General News

Trichoderma in Biological Control: a Taxonomist Reports

Some have estimated that fewer than 10% of the fungi have been described. Isn't it interesting that almost all of the reports of fungi used in the biological control of diseases caused by other fungi refer to one of about three species of *Trichoderma*, viz. *T. harzianum*, *T. virens* and *T. viride*? Why *Trichoderma* and why just these three?

Is *Trichoderma* a Super Hero of comic book proportions ('Mighty T, Super Fungus'), fighting an unceasing battle against the evil parasites that would deprive us of our daily ... chocolate, for example? Will fungi in other groups give effective biological control? Will other species of *Trichoderma* give effective control of fungus-induced plant diseases? Are all reports based on correct identifications? Are species waiting in nature to be discovered? These are questions that I have been investigating as a research scientist in the Systematic Botany and Mycology Laboratory of the United States Department of Agriculture, Agricultural Research Service (USDA-ARS).

What is a Species of Trichoderma?

In a recent brief article, Christian Kubicek and his collaborators compared DNA sequences of biological control strains identified as *T. harzianum*. Of the eight strains that they studied, half were reidentified as either *T. atroviride* or *T. asperellum*. The low success rate in identifying *Trichoderma* strains to species is not very surprising. This is a genus that presents few morphological highlights: defining and identifying species is difficult. The 'up side' of the lack of diagnostic characters is that few species have been described. Between its first description early in the 19th century and 1984 no more than nine species were included in the genus *Trichoderma*. In 1939 G. R. Bisby could recognize only one species, *T. viride*, and for much of its life as a genus, the vast majority of reports of *Trichoderma* (primarily in ecology) referred to only that one species. The diversity of activities attributed to *T. viride* in the older literature is certainly extraordinary!

In 1969 Mein Rifai reviewed *Trichoderma* and proposed a taxonomy with nine 'species aggregates.' He said of most of these aggregates that they most likely comprised more than one morphologically indistinguishable species. While this was an advance over Bisby's 'single species' taxonomy (which made identification very easy!), it still left the observant user unfulfilled. In order to make an identification, species boundaries had to be very elastic indeed and, given that, there was no confidence that a species name used by different researchers actually referred to the same species. Between 1984 and 1991 John Bissett again took up *Trichoderma* taxonomy. Critical microscopic observations led him to recognize about 35 species, which he distributed among five sections. Obviously there is a great difference between nine species on the one hand, and 35 on the other. Which taxonomy best reflected the species in nature?

In the mid 1990s DNA sequence analysis was first applied to *Trichoderma* taxonomy. DNA sequences provided the much-needed independently derived data that would enable a better understanding of species of *Trichoderma*. A series of papers from Katrin Kuhls and Christian Kubicek and their collaborators established that Bissett's view of *Trichoderma* was a good approximation: there are certainly more than nine species of *Trichoderma*, although only some of his morphology-based groupings, or sections, are monophyletic.

Today, DNA sequence analysis is absolutely essential for the description and characterization of *Trichoderma* species. It is possible that *Trichoderma* is the only genus for which every species is represented in GenBank, the international database of DNA sequences, by at least one partially sequenced gene and many species are represented by sequences of two or more genes. In truth, it turned out that even Bissett's species concepts were not finely enough drawn. Some of the species that he recognized on the basis of their morphology, such as *T. viride* and *T. koningii*, have been divided among two or more species following DNA sequence analysis and a re-evaluation of their respective phenotypes.

Trichoderma entered the modern era of taxonomy (i.e. as defined by DNA sequence analysis) with a small number of species, all of which were represented by correctly identified cultures and at least one gene of each species has been sequenced. Because of this, the likelihood of proposing new species that are the same as previously described species is very low. At least *Trichoderma* taxonomists will not be accused of being 'name changers' by pathologists. This contrasts to genera such as *Fusarium* in which hundreds of species have been placed in the genus since the early 19th century; the finding of older names to replace familiar *Fusarium* names in the literature has been upsetting to some, however necessary the changes. DNA sequence analysis has permitted us to uncover misidentified species: many reports in the literature are based on misidentifications (see, for example, the article mentioned above from Kubicek). While this can lead to confusion in the literature, the end result is an accurately defined species. For example, *T. aureoviride* is often cited in the biological control and ecology literature. However, Elke Lieckfeldt and her collaborators found that despite these many reports, the species is only found in northern Europe (UK and The Netherlands) and is only known in cultures derived from the ascomycete *Hypocrea aureoviridis*. In another example, *T. viride* has long been known as THE species of *Trichoderma* that has globose and warted conidia and, as such, is easily identified. Lieckfeldt and her collaborators examined cultures identified as *T. viride* and found a correlation between DNA sequences and the type of warts, which were more or less conspicuous depending on the strain. They separated *T. asperellum* from *T. viride*. Currently, the soil fungus *T. asperellum* is being evaluated in various biological control applications from head-blight of wheat in Russia, caused by a *Fusarium*, to black pod disease of cocoa (*Theobroma cacao*) in Cameroon, caused by *Phytophthora megakarya*. Pierre Tondje and his collaborators in Cameroon believe that cellulase enzymes produced by the *Trichoderma* might be responsible for degradation of *P. megakarya* cell walls.

Trichoderma stromaticum, which is effective in control of witches' broom disease of cocoa caused by the mushroom *Crinipellis perniciosa* in South America, is the heart of the commercial biocontrol product TRICHOVAB® . This *Trichoderma* was reported first in the literature as *T. viride* and then later as *T. polysporum*. A combined study of morphology and DNA sequence analysis in our lab revealed it to be a new species. Today this species is being applied in areas affected by witches' broom in eastern Brazil, where it reduces inoculum of the pathogen. Indications are that it is becoming established in the area and that sexual reproduction, with possible genetic recombination, is taking place. Prakash Hebbar and Jorge T. de Souza, at USDA-Beltsville and Masterfoods, are investigating this.

The most commonly reported biocontrol *Trichoderma* is *T. harzianum*. However, this species was implicated as the cause of the green mould epidemic of commercially grown mushrooms in North America and Europe. The consequences of *T. harzianum* being a pathogen of such an economically important crop as mushrooms would have been disastrous to biological control. However we demonstrated here at USDA-Beltsville, through study of the morphology and cultural characters of *T. harzianum* and the mushroom parasite combined with use of DNA sequence analysis, that the mushroom parasite is a morphologically similar but phylogenetically distinct new species of *Trichoderma*, *T. aggressivum*. The mushroom parasite can be distinguished reliably from *T. harzianum* by its greatly diminished ability to grow at 35°C.

The bottom line is that one must view reports of identified species of *Trichoderma* with some scepticism. While *Trichoderma* species can be identified using the microscope and cultural characters, the most secure way for most people to identify a species of *Trichoderma* is through DNA sequences. Keys to the identification of most species of *Trichoderma* described up to the year 2000 are available in printed form and an interactive key is available at:
http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm

Can We Predict Biological Control Ability?

DNA sequence analysis has permitted us to 'see' the interrelationships of species through the formation of phylogenetic trees. Are phylogenetically homogeneous groups predictive of biological ability?

Christian Kubicek and collaborators found that relatives of *T. reesei*¸ a strain of which is the industry standard for cellulase production, tended to produce cellulase in higher concentrations than did species outside the *T. reesei/longibrachiatum* group. Members of this group are also able to grow and sporulate at 40°C and have been isolated from humans who have compromised immune systems: the warning is that the use of *T. longibrachiatum* strains in biological control should be considered very carefully with special regard for the possibility that those who prepare or apply the fungus are at high risk of inhaling it. However, in one case a strain of *T. longibrachiatum* that was reported to have biocontrol ability in Costa Rica was actually *T. asperellum*.

One of the first volatile antifungal compounds isolated from a *Trichoderma* species is 6-pentyl-α-pyrone (6PAP). This non-toxic compound has the distinctive coconut odour that characterizes *T. viride* and its relatives (*Trichoderma* sect. *Trichoderma*). Production of 6PAP has been attributed to *T. harzianum*, which is not a member of the *viride* group, but we have never had a culture of true *T. harzianum* that has the coconut odour. The only isolates in which we have noticed this odour are members of the *T. viride* group, in confirmation of an observation that was made in 1971 by C. Dennis and J. Webster. Reports from New Zealand that *T. harzianum* produces 6PAP are in fact based on *T. atroviride*, a member of *Trichoderma* sect. *Trichoderma*. As 6PAP can inhibit oospore formation in isolates of *Phytophthora cinnamomi* and inhibit conidial germination in *Botrytis cinerea,* an effort should be made to assay the many members of *Trichoderma* sect. *Trichoderma* for enhanced ability to produce 6PAP.

From these preliminary observations, it is possible to predict some biological activity based on phylogenetic relationships. The phylogeny of *Trichoderma* is being revealed at a great pace. From this phylogenetic framework it is reasonable to ask whether some kinds of biological activity, such as production of certain chitinases or the ability to direct parasitism of another fungus, are phylogenetically based. Unfortunately, there have been as yet no concerted efforts in this direction.

Where Are New Biocontrol Strains and Species Found?

Trichoderma species are found in almost all soils. They have been considered to be at least partially responsible for the control effect of 'suppressive soils', soils on which crops or trees are unaffected by a given pathogen. Control induced by suppressive composts has been attributed in part to elevated levels of some *Trichoderma* species. But species reported from these habitats are typically identified as one of the usual soil species, viz. *T. harzianum* or *T. hamatum*. Whether these species have been correctly identified is, of course, another question but it is 'reasonable' to expect to find these common species in such soils. Several new species of *Trichoderma* from eastern and southeast Asian soils have recently been described by John Bissett and his collaborators but the biocontrol potential of these new species was not assessed. The high number of new species found in the Asian soils by scientists who were looking for new species was surprising. Reports of *Trichoderma* species in soils come from all around the world – these can be seen by a quick search of the Internet – and one must wonder how many new (or incorrectly identified) species are found in the normal course of searching for biocontrol fungi in soils.

Another 'source' of new species of *Trichoderma* is the DNA-based phylogenetic analysis itself. As mentioned above, phylogenetic analysis of the morphological species *T. viride* revealed that it comprises two or more new species, including the biocontrol species *T. asperellum*. Similarly, *T. koningii* is representative of a common morphology in *Trichoderma* sect. *Trichoderma* that is shared by at least four species, of which three are undescribed. Were it not for DNA sequence analysis, all would have been made to fit the *T. koningii* morphological concept. In the light of the sequence analysis, though, *T. koningii* can be defined in a strict sense by the length/width ratio of its conidia and by its growth rate. One of these *T. koningii* morphological species is being evaluated for biological control use against frosty pod rot of cocoa, caused by *Crinipellis roreri* (formerly *Moniliophthora roreri* and *Monilia roreri*), in Ecuador but the same species has been found in soil in Brazil, and Germany, and from mushroom compost in Canada (Ontario). It has been isolated as an endophyte from trunks of *Theobroma* species in Brazil, Ecuador and Brazil and its *Hypocrea* sexual stage has been found in Cuba, Puerto Rico and the USA (Kentucky).

Phenotypic and genetic diversity in *Trichoderma harzianum* has led some to question whether it represents a single species. Priscila Chaverri and her collaborators examined a wide range of isolates using DNA sequences of four genes and found *T. harzianum* to be a species complex. While some consistent lineages had developed within the species, there were no consistent geographic, biological or phenotypic characters associated with any of the lineages. She rejected the hypothesis that *T. harzianum* comprises more than one species.

A previously unexplored niche is the tissue of healthy cocoa trees where *Trichoderma* species are found as endophytes. CABI Bioscience scientists Harry Evans and his collaborators are using 'classical biological control' techniques in searching for biocontrol agents in the area of origin of the crop and/or pathogen. The object is to find biocontrol agents that coevolved with the pathogen but that probably did not follow it to new areas. In the case of cocoa, they have isolated endophytic fungi from asymptomatic cocoa and cocoa relatives (*Theobroma* and *Herrania* species) in the upper Amazon region, which is where cocoa is thought to have evolved along with one of its major American pathogens *Crinipellis perniciosa* and in the western Andes where the second major pathogen, *C. roreri*, evolved on its original forest host *T. gileri*. Coevolved antagonists of *Phytophthora megakarya* have been sought in the Korup National Park of western Cameroon, where the pathogen is thought to have evolved in association with *Cola,* a relative of cocoa. We have also taken soil samples from the Korup forest in the hope of finding more effective strains of *Trichoderma asperellum* than are currently being used in Cameroon by Pierre Tondje. Keith Holmes and Harry Evans have isolated endophytes from trunks of *Cola* species in the Korup National Park. The *Trichoderma* species found as endophytes of cocoa include well-known species, such as *T. harzianum*, as well as a high proportion of new species. In Ecuador, they have found an endophytic strain of *T. stromaticum*, the species referred to above that is used in control of witches' broom. However, the endophytic strain from Ecuador is more effective, in *in-vitro* studies, than the one currently used in TRICHOVAB. The finding of *Trichoderma* – and other soil fungi such as *Clonostachys rosea* (formerly *Gliocladium roseum*, a species often used in biological control) – living endophytically within asymptomatic cocoa trees is surprising. The substantial endophyte literature (exclusive of that on the grass endophytes) emphasizes leaves of dicotyledonous plants and not stems, and the fungal endophytes of leaves tend to be species that, outside of the leaf, are found on decaying leaves or as twig, leaf and fruit inhabitants but not soil fungi. We are in the process of describing several new *Trichoderma* species that are endophytic within trunks of *Theobroma* species. Keith Holmes from CABI Bioscience was able to reinfect cocoa seedlings with some of them and he could reisolate many from the shoot meristem of the cocoa seedlings. At least one of them, a new species, inhibited radial growth of *Crinipellis roreri in vitro*. It also persisted on the surface, and within the tissues, of cocoa pods in the field for at least 10 weeks.

Why Trichoderma?

One thinks of *Trichoderma* in biological control simply because most of the fungi used in biological control of fungus-induced plant diseases are species of that genus. *Trichoderma* species are successful in biological control because they have shown variously an ability to parasitize pathogenic fungi, or out-compete pathogenic fungi for nutrients, or produce compounds that are toxic to pathogenic, or enzymes that lyse the cell walls of pathogenic fungi. They may also enhance plant growth and vigour, enabling the host plant to successfully defend itself against attack. Many aspects of *Trichoderma* biology and biological control have been reviewed in a two-volume publication edited by Christian Kubicek and Gary Harman.

Recently, Betsy Arnold, Allen Herre and their collaborators studied the leaf endophytes of cocoa in Panama. Typical of endophytes of leaves of dicotyledonous plants, they have isolated many fungi in leaves of cocoa. They have found that the endophytes might be adapted to cocoa in preference to other tree species. Some of these endophytes can be reinoculated into, and reisolated from, cocoa seedlings. None of the isolates is a *Trichoderma* species but some, unnamed species, can protect seedlings from infection by *Phytophthora*.

Stem and leaf endophytes of cocoa, respectively, are represented by ecologically and phylogenetically different fungi. Nonetheless, each ecological group offers exciting prospects for biological control applications not only in cocoa but other crops. Moreover, the interactions of endophytic fungi with their host plants may offer insights into how plants defend themselves against attack by fungal pathogens.

In Conclusion

Trichoderma species are effective in biological control of fungus-induced plant disease. A search of the Internet will show literally hundreds of examples. New species will be found as different niches are explored and also as the phylogenetic species concept comes into greater use, which is certain to happen as more people utilize DNA sequences and the incredible GenBank database. The study of endophytic fungi in stems and leaves from a biological control perspective, especially when combined with exploration in areas of diversity of hosts and their pathogens, holds the promise of finding new or more effective biocontrol agents and not just in the genus *Trichoderma*. The study of the interaction between host plants and their endophytes, especially at a molecular level, will certainly give new insights into the resistance of plants to diseases causing fungi. It's all very exciting at this point!

Selected Further Reading

Although this news section does not habitually include references, in this instance many of them are very recent and many readers are likely to clamour for them.

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Origin of Florida Air Potato Disentangled

One of the most destructive invasive plants in Florida is air potato, *Dioscorea bulbifera,* a climbing member of the yam family, which ascends rapidly into tree canopies and smothers native vegetation. Air potato invades a variety of natural habitats, including hardwood forests and pine habitats. It is present in at least 23 Florida counties, extending from the northern panhandle to the southern peninsula, and is also a weed in Georgia and Alabama. Current control involves cutting the vine and pulling it clear of the underlying vegetation, and then removing all 'potatoes' (aerial bulbils) from the site – a laborious process – to prevent them from sprouting into new plants.

A more promising strategy for combating this invasive plant is classical biological control – the introduction into Florida of insect herbivores that feed on air potato in its native home range. Such biological control agents would have to be shown to feed only on the target plant and, thus, pose no threat to native or economically important plants. For air potato, this is a particularly important issue because there are two native yams in Florida: *D. villosa* and *D. floridana*. The screening for nontarget effects would be carried out in a highly secure quarantine facility.

The best biological control agents of air potato are likely to be found on plants that are genetically similar to those found in Florida. Air potato is not known to reproduce sexually in Florida, and thus has probably undergone little genetic change since arriving in the state. This means that it will still be genetically similar to the original source population in its native range, which would make that the first-choice location to survey for natural enemies. Unfortunately, until very recently this presented a stumbling block, as the source of Florida's air potato was unknown. What *was* known was that in 1905, the US Department of Agriculture (USDA) sent aerial bulbils of air potato to Henry Nehrling, a nurseryman based in Gotha, Orange Co., Florida, but the origin of these bulbils is unknown. It has been speculated that *D. bulbifera* was introduced into the USA on slave ships coming from Africa in the early days of the Atlantic slave trade. Yams store well, which made them ideal for long sea voyages. Nevertheless, whether this was the original (or only) pathway for introduction remained unclear.

The area of origin of the Florida material needed to be narrowed down (for both practical and economic reasons) before surveys could begin, as air potato is widely distributed in Asia and tropical Africa, where it occurs in wild populations and is a minor agricultural crop. The centre of origin of the genus *Dioscorea* is thought to be Asia, although a secondary centre of yam species diversity exists in West Africa. Inconveniently, from the point of view of the scientists trying to determine the provenance of the Florida material, *D. bulbifera* is the only member of the genus that occurs in the wild in both Asia and Africa, thus it could have been introduced into Florida from anywhere in this range.

Recently, the University of Florida (UF) and the University of Miami (UM) joined forces to solve the mystery. Previous work by Japanese scientists had shown that air potatoes from Asia and Africa are quite different at the molecular level, and can be readily distinguished by examining their chloroplast DNA. Using this technology, and with financial support from the Florida Exotic Pest Plant Council, the universities' scientists showed that air potato in Florida is most likely to be of African origin.

Now that mystery is solved, efforts are underway to explore for natural enemies. A trip to Africa in June 2003 identified the Crops Research Institute in Ghana and Makerere University in Uganda as collaborating institutions. Florida's Department of Environmental Protection recently awarded a grant to UF-IFAS (Institute for Food and Agricultural Science) and UM to support the work in Ghana and Uganda, and to allow further genetic studies to narrow the search in Africa to areas with genotypes similar to those found in Florida.

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Biocontrol of Aflatoxin Contamination of Groundnuts

The fruits of more than 20 years' research by US Department of Agriculture – Agricultural Research Service (USDA–ARS) scientists may provide a natural solution to a contamination problem affecting some of the world's major crops, including groundnuts and maize.

Groundnuts are a tasty, highly nutritious food enjoyed by people around the world, and it is one of the major cash crops of the southeastern USA. Nonetheless, aflatoxin contamination of groundnuts is a major concern from both food safety and economic points of view. Aflatoxin is a chemical produced by a fungus, *Aspergillus flavus*, which is naturally present in soils in which groundnuts are grown. Under certain environmental conditions (drought with high temperature, a not-uncommon combination in the southeastern USA), *A. flavus* can invade groundnuts as they develop in the soil and contaminate them with aflatoxin. Aflatoxin contamination can also occur during crop storage, while groundnuts are awaiting processing, if the right combination of temperature and moisture enables the fungus to grow.

Aflatoxin can be lethal in large doses, while prolonged exposure can cause liver disease and cancer, so groundnuts are closely monitored for its presence, and contaminated groundnuts must be diverted from the edible food supply. The US federal limit for food for human consumption is 20 p.p.b. (parts per billion), and the groundnut industry sets a limit of 15 p.p.b. It has been conservatively estimated that the cost of aflatoxin to the groundnut industry of the southeastern USA alone averages more than US$25 million per year.

There are many different strains of *A. flavus*, and not all have the ability to produce aflatoxin. However, while most of the strains that naturally occur in groundnut soils in the southeastern USA are toxigenic (i.e. have the ability to produce aflatoxin), the fact that nontoxigenic strains exist provided the key to developing a biological control solution. The strategy for reducing aflatoxin contamination of groundnuts developed at the USDA-ARS National Peanut Research Laboratory in Dawson, Georgia is based on competitive exclusion. A certain nontoxigenic strain of *A. flavus* was discovered that is highly competitive against other naturally occurring strains present in soil. When that strain is applied to the soil of the developing groundnut crop and conditions become favourable for *A. flavus* to invade the groundnuts, the nontoxigenic strain invades preferentially, and the result is a significant reduction in aflatoxin contamination, generally in the range of 70–90%. Studies have shown that not only is contamination reduced in the field, but a carryover effect also reduces contamination during storage.

Once the strategy had been shown to work, the next step was to develop an efficient application method, which led to the development of a unique formulation technique. Spores of the nontoxigenic *A. flavus* are coated onto the surface of a small grain, such as shelled barley. The barley serves both as a carrier to deliver the fungus to the field, using conventional farming equipment, and as a substrate for it to grow on after application. The coated barley is applied in a band over each planted groundnut row using conventional application equipment for granular materials. When the barley becomes moist in the field, the spores adhering to the surface germinate, grow, and produce more spores that become the competitive inoculum in the soil.

Circle One Global, Inc. (Cuthbert, Georgia) has licensed the ARS-developed technology and has given the trade name Afla-Guard to the product that it hopes to commercialize in the near future. The company is currently seeking registration of Afla-Guard as a biopesticide with the US Environmental Protection Agency.

The technology could have much wider application against other *A. flavus*-susceptible crops. It has shown promise for reducing aflatoxin contamination of maize already, and studies are planned to refine the methodology for this crop. Since there is no method currently available for preventing aflatoxin contamination of groundnuts and maize (except to control the environment through irrigation), the strategy has a lot to offer in combating a major agricultural problem in the USA. Further afield, aflatoxin contamination is a serious issue in the developing world, where contaminated crops may be eaten either in the absence of any alternative or through lack of awareness. The ease of use of this technology could make it suitable for resource-poor farmers in the developing world.

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Identifying Weak Links for OTA in Cocoa

Ochratoxin A (OTA) is a mycotoxin, a natural toxin produced by a number of fungal species in the genera *Aspergillus* and *Penicillium*. It is commonly associated with many food crops grown in semi-tropical and temperate climates, including cereals, vine fruits, coffee and cocoa. Growth of the fungus and production of the mycotoxin are dependent on factors such as temperature and humidity during crop growth, harvesting, drying, storage and shipment. Because it is stable to heat and other physical food processing, OTA can end up in food products made from these crops. However, recognition of the threat to health posed by this genotoxic carcinogen has led to limits being set.

CABI Bioscience (UK Centre), CABI-SEARC (South-East Asia Regional Centre) and ICCRI (Indonesian Coffee and Cocoa Research Institute) have recently been awarded funding by FAO (Food and Agriculture Organization of the UN) for a 2-year project to work on the management and identification of OTA-producing fungi in the cocoa commodity system in Indonesia. The project will apply a HACCP (Hazard Analysis Critical Control Point) approach to the whole production system.

OTA is of particular concern in cocoa, and especially in Indonesia, where there are increasing instances of rejection of cocoa beans by buyers because of unacceptable OTA levels. Data confirm that OTA is carried over from its source to the final retail product (i.e. chocolate). Sulawesi is the main cocoa-producing region of Indonesia, where the crop is predominately produced by smallholder farmers (see *BNI* 24(3) [September 2003] 55N–58N, Sulawesi setting for cocoa IPM initiatives) and exported to the USA. Generally the cocoa beans do not undergo on-farm fermentation but are sold directly to a middleman. The length of time the cocoa beans are stored before being sold on is highly variable. Thus there are many points in the supply chain where OTA contamination could occur.

Since OTA contamination can occur during any stage of the commodity system and current data are based entirely on examination of retail products, an epidemiological survey of OTA-producing fungi together with an analysis of OTA levels throughout the system is essential. The project will examine when the OTA-producing fungi start to appear, when they start producing OTA and if there is anything in the production system which augments OTA levels. In addition to the survey the project activities will include a technical questionnaire to collect data throughout the whole commodity system, i.e. harvesting, fermentation, drying, local storage, export, shipping and import storage. This information, combined with the results of the survey, will identify the critical sources of fungal and toxin production to determine where they are initiated and where OTA contamination occurs.

Other activities aimed at ensuring that the project has a sustainable impact include:

- Training local staff in sampling, isolation and identification techniques and data collection.
- Establishment and maintenance of a reference collection, an important part of the systematic development of a management process for cocoa bean production.
- Training-of-Trainers (TOT), covering participatory training techniques and communication skills, and how to prevent contamination of OTA at various points in the cocoa chain.

This is a timely project in that the European Union and other countries are likely to introduce regulatory limits for levels of OTA in imported cocoa from the end of 2004.

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Local Solution to African Armyworm

The African armyworm (*Spodoptera exempta*) is a major threat to food production in a large area of eastern and southern African. Outbreaks threaten the livelihoods of subsistence farmers, in particular, as the pest can inflict crop losses that represent a major portion of the families' annual food supply. However, the armyworm is now providing the means of its own destruction. A team of Tanzanian and UK scientists is developing cost-effective methods for harvesting diseased caterpillars to obtain naturally occurring *S. exempta* NPV (nucleopolyhedrovirus), and for multiplying and reapplying the NPV in the field.

The African armyworm appears between December and May as armies of black caterpillars covering many hectares with densities of up to 1100/m^2. As the crops grow, armyworms swarm across the countryside stripping the fields of grass, wheat, maize and other crops. Overall losses of 30% for crops have been estimated, but in major outbreak years losses of up to 92% in maize, a staple food in the region, are recorded. Outbreaks begin in Tanzania and are serious in 9 out of 10 years; in 2001 they covered 157,000 ha of crops and pasture. In major outbreak years the pest subsequently migrates to produce further extensive outbreaks in Kenya, Uganda, Ethiopia, Somalia, Mozambique, Zambia, Eritrea and may travel as far as Yemen.

Until now, the response to armyworm outbreaks has been to spray chemical insecticides; these are effective but are too expensive. In 2001, Tanzania could afford to control only some 30% of the outbreaks and in large outbreak years local stocks are exhausted early in the season. Poorer subsistence farmers, in particular, often cannot afford to purchase sufficient insecticide. Also weighting the balance against chemical insecticide use are growing concerns about their safety. Although future bans on older, cheaper chemicals on safety grounds may alleviate these concerns, their newer replacements, while less persistent and much less environmentally damaging, are much more expensive. An additional obstacle to chemical control is that many of the early armyworm outbreaks start in national parks, where widespread application of chemical insecticides is not desirable.

It has been recognized since the early 1960s that many armyworm outbreaks collapse late in the season as a result of a disease that kills up to 98% of the caterpillars. The disease-causing organism has been identified as a host-specific NPV. Late-season disease-associated population collapse is a recognized phenomenon in lepidopteran pests in a number of crops. Under natural conditions this does little to help the farmer as the rise in infection levels lags behind the growth of the pest population, and the disease spreads too slowly to prevent crop damage being inflicted. However, inundative application of NPV early in the season can cause sufficient early-season pest mortality to prevent populations reaching damaging levels.

In contrast to chemical insecticides, NPVs are highly selective, attacking only specific pest insects and posing few if any nontarget risks. They have been recommended by FAO (Food and Agriculture Organization of the UN) as pest control agents and a recent OECD (Organisation for Economic Co-operation and Development) report concluded that baculoviruses, which include the NPVs and closely related granuloviruses (GVs), are safe and do not present any health hazard. Several of these baculoviruses have been developed as commercial biological insecticides in the USA, Europe, China, Thailand and India. These are sprayed like chemicals onto pest outbreaks causing epidemics of disease that kill off the pests. They act as a natural insecticide and also the organisms multiply inside infected insects, which then serve as a source of disease inoculum for subsequent generations, thus the effect can be more persistent than that of chemical insecticides even though the persistence of the NPV particles outside the insect is generally shorter than that of many chemical insecticides.

One commonly suggested obstacle to biopesticide development is the cost of production (see *BNI* 24(4) [December 2003] 81N–82N, Harmonization sounds good for biopesticide business]. Production of NPVs currently relies on *in vivo* systems, which can make large-scale production of NPV products more expensive than the older, cheaper chemical insecticides, but NPVs are robust organisms and amenable to mass production, provided quality control issues are adequately addressed. Simple systems for producing and processing NPVs from infected insects have been developed in a number of countries. In India and Thailand locally produced NPVs are used to control a number of important pests such as *Helicoverpa armigera*, *Spodoptera exigua* and *S. litura* whose resistance to chemical pesticides has made them a serious problem for agriculture. In Brazil, 40 tonnes of NPV infected insects are harvested annually, largely by farmers. These are subsequently used to control the velvetbean caterpillar (*Anticarsia gemmatalis*) in soyabeans on over two million hectares at an average cost of US$1.26/ha.

The aim of the project in Tanzania funded by the Crop Protection Programme of the UK Department for International Development (DFID) is to develop a similar system for the armyworm NPV. A team led by Wilfred Mushobozi from the Tanzanian Ministry of Agriculture's Pest Control Services together with scientists from the Natural Resources Institute (University of Greenwich, UK), the NERC Centre for Ecology and Hydrology Oxford (UK), Stirling University (UK) and CAB International Africa Regional Centre (Kenya) has reported substantial progress following intensive studies to develop a low-cost, effective and safe armyworm control that can be produced in Tanzania. Field trials over the last 2 years in

Tanzania have shown the armyworm NPV to be highly effective in controlling the pest if sprayed early in the outbreaks. The NPV reduced armyworm populations as much as chemical treatment (sumithion) and was more effective than a neem preparation.

The system adopted involves producing NPV in the field from natural armyworm outbreaks. Outbreaks on low-value pastureland are sprayed with NPV. Subsequently, the dying insects, by now full of NPV, are collected and processed to produce more NPV. This system is an adaptation of the field production techniques developed originally at EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) Brazil, and Dr Moscardi who leads the Brazilian team has helped train Tanzanians in the low-cost NPV production technology. NPV is highly productive and one armyworm infected by a thousand NPV particles will contain some 2×10^9 particles by the time it dies. Recent research has indicated that larvae surviving viral challenge may carry a 'covert' viral infection with them as adults, which they may then pass on to their young. Under some circumstances, these covert viral infections may be triggered into lethal infections, which may infect other larvae and initiate viral epidemics at armyworm outbreaks beyond those that were initially sprayed. Thus, NPV potentially provides biocontrol beyond the originally targeted pest population. Ongoing studies are attempting to identify which cocktail of viral isolates best yields the most effective method of control.

One important future control strategy would be to strategically target the first outbreaks of the season in Tanzania, so as to minimize the number of insects migrating to initiate outbreaks in other parts of Tanzania and beyond. Predicting where these early outbreaks are going to occur, so NPV can be sprayed before pest populations begin to build up, is clearly key to successful control. Unlike chemicals, there is a time lag before NPVs begin to have an impact on the target pest. The NPV work is therefore progressing hand in hand with the development of a better armyworm forecasting system.

A successful centralized forecasting service has been operating in Tanzania based on a network of pheromone traps and rain gauges supplemented by satellite-derived rain cloud images. The pheromone traps specifically attract the armyworm male moths using an artificial female sex pheromone. However a problem with this centralized system is poor information flow to many intended recipients, especially poor subsistence farmers. To help overcome this, a community-based forecasting system is being developed. Village communities keep and operate their own pheromone trap and rain gauge, and use these to make their own forecast. Although some loss of forecasting accuracy might be expected compared to the national forecast, poor information flow ceases to be a problem: the forecast is generated by the people who need to act on it and this is in line with Tanzanian government policy to devolve responsibility for armyworm control to farmers.

This work builds on many years of armyworm studies by organizations such as the Desert Locust Control Organization for Eastern Africa, FAO and the old Centre for Overseas Pest Research (UK), which have provided a wealth of biological and population information that has been essential underpinning for efforts to develop improved forecasting and control of armyworm.

The first aerial applications of NPV are planned for Tanzania in early 2004 jointly funded by DFID and USDA–ARS (US Department of Agriculture – Agricultural Research Service) to confirm the efficacy of the NPV on a larger scale.

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Root Fungi Affect Leafminer Parasitoids

Increasing calls for scientists to predict the likely outcome of biocontrol releases make it important to be able to assess interactions between potential biocontrol species, and how they are likely to interact with organisms already present in the target environment. A recent study* is of interest because it tackled a previously unstudied relationship: parasitoids and soil organisms.

Interactions between fungi colonizing plant roots (arbuscular mycorrhizal (AM) fungi) and parasitoids attacking leafminers have been demonstrated in a common European meadow flower, ox-eye daisy (*Leucantheum vulgare*). A team of scientists from Royal Holloway College (University of London) and the University of Reading conducted studies in an experimental flower meadow using fungicide treatment to reduce soil fungal populations, and in controlled laboratory conditions using plants infected with one or more of three species of AM fungi and exposed to leafminers and parasitoids.

When experiments began in spring 1999, ox-eye daisy was the dominant plant in the meadow, which had been established 2 years previously, but it declined in density over the next 4 years. The results of field trials over this period showed that AM colonization was reduced by fungicide (iprodione) treatment (8.3% plants colonized compared with 36% for an untreated control), and treated plants were also smaller. Densities of the generalist leafminer *Chromatomyia syngenesiae* were highest in the first 2 years and then declined, but did not vary with treatment. Leafminer parasitism by the generalist parasitoid *Diglyphus isaea* fell over the 4 years of the experiment. However, in the first 2 years, when plant and leafminer densities were also highest, parasitism was lower in the untreated control where AM colonization was higher.

Laboratory experiments looked at colonization of ox-eye daisy roots by three AM species common in soil samples at the field site: *Glomus caledonium, G. fasciculatum* and *G. mosseae* (*Gc, Gf* and *Gm*, respectively). Complex interactions between species were apparent; for example, colonization by either *Gc* or *Gf* alone reduced ox-eye daisy leaf number, but dual colonization by both species together increased leaf number over uncolonized control plants. Single-species AM colonization reduced leafminer density, with *Gm* reducing it the most. However, the effects of multiple-species colonization varied was variable, with some leafminer densities higher and some lower than for the single species colonizations, and *Gc* and *Gf* together gave leafminer densities indistinguishable from the control. The impact of AM colonization on parasitism was even more variable: by themselves, *Gc* and *Gf* allowed higher and *Gm* lower rates of parasitism than an uncolonized control. Multiple colonization again gave variable results, but in general *Gm* decreased parasitism while *Gc* increased it. An interaction between these species was also evident: *Gm*'s antagonistic effect on parasitism was only evident in the absence of *Gc*.

The authors highlight the variation in the study, noting the changes over the course of the field study: parasitism was only significantly lower when the host plant and leafminer densities were highest. They suggest that multitrophic effects are related to the successional age of the community.

Three possible mechanisms (or a combination of them) could be involved in AM-mediated alterations in parasitism levels: plant size, plant chemistry and density-dependence between parasitoid and host insect. The authors suggest that the first two of these are important in the AM–parasitoid relationship:

• Decreased parasitism is correlated with the larger plant size of AM-colonized plants; the parasites have a larger leaf area to search for their leafminer hosts and are therefore less efficient.

• AM fungi have significant effects on the chemistry of host leaves, which may alter their attractiveness to parasitoids. Parasitoids react to plant volatiles emitted by herbivore-damaged plants, but AM-mediated reduced carbon availability may affect their production. Alternatively, they may (though not demonstrated) affect food quality by altering nitrogen availability.

The authors point out that the reasons for the variation found in this study need to be understood, "not just to enhance our knowledge of community structuring forces, but for the application of strategies involving natural enemies in pest control situations."

*Gange, A.C., Brown, V.K. & Aplin, D.M. (2003) Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. *Ecology Letters* 6: 1051–55.

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Code of Conduct Revision Proceeding

Several times last year, *BNI* flagged the imminent revision of International Standard for Phytosanitary Measures No. 3 (ISPM3), the Code of Conduct for the Import and Release of Exotic Biological Control Agents (e.g. see *BNI* 24(1) [March 2003] 1N–4N and 15N–27N). This process is now formally underway. An IPPC (International Plant Protection Convention) Expert Working Group meeting was held in Rome on 9–12 December 2003 and was comprised of eight biological control experts (scientists and regulators) from around the world.

The revised draft ISPM3 will be reviewed by the IPPC Standards Committee and then be released for country consultation, hopefully in 2004. It is during this country consultation phase that all interested parties can comment, but this needs to take place through the relevant National Plant Protection Organizations (NPPOs). The relevant contact people for the NPPOs can be found at:
www.ippc.int/IPP/En/nppo.jsp
ISPMs under consultation will also be posted on this website.

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IPM Systems

This section covers integrated pest management (IPM) including biological control, and techniques that are compatible with the use of biological control or minimize negative impact on natural enemies.

Saving the Cedars of Lebanon

The biggest stand of cedars (*Cedrus libani*) in Lebanon has been the site of a major pest outbreak for the past decade. The insect responsible, unknown from anywhere else in the world, is a web-spinning sawfly that was first identified in 1998 as a new species of *Cephalcia*, and named *C. tannourinensis* after the village of Tannourine in north Lebanon where it was first detected. The 600-ha cedar forest it had infested is the largest of the 12 stands on Mount Lebanon and represents about one-third of the total cedar forest in the country.

Historically, cedars have always been the symbol of Lebanon and their health is a public issue. Back in the 950s B.C., it was said that King Hiram of Tyre (Lebanon) provided King Solomon with cedar logs to build his temple. Lebanon's flag features a green well-spread *Cedrus libani*, a symbol of power and immortality. The threat to cedars from the previously unknown pest therefore prompted great concern.

For more than a decade, the villagers of Tannourine had seen the forest turning brown each July, looking as if it was swept by a fire. Year after year, the alarming symptoms of needle browning worried the local community to the extent of calling for the relevant ministries and municipalities of the region to declare an emergency. This led to a management programme for the problem being initiated in 1998 under the guidance of Mr Guy Demolin, a forest entomologist from INRA (Institut National de la Recherche Agronomique, France), who was urgently called upon to identify the insect and advise on methods that would best solve the problem with least adverse effects on the other organisms present in the forest. Mr Demolin worked closely with Mr Nabil Nemer, a researcher in the Entomology Laboratory at the American University of Beirut, on the life cycle of *Cephalcia tannourinensis* and the choice of treatment method. Field visits were made to assess the damage and learn about the habits of this previously unknown species. The work was financially supported by a number of French and Lebanese NGOs and the Lebanese Ministry of Agriculture, while technical and scientific collaboration was provided by entomologists from the American University of Beirut (Nasri Kawar and Nabil Nemer) and the Lebanese University (Linda Kfoury).

The wasp-like *C. tannourinensis*, described by Henri Chevin in 2002, is a hymenopteran in the family Pamphiliidae. Adults swarm from mid-April to mid-June and females have a high egg laying potential of up to 50 eggs per female. Eggs are placed on the needles of a new bud and as the buds open, the eggs hatch and the larvae start chewing on the needles. Each larva passes through three instars and eats about seven needles before it matures. The voracious appetite of the larvae causes the trees to turn rusty-brown and eventually die. After their last moult, the larvae drop to the ground in July and make a hole for hibernation. The duration of the pupal stage has not been determined yet and could extend for several years depending on climatic conditions.

Soil sampling has been conducted monthly since 1999. The number of prepupae in a 40 × 40 × 40 cm sample is counted in order to assess their density in the soil, and thus gain an accurate estimate of the population. Prepupae have the capacity to enter prolonged diapause and may emerge as adults the following spring or in later years. Thus regular monitoring is necessary to assess changes in the underground population of prepupae and predict outbreaks.

Aerial treatment using the insect growth regulator (IGR) diflubenzuron was carried out by a helicopter equipped with ultra low volume (ULV) sprayers. Diflubenzuron is particularly effective against insect larvae and also acts as an ovicide, killing insect eggs. It is used against lepidopteran caterpillars and sawfly larvae and is not harmful to bees. It has been the sole product used during the spraying activities in Tannourine for the past 4 years and has been successful in suppressing the insect population. However, other measures such as monitoring and integrated pest management (IPM) have also been explored during the implementation of the FAO (Food and Agriculture Organization of the UN) project, described below.

Two other insect pests were also identified in Tannourine, which multiplied as a result of the weakness of the attacked trees and contributed to their decline. *Ernobius* sp., a coleopteran belonging to the family Anobiidae, was found feeding on 'emergency' buds produced by defoliated cedar branches whose needles have been eaten by *C. tannourinensis*. The second pest was a cecidomyiid fly, *Dasineura cedri*, which completed the cocktail of fatal pests attacking the trees.

FAO had received a proposal for follow-up integrated management in Tannourine forest. After some coordination meetings in the presence of the FAO regional coordinator and FAO forest officers, FAO approved funding for a 2-year project. Achievements of this project include the development of a methodology for monitoring insect populations, the utilization of pheromones for monitoring, and the introduction of an IPM programme against *C. tannourinensis*. Workshops for training young scientists also took place and publications were prepared for use in public awareness campaigns on forest health issues.

The first phase of the project, which extended from July 2001 until January 2002, featured several activities which focused on the development of survey and sampling techniques for collecting data on the population of *C. tannourinensis*. A first inception workshop was held and specialized training in France was also organized for three engineers from the Ministry of Agriculture.

The second phase of the project, extending from 15 April until 15 October 2002, covered the pest control evaluations to appraise the efficacy and/or safety of the spraying operation, the training course in Lebanon and surveys to evaluate *C. tannourinensis* populations before and after treatment. Surveys to monitor the other forest entomofauna were also carried out during this phase. A second training course was held, attended by 26 participants.

The last phase of the project dealt with laboratory identification of the entomofauna collected from the cedars and the preparation of printed material on cedar forest insects to be distributed to relevant parties or people interested in forest problems.

In 2003, no spraying was undertaken against the previously harmful pest. The underground prepupal monitoring of *C. tannourinensis* carried out by the engineers from the Ministry of Agriculture in collaboration with the American University of Beirut and the Lebanese University showed an average of 70 prepupae/m^2. In comparison with the average in 1999 (626 prepupae/m^2), the population has been reduced by approximately 90%.

This FAO project has allowed the Ministry of Agriculture and particularly the Directorate of Rural Development and Natural Resources to deal with a new pest problem and has also provided the technical and scientific methodologies to manage the insect. This is a unique experience since the insect pest in question is new to science and the lessons learnt from its management could be used for the other cedar forests in the region.

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Malaria High on ICIPE's Agenda

Malaria causes over one million deaths worldwide each year, and 90% of these deaths occur in Africa. The announcement by ICIPE (International Centre for Insect Physiology and Ecology) in Nairobi that it is establishing Africa's first factory to produce *Bacillus thuringiensis* (*Bt*) for malaria control signals its commitment to the continent-wide drive to tackle malaria. Although both *B. thuringiensis* var. *israelensis* (*Bti*) and *B. sphaericus* (*Bs*) are successfully used against malaria in other parts of the world, they have not been widely used in Africa, partly because of the high cost of obtaining them from the industrialized nations where large-scale production is centred, and also because larval control has been a neglected area of mosquito control since the failure of malaria eradication in the 1970s.

In fact, there are very few facilities with the capacity for producing large volumes of biopesticide in Africa. *Bt* is potentially useful against a range of important agricultural pests, such as stem-borers, as well as public health pests, so ICIPE's venture is pioneering in potentially more ways than one.

The first phase of the project will see the setting up of a demonstration facility at ICIPE's Duduville headquarters at Kasarani near Nairobi, together with research into market access for the product. Equipment was supplied by Kernel (Wuhan Province) and financed by the Ministry of Science and Technology, China. Production know-how and the *Bt* strains to be produced will come from both ICIPE and Kernel gene banks. Additional funding for the plant's commissioning is being provided by UNDP (UN Development Programme), UNICEF (UN Children's Fund), Biovision – Switzerland and Kernel. For the test phase, ICIPE is hoping to obtain further funding from UN agencies, governments and other sources.

The search for the silver bullet for malaria is over. Experts now agree that an integrated management strategy, using old and new mosquito control methods combined with drugs (and perhaps in the future a vaccine), is more likely to succeed. This recognition is mirrored in the multidisciplinary research of ICIPE's Human Health Division, which includes eco-epidemiology, vector ecology and behaviour, and community-based intervention studies. The aim is to provide biologically based tools and strategies to control the vectors and break the cycle of transmission, which can be integrated with disease management.

Growing Populations

Who is most at risk? ICIPE's studies show that children are both most at risk and the largest reservoir of infection. A study of the dynamics of gametocytes of the malarial parasite, *Plasmodium falciparum*, in asymptomatic children in western Kenya revealed that children under 5 years of age constitute the most important infectious reservoir, with 68% harbouring gametocytes for more than a week.

Where do the mosquitoes come from? Knowing where they breed is a first step in developing effective interventions. Studies in various parts of Kenya have revealed natural and man-made breeding sites.

- Mapping of larval habitats during an outbreak in western Kenya revealed that over 90% of the larval sites were man made.
- In coastal Kenya, empty coconut husks, abandoned swimming pools and water collection points were major breeding sites.
- Tree ponds were found to be important in sustaining mosquito populations over the dry season in both urban and rural sites.

With more people migrating to the cities, it is important to know whether the ecology of the malaria vector changes in the urban environment. In addition, new migrants tend to move into unplanned housing areas, where drainage is often poor. Are these populations more at risk from malaria? The short answer is, 'yes'. A survey to map habitat types and vector distribution in Kisumu, on the shores of Lake Victoria, showed that although mosquito larvae were found in all habitats, they were predominant in poorly drained peri-urban areas. In truly urban habitats, 64% of larval breeding sites were man-made and therefore not sensitive to rainfall. It is therefore becoming increasingly difficult to control malaria in urban areas because of encroaching unplanned, poorly drained areas where malaria vectors have been noted to breed in polluted habitats.

What of the countryside? Agricultural production remains the backbone of the Kenya economy, and the health of the rural population is of prime importance to this. Do agricultural developments such as irrigation alter the prevalence of malaria? Surveys of villages in the Mwea Rice Irrigation Scheme (in the central highlands) and villages nearby showed that although the irrigation scheme villages had up to 130-fold higher densities of mosquitoes (*Anopheles arabiensis*) than villages outside the scheme, malaria cases were lower by 40% inside the Scheme. The difference was attributable partly to differences in agricultural practices (see below). Children were again found to be at greatest risk, with those under 10 years old having the highest gametocyte levels.

Understanding Mosquito Development

Equally useful is to know why mosquitoes choose particular breeding sites, what affects the development of the larvae, and what factors contribute to the ability of the adult mosquitoes to transmit disease (vector competence and transmission intensity). Behavioural and chemical studies are used in this context to assess how factors, both abiotic (e.g. soil composition) and biotic (e.g. soil microflora, presence of competing species), affect choice of oviposition site. Other research has shown that sugar composition is an important factor in plant-feeding behaviour by adult mosquitoes. Chemical signals have been identified that may be implicated in host preference or location: human foot odour analysis showed large difference in attractiveness and chromatographic profiles.

Genetic studies are being conducted on both mosquitoes and parasites to unravel the mechanisms regulating the successful development and transmission of the malarial parasite. Molecular analysis is also helping to elucidate the genetic structure of mosquito and parasite populations.

With the results from such studies, a picture begins to emerge of what constitutes the ideal (and less-than-ideal) breeding conditions for both mosquito and parasite, while potential intervention points can also be identified: useful information to feed into a developing integrated management strategy.

Prospects for Indigenous Solutions

Exploring the potential of native biodiversity has identified a number of promising control possibilities.

DEET (N,N-Diethyl-3-methylbenzamide) is the most commonly used commercial insect repellent, but there may be concerns about its toxicity when applied to the skin over a long period. Five new repellents with higher activity than DEET were identified during the screening of 250 indigenous plants, and a number of compounds are undergoing market testing. Six plants with potent repellent and fumigant toxicity discovered through bioprospecting show promise for extraction of essential oils by thermal methods, such as from small stoves.

Ten plants with promise as larvicides were also found, including neem (*Azadirachta indica*) and *Melia* spp., and are currently being tested in the western highlands of Kenya.

Bti and *Bs* are now being field tested in the Mwea Rice Irrigation Scheme (see below). Studies in Eritrea have shown these biopesticides to be as effective as temephos in causing larval mortality, with a duration of activity of 2-3 weeks, and they could provide effective mosquito control if applied to all larval habitats. The new *Bt* demonstration factory (see above) will provide volumes adequate for large-scale field testing and public health applications.

Another project soon to start will research the potential for natural herbal medicine in combating malaria. The project will involve dose-ranging clinical trials of *Artemisia annua* tablets for their efficacy, safety and tolerability in uncomplicated *Plasmodium falciparum* malaria. The *Artemesia* will come from pilot field production sites in Kenya and Ethiopia. A comprehensive bibliography (including Chinese sources and references) on the distribution, agronomy and utilization of *Artemisia* is also being compiled.

New Tools

Molecular tools developed at ICIPE allow rapid identification of field-collected material, including mosquitoes of the *Anopheles gambiae* complex and bloodmeal sources, which provides support to behavioural and epidemiological studies. However, biotechnology and the unravelling of the *Anopheles* mosquito genome may have more to offer in the future.

The potential for genetic modification as a tool in malaria control has been hotly debated. One mechanism could involve release of mosquitoes genetically modified to have a lower than normal ability to transmit the disease. Other avenues ICIPE will soon work on include the genes expressing odour reception, with a view to both potential manipulation and rational design of new attractants and repellents. Safety and social issues aside, could such an approach contribute to malaria management? A key question is whether introduced (parasite-inhibiting) genes would spread and fix in the population. Research at ICIPE is investigating whether the dynamics of introduced genes can be predicted on the basis of fitness traits and whether introgression of introduced genes happens at the same rate across the mosquito genome.

Helping Communities

While the research outlined above is expected to lead to new control measures that can be implemented as part of an integrated management strategy, ICIPE's community-based intervention and training are helping to alleviate malarial problems now. For example:

- In western Kenya, in conjunction with other research and development partners, ICIPE provided training on malaria management for local communities in Kisii and Gucha districts. This gives them the knowledge to be able to assess their health and environment and control malaria.
- Community groups in Malindi, on the Kenya Coast, have developed an awareness of malaria and mosquito control under the umbrella of the Greentown Movement, with various initiatives including a 'Malaria Mosquito Day'. ICIPE helped build on this with a Trainer of Trainers (TOTs) workshop on mosquito recognition (especially of larvae in water bodies) and effective facilitation and communication skills, giving trainees the skills to make decisions about control options. Community groups were also given training on how to impregnate bednets, and the use of neem and repellents.
- Farmers in the Mwea Rice Irrigation Scheme participated in a project, described in more detail below, in which various options were selected for integration in a mosquito control programme.

The potential for integrating the research outlined in this article is well illustrated by the ecosystems approach taken at the Mwea Rice Irrigation Scheme. Links between health, agriculture and the environment are particularly apparent in irrigation schemes. Baseline data collected by ICIPE indicated that almost a quarter of children had malarial parasites in their blood. The community and ICIPE came up with a strategy to tackle malaria through better management of the ecosystem. The first phase involved:

- Training farmers in better environmental management, including draining and maintaining previously clogged irrigation ditches to reduce mosquito breeding sites.
- Educating and training community-based organizations, farmers and National Irrigation Board staff to improve participation and information sharing.
- Encouraging farmers to keep cattle or other livestock, as the animals attracted mosquitoes away from humans. To this practice was attributed the lower incidence of malaria (despite higher mosquito densities) than in villages outside the scheme.

The second phase involves pilot studies on the use of *Bti* and *Bs* as larval control agents to limit mosquito populations. Training is being provided for farmers on their use, and their efficacy is being assessed.

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Announcements

Are you producing a newsletter, holding a meeting, running an organization or rearing a natural enemy that you want other biocontrol workers to know about? Send us the details and we will announce it in BNI.

Invasive Aquatic Species Conference

The 13th International Conference on Invasive Aquatic Species will be held at Ennis, County Clare, Ireland on 19–23 September 2004, hosted by the Institute of Technology Sligo. This annual 4-day conference is widely considered the most comprehensive international forum for the review of accumulated scientific knowledge on the impacts of aquatic invasive species, presentation of the latest field research and data, new technologies or advancements in control and mitigation, discussion of policy and approaches to effective public education and outreach initiatives to prevent new introductions.

Suggested topic areas include:

- Policy, legislation, monitoring: international cooperation and coordination; rapid response to new invaders; management and implementation plans; effective monitoring.
- Ecological and ecosystem impacts: species at risk; regions at risk; pathogen introductions and impacts; habitat/ecosystem changes; nutrient cycling; blue–green algal blooms.
- Socioeconomic impacts: impacts of invasive species on human health; economic impacts of invasive species.
- Information exchange: database compatibility; addressing data needs; early warning/detection techniques.
- Education and outreach initiatives: local monitoring initiatives; effective invasive alien species education and outreach programmes; raising awareness at the political and decision-making levels; volunteerism, stewardship and other grass roots initiatives.

• Vectors of introduction: ballast water; hull fouling; aquaculture and live food-fish industry; water garden industry.

• Control technologies for industrial and non-industrial settings: barrier technologies; biological control; chemical control; non-chemical control.

Contact: Elizabeth Muckle-Jeffs, Conference Administrator, 1027 Pembroke Street, East Suite 200, Pembroke, ON K8A 3M4, Canada
Email: profedge@renc.igs.net
Fax: +1 613 732 3386
Web: www.aquatic-invasive-species-conference.org

Golden Apple Snail CD

The golden apple snail (GAS), *Pomacea* spp., is an invasive alien pest of rice, taro and other plants that grow in aquatic environments. A new CD-ROM* collates information sourced from experts around the world to provide quick and easy access to some 30 years' literature on the ecology, damage, management options and utilization of GAS. The CD-ROM includes over 400 articles and over 100 images, and is designed to be a reference tool or a resource package for agricultural technicians, researchers, advisers and students, and for those engaged in GAS research, extension and training. Information is searchable by author, title, year of publication, journal title and keywords. All software needed to run the database is supplied on the disk.

Future plans include developing web wizards, establishing mirror sites for webpages and translating the CD-ROM content into Spanish, Chinese, Thai, Khmer, Vietnamese, Indonesian and other languages.

*Joshi, R.C., Baucas, N.S., Joshi, E.E.; Verzola, E.E. (2003) Scientific information database on golden apple snail (*Pomacea* spp.). CD-ROM. Baguio, Philippines; Department of Agriculture – Regional Field Unit – Cordillera Administrative Region (DA-RFU-CAR) in collaboration with Department of Agriculture – Philippine Rice Research Institute (DA-PhilRice), Agricultural Librarians Association of the Philippines (ALAP) and The Department of Agriculture – Cordillera Highland Agricultural Resource Management Project (DA-CHARMP). Price: Philippine Pesos 500 (local purchase) or US$50.00 + P&P (overseas purchase).

Order from: Ms. Salome Ledesma, President, Agricultural Librarians Association of the Philippines (ALAP), University of the Philippines at Los Baños, Main Library, College, Laguna 4031, Philippines
Email:
omecledesma@yahoo.com / ejoshi@philrice.gov.ph

Global Plant Clinic

The Global Plant Clinic is a new service from CABI Bioscience, drawing on its long experience and expertise in diagnostic and advisory services. The Global Plant Clinic offers comprehensive support in disease identification and management to growers, government and non-governmental organizations and private enterprises around the world. It deals with all crops in all countries and its staff has particular experience of tropical agriculture (including forestry and horticulture).

The Global Plant Clinic:

• Receives plant samples and cultures from all countries.

• Provides expert identification services for fungi, bacteria, nematodes, viruses and phytoplasmas.

• Has up-to-date access to information databases and electronic compendia.

• Makes practical diagnoses based on available evidence, including plant samples, cultures and photographs.

The Global Plant Clinic also provides quarantine services and will test plant material in transit.

A free service is available for eligible clients in developing countries through the support of the UK Department for International Development. See the website or contact the Global Plant Clinic for details of the charging policy and charges. Advice on collecting and submitting samples is also available.

The Global Plant Clinic can provide current information on a variety of issues affecting smallholder and commercial farmers throughout the world. These include:

- Global pest and disease information
- Advice on disease management strategies, including IPM and biocontrol
- Crop and pest management
- Quarantine and safe movement of germplasm
- Biopesticides and pesticide use
- Research planning

The clinic is based in the UK, but works closely with parallel services overseas. In Bolivia, Uganda and Bangladesh it is helping local partners to improve operation and delivery. It also collaborates with partners in Colombia, Brazil and Vietnam and supports PacificPestnet, a new electronic service for growers in that region.

Contact: Global Plant Clinic, CABI Bioscience UK, Bakeham Lane, Egham, Surrey TW20 9TY, UK.
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Fax. +44 1491 829100
Web: www.globalplantclinic.org

GISP News

The Global Invasive Species Programme (GISP) Secretariat, which arrived in Cape Town, South Africa in June, has been planning how to meet the challenges and diverse responsibilities it expects to meet in the coming years. Areas of work include national, regional and international cooperation; communication, education and training; information management; development of best practice in invasive alien species (IAS) management (including prevention and control); economics of IAS; evaluation and assessment; and law and policy.

New initiatives launched include:

- A new website at: www.gisp.org
- A newsletter – the introductory issue is on-line now
- The Invasive Alien Species Toolkit (see *BNI* 22(4) [December 2001] 100N–101N) in French and Spanish, as well as English, now available for download as pdfs from the website
- Collating information for an interactive map, which will link to information from all the countries in the world. Sample information can be seen in the Regional Resources section of the website. At this early stage, GISP is calling for information about projects on IAS management, control or research from all countries. Email: mcocks@uwc.ac.za

Contact: Ms Kobie Brandt, Communications Officer,
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International Trichoderma Workshop

The 8th International Workshop on *Trichoderma* and *Gliocladium* will be held on 20–23 September 2004 at Zhejiang University, Hangzhou, China, organized jointly by the university and the Mycological Society of China.

Topics will include:

- The role of *Trichoderma* and *Gliocladium* in natural ecosystems
- Applications: biocontrol; improvement of plant growth, disease resistance and yield; enhancement of plant resistance to abiotic stresses; enzymes for pharmaceutical production and for the food and fabric industries; bioremediation
- Taxonomy, proteonomics, genomics and metabolomics

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Conference Report

Have you held or attended a meeting that you want other biocontrol workers to know about? Send us a report and we will include it in BNI.

Ecological Impact of Genetically Modified Organisms

In January 2003, a study group of the West Palaearctic Regional Section (wprs) of the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) was established to consider the ecological impact of genetically modified organisms (GMOs). At its last meeting in September 2003, the council of the IOBC/wprs changed the status of the group to a working group. The first full meeting of this new working group was held in Prague, Czech Republic, on 26–29 November 2003. The local organizer was František Sehnal from the Entomology Institute of the Czech Academy of Sciences in Èeské Budìjovice. More than one hundred participants from 23 countries attended the meeting. During the first 2 days, two keynote papers, 32 oral contributions and more than 40 posters were presented.

In her keynote paper, Angharad M.R. Gatehouse (University of Newcastle, UK) gave an overview of the methods routinely used for plant transformation, presenting examples of this novel technology with an emphasis on its contribution to agriculture; she also reviewed the current global status of GM crops. The opportunities for unintended effects (both predictable and unpredictable) to occur during transformation were discussed in detail in the light of such events occurring during natural recombination or classical plant breeding. She suggested that such unintended effects did not necessarily imply risks to the environment or, in the case of food crops, consequences for human health. In the second keynote paper, Jonathan Gressel (Weizmann Institute of Science, Israel) discussed possible ways to prevent transgene introgression from GM crops to other varieties and to related weeds or wild species (containment strategies) as well as ways to preclude the impact of introgressions should containment fail (mitigation strategies). Such technologies will allow transgenic crops to be developed for situations where they are especially needed, e.g. herbicide-resistant rice and barley that will allow control of closely related weeds without risk of the transgene moving into the weeds.

A large proportion of the presentations and discussions focused on the possible impact of insect-resistant GM crops, and *Bt*-maize in particular, on nontarget arthropods such as parasitoids, predators, butterflies and soil organisms. This focus came about because (a) most participants had a background in entomology, (b) there is a vast amount of published information and on-going research on the ecological effects of this particular GM crop, and (c) *Bt*-maize is the only insect-resistant transgenic crop that is currently grown commercially in Europe (i.e. Spain) and likely to enter the market in other European countries. Other important agronomic traits such as herbicide resistance were also addressed, but not in such detail. The exception, however, was a series of three oral presentations with results from the UK farm-scale evaluation of spring-sown herbicide resistant crops. This study made quite clear that detected effects such as differences in weed biomass, seed rain or invertebrate abundance were due to changes in herbicide regimes and, connected to this, in weed abundance and management rather than to the GM trait itself.

In addition to laboratory studies, results from a number of field experiments with *Bt*-maize conducted in Germany, Hungary, Czech Republic, Spain and Turkey and with *Bt*-potato and *Bt*-canola (rape) conducted in Italy were presented. The studies were generally faunistic in nature, using a number of different sampling methods to record the population dynamics of a large range of arthropod species. None of the studies revealed any clear evidence for ecologically relevant differences between the *Bt*- and non-*Bt* crops. A strong positive 'side effect' was reported from a study in Turkey where 20 times less of the mycotoxin fumonisin was detected in *Bt*-maize kernels after harvest compared with non-*Bt* kernels.

Other ecological effects of GM crops that were covered in the presentations included (a) the potential for gene flow from GM crops to non-GM plants and the possible consequences of this event, (b) unintended effects of GM crops such as probiotic effects on nontarget herbivores and (c) regulatory issues, especially the importance of post-release monitoring. This latter topic was discussed in detail in relation to *Bt*-maize and nontarget butterflies.

Unfortunately some relevant fields of study were absent or only marginally represented. These included the impact of GM crops on soil function/organisms and studies on GM organisms other than plants. Furthermore, the need was felt to involve botanists/weed specialists whose knowledge would be important for the discussion of gene flow from GM crops to non-GM crops or wild relatives.

On day 3 of the meeting, the following seven half-day long workshops were held (workshop organiser in parentheses):

- Hybridization/fitness of hybrids (Detlef Bartsch, Robert Koch Institute, Germany and Hans C. M. den Nijs, University of Amsterdam, The Netherlands)
- Monitoring/bioindicators (Salvatore Arpaia, Italian National Agency for Energy and Environment, Italy)
- Biodiversity implications – off crop (Andreas Lang, Bavarian State Research Center for Agriculture, Germany)
- Impact of GM crops on natural enemies (Jörg Romeis, Swiss Federal Research Station for Agroecology and Agriculture, Switzerland)
- Impact of GM crops on soil organisms/functions (Wolfgang Büchs, Federal Biological Research Centre for Agriculture and Forestry, Germany)
- Resistance management (Achim Gathmann, RWTH Aachen, Germany)
- GM crops and pollinators (Stefan Kühne, Federal Biological Research Centre for Agriculture and Forestry, Germany and Dirk Babendreier, Swiss Federal Research Station for Agroecology and Agriculture, Switzerland)

There was a feeling expressed throughout the meeting that most risks associated with GM crops are similar to the risks of other agricultural innovations and should be evaluated in this context. The risks must be assessed on a case-by-case basis, i.e. separately for each crop, each type of genetic manipulation and, where appropriate, for the geographic region. Participants at the meeting agreed on the need to elaborate risk assessment methods that should be applied to all new agrotechnologies, GM crops included.

The proceedings of the meeting, including short workshop reports, will be published in 2004 as an IOBC/wprs Bulletin. The next full meeting of the working group will take place in the first half of 2005. If you wish to be included on the mailing list of this working group or need additional information, please get in contact with the convenor at the address below.

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