

The first scientific session (Session 2) involved presentation of work conducted in Brazil on witches' broom disease since 1989 when the disease was introduced into Bahia, the country's main cocoa producing area. Hearing about use of surveillance techniques, adoption of *cordon sanitaires* and attempts at eradication when the disease was first introduced, together with the subsequent development of resistant germplasm produced through breeding programmes enhanced via molecular techniques and used in conjunction with biocontrol as a management strategy, proved a very useful "lessons learnt" approach for participants from other regions.

In Session 3, there were five presentations concerning the current status of five more major constraints to cocoa production. Frosty pod, a devastating fungal disease confined currently to Latin America, probably represents the most serious threat to cocoa production globally, having replaced witches' broom in all countries where both now co-exist. The spores are long lived, and can travel long distances and withstand environmental extremes. The other constraints discussed were cocoa pod borer, a pest confined to Southeast Asia and Papua New Guinea, and a triumvirate of some mirid species, the aggressive black pod *Phytophthora megakarya* and CSSV (cocoa swollen shoot virus) which are confined to West Africa. Management practices are available for the insect pests but *P. megakarya* and CSSV remain as major challenges for West Africa and potentially to other cocoa producing regions, should they escape to them.

In Session 4, presentations examined how to prevent the globalisation of these pests and pathogens and included the concept of Pest Risk Analysis, with comparisons to other crops. Here, pathways by which alien organisms could invade countries can be identified and, once identified, management of the pathways can be undertaken. The concept of biosecurity planning was discussed, with countries now planning pre-emptive action to exclude pests rather than aiming to manage the situation after an alien pest arrives; cocoa industries within each country should develop their own Biosecurity Plans.

One potential pathway for introduction of alien pests and pathogens is via exchange of germplasm for breeding programmes, but mechanisms for the safe movement of germplasm are in place. Participants were alerted to the need for third country quarantine (often in Europe) which allows the safe movement of cocoa material from one cocoa producing area to another. This has been a crucial facet of some of the on-going global breeding programmes. Post-entry quarantine in-country is also needed, entailing phytosanitary certification under the auspices of national and regional phytosanitary organisations. The final presentations in this session dealt with the need for improved surveillance and quarantine for disease free countries, and for mass methods of information dissemination to sensitise farmers to the symptoms of major pests and diseases that threaten the region so that if invasion occurs, rapid detection will also occur. This is vital: if a pest is introduced then the more rapidly the authorities are alerted, the greater the chances that rapid action can be undertaken to eradicate it.

In Session 5, the potential of resistant germplasm in the management of the pests and diseases was discussed. Major advances have been made over about the last ten years with networks of breeders being established worldwide and improved material (for disease resistance, yield and other traits) being developed in the International Cocoa Collections at CATIE (Costa Rica), CRU (the Cocoa Research Unit, Trinidad) and in Brazil such that material is now being passed through third country quarantine and will be available to other national and regional breeding programmes. This improved germplasm provides an opportunity for preventative breeding, as it may be imported into cocoa producing countries to be tested, which would include assessing how material bred for resistance to alien pests and pathogens not yet present responds to local climatic and edaphic conditions and indigenous pests and pathogens.

In the final scientific session, participants took part in facilitated group discussions concerning the components of a strategy for preventing the globalisation of cocoa pests and diseases and how best to manage the situation if the worst case scenario should occur. Some of the key points are outlined below.

- Prior to any pest or disease incursion but in preparation for such an event, commitment and co-operation from all stakeholders in-country is considered essential and biosecurity planning by the whole cocoa sector is needed – including preparation of PRAs to identify key pest pathways into cocoa producing countries. Once identified, these risks can be managed. Linked to this is the urgent need for preparation of emergency response plans and contingency planning involving all cocoa stakeholders and the relevant government authorities.

- Linked to the above, improved surveillance is necessary for threats, both regionally and globally, with international collaboration needed to share expertise and experiences of management practices elsewhere.
- New legislation may be needed in-country to enforce any action that may have to be undertaken such as setting up cordon sanitaires, eradication of infected material, giving compensation to growers – and that would mean a commitment of finances in some sort of national action plan which would involve all key government agencies.
- Raising awareness of these issues with policy makers is essential so they recognise the threats and commit the necessary finance and resources both in advance of any incursions and after any that do occur.
- Raising awareness among producers and all stakeholders in the cocoa supply chain is important to alert them of the symptoms of pest and disease that threaten a country so that early detection is possible in the event of one of them being introduced.
- All stakeholders along the supply chain also need to be alerted that if a particular pest or disease invades, stringent measures such as eradication may be needed but that some form of compensation will be made available.
- Raising awareness is also needed to encourage producers to obtain improved material from certified sources and to discourage growers from using 'unofficial germplasm'.
- Preventative breeding needs to be implemented such that all local breeding programmes receive resistant material that can be tested under local conditions in conjunction with other management practices such as cultural, chemical and biological control, and proven material can be multiplied for distribution to growers.
- Raising awareness in each country's plant health authorities to the threat of alien pests and diseases is necessary. In particular they need information on how to recognise the symptoms and how to control the invasive pests and diseases. Port authorities, customs staff and border posts, etc., need to be alerted to potential introductions. Improvement of post-entry quarantine is also recommended.
- Finally a large amount of training and capacity building is needed – for producers, and right through the supply chain, to port and border officials, plant health officials, etc. Recognition of the symptoms to aid early detection and training in management techniques were two aspects highlighted.

The proceedings are available to participants on the ICCO website. The outputs of the workshop will be used as the basis for a project proposal to the CFC facilitated by the ICCO.

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DNA-based Monitoring of Disease Processes

DNA-based approaches to the detection, identification and monitoring of disease-causing organisms hold great promise for the future. But while field biologists may dream of a hand-held tool to give them instant identification of a morphologically unidentifiable specimen, a paper by John Darling and Michael Blum in the journal Biological Invasions[1] puts the potential for DNA-based methods in perspective by reviewing the prospects for molecular methods for monitoring invasive species. (From the perspective of the biocontrol scientist, this could be applied to natural enemies.)

In terms of traditional taxonomy, morphological identification has become more accessible by the development of dichotomous and multi-access (matrix-based) electronic keys. However, there are limitations to what can be achieved through morphological inspection; there may not be a key for the group you are interested in; knowledge of the group may be insufficient; morphology may not be sufficient (e.g. for identifying immature arthropod stages, plant samples without flowers, and the not uncommon situation where related innocuous and invasive taxa are morphologically indistinguishable). This is where DNA-based methods come into their own: an individual's DNA is the same in every cell, immature or mature, root or flower; it can be distinguished from that of another species or even strain. Bridging the gap between the theoretical possibilities and devising practical tools for identifying species in field-collected samples is a challenge for molecular biologists – and even more of a challenge for non-molecular scientists to understand.

This is the complex issue molecular biologists Darling and Blum set out to explain in a concise article reviewing current and 'in development' methods of DNA diagnostics, which we summarise here. They discuss what tool(s) would be most appropriate for a variety of purposes taking into account their cost-effectiveness. They give examples of the use of the tools (where this has happened), and where use is so far restricted to a few taxonomic groups, they consider its potential for other groups. The article is enhanced by a tabulated summary of the tools they describe, and charts illustrating how to decide the best tool(s) for a particular circumstance.

The table represents a summary of eight potentially useful DNA-based technologies, giving a simple description of the procedure and explaining how the information this generates might be useful for monitoring purposes. Many of these methods are already applied to disease identification questions within tropical agriculture and specifically to cocoa. Some typical cases are listed below.

- PCR/RFLP (polymerase chain reaction/ restriction fragment length polymorphism). PCR primers which specifically amplify DNA from a narrow range of target species, typically a group of relatively closely related taxa (e.g. all species in a genus). Diagnostic species-level nucleotide differences within the amplified region result in species-specific patterns of restriction enzyme digestion sites, which can be detected by agarose gel electrophoresis.
- DNA barcoding. This is a new terminology for using DNA sequence information from short (<1000 base pair) regions of the genome that are informative in terms of distinguishing between species. These regions show very little nucleotide variation between individuals within a species, but substantial variation between individuals of different, even closely-related, species. By placing sequence information derived from the unknown sample into phylogenies built from reference sequence databases, it is possible to assign unknown specimens to species.

  For example the two approaches described above were employed by Appiah *et al.*[2] who used DNA sequence data and restriction patterns from the internally transcribed spacer (ITS) regions of the ribosomal RNA (rRNA) gene cluster to differentiate isolates of *Phytophthora* species involved in pod rot, stem canker and leaf blight of cocoa, to provide a rapid identification procedure for these species, being able to distinguish isolates that had previously been misidentified by morphological methods. Detailed sequence analysis and comparison with published literature suggested that *P. capsici* isolates from cocoa may be closely related to *P. tropicalis*, a species recently described from *Cyclamen* and *Dianthus*. A similar approach was used in the identification of *Trichoderma*[3], and in the identification of possible biocontrol agents for witches' broom disease[4].

- SSP (species-specific PCR). PCR primers are designed to specifically amplify DNA from all representatives of only one target species. This approach has been applied to the detection of witches' broom disease.
- qPCR (quantitative PCR) accurately estimates the amount of DNA from a specific organism that is present in the sample. One example of the utilisation of this technique has been in the quantification of endophytic *Trichoderma* infection of cocoa seedlings linked to biocontrol of cocoa disease agents[5].

Importantly, Darling and Blum identify three variable characteristics of monitoring tasks that affect which type of DNA diagnostic tool is most useful:

- Sample complexity: are you working with samples of single individuals of unconfirmed identity, or complex biological communities drawn from environmental samples?
- Quantisation: is a simple presence/absence sufficient, or do you need more detailed information about species abundance; e.g. propagule pressure?
- Target specificity: do you need to detect and identify a single species, multiple species, or all species in a complex sample?

The methods and limitations of the identification and monitoring system coupled with the complexity, quantisation and specificity of the sampling inform the selection of the most appropriate methods.

Darling and Blum present a decision making flow chart, which acts as a guide to the most appropriate methodology given the questions about the sample that need answering. It contrasts simple to complex samples, presence/absence to quantitative abundance information, and a comparison of single to multiple species. Thus, depending on your need – whether you need to confirm the identity of a specimen, or have it identified; to screen for presence of various target species, or assess their propagule pressure; to have information on species composition, or on abundance as well – the various tools are designated as 'best', 'questionable' or 'inappropriate'.

The actual and potential uses of DNA diagnostics in each of the six options from the flowchart are discussed. For each, the authors identify the most appropriate tool(s) and describe the process of extracting and testing DNA, together with the problems and likely causes of error in results. Many of these technologies have been developed successfully for looking at microbial communities. In fact, microbial community ecologists often do the type of thing that might be most difficult: generating complete descriptions of the biodiversity present in a complex sample. There is an abundant literature devoted to this, which is well-referenced.

In writing this review, the authors are careful to distinguish between applications that are technically possible, and could be made available give initiative, time and money, from others that remain "hopeful science fiction". They conclude that currently available technology is probably sufficient in most cases to develop tools associated with confirmation of species identity, identification of unknown specimens, and targeted screening of complex environmental samples. However, they say that there are considerable technical challenges to overcome before tools can be developed for assessing species abundance, and (notwithstanding advances for microbes) characterising biodiversity in complex samples. Whether there will ever be the tools to answer the most complex questions remains to be seen but, the authors argue, the benefits of tools that can be developed will be huge and worth time and money spent on them.

[1]Darling, J.A. & Blum, M.J. (2007) DNA-based methods for monitoring invasive species: a review and prospectus. *Biological Invasions* 9, 751–765.

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Reviving Maya Cocoa

The Maya were the first people to discover culinary uses for cocoa. Comparatively little is known about the civilisation although it stretches back some 4000 years and the Maya were once the world's most advanced horticulturalists. Today there are some six million Mayas represented by a number of indigenous tribes living in Central America's Yucatan peninsula and spread across several countries, including Belize, Guatemala and Honduras where they still grow a range of crops including cocoa.

Drawing on a much larger plan which seeks to develop the 'Ruta Maya' (Maya Route) as an inter-country initiative to conserve the region's culture, history and environment and promote its sustainable development, the Toledo Cacao Growers' Association in southern Belize (TCGA) aims to establish and strengthen a consortium of indigenous cocoa producers in Honduras, Belize and Guatemala. As well as breathing new life into cocoa growing in this region, it could become a model for other ethnic groups separated by political borders.

Organic Growth

TCGA was registered in 1985 as a representative body responsible for promoting, fostering and encouraging the growth of cocoa, the orderly and proper delivery to buyers, and the expansion and welfare of the cocoa industry. Its members are small farmers who grow cocoa using a combination of traditional farming practices; poverty meant most of them had never used agrochemicals. These two factors were to prove significant in the development of an organic industry.

By 1991 the TCGA and its 170 members were facing an uncertain future – in company with cocoa farmers worldwide – as cocoa prices fell by more than half, making cocoa growing unprofitable and leading many to abandon the crop and seek work elsewhere. During this bleak period TCGA was approached by the UK-based company Green & Black's (now part of Cadbury Schweppes) who had been the pioneers of organic chocolate. At the time they were importing their beans from various suppliers and were seeking additional sources. In 1993 an agreement was reached between TCGA and Green & Black's which had four elements.

1. A five-year rolling contract as primary supplier of organically grown cocoa for use in Maya Gold chocolate, paying above the prevailing market price under a Fair Trade agreement.
2. Help from Green & Black's in obtaining Organic Certification, which would be a

Organic or Fairtrade?

Sustainability is as often cited as critical for the cocoa industry (sustainability of supply) as for cocoa farmers (sustainable livelihoods). Organic and/or Fairtrade cocoas are growing in importance as niche commodities in this respect. The approaches may seem superficially similar, especially to the consumer who pays a premium for both. Nonetheless, they have different philosophies – but they are largely operationally compatible.

Organic cocoa is a way of farming. It is:

- Produced according to defined international standards.
- Grown without applying synthetic pesticides, artificial fertilisers, and human waste/sewage, relying instead on ecological processes and cycles adapted to local conditions.
- Processed without ionising radiation and additives.

Encompassed within a broader definition of 'organic' is commitment to preserving the environment, a good quality of life and food, and fairness to all involved.

Fairtrade cocoa is a business ethic. It is:

- An alternative approach to conventional international trade.
- A trading partnership that aims to provide sustainable development for excluded and disadvantaged producers.

Fairtrade focuses on trading conditions, awareness raising and campaigning which includes:

- Market participation through direct links with producers, long-term relationships, minimum price + Fairtrade premium, pre-finance support and development of producer organisations.
- Advocacy.
- Resource redistribution, development & empowerment.
- Accountability, transparency & verification.

Further information:

IFOAM (International Federation of Organic Agriculture Movements): www.ifoam.org

Soil Association: www.soilassociation.org

IFAT (International Fair Trade Association): www.ifat.org

Fairtrade Foundation: www.fairtrade.org.uk

Fig. 1. Recovery after Hurricane Iris: trees are still awry but bearing again. (Gregor Hargrove)

valuable asset in the northern European market, with or without Green & Black's in the picture.

3. A cash advance to guarantee members on-the-spot cash for cocoa brought in.
4. Training for key TCGA members in management accounting, correct fermentation and quality control.

TCGA farmers were awarded organic status following an inspection by the Soil Association, and in 1994 Maya Gold was launched, the first British product to be awarded the Fairtrade mark. Since then the TCGA has grown to 1000 members, who have started to replant their traditional Criollo varieties. Annual production of organic beans has grown to some 40 tonnes. Green & Black's still buys all the organic cocoa produced in the Toledo district, paying market prices and guaranteeing a minimum price of US$1600/tonne, a Fairtrade premium of US$150/tonne which is invested in social projects, and an organic premium of US$200/tonne to cover the costs of Soil Association certification. It also funds a cocoa agronomist and local extension officers trained in agronomy and nursery management, and a compliance officer to meet the IT and administration requirements of the certification schemes.

Benefits on top of the economic ones include:

- Strengthening the position of women through their role in postharvest processing.
- Increasing migratory bird populations, through more forest cover and reduced pesticide residues.
- The emergence of TCGA as a main uniting force for the distinct Kekchi Maya and Mopan Maya.
- The respect accorded to leaders of the TCGA in their communities.

The growth of Toledo's cocoa industry is remarkable considering other events that could have derailed it, including drought, Hurricane Iris in 2001, and frosty pod disease (*Moniliophthora roreri*) which arrived in Belize in 2004. The active intervention of farmers in orchard management assured recovery from the hurricane damage and has kept the frosty pod threat well controlled. Issues of land tenure remain challenging but the Government of Belize has promised cooperation in appropriate resolution of the issues in a manner that will give farmers the necessary confidence to continue their investment in cocoa production.

Reaching Across Borders

From its origins as a ceremonial crop, cocoa's importance as a cash crop in Toledo has grown in the last two decades through supplying the lucrative organic cocoa market. Now the growth of the world organic cocoa sector is providing impetus for expansion: an estimated ten-fold increase in supply will be necessary by 2012 to meet demand for Maya Gold alone. Significant planting has been undertaken in the Toledo district, but these trees will take six years to come into full production, and in the meantime TCGA is looking farther afield for sources of organic cocoa. The Maya clans in adjacent Guatemala and Honduras speak the same Maya language and are in many cases directly related to the Maya in Belize. Many of these Maya also produce cocoa but are challenged by the lack of a market in the remote regions they inhabit. However, they grow cocoa using methods similar to the traditional methods that were so easily adapted for Organic Certification in Belize. TCGA is exploring the potential for establishing and merging the efforts of cocoa associations in Guatemala and Honduras. It is extending administrative guidance and technical assistance on a

Fig. 2. Orchards are dark unless, as Agronomist Marco Figueroa is explaining, they are opened up. (Gregor Hargrove)

borderless basis in order to determine the prospects for reviving the cocoa industry in adjacent areas. It is providing technical services for certification and improved quality, and for standardising quality certified cocoa beans from the region for organic markets.

An analysis conducted by the Government of Guatemala and a follow-up study by Green & Black's revealed areas largely contiguous with the Belize border where several indigenous groups are again looking to cultivate cocoa as a cash crop, as it is now perceived to be less affected by price fluctuations than coffee or the other crops they produce. This is a reversal of the recent past when a number of factors led to the neglect of the cocoa orchards in both countries – the decrease in the price of cocoa in an irregular market being critical, alongside damage to trees from Hurricane Mitch. Areas totalling some 600 ha were identified which, with rehabilitation, could support organic cocoa. However, despite the extraordinary size of cocoa beans, favoured by many chocolate companies, postharvesting methods and quality control were considered to need significant improvement. In addition, although keen to re-enter cocoa production, farmers need the reassurance of a guaranteed market before they do so. Green & Black's study revealed strong familial and cultural links uniting people across the border; cocoa production plays the same cultural and ceremonial role in both countries and there is already regular cross-border trade and traffic despite the fact that there is no legal entry point for more than 160 km.

As a first step, TCGA, funded by an organisation working in cooperation with Oxfam UK, entered into an agreement to provide technical assistance in Peten District on the Belize border; farmers have been registered as TCGA members and have been added to TCGA's Organic Certification schedule. This small project forms a model for much larger scale revival of cocoa, under Organic Certification, in the Maya areas of Belize, Guatemala and Honduras.

TCGA hopes to strengthen and provide more services to farmers of the cocoa growing areas of Belize, Guatemala and Honduras through a consortium. With the Peten project underway, TCGA is seeking to realise this aim through:

- Consolidating its own technical and managerial expertise and adding extension officers to work locally in the expanded area.
- Forming linkages with other cocoa growing associations so the initiative can be expanded to other provinces of Guatemala – with the eventual aim of including the Maya cocoa growers of Honduras where extensive areas of cocoa remain untouched since devastated by Hurricane Mitch in 1999.
- Using TCGA technical expertise to help the interested cocoa associations of Guatemala and Honduras revive cocoa production and achieve internationally recognised Organic Certification and Fairtrade status. This will include developing a farmer-friendly training manual and training extension officers in orchard management and postharvesting (fermenting and drying), and will draw on successful methods used in Belize.
- Encouraging strict orchard sanitation and pruning measures with improved air flow combined with prompt removal or diseased pods, which has done much to limit and aid recovery from the impact of frosty pod disease (this is now a serious constraint to production in the region's cocoa, with losses reaching 80% of the crop in Honduras). Farmers are now observing that these improved orchard management practices not only help control disease but also improve yields, giving a return on the investment that is more than just the management of frosty pod.
- Identifying market opportunities and looking at the logistics of buying, assuring quality control, and providing warehousing and shipping for organic cocoa beans from the region; neglect of cocoa in some remote areas has been precipitated in part by lack of access to markets.
- Providing continuing capacity building and technical services into the future, including training and support, for farmers in order to assure and improve the quality of organically certified cocoa beans and ensure compliance with regulations and principles.

Fig. 3. Extension officer Pablo Bol with cocoa pruned after hurricane damage, now showing results with pods on the trees. (Gregor Hargrove)

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