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General News

India to Release First Fungal Pathogen for Classical Biological Control of a Weed

Mikania micrantha (mile-a-minute weed) has been a persistent problem in the tropical moist agroforests and natural forests of India, especially in Kerala and in the north-eastern states. It is also a serious invasive alien weed in several agricultural and horticultural ecosystems, tea being one of the major crops affected. Between 1996 and 2000, an international collaborative project, funded by the UK Department for International Development (DFID), investigated the classical biological control (CBC) potential for this weed in India. Natural enemies from the Neotropical native range of the plant were studied and the autoecious, microcyclic rust, *Puccinia spegazzinii*, which produces only teliospores and basidiospores, was identified as the prime candidate for introduction into India[1]. In the Implementation Phase (2003–2005) of the project, which is a collaborative venture between CABI Bioscience, UK and the Project Directorate of Biological Control, National Bureau of Plant Genetic Resources (NBPGR), Kerala Forest Research Institute and Assam Agricultural University in India, *P. spegazzinii* (isolate W1761 originating from Trinidad) has been imported into the National Containment-cum-Quarantine Facility at NBPGR, New Delhi, and established successfully on several Indian ecotypes of *M. micrantha*.

India has considerable experience in the use of arthropods as CBC agents against weeds. *Neochetina eichhorniae*, *N. bruchi* and *Orthogalumna terebrantis* against *Eichhornia crassipes* (water hyacinth), *Cyrtobagous salviniae* against *Salvinia molesta* (water fern), and *Zygogramma bicolorata* against *Parthenium hysterophorus* (parthenium weed) have been notable examples of CBC of weeds in India. The stem-gall fly, *Cecidochares connexa* is the most recent weed insect introduction (imported in 2002), and is ready for field release against *Chromolaena odorata* (siam weed) in south India. However, no pathogen has ever been introduced by the Indian government for weed control, prior to *P. spegazzinii* importation.

Puccinia spegazzinii was imported into India after establishing its host specificity in the CABI Bioscience Quarantine in UK, where the universally-followed centrifugal phylogenetic system was employed to aid test plant selection. The results formed the core of a Dossier drawn up for the regulatory authorities in India. However, in consultation with the Indian economic botanist, mycologists and plant pathologists, an additional list of economically important crops, as well as plant species taxonomically related to *M. micrantha* that grow in India, was drawn-up for further testing of the rust under the Indian quarantine conditions.

In the additional host-specificity tests done in India during 2004–05 *P. spegazzinii* isolate W1761 did not infect any of the 74 plant species tested. However, the rust was highly pathogenic to different populations of *M. micrantha* from both Assam and Kerala, indicating that the rust fungus has considerable potential as a CBC agent for *M. micrantha* in India. The repeated testing of 18 plant species that had already been screened in CABI quarantine confirmed their immunity to the rust, and hence the integrity of the UK tests. The additional host-specificity tests have confirmed that the rust is specific to *M. micrantha* with reference to the range of plant species tested and under Indian quarantine conditions.

Based on a Supplementary Dossier summarizing these results, which was submitted with the application for limited field release of the rust, the Plant Protection Advisor to the Government of India, from the Ministry of Agriculture, has given the permit for release of *P. spegazzinii* in four identified areas, two each in Kerala and Assam.

The teliospores of *P. spegazzinii* are embedded in the host tissue, and it is the basidiospores that are released to infect new plant tissue. Hence, the release strategy is to be based on placing pots of rust-infected plants, with young pustules, at strategic points within severely weed-infested areas, and allowing the infection to spread naturally. In agricultural areas with high value crops affected by the weed, such as tea, the inoculation process will be intensified. Large numbers of rust-infected plants will be mass-produced early in the growing season, and placed at close intervals in the infested crop. The aim of this approach is to speed up the infection process, and hence minimize the impact of the weed within one season.

According to a recent estimate[2], 26 species of fungi originating from 15 different countries have been used as CBC agents against more than 26 weed species in seven countries. With the release of *P. spegazzinii*, India would become the eighth country in the world and the first continental Asian country to deliberately and scientifically introduce an exotic fungal pathogen for weed biological control. This is also the first time that a pathogen is being used as a CBC agent against *M. micrantha*.

[1]Ellison, C.A. 2001. Classical biological control of *Mikania micrantha*. In: *Alien weeds in moist tropical zones, banes and benefits*, Sankaran, K.V., Murphy, S.T. & Evans, H.C. (eds). Kerala Forest Research Institute, India & CABI Bioscience, UK Centre (Ascot), UK, pp. 131–138.

[2]Barton, J. 2004. How good are we at predicting the field host-range of fungal pathogens used for

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classical biological control of weeds? *Biological Control* **31**, 99–122.

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Soyabean Aphid: Foreign Agents May Apply

Scientists spread across six states in the US Midwest together with USDA-ARS (US Department of Agriculture – Agricultural Research Service) are collaborating to tackle the soyabean aphid, *Aphis glycines*, through classical biological control. The new programme is supported by soyabean producers through the North Central Soybean Research Program (NCSRP). This work follows on from previous initiatives that did much to define the key elements of the aphid's management, which included research on the basic ecology of the soyabean aphid, sampling, threshold development and conservation of native natural enemies.

The soyabean aphid is native to Asia, and was first recorded in the USA in 2000 in Wisconsin. From there it spread rapidly and is now found in 22 US states and three Canadian provinces, putting 60 million acres (>24 million hectares) of soyabean crops at risk. Direct feeding is estimated to result in a 14% average yield loss, which translates into costs of millions of dollars through damage and/or control costs. Figures for lost production and acreage sprayed in 2003 indicated that, just 3 years after its arrival, the soyabean aphid had become the most costly insect pest ever for this industry. In addition, indirect damage through pathogen transmission is a distinct threat as soyabean pathogens now have a widespread and abundant vector, something they lacked in the past. Its role as a mechanical vector of several diseases in snap beans (*Phaseolus vulgaris*) has already been demonstrated, and threatens continued production of this crop in Wisconsin.

Initial studies indicated that the situation could have been far worse, for natural biological control was limiting the damage. Surveys revealed that over 45 species of natural enemies attack the aphid in US soyabean fields. By comparing aphid populations protected and unprotected from these native natural enemies, it was shown that the natural enemies have a significant impact on the aphid's population growth and pest status:

- In Michigan in 2002, aphids protected from natural enemies reach a population 100× higher than unprotected aphids
- In Indiana in 2004, aphids protected from natural enemies typically quadrupled their population size in a week

In fact, in 2004, although the aphid arrived early in the season and found temperatures near-optimal for growth, few crops suffered economic damage – a situation attributed to the impact of natural enemies. However, this contrasted with the outbreaks of the previous year.

With the realization that native natural enemies were having an impact, work had focused on integrating biological control into the broader management strategy. To conserve these naturally-occurring natural enemies the conservation growers are encouraged not to spray insecticides until the aphid reaches threshold (250 aphids/plant) and not to use 'tank mixes', especially early in the season (e.g. adding insecticide to applications of glyphosate (Round-up)). The outbreak in 2003 showed this approach had limitations, and the logical next step was to investigate whether introducing additional natural enemies from the pest's area of origin could improve the consistency of control. The objectives of the classical biological control programme are to:

- Assess the suitability of exotic natural enemies of the soyabean aphid
- Educate growers on the potential of biological control for managing soyabean aphid
- Develop a region-wide release programme

Finding New Natural Enemies

Field surveys were conducted in China, Japan and South Korea to study the ecology of the aphid in its native range, where previous research by national scientists had shown that natural enemies are key to soyabean aphid's non-pest status (except sporadically in north-east China). Dozens of natural enemies were found.

The process of selecting safe species to release from among such a large number of possible candidates is a complex process. Nine species are currently being studied in quarantine facilities in USDA-ARS facilities in Delaware (New Jersey) and at the University of Minnesota, and this work will be supported by further studies in the field in the USA and in the areas of origin of the natural enemies. Making a successful classical introduction (not to forget making most effective use of time and funds) means selecting a natural enemy that can establish and thrive (in particular overwinter) in the Midwest, has an impact on the soyabean aphid, does not adversely affect other species, and does not suffer undue adverse effects from other natural enemies of the aphid.

Native US aphids that could be at heightened risk to nontarget effects of released (Asian) natural enemies are those that exist at low numbers and temporal and spatial proximity to potential release sites. They will be identified by surveying aphids in habitats near those the soyabean aphid uses throughout its life cycle. In addition, field studies in Asia will provide insights into potential nontarget effects by looking at what aphid species are being attacked by soyabean aphid natural enemies there, again using the stages of the soyabean aphid life cycle as the basis for surveys (with the added prospect that this may uncover as-yet unknown natural enemies.)

Compatibility with native (and previously established) natural enemies means looking at intraguild predation both in quarantine and post-release in the field. The effects of insecticides will also be assessed. Natural enemies most resistant to attack by natural enemies already present and tolerant to pesticides used in soyabean crops will be given priority.

Of the species currently under study, studies on one promising candidate have progressed to the point where an application for permission to release is being considered: the parasitoid *Lipolexis gracilis* was collected in a region of China with a climate similar to that of the North-Central region, is highly specific, and can attack 30 soyabean aphids per day. Other species are expected to be ready for approval later on.

Staging Releases

Once a candidate gains approval for release, the first releases will be made in 2 m × 2 m cages. These will allow researchers to measure the natural enemy's ability to attack aphids, increase in density and (crucially) protect soyabean yield. Next, field releases will be made at university research stations. Natural enemy population dynamics, overwintering ability and impact on nontarget species will be monitored, together with effects on soyabean yield. The final stage will be scaling up to region-wide releases involving soyabean growers as active participants, following the model established by the purple loosestrife biological control programme, see: www.seagrant.umn.edu/exotics/purple.html

The success of the soyabean aphid programme will ultimately depend on engaging with the growers. Biological control is more knowledge intensive than many other management practices. Delivery of information about existing and imported natural enemies will be critical. The research team will work with extension agencies on a range of educational materials including PowerPoint presentations, fact sheets on soyabean aphid and common natural enemies, a pest and natural enemy pocket guide, a 30 – 40 page colour booklet on biological control of insect and mite pests of soyabeans, and a soyabean aphid biological control website, as part of the University of Wisconsin's existing Soybean Plant Health website, see: www.plantpath.wisc.edu/soyhealth/

Workshops, field days and distance learning will all have a role to play.

With useful native natural enemies already impacting on soyabean aphid in North America, no one is even looking for a 'silver bullet'. Carefully selected exotic agents that can enhance and stabilize the control afforded by the biological control strategies put in place by earlier initiatives will benefit growers with savings in control costs and preserved yields that will quickly run into hundreds of millions of dollars.

Collaborating institutions in this project include Purdue University in Indiana, the University of Wisconsin, Michigan State University, the University of Minnesota, Iowa State University, the University of Illinois, the Illinois Natural History Society and the US Department of Agriculture.

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Hieracium Biocontrol: Hands across the Pacific

Hawkweeds (*Hieracium* spp.) are perennial rhizomatous herbs, with up to 1000 species occurring worldwide. Several hawkweed species of Eurasian origin have become major weeds in pastures, and clear-cut and conservation areas in New Zealand and North and South America. Here we look at biological control programmes from either side of the Pacific – New Zealand and Chile – against *H. pilosella* (mouse-ear hawkweed). This is a hawkweed species that can reproduce vegetatively through stolons resulting in dense, carpet-like ground cover. It displaces native plants and forage species, and reduces biodiversity and food available for livestock.

New Zealand: Hieracium Agents Settle in for the Long Haul

The biological control programme against *H. pilosella* in New Zealand is managed by Landcare Research. *Hieracium pilosella* is a major weed in the highland areas of South Island, where it can form dense mats that exclude almost all other vegetation. Its success is attributed to tolerance of low soil fertility and overgrazing, although it is given an edge over plant competitors because it is one of the few plants that rabbits do not eat. It is now becoming more common in some areas of North Island. Over the years, five insect natural enemies found during surveys in Central Europe by CABI Bioscience European Centre (Delémont, Switzerland) have been approved for release in New Zealand.

There are no native *Hieracium* species in New Zealand, but of the nine introduced species (plus one hybrid), five are regarded as weedy (*H. pilosella, H. praealtum, H. caespitosum, H. aurantiacum* and *H. lepidulum*). The biocontrol programme primarily targeted *H. pilosella* as the most problematic and widespread of the hieraciums. All of the five introduced insect biocontrol agents attack *H. pilosella* but varying combinations of these agents also attack varying combinations of the other four weedy hieraciums.

As far as the principal target, *H. pilosella*, is concerned, the signs seem promising for two gall-forming species, but the other three introduced insects are proving less tractable.

The hieracium gall midge (*Macrolabis pilosellae*) was released 3 years ago and has established at 60% of all release sites. This insect seems to establish well as galled leaves were found at 70% of sites where they were first released only a year ago. Establishment is likely to have been aided by the scale of releases with a mammoth rearing programme having allowed a significant increase in insect numbers per release.

The hieracium gall wasp (*Aulacidea subterminalis*) was first released in 1999. A survey this year showed that it has established at 20% of sites checked. This may be an underestimate as a wet spring and summer have led to lush growth that made the pea-sized galls hard to find. Two sites have been lost this season due to changes in land management making the overall establishment success rate for all release sites to date 38%. In total, 98 gall wasp releases have been made, including four this year. All galls found were within 50 m of the release site and at one site about six gall wasp larvae per square metre were found. Obviously higher levels than this are needed to make any impression on hieracium, but it is a start.

Next year, Landcare hope to look at midge and wasp density and spread in more detail.

Five new releases of hieracium root-feeding hoverflies (*Cheilosia urbana*) were made early this year, from a shipment received from Switzerland. Unfortunately the flies' emergence was protracted after a prolonged pupation period so only small numbers could be gathered together for each release. There have been nine releases of this agent since 2002 but first signs of establishment are yet to be found. Investigations are looking at whether the flies can be mass-reared in glass and shade houses, and how successful this is will be found out in the spring. Work with the closely related crown feeding hoverfly (*C. psilophthalma*) was put on hold in New Zealand while further research into its rearing is undertaken in Switzerland.

The hieracium plume moth (*Oxyptilus pilosellae*) is proving to be an even greater source of frustration. The insect was first imported in 1996, but has yet to be observed mating in New Zealand. CABI Bioscience in Switzerland has been asked to try and unravel the mysterious cues that trigger the plume moth's mating and oviposition.

This project is funded by the Hieracium Control Trust and the Sustainable Farming Fund.

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Chile: Hieracium Biocontrol Programme Launched

In 1987, the presence of *Hieracium pilosella* was detected for the first time in Chile, following a report from a rancher in the south of the country, near to the city of Punta Arenas (at a latitude of around 53º South). The weed poses a serious risk to the livestock industry in this region, where the harsh climate and poor soil mean sheep rearing is one of the few economically viable land uses.

Quite how *H. pilosella* reached Chile is not clear, although it might have entered with the import of animals from the Falkland Islands or New Zealand, or through the import of contaminated forage seeds. Whatever its origins, this weed has proved very aggressive, with a high index of vegetative spread and excellent longevity (plants up to 13 years old have been found) in spite of the extreme conditions of climate and soil present in the affected zone.

The areas affected by the weed are in Porvenir and Primavera counties (Tierra del Fuego Province) and San Gregorio and Punta Arenas counties (Magellan Province), with a total area of some 86,000 ha of land infested at present. However, the area at risk of being colonized could be as high as one million hectares.

In 1989 both the Chilean Agricultural and Livestock Service (SAG) and the Chilean Institute of Agricultural Research (INIA) initiated chemical control trials in the area of Pecket Harbour, Punta Arenas County. The most effective herbicide was found to be Tordon 24 K (picloram) at a dose of 2.2 l/ha. Control measures recommended to producers consist of strict localized preventative control, which means eradicating initial patches of hieracium using chemical or mechanical means. However application of herbicides alone does not eliminate the danger of re-infestation. In addition chemical control of this weed in this region of extensive sheep rearing is economically impractical in view of the cost of control and the profitability of the land. Other management options are clearly needed.

In the search for alternative ways of controlling the weed, in 2004 SAG signed an agreement with Landcare Research of New Zealand, an institution with vast experience in biological control, to provide biocontrol agents together with advice in biological control methods. As a result, 100 specimens of the hieracium gall wasp, *Aulacidea subterminalis,* were sent from New Zealand to Chile in November 2004. This cynipid wasp causes galling of the tips of *H. pilosella* stolons, which reduces their ability to spread vegetatively by producing daughter plants at the tips. The imported wasps are currently being multiplied under quarantine conditions in greenhouses of SAG located in the central zone of Chile, using hieracium plants brought from Punta Arenas. Specificity tests will be carried out, exposing *A. subterminalis* to different hosts plants to confirm that this wasp will only attack *H. pilosella* and would pose no threat to crops or other native plants (there are a number of native *Hieracium* species in South America). Once this has been done, the wasp could feasibly be approved for release in 2006, with releases initially planned for the worst-affected zones.

At the end of 2005, a second biocontrol agent, *Microlabis pilosellae*, the hieracium gall midge, will be imported from New Zealand under the same agreement. This cecidomyiid fly lays its eggs in the centre of rosettes, in stolon tips and in leaf axils, but sometimes in the flowerheads too. It thus complements perfectly *A. subterminalis*'s activity. The same steps will have to be carried out with this species to ensure it is safe to release. If all goes well, first releases are anticipated at the end of 2007.

If only 30% of the livestock in the region suffers from the action of this weed in the next 15 years, to the extent that this proportion of lambs would not achieve the goal of 12 kg cold carcass weight the European market demands, the loss for the producers would be almost US$2 million per year. This is because the differential price for a carcass of smaller size, which must compete for alternative markets, is more than $10 per animal. Undoubtedly, if successful, the project will provide a viable alternative for managing one factor in the deterioration of the productivity of the natural grassland of this region, caused by high populations of *H. pilosella*, overgrazing and previous deficiencies in management. This would lead to a significant improvement in the productive potential of the sector, resulting in a greater quantity of lamb and wool for the market, thus raising the income of the producers.

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Negating Noogoora in Australia

The Department of Agriculture, Western Australia, the Northern Territory Department of Infrastructure Planning and Environment and CSIRO have combined to launch a renewed attack on noogoora burr (*Xanthium occidentale*) in northern Australia, including the Kimberley. An annual weed, it mainly infests the banks of waterways and regularly flooded areas of the Top End.

Noogoora burr was one of the first weeds to be targeted for biological control in Australia but agents officially released on it have had little impact. However, infestations in south-eastern Queensland have been largely controlled by the rust fungus, *Puccinia xanthii,* that was accidentally or illegally introduced into Australia in 1975. Unfortunately the fungus has not been successful at controlling the massive infestations of the weed in the northern areas.

Now, in a 2-year project, Dr Louise Morin, from CSIRO Entomology and the CRC for Australian Weed Management, is leading a team which will use the latest molecular science techniques to confirm suspicions that the rust fungus is not genetically diverse in Australia, a possible reason to explain the poor performance of the rust in the northern, more tropical conditions. Data will also be collected to assess the threat that Noogoora burr poses to northern Australia and document the prevalence of the rust fungus there. The results of this work will help facilitate any future searches for additional strains of the rust elsewhere in the world that might be better adapted to the northern Australian conditions.

Noogoora burr is a major pastoral and environmental weed. It outcompetes native and pasture plants, its seedlings are toxic to livestock, burrs reduce wool value and it poses quarantine issues. Thick infestations of the burr along riverbanks prevent access for stock and recreational users. It is also less effective at preventing erosion than native plants. The seeds (burrs) are easily spread by stock, feral animals, floods and vehicles.

The funding for this initial pilot project has come from the Department of Agriculture, Western Australia.

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Toward Biological Control of Cabomba in Australia

Cabomba (*Cabomba caroliniana*), or water fanwort, is a fast-growing submerged weed with the potential to spread throughout Australia's aquatic habitats. It was originally introduced into Australia by the aquarium trade and thrives in slow-moving water, particularly where there is a high nutrient content. Cabomba prefers areas of permanent standing water less than 3 m deep and is often found along the margins of lakes and reservoirs. However, it can grow in deeper water.

Once cabomba is established control is difficult. Herbicides are largely ineffective and should not be used in or around public water supplies. Some use is made of floating mechanical harvesters to remove the weed but they can only be used in deep water and wide channels. They are also expensive to purchase and operate. And because they only remove the tops of the plants, the stems soon reshoot.

Biological control offers effective, long term and sustainable control of cabomba. Funded by the Natural Heritage Trust (NHT) through the Australian Government's Department of Environment and Heritage, and with support from a number of community groups, CSIRO Entomology has made significant progress in its search for potential biocontrol agents in cabomba's home range, Argentina and adjacent countries.

Extensive surveys were conducted in South America to map cabomba's distribution and once its native range was known, the search for natural enemies began in Argentina and Brazil. Already, several insects, including a weevil and a moth, show promise as biocontrol agents.

The South American surveys of cabomba are continuing in order to discover as many potential agents as possible. Once ways of rearing the most promising agents in quarantine have been developed, further research will be conducted on them. After preliminary trials overseas, host-specificity testing of potential agents that have been approved for import by the Australian Quarantine Inspection Service will be conducted in quarantine in Brisbane, Queensland.

Meanwhile, studies on the ecology of the weed in Australia will help in:

- Understanding the growth and spread of the weed
- Devising integrated management strategies
- Allowing assessment of management strategies
- Guiding selection of appropriate agents
- Selecting appropriate release sites for approved agents

These studies are being done in collaboration with the Queensland Department of Natural Resources and Mines and the Lake MacDonald Catchment Care Group and the Noosa Shire Council, both in Queensland, along with numerous other stakeholders.

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On the Road for a Rapid Response

Following on from articles in the June 2005 issue on new state-of-the-art facilities in the USA for work on invasive species and their biological control [*BNI* **26**(2), 51N, 'US building capacity'], we report on another innovation, but this time one on wheels.

Lack of functional field laboratory and greenhouse space can be particularly acute when a new invasion is being tackled. Time is often of the essence if an eradication effort is to stand a chance. In the absence of suitable local facilities, and with no time to build them, tackling the invasion can be hampered by the time it takes to travel or transfer material to and from laboratories and restrictions due to quarantine regulations. The answer is to take the facilities to the invasion site.

The idea of a mobile containment greenhouse/laboratory was originally conceived 10 years ago by two USDA-ARS (US Department of Agriculture – Agricultural Research Service) scientists, Tim Gottwald and Steve Garnsey. A prototype was designed, constructed, improved and road-tested to prove that it worked – and has long been in use by the Citrus Canker Eradication Program[1].

The new, second-generation Mobile Containment Greenhouse Laboratory (MCGL) was rolled out in March 2005 at the US Horticultural Research Laboratory (USHRL) in Fort Pierce, Florida. The mobile unit will further the joint efforts of USDA-ARS and USDA-APHIS (Animal and Plant Health Inspection Service) to protect agriculture from pests and diseases.

The new MCGL is 15 m long, built on a trailer-type chassis, houses a laboratory and greenhouse, and can be pulled by a standard semi tractor or large truck. It is the result of a collaborative project involving USDA-ARS and USDA-APHIS-PPQ (Plant Protection and Quarantine) intended to provide new mobile field facilities for the Citrus Canker Eradication Program. The laboratory is equipped with metal cabinets, stainless steel counter tops, a refrigeration/freezer unit, a biosafety cabinet, an incubator, an autoclave and an HEPA air filtration unit. The self-contained greenhouse has temperature, humidity and CO_2 controlled by a computer data acquisition and feedback control system. The control system can be monitored remotely via wireless communication. There is also a manual back-up control system should the primary system fail. Temperature is regulated by means of two redundant Bard heating and cooling units. Light intensity and duration are controlled using a mixture of eight metal halide/high pressure sodium lamps (with adjustable heights). A fertigation injection system allows automatic fertilization of plants. The sidewalls of the facility are of custom-extruded aluminium framing sheathed with double walls of Lexan panels. It has an aluminium diamond plate floor, and a central drain channel empties into a holding tank, and to complete the self-contained status of the unit, an emergency diesel generator provides automatic back-up for the laboratory and greenhouse in the event of a power failure, or in the absence of a convenient electricity supply. The fuel and holding tanks levels are monitored, and the entire unit has a hydraulic self-levelling system. The MCGL was built by Clegg Industries (Victoria, Texas) and Competition Trailers (Henderson, Texas), with final outfitting by the Aircraft Equipment Operations group of USDA-PPQ at Mission, Texas.

The MCGL facility can be used for a number of pathological, entomological and horticultural tasks. It is currently deployed in the Miami (Florida) area, where it is being used routinely in the Citrus Canker Eradication Program, and is indeed a cornerstone of this programme. It is equipped to allow a wide range of tasks including pathogen isolation and culture, pathogen manipulations, host inoculations, tests of bacterial survival, detection assays, etc. Without it, much recent work that has supported the eradication programme against citrus canker could not have been carried out. However, this second-generation MCGL, which cost some US$250,000–300,000 to build, can be replicated and deployed as needed against outbreaks of significant new quarantine pests in the USA – and may elicit interest in other countries seeking to develop similar capacity.

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[1]For more information on the Citrus Canker Eradication Program and background see:

www.apsnet.org/online/feature/citruscanker/

www.doacs.state.fl.us/pi/canker/

IPM Systems

In this issue, this section is devoted to articles on microbial biocontrol and biopesticides, and specifically those targeting arthropod pests, as the introductory note explains.

Bioinsecticides: Set to Take Off?

Despite much promising research on entomopathogens, *Bt* continues to dominate the world's small bioinsecticide (indeed, biopesticide) market. But is there a rival in the wings? In recent weeks, *Metarhizium anisopliae* has stolen the headlines following field trials of a biopesticide based on the fungus against desert locust in North Africa, and the publication of research indicating its potential against adult anopheline malaria vectors. Given the size of the locust and mosquito control markets, is a commercial breakthrough for *Metarhizium* on the horizon? As news of other entomopathogens filtered in from around the world this quarter, it seemed a good opportunity to look at progress in and enduring obstacles to biopesticide development and microbial control of insects.

This is not an overview, but a snapshot from which some common themes emerge. A common hurdle is the progression from experimental efficacy to field success, with formulation and application singled out as needing particular attention. In this context, attention is drawn to the Proceedings section of this issue, which highlights a volume that synthesizes a wealth of information on microbial control in India and identifies gaps in formulation and application technologies. The argument is that, for biopesticides, these technologies are still in their infancy, and progress is essential for the future of the sector. Technology transfer is another recognized stumbling block. In Training News in this issue, an article looks at how a curriculum centred on microbial control-based IPM of rhinoceros beetle (*Oryctes rhinoceros*) in coconuts was developed for farmer field schools.

Green Muscle has a Field Day

For the first time, Green Muscle® has been successfully tested against the desert locust (*Schistocerca gregaria*) under large-scale field conditions. FAO (Food and Agriculture Organization of the UN) calls this a major breakthrough. During a field trial organized jointly by the plant protection authorities of Algeria and FAO near El Oued in eastern Algeria, the biopesticide was sprayed on more than 1400 ha of land infested by desert locust nymphs. Green Muscle® , a formulation of the fungus *Metarhizium anisopliae* in mineral oils, infects the insect and slows its feeding, eventually killing it in 1-3 weeks. In the trial, locusts were visibly weakened and started moving slowly after 4 days and were then eaten by birds, lizards and ants. By demonstrating that Green Muscle® can be applied successfully by professional plant protection teams over a large area, the trial in Algeria proved that the biopesticide can be a realistic alternative to conventional pesticides.

This is not the first large-scale trial of Green Muscle® , which has the potential to contribute to the management of a range of pest locusts and grasshoppers. Articles in the September 2005 issue of *BNI* [*BNI* 25(1), 47N–51N] include a report of operational-level trials against red locust (*Nomadacris septemfasciata*) adults and nymphs in Tanzania and Zambia. Nonetheless, it is the first extensive trial of the biopesticide against desert locust. Plagues of desert locusts in northern Africa and the Sahel in 2004-05 led to insecticides being applied on some 12.8 million hectares across 16 countries, and untold crop losses.

It is significant that the biopesticide was shown to be effective against desert locust under realistic field conditions. The length of the fungus' incubation period depends on the environmental temperature – it grows well between 15°C and 35°C. In the countries that are most affected by desert locust infestations, this temperature range prevails during parts of the winter and the rainy season when locusts breed. The trial in Algeria was conducted under optimal temperatures that favoured the development of the fungus and more field testing under less favourable conditions is required to explore the potential and limitations of the product.

Green Muscle® is not expected to replace synthetic pesticides. It is not a knock-down insecticide. Conventional, rapidly acting products will continue to be needed to protect crops from imminent locust attacks by hoppers and swarms. Though the biopesticide can be applied to swarms and will eventually kill the locusts, monitoring these swarms over a period of several weeks to assess the effect of the treatment is difficult and costly and cannot be expected of control operators in the heat of the battle.

There are also operational challenges to Green Muscle® deployment. The biopesticide is currently being produced commercially by only one company in South Africa with limited production capacity. Efforts are being made to develop production in Senegal, West Africa. The cost of Green Muscle® is still high when compared with some of the chemical pesticide alternatives. However, a larger market could lower production costs significantly. Unlike conventional pesticides, the application of Green Muscle® requires minimum safety measures and less personal protection equipment.

Future adoption of Green Muscle® now depends on (some) continued testing and demonstration trials and, ultimately, commitment from national governments, agencies (such as FAO) and donors to support the use of the technology to develop more sustainable, integrated strategies for locust control. The current contrast with Australia and the anti-locust *Metarhizium* product Green Guard® is marked. There, guarantee of uptake of the biopesticide by the Australian Plague Locust Commission provided the financial climate for the manufacturer to expand capacity, and the biopesticide is now in operational use alongside chemical pesticides. Funding commitment for Green Muscle® would allow producers in Africa to plan too, and give them the confidence to increase production capacity. Specifically, quantities of the product ordered and paid for well in advance of locust upsurges (and these are forecast with grim accuracy by FAO's locust monitoring system) could be manufactured and stored for rapid delivery when the need arises, in the same way that chemical pesticides are stockpiled by agrochemical companies.

Main source: FAO Press Release, 28 June 2005
www.fao.org/newsroom/en/news/2005/103849/index.html

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Breaking the Biopesticide Ceiling

Biological Control Products (BCP) was founded in 1995 by Dr Di Neethling, with the express intention of producing a biopesticide based on a fungus, *Paecilomyces lilacinus*, to control nematodes. The challenges facing the company at this early stage were enormous – including the generation of business plans, raising funds, attracting investors, registration trials, refining product application and developing a suitable manufacturing technology from scratch. Most of this had never been done before for a fungal biopesticide. The resultant product, PL Plus®, was launched in 1997 with full regulatory approval by the South African authorities for its use as a nematicide.

It was precisely the progress that had been made in these areas that lead BCP and CABI Bioscience to cross paths in 1998, under the banner of LUBILOSA and the helpful eye of Dr Nina Jenkins. A virulent strain of the fungus *Metarhizium anisopliae* var. *acridum* had been isolated by the LUBILOSA (Lutte Biologique Contre les Locustes et les Sauteriaux) team with the aim of controlling locusts in the famine-ravaged African continent in an environmentally responsible manner. Following the identification of manufacturing parameters and a novel formulation by the researchers, the product was ready for commercialization. Based on the progress made with PL Plus®, BCP was identified as a suitable commercial partner. Although still in its infancy at this stage, the Green Muscle® brand was born and the next step in the exciting life of Green Muscle® and BCP began.

After many years of patiently lobbying by both CABI Bioscience and BCP, supplying product for trials, optimizing the manufacturing process and refining the application methods, the concept of using fungal-based biopesticides began to garner recognition. With the support of the UN Food and Agriculture Organization, FAO – Sir Clive Elliott, Dr James Everts and many others – together with funding from the donor community, Green Muscle® has now been used to successfully control locusts (>97% mortality) in large-scale field trials in northern Africa [see 'Green Muscle has a field day', this section].

Since 1998, BCP has achieved registration in South Africa and many other African countries not only for Green Muscle® (*Metarhizium anisopliae*), but also for PL Plus® (*Paecilomyces lilacinus*), Tricho Plus® (*Trichoderma harzianum*), Bb Plus® and Bb Weevil® (*Beauveria bassiana*).

BCP has maintained strong ties with CABI Bioscience, Imperial College Wye and Rothamsted Research in the UK as well as with the Universities of Pretoria and Witwatersrand in South Africa. It has also forged strong links with many international companies involved in biological control.

Today, after a decade of operations, BCP counts among its shareholders British Aerospace Systems, SAAB and the IDC (Industrial Development Corporation of South Africa). BCP has a wide range of biologically based products in its range, with a very large portion of its expenses directed into researching and commercializing new products. But BCP's core focus still remains the fungal-based biopesticides it began with and it has recently upgraded its manufacturing capacity by 500% to cope with the increased demand for these products.

Commercialization Challenges

1. Customer acceptance and expectations

A good question to begin with is 'who is the customer?' and the answer is not as cut-and-dried as one would initially believe. If one takes a broad definition of the 'customer' as someone influencing the purchasing decision, then, in the case of Green Muscle® for example, BCP's purchase history has shown that the customer can be any one of the following:

- A research organization, wanting to undertake trials on target and nontarget organisms, or wanting to broaden the registration range
- A donor organization with a mandate to use a more environmentally friendly product in the fight against locusts plagues and famines in Africa
- A governmental department implementing a sustainable, preventative programme to control locusts at traditional breeding sites
- An international agency tasked with managing food security across borders and geographical boundaries
- A traditional chemical agent, wanting to carry the product in his portfolio
- A farmer wanting to buy direct so that he can control locusts in his citrus orchard without exposing himself or his workers to toxic products
- A consumer wanting fresh produce that is free from pesticide residues.

Every one of these customers would have a different set of expectations, ranging from high efficacy, low environmental impact, quick speed of kill, low cost of treatment, good shelf life, high profit margin to low eco-toxicity. Adding to these expectations are those of the researchers and the commercial partners, which include: specifications, quality, consistency of supply and demand, profit margins and the long-term future of the product. The challenge in ending up with a truly successful product is that most, if not all, of these expectations need to be met.

2. Educational challenges – breaking the 'pesticide mentality'

Without detracting from some of the successes of the chemical industry in agriculture over the last few decades, a 'pesticide mentality' has become prevalent. This culture carries the expectation that a 'good' product is robust, 'fire-and-forget', broad spectrum and immediately effective. This is obviously not true for the biological products, comprised of living organisms that have a life cycle to complete in order to be effective and need more careful storage and handling. Experience has shown that, although the concept of a natural growth cycle is not foreign to the average farmer, a strong educational programme is necessary to realign his expectations. Some are open to a novel approach and others are 'dyed-in-the-wool'. Those that are open to it are often surprised by spin-offs that come with a more natural approach, in the form of increased yields from healthier soil.

3. Pricing and volume dependency

There is clearly a case of emerging industries with comparatively high R&D and overhead costs having to compete with mature, large volume chemical products in the run-out phase of their lifecycle. In addition, registration requirements have been geared towards the chemical industry and in many cases are too restrictive and, more importantly, too expensive for biological products. It is no surprise to learn that many large chemical companies have, at some stage, investigated biological products, but, having considered the registration costs and the sales potential, decided not to pursue them. It becomes a volumes game and, once sales volumes increase beyond a certain level, then the manufacturing costs (and market price) drop quickly, also helping to absorb the significant start-up costs.

4. Traditional distribution channels – are they throttling supply?

In the case of many biopesticides, the only distribution channels available to take the product to the farmer are those of the traditional chemical agencies. Without a doubt, there are divided loyalties and differing agendas. Products are placed on the backburner, supplied only if requested. Some chemical agencies place a high mark-up on the products, pricing them out of the market. Other chemical agencies become glorified delivery boys, with no real understanding of the product and ill-equipped to offer a decent educational programme and after sales service. The challenge is to find a distribution channel that shares the vision and is committed to nurturing both the products and the end user.

5. Scale up

Some of the difficulties these fungal-based products are facing in market penetration include cost–benefit, consistent performance, efficacy and shelf life. Looking at these, one can see BCP's motivation for taking these fungal bioproducts from a traditional backyard type operation into a high quality GMP (good manufacturing practice) production facility. In doing so the costs of manufacture are initially higher as capital equipment needs to be procured and funds channeled to process research, but they quickly drop as volumes increase. Having a well equipped QA/QC (quality assurance/quality control) laboratory with suitably qualified staff means that not only can process optimization can be done but with strict quality assurance procedures in place, a more repeatable, controlled process is obtained with associated commercial benefits. Quality can be tightly monitored and shelf life improved. BCP's entomology division with plant and insect culture and testing facilities allows the essential monitoring of each batch for efficacy and allows product improvements to be easily tested *in vitro*. A more effective product can lead to reduced dose rates and better cost–benefit.

There is an invisible ceiling that needs to be broken through, below which it is increasingly difficult to succeed in the biopesticides industry and ultimately the only race is against time as start-up capital is depleted.

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Malaria Biocontrol Grows Up

Beating malaria is one of the world's biggest health and development challenges, with some high-profile funding initiatives currently backing the drive. Two papers in a recent issue of *Science* highlight the potential for fungal pathogens to reduce malaria transmission. *Bacillus thuringiensis* ssp. *israelensis* (*Bti*) is an effective biocontrol agent against larval mosquitoes, used in many countries, but these two studies looked at the potential of *Beauveria* and *Metarhizium* against adult *Anopheles*. Targeting adults in this way is an exciting development because malaria rates are far more sensitive to changes in adult than larval mosquito survival.

Strategies for controlling the adults rely heavily on internal treatment of buildings – spraying or impregnating surfaces with insecticides. This can be very effective and has been shown to lead to substantial reductions in the incidence of malaria. Biopesticides based on fungi could be used in a similar fashion, with spores formulated for spraying walls or ceilings or impregnating cloth or netting. The spores germinate on contact with a mosquito, penetrate the cuticle and grow inside the insect body, eventually causing death. The slowness of this process compared with a knock-down insecticide is a well-known drawback of bioinsecticides but, as the studies described below found, not necessarily in the case of malaria mosquitoes. Species of the malaria parasite, *Plasmodium*, take up to about 14 days (depending on ambient temperatures) to develop infective sporozoites, so mosquitoes cannot transmit the disease until some 2 weeks after an infectious blood feed. If a newly hatched malaria-uninfected mosquito picks up a fungal infection, that gives a large window of time for the pathogen to act. This does not hold for a mosquito that comes into contact with the fungus only after it has been infected with *Plasmodium,* in which case the speed of action of the pathogen may limit its efficacy (although the effects of fungal infection on mosquito feeding may hinder transmission – see below).

The authors of the first paper[1] began by testing residues of oil-based sprays containing spores of eight isolates of *B. bassiana* and *M. anisopliae* against *A. stephensi* carrying rodent malaria, *P. chabaudi. Beauveria bassiana* isolate IMI 391510 was selected for further study, not because it was the most virulent, but because it is already used in an agricultural product, and the existence of a registration dossier would make applications for subsequent field testing easier. Tested by topical application and as a spray residue on cage mesh (partially simulating treatment of bednets), it achieved mortality by day 14 in exposed mosquitoes of 91% and 93%, respectively. By varying the period of exposure to the spray residue to simulate resting times after a blood meal, 6 hours was shown to be sufficient to cause high levels of infection, well within the post-feeding resting period for most *Anopheles* malaria vectors.

Next, the authors assessed how this might affect malaria transmission by looking at the effects of *B. bassiana* exposure on mosquitoes carrying rodent malaria. They found that 95% of the mosquitoes exposed to *B. bassiana* died by day 14, a mortality 65× that of the unexposed mosquitoes. Furthermore, on day 14, 35% of the unexposed mosquitoes were sporozoite positive (i.e. infective) compared with only 8% of mosquitoes that had been exposed to *B. bassiana.* Of the original mosquitoes, 31% of unexposed mosquitoes had survived to become infective, while only 0.4% of those exposed to *B. bassiana* had done so, which translates into a reduction of transmission risk of some 80×.

The authors note that although *B. bassiana*'s primary impact is to kill most mosquitoes before they become infective, there are probably other synergistic effects. They suggest that fungal infection may adversely affect *Plasmodium* development and maturation. They also observed that mosquitoes infected with the fungus fed less on days 8–14, which would reduce transmission yet further.

The second paper[2] tested the impact of exposing mosquitoes to *M. anisopliae*-impregnated 3-m^2 black cotton sheets, and in particular the mortality effect on *A. gambiae s.l.* The trials were conducted in houses in a village near Ifakara in central Tanzania. About 23% of *Anopheles* mosquitoes caught in houses containing impregnated sheets were infected with the fungus (and none in untreated houses). The fungus-infected mosquitoes proved to have a significantly shorter lifespan than mosquitoes not infected with the fungus.

Using an epidemiological malaria transmission model[3], it was shown that this relatively low fungal infection rate could have a major impact on disease transmission. The probability that females reach the minimum age for transmitting malaria was reduced drastically. Based on 23% fungal infection of mosquitoes and extrapolating over time, the number of malaria-infectious bites per person per year would be reduced from 264 to 62, i.e. a 75% reduction in transmission intensity.

Modifications to the system, some simple, could increase efficacy further; for example, increasing the size of the impregnated sheets, using higher spore dosages and improving formulation efficacy. Increasing the coverage of resting sites could feasibly increase the proportion of infected mosquitoes to 50%, which equates to a 96% decrease in malaria transmission.

With the drive to reduce deaths from malaria high on the world's agenda, these papers received a good deal of publicity and a commentary in *Nature*[4] pointed out some possible shortcomings. It queried how the first study, on rodent malaria in the lab, would equate to human malaria in the field, but acknowledged that parallel findings in the second study suggested the results might be more generalized. It considered ecological and evolutionary issues to be of greater concern, beginning with the lack of fungal specificity. The isolates used in the two studies were of beetle and moth origin. While it could be argued that killing nontarget insects inside houses is likely to be a good rather than bad thing (and perhaps of little significance in countries where malaria is a major factor in high infant mortality), the potential impact on biodiversity should not be ignored. The possibility of the mosquitoes developing resistance to the fungal products was raised – a problem shared by chemical pesticides. Mosquitoes might develop mechanisms to combat the fungi, although these would be unlikely to increase *Plasmodium* transmission or virulence. But would the widespread use of the fungal pathogens to control malaria have an impact on other systems? Possible behavioural evolution, with the mosquitoes shifting to outdoor resting sites and domestic animal hosts was considered in this analysis as beneficial, as human malaria develops only in humans; the economic impact of increased transmission of animal diseases was judged a lesser evil than the human health impact of malaria. Of potentially greatest significance was the evolutionary pressure the fungal pathogens would place on *Plasmodium* to develop faster and produce offspring before the fungus kills the host. This speeding up of the generation time could increase malaria cases per unit time, and speedier development in the mosquito host might conceivably equate to greater virulence in humans too (although there is no evidence from the rodent model that this would be so; why malaria has not evolved to develop more quickly within the mosquito in the first place remains one of the mysteries of malariology). The commentators returned, however, to the promise of mosquito-killing fungi and welcomed "the prospect of opening a new front in the war on malaria."

The authors of the *Science* papers agree limitations need to be addressed. The isolates tested are not necessarily the best ones, and the formulation used in the trial in Tanzania, while stable in the 'bottle', was of limited viability in the field. As experience with other biopesticides has shown, moving from experimental to operational is a laborious process. The practicalities of good formulation, best dosages and application regimes, scaling up, quality control, and so on, may seem mundane steps compared with the scientific breakthrough, yet they are key to a successful product. Long-term commitment will be vital if this exciting work on malaria is to have a chance of making a real contribution to disease control.

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[3]Part of [2], as online material:
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Turning the Tables on Formosan Termites in New Orleans

The appearance of alates of the Formosan subterranean termite (FST), *Coptotermes formosanus*, in the US southern city of New Orleans each spring is one of the season's most unwelcome sights. However, research at the USDA-ARS (US Department of Agriculture – Agricultural Research Service) Formosan Subterranean Termite Research Unit in New Orleans (part of the Southern Regional Research Center) has led to the discovery of a new strain of the fungus *Metarhizium anisopliae* with the potential to reduce swarms.

The FST is the world's most widely distributed and economically important termite species. First described from Taiwan in the early 1900s, it is probably native to southern China. Although less widely distributed in the USA than other subterranean termites, it costs the nation more than any other such species. Termites are estimated by the National Pest Management Association to cause property damage valued at US$2.5 billion annually. According to USDA, the FST causes $1 billion damage yearly, a figure which includes preventive control, remedial control and costs of repairing damage.

The FST first arrived in the USA in Hawaii in the late 1800s and is now ranked as the most economically important pest species in the island state. It has spread further since then and has been discovered in 11 US states, but is now known to infest only ten (including Hawaii). In the southern city of New Orleans, Louisiana the density of the termite is one of the highest in the country. It was first identified in Louisiana in the mid 1960s, although it had probably arrived two decades before. It flourished in the warm humid climate, and particularly on the older wood-based buildings. Termite control and damage is estimated to cost the city $300 million a year.

An individual FST worker termite consumes no more wood than a native subterranean worker termite. The secret of the FST's success is based on sheer numbers. Individual FST colonies can contain several million termites, larger by a factor of ten than native subterranean termite colonies, and the termites can forage up to 100 m from home in soil. The combination of population size and range means FST colonies pose a serious threat to nearby structures, and colonies spawn new colonies only too well. A single mature colony of FST may produce over 70,000 alates per year. The survivors among these can establish new colonies which in turn become large colonies in 5–8 years. Large colonies can cause severe damage and begin producing more alates. Once established, the termite has never been eradicated from an area.

The strain of *M. anisopliae* discovered by the USDA–ARS researchers in New Orleans may provide a new pathway for tackling the termite. The strain is highly effective against the alate form, killing 100% of them in the laboratory within 3 days. Current control strategies are aimed at colonies, but the new find opens up the prospect of targeting alates, thus reducing new colony formation and slowing population spread and growth.

Metarhizium anisopliae has already been shown to be an effective agent for termite control, and a strain has previously been commercialized as a biopesticide for above-ground use. Nonetheless, there is a long way to go before a product based on the new strain is ready for use. An important factor that can contribute to the successful use of this strain is the fact that alates once formed stay in their nest for 1–2 weeks before swarming. During this period they would be very vulnerable to the deadly fungus. The USDA–ARS researchers are currently seeking collaborators to develop a formulation to deliver the spores of the new strain. Application is another hurdle, but they envisage applying the fungus to buildings and trees known to harbour the termites before the swarms are in full swing.

The proposed biopesticide is not expected to provide a complete answer to control of the Formosan subterranean termite, but will join a growing number of technologies in an integrated pest management system for combating this devastating pest.

Sources: USDA–ARS press releases: 'Termite researchers find a source of calm before the swarm' (13 May 2005)
www.ars.usda.gov/news

http://creatures.ifas.ufl.edu/urban/termites/formosan_termite.htm

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Biopesticide Project Scoops Prize

Biopesticide production technology for resource-poor farmers in India led to the 'Traditional technology with a modern twist' project receiving the accolade of the World Bank's Development Marketplace Award for 2005. The project, a partnership between ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) and the Center for World Solidarity (a Hyderabad-based NGO working on IPM) developed the production and use of the *Helicoverpa armigera* nuclear polyhedrosis virus (HaNPV) for managing the pest, locally known as the pigeonpea pod borer. This pest attacks nearly 200 crops worldwide, including cotton, pulses, cereals, vegetables and fruit, with annual global costs amounting to some US$2 billion in crop losses and $500 million spent on insecticides.

The prize is a grant of US$150,000, which will be used to establish 100 community-based rural NPV production facilities. The project's sustainability is based on a programme to train villagers on NPV production and utilization, and thus spread awareness on the use of biopesticides. The project will also establish knowledge hubs and shared management systems for podborer.

The technology ICRISAT developed built on the traditional farmers' practice of vigorously shaking pigeonpea plants to dislodge caterpillars (podborer larvae), which they collected and used for virus multiplication. This cost-effective and environmentally friendly method is known to reduce infestations by up to 85%. However, nowadays dislodged larvae are generally collected and burnt.

NPV infection causes heavy mortality in pod borers without any adverse impact on nontarget organisms. The technology developed by ICRISAT involves collecting the larvae, as above, and feeding them with an NPV-infected diet until they die from the infection. The NPV biopesticide is extracted from the dead larvae, and can be sprayed on crops to prevent podborer damage. The necessity for an artificial rearing system for host larvae can greatly inflate production costs for NPV production, but in this system, farmers can collect larvae dislodged by shaking, so rearing costs are eliminated.

ICRISAT not only developed and pilot-tested the technology for producing the NPV biopesticide. The institute's research also addressed problems related to mass production, storage and utilization of the virus and application timing. The package is now ready for dissemination, and the grant that comes with the prize will help facilitate this process.

The village-level NPV production facility will not only serve the needs of the immediate community, but because excess product can be sold, will provide additional income for the farmers. In this way, a project with a firm scientific basis has created a sustainable technology to help improve the livelihoods and well-being of the resource-poor farmers of the semi-arid tropics.

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Coconut and Taro Beetles: Tailoring Biocontrol for the Pacific

Taro and coconut are important crops in the Pacific region for food security, cultural reasons, manufacture of value-added products and export. Two key beetle pests can affect production of these traditional crops, the palm rhinoceros beetle (*Oryctes rhinoceros*) on coconut and taro beetles (*Papuana* spp.). For the first, biological control is an established management method, while for the second the technology is emerging from the field trial stage.

A regional workshop held in April 2005 in Suva, Fiji highlighted the importance of managing these beetle pests, reviewed current practices, provided training, and made recommendations for the future. The 4-day workshop was organized by SPC (Secretariat of the Pacific Community)[1] Land Resources Division in collaboration with the Ministry of Agriculture, Sugar and Land Resettlement (MASLR) and funded jointly by the Australian Centre for International Agricultural Research (ACIAR) and the EU (European Union). The aim of the workshop was to strengthen technical capacity in the island countries to maintain and promote sustainable pest management practices using biological control. Biological control is seen by SPC as having a key role in pest management in the fragile island environments of the Pacific Islands. Participants shared experiences on the effectiveness of biocontrol agents and reported on their coconut and taro industries, while participants from New Zealand and Australia provided technical input. This article synthesizes the activities of the workshop with additional information on some microbial options for control of coconut pests drawn from the work and publications of the Asian and Pacific Coconut Community[2].

Palm Rhinoceros Beetle

Oryctes rhinoceros is native to South Asia but has spread to 37 other countries, including many Pacific Islands. Adults attack the crowns of palm trees such as coconut, oil palm and date palm. Characteristic V-shaped damage is apparent when the fronds open, while feeding on the spathe often destroys the inflorescence and therefore nut production. In coconut, the beetle reduces yield, and can kill seedlings and young and old trees. The larvae develop in rotting palm logs (which includes the tops of dead standing palms killed by adult beetles or other causes). The discovery of a disease of the beetle in Malaysia in 1963 led to a biological control programme using the causal virus (baculovirus), which has been a major regional success.

A virus mass-production system gives a product (larval cadavers containing the virus) that can be stored indefinitely at 4°C. Field-collected adult beetles are inoculated by submerging in a solution of the ground-up cadavers for 10–30 minutes and are then incubated together for 24 hours to maximize the infection rate. They are released during the evening/night at a rate of 10–15 beetles per hectare. (A novel method of disseminating the virus is by using baited traps.) Autotransmission (mainly through faecal matter) means the virus can set up epizootics in beetle populations in breeding sites and palm crowns. Infection can be detected at 3 days post-infection and is released from day 7 until death. Infected adults spread the disease through feeding. The disease can spread at about 1 km/month in the field. Recently, however, observations indicate that the pest is not under control in some areas, suggesting a breakdown in virus control. Given the importance of coconuts in the region, this is potentially disastrous.

Although much research underpinned the original biological control programme, little has been done since the 1980s. In view of the advances in technology and knowledge since then, and the apparent patchiness of control now, it was judged useful to review and update practices, and identify areas for research and other activities.

The SPC workshop sessions included lectures and training sessions on the history, biology and use of *Oryctes* virus for rhinoceros beetle control in coconut plantations, the background to microbial control, and virus biology and epidemiology, with a focus on *Oryctes* virus pathology. Laboratory sessions concentrated on disease diagnosis; handling, infection and release of the virus; monitoring beetle populations for establishment and the long-term effect of the virus. Discussions were held on adapting new technologies for SPC use and development of improved management systems for rhinoceros beetle, with the recommendations summarized below.

- The central question is why control is breaking down – is it because the beetles are not becoming infected, or because the virus is not sufficiently pathogenic in some situations, or something else? The status of *Oryctes* virus in rhinoceros beetle-infested areas needs to be monitored. Techniques such as PCR (polymerase chain reaction) which open the door to new detection tools and monitoring systems may help. Tools for identification of virus infection in *Oryctes* need to be developed and validated for the Pacific. If successful, a regional *Oryctes* virus identification system should be established in Suva. A simple, 'low-tech', immunodetection system would also be a valuable development for use in smaller countries and outer islands. Together these would allow data on the occurrence of the virus throughout the region to be collected and collated.

- There is a suspicion that the breakdown in control may be the result of mutation and adaptation, but how such variations affect the virulence and long-term persistence of the virus is unknown. To fill the gap in knowledge, SPC needs to link with work in New Zealand and Europe on sequencing the *Oryctes* virus. A full sequence would provide a template for examining virus variation in the Pacific. Once virus strains are characterized, it may be possible to select strains on the basis of specific environmental or management needs. A standardized bioassay system would allow comparison of different virus strains.

- The new detection technologies and improved understanding of the virus strain will enable an improved management system for the rhinoceros beetle to be developed, and this should be assessed in a pilot project. The advances in understanding and detection will also allow the role of the rhinoceros beetle in intensive coconut production to be re-examined and management options modified for these conditions as necessary.

- Technology transfer is key to ensuring that scientific breakthroughs translate to improved rhinoceros beetle biological control. The final call was for technical bulletins to communicate information on disease diagnosis, and infection and monitoring of *Oryctes* virus in the management of rhinoceros beetle.

The *Oryctes* virus is one of a number of management measures described by APCC for managing the rhinoceros beetle. Another microbial control option with potential for the Pacific Islands is *Metarhizium anisopliae*, which has been shown to be effective as an area-wide approach against third-instar larvae. Cost-effective methods used for pilot-scale production of preparations include broken maize grains (Philippines and elsewhere), cassava chips mixed with rice bran supplemented with urea or fish meal extract, and coconut water waste from the copra industry – the latter is used successfully in Kerala, India and needs no supplement or modification. The products are generally applied to breeding sites as powder, spray or by injection, and by inoculation of larvae which are then released into breeding sites.

A novel technique for infecting beetles and disseminating the fungus uses a pheromone trap containing sawdust mixed with *M. anisopliae* spore preparations in powder or liquid form. The traps are set out at a rate of five traps per hectare, covered with leaves to preserve humidity and deter birds. Spores remain viable for 2 years, but boxes are renewed every 6 months.

Farmer innovation also offers ideas. Manure heaps are common breeding sites for rhinoceros beetle. Farmers macerate dead *M. anisopliae*-infected grubs in water with a small amount of detergent ('Teepol') and stir vigorously. They pour the liquid suspension into holes made in a manure heap, at the four corners of a metre-square.

This is a technology still in development. Priorities for research are quantifying the diversity of *M. anisopliae* strains and selecting virulent ones, identification of UV protectants, development of economically viable production and packaging technology, application regimes for different ecological niches, toxin identification and the impact of different media on their production, and development of fungicide compatible strains.

For the last 18 months, APCC has been unable to mass rear rhinoceros beetle in the laboratory at its centres in PNG and the Philippines owing to high mortality of field-collected larvae, due to the virus in PNG and *M. anisopliae* in the Philippines. Although this is not good news for those responsible for the laboratory cultures it indicates that good biological control of rhinoceros beetle is happening in the field.

Taro Beetles

Taro beetles are native to eastern Indonesia and Papua New Guinea (PNG). Eighteen species are known in PNG and some of these have spread eastwards. Taro beetles now occur in a wide sweep from Indonesia through PNG, the Solomon Islands, Vanuatu to Fiji and New Caledonia, with invaded countries having anything up to five species present. Planting material and taro's use in traditional ceremonies are important pathways for the movement of this invasive pest. Quarantine measures are being implemented to try and prevent its further spread, while measures to control it in affected countries are urgently sought under the Taro Beetle Management component of the Pest Management in the Pacific Project with funding from ACIAR and the EU. To add to the problems of effective quarantine, the beetles have been identified attacking 15 economic plant species in PNG, including banana, sugarcane, sweetpotato and yam.

The adult taro beetle lives in the soil and burrows into the taro corm, leaving a maze of tunnels, and the wounds can later rot. Feeding may cause plants to wilt and die. The result can be catastrophic. In Fiji, for example, smallholders may lose up to 25% of their crop to the beetle. Although not yet present in the major taro-growing areas of the country, it also poses a threat to the export trade to Australia and New Zealand. After feeding for about 2 months, the adults leave the fields and find alternate hosts (of which there are many) where they mate and lay eggs from which the next generation emerges to feed on the plant roots and detritus, eventually returning to the taro fields as adults.

Mulching and other physical barriers have been only partially effective, although imidacloprid (as Confidor) applied to the soil in planting holes at planting gives very good control. Although an effective insecticide, imidacloprid can have nontarget effects on birds, so a strategy to reduce its use would be beneficial. Over the last 4 years, a project managed and coordinated by SPC has been targeted at finding a biological control solution, with attention currently focusing on *Metarhizium anisopliae*. Research at NARI (National Agricultural Research Institute), PNG assessed a number of candidate biological control agents against *P. uninodis*, and identified a highly virulent one, *M. anisopliae* TB 101. Studies since then have revealed further isolates with potential.

The next challenge was to develop locally adapted technology to facilitate widespread use of microbial control, and it was this that participants at the SPC workshop learnt about. The aim of the workshop was to give them the latest information and procedures for laboratory mass production and field application of *M. anisopliae*.

Researchers in PNG and Fiji (MASLR Plant Protection Section), with the help of consultant Richard Milner, have developed a mass production technique for *M. anisopliae* appropriate for the Pacific Islands and suitable for community use. The fungus is grown on rice, which is cheap and readily available. Sodium metabisulphate is used to sterilize the culture medium. Culturing is done in 'breather bags', and the product is air dried at room temperature. Stored in a cool, dry environment and out of direct sunlight, it has a shelf life of about 6 months; it can also be stored in a refrigerator at 4°C. The product is applied to the taro planting holes at time of planting.

However, field experiments showed that *M. anisopliae* on its own cannot give the required level of control, so trials were conducted to see whether it would act synergistically with an insecticide. Used in combination with Confidor, over 90% control of the beetle was achieved in the field in PNG and Fiji (i.e. equivalent to the Confidor alone).

Confidor 35% SC alone gives over 90% control of taro beetles (at 3 ml/l H_2O) with 125 ml solution applied to planting holes. In synergy trials, the same level of control was achieved with Confidor at 1 ml/l. Further trials are underway in PNG and Fiji, and trials are also being conducted by SPC in the Solomon Islands and Vanuatu, to test dosages and frequency of applications. Indications are that when used in combination with *M. anisopliae,* rates of Confidor could be further reduced without affecting the level of control.

Clearly, this work is still at the developmental stage and research needs to continue to find the best and appropriate control for the beetle. Fine-tuning of application regimes for *M. anisopliae* needs to be accompanied by formulation improvements to enhance activity, quality and shelf life. Nonetheless, in 4 years the project has made great strides in developing a locally adapted sustainable control method for the taro beetle which will allow insecticide use to be reduced. For the first time two insecticides (imidacloprid and bifenthrin) will be recommended to growers for the management of taro beetle in the Pacific, but greater emphasis will be placed on using low rates of imidacloprid with *M. anisopliae*.

Further information

[1]SPC website: www.spc.int

[2]See: 'Prolific coconut IPM project', Announcements, this issue. The above article draws on:

- Singh, S.P. & Rethiman, P. (2004) Baculovirus – a key component of biointensive integrated pest management of coconut rhinoceros beetle. Cocoinfo International **11**(1), 117–20.
- Singh, S.P. & Rethiman, P. (2004) Use of green muscardine fungus in BIPM of rhinoceros beetle. Cocoinfo International **11**(2), 19–24.

Also see: 'Planning farmer field schools in coconuts', Training News, this issue.

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Biopesticides a Hot Topic for Sunn Pest

The sunn pest (a complex of mainly pentatomid bugs, although the most serious species, *Eurygaster integriceps* is a scutellerid) is a widespread and major pest of wheat and barley in North Africa, the Middle East and western Asia. An estimated 10 million hectares were affected in 2004, with damage as little as 2% enough to render a crop useless. Government polices have relied on (often ineffective) blanket pesticide spraying. A 3-year collaborative project funded by DFID (UK Department for International Development) involving organizations in Syria, UK, Turkey, and Iran (as well as the University of Vermont who were separately funded) to develop IPM for sunn pest came to an end last year. The project has already had a major impact on policy in Turkey, where the Government has initiated a national farmer training programme, has scheduled a series of parasitoid releases and, more significantly, will no longer be spraying fields with pesticides, which will become the responsibility of the farmers.

Trials during 2004 had given strong indications that a biopesticide based on a Syrian strain of *Beauveria bassiana* could be an effective IPM component for control of the summer generation of sunn pest. Very effective laboratory kill had been achieved, as well as minor reductions in field numbers. Funding in 2005 from the CABI Partnership Facility allowed CABI scientists to return to ICARDA (International Center for Agricultural Research in the Dry Areas), who provided accommodation and logistical support, so trials of the biopesticide and biological studies could continue.

Visits covered much of the infestation season, from the appearance of new generation nymphs in early May to the final migration of the adults in mid June. The impact of the biopesticide was assessed in the field for the first time. The size of the application plots was increased from 1000 m^2 to 2500 m^2, incorporating a 400 m^2 centrally sited sampling area in the hope that a buffer zone would reduce the effects of sunn pest movement on the trial results. Plots were sprayed twice and sunn pest numbers assessed every 5–7 days thereafter; grain yield from treated plots was also recorded.

Studies on the pest biology were carried out to find out more about sunn pests' movements upon and through the crop on a daily and seasonal basis. These included monitoring population structure during May–June, and daily movement of sunn pest individuals in the crop (including the effects of early *B. bassiana* infection), and mark-and-recapture studies.

Biopesticide applications did not reduce sunn pest numbers in the field this year, but much was learnt that was invaluable in understanding the pest and possible control strategies. Like last year, there were indications that mortality of individuals brought back to the laboratory was significant, but this was not reflected in field results. Dorsal deposits of the biopesticide had little impact, but ventral deposits caused more than 90% mortality, probably reflecting the different natures of the cuticle. Diurnal activity of the sunn pest was considerable and this would allow considerable pick-up of inoculum to be achieved, which has implications for the isolate selected and underlines the importance of the basic biological studies. Persistence of the isolate may also be much more important than previously considered. Temperatures during the study period were above 30°C (but usually below 40°C) for much of the day, so the search for an isolate with higher temperature tolerance is indicated. An alternative (or additional) strategy is for the biopesticide to be applied earlier in the season – probably around March when adults first reach the fields. The idea would be to create a *cordon sanitaire* by treating the edges of fields so the sunn pests are infected as they walk through.

Ambient temperatures would be lower, but persistence would have to be much higher than at present (as much as 4–6 weeks) or a second application may be necessary.

The search is underway for funding for a follow-on project, which, with the experience and knowledge generated by the first project to draw on, has excellent prospects for achieving a sustainable reduction in sunn pest damage to cereals in the Middle East. For the biopesticide, the basic research has been done, but as other articles in this section show, there is still hard work ahead.

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Novel Application Methods for Microbial Control Products: IITA's Research against Banana Weevil and Burrowing Nematode

Despite the vast potential of biopesticides, their development, commercialization and use have not yet lived up to expectations. It is not difficult to demonstrate the beneficial effect of biological control agents in the laboratory or in controlled *in vivo* experiments. Especially in developing countries, expertise among researchers is often lacking in the areas associated with the later but equally important steps in biopesticide development. Major limitations with biological control are lack of ecological fitness and cost-effective production systems, coupled with poor formulation.

Bananas (*Musa* spp.) are an important fruit commodity and a main starchy staple in parts of Asia, the Americas and Africa. A third of the annual world production of 90 million tonnes is in Africa; Uganda alone produces 10 million tonnes and is considered one of biggest banana producers in the world. The East African Highlands has the highest per capita consumption of banana in the world. The banana weevil *Cosmopolites sordidus* is an important production constraint on East African highland cooking banana and plantain. It causes reduced bunch size, snapping, toppling and complete mat die off. The adults are usually found in leaf sheaths or in soil at the base of the banana mat. Eggs are inserted singly into the leaf sheaths or rhizome at a rate of up to two eggs per week. The larvae feed within the rhizome cortex, central cylinder and, occasionally, the pseudostem. Population build up is slow and banana weevil problems are most pronounced in ratoon crops. Crop losses due to banana weevil attack can exceed 60%.

A complex of different nematode species causes destruction of banana roots. The burrowing nematode (*Radopholus similis*) is considered the most destructive nematode species worldwide. *Radopholus similis* is a migratory endoparasite and completes its life cycle in 20–25 days within the root cortex. Roots invaded by nematodes are frequently colonized by soil microorganisms, become necrotic, atrophy, and may finally die. The damaged root system results in reduced water and nutrient uptake, and poor anchorage of the plant, resulting in toppling. Yield is reduced and the vegetative cycle is lengthened. Crop losses due to nematode attack in banana under Ugandan conditions can reach 50%. The use of nematicides is a common practice where it is necessary to maintain productivity in commercially managed banana plantations, but they are unavailable and economically unfeasible for resource-poor farmers.

Soil-borne banana pests such as banana weevils and *R. similis* are mainly dispersed through contaminated planting material. Propagation of pest-free banana planting material is therefore vital. Pest-free banana can be easily micro-propagated in tissue culture. *In vitro* propagation of banana is common in other parts of the world, but has not yet been used on a large scale in tropical Africa. Nevertheless, *ex-ante* studies suggest that tissue culture can dramatically increase banana productivity for resource-poor farmers in the region. Tissue culture bananas are more uniform, grow faster and are more vigorous than conventional planting material, especially during the first crop cycles. This results in a higher and more marketable yield. The use of tissue culture plants has other advantages as well. Superior new germplasm, arising from breeding programmes, can be rapidly multiplied and disseminated to local farmers at reasonable costs.

Pest-free tissue culture plants are often introduced to an environment burdened with biotic pest pressures, such as banana weevils moving from neighbouring fields and *R. similis* already present in the soil. Banana weevil and *R. similis* attacks can easily destroy tissue culture plants during the months after field transplantation, as they initially possess fewer nutrient reserves than conventional planting material.

Suppressive Soils

Soils exist that are naturally suppressive to diseases caused by soil-borne pests and pathogens. In nature, soil suppressiveness can be detected by the observation that disease incidence remains low despite the presence of a susceptible host plant, climatic conditions favouring disease expression and ample opportunity for the pathogen to have been introduced. It is now clear that disease suppressiveness is microbial in nature. Soil suppressiveness provides a good example of cost-free biological control already working effectively in nature. However, since rhizosphere competence is an important characteristic, one of the major hurdles for an effective biological control agent of soil-borne pests and diseases is to overcome the native soil microflora that can inhibit the establishment of the introduced organism in the rhizosphere. Therefore, beneficial microorganisms from soil suppressive soils can be used for more extensive biological control purposes by isolating them and using them as artificially introduced biological control agents.

An important component of the microbiota in suppressive soils is endophytes. An endophyte is an organism that can colonize internal plant tissues without causing apparent harm to the host. Initially, endophytes were considered neutral, but as they have been studied in the last 20 years in more detail, an important role in host protection against pests and diseases has been revealed. This has led to research into potential use of endophytes for biological control of pests and diseases. A vast amount of research has been conducted on endophytes for crop enhancement in temperate regions. The beneficial effect of endophytes in grazing grasses, for example, has been demonstrated extensively. Unfortunately, very little work has yet been done of relevance to the tropics.

Endophytes of Banana

Since 1997, research at the International Institute of Tropical Agriculture (IITA) has focused on isolation and re-inoculation of endophytes into tissue culture plants for the control of banana weevils and *R. similis*. (This work is being done with partners including the Uganda National Banana Research Programme, the University of Bonn, Germany, and the University of Pretoria, South Africa). As a first step, endophytes were isolated from healthy bananas in plots that were diseased. Microorganisms isolated from a specific crop may be better adapted to that plant (or plant part), as well as to the particular environmental conditions. Such organisms may provide better control of diseases (and thus make better biological control agents) than organisms originally isolated from other crops. Also, apparently healthy banana plants were chosen because it was hypothesized that these plants contained intrinsic protection, such as endophytes, against the abovementioned pests. The predominant taxa isolated were *Fusarium* spp., and among these *F. oxysporum* was most common.

Fusarium oxysporum is one of the more common soil fungi and has a cosmopolitan distribution. Some strains of *F. oxysporum* are wilt-causing pathogens on many crops, including banana, and therefore the fungus has a bad reputation. However, most *F. oxysporum* strains are demonstrably non-pathogenic on the plant from which they are isolated. The genus *Fusarium* contains species associated as endophytes or plant diseases of virtually every plant family. For example, *F. oxysporum* is among the predominant endophytes in tomato, sorghum, maize and soyabean. Although not identifiable using morphological characters, individual strains show a high level of host specificity. Strains are separated in *formae speciales*, that are further subdivided into races. The *formae speciales* classification of *F. oxysporum* is based on the host species in which a fungal strain is pathogenic, while race is determined by the ability of a fungal strain to cause disease on particular cultivars of the host species.

The delimitation between saprophytic and parasitic fungi is by no means sharp. Similarly, the distinction between pathogenic and non-pathogenic *F. oxysporum* might be small. For instance, pathogenic *F. oxysporum* colonize the cortex but can also move past the cortical tissue and invade vascular tissue, causing wilt. Non-pathogenic, endophytic *F. oxysporum* stay in the cortex, a phenomenon that has been demonstrated in banana by IITA researchers. Vegetative compatibility grouping (VCG) is being used to distinguish endophytic *F. oxysporum* strains from pathogenic ones. Isolates within one VCG represent a genetically isolated population, sharing the same genes for pathogenicity.

Endophyte-enhanced Tissue Culture Banana

Due to the sterile nature of the production system, tissue culture bananas lose the endophytes they are naturally associated with. Since pest infestation remains a barrier to banana establishment, tissue culture plants must be protected against banana weevils and *R. similis* during this vulnerable period. Incorporation of endophytic biological control agents can provide this protection, extend the benefits of clean planting material and offer the potential for sustained pest control.

A variety of problems and bottlenecks plaguing augmentative biological control strategies can be avoided with the use of a biological control agent that can be maintained inside the plant tissue. Development of *F. oxysporum*-enhanced tissue culture plants has the following benefits:

- Endophytes are situated within the roots and rhizome of the plant, where they are ideally suited for control of cryptic pests such as banana weevils and *R. similis*.
- Incorporation of a biological control agent into tissue culture plants entails limited additional costs because little inoculum is needed due to the targeted application directly into the plant.
- The endophytes are inoculated into the plant by the tissue culture supplier prior to distribution to farmers. Hence, the farmer will not have to worry about storage, application and efficacy of the biological control product.
- Inoculation of endophytes into tissue culture banana can easily be integrated in the normal process of tissue culture plant production.
- Containment of the endophytes within the plant minimizes environmental and nontarget effects.

In brief, many disadvantages of other potential biological control products are avoided and the prospects for farmer acceptance are therefore high.

Under normal field conditions, endophytes are usually not effective because their densities are low and they must compete with other microorganisms. Pest control by endophytes can be greatly enhanced when they are artificially inoculated. High colonization and subsequent persistence of *F. oxysporum* endophytes is necessary for effective biological control of soil-borne banana pests. At IITA, a relatively rapid and simple inoculation method has been routinely used that consists of dipping post-flask tissue culture plants in an endophyte spore suspension. The method can be improved by growing the tissue culture plants in a nutrient solution to enhance root growth prior to inoculation. However, the use of a solid substrate inoculation method results in the highest root colonization, but this method might not be economically feasible. Rhizomes are colonized to a greater extent (91%) than roots (63%) but endophyte persistence in rhizomes is much lower than in roots. The persistence of endophytic *F. oxysporum* strains in the roots of inoculated plants can be sustained for up to 25 weeks. In contrast, rhizome colonization decreases rapidly. The pattern of root and rhizome colonization by these *F. oxysporum* strains was further investigated using light microscopy. Percentage colonization in the rhizomes (93%) was higher than in the roots (56%), but hyphal density in the roots ($1.73/mm^2$) was higher than in the rhizomes ($0.21/mm^2$). The latter result might explain the difference in persistence: although roots are initially colonized to a greater extent, hyphal density is much lower, presumably allowing other microbes to occupy available niches.

F. oxysporum-enhanced Tissue Culture against Banana Weevils and *R. similis*

In vitro bioassays demonstrated that, depending on the *F. oxysporum* strain used, banana weevil egg mortality caused by spore suspensions was 32.8–50.3%. In *in vitro* bioassays against *R. similis*, fungal filtrates caused 84.2–100.0 % mortality. These mortality rates are a strong indicator that fungal endophytes have the potential to be used as microbial antagonists against banana weevils and *R. similis*. Furthermore, some strains were equally effective against both pests, creating the possibility of using a single endophyte strain to target the two pests simultaneously, as they usually occur together in the field. Tissue culture plants enhanced with various *F. oxysporum* strains are currently being tested in screenhouse and field experiments, with very exciting results. For example, the effect of three different banana tissue culture cultivars enhanced with *F. oxysporum* strain *V5w2* on two different population densities of *R. similis* was investigated. Nematode population densities in roots were much higher in control plants than in endophyte-treated plants. In none of the experiments was the size of the nematode dose associated with any difference in nematode population densities in roots, demonstrating that endophytes have the potential to be equally effective against a wide range of pest densities.

Combinations of multiple endophyte strains may enhance the level and consistency of control by providing multiple mechanisms of control, and effectiveness over a wider range of environmental conditions and different locations. Combinations may also be useful even if there is no direct synergistic effect since different strains could target different pests that occur within the same plant. For example, two *in vivo* experiments were carried out to investigate the effect of combinations of different endophyte strains on *R. similis* reproduction. After 63 days, endophyte-free plants and plants that had been inoculated with a single endophytic strain supported higher *R. similis* densities than plants that were simultaneously inoculated with two strains. Lowest *R. similis* densities were recorded when plants were inoculated with a combination of all three endophytic strains. We therefore conclude that combining two or three endophytic strains appears to enhance their potential to lower *R. similis* densities in a synergistic way. In rice and maize, it has been shown there is a marked interspecific tissue specificity among endophytes, and likely the same is the case among *F. oxysporum* endophytes in banana.

Based on research in other crops, endophytes seem to have four types of modes of action against pests and diseases: (1) antibiosis and antixenosis, (2) direct parasitism, (3) competition and (4) induced plant resistance. We found that direct parasitism and competition are unimportant for control of banana weevils and *R. similis* in banana. *Fusarium oxysporum* endophytes produce mycotoxins that cause antibiotic and antixenotic effects such as oviposition, penetration or feeding deterrence. Most interestingly, *F. oxysporum* endophytes can also induce banana resistance. Thus, not only do they have direct effects against both pests, but they can also act indirectly by changing the banana's physiology and thus induce its pest tolerance and resistance. Reinforcement of the cell wall through complex phenylpropanoid biosynthetic pathways is a common resistance strategy applied by higher plants. In the phenylpropanoid biosynthetic pathway, esterification of phenols to cell wall materials takes place, leading to accumulation of lignins. Phenylpropanoid biosynthetic pathways also set in motion a cascade of production of other secondary metabolites that aid in the plant's defence against pests and pathogens. Research at IITA has demonstrated that upon enhancement of banana with endophytic *F. oxysporum*, phenol production is enhanced.

Fusarium oxysporum, through induction of systemic resistance, protects the banana plant in a way that is similar to mechanisms currently present in resistant banana cultivars. Banana pests and diseases are most effectively controlled through the use of disease-resistant cultivars. However, conventional banana breeding is a lengthy and difficult process due to the intrinsic characteristics of the crop (parthenocarpy, polyploidy and a long vegetative cycle). Pathogens, in particular, are quick to adapt to new genotypes and, therefore, this form of control is potentially compromised by the appearance of a new pathogenic race, capable of causing disease in a previously resistant cultivar. *Radopholus similis* populations show differences in reproductive fitness and specific pathogenicity. Also among banana weevils, considerable variation seems to exist between populations. Endophytes such as *F. oxysporum* can be considered intrinsic components of banana plants that are adapted to local pest and disease pathotypes. As such, the use of endophyte-enhanced tissue culture plants can be seen as a complement to current breeding programmes, and a highly flexible tool to tackle the diversity of existing pathotypes and counter emergence of new pathotypes.

In the future, molecular techniques could be employed to modify endophytes for enhanced performance. Desirable genes, such as those coding for *Bacillus thuringiensis* toxins and protease inhibitors against insects, can be inserted more easily in a microorganism than in a plant. Since endophytes are by nature such good colonizers, they would then basically be used as vectors for delivering genes within the plant.

B. bassiana as an Endophyte

The entomopathogenic fungus *Beauveria bassiana* is one of the most commonly studied and promoted microbial control agents against insects. *Beauveria bassiana* has been identified as a virulent pathogen of banana weevils, and many strains have been tested using a wide variety of formulations, dosages, application methods and delivery systems under a wide range of conditions. Laboratory infectivity tests carried out at IITA resulted in adult banana weevil mortalities of more than 90%. However, promising laboratory results did not necessarily translate into equally effective field control. In addition, viable mass production and formulation systems still need to be developed as the application rates currently being employed are not economically feasible. Artificial inoculation of *B. bassiana* in plants, rendering them artificial endophytes, might circumvent these problems.

Recently, IITA has initiated research into the use of *B. bassiana* as an artificial endophyte in banana. In a series of experiments, three Ugandan *B. bassiana* strains were inoculated into tissue culture banana plants using three different inoculation methods: (1) root and rhizome dip in a conidial suspension, (2) injection of a conidial suspension into the plant rhizome, and (3) application of sterile soil mixed with *B. bassiana*-colonized rice substrate. After 4 weeks, plant tissue colonization was assessed through re-isolation of *B. bassiana* and plants were evaluated for growth. All *B. bassiana* isolates were able to colonize banana plant roots, rhizomes and pseudostem bases. However, colonization by *B. bassiana* varied among inoculation methods and among the different plant parts. Plants inoculated by dipping the roots and rhizomes in a conidial suspension showed higher colonization (up to 50.6%) than plants injected with a conidial suspension (up to 44.4%) or plants planted in soil mixed with the solid substrate inoculum (up to 31.5%). None of the three *B. bassiana* isolates affected tissue culture plant growth. However, inoculation methods themselves had significant influence on plant growth. The dip inoculation method differed significantly and was better than the other two methods for all parameters assessed. This study demonstrated the potential of *B. bassiana* to grow endophytically within tissue culture banana plants, causing no harmful effects, and is currently being followed up with pathogenicity tests against banana weevil larvae.

B. bassiana-baited Pheromone Traps

The use of trapping to control banana weevils has been recommended for a long time by many workers. The effectiveness of trapping is determined by many factors, such as trap density, trapping frequency and the types of materials used. Adoption of systematic trapping requires discipline and commitment on the part of the farmer. Also, the amount of material and labour required for trapping might be economically unrealistic. In addition, it is likely that trapping in established fields will only result in a gradual decline in banana weevil numbers, and it may be some time before effects are manifested in reduced damage. Olfactory cues are used by banana weevils in locating host plants, conspecifics and mates. The males release an aggregation pheromone via the hindgut that is attractive to both males and females. This compound, named sordidin, has been identified and synthesized, and is currently produced by a private company (Chemtica International, Costa Rica). The use of enhanced trapping with semiochemicals results in higher rates of banana weevil removal at lower trap densities and with reduced labour.

Addition of biopesticides and semiochemicals to current trapping methods is currently being investigated by IITA. Research has shown that *B. bassiana* can be transmitted horizontally among banana weevils. Furthermore, *B. bassiana*-infected adults exhibit the same behaviour as healthy individuals. Banana weevils are strongly attracted towards pheromone-baited traps, and therefore these traps can be modified so that they act as very efficient *B. bassiana* delivery systems.

Conclusion

Research results suggest that no single method will bring about complete control of banana weevils and *R. similis*. Therefore, a broad integrated pest management strategy appears to be the best approach. This includes cultural control, biological control, host plant resistance, the use of botanicals and chemical control. The way forward appears to be through improved management in small farmer systems, the refinement of microbial control production and delivery systems, and the development of both conventional and non-conventional breeding for host plant resistance. New technologies, such as the use of *B. bassiana*-baited pheromone traps and endophyte-enhanced tissue culture, are being developed by IITA that elegantly integrate microbial control options with other management techniques, with the ultimate purpose of circumventing bottlenecks for traditional biological control options.

By: Clifford S. Gold and Thomas Dubois

Work described in this article was conducted by Juliet Akello, Shahasi Athman, Daniel L. Coyne, Thomas Dubois, Clifford S. Gold, Sinnia Kapindu, Erisa Mukwaba, Caroline Nankinga, Bjoern Niere, Pamela Paparu, Paul Speijer and William Tinzaara (IITA), and Matthias Griesbach, Ralf-Peter Schuster and Richard Sikora (University of Bonn) and funded by the German Federal Ministry for Economic Cooperation and Development (BMZ) and by the Rockefeller Foundation.

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Fruit and Nut Case Studies from the Western USA

The fruit tree industry of the western USA is home to a long-running area-wide IPM programme that pioneered many approaches now used in other places and crops. No survey of bioinsecticides, however cursory, is complete without an update from there. We include reports on research in recent years on using granuloviruses and nematodes for pest control in fruit tree crops.

Could Viruses Catch On?

The codling moth, *Cydia pomonella*, is the most serious pest of pome fruits worldwide. The larvae bore into the fruit, making them unmarketable, and there can be several generations per growing season, making control difficult. Control via mating disruption with synthetic lures has been widely commercialized, but its efficacy declines at high pest pressure. When this happens, the majority of growers turn to chemicals, with detrimental effects on IPM, and risks to environmental and grower safety.

One alternative that showed scientific promise but met with earlier grower scepticism was the *C. pomonella* granulovirus, (CpGV). The virus is usually applied as an aqueous suspension to neonate larvae before or during entry to the fruit, and must be ingested. Over 30 years and in several continents, field trials have shown good activity against the codling moth. Its limited host range and absence of nontarget effects have also been thoroughly documented, a bonus for the conservation of natural enemies suppressing secondary pests. Nonetheless, commercial development and adoption of CpGV has been limited in North America, with growers expressing a variety of concerns, some of which mirror shortcomings of similar products in other systems:

- Short residual activity and need for multiple applications
- Slow speed of kill and increased numbers of shallow stings
- Limited efficacy at high codling moth density
- Cost and quality of the product

There have been considerable advances with formulations in recent years, and the suspicion was that growers' misgivings might be based on experience with older products. Trials on apples in an experimental orchard and evaluations in commercial organic orchards looked at how three newer formulations measured up against some of these criticisms.

It has to be admitted that, although the products evaluated all showed consistent high quality, the trials indicated that short-term efficacy remains a major obstacle to CpGV use. Initial control (94% at 24 hours) is comparable to chemical pesticides, but larvicidal activity declined by 50% in about 8 days (and more rapidly later in the season). This could be overly pessimistic as virus in (UV-) protected microenvironments may remain active for much longer and significant activity was recorded for up to 14 days, but this does not translate into prolonged larval control. Studies elsewhere, however, suggest adjuvants can provide protection from UV and increase its ingestion, which provides avenues for future work.

The short persistence means CpGV has to be applied repeatedly throughout the peak hatch period (7–10 day intervals are recommended on labels). In areas with only one codling moth generation per year (e.g. Nova Scotia and parts of Europe), two applications annually may give adequate control. The greater challenge is further south with longer growing seasons. In the study area, there are at least two generations per year. Local information on the development of each generation (based on degree–days) allows these multiple interventions to be timed to coincide with egg hatch of successive generations. The window for treatment is small, as larvae bore into the fruit within 24 hours of hatching. Is this knowledge- and labour-intensive business worth it? In the orchards, optimally timed interventions against the first generation reduced or eliminated fruit damage during the second generation and reduced second generation moth catches by 66–94%, while evidence indicated that numbers of prediapause larvae were also drastically reduced.

Slow speed of kill and resultant damage also persist. Even though larvae may pick up the virus *en route* to the fruit, they may take days to die. First and second instar larvae are often found dead in shallow entries. Current research in this area is focused on attempting to induce feeding by neonates on leaf surfaces, which may hasten their demise before fruit entry. However, fruit with shallow/slight damage may be marketable for some purposes (juice, sauce), or may even recover as the apple epidermal cells continue to divide for several weeks. Damaged fruit may also be removed during selective thinning.

The trials included application rates both lower and higher than the recommended label dose. The lower doses showed no less efficacy than those recommended, and applied operationally would lower costs.

So, overall, should the verdict on the granulovirus be 'still much room for improvement'? This may be an unhelpfully narrow view. Increasing stringency in pesticide legislation and development of new forms of insect resistance to pesticides mean direct comparisons, like speed of kill and persistence, are not the only factors to consider. In addition, in an IPM system the knock-on effects of chemical pesticides on natural enemies and secondary pests can diminish the usefulness of chemical insecticides.

In orchards where CpGV was part of management programmes, codling moth damage was acceptable for organic production and CpGV provided a workable part of a strategy for season-long control. In one orchard, CpGV was the sole intervention against codling moth. In others, where additional measures were used, growers considered CpGV to have played a key role in protecting the crop. Another orchard, where total crop loss occurred, reinforced the importance of well-timed early-season intervention.

The conclusion of the study was that weekly applications of commercial CpGV formulations timed during peak egg hatch and integrated with appropriate strategies such as mating disruption or application of other ‘soft’ pesticides do provide a valuable alternative for codling moth management at low to middling pest pressure, with reduced doses translating into cost savings. Work is continuing to optimize application regimes for CpGV, as well as on improving its formulation. Other recent work, which looked at the impact of different CpGV application rates and spray intervals in full-season programmes, indicated that programmes can be tailored to local pest pressure. Nonetheless, the main caveat of these studies on CpGV use is that at high pest pressure granulovirus, like mating disruption, is insufficient by itself to prevent economic damage.

So often the comparison is made between CpGV and chemical pesticides from the perspective of cost and immediate kill. Besides efficacy and lack of effect on beneficials, it is also safe for applicators and there is zero pre-harvest interval – one could spray today and harvest tomorrow if necessary (guthion on the other hand is toxic to all organisms and has a 21-day pre-harvest interval).

Carrying on the Good Work

Having worked hard to bring codling moth populations down over the summer months, it makes sense to keep up the pressure over the winter. Fruit bins placed in the orchards for harvest have been identified as a prime site for codling moth larvae to overwinter, from where they re-infest orchards the following season. Left untreated, they can jeopardize the success of IPM strategies.

The potential of entomopathogenic nematodes for use against codling moth indicated that two species, *Steinernema carpocapsae* and *S. feltae,* have significant activity against diapausing and non-diapausing larvae (which are both more susceptible than pupae) under a variety of environmental conditions.

Other work showed that diapausing larvae in miniature fruit bins were very susceptible to infection by *S. carpocapsae* and two other species when bins were immersed in solutions containing infective juveniles for the kind of periods used for floating fruit from bins in commercial operations. An alternative strategy would be to include the nematode suspension in sprays used for treating or cooling fruit. Research since has indicated that adjuvants can improve penetration of the cocooned larvae and decrease desiccation of the nematodes, with codling moth mortality of up to 85% recorded. The work is still at the experimental stage, but used operationally, it could lead to the reduction or elimination of this overwintering ‘captive audience’ of cocooned, diapausing codling moth larvae in the autumn after fruit is harvested, which could markedly reduce or eliminate fruit damage the following spring.

Worms Eat Worms

The navel orangeworm (*Amyelois transitella*) is a key pest of pistachios in California, controlled by applying organophosphate, carbamate and other insecticides. The search for a biological control solution took researchers to the pyralid pest’s overwintering site, in fallen pistachio nuts.

Sanitation, by removing nuts from trees and tillering fallen nuts into the soil, reduces re-infestation in the spring. How many navel orangeworms survive depends on how deeply the nuts are buried and how many decompose over the winter. Entomopathogenic nematodes, which provide effective control of a wide variety of insects in soil as well as other cryptic habitats, could add another mortality factor. Earlier work evaluating steinernematid and heterorhabditid nematodes in almonds on trees suggested they were certainly worth assessing for the navel orangeworm in overwintering pistachios.

Trials during winter 2002–03 were designed to mimic the situation in orchards. They looked at the ability of two *Steinernema* species to control orange navelworm infesting pistachios, on the ground or partially buried, in bare soil or covered with fallen pistachio leaves. The nematodes were applied by backpack sprayer at rates of 50,000–100,000 infective juveniles per square metre with two volumes of water.

Steinernema carpocapsae was the more effective nematode in pistachios in these trials, producing >72% mortality at the highest application rate when nocturnal temperatures remained above 0°C. It was equally effective applied on bare and leaf-covered plots, and persisted longer in more sandy soil than *S. feltiae.* It can also multiply in the soil (although there was no clear evidence that it had done so in these trials).

The better performance of *S. carpocapse* in these trials could result from a combination of greater tolerance to various abiotic factors and foraging behaviour more suited to locating navel orangeworm in the fallen nuts. However, it could equally result from differences in formulation. More work is needed to unravel these contributory factors, and far more still if a product is to reach the market. Nonetheless, the conclusion is that entomopathogenic nematodes can play a role in postharvest control of navel orangeworm and thus could contribute to a reduction in insecticide use in pistachio crops.

Updated from main sources:

Arthurs, S.P. & Lacey, L.A. (2004) Field evaluation of commercial formulations of the codling moth granulovirus: persistence of activity and success of seasonal applications against natural infestations of codling moth in Pacific Northwest apple orchards. *Biological Control* **31**, 388–397.

Lacey, L.A. & Chauvin, R.L. (1999) Entomopathogenic nematodes for control of diapausing codling moth (Lepidoptera: Tortircidae) in fruit bins. *Journal of Economic Entomology* **92**, 104–109.

Siegel, J., Lacey, L.A., Fritts, R., Jr., Higbee, B.S. & Noble, P. (2004) Use of steinernematid nematodes for post harvest control of navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in fallen pistachios. *Biological Control* **30**, 410–417.

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Training News

In this section we welcome all your experiences in working directly with the end-users of arthropod and microbial biocontrol agents or in educational activities on natural enemies aimed at students, farmers, extension staff or policymakers.

Planning Farmer Field Schools in Coconuts

Farmer field schools (FFSs) have an air of informality, but success lies with a rigorously planned curriculum and carefully selected participants.

Establishing FFSs in tree crops like coconuts presents challenges that differ in some respects to those found in annual crops like rice, where the FFS movement began. With coconuts a crop of key economic and cultural significance in many countries of the Asia–Pacific region, national organizations, and international bodies such as APCC (Asian and Pacific Coconut Community), have invested heavily in research. This has created a strong knowledge base, and many promising advances in combating production constraints have been made [e.g. see: 'Coconut and taro beetles: tailoring biocontrol for the Pacific', IPM Systems, this issue]. Now these need to be communicated to the region's millions of coconut growers.

A Workshop on Curriculum Development for Farmer Field School of the CFC/DFID/APCC/FAO[1] Project on Coconut Integrated Pest Management, held in Kerala, India on 3–5 February 2005 sought to address the issues specific to coconut poduction. Participants were representatives of national coconut research organizations (including India and the Philippines which have on-going coconut FFS programmes), representatives of APCC and FAO, and resource people drawn from Indian organizations and universities with experience in participatory methods in coconuts and other crops. In depth discussions were held on the type of curriculum required. A dynamic curriculum was developed, which will be implemented in 25 FFSs, five each in the Philippines, Papua New Guinea (PNG), Sri Lanka, Tanzania and India. (Since then it has been decided to run four additional FFSs in Malaysia, Thailand, Samoa and Indonesia, this will cover all nine centres of the project.)

Acceptance by all partners of the FFS model with farmers at the centre was agreed to be essential. An FFS is seen as an institution without a structure, serving the purpose of a research-oriented scientific institution. There should be a good mix of dynamics, expertise and decision making, with community development the priority. Only economically viable and sustainable technologies should be included. But farmer involvement is needed if the practices disseminated are to be sustainable, so participating farmers need to have ownership of the FFS from the outset.

All with experience in FFSs felt that selecting the right kind of farmers was essential for a successful programme: they need to have aptitude and enthusiasm for coconut farming, and be interested in adoption of technology disseminated through the FFS. The workshop agreed on a selection system beginning with a community-based gathering followed by a discussion with the candidate farmers that identified, also obtaining feedback through a structured questionnaire.

Each FFS will run for 9 months (12 sessions) to allow participants to work through the IPM processes based on the life cycle of the key pest of coconut, the rhinoceros beetle (*Oryctes rhinoceros*). This pest is native to the region, is often kept in check by natural enemies, and has had a range of IPM options validated for its control. It thus provides a good central model for farmers to learn from. The FFS curriculum covers the ecology of the coconut ecosystem, natural enemies of common pests, sustaining natural control and understanding agronomic practices that maintain a healthy crop. The half-day sessions are fortnightly for the first 3 months, then monthly.

Preparatory sessions concentrate on building community relationships, selecting farmers for the FFS and identifying field sites. Field studies form the core of subsequent sessions. Throughout the FFS, alongside each curriculum activity are learning objectives and quality indicators. For example, in one of the first activities, farmers collect specimen material and set up ballot boxes: a series of stations, each bearing a coconut-related question – e.g. 'Which one [of three insects on show] is a pest?' or 'This insect [on show] damages which of these?' [with three answers for the farmers to choose from]; the answer papers are posted in the appropriate boxes. The objectives of the activity are (i) for farmers to learn about sampling and to collect arthropods in the field and (ii) to prepare them for agro-ecosystem analysis (AESA); (iii) for facilitators to collect information on the general field situation and (iv) to initiate discussion of farmers' field problems. The quality indicators are that farmers understand how to take samples, they ask questions about what they see, they draw on what they observe, and they are able to identify key issues facing them in their farms.

As the FFS progresses, the farmers learn how to undertake field observations, set up experiments, record and analyse results, discuss what they have done, prepare reports, and so on. They experiment on pests, diseases and abiotic factors, investigating ways of managing them through IPM technologies, and even look at livelihood diversification possibilities. Learning the importance of team work is also emphasized and one of the later activities is organizing a field day. The goal is that at the end of the FFS, farmers will understand their crop, be confident in conducting research, be able to translate the results into improved production methods, and (an axiom of the FFS philosophy) be willing and able to communicate their knowledge to other farmers, thus ensuring a horizontal spread of the FFS outputs.

[1]Common Fund for Commodities/UK Department for International Development/Asian and Pacific Coconut Community/UN Food and Agriculture Organization

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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.

Parthenium Discussion Group

Parthenium hystophorus is acknowledged to be one of the world's ten worst weeds. As an extension of the International Parthenium Research News Group (www.iprng.org), a discussion group has been formed to address different aspects of parthenium management. See:

http://in.groups.yahoo.com/group/IPRNG

Contact: Pankaj Oudia, Convenor, IPRNG
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Arthropods Against Weeds

A manual developed by USDA-ARS (US Department of Agriculture – Agricultural Research Service) is now available online to help scientists, resource managers and others identify biological control insects that play a key role in helping to control aquatic weeds. It was originally published by ARS scientists at the Invasive Plant Research Laboratory (IPRL) in Fort Lauderdale, Florida, in cooperation with colleagues from the Florida Department of Environmental Protection and the US Army Corps of Engineers. It covers more than 50 of the most common insects and mites found in aquatic environments in the USA.

The manual is organized alphabetically by plant name, and within these sections alphabetically by the various insects that attack them. These subsections include a history of each insect, its host plants, and its biology and ecology. There is a separate section for polyphagous insects.

Center, T.D. Dray, F.A., Jr., Jubinsky, G.P. & Grodowitz, M.J. (2002) *Insects and other arthropods that feed on aquatic and wetland plants.* USDA-ARS Technical Bulletin 1870, 200 pp. Download: www.ars.usda.gov/is/np/aquaticweeds/aquaticweedsintro.htm

Neobiota Bern Conference

The proceedings of this conference, organized by the German Group on Biological Invasions (29 September – 1 October 2004), have been published.

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Or order online:
Web: www.tu-berlin/de~neobiota

Oilseed Rape IPM Symposium

An international symposium on IPM in oilseed rape will be held in Göttingen, Germany on 3–5 April 2006. The meetings is being organized by BCPC (British Crop Protection Council) on behalf of the EU (European Union) Master (Management Strategies for European Rape Pests) project. The project is developing IPM strategies incorporating biocontrol for European oilseed rape pests. Scientific sessions will cover:

- Biocontrol agents (parasitoids, predators and pathogens)
- IPM and integrated crop management (ICM)
- Oilseed rape pests
- Crop protection
- Socioeconomics and policy

Contact: ipm@bcpc.org
Web: www.symposium-ipm-oilseed-rape.de/

Forest Invasive Species Network for Africa

A new website is facilitating information flow on invasive species in forests between countries in Africa. The FISNA (Forest Invasive Species Network for Africa) website was developed during a workshop held in December 2004 in Zomba, Malawi. This network is open to all countries in subSaharan Africa. The website, which is hosted by the Forestry Department of FAO (UN Food and Agriculture Organization), was launched in March 2005. See:

www.fao.org/forestry/site/26951/en

All types of invasives, from pests of trees to trees that are pests, are included. The site provides information on new outbreaks, quarantine issues, FAO's Global Information System and woody invasive species. Usefully, it also lists projects on invasive species in forestry and forests in Africa (with a section for those on biological control), together with contact information.

How the website can help meet the objectives of the network is illustrated by visiting 'New Outbreaks':

- The blue gum chalcid (*Leptocybe invasa*) was found damaging young eucalyptus trees and nursery seedlings in Uganda and Kenya last year. The website has posted news that it was found on a number of host trees in Tanzania in May 2005, alerting neighbouring countries to its spread. Links take visitors to further information on identification of the pest and management strategies.
- Also in May 2005, an alert from South Africa drew attention to the discovery of the cossid moth *Coryphodema tristis* there. Host plants are listed, and a link is given for more information on this pest.

Prolific Coconut IPM Project

Information provision for stakeholders needing to know about management of coconut pests in the Asia–Pacific region, and beyond, has had a boost through the prolific publishing outputs of the CFC/DFID/APCC/FAO[1] Project on Coconut Integrated Pest Management in the last year, all published by APCC. Amongst the publications are:

- Bibliographies containing 860 references for rhinoceros beetle (*Oryctes rhinoceros*) and 398 references for coconut mite (*Aceria guerreronis*), which form Appendixes 1 and 2, respectively, in the Annual Report 2004.
- Singh, S.P. & Rethiman, P. (2004) Baculovirus – a key component of biointensive integrated pest management of coconut rhinoceros beetle. *CocoInfo International* 11(1), 17–20. An illustrated fact sheet including a description of this pest together with its host plants, damage and life cycle. The history of the use of the rhinoceros beetle virus (baculovirus) in biological control is summarized. Its mass production is described in detail, together with methods for infecting and incubating beetles, and their field release (including novel methods using traps). The impact of the virus on larval and adult beetles and palms is described. Pathways of virus dispersal are identified and its field persistence noted. A key to diagnosing virus-infected larvae is included. Countries where natural infections exist in rhinoceros beetle populations are listed. Biointensive IPM practices that can be integrated with the use of the virus to control the pest are also listed.
- Singh, S.P. & Rethiman, P. (2004) Use of green muscardine fungus in BIPM of rhinoceros beetle *CocoInfo International* **11**(2), 19–24. An illustrated fact sheet including an outline of the pest's life cycle and possible management options. The use of entomofungal pathogens as biological control agents is discussed. Methods of producing *Metarhizium anisopliae* are described in detail, focusing on cost-effective methods, some suitable for community use, developed in the Asia–Pacific region (including farmer innovations). Various options for applying the fungus as spray and powder and via pheromone trap boxes are also described. A protocol for assessing the impact of treatment is outlined. Key points for successful use of the technology are listed. This is a developing technology, and future research needs are identified.
- Singh, S.P. & Rethinam, P. (2004) Coconut eriophyid mite (*Aceria guerreronis*). This 82-page bulletin, starts with an outline of the problems of controlling the pest and a summary of the eriophyid mites recorded from coconut palms. A description of *A. guerreronis* is followed by notes on its host plants, feeding habits, the nature and extent of damage it causes, distribution, bioecology and dispersal. Methods used in research on the pest are described, including rearing, monitoring and counting techniques. The results of population studies in different regions of India are summarized. Natural enemies and predators found associated with *A. guerreronis* or on coconut are listed, including some 28 predatory mites found associated with the pest beneath the perianth of coconuts, and their role in providing some control is discussed. Mass production and release techniques for predatory mites are outlined. Entomopathogenic fungi are considered, and in particular *Hirsutella thompsonii*. Its life cycle is described in relation to its potential as a biological control agent. The need for better understanding of its taxonomy and diversity is noted. Its use in biological control is reviewed, including its formulation as a biopesticide in India. Other management options considered include host plant resistance, cultural management and chemical control (including different application technologies). The efficacy of integrated management initiatives in India is discussed and future needs for *A. guerreronis* control are identified. A summary includes recommendations for direct and indirect (stem injection and root feeding) acaricide applications. The volume is completed by a bibliography containing some 400 references.
- Singh, S.P. & Rethinam, P. (2005) Coconut leaf beetle, *Brontispa longissima*. This 35-page bulletin begins by outlining the importance of coconut as a crop in the Asia–Pacific region, and the history of the coconut leaf beetle's emergence as a pest. Its taxonomic history has been complicated by colour variation, and there is a call for a revision of *Brontispa* species in the Asia–Pacific region. Its current geographical distribution is given, together with the risk and potential impact of further spread. Aspects of its biology dealt with include host range, life cycle and factors affecting abundance. An account of the history of the spread of the pest includes the nature and extent of damage inflicted across the region.

The history and prospects for biological control are discussed. Natural enemies and details of introductions for biological control to date are listed. The use of *Tetrastichus brontispae* as a biological control agent is described in detail, with an outline of mass-rearing methods and a history of its use. *Aescodes hispinarum* is reviewed more briefly. The identification of a local strain of *Metarhizium anisopliae* and methods for production and application are described. Other management options considered are varietal resistance, quarantine measures, cultural control, attractants, and chemical control (spraying, trunk injection, 'tea bags' containing insecticide). Finally FAO and APCC initiatives to tackle the pest are noted, the role of pest risk analysis in informing plant protection strategies is discussed, and future needs for tackling the pest are identified.

The proceedings of a Farmer Field School Curriculum Development Workshop are dealt with in Training News, this issue. APCC also publishes the *Cord*, the International Journal on Coconut R&D.

[1]Common Fund for Commodities/UK Department for International Development/Asian and Pacific Coconut Community/UN Food and Agriculture Organization

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Japan's Invasive Act

Japan has enacted The Invasive Alien Species Act, which came into force on 1 June 2005. In total, 37 species have been designated as invasive. For information in English, see:
www.env.go.jp/en/topic/as.html

Brazil Outlaws Biopirates

In a move that may impact on biological control, Brazil has introduced a law to regulate the development of commercial products from its native species. The new law has a two-fold aim: to ensure Brazil retains sovereignty over its biological resources, and to educate people about conservation. Anyone using Brazil's indigenous resources will be required to obtain permission and share the benefits. Unauthorized export of Brazilian species is prohibited by this new law, and the money from fines (maximum penalty US$20 million, although 'first offenders' may meet leniency) will be used to fund conservation of biodiversity.

Conference Reports

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.

Hemlock Woolly Adelgid Symposium

Over 200 people attended the Third Symposium on Hemlock Woolly Adelgid (HWA) in the Eastern United States in Asheville, North Carolina on 1–3 February 2005. The first symposium was held in 1995 in Charlottesville, Virginia and the second, in 2002, was held in East Brunswick, New Jersey. Unlike the previous conferences, the Asheville meeting included presentations on two related invasive pests, the balsam woolly adelgid, *Adelges piceae* (BWA), and the elongate hemlock scale, *Fiorinia externa* (EHS). There were 83 presentations (43 papers and 40 posters), including six on the EHS and six on the BWA. The remaining presentations were on HWA, *Adelges tsugae*. On the first day of the symposium, the presenters discussed challenges of adelgid systematics, economic and ecological impacts of the three invasive pests, and survey, impact, and detection methodology. On day 2 the topics included research and technology development, and a forum discussion on biological control of HWA. On the morning of day 3 various management strategies were covered. The symposium concluded with a discussion on where we are now and where do we need to go in our understanding of these three pests.

EHS was first reported in 1908 on Long Island, New York. It is native to Japan where densities are much lower. The scale has at least 57 host species including species of hemlock, cedar, fir, pine, spruce and yew. It is found from North Carolina to southern New England and west to Ohio. The insect infests the underside of needles and causes a yellow banding on top of the needles and premature needle drop. Infestations of both the scale and HWA hasten hemlock decline, and hemlock borer or *Armillaria* root rot may attack these weakened trees. Topics on EHS included: (i) classical biological control of the scale, (ii) natural enemies of the scale in the southern Appalachians, (iii) management with entomopathogenic fungi, (iv) effects of systemic insecticides, a growth regulator, and oil on the scale and associated natural enemies, and (v) reproduction, mass release and recovery of a scale predator.

BWA, like HWA, was discovered in the southern USA in the 1950s. Unlike HWA, widespread mortality of its host by BWA was immediately apparent. About 90% of all mature Fraser firs (*Abies fraseri*) in the southern Appalachians have been eliminated. Fortunately, BWA prefers mature trees, and in many cases the old growth trees have been replaced by the next generation of Fraser fir. Time will tell how well the second generation will fair against BWA. The early research on BWA emphasized classical biological control. While a number of predators were successfully established, none have had a significant impact on BWA populations. Recent and current research has emphasized host factors. BWA topics at the symposium included: (i) impacts in the southern Appalachians, (ii) host interactions, (iii) effects of BWA on the chemical composition of the wood and bark, (iv) some preliminary results of metabolic profiling and microarray analysis of Fraser fir infested by BWA, (v) isolation and characterization of microsatellite markers in Fraser fir, and (vi) microfeeding sites of both BWA and HWA in artificial feeding systems that are being researched.

The majority of the presentations dealt with HWA. Unlike BWA, HWA will attack all ages of its host in natural stands and represents a more serious threat to eastern and Carolina hemlock (*Tsuga canadensis* and *T. caroliniana*, respectively) than BWA does to Fraser fir. However, western (*T. mertensiana* and *T. heterophylla*) and Asian (*T. chinensis, T. diversifolia* and *T. sieboldii*) species of hemlock are not as susceptible to HWA. A fundamental question that is not yet fully understood is: are the hemlock species that survive HWA surviving because of host resistance, HWA natural enemies, site factors, or a combination of several these factors? HWA topics that were covered included: (i) impacts on residential landscapes, recreation areas, and headwater streams, (ii) binomial sequential sampling, (iii) sampling within stands, (iv) branch sampling, (v) use of hyperspectral technology for monitoring, and use of satellite imagery and environmental data, (vi) use of landscape scale models, (vii) GIS-based risk assessment, (viii) biological control, (ix) chemical control, (x) host resistance, (xi) gene conservation of hemlock, population dynamics of HWA, (xii) use of mitochondrial DNA for determining the geographic origin of HWA, (xiii) silvicultural options for minimizing HWA impact, (xiv) state and national forest guidelines, (xv) national park strategies, and (xvi) environmental impact of the insecticide imidacloprid.

The preponderance of HWA presentations at the symposium dealt with biological control (16 presentations), management (18), or survey, detection and monitoring (14). This reflects that great progress has been made in these areas, although there is still much to be done. For example, one aspect of classical biological control that is just now being addressed is long-term studies of HWA and its natural enemies in Asia. Up to the present, our knowledge of the natural enemies of HWA in its native range has been based on very short-term visits. This has provided an inadequate assessment of the role of natural enemies in the native range of HWA.

The symposium had only a few presentations on host resistance (2), genetic conservation of host material (3), population dynamics of HWA (3), and genetics of HWA (1). This is a reflection of the fact that support for a more comprehensive research programme has not been forthcoming. Recently, the US Department of Agriculture (USDA) Forest Service has begun to address this by establishing a small programme with a request for proposals on host resistance to HWA. This is a good beginning. The strategic plans for a comprehensive research programme on HWA have existed since 1997. Unfortunately, the financial support has been limited to a more narrow focus. It is this reviewer's fervent hope that one outcome of the Asheville symposium will be a recognition that far more resources are needed to establish a comprehensive programme on invasive pests if we are to have any hope for saving the threatened host trees.

Published proceedings of the symposium should be available by the end of 2005.

By: Fred P. Hain, Department of Entomology, North Carolina State University.

European and Mediterranean Moves on Invasives

Two more meetings this summer signalled that there is growing awareness and concern about invasive alien species (IAS) in Europe and the Mediterranean Region. While the long experience of other regions in biological control was recognized as an important resource to draw on in the first meeting, the potential for classical biological control to contribute as a management tool for IAS came as something new at the second.

Call to Mediterranean Region Countries

Whilst many regions of the world, including many countries in Mediterranean-type ecosystems (California, Australia, South Africa and Chile), have considerable experience in dealing with invasive plants, the same cannot be said of the Mediterranean basin countries themselves (or indeed anywhere in Europe). The 'Invasive Plants in Mediterranean Type Regions of the World – International Workshop' was held in Mèze, France, on 25–27 May 2005 and organized by the Conservatoire Botanique National Méditerranéen de Porquerolles, the Council of Europe, The World Conservation Union (IUCN) and the European and Mediterranean Plant Protection Organization (EPPO). It brought together 110 participants from over 20 countries with the aim of producing a declaration to further the cause of invasive plant management.

The workshop followed publication of a 48-page booklet, 'Plantes envahissantes de la région Méditerranéene' [Invasive plants of the Mediterranean region'] and the recently published European Strategy on Invasive Alien Species.

There were 26 oral presentations and 52 posters. Eleven working groups, which dealt with the broad themes of checklists/databases, prevention, communication/education and management, included one on biological control. Interest in this was high, and there are many potential targets for which 'off-the-shelf' agents exist.

Considerable time was spent drafting the Mèze Declaration, which represents the findings of the working groups and calls for countries of Mediterranean-type ecosystems to contribute to a greater awareness of the problems and encourage action at the Euro-Mediteranean scale, drawing on information and experiences elsewhere – a point that had been reinforced by the presence at the meeting of biological control experts from organizations such as the USDA-ARS-EBCL (US Department of Agriculture –

Agricultural Research Service – European Biological Control Laboratory) and the CSIRO (Australian Commonwealth Scientific and Industrial Research Organisation) European Laboratory, both in Montpellier, France, and CABI, UK.

By: Richard Shaw and Djamilla Djeddour, CABI Bioscience.

How to Reach a United European Approach

The symposium 'Plant Protection and Plant Health in Europe: Introduction and Spread of Invasive Species', held in Berlin on 9–11 June 2005 and organized jointly by DPG (German Phytomedical Society) and BCPC (British Crop Protection Council), had the aim of bringing together specialists from research, consultancy, trade and administration in order to address the subject of IAS from different angles and to identify appropriate ways of handling associated issues. Although there were attendees from outside the EU (European Union), including some from non-member states in eastern Europe/the Balkans and from outside Europe, delegates predominantly came from EU member states, with a strong representation from BBA (the Federal Biological Research Centre for Agriculture and Forestry in Braunschweig and Berlin) as the main organizing institution.

The mood of the meeting was set by the opening lecture. Stephen Hunter, Head of Plant Health at DEFRA (UK Department for the Environment, Food and Rural Affairs) addressed the challenges presented by IAS at a time of increasing globalization. He noted that 25% of UK resources on plant quarantine and health are currently directed at dealing with *Phytophthora ramorum*, the causal agent of sudden oak death (SOD). This highlighted the two-fold impact of *Rhododendron ponticum* in the UK: as an invasive alien shrub and as a host of the destructive SOD pathogen, and thus the frequently inter-related nature (and threats) of many IAS problems.

One and a half days of the symposium were devoted to platform presentations, with sessions on 'Trade as a pathway for introduction and spread of alien species', 'Risk management and regulatory frameworks' and 'Monitoring alien invasive species'. Some talks addressed regulatory issues, risk assessments and specific activities undertaken by organizations such as EPPO and IPPC (International Plant Protection Convention). Others presented case studies, such as SOD, the horse chestnut leaf miner (*Camereria ohridella*) and the invasive weed *Ambrosia artemisiifolia.* Three subsequent parallel workshops dealt with 'Climate change and its effect on invasive species', '*Diabrotica* – case study of an invasive species' and 'Trade with beneficials and useful organisms – a risk?'. The last half day was taken up by poster sessions, followed by plenary reports of the three concurrent workshops. The final plenary session, chaired by Stephen Hunter, dealt with a number of topics, including how resources can be optimized across Europe to address IAS, how the costs of dealing with IAS could be shared between government and industry (i.e. the horticultural trade), the need for close collaboration between EPPO and the EU, the need for regional cooperation in Europe, as well as for a network of scientists and other staff working in diagnostic and regulatory affairs, and (last but not least) how theory might be turned into practice, taking into account potential impacts on trade – a question that was left largely unanswered.

By: Marion Seier, CABI Bioscience.

New Books

Genetics, Evolution and Biological Control

Within the covers of this book the editors have collected together a veritable gold mine of information on recent advances and applications of genetic and evolutionary biology in the field of biological control by use of natural enemies. Biological control technologies, particularly classical biological control (CBC), have a proven track record of providing long-term sustainable solutions to the growing global concern about invasive alien species. Nonetheless, on the other side of the coin, the generally low rates of successful establishments that lead to economic controls in CBC projects is a forceful reminder of the need for researchers to keep focussed on key topics of relevance. The book is very timely because many of the cutting-edge issues within biological control require inputs from genetics and evolutionary biology (at least in this reviewer's mind).

The book arises from the International Organization of Biological Control (IOBC) Third International Symposium on the subject area, which was held in Montpellier, France in 2002; this was done in collaboration with the Complexe International de Lutte Biologique–Agropolis (CILBA). There is good international representation in the authors, reflecting that advances are being made across the globe at an exciting pace – most notably in the USA, western Europe and Australia.

Six themes within biological control have been chosen for the book (as described below), although the main emphasis for a large part of the book is in the context of CBC. Two chapters are devoted to each theme.

The first three themes – the genetic structure of pest and natural enemies, molecular diagnostics, and tracing the origin of pests and natural enemies – include topics that have hugely benefited from recent 'thinking' and advances in population genomics. This is the genetic determinism of phenotypic variations and involves genetic mapping to identify important genes associated with particular traits.

The first two chapters cover the genetic structure of populations. Wajnberg (France), focussing on insect parasitoids, discusses the importance of understanding intrapopulation genetic variability in the context of natural enemy rearing and release strategies. The chapter contains a useful review of experimental and statistical methods for quantification of qualitative biological traits. Overall, intrapopulation variability is a little-studied research area that could make more use of the powerful molecular tools now available. On the plant pathogen front, Burdon & Hall (Australia) provide a thought-provoking essay on temporal and spatial variability in host plant resistance and pathogen virulence and the implications for improving biological control strategies. The authors illustrate how the life history features of the biological control agent and plant target do have a major impact on the ecological and evolutionary dynamics of the system but the subject suffers again from the lack of experimental studies. For example, there is still a paucity of information on actual patterns of resistance within invasive plant populations and the patterns of virulence of pathogens in the endemic ranges of the plants.

The second theme, molecular diagnostics, is covered in the next two chapters. This is a very exciting branch of research where molecular tools are clearly revolutionizing thinking. Evans & Gomez (Australia) provide a nice complementary chapter to that by Burdon & Hall by reviewing the genetic tools (markers) that can be used to characterize rust strains or populations and how these tools can be applied during the course of a weed biological programme. Genetic markers can be usefully used to characterize the plant–pathogen interaction and thus 'map' the fate of pathogen strains after release. The authors include the skeleton weed (*Chondrilla juncea*) biological programme in Australia as a case study as this provides a practical example of the impact of genetic diversity in an apomictic weed on the success of biological control. Heraty (USA) covers the use and application of molecular methods for the recognition of species and understanding the relationships of Hymenoptera; particularly the Chalcidoidea. Most effort to date on this superfamily has been on species diagnostics but recently researchers have been putting more emphasis on higher-order relationships because of advances in DNA sequencing as a marker-based technology. The latter research thrusts are very important for biological practitioners as the work is allowing a better resolution of species groupings within complicated groups such as *Encarsia.*

Genetic and statistical approaches to tracing the origin of pests and natural enemies are the subject of the next two chapters. Roderick (USA) provides a general overview of the issues associated with determining origins of non-native species, while Brown (USA) focuses on the issues related to cryptic species (introduced species that are similar morphometrically to native species – e.g. *Bemisia* species) and their natural enemies. Here again, the use of molecular markers has enabled tremendous advances to be made. Indeed, computer programmes are now available on the Internet that allow the processing of data if markers have been measured. The main constraint facing researchers working on new pests is access to source material and the development of new markers.

The fourth theme of the book is evolutionary change in species and the consequences for host–natural enemy interactions. Müller-Schärer & Steiner (Switzerland) discuss invasive alien plants and the recent developments in evolutionary theory, genetics and molecular tools that offer a new integral approach to investigating genetic factors that affect invasion success and its consequences for CBC. These authors provide a useful summary of emerging research topics and how some of this research might be achieved during a biological control project, e.g. using host specificity studies to examine trade-offs between plant defence and fitness for native and introduced plant genotypes. Kraaijeveld (UK) discusses house fly/fruit fly – parasitoid systems. The focus is on the evolution of resistance to parasitoids versus pesticides and why the former is much rarer then the latter.

The last two themes cover the impacts of transgenic crops on natural enemies and the genetic manipulation of natural enemies for improved performance in biological control. Obrycki, Ruberson & Losey (USA) provide a general overview of the interactions between natural enemies and transgenic crops and thus bring together much useful information in a key subject area. Trends are emerging such as lower densities of host-specific species versus generalist predators in areas where insecticidal crops have a high dosage of insecticidal toxin(s). The authors discuss this and other effects in the broader context of whole pest spectrums in crop systems. The following chapter by Hilbeck (Switzerland) covers the GMO Guidelines Project which is the development of international scientific environmental biosafety testing guidelines for transgenic plants. The project has an international steering committee and is welcoming inputs from the wider scientific community in the development of the guidelines.

The selective breeding of natural enemies for particular traits is not a new subject. As with some of the other themes, the chapters covering this subject provide excellent reviews of progress to date, how the technology is being used, and a discussion of 'what next?' Poppy & Powell (UK) review parasitoids of plant pests (and also genetic manipulations to the plant). Stouthamer (USA) reviews sex-ratio distorters (mainly bacteria) and other selfish genetic elements and the implications for biological control rearing and release. Not all heritable bacteria are bad, as those inducing parthenogenesis in natural enemies may be good for biological control. Manipulating such bacteria is complex, and how such modified bacteria may play a role in improved biological control is still unknown.

Ehler, L.E., Sfora, R. & Mateille, T. (eds) (2003) *Genetics, evolution and biological control.* Papers from an International Organization for Biological Control symposium, Montpellier, France, 2002. CABI Publishing, Walllingford, UK & Cambridge, MA, USA. 260 pp. Hbk. UK£55/US$100.
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Proceedings

Biopesticide Formulation and Application in India

The proceedings have been published of the joint ICAR (Indian Council of Agricultural Research) – CABI Workshop on Biopesticide Formulations and Application, held in Bangalore, India in December 2002. Agricultural policies in post-independence India, thanks to the Green Revolution and policies pursued by ICAR, have led to a surplus in food grain production. The challenge now is to sustain an Evergreen Revolution to ensure the food and health needs of the still-growing population continue to be met. Growing crops without chemical pesticide residues and contamination of the environment has become a major concern.

While considerable progress has been made by PDBC (Project Directorate of Biological Control) and a few other ICAR institutions and state agricultural universities in developing microbial control, biopesticide formulation and application technology are still in their infancy and current production does not meet potential demand. ICAR approved the organization of this workshop to synthesize the current status of microbial pesticides in India. In order to boost adoption of this technology, it is essential to develop formulations with good efficacy, field persistence and storage stability, as well as appropriate field application technologies.

The proceedings open with an assessment of the current status of production and use of microbial pesticides in India, which also identifies a way forward (R.J. Rabindra), followed by a consideration of prospects and future uses (Dilip K. Arora & Ratul Saikia).

Subsequent papers review the current status in India of baculovirus formulations and applications (K. Narayanan), granulovirus formulations in pest management (S. Easwaramoorthy), microbial control of *Helicoverpa armigera* (N. Sathiah, R.J. Rabindra, S. Palaniswamy, K.P. Jayanth, S. Subramanian, C.M. Senthil Kumar, B. Rajasekharan & J.S. Kennedy), microbial agents for vegetable and fruit IPM (C. Gopalakrishnan), microbial control of forest insect pests (V.V. Sudheendrakumar), and entomopathogenic fungi in rice pest management (L.K. Hazarika & K.C. Puzari).

With these reviews are two papers presenting case studies; first, on how *Bacillus thuringiensis* was brought within reach of India's dryland farmers (P.S. Vimala Devi & M.L.N. Rao) and second, on the development of products based on *Metarhizium anisopliae* (Roy Bateman). Attention focuses strongly on production and application issues, with papers on formulations and applications of mycopathogens (M.V. Despande), formulations of entomopathogenic nematodes (S.S. Hussaini), formulation and delivery of fungal and bacterial antagonists for disease management in India (U.S. Singh & Najam Waris Zaidi), formulations of bacterial antagonists in disease management in India (R. Samiyappan), and formulations and applications of *Trichoderma* species for control of plant diseases (R.D. Prasad). There is also a look at the scope for fungal pathogens in weed control in India (P. Sreerama Kumar). The contribution of the Andra Pradesh Netherlands Biotechnology Programme is assessed (M.L.N. Rao & G. Pakki Reddy). Three papers by David Dent and Nina Jenkins deal with issues surrounding the registration of biopesticides, licensing microbial pesticide technologies to commercial companies, and a European perspective on the economics of biopesticides. The final two papers cover quality control and registration requirements (A.D. Padwar) and commercial production and market prospects (K.P. Jayanth) for microbial pesticides in India. The volume ends with the recommendations of the workshop.

As a synthesis of the status of the microbial pesticides in India at the end of 2002, this is an invaluable publication. Facts and figures, neatly tabulated and clearly summarized in the text, abound in its pages, together with well-informed discussion of the issues, and identification of the gaps in knowledge. The papers are well referenced making it also a useful bibliography. This is a proceedings that has a place on the shelf for anybody with an interest in microbial pesticides, not just in India, for it contains a wealth of information of global value.

Rabindra, R.J., Hussaini, S.S. & Ramanujan, B. (eds) (2005) Microbial biopesticide formulations and application. Proceedings of the ICAR–CABI Workshop on Biopesticide Formulations and Application, 9–13 December 2002, Bangalore. Technical Document No. 55, Project Directorate of Biological Control (Indian Council of Agricultural Research), Bangalore 560 024, India, 269 pp.
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