Biocontrol News and Information **28**(4), 67N–83N
pestscience.com

General News

Advances and Challenges in Biocontrol Efforts against Fire Ants in Texas

It is nearly ten years since parasitoid phorid flies were first released as prospective biocontrol agents for fire ants (*Solenopsis invicta*), starting with small trial releases in Florida and Texas (USA) that set the stage for releases at multiple sites that now include several phorid species. Along the way, much has been learned about the complexities of this host–parasitoid relationship, and about the practical challenges of establishing these phorid flies in arid Texas conditions. With successful establishment of the flies across the southern USA, several studies are now examining the possible impacts of these biocontrol agents on fire ant populations in the wild. This article provides an overview of the ongoing biocontrol effort with an emphasis on developments and challenges in Texas.

Background on Phorids as Biocontrol Agents

The possibility of using phorid flies for biocontrol was inspired by Don Feener's demonstration in the 1980s that phorid flies alter the ability of a *Pheidole* sp. to compete for food. Early work by Sanford Porter (US Department of Agriculture [USDA]) and others on fire ants in South America found that fire ants were attacked by multiple species of phorids and occurred there in much lower densities. While direct mortality from phorid infections is low (generally less than 1%, sometimes up to 4%), it is the potential of indirect effects on foraging ant behaviour that forms the basis for using phorids as biocontrol agents. Other possible biocontrol agents that have been reviewed by USDA include microsporidian and viral pathogens, mermithid nematodes and eucharitid wasps. Based on studies of fire ant populations in their native range in South America, it may be necessary to introduce a suite of pathogens and parasites for effective biocontrol in North America.

Release History

Several years of effort were required to bring phorid flies into culture and assess their host specificity before the first releases of *Pseudacteon tricuspis* occurred in Florida in 1997 (USDA – Porter) and in Texas in 1995 (University of Texas [UT] Austin – Larry Gilbert). Releases at the UT Austin Brackenridge Field Laboratory were repeated until establishment was confirmed in 1999. The difficulties encountered during these early releases were possibly on account of adverse harsh weather or a biotype mismatch, and similar challenges have been faced at other sites in dry areas of southwestern Texas.

USDA (Porter) and UT Austin (Gilbert) have continued assessments of several species and biotypes, resulting in the introduction of *P. curvatus* in 2004 and *P. obtusus* in late 2006. Additional candidate species are being evaluated from a pool of about 20 *Pseudacteon* species that vary in host-location cues, size and temporal activity. The *Pseudacteon* species group appears to contain several cryptic species and biotypes that provides a rich area for studies, but has also contributed to difficulties in rearing several lineages.

Release Strategies in Texas

In comparison to relatively rapid establishment at release sites in Florida and other coastal states, many of the early Texas releases failed. This forced a review of methods and strategies, building on experience as we proceeded. Many of the failed releases can be attributed to the harsh weather conditions and ongoing drought conditions of the past decade. In arid areas, the fire ant populations are fragmented and often restricted to moist areas, both natural and man-made. Another key observation has been the effect of wind-assisted dispersal from the successful release sites, where subsequent spread has been supported by prevailing onshore Gulf winds. Our recent strategy in southern Texas has therefore been to focus on introductions along major drainage systems close to the Gulf Coast, with the potential for spread inland during favourable conditions while providing moister refuges during arid times.

Another change in release methods has been the use of the 'Trojan colony' method, to supplement the direct release of adult flies. This method entails collecting a portion of a colony and bringing it to Austin where workers are infected by exposure in mass-attack chambers in our laboratory facility, or outdoors where strong fly populations occur. A sequence of multiple attacks allows infection across a ten-day time horizon, laying the basis for overlapping fly generations. The infected workers are then returned and accepted by the parent colony before pupation has occurred. This enables infected workers to follow normal developmental steps with lower risk of weather related mortality.

Monitoring the Spread of Phorid Flies

Early techniques for monitoring flies were based on numbers of flies observed at a disturbed ant mound within a given time. We quickly found that this method was not sensitive for detecting low density populations and we developed a trap using commercial sticky fly-paper. Flies are attracted by alarm pheromones, foraging pheromones and midden odour, and traps can be modified to incorporate particular attractants. Traps can be left in place for 24 hours to detect very low fly densities; on the other hand they can be overwhelmed by hundreds of flies when densities are high.

We use these traps on transects originating from the release sites, and have mapped the spread of flies from all established sites. After a slow establishment phase, *P. tricuspis* spread dramatically between 2003 and 2006 to cover over 2.8 million hectares of central and coastal Texas. More recently, *P. curvatus* has also shown accelerated spread and it now extends across about 0.6 million hectares. In both cases, the rates of spread approach 40 km/year, well beyond the expected flight capacity of these small flies and indicative of wind-assisted long distance dispersal. Of interest, we have observed a dramatic decline in *P. tricuspis* densities in areas now occupied by *P. curvatus* and studies are underway to explore possible mechanisms. The introduction and spread of phorid flies have allowed careful observation of a 'controlled invasion' with wider lessons for how invasions proceed.

False Negatives and Harsh Weather

At several release sites in south Texas, tens of thousands of flies were released at various times between 1999 and 2004. Almost without exception, these populations appeared to have failed and so we were surprised to find them still viable 3–5 years later. For example, in Brownsville, over 12,000 *P. tricuspis* flies were released and established in 2002 and 2003, but appeared to have been extirpated by a severe drought. However, in 2007 they were detected throughout the release area and about 30 km along the Rio Grande valley. This was particularly important since fire ants had recently been detected across the border in Mexico and the flies may impede their further spread.

Another site near Laredo exhibited a similar pattern during the same period, reinforcing the prospect that if flies establish in local refuges, despite becoming extinct or undetectable at the release site, they can subsequently become source populations in a fragmented metapopulation structure.

Impacts on Fire Ants

While further phorid species are being introduced, a burning question remains as to whether the current two or three species are having an impact on fire ants, and there are several aspects to consider. First, we will re-evaluate the direct effects of mortality on fire ant populations when multiple phorid species are present. Next, we have begun baseline studies to examine how indirect effects on fire ant foraging affect their populations, but we need to allow sufficient time for these effects to work. Our working hypothesis holds that one phorid species alone may not suppress fire ants, but that some combinations of pathogens, parasitoids, competition from native ants and severe weather may cumulatively level the playing field and help bring fire ants under control.

Further Information

UT Fire Ant Laboratory: http://uts.cc.utexas.edu/~gilbert/research/fireants/

USDA Fire Ant Program: http://fireant.ifas.ufl.edu/

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DNA-based Monitoring: Fact or Fiction?

DNA-based approaches to species identification are currently the most widely adopted non-traditional methods, and hold most promise for broad application in the future. But while field biologists may dream of a hand-held tool to give them instant identification of a morphologically unidentifiable specimen, a paper by John Darling and Michael Blum in the journal *Biological Invasions*[1] puts the potential for this in perspective in a review of the prospects for DNA-based methods for monitoring invasive species (from the perspective of the biocontrol scientist, this could be applied to natural enemies).

In terms of traditional taxonomy, Lucid software has done a great deal to make morphological identification more accessible by providing the ability to develop dichotomous and multi-access (matrix-based) electronic keys. But there may not be a Lucid key for the group you are interested in; indeed, there may be no accessible taxonomists specializing in it, or knowledge of the group may be limited. Morphology may not, in any case, be sufficient: e.g. for identifying immature arthropod stages, plant samples without flowers, and the not uncommon situation where related innocuous and invasive taxa are morphologically indistinguishable. This is where DNA-based methods come into their own: an individual's DNA is the same in every cell, immature or mature, root or flower; it can be distinguished from that of another species or even strain. Bridging the gap between the theoretical possibility and devising practical tools for identifying species in field-collected samples is a challenge for molecular biologists – and even more of a challenge for non-molecular scientists to understand.

This is the complex issue molecular biologists Darling and Blum set out to explain, and do so in a concise article reviewing current and 'in development' methods in DNA diagnostics. They discuss what tool(s) would be most appropriate for a variety of purposes taking into account their cost-effectiveness. They also discuss prospects for tools still in the early stages of development and indicate needed research. The article is enhanced by a tabulated summary of the tools they describe, and charts illustrating how to decide the best tool(s) for a particular circumstance.

They begin by summarizing eight potentially useful DNA-based technologies, giving a simple description of the procedure and explaining how the information this generates might be useful for monitoring purposes.

They then identify three variable characteristics in monitoring tasks that affect which type of DNA diagnostic tool is most useful:

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

They depict these three variables as axes of a three-dimensional array with monitoring tasks of least difficulty closest to the origin, and those most difficult farthest from it along each axis (simple to complex sample, presence/absence to quantitative abundance information, single to multiple species).

They also present variation in the questions that may need answering using DNA diagnostics as a decision-making flowchart. Thus, depending on your need – whether you need to confirm the identity of a specimen, or have it identified; to screen for presence of various target species, or assess their propagule pressure; to have information on species composition, or on abundance as well – the various tools are designated as 'best', 'questionable' or 'inappropriate'.

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

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

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

IPM Systems

This section covers integrated pest management (IPM) including biological control, and techniques that are compatible with the use of biological control or minimize negative impact on natural enemies. As in many recent issues, we are featuring biopesticide stories, beginning with a novel use of endophytes.

Endophyte Spices Up Biofumigation

Muscodor albus, a new genus and species of endophytic fungus first isolated and described from the non-native cinnamon tree (*Cinnamomum zeylanicum*) in Honduras by Gary Strobel and his team at Montana State University (USA), could provide a fresh approach and tool for biological control with applications in areas from agriculture to human health. Its discovery in 1997 – and the subsequent discovery of related taxa – also provides a salutary reminder of the untapped resources lying undiscovered in the world's fast-disappearing tropical rain forests.

Endophytic fungi are commonly found in virtually all plant species. However the diversity of species recovered from rainforest plants is especially great. Their role in plants remains rather mysterious although it seems likely that they confer some benefit on the plant in return for it acting as a host; protection from pests and diseases is one suggested benefit.

The Gaseous Fungus

The novel fungus was spotted when endophytic fungi plated from cinnamon tree limb samples were all placed in a plastic box to avoid contamination by phytophagous mites. After 12 days of incubation in the box all endophytes had died off except one. Strobel recognized that in 'isolate 620' he had something new that had killed its plate-mates through production of volatile organic compounds (VOCs). Although many fungi are known to emit low concentrations of gases, this was the first time their widely lethal properties had been recorded for an endophyte. Analysis showed that the fungus, subsequently described as *M. albus,* produces a cocktail of some 28 VOCs (mainly alcohols, acids and esters) which kills or inhibits a broad range of plant and animal pathogenic fungi and bacteria. Its practical potential was demonstrated in greenhouse experiments where it gave complete control of the smut *Ustilago hordei* on barley seed. More recently, nematocidal and insecticidal activity has been demonstrated.

Various VOC-producing fungi in other genera have been isolated and studied over past decades (e.g. *Trichoderma* spp.), but none has shown the comprehensive spectrum of antimicrobial activity of *M. albus*. However, since *M. albus* was isolated, more strains have been recovered from nutmeg (*Myristica fragrans*) and some rain forest vines, and a number of other VOC-producing *Muscodor* species have been identified in tropical tree and vine species in the Neotropics, Southeast Asia and Australia. The genus *Muscodor* has now been found in plants from at least eight tropical plant families (discovery has been facilitated by using *M. albus* as a selection tool in screening endophytes from samples following the discovery that VOC-producing fungi are immune to those from related species).

It is not yet clear just how widespread VOC-producing endophytes are, but the species and strains found so far seem to produce unique spectra of VOCs and different spectra of pathogenic activity – and consequently varied potential applications. For example, *M. vitigenus* from the Peruvian Amazon produces solely naphthalene and has insect-repellent properties. The variation may prove crucial: although the first *M. albus* isolate, 620, killed most pathogens tested, there were notable exceptions including wilt- and root rot-causing *Fusarium solani* and the sugarbeet pathogen *Cercospora beticola*; isolates yet to be found may be effective against these. An Ecuadorean isolate, *M. albus* E-6, from the agroforestry species *Guazuma ulmifolia* produced the first VOC mixture to kill a gram-positive bacterium (*Bacillus subtilis*), although *Rhizoctonia solani*, which is very susceptible to isolate 620, survived.

Comparison of the activity of *M. albus*' VOC cocktail with artificial compounds and mixtures demonstrated that its activity is related to the synergistic activity of the compounds. Although the most active substance was 1-butanol, 3-methyl-, acetate, – no single artificial compound was lethal to any of the pathogens killed by *M. albus*. However, it has also been shown that not all the VOCs are necessary for biological activity: just three – naphthalene, propanoic acid and butanol-3-methyl – approach the lethal action of *M. albus* although they are not as effective as the complete cocktail of the fungal VOCs.

Strobel and his team are continuing their concerted search for more undiscovered endophytic microbes that produce novel bioactive products and/or processes that might prove useful. However, there is also a pressing need to understand the mode of action of the multitude of VOCs discovered already and how they interact synergistically to kill microbes and this work is underway. In addition, there is a plan to do the complete sequencing of the *M. albus* genome.

Some understanding of host–endophyte relations and factors underlying host preferences is needed to pave the way for the use and development of these endophytes for non-natural host plants. Some progress has been made with isolate *Muscodor albus* E-6. This was found to possess a tough mycelial mat, not so-far observed in any other endophyte, which can be lifted off an agar plate with a scalpel. This and ease of propagating its agroforestry host species provided a system that allowed a *Muscodor* endophyte to be re-established in its host for the first time. Thus, it may eventually be possible to inoculate seedling tree species and get some benefits to the plant throughout the course of its life via endophytic *M. albus*.

Expanding Use of Volatiles

Application of endophytes – for research or the few existing practical/commercial purposes – involves either incorporating them in the plant tissues or applying them externally, e.g. onto leaves or by root dipping. They rely on the presence of the endophyte in (or on) the plant for its antagonistic or (hyper) parasitic properties to protect the host. In contrast, the mode of action of *M. albus* does not require contact with the plant – and, importantly, neither does it restrict its use to plant protection. However, practical applications demand a method for mass-production, and preferably on a host more amenable to a commercial production system than the cinnamon tree. The fungus grows as a white mycelium, but is sterile and does not produce asexual or sexual spores, or spore-producing structures. The challenge is to find ways of preparing and 'packaging' the mycelium so it can produce VOCs when and where needed. The remainder of this article reviews current applications and on-going research into new ones.

Gas Plants to Kill Pathogens

As a biofumigant, *M. albus* shows promise for treatment of soil, seeds and other propagules, and for postharvest treatment of food commodities and animal feeds, as well as in ornamentals and cut flowers. It has demonstrated extremely effective activity against many crop, postharvest and soilborne diseases, notably species of *Botrytis, Colletotrichum, Fusarium, Geotrichum, Monilinia, Penicillium, Phytophthora, Pythium, Ralstonia, Rhizoctonia, Rhizopus* and *Sclerotinia*

Its emergence as a contender in methyl bromide replacement is timely. Methyl bromide fumigation was used for some 50 years to control a wide variety of pests and diseases, but because it depletes the ozone layer it has been phased out under the Montreal Protocol, apart from some exemptions for which replacements are being sought. (And as methyl bromide is toxic there were risks to applicators, as well as food residue and soil and groundwater contamination issues to contend with.) In contrast, *M. albus'* biological activity resides in sub-micromolar concentrations of its volatiles and it is unlikely that significant residues would be found in treated produce.

Researchers have been achieving promising results using *M. albus* culture and, more commonly, in grain-based preparations; biofumigation is achieved by placing reactivated grains in an open container or inside permeable packaging among the fruit to be treated (thus avoiding direct contact and potential contamination). While the control achieved has often been phenomenal, the progress in developing delivery systems for the fungus with a view to commercial operations is also of great interest.

A commercial formulation developed by AgraQuest (Davis, California, USA) has received US Environmental Protection Agency and the California Department of Pesticide Regulation approval and has been registered as a natural biofumigant and an alternative to methyl bromide for agricultural applications. Research now is focused on determining optimum production methods and formulation for best efficacy and cost–benefit.

Preventing a Rotten Apple from Spoiling the Barrel

Harvested fruit are particularly prone to fungal diseases, especially damaged fruit however minor the wound. Some diseases can spread from a single infected fruit to affect an entire shipping carton. Postharvest losses can consequently be high. Use of synthetic fungicides has led to the development of resistance, and some products have been withdrawn from the market, leaving growers few options. Of a number of biological alternatives investigated by various workers, *M. albus* biofumigation is an attractive option because the fruit does not have to be handled and risk damage, and the control agent does not have to be in direct contact with the fruit or colonize wounds (which antagonistic yeast and bacteria do), so contamination issues are unlikely. Research at AgraQuest by Julien Mercier and co-workers, as well as collaborators Joe Smilanick (US Department of Agriculture – Agricultural Research Service [USDA-ARS], Parlier, California) and Guido Schnabel (Clemson University, South Carolina) has shown the exciting potential of this approach in a range of fruit.

Pome and Stone Fruit

Pome and stone fruit are both easily damaged during and after harvest, which makes them very vulnerable to a number of fungi, which grow and spread rapidly to the other fruit in a crate or case.

Initial research showed that biofumigation with *M. albus* (on colonized grain) gave excellent control of *Penicillium expansum* (blue mould) and *Botrytis cinerea* (grey mould) on apple cv. Gala, and complete control of *Monilinia fruticola* (brown rot) on peach cvs. Ross and Zee Red when the fruit were biofumigated for 24–72 hours in plastic boxes with tight (but not air-tight) lids beginning up to one day after the fruit had been wound-inoculated with the pathogens.

No lesions developed on infected fruit exposed to *Muscodor albus*, suggesting the pathogens had been killed rather than inhibited by the VOCs; in vitro experiments supported this conclusion – and similar results were obtained later in lemons. This contrasts with biofungicides based on antagonistic yeast or bacteria, which have generally not shown curative activity against postharvest fruit diseases.

Subsequent work assessed a possible delivery vehicle for *M. albus* in peaches. Pads made from heat-sealable tea-bag paper and filled with desiccated *M. albus*, which were then dipped in water to reactivate the fungus, proved a promising method for protecting bagged cartons of peaches cvs. Coronet and Red Globe from brown rot (the method was less effective with unbagged cartons and it was presumed that VOCs escaped).

Citrus

Harvested citrus are particularly susceptible to infection by *Penicillium digitatum* (green mould), and conventional fungicide treatments have led to emergence of multiple fungicide resistance. In addition, many countries have low maximum residue limits for imazalil, the key fungicide.

Complete control was recorded in immature lemon cv. Eureka exposed in closed plastic boxes immediately after wound-inoculation to 24–72 hours biofumigation with *M. albus* (in grain culture). The discovery that more-mature lemons, while still well protected, succumbed to the disease more after similar exposure to *M. albus* suggested that penetration of the pathogen into the fruit skin and consequent protection from VOCs could limit disease control in ripe fruit. Biofumigation gave some control of another citrus disease, sour rot (*Geotrichum citri-aurantii*) in the lemons. Although this was not as successful as green mould control even when applied immediately post-inoculation, the results were far from disappointing as there is currently no effective control for this disease.

The practice of picking citrus fruit 'green' and then 'degreening' with ethylene (to develop the orange or yellow colour that appeals to consumers) can provide a challenge for postharvest fruit protection. Degreening, conducted at around 20ºC – perfect conditions for disease development – and before conventional fungicide treatments are applied at the packing stage, can lead to significant disease development, especially for fruit harvested during wet weather. Combining room biofumigation with ethylene and *M. albus* for lemons in open plastic storage boxes or vented fibreboard cartons had no adverse effect on colour development in the fruit and gave good control of green mould by providing early protection from the pathogen. An emerging advantage of *M. albus* for postharvest citrus protection is that it has the flexibility to be integrated into the various phases of degreening, cold storage, packing, etc.

Grapes

Grey mould is a particular problem on table grapes because it grows rapidly and spreads from fruit to fruit even at low temperatures. Conventional treatment of sulphur dioxide fumigation is associated with a number of problems including tainting, bleaching, residues and sulphur dioxide emissions. On the other hand, measures involving handling or manipulating bunches risk detaching grapes.

Continuous biofumigation of table grapes cv. Thompson Seedless with *M. albus* (in grain formulation) significantly controlled grey mould in the various grape packing systems tested (clamshell boxes and ventilated polyethylene cluster bags). However, variations occurred which have practical implications.

- The lower dose used controlled the disease better at 20ºC than at 5ºC (the higher dose controlled it completely at both temperatures), indicating that higher doses will be needed to generate sufficient VOCs at prevailing (low) storage temperatures.
- The treatment was most effective on single grapes (compared with ~100-g clusters) which suggests that the degree to which the VOCs circulate through the fruit is a significant factor in the treatment's effectiveness. A similar conclusion was reached during the lemon work, indicating the importance of storage and packing arrangements.
- Biofumigation was most effective immediately after wound-inoculation; a delay in treatment presumably allows the pathogen to penetrate the fruit where the volatiles do not penetrate.

As with peaches, the use of reactivated *M. albus* pads provided significant decay control in commercial cartons.

Greenhouse Culture: See How They Grow

Damping-off and root rot are serious constraints in both soil-based and soilless greenhouse production. Although sanitation and the use of clean growing medium and planting material are widely practised, contamination still occurs. Methyl bromide is being phased out. Fungicide drenches can have adverse side-effects. Use of biological antagonists has had some success but most species control a limited spectrum of soil/root pathogens.

Mercier and his team found that in soilless culture, *M. albus* (in grain culture) achieved complete control of damping off (*Rhizoctonia solani*) in broccoli, and of *Phytophthora* root rot in bell pepper cv. California Wonder at 4–22ºC and at disease inoculum levels that would normally cause almost complete mortality; seedling emergence levels were similar to those obtained in disease-free conditions. However, results indicated that the VOCs did not diffuse through the medium: to be effective *M. albus* needs to be thoroughly mixed with the growing medium.

Further work using *R. solani* indicated that the ability of *M. albus* to control damping off declined over time once incorporated with the growing medium. Comparison of sealed and non-sealed pots provided no evidence that the VOCs were escaping from the potting mix and it seems they may become bound and inactivated by the medium.

Unexpectedly, the weight of bell pepper seedlings was almost doubled by *M. albus* treatment compared with disease-free controls; this was also observed in an ornamental flower, cosmos cv. Sensation. Investigations indicated that this was probably the result of killing deleterious soil organisms while others beneficial to plant growth (*Bacillus subtilis, Trichoderma* spp.) survived and presumably thrived in the absence of competition.

Closing the Window on Potato Tuber Moth

The most recent development is the demonstration of *M. albus*' insecticidal activity. PTM (*Phthorimaea operculella)*, a pest of potatoes in most potato-growing countries, recently established in the Pacific Northwest USA and is now a major pest. Yield loss at harvest is less important than the effects of infestation of tubers before storage. Current control methods (broad-spectrum insecticides and cultural measures) leave a window of opportunity for the moth: just before and during harvest when chemicals cannot be applied. If egg and larval infestations are not detected, further tubers may be damaged before the storage facility can be cooled (to 3–10ºC), which may lead to entire loads of potatoes being rejected.

Lerry Lacey and his team (USDA-ARS, Yakima, Washington State) showed that exposing adult PTM to VOCs from *M. albus* grain culture gave mortalities of up to some 90%, and exposing neonate larvae reduced numbers reaching the pupal stage by up to nearly 73%. Subsequent work investigated the practicalities of using *M. albus* to protect stored potatoes by testing it on 3-day-old PTM larvae in tubers. Mortality of larvae within the tubers (% failing to reach the adult stage) biofumigated at 24ºC increased with exposure to *M. albus* up to 14 days – from 84% at 3 days to over 99% at 14 days – but longer incubation produced levels of CO_2 unacceptable for tuber storage(which may, though, have contributed to larval mortality). Low temperatures (10ºC and 15ºC) significantly reduced mortality; this mirrors the results from grapes, above, and may indicate that higher doses of *M. albus* are needed.

Although storage temperatures for potatoes depend on their ultimate use (seed, fresh market, processing) and range from 3–10ºC, they are all initially kept at 10–16ºC for 3–5 weeks to allow wound healing, and thereafter the temperature is slowly lowered. Biofumigation with *M. albus* during the first few days of wound healing may be sufficient to control hatching and young PTM larvae (and indeed, any pathogens and nematodes present); the frass produced by older larvae would allow infested tubers to be identified and removed before storage. Good ventilation may solve the problem of CO_2 build up, but if not, *M. albus* could be incubated in a separate chamber, and the VOCs from it circulated through the potatoes via the ventilation system.

Muscodor albus may offer further promise for the potato industry in the Pacific Northwest, and specifically against plant-parasitic root-knot nematodes, lesion and stubby root nematodes that attack potatoes in Washington State. Ekaterini Riga and coworkers reported at this year's APS (American Phytopathological Society) annual meeting (San Diego, California) that they had recorded mortality in the range 70–86% for three species that had been biofumigated for 72 hours with *M. albus*; only 7% control of a fourth species was achieved but over 80% of the surviving juveniles showed signs of nematostasis. Subsequently, 80–100% control of a mixture of all four species was achieved in pot experiments.

The Human Dimension

Muscodor albus is a very unusual biocontrol agent insofar as it does not need to have physical contact with the products being treated. This makes it particularly attractive in some agricultural situations; for example, for treatment of harvested commodities that are too fragile to sustain a postharvest fungicide treatment, such as berries. However, this 'remote action' and the broad antimicrobial activity spectrum of the VOCs it produces allow for a large number of potential uses, not just in agriculture but in areas remote from plant protection such as human health. We end this article with a look at two such applications.

WAG Bags Clean Up

The first product to be based on *M. albus* was developed by Phillips Environmental Products Co (Montana, USA), and is designed to clean up human wastes and bring about decontamination of *E. coli* and other microbial pathogens. Containment and decontamination of human waste is vital for reducing infection and disease. The company formulated *M. albus* as 'EarthPure™', a grain-based desiccated culture, which is re-activated by water. It is packaged in WAG BAG® (waste alleviating and gelling) waste kits, as an additive to existing Pooh Powder™ waste treatment powder for their commercial portable toilet systems (used alone EarthPure is most effective over a period of time).

WAG BAGS are issued free of charge at a number of US national parks that have dispensed with traditional pit toilets (which are covered when full) and encourage campers to remove waste. Others add EarthPure to pit toilets; preliminary test results indicating a significant reduction in volume and odours from waste are borne out in these parks. This also signals a much larger and important potential market for *M. albus* in the developing world where poor sanitation lies behind much ill health and premature death and is a major factor in high child mortality. Moreover, *M. albus* could play a vital role in protecting victims of natural disasters from the ensuing disease epidemics, which often kill more people than the disasters themselves.

Building on the Foundations

Fungi in several genera such as *Aspergillus, Penicillium* and *Stachybotrys* can colonize cellulose-containing building materials that have been exposed to water or held under damp conditions. Such building mould outbreaks can cause malodours and have been associated with human health problems such as allergies. Julien Mercier and Jorge Jiménez have shown recently that biofumigation with *M. albus* can provide preventative and curative control of building moulds on walls subjected to water damage.

Further Information

Agraquest: www.agraquest.com/

Phillips Environmental Products: www.thepett.com/

EPA *Muscodor albus* QST 20799 (006503) Fact Sheet.
www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_006503.htm

UNIDO methyl bromide page: www.unido.org/doc/29643

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Use of Biological Control against a Forest Pathogen in the UK

By far the most significant pathogen facing forestry in the Northern Hemisphere is the basidiomycete fungus *Heterobasidion annosum* (formerly *Fomes annosus*). It affects many conifer species of commercial significance causing huge losses through tree mortality and decay. The pathogen enters forestry crops when air-borne spores infect wounded stems or the surface of freshly-cut tree stumps created during crop thinning and clear-felling operations. These spores are released throughout the year from fruit bodies growing on old stumps and decaying timber, and are capable of travelling long distances, in some cases hundreds of kilometres[1]. Spores landing on the surface of stumps germinate, and the fungus grows down into the stumps and associated root system. This phase in the life cycle of the fungus is of no economic importance *per se*. However, where adjacent trees' roots are in contact, *Heterobasidion* can spread from an infected stump root into the root system of a nearby healthy tree. Healthy roots can then be colonized by the pathogen, resulting in decay which extends progressively from the root system into the base of the standing tree. Over time *Heterobasidion* infection extends into other surrounding trees and, in affected stands, it is sometimes possible to see distinct 'disease-centres' where dead trees infected early on in a rotation are surrounded by radiating rings of dying and declining trees.

Economic Significance

The economic significance of *Heterobasidion* is considerable: losses due to tree mortality, reduction in growth, wind-throw and downgrading of timber due to staining and decay are estimated to approach €800 million per annum across Europe[2]. It is particularly problematic as it is extremely long-lived and can persist from one crop rotation to the next, with disease incidence increasing on some sites to such an extent that commercial forestry is no longer practical or economic.

Heterobasidion can be found at low levels within old-growth conifer forests, but the disease is especially significant in commercial forestry stands. This is because the normal practice of clear-felling followed by re-planting creates an abundance of fresh stumps which present ideal entry points for the pathogen. Once established within the stumps and living trees there are no methods of replacing or killing *Heterobasidion in situ*, so badly diseased crops may be felled early to salvage marketable timber before the decay extends too far. The only reliable method for eradicating the pathogen once established on a site is to dig up all infected stumps before replanting. This is an expensive procedure and, depending on terrain, often impractical. The remaining option is to replant with resistant species, and these are mainly broadleaved species.

Control through Stump Treatment

In the absence of remedial treatments, by far the best option for managing the disease is to prevent infection in the first instance. As *Heterobasidion* mainly gains access through infecting stumps, it can effectively be excluded from healthy stands by treating the surfaces of stumps as long as this is done immediately after felling. The technique of 'stump treatment' has proved extremely successful at controlling *Heterobasidion* in the UK. In contrast with much of northern Europe, the UK has a relatively short history of commercial conifer forestry. This, combined with an extensive programme of prophylactic stump treatment from the 1960s onwards, has resulted in historically low levels of the pathogen compared with elsewhere in Europe. Two products are licensed for stump treatment use in the UK. One is the commodity chemical urea, used on both pine (*Pinus* spp.) and spruce (*Picea* spp.). The other is a biological control agent called PG Suspension® (manufactured by Forest Research, Surrey) which is available for use on pine[3]. These are the only anti-fungal treatments used in UK forestry.

Biological control of *Heterobasidion* root and butt rot is very much encouraged: a code of practice informed by European policy and the certification standard known as the UK Woodland Assurance Standard (UKWAS) encourages forest managers to minimize the use of pesticides, and to use non-chemical alternatives where available. A common, native saprotrophic fungus called *Phlebiopsis gigantea* (previously *Peniophora gigantea*) is known to cause rot within fallen branches and stumps, but it does not attack living trees. Extensive research conducted in East Anglian forests in the 1950s and 1960s demonstrated that it was highly effective at excluding *Heterobasidion* if it was applied to stumps immediately after cutting[4]. *Phlebiopsis gigantea* has the virtue of readily producing spores in culture through hyphal segmentation, lending itself easily to commercial formulation, and it has now been used as a treatment to combat *Heterobasidion* in UK pine forestry for more than 40 years.

PG Suspension, the product currently available in the UK, is a suspension concentrate of *P. gigantea* spores. When stored and applied according to manufacturer's instructions it provides 100% protection against the pathogen on pine. It is formulated in 8- to 10-ml sachets of liquid and, provided these are kept refrigerated to maintain viability, the product has a six-month shelf-life. Each sachet contains enough spores to make 25 litres of working solution. Two other *P. gigantea*-based biological control agents are used elsewhere in Europe. Rotstop®, a wettable powder manufactured by Verdera Oy on a relatively large scale in Finland, is widely available across Scandinavia and the Baltic states. The other, known as PG IBL®, is a sawdust-based product used in Poland and manufactured by several companies, e.g. Biofood s.c., Walcz.

Application of Stump Treatment

Both UK stump treatments, urea and PG Suspension, can be applied to stumps by hand using a simple applicator such as a polythene bottle with holes drilled in the stopper, or a brush fitted into the lid. However, the demands of commercial forestry require quick, automated application as part of the harvesting process. Therefore, PG Suspension, like urea, is more commonly applied directly during felling using mechanized forestry harvesters. Although extremes of temperature and pressure can damage spore viability, *P. gigantea* is a very robust organism, and the conditions experienced during passage through a typical harvester cause no deterioration in viability.

Most new harvesting machines are supplied with a stump treatment option consisting of an additional reservoir (tank), spraying pump and associated valves and hydraulic lines. (These can be retro-fitted if necessary.) Such systems are usually set up to treat stumps automatically on felling, but can be switched off when processing the timber. Older machines may utilize one or two spray nozzles mounted beside the bar. However, most new machines apply the product directly through a specially designed cutting bar. In this case the product is pumped onto the stump surface through holes drilled along the length of the partially hollow bar. Many operators actually choose bars designed with part-drilled holes as this allows the position of the open delivery holes to be customized by the user according to the stump size on site (e.g. small thinning stumps versus larger clear-fell stumps). This reduces wastage without compromising levels of coverage.

Full coverage of the stump with any stump treatment agent is essential, as any gaps in coverage can allow the pathogen into the stump. The recommended application rate for urea and PG Suspension is one litre of product per square metre of stump surface, although in practice application rates vary depending on the set up of the harvester and experience of the operator, and application rates of around 2.5 litres/m^2 are more typical. In line with UKWAS guidelines, however, every effort is made to minimize wastage and to target application to the stump.

Chemical versus Biological

Both stump treatment products have advantages and disadvantages. Not surprisingly urea is readily available as its other main use is as a fertilizer in both forestry and agriculture. It also has relatively simple storage requirements. However, it is corrosive to most metals and also has a tendency to crystallize at both high and low temperatures, causing blockages in the harvester delivery systems. Costs of maintaining a harvester head used to apply urea are consequently estimated to be three times higher than when using the alternative biological agent (or indeed no treatment). PG Suspension has more exacting cold storage requirements and there are additional labour costs associated with having to prepare fresh working solutions daily. However, the main drawback associated with PG Suspension is that it is currently restricted for use on pine species. This tends to limit the use to growers in eastern England where the commercial conifers are predominantly pine species. In areas where a mixture of conifer species are grown, urea tends to be used as it can be applied to any conifer. However, switching between the two products is possible so long as the harvester tank is thoroughly drained and cleaned, and any residual urea solution is flushed through the hoses before switching to PG Suspension.

Future Prospects

As users become more aware of European and UK incentives to reduce chemical usage within our forests we expect an increase in uptake of PG Suspension. However, the fact that the product is only approved for use on pine remains a major limitation. Consequently, the Forestry Commission Research Agency is conducting studies to establish whether PG Suspension could be effective on other species, particularly our main commercial conifer, the North American Sitka spruce (*Picea sitchensis*). These experiments also include the Finnish product

Rotstop, as this is licensed for use on Norway spruce (*Picea abies*) as well as pine in Scandinavia, and it is possible that Rotstop may prove to be effective on our exotic spruce species.

The future of all licensed stump treatment products is currently a little unclear in light of EU Directive 91/414. Commodity chemicals used as plant protection products (PPPs), and all biological PPPs are under evaluation by a European review process. This dictates that no active substance can be used unless it is included in a European Union (EU) list known as Annex 1. An EU programme of evaluation to create this list has been underway since the early 1990s, first examining chemical actives and now, in Stage 4, biologicals and commodity chemicals. The main interested parties across Europe have submitted collaborative dossiers on urea and *P. gigantea* and are currently awaiting the decision on whether these two active ingredients will be included on this list. The review process is likely to take a number of years, and in the meantime it is important to continue to monitor forestry crops and prevent infection through rigorous stump treatment, and particularly using biological treatment whenever possible!

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Death Leads to Life

Helicoverpa armigera, the insect pest known for attacking nearly 200 crops worldwide including cotton, pulses, vegetables and fruits, causes annual global losses amounting to US$2 billion despite US$5 million spent on insecticides. Due to its multiple generations, polyphagy, wide distribution and high insecticidal pressure over several years, this species has developed resistance to a wide range of insecticides making the problem a tough nut to crack – until we developed feasible production technology for nuclear polyhedrosis virus (NPV).

The International Crops Research Institute for Semi-Arid Tropics (ICRISAT), in partnership with the Centre for World Solidarity (CWS – a Hyderabad-based NGO working on integrated pest management (IPM), State Agricultural Universities and the Department of Agriculture, established the production and use of *Helicoverpa* nuclear polyhedrosis virus (HaNPV) at the village level through a World Bank funded project. The project proposal won the World Bank Development Marketplace award for 2005. Farmers in villages of India and Nepal have traditionally shaken crop plants, particularly pigeonpea, to dislodge and destroy *H. armigera* larvae. ICRISAT has added a valuable twist by using these dislodged larvae for the multiplication of HaNPV, a biopesticide that kills *H. armigera.*

The technology for NPV production involves collection of larvae and rearing them on an NPV infected diet (pre- soaked chickpea seeds) until they die. The carcasses are ground up and the extract from the resulting liquid is sprayed on crops as a biopesticide that infects and kills other *Helicoverpa* that infest the crop. Under this project, ICRISAT, in collaboration with the National Agricultural Research Systems (NARS) and NGOs in India and the Nepal Agricultural Research Council (NARC) and an NGO in Nepal, Forum for Rural Welfare and Agricultural Reforms for development (FORWARD), has been able to establish biopesticide production units in India (76 villages) and Nepal (20 villages).

Through developing this biopesticide production technology in India and Nepal, ICRISAT and partners have provided the villagers with the know-how for the production of an effective biopesticide. The villagers were more than happy to learn about an effective alternative to expensive chemical pesticides. The fact that they could be self-sufficient in this venture made the proposition more attractive. These village units commissioned production and produced from 500 to 20,000 larval equivalents (LE = 6×10^9) of the virus and utilized it on a number of crops including cotton, vegetables, chickpea and pigeonpea with satisfactory results.

During this project period (2005–07), 201 researchers from NARS and 983 farmers in India and Nepal were trained on NPV production and IPM at the village level, with special emphasis on the importance of residue-free food and the environment. This project gave high priority to technology dissemination of an eco-friendly approach with the active participation of all partners at various locations. The high impact of the biopesticide strengthened the other eco-friendly activities ongoing in these 96 villages in India and Nepal in the past two years. The farmers reduced application of chemical pesticides by 65% in cotton, 24% in pigeonpea and 21% in chickpea, thereby reducing financial costs, and protecting the environment and crop-friendly insects.

Interestingly, what would otherwise have been pest *H. armigera* larvae are now providing a priceless environmental service through their use in producing biopesticide that can be used to protect crops from *H. armigera* itself. Nonetheless, although HaNPV is effective and a safe alternative to chemicals, it has still not been able to totally replace chemical insecticides. This is especially true for large-scale production that requires large numbers of the larvae, which are not always available. A solution could be achieved through organizing biopesticide networks across locations to meet large-scale requirements. The networks could also further strengthen livelihood and self-sustainability aspects amongst farming communities as farmers would be able to sell excess biopesticide production to neighbouring farmers.

The project not only provided farmers with an alternative to chemical pesticides to deal with endemic problems but also promoted awareness and infrastructure, and helped the farmers to develop contacts. It has greatly assisted in strengthening the health and environmental related aspects of agriculture towards achieving the Millennium Development Goals.

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Announcements

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

Biorational Mosquito Control

The publication of a new book, *Biorational control of mosquitoes*[1] by the American Mosquito Control Association is timely, for mosquitoes are an increasing threat to human and animal public health worldwide as we see upsurges of mosquito-borne diseases in traditional areas, their spread to new countries and even continents, and the emergence of new or previously unrecognised diseases.

The chapters of this book cover population dynamics, semiochemicals, molecular genetics, transgenic technologies, methoprene, modelling, recombinant technology, and federal, state and international regulations governing the use of biorational control agents against mosquitoes. It will be of interest to all mosquito workers, within and well beyond the borders of the USA:

- Introduction (James J. Becnel & Thomas G. Floore)
- Microsporidian parasites of mosquitoes (Theodore G. Andreadis)
- Ascogregarine parasites as possible biocontrol agents of mosquitoes (Michelle Tseng)
- Mosquito pathogenic viruses – the last 20 years (James J. Becnel & Susan E. White)
- Oomycetes: *Lagenidium giganteum* (James L. Kerwin)
- Mermithid nematodes (Edward G. Platzer)
- Cyclopoid copepods (Gerald G. Marten & Janet W. Reid)
- Insects and other invertebrate predators (Motoyoshi Mogi)
- Aquatic insects as predators of mosquito larvae (Humberto Quiroz-Martínez & Ariadna Rodríguez-Castro)
- *Toxorhynchites* as biocontrol agents (Dana A. Focks)
- *Toxorhynchites* (E. T. Schreiber)
- *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control (Lawrence A. Lacey)
- Developing recombinant bacteria for control of mosquito larvae (Brian A. Federici, Hyun-Woo Park, Dennis K. Bideshi, Margaret C. Wirth, Jeffrey J. Johnson, Yuko Sakano & Mujin Tang)
- Larvicidal algae (Gerald G. Marten)
- Larvivorous fish including *Gambusia* (William E. Walton)
- Turtles (Gerald G. Marten)
- Methoprene (Clive A. Henrick)
- Semiochemicals, traps/targets and mass trapping technology for mosquito management (Daniel L. Kline)
- Modeling and biological control of mosquitoes (Cynthia C. Lord)
- Population dynamics (Steven A. Juliano)
- Competitive displacement and reduction (L. P. Lounibos)
- The molecular genetics of larval mosquito biology: a path to new strategies for control (Paul J. Linser, Dmitri Y. Boudko, Maria Del Pilar Corena, William R. Harvey & Theresa J. Seron)
- Regulatory considerations with biological control of public health pests (Karl Malamud-Roam)

[1]Floore, T.G. (2007) *Biorational control of mosquitoes*. American Mosquito Control Association, Bulletin No. 7, 330 pp.

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Insect Pathology in the English Midlands

The next annual meeting of the Society of Invertebrate Pathology will be held at the University of Warwick, Coventry, UK on 3–8 August 2008. More information will be available shortly on the SIP website:
www.sipweb.org/

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CPC 2007

The 2007 Edition of CABI's Crop Protection Compendium has been published on CD-ROM and the Internet.

This gives us a small chance to plug a report from the Australian Centre for International Agricultural Research (ACIAR) which revealed that the CAB Abstracts Database and the Crop Protection Compendium (CPC) save Australian researchers between Au$1.2 and Au$2.2 million in research time every year. The CPC alone saves each researcher between 37 and 54 days per year which equates to between Au$940,000 and Au$1,380,000 per year for time savings across all researchers.

New features include revised/updated and new full datasheets on pests and crops making the total number of full datasheets more than 2410. The library has been enhanced. The CPC 2007 includes new data and/or documents from the International Plant Protection Convention (IPPC), the European and Mediterranean Plant Protection Organization (EPPO), FAOSTAT, the World Bank, the British Crop Protection Council (BCPC) e-Pesticide Manual v. 4 and CAB Abstracts.

Crop Protection Compendium 2007 edition. CABI, Wallingford, UK. ISSN: 1478-7040 (online), ISSN: 1365-9065 (CD-ROM), ISBN: 978-1-84593-357-9

Another Perspective

It's generally a good thing to know what the 'other side' are saying, and for biocontrol that is often the pesticide sector. Recognizing the public's perception of pesticides as hazardous rather than appreciating the benefits, a recent publication from the University of Greenwich (UK) explores and analyses "the many benefits of using pesticides, in order to inform a more balanced view", identifying 26 primary benefits (described as immediate and incontrovertible), and 31 secondary benefits (longer term, less intuitive and harder to establish causality for). It cites benefits such as increased crop and livestock yields, improved food safety, human health, quality of life and longevity, and reduced drudgery, energy use and environmental degradation. It examines how these interact at different levels (local, national and global) in social, economic and environmental domains.

Cooper, J. & Dobson, H. (2007) The benefits of pesticides to mankind and the environment. Natural Resources Institute, University of Greenwich. 75 pp.

Conference Report

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

SIP in Quebec

The Society for Invertebrate Pathology (SIP) held its 40th Annual Meeting in Quebec on 12–16 August 2007. The meeting was also the 1st International Forum on Entomopathogenic Nematodes and Symbiotic Bacteria (IFENSB). The conference consisted of oral presentations and poster sessions on a range of areas such as types of organism, modes of action of toxins, genomics, use strategies, etc. The Founders' Memorial Lecture honoured Albert K Sparks as 'A pioneer and visionary in non-insect invertebrate pathology'.

The divisions (based around the organism of interest) had various symposia:

- Bacterial division: Mode of action of toxins
- Virus division: Insect cells and baculoviruses; Baculovirus bounty
- Fungi division: Are entomopathogens only entomopathogens?
- Fungal secondary metabolites: Knowns and unknowns
- Microsporidia: Microsporidia of beneficial and pest insects in greenhouse, nursery and pollination systems.

There were also a number of cross-divisional symposia:

- Advances in the use of microbial agents for control of orchard pests
- Current situation on the biological control of turfgrass insects
- Battling alien invaders: development and use of entomopathogens to control invasive insect pests
- So many strains, so few products! opportunities and constraints to commercial development of new *Bt* products

The International Forum on Entomopathogenic Nematodes and Symbiotic Bacteria had four sessions:

- Symbiosis
- Virulence
- Infection and Stress Biology
- Ecology

The conference was, as always, extremely stimulating and a great deal of activity devoted to student participation. With concurrent sessions it is impossible to appreciate the full range of topics, but the convenience of the presentation halls and good timekeeping in the sessions, allowed movement between talks.

One impression gained, however, was a growing lack of faith that insect pathogens, acting as biopesticides, can make a major impact in global pest control. There were a number of presentations on synergistic combinations, the use of metabolites rather than the whole organism and, more positively, an overdue recognition that these agents can offer more than just a superior pesticide approach. In one session, 'Are entomopathogens only entomopathogens?' a series of quotes were displayed, spanning a number of decades. These all talked of the potential of mycoinsecticides (this reporter was not the only participant to think they recognized one of these quotes as their own!); this potential needs to be realised. It is undoubtedly true that using entomopathogenic fungi as mycoinsecticides is only using one facet of their biology (in a rather unnatural way) and that examining the possibilities of, for example, systemic protection via endophytic growth of entomopathogens may be more fruitful.

The next SIP will be held at the University of Warwick, Coventry, UK on 3–8 August 2008.

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IWBCISF in Beijing

The International Workshop on Biological Control of Invasive Species of Forests (IWBCISF) was held on 20–25 September 2007 in Fragrant Hill Empark Hotel, at the southeast foot of the Fragrant Hill, Beijing, P. R. China. The workshop was organized by the USDA (US Department of Agriculture) Forest Service – Forest Health Technology Enterprise Team (FHTET), the Chinese Academy of Forestry – Research Institute of Forest Ecology, Environment and Protection, the Asian-Pacific Forest Invasive Species Network (APFISN), and the Asian-Pacific Association of Research Institutions.

This international workshop was the first of its kind. The intent of the IWBCISF meeting was to create a forum for presentations to exchange information and to foster discussions of issues affecting the use of natural enemies for control of invasive species of forests. The original focus of the IWBCISF workshop was to summarize 15 years (1993–2007) of cooperation between the USDA Forest Service and various organizations in P. R. China, but has expanded to include the Asian-Pacific Region and beyond.

The workshop was widely considered a successful meeting. It was attended by 129 delegates, including 97 from P. R. China and 32 from 14 other countries (in alphabetical order): Bhutan, Cambodia, Canada, Finland, India, Indonesia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, UK, USA and Vietnam). It was sponsored by three organizations, the USDA Forest Service, the China State Forestry Administration, and the Asia-Pacific Forestry Commission, and co-sponsored by five organizations, the Chinese Academy of Forestry, Beijing Normal University, the Chinese Academy of Agricultural Sciences, IUFRO 7.03.08 – Forest Protection in Northeast Asia, and the USDA Forest Service Northern Research Station, NRS-03, Ecology and Management of Invasive Species and Forest Ecosystems.

Sixty-five oral presentations (seven at the Opening Ceremony, eight in Session I 'General Session', 16 in Session II 'Arthropods', 13 in Session III 'Plants', 12 in Session IV 'Phytopathogens & Biopesticides', and nine in Session V 'Strategy and Technology for Monitoring and Controlling Invasive Species for a Green Olympics 2008'), and nine posters were presented addressing the six objectives:

- To summarize 15 years (1993–2007) of cooperation between USDA Forest Service and various organizations in China to manage invasive species of forests;
- To plan for future cooperation among the USDA Forest Service and various organizations in China, as well as other countries in the Asian-Pacific region, to manage invasive species of forests;
- To develop/strengthen strategies for minimizing introduction of invasive species of forests between USA and China, as well as other countries;
- To encourage informal exchanges of scientists and workshops covering biological control of invasive species of forests;
- To harmonize the shipment of natural enemies; and
- To document the biology, life history, natural enemies, etc., for a prioritized list of potential invasive species of forests between USA and China, as well as other countries.

Setting the Scene

An opening ceremony was held during the morning of 21 September, presided over by Shirong Liu, Vice President of the Chinese Academy of Forestry. Welcome speeches were given by: Lieke Zhu, Deputy Chief, Chinese State Forestry Administration (CSFA); Richard Reardon, USDA Forest Service – Representative for the Washington Office, Forest Health Protection and International Programs; Yuyuan Guo, Academician of the Chinese Academy of Engineering, Representative of the Chinese Society of Plant Protection, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS); Patric Durst, Senior Officer, FAO Regional Office for Asia and the Pacific; K. V. Sankaran, Coordinator of the Asia-Pacific Forest Invasive Species Network; Xurong Mei, Director of the Institute of Environment and Sustainable Development in Agriculture, CAAS; and Dianmo Li, Beijing Program Director, The Nature Conservancy. Also present at the opening ceremony: Diansheng Wei, Director General of the Department of Silviculture, CSFA; Jian Wu, Senior Engineer of the Department of Agriculture, CSFA; and Jianbo Wang, Director of Forest Protection Division, Department of Silviculture, CSFA.

In the welcome speech, Lieke Zhu said "the Chinese government highly emphasizes prevention and control of invasive species and not only has included it in the '*National Economic and Social Development for the 11th Five-Year Plan*', but also has implemented sustainable prevention and control strategies, as well as developed many very effective pest preventions, controls, and ecological protections. We have gained practical experience by doing so".

In Richard Reardon's welcome speech he said that the USDA Forest Service had developed strong professional and personal relationships over the years in supporting international collaboration for invasive species from many people, for instance Bov Eav, Ann Bartuska (USDA Forest Service Research), Bill White, Ken Knauer, Allan Bullard, Rob Mangold (USDA Forest Service Forest Health Protection), Gary Man, Cynthia Mackie (USDA Forest Service International Programs). Richard Reardon also suggested the workshops continue being held every three to four years, as these workshops help to improve the coordination of activities and understanding of the roles of organizations in finding ways to instigate efforts against invasive pests.

Following the opening ceremony seven speakers presented at the General Session.

In the keynote address 'Biological invasions: recommendations for US policy and management', Richard Mack (Washington State University, USA) spoke about the Ecological Society of America (ESA) report in 2006 about its evaluation of current US national policies and practices on biological invasions in light of current scientific knowledge. Invasions by harmful non-native species are increasing in number and area affected. Without improved strategies based on recent scientific advances and increased investments to counter invasions, harm from invasive species is likely to accelerate. Federal leadership, with the cooperation of state and local governments, is required to increase the effectiveness of prevention of invasions, detect and respond quickly to new potentially harmful invasions, control and slow the spread of existing invasions, and provide a national centre to ensure that these efforts are coordinated and cost effective. Specifically, the ESA recommends that the federal government take action in six areas: (1) Use new information and practice to better manage commercial and other pathways to reduce the transport and release of potentially harmful non-native species; (2) Adopt more quantitative procedures for risk analysis and apply them to every species proposed for importation into the country; (3) Use new cost effective diagnostic technologies to increase active surveillance and sharing of information about invasive species so that responses to new invasions can be rapid and effective; (4) Provide emergency funding to support rapid responses to emerging invasions; (5) Provide funding and incentives for cost effective programmes to slow the spread of existing invasive species in order to protect still uninvaded ecosystems, social and industrial infrastructure, and human welfare; and (6) Establish a National Center for Invasive Species Management to coordinate and lead improvement in federal, state, and international policies on invasive species.

In 'Restoring the natural balance: biological control of invasive forest pests in the UK', Hugh Evans (Forestry Commission Research Agency, UK) described the processes that help protect the UK from potentially damaging exotic pests. Other than the natural barrier of its island status, there are a number of phytosanitary measures in place to reduce the incidence of pests and pathogens arriving and establishing in the country. Some pests have been managed using classical biological control. Two sawfly species, *Cephalcia lariciphila* and *Gilpinia hercyniae*, have been reduced to negligible levels by introduced parasitoids and an introduced baculovirus, respectively. An integrated management regime based on internal quarantine, selective felling and, particularly, introduction, rearing and release of the specific predator *Rhizophagus grandis* has been a great success against spruce bark beetle, *Dendroctonus micans*, which is now at low levels. The confidence in the biological control agent is so great that internal quarantine has now been dropped. Intensive studies on the biology of *R. grandis* have enabled an artificial attractant to be developed. This is now being used as a monitoring tool to assess the presence of the predator and, by implication, its specific bark beetle prey. However, not all bark beetles are so susceptible to natural enemies and a contrast between *D. micans* and the European spruce bark beetle *Ips typographus* indicates that biological characteristics of the pest species can influence natural enemy performance considerably. Key lessons from studies of invasive pests included: (1) Pest Risk Analysis tends to be retrospective – often providing insufficient early warning; (2) Pathway-based strategies need to be developed, especially for plants for planting; (3) The best strategy is to keep pests out! (4) Early detection of pioneer pest populations – methods need to be improved; and (5) International collaboration is essential to increase knowledge transfer and data on potential pests and their natural enemies.

Jerry Beatty (USDA Forest Service, Western Wildland Environmental Threat Assessment Center [WWETAC]) introduced the centre: a new unit of the Pacific Northwest Research Station has been created to generate and integrate knowledge and information to provide credible prediction, early detection, and quantitative assessment of environmental threats (e.g. invasive plants, potential insect outbreaks, the appearance of invasive insect threats, the appearance of new pathogens, and other threats, such as sudden oak death) in the western USA. The goal is to inform policy and support the management of environmental threats to Western wildlands.

A presentation by K. V. Sankaran and co-workers (Kerala Forest Research Institute, India) gave early results of biological control of *Mikania micrantha* using *Puccinia spegazzinii* in India. The rust fungus, *P. spegazzinii,* was imported from Trinidad and its host specificity confirmed before release. In Kerala State (southwestern India), releases of the rust were made in agricultural systems and degraded moist deciduous forests in August 2006 and June–August 2007. These releases are considered successful since the disease has spread from source plants to field populations of *Mikania* at all release sites and is still persisting in some of the sites. The fungus is apparently intolerant of high atmospheric temperature and low relative humidity. So, it might be necessary to make frequent releases involving large loads of inoculum when the climatic conditions are most favorable (June–August) for the fungus to spread and multiply. Release of the rust is continuing.

Zhongqi Yang (Chinese Academy of Forestry, Beijing, P. R. China) gave a presentation on 'Recent advances in the biological control of forest invasive pests in China'. Recently, a number of invasive species have particularly caused great losses and become serious problems in forests in China, i.e., fall webworm (*Hyphantria cunea*), red turpentine beetle (*Dendroctonus valens*), pine wood nematode (*Bursaphelenchus xylophilus*), coconut leaf beetle (*Brontispa longissima*), pine needle scale (*Hemiberlesia pitysophila*) and emerald ash borer (*Agrilus planipennis*). Biological control, by applying beneficial ecological factors, i.e., natural enemies, is the best choice for effectively bringing the exotic pests under control and achieving sustainable control results without polluting the environment. Zhongqi Yang summarized recent years' classical biological control programmes and achievements made by Chinese scientists in forest protection.

Zhengqing Peng (Chinese Academy of Tropical Agriculture Sciences, Hainan, P. R. China) gave a talk entitled 'Import and application of two parasitoids of coconut leaf beetle, *Brontispa longissima* (Gestro)'. He pointed out that coconut leaf beetle is one of the most important insect pests of palm plants in Southeast Asia and the Pacific. It is also in the second category of quarantine risk insect pests in China. Its larvae and adults feed on the unemerged leaves, and can even cause plant death. The coconut leaf beetle was first discovered in Haikou City in June 2002 and shortly afterwards in Sanya City. It has now become the most serious threat to Hainan's smallholder coconut industry. In order to control the pest, two parasitoids were imported in 2004, and in-depth studies were undertaken on their biology, ecology, rearing and release techniques, tracking techniques, control effects assessment and effects of insecticides on parasitoids release. The parasitoids have had a great effect on controlling the dispersal of coconut leaf beetle. Zhengqing Peng listed nine important outcomes from importing and application of the two parasitoids.

Kari Heliövaara (University of Helsinki, Finland) stated that there are "No invasive alien species in Finnish forests". Though exotic phytophagous insects are invading forest ecosystems worldwide, the number of terrestrial introduced alien species has remained relatively low in Finland: ca 550 plants, 25 invertebrates, five birds and 16 mammals have been reported. So far Finland is one of the few countries in the world where not a single exotic forest insect species has been observed. However, increased timber transportation, especially from Russia, involves a risk of introducing new, exotic forest pests to Finland, e.g., bark beetles (Scolytidae). Some potential pests may also spread naturally. An example is the main defoliator in North China and Siberia, the Siberian moth (*Dendrolimus superans sibiricus*). Because alien species may strongly affect local biotas, more action is needed to prevent unintentional introductions. The pine wood nematode (*Bursaphelenchus xylophilus*) has already been under surveillance by Finnish plant health officials since 1984. Out of hundreds of inspected lots of coniferous packing wood, 3% have contained living pine wood nematodes. Though Finnish pine forests would meet the nematode's biological requirements, no successful establishment has taken place. He concluded that the lack of invasive alien species in Finnish forests is mainly due to harsh climate, high level of forest hygiene, legislation and good luck.

Four concurrent sessions were held on 22–23 September after the general session.

Arthropods

Session II 'Arthropods' consisted of fifteen 30-minute presentations. Ten of these focused on arthropods native to China that have invaded the USA. Only one presentation in this session focused on a pest native to USA that has invaded China, but a paper in the plenary session discussed three arthropods introduced from USA to China. Three papers discussed species from Southeast Asia that are invasive in China. In two cases, *Adelges tsugae* and *Tomicus piniperda*, China was not the source of the introduction to the USA but is a resource for natural enemies for biological control. The session provided many examples of the high diversity of natural enemies in China and greater control of invasive species in China by host shifts of polyphagous, native parasitoids than by introduced parasitoids.

In addition to the above two forest pests, other biological control targets discussed included *Agrilius planipennis*, *Anoplophora glabripennis, Hemiberlesia pitysophila*, *Oracella acuta* and *Halyomorpha halys*. More than half of the presentations included information on the behavior, ecology and spatial dynamics of the host–natural enemy systems; this was stimulating and, at the end of the session, more work on behaviour was suggested. Other suggestions included more taxonomic support (both traditional morphological and molecular) in identifying natural enemies and DNA barcoding of natural enemies.

Plants

Presentations at Session III 'Plants' were given on both plants in the USA that are originally from Asia, for which biological control agents may occur in China, and also on plants invasive in China, some of which came from the USA and others from various tropical areas. As with the Arthropod session, more were in the first category than in the second.

Notable in the first category was the temperate 'mile-a-minute' weed, *Persicaria perfiliata (=Polygonum perfoliatum)* (in the Polygonaceae, not to be confused with the tropical 'mile-a-minute' weed [*Mikania micrantha* – see above], a different invasive species). This species can develop dense monocultures in the eastern USA, preventing tree regeneration and reducing biodiversity. Judy Hough-Goldstein described release of a host-specific weevil from China, first collected by Jianqing Ding and Weidong

Fu (who also did much of the initial host-specificity work), which seems to be quite successful, and Yun Wu described her study looking for fungal pathogens of the species. Japanese knotweed, another Polygonaceae, is a perennial invasive species that is widespread in the USA. Ding summarized his work with a leaf beetle that feeds on both mile-a-minute (*Persicaria perfiliata*) and Japanese knotweed.

Tree-of-heaven (*Ailanthus altissima*) is the focus of efforts with a large weevil, and studies in China were reported concerning the insect's response to environmental conditions, reported by Fushan Zhu. Donald Davis reported that, in the USA, tree-of-heaven was observed being attacked by two species of *Verticillium*, whose provenance is unknown. Studies on kudzu (*Pueraria lobata*) were also reported, but the insects that have been studied so far are potentially damaging to soybean. However, a fungal disease that may be host specific is under investigation, reported by Zide Jiang. There are several other invasive weed species from Asia that have invaded Hawaii, and Tracy Johnson will be looking for insects on a *Rubus* species while in China.

The session also heard about plant species that are invasive in China and efforts to control them. Defu Chi talked about standards for determining whether a plant is 'native', which is difficult in China, given its lengthy history of human habitation. Wanzhong Tan gave many references with various estimates of the number of alien invasive plant species in China, ranging from about 50 to more than 180. He also talked about his research programme on the use of plant pathogens as mycoherbicides, or using toxins produced by these pathogenic fungi as herbicides.

In addition, Yan Chen discussed the alien plant 'yellow top', which shows some allelopathic properties. Yina Yu talked about controlling *Spartina* by planting mangroves, thus creating shade, which eliminated the grass. The director of the Kingho Biotech Company talked about botanical pesticides, and Mr Li Zhigang discussed his work with insects that feed on *Mikania micrantha*, including observations of a native parasitic dodder, which suppresses this plant.

The session highlighted valuable instances of collaboration between the USA and China and with the other countries represented here, and indicated the potential for many more.

Phytopathogens and Biopesticides

George Newcombe's presentation 'Endophytes influence growth, competition, and protection of an invasive plant' began the morning of Session IV 'Phytopathogens and Biopesticides'. Invasiveness may be the result of 'novel weapons' that an invasive plant employs against evolutionarily naïve neighbors in its invaded range. According to this hypothesis, the same weapons are neither novel nor effective in the plant's native range because its neighbours there are adapted to its weapons. For example, much evidence indicates that invasive *Centaurea* spp. possess novel weapons because naïve plants from their invaded ranges (e.g. North America) grow more poorly in their presence than adapted plants from their native range (i.e. Eurasia). Although allelochemicals have been proposed as the putative weapons, he described results from pot experiments indicating that the only novel weapons possessed by *C. stoebe* are fungal endophytes. Endophytes inoculated into *C. stoebe* plants reduced the growth of the naïve neighbours (i.e. *Festuca idahoensis*, Idaho fescue) of its host, but increased the growth of adapted neighbours (i.e. *F. ovina,* European fescue). Competitive advantage of *C. stoebe* over *F. idahoensis* relative to that of *C. stoebe* over *F. ovina* was significantly increased by five of the six most common endophytes, whereas endophyte-free *C. stoebe* had no greater competitive advantage over *F. idahoensis* than over *F. ovina.* Given the relatively high isolation frequencies of endophytes from seed, these novel weapons could easily have been co-introduced into their invaded range with their host.

The next couple of presentations were on *Phytophthora* spp. (primarily *P. ramorum*), their impacts on forested communities and the critical need for work on the taxonomy of this important group of plant pathogens. There was also a report on the search in southwestern China for the site of origin of *P. ramorum;* the results being negative for *ramorum,* but revealing many unknown species of *Phytophthora.* The following presentation was on the management of pine nematode in southeast China, primarily using species (tree) manipulation, sanitation of dead, infected trees, and other silvicultural methods. The last presentation of the morning was on the history of invasive species in California, USA; primarily the work done on identifying, monitoring and resistance breeding to manage white pine blister rust.

The most important conclusion, in the morning session, for future workshops and research needs in this area, is the need for a focus on the genus *Phytophthora.*

The afternoon session included four talks: The first, by Zhong Yang, 'Rapid detection of wood biological decay by near infrared spectroscopy', focused on the use of near infrared spectroscopy to detect reduction in strength before weight loss of wood. A portable, battery-powered unit was described that could be brought to the field. The research had been conducted at a USDA site in Pineville, Louisiana where slash pine could be affected by either brown rot or white rot. Zhong Yang also explained the statistical methods that he used.

The second talk 'Poison mushroom and their usage in biological control' was given by Ruiqing Song. Many mushroom-producing, basidiomycete fungi are toxic. Their toxins are of potential use in combating plant diseases. Her assay system was based on three plant pathogens: an isolate of *Alternaria* that causes poplar blight in China, a *Cytospora* that causes poplar canker, and a *Sphaeropsis* that causes a blight of pines. Filtrates and mycelial extracts of toxic fungi such as *Amanita*, *Leucopaxillus* and *Lepiota* were used to treat the spores of the three plant pathogens growing in Petri dishes. Spore germination was often inhibited by particular filtrates and extracts, the safety of which was being assayed with white mice.

The third talk 'Latest progress, problems, suggestions and expectations on biopesticide research' was given by Changxiong Zhu. He compared and contrasted the commercialization of biopesticide research in China with that of foreign countries including the USA. He forecast a huge market in the future in China for biopesticides that he thought would take 20–30% of the market away from chemical pesticides in the next ten years. Even at present, China's biopesticide products account for 10% of the pesticide market.

The fourth talk 'Prospect of using *Beauveria bassiana* to control long-horned beetles and other forest pests' was given by Chunsheng Deng. The host range of *Beauveria bassiana* is huge with some 700 insect pests that can be controlled by products containing this entomopathogenic fungus. Many strains of *Beauveria*, all from insects collected in China, have been investigated. Different formulations were described, and examples of specific usage were covered.

A Green 2008 Olympics

In brief, Session V 'Strategy and Technology for Monitoring and Control Invasive Species for a Green Olympic 2008' was very successful. Rumei Xu, the co-moderator, reported that Wanqiang Tao, Director, the Beijing Forest Protection Station, and Linjun Zhang, Director, the Beijing Plant Protection Station gave knowledgeable and informative presentations on the occurrence, monitoring, prevention and control of forest and plant invasive species in Beijing. These were followed by reports from Richard Mack and Hugh Evans, which included many very useful suggestions on preparing for the Olympics in 2008, based on their long experience. Other reports also concentrated on biological control of the most important invasive species in Beijing.

The most successful outcome was that participants realized that bilateral long term cooperation is needed; therefore, a memo was drafted and presented during the session.

Final Events and Looking Ahead

At the closing of the workshop, Yun Wu and Zhongqi Yang expressed their appreciation to the sponsors and co-sponsors for supporting the workshop; Yun Wu, especially, expressed her sincere thanks to the working group, people mostly from the Chinese Academy of Forestry, with a few from the Chinese Academy of Agricultural Sciences. Then she seconded one of Richard Reardon's suggestions in his speech at the opening ceremony, to have the next IWBCISF meeting in three to four years, and asked for potential organizers to take responsibility. Since then several parties have contacted the organizer, including Richard Reardon (USDA Forest Service – FHTET), Jerry Beatty (USDA Forest Service – WWETAC), K. V. Sankaran (APFISN), and Pham Quang Thu (Forest Protection Research Division, Forest Science Institute of Vietnam), to express their interest in cooperating in the organization of the second workshop.

A one-day field trip was arranged the day after the sessions. In the morning, the workshop participants visited four laboratories at the Chinese Academy of Forestry (the Biological Control Laboratory, Forest Quarantine Laboratory, Remote Sensing Laboratory and Forest Molecular Genetics Laboratory) followed by a visit to the Biological Control Laboratory at the Xishan Forestry Center. In the afternoon, the participants toured Beijing Botanic Garden which is also located at the southeast foot of Fragrant Hill.

And finally, the Proceedings of the International Workshop on Biological Control of Invasive Species of Forests will be published by the USDA Forest Service – FHTET who also have a series of publications regarding biological control of invasive species of forests (see www.invasive.org/ for more information). Contact: Yun Wu at ywu@fs.fed.us and Zhongqi Yang at yzhqi@caf.ac.cn.

This report was compiled by Yun Wu (main co-organizer, USDA Forest Service – Forest Health Technology Enterprise Team) with the summary information for con-current sessions provided by Mike Montgomery (Co-moderator for the Arthropod Session, USDA Forest Service – Northern Research Station), Judy Hough-Goldstein (co-moderator for the Plants Session, University of Delaware, USA), George Newcombe (co-moderator for the Phytopathogen & Biopesticides Session, University of Idaho, USA), Jerry Beatty (co-moderator for the Phytopathogen & Biopesticides Session, USDA Forest Service – Western Wildland Environmental Threat Assessment Center), and Rumei Xu (co-moderator for Strategy and Technology for Monitoring and Control Invasive Species for a Green Olympic 2008, Beijing Normal University). The presentation information for the Opening Ceremony and the General Session was provided by the speakers.