General News

Mimosa Leaves Niche for New Agents

A leaf-feeding geometrid moth, *Macaria pallidata* (= *Xenoecista pallidata*), released in Northern Territory in September this year is the latest in a long line of biocontrol agents introduced into Australia against *Mimosa pigra* (mimosa or giant sensitive plant). In addition to the promise this agent holds, encouraging results reported for previously released agents suggest that the sustained biocontrol programme against mimosa is paying off.

Mimosa currently occupies some 850 km^2 of wetlands in Northern Territory and threatens a great deal more. It has been found recently for the first time in Queensland, about 2000 km from the nearest infestation. Its potential distribution extends from the north coast of Western Australia across the country and down as far as northern New South Wales. *Mimosa pigra* was declared one of Australia's 20 Weeds of National Significance in 1999. It has been the target of a biological control programme for the last 20 years, and since 1983 a suite of biocontrol agents has been introduced against it.

Mimosa pigra is native to Central and South America, extending from Mexico to northern Argentina, but it has become widespread as a weed in Africa and Asia as well as Australia. Although it occurs in small patches of inconspicuous and straggly plants in its home range, in Australia it grows to 6 m tall and forms impenetrable thorny thickets, which compete with pastures, hinder mustering and prevent access to water. It also poses a serious conservation problem as it can completely alter the natural grass and sedge floodplain landscape, replacing it with dense monospecific stands, and it threatens tourist income in areas such as Kakadu National Park. The weed grows at an alarming rate. Infestations in a river system can double in area in just over a year following good rainfall, and a single plant can produce more than 200,000 seeds per year. Seeds are dispersed mostly by water: some germinate soon after release, others in subsequent seasons.

In its home range, *M. pigra* is attacked by some 440 insect herbivores and a number of fungal pathogens, and it has been the task of the biocontrol programme to select the most promising of these and screen them for introduction. Eleven insect and two pathogen species have been released to date [see also *BNI* **20(1)**, 6N-7N (March 1999), Preventing wetland wipe-out].

One agent, the leaf-beetle *Chlamisus mimosae* (introduced in 1985), only established on one river system and has had only minimal impact on mimosa. The impacts of a flower-feeding weevil (*Coelocephalapion pigrae,* introduced in 1994) and a seed-feeding bruchid (*Acanthoscelides puniceus*, introduced in 1983), which are widespread and sometimes abundant, are currently being assessed. However, two stem-boring moths (a gracillariid (*Neurostrota gunniella*) and a sesiid (*Carmenta mimosa*) first released in 1989) are undoubtedly the most successful to date. *Neurostrota gunniella* dispersed rapidly and is abundant in many locations. *Carmenta mimosa* is spreading more slowly, but is already widespread on two river systems and firmly established on two others. The two moths have been credited with reducing seed production by up to 87% at some sites and for a large amount of leaf drop and stem dieback. *Neurostrota gunniella* abundance has been correlated with reduced seed output and canopy growth, and *C. mimosa* has been shown to further reduce seed production and has even caused plant death in localized areas. Subsequent regeneration of grasses and sedges beneath heavily attacked plants, in turn, means it is more difficult for mimosa seeds to germinate and grow. Furthermore, biological control integrates well with alternative management options. Damage from biocontrol agents is greater on plant regrowth following burning, crushing or spraying.

Despite these promising results, control was still not adequate, and biocontrol scientists continued to search for agents to complement those already at work. The agents that had established by 2000 fed and reproduced on flowers (1 sp.), seeds (1 sp.) and stems (2 sp.), with only the ineffective *Chlamisus mimosae* attacking the leaves. The best-performing agents were having a good impact on seed production of mature plants, so interest was focused on species that fed on other plant parts; in particular, those that fed on foliage and/or seedlings and would further reduce the degree of shading beneath stands, whilst also reducing seedling regeneration, hastening the replacement of mimosa with native vegetation.

A chrysomelid beetle (*Malacorhinus irregularis*) from Mexico was one such candidate. It has an adult leaf-feeding stage, but its soil-dwelling larvae feed on various plant parts, particularly seedlings and germinating seeds. Adult beetles were shown to prefer young, growing leaves. For larvae, survival to adult was best on seedlings, with the cotyledons preferred by all larval stages. By consuming leaves the adults reduce the vigour, growth rate and seed production of the plant, while by consuming seedlings the larvae reduce stand regeneration. This 'double-barrelled' strategy meant the potential for added impact from introducing this species was great.

Host-specificity testing on 82 plant species showed that larvae survived and completed development only on *Mimosa pigra* (on which survival to adult averaged 85%). Minimal adult feeding was recorded on a few *Acacia* and *Mimosa* species, but the results of further screening of these and other related species indicated that introduction of the beetle would not impact on non-target species in Australia. Oviposition normally occurs in the soil, so oviposition testing was considered not to be applicable.

With testing completed successfully, *Malacorhinus irregularis* was introduced to Australia in 2000. It is now reported to have established successfully and to be inflicting significant damage to mimosa at release sites. At one site, last year's seedlings were almost totally stripped of leaves by the adults. It is too early to be able to assess larval impact, and plots continued to be monitored, but the signs are that this agent will indeed complement existing biocontrol agents by further reducing mimosa regeneration.

The latest agent to be released, *Macaria pallidata,* was another promising candidate as its larvae feed voraciously on *Mimosa pigra* leaves. They feed on both young and mature foliage, first stripping the top surface of leaves. At high densities they can completely strip foliage, thus reducing plant growth and seed production.

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Although exposed when feeding, they rest on leaf tips during the day in positions that imitate stems, and they tend not to leave their host plant unless forced to look for a new food source. They are abundant and damaging in *M. pigra*'s native range in Mexico and museum records indicate they are widespread across tropical America. Only genetic material from Mexico was tested for host specificity. Because of the wide geographic range and the possibility of the existence of a species complex, only this Mexican genotype was released into Australia.

Adult female *Macaria pallidata* oviposit indiscriminately on most surfaces when confined in a cage, so host specificity assessments focused on larval development testing, which was carried out using 70 plant species at CSIRO's Long Pocket Laboratories in Queensland. In Mexico, open field tests on 28 plant species were also conducted near the CSIRO Mexican Field Station in Veracruz to determine oviposition acceptability and larval development suitability under natural conditions. As is common amongst moths, adult *M. pallidata* do not feed destructively but only on substances such as nectar, so it was not appropriate to assess adult feeding specificity.

The results of testing demonstrated that survival rates on all plant species apart from *Mimosa pigra* were too low for any of them to support a population of *Macaria pallidata*. Only six species supported development to adult, with a highest survival rate of 1.1% on *Acacia decurrens* (compared to 64% on *Mimosa pigra*). Adults that did emerge from the test species were often deformed, but viability could not be assessed as so few emerged, and these over a protracted period. However, an adult emerging (albeit deformed) from *Neptunia plena* (a native Mexican species) in the open field trial indicated that this is a host in the native range, so *Neptunia* species occurring in northern Australia (including *N. major*, which is sympatric with mimosa) will be monitored by the biocontrol programme. On balance, however, it was concluded that the moth was safe to release in Australia. This conclusion was supported unanimously by the authorities that regulate the importation of biocontrol agents who granted permission to release.

From a rearing perspective, *Macaria pallidata*'s credentials are a biocontrol practitioner's dream: they have a short generation time, high fecundity, and are easy to rear. Releases of thousands of moths each month are underway.

The mimosa biocontrol programme is a joint programme of the Northern Territory Government (Department of Infrastructure, Planning and Environment) and CSIRO, supported by the Australia Government's Natural Heritage Trust and previously by the Australian Centre for International Agricultural Research. The slow but sure success of the programme against this dangerous wetland weed proves the value of taking a collaborative, sustained approach against intractable invasive weed problems.

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Biocontrol to Combat Kudzu

Kudzu, *Pueraria montana* var. *lobata* (= *P. lobata*), was introduced to the USA during the middle of the 19th century as an ornamental. During first half of the 20th century, approximately 134,760 ha were planted to feed livestock and for erosion control. Presently, extension agents report more than 404,280 ha of kudzu distributed among approximately 700 counties. Estimates suggest 10% of some lands administered by the Army Corps of Engineers are occupied by kudzu. Although the largest and most dense infestations have been documented in the southeastern USA, small infestations have appeared recently in the Pacific North West, the Great Plains, and the North East. This development supports predictions concerning the range to which kudzu may expand as a function of increased carbon dioxide concentration and of increased winter temperatures resulting from global climate change.

Kudzu kills trees by climbing up their boles and into their canopies, out-competing them for light. Infestations cost commercial forests approximately US$119.00/ha annually and compromise the integrity of valuable natural resources. Recently, dense infestations of kudzu have been reported to interfere with military exercises in North Carolina, South Carolina, and Virginia.

Herbicides are generally used to manage small, isolated populations of kudzu, but obstacles exist to their application. The most important are concerns for applicator safety and cost. Evaluating potential hazards for application equipment and operators on land occupied by kudzu is difficult. Also, repeated application over several years is required to kill the large corms, which can weigh up to 75 kg. Use of expensive herbicides against infestations on land of marginal economic value is generally not cost effective. Furthermore, application of herbicides over large areas threatens contamination of ground water. While several herbicides are effective against kudzu, application of the most effective in proximity to aquatic habitats is discouraged.

In response to these obstacles, an integrated approach including biological control is being studied. Biological control is almost never implemented by itself in the USA, but instead is usually part of an integrated management programme that may include herbicides, mechanical removal, livestock grazing, and more responsible land stewardship. The goal of a biological control programme is balance between kudzu and its new habitat. Natural enemies are the means by which this goal may be achieved and these may be found either in the habitat to which kudzu is native or in its new habitat. Pursuit of biological control agents in kudzu's native range in China against populations of the weed in the USA commenced during 1998.

Presently, the USDA (United States Department of Agriculture) Forest Service is sponsoring exploration for biological control agents through cooperative agreements with the Chinese Academy of Sciences and three universities: Anhui Agricultural University, South China Agricultural University, and the South China Institute of Botany. Eleven sites in three provinces (Shaanxi, Guangdong, and Anhui) have been surveyed for natural enemies. China is a vast country, and kudzu occupies a large area within its borders. To optimize use of limited resources and increase chances of encountering potential biological control agents at our sites, important parameters like defoliation, seed damage, and stem and root boring were monitored continuously to identify the best opportunity for comprehensive surveys.

So far, approximately 240 insects associated with kudzu have been identified. Among them are defoliators, root and stem borers, and gall makers. Only a few, however, satisfy the important criterion of host-specificity in preliminary experiments. More refined study of these insects continues, which includes preliminary host-testing, identifying life cycle parameters, and quantifying their impact on kudzu. Also, a comprehensive list of economically and ecologically important plants on which the host range of these insects will be tested in quarantine in the USA is being compiled (we gratefully acknowledge the efforts of George Markin and Jennifer Birdsall – USDA Forest Service, Bozeman, Montana – for their efforts). So far, the most promising potential biological control agent encountered during surveys of kudzu in China is a sawfly, *Arge similis*. Results of preliminary experiments in China testing its host range suggest it completes its life cycle only on kudzu.

Pathogens (formulated as bioherbicides) also may be used as biological control agents. Bioherbicides generally cost as much as chemical herbicides to formulate and apply, but they are less likely to contaminate ground water and nearby aquatic habitats. One pathogen encountered during surveys of kudzu in China is an imitation rust, *Synchytrium puerariae*, which interferes with translocation of water and nutrients throughout a plant. Impact of this fungus on kudzu is being studied.

Systematic resolution has been an obstacle to developing integrated management programmes for many invasive, exotic plants, including hoary cress, leafy spurge, and spotted knapweed. Distinguishing between kudzu and related taxa in the field by their morphology is unreliable. Other plants for which the taxonomy and systematics are poorly refined are frequently mistaken for kudzu, including *P. montana* var. *chinensis* (= *P. thomsoni*) (= *P. montana* var. *thomsoni*) and *P. montana* var. *montana* (= *P. montana*). Also, evidence from herbarium specimens suggests the hybridization of kudzu and related taxa. For this reason, molecular tools are being developed to distinguish between specimens. So far, results of preliminary experiments support the use of randomly amplified polymorphic DNAs (RAPDs). Continued study of this tool is necessary before it may be used to evaluate the composition of populations in China and in the USA. More convenient and reliable differentiation of kudzu and related taxa in the field will expedite surveys for biological control agents by allowing professionals to reconcile identity of potential agents with identity of their targets.

Over the next 3 years the completion of host testing against leguminous crop plants in China is anticipated. Rearing methods will be developed for those insects with some promise as biological control agents, and their study will continue in quarantine facilities in the USA.

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Mass-Rearing Weevils to Lick Houndstongue

Help is on the way for western Canada’s cattle ranchers unarmed in their fight against the invasive, noxious weed houndstongue (*Cynoglossum officinale*). A new industry-supported project led by Agriculture and Agri-Food Canada's Lethbridge Research Centre will rear tens- to hundreds-of-thousands of a European root-feeding weevil, *Mogulones cruciger,* that targets and kills the weed. Researchers will then distribute the weevils to ranchers and land managers in interior British Columbia (B.C.), where houndstongue has become a serious problem of cattle production and a blight on rangeland.

As biocontrol researcher Rose De Clerck-Floate puts it, the weevil is likely to be the best way to control houndstongue, and will allow ranchers and land managers to control their own weeds in an economically feasible way. She reports that the project is generating a lot of excitement within the ranching community – the weevil has started to become available in the past 2 years, and there is a bottleneck in demand as more ranchers hear about its success.

Houndstongue is a short-lived perennial weed of mountainous rangelands in northwestern North America, probably introduced accidentally from Eurasia in the 1800s. It has become widespread because of a lack of the natural enemies that keep it in check in its area of origin. It is a colonizer of disturbed areas, and often infests abandoned cropland, and forest sites cleared for cattle grazing, mining operations and road construction. Houndstongue hinders forage establishment, which affects both cattle and wildlife, and has potential to poison the animals. Its burrs irritate cattle and reduce their market value. It also contains pyrrolizidine alkaloids which make it highly toxic to livestock. B.C. cattlemen rank houndstongue second only to knapweed (*Centaurea* spp.) as a priority for control.

A biological control programme began in 1988 with European exploration for potential agents against houndstongue (by CABI *Bioscience* Switzerland Centre). After 9 years of host-specificity testing, the weevil *M. cruciger* was introduced to Canada in 1997 [see *BNI* **22(1)**, 2N (March 2001), Weevils' success against Canadian rangeland weeds]. The screening project received support from funders in British Columbia and in Montana and Wyoming, USA.

The European root-feeding weevil was first released to target the weed at B.C. research sites in 1997 and, says De Clerck-Floate, has been very effective. On experimental sites where the release rate has been 200 to 400 insects, the weevils controlled the weed within 2 years. It has also been encouraging to see that the weevils seek new patches of houndstongue.

Chemical control is another option, but one that is impractical and costly on rangeland. In addition, explains De Clerck-Floate, houndstongue is a sneaky weed that will grow under canopy, which makes it hard for ranchers and weed managers to find and selectively kill the plants.

The need for a cost-effective way to mass-rear the weevils is what prompted the new project and propelled its strong support from industry, says De Clerck-Floate. She points out that insectaries and specialized laboratories are standard sites for mass-producing beneficial insects, but these require costly overheads and expertise. The approach they have decided upon is potentially far less costly, and is truly novel in the field of weed biocontrol.

As a first step, researchers will farm houndstongue as a row crop in select areas, De Clerck-Floate says. Then they will seed the insects into the weed and harvest the offspring. She emphasizes that proper steps will be taken to prevent the weed crop from spreading, and it will be grown specifically in areas where houndstongue is already a problem, so any insects that move beyond the row crop will be welcome. In addition to work at the Lethbridge Research Centre, researchers are conducting the project on private rancher and farmer properties, at their request. The weevils produced from this activity will be ready for distribution to the various partner groups in 2004 or 2005.

Eventually, the project may evolve to the point where ranchers and land managers can cost-effectively produce their own weevils, says De Clerck-Floate. She explains that as part of the initial phase of this project, they will examine the best strategies for rearing the weevils, looking at variables such as fertilization and row spacing that ultimately result in more, bigger and healthier weevils. This will improve future rearing efforts.

With promotional help from the Boundary Weed Management Committee of B.C., the project obtained one year of funding from the Cattle Industry Development Council of B.C., the Kettle River Stockman's Association, and the Southern Interior Stockman's Association. De Clerck-Floate hopes to continue the project for another 3 years and will be looking at bringing more groups on board for funding support.

The Boundary Weed Management Committee and interested ranchers have provided houndstongue seed for planting. The B.C. Ministry of Forests will provide some of the needed 'seed' insects from their insect propagation facilities in Kamloops, and the remaining insects to be seeded into the weed crop in June 2003 will be produced at the Lethbridge laboratory this winter.

Biocontrol research at Agriculture and Agri-Food Canada's Lethbridge Research Centre illustrates the government's commitment to promote innovation for growth, maintain security of the food system and protect the health of the environment, as proposed in the new Agriculture Policy Framework. The framework aims to increase profitability for producers by giving them the tools and capabilities to respond to constantly changing consumer demands for safe food produced in an environmentally responsible way.

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Impasse Dogs Houndstongue Biocontrol in the USA

Invasive weeds do not recognise national boundaries, so the cattle ranchers of Montana, USA have the same problems with the weed houndstongue (*Cynoglossum officinale*) as their Canadian neighbours in British Columbia. However, due to differences between the two countries in how applications to introduce biocontrol agents are processed, US scientists have been unable to introduce some biocontrol agents that have been approved and released against houndstongue in British Columbia.

Although the majority of host specificity testing of houndstongue biocontrol agents has been funded by British Columbia, Montana has also provided substantial funding to this effort. Following completion of host specificity tests on the houndstongue root weevil, *Mogulones cruciger*, Montana and British Columbia jointly submitted a petition to introduce the weevil in 1996. The petition was reviewed by the US Technical Advisory Group for Biological Control Agents of Weeds and the corresponding Canadian review committee. In April 1997, both committees recommended the petition be approved. The weevil was released in British Columbia in 1997 and is proving to be an excellent biocontrol agent.

However, similar progress was not made in the USA. The US Fish and Wildlife Service, which also reviews all US biocontrol agent petitions due to its mandate to protect endangered species, expressed concern about the potential impact of this insect on a related, endangered plant species in southern Texas. This concern has stopped all progress on the *M. cruciger* petition in the USA. Progress with other weed biocontrol agent petitions will likely be delayed or stopped for similar reasons. In view of this, Montana recently suspended the funding of biocontrol agent screening on most weed projects until the bureaucratic impediments are reduced. The fact that Montana, a national leader in biocontrol of weeds, has suspended most biocontrol agent screening activity says a lot about the seriousness of these bureaucratic problems.

Ironically, the weevil was introduced just north of the Montana border, so the insect will undoubtedly enter the USA on its own in the near future. While the weevil's dispersal into the USA will be welcome to weed managers, the bureaucratic conflict remains. It is obvious that for biocontrol to proceed in the future, the agencies reviewing the petitions will have to consider risk assessment. The damage done by the weed and the benefits of a biocontrol agent must be weighed against any potential risks if we are to implement long-term, cost-effective weed management and support healthy ecosystems.

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Banana Endophytes: Potential for Pest Biocontrol

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 banana of 90 million tonnes is in Africa; Uganda alone produces ten million tonnes and is considered one of biggest banana producers in the world. In the East African Highlands, the highest per capita consumption of banana in the world is found. Edible bananas include diploids, triploids and tetraploids with a range of end uses including dessert, cooking, roasting and brewing bananas. In East Africa, for example, cooking banana is a leading staple food, while brewing bananas are also widespread.

The above-ground part of the banana plant consists of a pseudostem, which is formed by the leaf petioles, the leaves themselves and the inflorescence. The underground part of the banana is a corm that can clearly be differentiated into the central cylinder and the cortex. Primary roots emerge in groups of three or four from the central cylinder and spread horizontally to a distance of up to 5 m. Most roots are found in the top soil at depths between 15 cm and 60 cm. The main function of the roots, beside water and nutrient uptake, is anchorage of the plant. The corm produces roots and lateral shoots which can be classified as peepers and sword and maiden suckers. The last two are the preferred planting material. Bananas are propagated vegetatively; therefore, all distinct bananas are clones. A banana mat usually consists of a mother plant and one to several suckers.

Banana is a perennial plant and produces several generations of growth cycles; the first is called the plant crop, and the succeeding cycles the ratoon crops. In Uganda, fruit is available year round and besides being an important staple and food security crop, banana is also an important cash crop. Banana is considered a hardy crop; nevertheless, declining yields in the East African Highlands have been reported in recent years. Major biotic production constraints of cooking banana ('matooke') as identified by the Uganda National Banana Research Programme (UNBRP) in collaboration with the International Institute of Tropical Agriculture (IITA) are the banana weevil (*Cosmopolites sordidus*) and plant-parasitic nematodes.

The banana weevil is considered the most important pest of cooking banana and 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. Weevil eggs are inserted singly into the leaf sheaths or corm at a rate of up to two eggs per week. The larvae feed within the corm cortex, central cylinder and, occasionally, the pseudostem thereby destroying the banana plant. The insect passes from egg to adult in 6-8 weeks. Population build-up is slow because of low fecundity and, therefore, weevil problems are most pronounced in ratoon crops. Crop losses due to weevil attack can reach 44% in the third ratoon. Weevil population studies found only a weak relationship between adult weevil density and damage. This suggests that control strategies targeting weevil immature stages may be more effective than those directed at adults.

A complex of different nematode species causes destruction of banana roots. The burrowing nematode (*Radopholus similis*) is considered the most destructive nematode species to banana roots worldwide. Disease severity is affected by a number of environmental factors but usually correlates with the number of nematodes present in the roots. *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. Yield is reduced and the vegetative cycle is lengthened. Uprooting or toppling is the most obvious symptom and it is the major cause of yield loss in banana and plantain. Toppling can be confused with snapping, caused by the banana weevil, and nematode damage is therefore often underestimated. Crop and ratoon losses due to nematode attack in banana under Ugandan conditions can reach 50% in on-station trials. The banana weevil and nematodes are moved from one field to another with infested planting material. The best way to start a new plantation is therefore to use clean planting material. Tissue cultured bananas are the best source of clean planting material and are now widely used in commercial plantations all over the world. Plants derived from tissue culture can be produced in large quantities on demand and they have higher yields because of bigger bunches and earlier maturation than conventional sucker-derived material. Because they are produced under sterile conditions, all pests and most pathogens, except for some banana viruses, are eliminated. However, in most cases bananas are planted in infested soils and reinfestation with weevils and nematodes remains a concern. Therefore combining the benefits of clean planting material with a biological control agent seems favourable.

Hidden Strengths of Endophytes

The use of endophytic fungi may offer such an approach and, from both an environmental and an economic point of view, has a major advantage over other biological control agents that are applied directly to the soil. The latter, due to the high levels of inoculum needed to treat the soil, are more costly, have to be applied more frequently, and their efficacy is often strongly influenced by environmental factors. Another advantage is that endophytic fungi live in plant tissue, thereby reducing the risk of side-effects on non-target organisms including crops and humans. The application of indigenous strains further limits environmental risks.

Endophytes are defined as organisms that, at some time in their life cycle, live symptomlessly within plant tissues. Clearly, latent pathogens and opportunistic fungi that occur alongside mutualistic fungal endophytes must not be included in a biological pest control approach using endophytes. Mutualism is the symbiosis of two organisms in which both organisms benefit from each other, either through nutrient cycling or protection. It is assumed that mutualistic endophytes have evolved from plant pathogenic fungi and that most if not all higher plants host endophytic fungi. Of these, the grass-endophyte associations have become the best-known of interactions between endophytes and plants. Grass endophytes have in many cases developed mutualistic relationships with their hosts and in some cases can protect the plant from herbivory.

Endophytes in the fungal family Clavicipitaceae are important organisms in grasses and produce a number of toxic substances *in vitro* as well as *in vivo* that are suspected to confer insect resistance. From that point of view, these associations can be termed mutualistic. However, some of the compounds that have an effect on insects such as the ergot alkaloids also have detrimental effects on mammals. Rye grass staggers, fescue foot and sleepygrass are some of the conditions caused. This has been a limiting factor for the utilization of these fungi in biological control.

Endophytes of plants other than grasses are substantially different. They generally belong to different fungal families, orders, or even classes to the grass endophytes and consequently exhibit different lifestyles. Their presence has been proven in all plants investigated including important crops, e.g. rice, maize, tomato and banana. Interactions of these fungi with their host plants and other organisms are generally less understood than for grass endophytes. The presence of systemic infection by endophytic fungi has not yet been demonstrated in these plant groups but must not be excluded. Similarly, the involvement of secondary metabolites in the control of plant pests, as shown for grass endophytes, has not been elucidated for endophytes of crop plants. However, fungal endophytes have been shown to produce more biologically active secondary metabolites than fungi from other habitats.

Endophyte Research to Support Banana IPM

Whereas most studies of endophytic fungi have concentrated on aerial parts of plants, research at IITA in Uganda on banana endophytes focuses on fungal endophytes of roots and corms, which are subject to devastating attack by banana weevil and nematodes. Biological protection of these plant parts would therefore considerably increase banana plant health.

In a study on fungal endophytes from roots and corms of the important banana clone Pisang Awak (*Musa* ABB) originating from Thailand, it was found that the most frequently isolated – and maybe the most important – genus appears to be *Fusarium,* followed by *Acremonium*. The dominant species in this study was *F. oxysporum.* This species was reported as an endophyte of many crop plants and as an effective colonizer of plant roots. However, the fungus is also notorious as the causal agent of *Fusarium* wilt of many crops; these are distinguished as specialized forms and physiological races. Nevertheless, the majority of isolates of *F. oxysporum* are non-pathogenic.

It was further found that in root, and to a lesser extent corm, tissues typical soil fungi belonging to the genera *Penicillium*, *Aspergillus* and *Gongronella* constitute large proportions of the fungi isolated. Some fungal genera with biological control potential such as *Trichoderma* were infrequently isolated from plant tissue. In this study, no entomopathogenic fungi (i.e. fungi such as *Beauveria bassiana* that directly attack insects) were isolated from banana. It appears that few fungal genera and species dominate the endophytic fungal community of banana.

Vegetative compatibility grouping (VCG) has been used to determine the relationship of selected endophytic isolates belonging to *F. oxysporum* with tester strains for different specialized forms including the ones for the banana wilt fungus, *F. oxysporum* f. sp. *cubense*. Isolates within one VCG represent a genetically isolated population and they usually share the same genes for, e.g., pathogenicity. None of the endophytic isolates was vegetatively compatible with the pathogen or caused disease in susceptible banana clones and other crops such as sweet potato and tomato. Using VCG, it was also found that non-pathogenic isolates form a heterogeneous part of the *F. oxysporum* population and that most isolates tested for activity against the banana weevil and nematodes were not closely related. From these studies it was also concluded that different strains of the fungus can colonize the same plant, although different plant tissues. The extent to which the same plant tissue, e.g. the corm, is colonized by different strains of *F. oxysporum* is currently being investigated.

A number of unidentified fungal isolates from healthy roots were used in *in vitro* screening experiments at IITA and the University of Bonn, Germany. All isolates originated from Uganda and were mainly isolated from East African Highland banana clones. In those experiments, culture filtrates of the endophytic isolates were tested for their activity against weevil eggs and larvae and vermiform stages of *R. similis*. Inactivation or mortality of nematodes or weevil larvae exposed to these culture filtrates were recorded at certain intervals. Secondary metabolites produced by the fungi had inactivating or killing effects on both weevil larvae and nematodes. Spore suspensions applied to weevil eggs resulted in mortality rates of up to 80-90 %. In most cases, effective isolates were identified as *F. oxysporum*. Other isolates that produced entomotoxic or nematotoxic metabolites belonged to *F. solani*, *F. concentricum* and *Acremonium* spp. Out of 15 isolates that produced highly toxic culture filtrates to nematodes, eight belonged to *F. oxysporum*. All these isolates could be identified as distinct strains on the basis of vegetative compatibility. Although these screening experiments do not necessarily reflect the situation *in planta*, they were a necessary and useful step in the selection process of candidate strains.

In pot experiments, endophytes were inoculated onto tissue cultured banana plants and plants were subsequently challenged with weevils or nematodes. Inoculation of endophytes into tissue culture banana can easily be integrated in the normal process of tissue culture plant production. Endophytes are usually inoculated at an early stage when plants are removed from the culture vessels and prior to planting in pots with soil. From there the normal weaning process is followed.

Inoculation of endophytes into tissue cultured banana and subsequent challenge with the banana weevil did not produce conclusive results. Although spore suspensions in *in vitro* experiments caused high mortality of weevil eggs, total numbers of weevil larvae were in most cases not affected. However, it was observed that the ratio of small to large larvae was higher in endophyte treated plants compared to the control plants. Endophyte treated plants also showed less weevil damage expressed as percentage of destroyed corm tissue. From this it may be concluded that weevil development is affected by the endophyte treatment. There seems to be a cultivar influence on endophyte performance or host plant reaction towards endophyte inoculation. *In vivo* screening experiments are on-going at IITA in Uganda to answer these questions.

More information is available on endophytes for the control of banana nematodes. Endophyte inoculation into tissue cultured banana resulted, depending on the banana cultivar and the strain used, in reduced nematode reproduction in pot experiments. Plants in these experiments were usually between 4 and 6 months old. Nematode numbers in endophyte treated plants were reduced by more than 30% over the controls in some but not all banana clones. Nematode damage, expressed as a percentage of necrotic root tissue, was also reduced depending on the clone and strain used. The number of nematode females was reduced in some East African Highland banana cultivars inoculated with fungal endophytes. The reasons are not yet established but it may be speculated that development of nematode juveniles into females is affected by the endophyte inoculation. The fact that not all clones responded equally to the same isolate is an indication that endophytic strains need to be compatible with their host. Therefore, the best combination of cultivar or clone with an endophytic isolate needs to be identified and remains a research priority.

In a few cases, increased plant growth was observed although this should not be seen as the principal effect of endophytes on banana plants. The mechanisms leading to the effects on weevil development and nematode control have not been clearly identified. Production of secondary metabolites, competition for nutrients or induced resistance may play a role. Fungal infection of weevil larvae and nematodes or their eggs has not been observed *in planta* but must not be excluded. Long-term control will require persistence of the fungal endophyte in the plant or the activation of effective and durable plant defence responses. Currently the mechanisms leading to reductions in pest levels are being investigated at IITA in Uganda.

Since the frequency of re-isolation of the strains tested on banana plants so far decreases over time, possibilities for long-term protection in a perennial crop may not be strong. Initial results suggest, however, that endophytes can provide protection in the first growth cycle. This may be sufficient to give a perennial crop such as banana a head start. Until endophytic isolates are found that effectively colonize the suckers of the ratoon crop, and until the extent to which the surrounding soil influences the composition and distribution of root and corm endophytes is known, durable biological control in banana will not be possible with the use of fungal endophytes alone. The use of fungal endophytes for the biological control of the banana weevil and nematodes must be seen as one component of Integrated Pest Management (IPM).

By: Bjorn Niere, Cliff Gold and Danny Coyne

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Insider Knowledge: Endophytes for Cacao Disease Biocontrol

Cocoa is a major global commodity crop, with 3,410,022 tonnes of beans, with an estimated value of US$2,259,401,000, produced annually. The main cocoa production takes place within 8° of the equator. The major cocoa producing regions are West Africa, Latin America and Malaysia; 60% of world cocoa production is in West Africa with Côte d'Ivoire accounting for approximately 40% of global output.

Cocoa is a product of the cacao tree (*Theobroma cacao*) which is considered to have evolved in the Upper Amazon region of South America. It grows as an initial single stem which branches at 1-2m to form a jorquette and if unpruned can reach a height of 15 m. It is cauliflorous in habit with the flowers and fruit (pods) borne on the stem and branches. The pods reach maturity after 4-6 months and contain seeds (beans) inside a white mucilage; these beans are processed to produce cocoa.

Threats to Production

There is an increasing demand for cocoa in the food and cosmetic industries in developed countries. Current land under cultivation is unlikely to be extended into new areas; any increase will require increased productivity. One of the main biological constraints to increased cocoa production is disease, in particular fungal diseases. The three major fungal diseases of cacao are black pod, witches' broom and frosty pod.

Black pod is caused by various *Phytophthora* species. It is characterized by rotting pods and associated cankers and results in an estimated 44% loss in production worldwide. The main pathogen globally is *P. palmivora*, with the more aggressive *P. megakarya*, which is still in an invasive phase, threatening cocoa production in West Africa.

Witches' broom disease caused by *Crinipellis perniciosa* is presently restricted to Latin America and is found in all cacao growing areas of South America, Panama and Trinidad. It results in 21% loss of cocoa production globally. It is still in an invasive phase, threatening the cacao growing areas of Central America. *Crinipellis perniciosa* infects meristematic tissue resulting in hypertrophy and hyperplasia in vegetative parts of the plant (vegetative and cushion brooms) and rotting of the pods.

Similarly, frosty pod rot due to *Moniliophthora roreri* is currently restricted to Latin America – Ecuador, Peru, Colombia, Venezuela, Panama, Costa Rica, Nicaragua, Guatemala and Honduras. It is estimated to cause a 5% loss of world production. Frosty pod is still in an invasive phase, and threatens the cacao growing regions of Brazil, Bolivia and Mexico. The disease appears as a mass of cream-coloured spores on the pod surface. Again it is similar to witches' broom disease in infecting the meristematic tissue of the pod resulting in destruction of the beans.

Control of black pod caused by *P. megakarya*, frosty pod rot and witches' broom disease through conventional cultural and chemical control has been woefully inadequate. These pathogens are still in an invasive phase locally and pose a potential threat internationally. Although plant breeding offers a means to control the diseases in the long term, in the short term an alternative strategy is required. Biological control, in particular the use of endophytes, is being pursued to fulfil this role.

Endophytes: a Novel Strategy

Endophytes are known to occur in all plants so far studied. Mutualistic endophytes are of interest as potential biological control agents as they inhabit the host plant's tissues for all or part of their life cycle, asymptomatically. The relationship between these endophytes and their host is as yet not well characterized. However, it is considered that they can confer resistance to pathogens through a number of mechanisms: competitive exclusion, parasitism, metabolite production and induced resistance. A number of research groups are actively investigating the use of endophytes to control diseases of cacao.

Ideologically different approaches to the identification of sources of endophytic biocontrol agents, for the control of cacao diseases, are currently being studied: isolation from:

- Exotic agroecosystems
- The centre of origin, or diversity, of *T. cacao* and its pathogens

Seeking Old Allies

CABI *Bioscience* (led by Harry Evans), as part of a USDA-ARS (US Department of Agriculture – Agricultural Research Service) funded programme, is working within the principles of classical biological control in the pursuit of an endophytic biocontrol agent. This involves returning to the centre of origin or diversity of the host plant and its pathogens to identify co-evolved endophytes. It is hypothesised that, as endophytes do not appear to be transferred horizontally within woody perennials, once removed from the centre of origin to exotic agroecosystems the cacao loses its co-evolved endophytes and is colonized by local endophytes. However, the co-evolved endophytes may confer resistance to disease, while their local replacements do not. The classical approach has been successfully used in the past to control invasive weeds and alien insect pests, but this is the first time it has been deliberately employed to control plant disease.

Surveys have been undertaken in the centre of origin of *T. cacao* and *C. perniciosa* in the upper Amazon. Fungal endophytes have been collected from the woody stem tissues of *Theobroma* and the closely related *Herrania* species. Endophytes, from both woody stem tissues and healthy pods, have also been collected in western Ecuador from *T. gileri*, the purported original forest host of *M. roreri*. In addition, collections were made in exotic agroecosystems in Mexico, Costa Rica, Brazil, Ecuador and Peru.

Control of frosty pod rot and witches' broom is the primary aim of the studies being undertaken. However, an effective endophyte may confer resistance to all the major fungal pathogens of cacao.

Approximately 2000 fungal isolates have been collected and identified, with a high proportion of new species and even new genera being identified. This is an indication that these novel organisms could be a source of potential new biocontrol agents. Of particular interest are *Trichoderma*, *Clonostachys*, *Acremonium*, *Verticillium* and *Cylindrocarpon* species which have proven biocontrol activity.

Initial comparisons of isolates from *Theobroma* species in natural forest ecosystems and exotic agroecosystems, by ordinal analysis, have demonstrated that endophyte populations are more diverse within the natural forest ecosystem. This also suggests the endophytes from natural systems may be a source of novel biocontrol agents.

However, screening of the endophytes as biocontrol agents of *M. roreri* and *C. perniciosa* is problematic. Due to the potential mechanisms that an endophyte may employ to confer resistance, screening should, where possible, be undertaken *in planta*. However, this is complicated by the biology of the pathogens themselves; both are infective only in actively growing meristematic tissue.

For frosty pod this necessitates screening the endophytes on the actively growing pod, a limitation compounded by the window of infection being restricted to the first 3 months of growth. Small-scale field screening is required, and this is now being initiated in Ecuador in collaboration with INIAP (Instituto Nacional Autónomo de Investigaciones Agropecuarias) at Estacion Experimental Tropical, Pichilingue, Quevedo, Ecuador. These studies will look to identify those isolates with the ability to colonize pods and reduce frosty pod incidence.

Crinipellis perniciosa infects meristematic tissues in the pod and vegetative parts of cacao. This allowed the development of a model bioassay using endophyte-inoculated seedlings, which are subsequently challenged with basidiospores of *C. perniciosa* and assessed for the production of brooms. Screening of pre-selected isolates in the UK and in Brazil, in collaboration with Almirante Cacau demonstrated that the endophytes exhibited varying degrees of control. One isolate identified as *Gonytrichum* aff. *macrocladum* reduced witches' broom incidence by 100%. This is now undergoing further assessment.

Initial studies will involve application of the endophytes by spray application. In the future it is hoped that this inundative approach will be replaced by an inoculative method, the endophytes being applied to nursery or grafting materials to provide life-long resistance to disease.

Assessing Local Talent

Almirante Cacau (led by Alan Pomella), which is Masterfoods' cocoa research facility in Brazil, is investigating the potential of local endophytes from the exotic agroecosystem in Bahia, the main cocoa producing region of Brazil. In collaboration with the University of São Paulo (led by Dr João Lúcio Azevedo) a study has been investigating the fungal and bacterial endophytes on cacao in Bahia assessing the community diversity and its composition over time. Endophytes (134 fungi and 135 bacteria) have been isolated from branches of infected and non-infected trees. The bacterial isolates were grouped on morphological features, with certain groups being consistently recovered only from highly resistant cacao trees, an indication that these may be associated with disease resistance. In *in vitro* assays, 41 fungi and 24 bacteria were antagonistic to *C. perniciosa*, and subsequent glasshouse trials of these pre-selected endophytes demonstrated that three fungi and five bacteria provided 70% and 50-62.5% reduction in disease incidence, respectively.

In addition to this study isolates from Almirante Cacau, in particular isolates of *Trichoderma*, have been assessed in laboratory and glasshouse studies, using the model system developed with CABI *Bioscience*. Of 350 endophytes screened a *T. stromaticum* (FA 323) isolated from *Theobroma grandiflorum* has proved the most successful, consistently providing more than 70% reduction in disease incidence under controlled conditions. Its mode of action may in part be due to induced systemic resistance as enzymatic studies have demonstrated that the amount of total protein expressed in asymptomatic plants inoculated with this endophytic *Trichoderma stromaticum* and *C. perniciosa* is much higher than in control plants inoculated with *C. perniciosa* or water.

A new phase of research has been initiated with the recent collection of fungal and bacterial endophytes from *Theobroma cacao* and related species in Amazonia. These will be evaluated as part of a classical biocontrol strategy.

The ultimate aim of the research on endophytes is to introduce them into the agroecosystem. At Almirante Cacau studies are already underway to assess various application systems. Propagation of cacao is commonly achieved by the grafting of new genetic material, which is supplied to the farmer as budwood. The production of endophyte-colonized budwood could aid reductions in disease incidence as part of an integrated crop management package. Almirante Cacau have succeeded in inoculating budwood by dipping in an endophyte suspension for 60 minutes. An alternative means of propagation is with seed. It has been demonstrated that seeds can be inoculated with fungi in the model system currently being utilized by CABI *Bioscience* and Almirante Cacau. Almirante Cacau has also shown that it is possible to inoculate seed with bacteria by immersion in a bacterial suspension for 24 hours. For mature trees, application of endophytes through injection into the stem, using a high-pressure syringe at 20 psi, has been assessed. Early studies have shown introduced endophytes to move some distance from the point of injection. Further studies will assess the impact of the introduced endophytes on disease incidence in the field.

More research is being conducted in other parts of the world to assess the ability of local endophytes to control diseases of cacao.

The Smithsonian Tropical Research Institute (STRI) (led by Allen Herre) in Panama has been studying indigenous endophyte communities within the leaves of *T. cacao* on Barro Colorado Island, and in exotic agroecosystems in Bocas del Torro and Nombre de Dios, in Panama. They isolated more than 500 morphospecies from leaves. Pre-selected isolates have been assessed in glasshouse and field trials. In glasshouse studies application of endophytes to leaves was observed to reduce incidence of *Phytophthora* in those leaves inoculated, but no systemic resistance was observed. In field studies in Bocas del Torro, monthly application of endophytes to cacao significantly reduced the incidence of black pod caused by *Phytophthora* but failed to impact upon frosty pod rot caused by *M. roreri*. New trials are being initiated using new agents and mixtures to attempt to reduce the incidence of frosty pod rot.

The Institut de Recherche Agricole pour le Développement (IRAD) (led by Pierre Tondje) in Cameroon is investigating the possibility of using local endophytes to control *Phytophthora* disease, in particular the aggressive *P. megakarya*. They have collected fungal (1000) and bacterial (274) endophytes from the pods and leaves of cacao in cocoa farms. These endophytes have been screened in leaf disc and pod assays developed in collaboration with USDA-ARS. From this screening programme a number of potential endophytic biocontrol agents have been identified and are currently being assessed in field trials.

The Future

The development of endophytes as a control strategy is still in its infancy, but much basic research is underway. Identification of effective endophytic biocontrol agents for the economically important fungal diseases of cacao is being pursued. Indeed, some potential biocontrol agents have already been identified and are presently undergoing further assessment. A network of research groups is working towards the development of endophytic biocontrol as an environmentally sustainable option as part of future integrated crop management packages. Initial application may be augmentative/inundative but the long-term hope is to produce inoculated vegetative material from which the endophyte will provide life-long protection for the cacao.

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Brazilian Wasp Has Sweet Tooth

An exotic wasp from Brazil, which has a taste for honey and lays its eggs in maggots, may be a new weapon in the arsenal against nuisance and disease-causing flies for livestock and poultry farmers in the USA.

In the USA, biological control of filth-feeding flies (synanthropic Diptera) such as houseflies and stable flies has until now relied largely on a complex of native parasitoids in the genera *Muscidifurax* and *Spalangia*. Farmers can buy the parasitic wasps from commercial insectaries. Research suggests that such an approach could be successful if the right species and strains were applied in the right locality. One drawback of these native agents, however, is that they are all pupal parasitoids, feeding and laying their eggs only in the pupal stage. In theory, complementing these with species that attack other (egg and/or larval) stages could increase the level of control. Other approaches have been tried, including the use of pathogens and predatory mites, and inundative releases of parasitoids and predators.

Although partially successful, none of these strategies has become the sole method for fly control, and the wrong choice of a parasitoid strain may even have detrimental results. Instead, the focus has been on integrated controls including methods such as cultural control, adult baiting and aerosol treatments with short residual insecticides. However many biological control agents have not been thoroughly surveyed, nor their potential adequately assessed.

A gregarious larval parasitoid, *Tachinaephagus zealandicus*, from Brazil has been exciting increasing interest. Over the last few years USDA-ARS (US Department of Agriculture – Agricultural Research Service) scientists at the Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida, and cooperators at the University of Campinas in Brazil have been evaluating this wasp as part of an effort to screen Brazilian wasp species that may be biocontrol candidates against flies in the USA. *Tachinaephagus zealandicus* may have considerable potential for biological control as the range of habitats it utilizes is considered unparalleled by any other fly parasitoid, and it is particularly promising in the US context as it attacks flies in the larval stage. It does not thrive in hot conditions (>35ºC), but could be useful in the cooler months in the southern USA, or throughout the summer in the north.

Unlike the native US species, which feed on their immobile pupal hosts in order to develop their eggs, *T. zealandicus* does not host-feed. Females have viable eggs when they emerge, but they need a further energy source to be able to sustain further attacks. Identifying the food source of a parasitic wasp is often difficult and can create an obstacle to rearing and mass-rearing which sometimes proves insurmountable – but not in this case. The team working on *T. zealandicus* has found that feeding the wasps on honey tripled their attack rate on target flies in the USA and increased the number of progeny developing in them. Interestingly, the team found that honey added for 2-3 days to the diet of native wasps increased their attack rates and longevity compared to those just exposed to pupae.

Honey also proved to be a useful vehicle for administering medicine: honey treated with the antibiotic rifampicin helped *T. zealandicus* ward off disease caused by a newly identified *Nosema*, which is transmitted from females to their offspring, and spreads between larvae feeding on the same host. Infected wasps take longer to develop into adults and lay substantially more male eggs – a real barrier for rearing wasps for biological control, and especially in a commercial environment. Treatment allowed new, disease-free colonies to be established using uninfected females. It is harder to treat the native wasps in this way because, although they will feed on honey, they are not obligate feeders on anything but the hosts.

Although it has been found in the wild in the USA before, and no problems were reported, *T. zealandicus* is currently being evaluated in quarantine to rule out non-target effects, before an import permit is sought so that it can be used as a biological control agent against muscoid flies.

Sources: Agricultural Research Magazine, August 2002, Attacking flies with wasps.
www.ars.usda.gov/is/AR/archive/aug02/flies0802.htm

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Agricultural Bioterrorism: Reconciling Security and Research

As USDA-APHIS (US Department of Agriculture – Animal and Plant Health Inspection Service) issue a 'select list' of nine plant pathogens being considered for regulation under the Agricultural Bioterrorism Act 2002, the October feature article on the American Phytopathological Society (APS) website calls for a balance to be struck between strategies for countering agricultural bioterrorism. In addition, while it recognises positive developments, it also outlines continuing unmet needs for increasing crop biosecurity.

In 'Crop biosecurity and countering agricultural bioterrorism: responses of the American Phytopathological Society' the APS notes that sessions at its 2002 annual meeting revealed two fundamentally different strategies for assuring crop biosecurity: *prevention* (increased security, secrecy and border protection) and *preparedness* (early detection, rapid diagnosis and rapid recovery). It argues that while both are important, too much focus on security and secrecy will impair the necessary free and open conduct of science and information exchange fundamental to both prevention and preparedness. It notes that the new US legislation focuses mainly on prevention, whereas the scientific community has and continues to focus on preparedness, including preparedness as a means of prevention.

APS calls for the select list of plant pathogens to be limited to currently exotic pathogens of US crops, and be subject to periodic review based on scientifically based risk assessment procedures. (It offers to examine its editorial policies with respect to new information on pathogens on this list submitted for publication in its journals.) However, APS declares itself against including on the list any pathogen already present in the USA and under some level of management, including exotic or genetically modified strains of endemic pathogens. It argues that this would have counterproductive effects on the very research and international cooperation needed to limit existing and naturally occurring crop damage. It adds that although determination of a broader threat list of exotic and endemic plant pathogens for preparedness purposes would be valuable, there should not necessarily be any restrictions on many of these agents.

APS also identifies capacity building initiatives crucial to improving preparedness, including:

- A regionalized system of diagnostic laboratories
- A national plant disease centre providing a service analogous to that provided for human health by the Centers for Disease Control and Prevention
- Personnel training
- Continued enhanced funding for plant pathogen genomics

It argues that such institutions and initiatives will be invaluable to US agriculture regardless of whether the pest or disease threat comes from criminal activity or from the multitude of naturally occurring, emerging, or re-emerging plant diseases or pests that farmers and scientists come across each year.

APS acknowledges that a balance needs to be struck on the level of detail and timing of information sharing, confidentiality and public access to information on organisms of bioterrorist potential, but it concludes that the risk that such knowledge might increase the vulnerability of agriculture to bioterrorism is small compared to the enormous contributions to both the prevention and preparedness strategies of continued free and open exchange of the information produced from genomics and all other basic research on plants and microorganisms.

American Phytopathological Society
www.apsnet.org/

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Rabbits and Possums in GMO Potboiler

Advances in biotechnology mean that the dream of creating 'designer solutions' for intractable problems in many fields, including agriculture and the environment, is rapidly becoming a reality. However, unless action can be agreed to resolve conflicts of interest, some of the dream could fade. Recognising this, researchers into biotechnological methods for managing wild European rabbit (*Oryctolagus cuniculus*) populations from around the world are coming together at a symposium (see final section) to try and agree on ways of reconciling conflicting needs to suppress or enhance rabbit populations in their respective countries. In the process, they may both draw on the experience of classical biological control and contribute fresh ideas.

This article highlights inter-country (transboundary) issues raised by the development of disseminating GMOs with reference to rabbits and possums, and discusses how they relate to experience with classical biological control and invasive alien species. It looks at guidance on assessment of risk in these two areas and considers how that might be developed for disseminating GMOs. It also discusses obligations under international agreements.

Conflicts of interest have featured previously in the *BNI* news section, notably in relation to classical biological control of invasive alien species (IAS) such as black wattle in South Africa and cactus in the New World. Current interest in developing genetically modified (GM) biocontrol agents brings a new dimension to the topic. Research into the development of disseminating (i.e. self-replicating and self-spreading) genetically modified organisms (GMOs) to manage populations of exotic vertebrate pests is being undertaken in a number of countries. In Australia, for example, a variety of targets includes European rabbit, house mouse, European fox, cane toad and carp [also see *BNI* **21(4)**, 89N-93N (December 2000), Mammal biocontrol: the hunt continues; and *BNI* **22(3)**, 58N-59N (September 2001), Cane toads, possums and GM].

There are parallels between the new technology and classical biological control, which over the last century has chalked up some striking successes against introduced pests through the introduction of natural enemies, mostly from the area of origin of the pest. Both technologies involve the potentially irreversible introduction of an organism. Thus, before an introduction, both need comprehensive assessment of the likely outcome of the introduction compared with alternative options (doing nothing or using other control measures). Both also would benefit from international agreement on methodologies and criteria for assessment of what is safe and acceptable. Classical biocontrol, as the more established method, may have some useful experience, borne sometimes of hard lessons, to impart to the emerging technology. There are also differences, which mean that protocols developed for evaluating the safety of classical biological control agents are not always applicable to disseminating GMOs. Classical biological control, in the large majority of cases, uses a natural enemy from the area of origin of the target species (an 'old association'). The confidence with which scientists can predict the likely outcome on the target population of introducing such an organism is based in part on field evidence from their common area of origin. Even in the rarer case where a natural enemy is introduced from an area outside the target's native range (a 'new association'), there is generally field data on its ecological interactions with its natural host, which is usually a close relative of the target species (and if not, this has to be taken into consideration in the risk analysis process, which is discussed further below). In the case of a GMO, the association with its host is also 'new'. The genetic modification may have little or no impact on the biology of the host-parasite interaction at the level of the organism (extensive testing is conducted to examine this). However, a GM agent that reduces natality or increases mortality in the target population could (and should if the agent is to be effective) have a significant impact on the target's population dynamics, because these are two of the fundamental processes affecting recruitment or loss of individuals to a population. The complexity of the interactions between the processes means that a (more or less subtle) alteration to one of them could have unpredictable consequences on population levels. The effects of the genetic modification on the ecology of the target, and to some extent ecosystem, can be modelled, and studied in lab and field trials (necessarily small-scale, to permit appropriate levels of quarantine), but there is no field experience to draw on to establish the realism of the models or the trials in the context of the more complex entire ecosystem.

In addition, risks associated with onward spread from the area of introduction differ. Natural enemies for classical biocontrol are generally sought in the native range of the target pest, but a GM agent has no native range because it does not occur in nature – although its potential range is the same as that of the 'natural' (unmodified) species. Both classical and GM biocontrol agents would have a novel impact in the area of introduction, and in areas they spread to from there. In areas of onward spread, the effects of either might or might not be desirable, depending on the status of the target species in these new areas. However, for a GM agent the area of onward spread includes the area of origin of the target species, whereas that would be (in) the home range of an 'old association' classical biocontrol agent. The key difference between the risks of introducing a GM agent and a classical biological control agent taken from the native range of the host species is the potential impact of the GM agent if it reached populations in the area of origin of the target. For example, many of the GMOs under development reduce the fertility of females by inducing immunocontraception or immunosterility. While this may be desirable in a country where the species is a pest, it is not desirable everywhere, and could have disastrous consequences should the GMO spread to the native range of the target species. Consequently, although in most cases the area of origin of a species and the area where it is an introduced pest are sufficiently isolated from each other to prevent natural spread, export prohibitions on both the pest (in case it harbours the introduced GMO) and the GMO itself are crucial. However, the illegal introduction of the (naturally occurring) virus that causes rabbit haemorrhagic disease (RHD) into New Zealand following its government's decision not to introduce RHD shows that, like all quarantine measures, movement controls may not be able to provide complete protection against the spread of organisms, GM or otherwise. Scientists must also get the science right in order to minimize the consequences of accidental or deliberate spread. For example, scientists researching a disseminating GMO to protect rabbits against disease in Spain (see below) showed in the laboratory that the GMO was capable of only a limited transmission, so an ever-expanding epidemic would not be expected. Such a tool could be considered for managing populations on a local level. The researchers note, however, that this would need to be demonstrated in natural field conditions too (and other risks, dealt with below, would also need to be addressed).

The case of the European rabbit provides a good illustration of conflicts that need to be addressed during the development of disseminating GMOs. The rabbit is native to Spain, where it is conserved and managed as a resource for hunting and as a natural prey for endangered predators. In some other parts of Europe and in many southern hemisphere countries (Australia, New Zealand, Argentina and Chile, for example), it is a serious introduced pest. The potential for conflict is revealed starkly by recent interest in developing disseminating GMOs to manage wild rabbit populations. In Australia, research is being conducted on using a modified myxoma virus to disseminate immunosterility in female rabbits, while in Spain research on a different disseminating

GMO (but again a modified myxoma virus) to disseminate protective immunity to RHD and myxomatosis gave promising results in an island field trial. A report on the Spanish GMO is currently with the European Agency for the Evaluation of Medicinal Products (EMEA), and the timescale for further research is not known. The work raised awareness in both countries of the international implications of deploying such agents. The undesirable potential consequence of a Spanish GM virus entering Australia could be the incapacitation of the only effective broadscale controls on the rabbit: myxomatosis and RHD, both vitally important tools for managing rabbits over huge areas. Similarly, should the Australian GM virus reach Spain, the already-threatened rabbits and endangered predators dependent on them could be further threatened.

In Australia, the rabbit is famously responsible for the widespread destruction of native ecosystems, and its control is seen as crucial both to improving rangeland and to restoring and preserving the country's unique indigenous flora and fauna. Mulga (*Acacia aneura*) is an important and widespread tree in Australia’s semi-arid and arid rangelands, but the species is threatened with a marked reduction in distribution and abundance by the combined effects of rabbits and domestic and feral livestock. Mature mulgas are generally too tall to be killed by this onslaught, but seedlings and juveniles are not. Seedlings are especially vulnerable as they have very little resistance to grazing and in addition are sought out by rabbits. The result in some areas is a complete or partial absence of regeneration, and mulga will disappear from landscapes where existing trees senesce and die without adequate replacement. Many other palatable shrub and tree species are similarly affected in Australia’s rangelands. RHD (introduced – prematurely through accidental escape – as a biocontrol agent in Australia) has significantly reduced rabbit populations in Australia as a whole. However, research in the Gammon Ranges National Park in the Flinders Ranges of South Australia investigated the threshold density of rabbits that would allow palatable perennial trees to regenerate. Domestic livestock are excluded from the park, although some feral goats and feral donkeys are present. Results collected over 25 years from fenced exclosures that allow only rabbits inside indicated that average densities may need to be below one rabbit per *square kilometre* to stop them eating more than 50% of newly germinated mulga seedlings in their first 6 months of life. Such an extraordinarily low density may not be necessary in all areas, but where it is, clearly more effective layers of control are still needed. GM agents might prove a powerful tool in this regard, and one that could be integrated with existing forms of control.

Paradoxically, in Spain the rabbit is considered fundamental to the conservation of threatened species, and the emphasis is on restoring rabbit populations decimated by myxomatosis and RHD. The Iberian lynx, *Lynx pardinus*, has disappeared from two-thirds of the territory it occupied 30 years ago as a result of habitat loss, a reduction in rabbit populations (its main prey), progressive fragmentation of its populations and human-induced mortality. Only about 400 are believed to survive, and IUCN (the World Conservation Union) has identified it as the most critically endangered cat species worldwide. As part of a package of measures to protect it, the Council of Europe (CE) Standing Committee of the Convention on the Conservation of European Wildlife and Natural Habitats (the Bern Convention) recommended in 1991 that the density of rabbit populations throughout the lynx's range should be increased, and subsequently an action plan has been formulated. Similarly, the western Mediterranean endemic and globally threatened Spanish imperial eagle, *Aquila adalberti,* has only some 150 breeding pairs remaining in the wild, all in Spain. The population decline in Spain is attributed to a number of factors, in which habitat destruction, poisoning and electrocution on power lines figure highly, but also significant is population reduction of rabbits, which form 50-70% of prey. Bird Life International, on behalf of the EU (European Union), has drawn up an action plan which includes increasing rabbit populations, as number of rabbits was shown to be one of two factors (with age of birds) most affecting the eagle's breeding success.

Countries with rabbit management concerns share a common interest in developing an understanding of the biology and ecology of the rabbit and of the interactions between rabbits and their resources, diseases and parasites. Such knowledge may be applied in Spain to conserve rabbits and in Australia to reduce their numbers, and this divergence may result in conflict over methods developed to achieve these ends. In considering the development of a management strategy based on disseminating GMOs, countries would need to take into account the possible spread of the GMOs beyond their borders, and the impact they could then have on rabbit management elsewhere. In the case of rabbit GMOs, Australia and Spain would have an interest in protecting themselves against any GMO being developed by the other, but neither could expect the other to assist it in this regard unless each were prepared to reciprocate. The countries would therefore have a mutual interest in developing a workable solution.

What Does 'Safe' Mean?

Deliberate introduction is the bread-and-butter of classical biological control. Risk analysis to develop appropriate management options for introductions is a familiar process for anyone working in this field. Many issues that have to be addressed for disseminating GMOs are fundamentally similar to non-GM biocontrol: what are the likely hazards from the introduction of the novel organism, what would be the consequences and what mechanisms are needed to minimize the risk? Others, such as acceptability, are similar but probably more significant for GM biocontrol.

Some of the risks posed by GM biocontrol agents are not unique to biocontrol and are already being considered comprehensively during development. These relate mainly to the potential for a GMO to exchange its genetic material with taxonomically similar sympatric species through reproductive means. Stability of induced genetic change is perceived to be of more importance for GMOs in general than for traditionally bred organisms. If there is judged to be a high risk of adverse consequences in this context, a decision not to continue to develop or release a disseminating GMO could be taken. Another area of risk, for both GM and classical biological control, is the potential for biocontrol agents to attack/infect/feed on non-target species (notably species related to the target pest). This is assessed by host-specificity testing (see below), and many initially promising biocontrol agents fall at this hurdle. Biocontrol researchers accept that risk analysis is a key element in development of agents for release. Accepting that safety comes first is one thing; agreeing what is safe is another.

Central to the issue of safety is the interpretation of risks. Because risk implies uncertainty, it is likely that different countries, made up of independent stakeholders with potentially conflicting interests, will reach different conclusions when conducting risk assessments. Even when the findings of the risk assessment are the same, for example in similar climatic conditions with similar host availability, countries may interpret the results differently. Some types of risk are more acceptable to one country than to another. The right to sovereignty in setting acceptable level of risk is a fundamental principle of the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO/SPS).

However, the need for more detailed harmonized guidance on assessment of risk for biological control has been recognised. Initiatives for crop pests (including weeds and diseases) may provide models that could be adapted for vertebrate pests.

The WTO/SPS recognises three pre-existing international technical organizations as the standard-setting bodies providing details to the conceptual Agreement. One of these, the International Plant Protection Convention (IPPC), with a Secretariat in FAO (Food and Agriculture Organization of the UN), Rome, develops technical guidance through International Standards for Phytosanitary Measures (ISPM) but only in regard to phytosanitary risks. Since 1996 biocontrol has had a general international protocol, Code of Conduct for the Import and Release of Biological Control Agents (ISPM No. 3), for countries implementing classical biological control to apply directly or use as a model for national regulations. This lists the responsibilities of government authorities and exporters and importers of a biocontrol agent, but does not set out in detail how to assess whether a proposed introduction is safe (although publication of detailed guidelines for conducting a biological control programme has been proposed). Host specificity testing is central to assessing the safety of a proposed classical biocontrol agent, and has long been fundamental to weed biocontrol because of the early acknowledgement of potential risks to non-target plants, especially crops. Protocols for weed host-specificity testing have been developed over the decades by experts in the field and are now being refined for specific conditions. More recent has been the development of similar protocols for insects, which are now well-advanced, but consideration of microbial biocontrol agents is still at an early stage. The ISPM No. 3 is timetabled for revision in 2003-2004 and may provide greater detail on some of these issues. Examples of biocontrol of plant pests and weeds do not apply directly to vertebrate pests, but may offer useful ideas.

The Office International des Epizooties (OIE), another of the three standard-setting bodies recognised by the WTO/SPS, develops guidance on pests and diseases of animals. Its International Animal Health Code contains standards, guidelines and recommendations designed to prevent the spread of infectious agents and diseases pathogenic to animals and humans into the importing country during trade in animals, animal genetic material and animal products. The OIE Animal Code does not deal with the introduction of mammals that are themselves 'pests' nor with the biological control of such pests. It does refer to biologicals for veterinary use (Chapter 1.5.3), including the exemplary category of conventional or genetically modified microorganisms (no. 15 in 1.5.3.2). While the Animal Code addresses the need for quality assurance in manufacturing practices for production of vaccines (Chapter 1.5.2.3), this section focuses on other biologicals and recommends quality assurance of all stages of manufacture, not only testing of the final product.

A new or revised standard may be requested by a member country, its Regional Commissions, the International Committee of OIE, or an international organization with which OIE collaborates. Guidance on any form of control of unwanted mammals that are pests would be a whole new area for OIE but inclusion of this concept has been considered by OIE because no other convention or international body comprehensively covers this area.

Beyond that overall topic of biocontrol of mammals that are pests, the OIE has not yet tackled GMOs as a category or provided guidance related to GMOs within its existing topics. Any code it established relating to GMOs may need to deal with the development/constitution of the GMO itself (as with the manufacturing process for vaccine and biologicals referred to above) in addition to the sanitary and trade-related issues that occupy much of its present business. Although the GM biocontrol issue raised here does not fit easily into the OIE's current expertise, there has been increasing interest in guidance regarding wildlife diseases, and the issues of development and shipment of other biologicals may provide some interesting points regarding safety in the production process.

All GMOs that could cause injury to plants may soon be subjected to an additional process under the IPPC through a supplement to ISPM No. 11 on Pest Risk Analysis for Quarantine Pests. ISPM No. 3 also may cover GM biocontrol agents, although no detailed guidance is included in the original version. The OIE may decide to fill out the sections that cover GM microorganisms affecting animal health. European Directives and national laws regarding release of exotic and/or GM organisms will also have a role in decisions regarding use of biological control against rabbits.

After taking into account the available WTO/SPS related guidance on risk analysis to support decisions regarding import and/or intentional introduction of an organism, however, it remains possible (even if not in the spirit of the text) to ignore the impacts of (re-)export of an organism, very possibly unintentionally. Responsibilities of a country planning to introduce an exotic disease, including for the purpose of biological control, are highlighted by the Convention on Biological Diversity (CBD). These responsibilities (even to the point of liability) appear specifically under the initiative on invasive alien species (IAS), the essence of which is encapsulated in Article 8 (h). Subsequent decisions taken by the Conference of Parties (countries contracting to the CBD) on how to go about fulfilling their obligations under the CBD emphasise prevention of unintentional introductions and the use of precaution when considering intentional introductions. The CBD also invites international bodies to consider incorporating criteria related to the threats to biological diversity (posed by IAS) in amending or elaborating standards and agreements.

Some help for implementation of the general responsibility stated in the CBD may be found in the growing body of work on IAS. Threats from IAS have grown over the last century, with an alarming increase in recent decades owing to rapidly accelerating trade, tourism, transport and travel, which have dramatically enhanced the spread of IAS and allowed them to surmount natural geographic barriers. Recognition of the threats they now pose to biodiversity and livelihoods has led to substantial work by national and international bodies, including GISP (the Global Invasive Species Programme), which has produced 'A Toolkit of Best Prevention and Management Practices'. After review of many countries' experiences, GISP identified four major steps by which a country can deal with alien species: prevention, early detection, eradication and control. GISP also notes that deliberate introductions of non-indigenous species should all be subject to an import risk assessment as a step in choosing and developing these management options.

A logical step for countries releasing disseminating GMOs would be to have mechanisms to prevent their exit. This will be difficult to do, even though it may be easier in some cases than others: Australia, with no land borders, might find it easier than Spain to secure them against would-be emigrant rabbits or control agents, but even Australia was unable to prevent RHD being exported. One safeguard for a GMO would be to incorporate a marker gene to allow its spread to be monitored – the technology to do this already exists. An additional safeguard would be some kind of safety device to allow its effects to be countered. This is likely to be a very active area of future research as commercial companies recognise the importance of safety issues, which will have a spin-off in safety options for GM biocontrol.

One rabbit disease in particular (RHD) has manifested both invasiveness and unpredictability, and illustrates some of the difficulties of being certain about the safety of disseminating organisms, whether GM or naturally occurring. During the 1980s and 1990s RHD spread rapidly from China to Europe and elsewhere, probably through trade in rabbits or their meat and fur. In 1991 RHD was imported into quarantine in Australia for evaluation as a biological control agent. In 1995, during field trials being conducted on Wardang Island near the Australian mainland, RHD eluded the strict quarantine measures established to confine it to the island and escaped to the adjacent mainland. Two weeks later it was detected over 300 km away, almost certainly as a consequence of spread by flying insects. It subsequently spread (both naturally and deliberately as a control tool) throughout most of southern Australia. It was introduced illegally into New Zealand [see *BNI* **19(4)**, 99N-101N (December 1998), RHD after one year in New Zealand]. Discussion of these issues in relation to GMOs may help to find a way to avert such problems by technical means or political agreement, or both. It is essential that development of precautions for the safe management of risks of international movement of disseminating GMOs between the native and pest ranges of the target species progress at an equivalent rate to those for other risks.

From the IAS perspective, the development of GMOs presents groundbreaking opportunities, and biotechnologists are in an enviable position. For IAS, risk analysis to date has been largely retrospective, driven by the need to combat the result of centuries of sometimes accidental but often deliberate introductions of species that have subsequently become invasive pests. Classical biocontrol has played a role in some notable control successes against IAS, but it is also facing the aftermath of a less-regulated youth: cases where there has been some unanticipated movement and/or behaviour by agents introduced in earlier decades. For example, *Cactoblastis cactorum*, an Argentinian moth introduced to Australia in the 1920s against the introduced invasive prickly pear (*Opuntia*), was the spectacular and earliest success of weed biocontrol. This success has been repeated in South Africa and a number of other countries, including some Caribbean islands in the 1950s. However, *Cactoblastis* has now spread from the Caribbean to mainland North America where the threat it poses to native cactus species has significant economic, ecological and social dimensions. [see *BNI* **23(3)**, 63N-64N (September 2002), *Cactoblastis*: classical beauty or invasive beast]. Although some argue that these unanticipated impacts should have been predicted, biocontrol scientists also are grappling with the realization that priorities and values may change, nationally, regionally or globally, so that what once seemed like a sound decision to introduce an agent may now be questioned. With the benefit of a hundred years of hindsight, biocontrol has some valuable lessons to share, including the need for more recognition of transboundary issues and greater regional and international consultation on proposed introductions. Notwithstanding the existence of the IPPC Code of Conduct and more recent adherence to the CBD, it is recognised that countries still rarely consult each other about the introduction of exotic biocontrol agents, but such consultation needs to become the norm. As countries develop legislation to regulate GMO releases, they will be able to incorporate proactive measures to take into account national safety concerns and international obligations in a way that the IAS and biocontrol sectors only belatedly began to do.

Some concepts of safety address the lack of consultation and coordination among countries specifically on transboundary movement of GMOs. The Cartagena Protocol on Biosafety to the CBD establishes an advance informed agreement (AIA) procedure, similar to that used for pesticide trade, for ensuring that countries are provided with the information necessary to make informed decisions before agreeing to the import of such organisms into their territory. The Protocol reaffirms the precautionary approach contained in the Rio Declaration on Environment and Development, but also requires the use of risk analysis to develop science-based decisions. Over 100 countries have become signatories to this Protocol since January 2000, and 37 have taken the steps of national adherence in order to become contracting parties (including Spain, but not Australia). The Protocol comes into force once the 50th Party to the CBD ratifies or approves it through the national procedures. This could easily take place in 2003.

Although there is currently no global convention for the protection of animals that corresponds to the IPPC convention, and measures to deal with issues of releasing animal-related GMOs have not yet been considered, the pioneering of this process by IPPC partly reflects the more advanced status of crop-related biotechnology. The international efforts to harmonize methodologies and criteria for assessment of biocontrol agents and GMOs that impact on plants may provide a useful model for vertebrate biocontrol. The gains may be two-way, however: work on vertebrate pests and GM biocontrol agents and their regulation could advance the protection of other taxonomic groups against disease.

Possums Fuel Debate

Whilst development of disseminating GMOs involves cutting-edge science, the safety challenge is no less difficult. Australia and New Zealand, amongst leaders in this field, are both undertaking consultative, transparent processes to draw up biotechnology legislation, based on the precautionary approach. The New Zealand Hazardous Substances and New Organisms Act (1996) was followed by a Royal Commission of Inquiry to consider strategic options for New Zealand with respect to genetic modification, and the New Zealand Government has just (October 2002) issued a draft Biotechnology Strategy for comment.

In Australia, the Gene Technology Bill (2000) is the key component of the new national regulatory framework. It included the establishment of a Gene Technology Regulator, who prepares a risk assessment and risk management plan (RARMP) for every proposed intentional release of a GMO into the environment, in consultation with expert groups and key stakeholders, including the public. Amongst specific issues to be addressed are the potential of the GMO to affect the environment and the potential for dissemination or persistence of the GMO or its genetic material in the environment. Transboundary issues are not explicitly stated, but would be expected to be included. However, the legal position is presently unclear, and their inclusion might be challenged in the courts. The Regulator is also tasked with monitoring international practice in relation to the regulation of GMOs, and maintaining links with international organizations that deal with the regulation of gene technology and with agencies that regulate GMOs in countries outside Australia.

Despite these legislative advances, safety obstacles to introducing disseminating GMOs for vertebrate control are well illustrated by a conflict of interest over possums emerging between Australia and New Zealand. Australian brushtail possums (*Trichosurus vulpecula*) introduced into New Zealand have colonized more than 90% of its land area and are the most significant source of bovine tuberculosis, the country's most important disease of cattle and farmed deer. Possums are also the most important mammalian pest in the New Zealand environment contributing to the progressive loss of indigenous flora and fauna biodiversity. New control measures are needed, not only to avoid high-cost poisoning and trapping in perpetuity, but because present use of poisons for vertebrate control poses sometimes unacceptable environmental and trade risks, and faces growing public opposition, both at home and abroad. Although risks to human health have been minimized, and there has been a dramatic decline in the number of tuberculosis-infected cattle and deer herds since possum control was stepped up in 1994, there is a desire to minimize the risk of imposition of technical international trade barriers. These would threaten NZ$1.3 billion of export returns. The New Zealand Government is investing in research into a number of alternative strategies, and GM biocontrol is one such avenue.

Given that the whole point of a disseminating GMO is that it spreads through a population, and given the possible irreversibility of this once a GMO has been released, there are likely to be instances where their use would be highly risky – for example, if one could spread to the native host range of the target. Whilst technology may yet provide an answer, an alternative would be to develop a non-disseminating GMO, together with methods of application and distribution (as is necessary for microbial biopesticides and inundative use of insect agents). Landcare Research has purposely limited research on a GMO to control brushtail possums to non-disseminating systems because of both national and international public concerns, including fears about possible spread to Australia. AgResearch is being funded by MAF (Ministry Agriculture & Fisheries) and FRST (Foundation for Research, Science and Technology) to work on transgenic nematodes and marsupial virology, with the aim of evaluating disseminating delivery systems for biocontrol. Landcare Research is assisting it with studies of the ecology and epidemiology of the naturally occurring (unmodified) parasite, to provide baseline data for future risk analysis of a GM parasite. The draft National Science Strategy for Possum and Bovine Tuberculosis Control, which has been circulated for comment to stakeholder organizations, outlines plans to develop non-disseminating and disseminating delivery systems. The target date for having a disseminating GMO for possums ready for evaluation is 2010.

The development of a disseminating GMO must necessarily advance hand-in-hand with progress on safety. The New Zealand regulatory approval system for release of GMOs is under consideration for revision. Under current procedures, risk is assessed when researchers go to ERMA (Environment Risk Management Authority) to seek approval for any field trials. Following the advice of the Royal Commission, the New Zealand Government placed a 2-year moratorium (until October 2003) on the release of GMOs that can persist in the environment to allow this consideration to take place. The Ministry for the Environment has just released a draft Biotechnology Strategy for discussion. This declares as one aim to put arrangements in place to give adequate consideration to ethical and safety issues.

Although now facing a conflict of interest over possums, Australia and New Zealand have a history of working together on many issues, including safety, quarantine and biocontrol. Indeed, the New Zealand programme on biological management of possums has been complemented through investment in a number of Australian research institutions, including the Marsupial Cooperative Research Centre (CRC), and Standards New Zealand and Standards Australia developed a joint risk management standard (AS/NZS 4360: 1999 Risk Management). They are well placed, therefore, jointly to address issues created by the new technology.

One of the candidates for a possum GMO demonstrates how GM biocontrol can raise transboundary issues relating to the target in its area of origin – in this case a potential threat to Australia's indigenous possum fauna. The New Zealand Hazardous Substances and New Organisms Act (1996) makes it incumbent on applicants applying for permission to release GMOs to consider their international obligations. The possum GMO also provides an example of how control using a disseminating GMO differs from classical biocontrol, illustrating the danger of direct transfer of concepts such as host specificity.

AgResearch is looking at the potential for producing a nematode containing an inherited transgene that disorders reproduction (by compromising fertility of females) that could spread through the New Zealand possum population. Infected animals may survive several years without reproducing and thus continue to spread the GM nematode. Researchers are looking to induce a subtle and hopefully relatively gradual reduction in populations, which will minimize the selection of host resistance to the parasite, but will have a major impact in the long term. The selection of a nematode was partly because the relationship between hosts and nematode parasites and the determinants of specificity and pathogenicity have many components, so that altering one aspect of that relationship is, the researchers believe, less likely to result in fundamental changes to the relationship as a whole. As noted above, field research is underway to determine the host-parasite relationship between the naturally occurring nematode and the possum, to provide baseline data for future studies on a GM nematode.

The prospect of a disseminating GM agent for possum control being released in New Zealand has provoked some opposition in Australia: if the GM nematode reduces populations in New Zealand (as it is designed to do), it would have the potential to do the same to indigenous Australian host populations. The chosen vector, the parasitic nematode (*Parastrongyloides trichosuri*), is specific to the brushtail possum and a close relative in Australia, the mountain brushtail possum (*T. caninus*). While this limited host range would make it a promising classical biocontrol agent, a GM version of it could be, quite literally, a different beast in ecological terms. If it were to reach the home range of its *Trichosurus* hosts, it would be a ‘new association’ (and if the transgene does its job, an effective natural enemy), which would upset the ecological balance between the possums and their native, co-evolved natural enemies (including naturally occurring *P. trichosuri*). Coincidentally, recent taxonomic research, which has shown *T. caninus* to be two species, highlights another dilemma for biocontrol: the goalposts can move as more information becomes available. In this case, new research is needed into the effects of *P. trichosuri* on *T. caninus*, as now defined, and the newly described *T. cunninghami*. Any assessment of a GMO for possums must consider the potential impact on the three species in Australia, including aspects of population distribution and ecology of *T. caninus* and *T. cunninghami* on which current knowledge may be rather confused.

Host specificity in the target range is, as for any biocontrol agent, an essential characteristic for a parasitic species being considered as a transgenic vector. Clearly, however, for a GM agent the target species outside the target area is a non-target species and the risk of creating a new and highly damaging host association in the target’s home range must also be addressed. Biocontrol terms such as 'host specificity' and 'non-target species' need to be re-interpreted for this emerging technology. In summary, a host range restricted to brushtail possum and ‘*T. caninus*’ does not make the nematode acceptable for development as a GM biocontrol agent unless the risk of it reaching Australia can be demonstrated to be acceptably low.

These are the kinds of issues that a number of countries are becoming concerned about and are asking to be addressed.

The use of a nematode like *P. trichosuri* that has a free-living stage may raise the risk stakes of introduction to Australia. The eggs are passed out in possum faeces where they hatch and develop to an infective larval stage that may remain viable for up to 2 months. Although this makes it a good candidate for dissemination, it also raises the possibility that it could cross the Tasman Sea from contamination of soil, foodstuffs or camping equipment, or from a deliberate release (as with RHD in New Zealand). At present, insufficient is known about the nematode's behaviour in the free-living phase of its life cycle to know how to assess the risk. It will be possible to model the risk of accidental introduction once the necessary ecological data on the nematode have been collected. There is some reason for optimism: some plant-parasitic nematodes are also disseminated in soil but there are notable absences in the plant parasitic fauna of Australia despite ample opportunity for introduction. Their absence may be largely attributable to Australia's stringent and effectively applied quarantine regulations, but even they are not 100% effective: a number of other damaging plant-parasitic nematodes have managed to evade detection and enter the country. In addition, risk of deliberate introduction cannot be dismissed: possums are a significant pest to some Australian householders, inhabiting roof cavities and creating a nuisance through noise, defaecation/urination, etc.

Another reason for optimism is that there are differences in prevalence between Australia and New Zealand, which could be due to marked differences in environmental conditions; the range of *P. trichosuri* in Australia appears to be more restricted than that in New Zealand. These differences may constitute a mitigation of risk, but that has yet to be verified. Further research including extensive and detailed surveys of the nematode fauna of Australia's possums would be useful in this regard.

There is a strong imperative to resolve the issue for possum nematodes. As a substantial importer of New Zealand produce, Australia could feasibly feel forced into banning the (substantial) import of fruit, vegetable, cereal crops, etc. from New Zealand if it were to perceive the risk to its possums as too high. New Zealand, also possessed of one of the world's most effective quarantine services and a leader in implementing biodiversity conservation legislation (Biosecurity Act 1993), would probably be able to understand Australia's dilemma.

Failure to understand or take account of the ecological significance of proposed developments is widespread in the biotechnology sector, and researchers into mammalian biocontrol could play a pioneering role in breaking down inter-disciplinary barriers. With their history of inter-country collaboration and strong capacities across the board in ecology, invasive species biology, biocontrol and biotechnology, together with the lead they have given in legislation on national biosafety and GMO regulation, New Zealand and Australia should be ideally placed to foster a meeting of genetic and ecological minds, and turn this current conflict into a model resolution for others to follow.

Although the scientists working on the development of disseminating GMOs for mammal pest control have discussed safety issues at national and international meetings, there is as yet no formal inter-country initiative to tackle them. In this case, appropriate steps would be for the New Zealand government formally to recognise Australia's interest in the release of disseminating GMOs to manage possums in New Zealand, and to develop policy, consultative, technological and regulatory frameworks to deal with the issues involved in assuring the safety of a GM biocontrol agent. New Zealand researchers need funding to investigate the possibility of developing safety devices that will allow Australia to protect its possums against the introduction into Australia of disseminating GMOs released in New Zealand. The question of how, by whom and at whose expense research should be conducted on the possums in their home range in Australia has also to be resolved. However, international issues need to be addressed sooner rather than later. Any notion that another country's interests (in this case Australia's) are subsidiary should be disavowed by the New Zealand Government and the organizations conducting this work. AgResearch researchers call for a mapping out of the research that needs to be done and the establishment of the necessary funding and a scientific basis for that research.

On the many previous occasions when New Zealand and Australian cooperation has been fruitful and mutually beneficial, their interests have coincided. This time their interests are at odds with each other. With New Zealand's bargaining position apparently the stronger of the two, it may be more difficult to achieve an equitable solution. In the case of rabbits, the bargaining positions of the main protagonists (Spain and Australia) are more equal. Both are exploring the potential of GMOs to manage rabbits, and both also have an interest in protecting their ecosystems against a GMO developed by the other. This mirror-image symmetry was the chief reason that the rabbit was chosen as the main focus for a forthcoming international symposium.

Can Rabbits Show the Way?

Recognising the urgency and importance of moving transboundary issues relating to disseminating GM biocontrol forward, the organizers of the 3rd International Wildlife Management Congress (to be held in Christchurch, New Zealand, 1-5 December 2003) have accepted a proposal to hold a symposium: 'Rabbits, RHD, disseminating GMOs and conflicting international objectives'. The symposium will examine the need to regulate the use of these GMOs in order to minimize the risk of adverse consequences should they spread outside the country of their release, and the need to build safety devices into them that would allow the developers to achieve their objectives whilst also allowing other countries to protect their ecosystems. The goal is to assist with progression towards agreement on appropriate management of the risks from disseminating GMOs in the interests of all affected countries, which may be applied in the future to other disseminating GMOs being developed to control other pests in other countries.

It was because of the advanced state of research with disseminating GMOs for rabbits and, as importantly, the common interest of Spanish and Australian scientists in working together that rabbits were chosen as the model. As Spanish researchers have written, "Since the proposed use [of a GMO] involves the environmental release of a recombinant virus, considerations regarding safety are as important as the potential efficacy of the candidate [GMO]." The organizers note that support needs to be generated for taking the concerns further and developing mechanisms (regulatory and technological) to deal with them equitably.

The purpose of the symposium is to further explore three broad areas of common, and conflicting, interest:

- Epidemiology of RHD
- Consequences of RHD for rabbit populations and biodiversity
- International issues related to the possible spread between countries of disseminating GMOs used to manage rabbits, possums and other pests

Clearly many of the issues that will arise are relevant to future developments of GMOs for other pests in other countries – as indeed they mirror issues of classical biological control and IAS. Broadly, the conference will address:

- Potential sources of conflict
- Reducing conflict through technical innovation (in biocontrol agents)
- Political mechanisms to resolve conflict: can international agreement regulate the use of GMOs?

The organizers hope to focus on the rabbit to develop a model solution, which may provide a lead to solving the more general problems of using disseminating GMOs for pest control or protection of native species against disease. Spain and Australia have an incentive to negotiate and arrive at a workable solution, as both have an urgent, if diametrically opposed, problem to solve. Spain and Australia would gain mutually from achieving a solution, whether technical or political, and each country may need to compromise. Reciprocity offers the best prospect for developing workable solutions to this emerging issue. As a landmark meeting, this symposium has the task of doing the groundwork on which a coherent regulatory strategy can be built.

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Further Information

3rd International Wildlife Management Congress
www.conference.canterbury.ac.nz/wildlife2003/

CBD COP Decision VI/23, see:
www.biodiv.org/decisions/?ac-cop

Cartagena Protocol on Biosafety
www.biodiv.org/biosafety/

IPPC
www.fao.org/ag/agp/agpp/pq/default.htm

GISP Toolkit
www.cabi-bioscience.ch/wwwgisp/

Australian Office of the Gene Technology Regulator
www.health.gov.au/ogtr/index.htm

Australian Pest Animal Control CRC
www.pestanimal.crc.org.au/

Environment Australia:
www.ea.gov.au/index.html
Biodiversity>Invasive Species>Feral Animals>Rabbits

South Australian Animal and Plant Control Commission
www.pir.sa.gov.au/pages/sus_res/animal_plant/rabimpact.htm

NZ AgResearch
www.agresearch.cri.nz/

NZ ERMA
www.ermanz.govt.nz/

NZ Landcare Research
www.landcareresearch.co.nz/

NZ MAF, Biological Control of Possums
www.maf.govt.nz/mafnet/rural-nz/research-and-development/pest-control/biological-management-of-possums/httoc.htm

NZ Parliamentary Commissioner for the Environment
www.pce.govt.nz/
'Caught in the headlights'

NZ NSSC Possum & Bovine Tb Control
www.frst.govt.nz/about/possum.cfm

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IPM Systems

Stemming a Coffee Pest

Coffee stem borers (*Xylotrechus quadripes* and *Monochamus leuconotus*), both cerambycid beetles, are now the major pest constraint to arabica coffee production in India (*X. quadripes*) and Zimbabwe and Malawi (both *M. leuconotus*) and are inflicting severe economic losses. A new project funded by the CFC (Common Fund for Commodities) with co-financing from national governments aims to meet the urgent need for an effective integrated management package for the pest. The new project, which is being implemented by CABI *Bioscience,* the Natural Resources Institute, UK (NRI), the Coffee Board of India, the Coffee Research Centre, Chipinge, Zimbabwe and Lunyangwa Research Station, Malawi, recently held its inauguration workshop in Chikmagalur, India.

Draining Losses

Losses from the stem borer are cumulative. In addition to crop losses, investment has to be made to establish new plants in place of uprooted ones. As coffee trees take time to come into production, the effects of borer damage are felt for at least 5 years. A survey of the three countries reveals the extent of the problem.

- In Malawi, where coffee is the fourth most important export earner, the borer is the major pest constraint for smallholder coffee farmers, with incidence exceeding 90%.
- In Zimbabwe the borer has led to the uprooting and subsequent replanting of many plantations.
- In south India, where the borer has existed in coffee estates for more than a century, uprooting an average of one infested plant per hectare per year accounts for an annual loss of about US$8-10 million. The borer is the most destructive pest of arabica coffee in the country, and a growing trend to convert from arabica to robusta threatens export earnings.

Potted History

Damage is caused by larvae, which hatch from eggs deposited in cracks and crevices and under loose scaly bark of the main coffee tree stem and thick primaries. The beetles show an oviposition preference for plants exposed to sunlight. Young larvae feed on the corky tissue just under the bark, which splits making the stem appear ridged. Later, larvae enter the hardwood and tunnel in all directions, even into the roots. Infested trees normally have yellow, wilting leaves, and ejected larval frass and emergence holes can be found on the stems. Severe ring barking of the main roots occurs below ground level, and extends above ground in rejuvenated coffee.

Attack often causes death of young (1- to 2-year-old) coffee and severe wilting of older (3- to 4-year-old) trees. Mature trees are not necessarily killed but, especially in Africa, they become susceptible to termite attack, particularly in the absence of supplemental irrigation during the dry season. In general, infested trees are of poor vigour and yield poorly.

Current management strategies are generally ineffective, or rely on undesirable chemical applications. Cultural control methods include uprooting and burning infested trees, treating the stems during the oviposition period to kill or dislodge eggs and young larvae, catching and killing adults during their period of activity, and maintaining shelter belts in order to shade the coffee bushes. Chemical control includes treatment of stems and primary branches with BHC (or other general insecticides) to reduce the pest incidence.

Biological control of *X. quadripes* was attempted in Vietnam during the 1920s. Mass rearing and release of the two most easily reared parasitoids found in plantations (*Doryctes strioliger* and *Sclerodermus domesticus*) led to an increase in parasitism rates, but this was not maintained after releases ceased. The high cost of developing a continuous mass rearing programme led to the work being abandoned.

A variety of control measures are under trial on a local basis in the different countries:

- Maintaining optimum shade.
- Tracing infested plants before flight periods each year by looking for ridges on main stems and thick primaries. Identified plants are collar pruned (and uprooted if the borer has entered the root) and infested material is burned.
- Removing loose scaly bark of the main stem and thick primaries using coir glove or coconut husk, but avoiding damage to the stem, which could kill the coffee or facilitate pathogen entry.
- Spraying/swabbing the main stem and thick primaries during flight periods with Lindane 20EC and a wetting agent.

Blending Best Measures

Given the urgency of the situation and the need to alleviate losses as quickly as possible, the project, in partnership with national programmes and coffee farmers, will assess current measures to identify shortcomings and optimize good practice. It also aims to research and develop new technologies in pest management, concentrating particularly on the potential of biocontrol agents (parasitoids, fungal pathogens and nematodes) and other biologically-based methods including improved agronomic practices, safer pesticides, botanical repellents and pheromones. A participatory approach will be used to maximize dissemination of the knowledge generated in this project.

To achieve the project aims, the collaborating scientists will conduct socio-economic and biological surveys, screen coffee varieties, identify and evaluate potential biological control agents and initiate rearing programmes, and establish field trials to quantify the efficacy and potential of control methods. The dissemination of the project outputs will be enhanced by developing and facilitating improved extension mechanisms through training of trainers and extensionists in farmer participatory approaches.

Good Grounds for Development

The project was formally launched on 11 September 2002 at a 4-day Knowledge Workshop held in Chikmagalur in India's main coffee-growing state of Karnataka. Delegates from all the countries involved in the project, including scientists, extensionists and growers from India heard presentations on the history and technical aspects of the stem borer problem and its management with overviews of the problem in each country. All delegates were invited to share their views and participated in group discussions which led to finalized workplans for the first 2 years of the project.

The vast amount of knowledge, experiences and ideas imparted to all at the event will be made more widely available. The proceedings of the workshop will be published and distributed to assist the dissemination of technologies to help other coffee-growing countries not directly participating in the project, but where stem borers are also a problem (e.g. China, Sri Lanka and Vietnam).

With the world coffee industry in crisis, and prices at an all-time low, smallholder livelihoods are under threat as never before, and if the smallholder farming sector is to survive and compete with large plantations, it needs help to solve problems and develop new strategies. In the prevailing conditions, smallholder farmers tend to reduce all inputs including pest control efforts, which leads to reduced quality and quantity, thus further affecting their economic position. The best response to the situation, however, is to concentrate on increasing quality, and adopt tactics that help reduce inputs of dangerous and costly chemicals. Such a strategy will also facilitate entry to new markets, which pay more for environmentally friendly, sustainably produced coffees. This project aims to help smallholders achieve this, and protect them from any future claims that they are insensitive to the increasing environmental awareness of consumer countries.

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Announcements

Mass Gathering

The 10th Workshop of the IOBC Global Working Group on Arthropod Mass Rearing and Quality Control (AMRQC) will take place in Montpellier, France on 21-24 September 2003.

The workshop will focus on all issues related to the rearing of entomophagous and phytophagous insects and mites, and to principles and practices of quality control. The programme will consist of invited papers presenting an overview of selected topics and contributed presentations on the different aspects of arthropod rearing as it relates to quality control. Papers will serve as a basis for discussion and exchange, with the final aim of improving collaboration among scientists and practitioners.

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Further information,
including a pre-registration document,
can be found on the AMRQC website:
www.AMRQC.org

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Canberra Bioherbicide Workshop

The next workshop of the International Bioherbicide Group (IBG) will be held as a satellite meeting during the International Symposium of Weed Biocontrol in Canberra, Australia in 2003 [www.ento.csiro.au/weeds2003]. Abstracts are requested from those interested in attending the IBG workshop.

International Bioherbicide Group
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Giant Hogweed Unites Europe

An international workshop on *Heracleum mantegazzianum* (giant hogweed), to be held on 5-7 March 2003 in Riga, Latvia, will bring together people from across Europe with an interest in this weed, including practitioners, policy makers and researchers from governmental and non-governmental organizations.

The meeting will be an opportunity to draw together knowledge of giant hogweed, and thus to provide an overview of its status in Europe, focusing in particular on its identification and distribution and control measures. Country status reports will be used to generate an initial set of best practice guidelines. These will be disseminated more widely by making them available for publication in delegates' own languages in literature available to local practitioners.

The specific aims of the workshop are to:

- Produce status reports on *H. mantegazzianum* for every country in which the species occurs, including Canada and the USA, which can be used to inform discussion and planning at the workshop
- Use the status reports to disseminate country information to practitioners, together with the overview of best practice
- Solicit feedback on the first draft of a giant hogweed management manual
- Disseminate further information on *Heracleum mantegazzianum*
- Establish good contacts across Europe and with North America

The status reports and a draft giant hogweed manual will be available at or just before the workshop. The status reports will be used as the basis for the proceedings of the workshop, which are to be published separately.

For further information and registration, see the giant hogweed homepage:
www.flec.kvl.dk/giant-alien/

Or contact:
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Cocoa Conference

The 14th International Cocoa Research Conference will be held in Ghana in October 2003 with the theme, 'Towards a Sustainable Cocoa Economy - What Strategies to this End?' One of the aims of the conference is to increase productivity and quality through production and distribution of improved planting material and promotion of IPM. It will include sessions on pests and diseases, agronomy and physiology, breeding, utilization of cocoa by-products and extension-transfer and efficient utilization of the results of cocoa research.

The conference is being organized by the Cocoa Producers' Alliance (COPAL).

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Conference Reports

Pathologists Converge on Brazil

The VIII International Colloquium on Invertebrate Pathology and Microbial Control, the 35th Annual Meeting of the Society for Invertebrate Pathology, and the VI International Conference on *Bacillus thuringiensis* (ICBt) were held at Fos do Iguassu, Brazil on 18-23 August 2002.

The International Colloquium on Invertebrate Pathology, sponsored by the Society for Invertebrate Pathology, is held every fourth year in conjunction with the society's annual meeting. Over 380 scientists from 31 countries participated in the combined 2002 meeting held at Iguassu Falls.

The scientific sessions encompassed a wide variety of topics, beginning with a Plenary Session on Baculoviruses and the presentation of the Founders' Lecture by Dr Peter Luthy, in honor of Dr Huguette de Barjac. The Divisions on Viruses, Bacteria, Fungi, Microsporidia, Nematodes, and Microbial Control sponsored a variety of symposia, contributed paper sessions, and workshops, and held annual business meetings. The symposia sponsored by the Divisions and meeting organizers are listed below:

Fungi:

- Microecology of entomopathogenic fungi
- Toward the integration of fungal entomopathogens with other biological control agents
- Genetic structure of fungal populations

Viruses:

- Prospects for the use of viral pesticides
- Arthropod-borne viruses

Bacteria:

- Bacterial insecticidal proteins: specificity, improvement and novel toxins

- *Bt* transgenic plants and insect resistance to *Bt* toxins
- *Bti* and *Bsh* mosquiticidal strains: use and necessities

Nematodes:

- Entomopathogenic nematodes: research trends

Microbial Control:

- Solar irradiation of fungal pathogens: deleterious effects, and mitigation through genetics and formulation
- Microbial control of insect pests of potato – from Tierra del Fuego to the Great White North

Cross-Division:

- Bacteria/insect interactions: virulence aspects
- Microbial germplasm repositories: the legacy, the problem, the future
- Microsporidia within Entomophthorales

Meeting participants enjoyed several social activities including a poolside welcome reception at the Hotel Bourbon, a traditional Brazilian barbeque with entertainment and dancing, a trip to Iguassu Falls (raincoats provided), and the annual 5 km race which was held in the Iguassu National Park. The banquet at Hotel Bourbon honoured student award winners (travel awards, paper presentations, and posters) and featured an excellent Brazilian band and dancing.

Information on obtaining copies of the Abstracts and Proceedings will soon be made available at the Society's website: www.sipweb.org

The 36th Annual Meeting of the Society for Invertebrate Pathology will be held on 26-31 July 2003 in Burlington, Vermont, USA. All scientists interested in the biology of invertebrate pathogens as well as their use in biological control are welcome. Watch the SIP website for details regarding this and future meetings.

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