What's New In Biological Control Of Weeds?

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Infidelity Ends Hopes of a Passion-Filled Relationship

We had hoped that the leaf spot fungus (*Septoria* sp.) used successfully in Hawai'i to control banana-poka (*Passiflora tarminiana*) might have the potential to control weedy banana passionfruit species in New Zealand (see *Honey, I Shrunk the Weed*, Issue 19). Unfortunately however, recent studies have suggested that this fungus is something of a wolf in sheep's clothing.

"We always thought there was something a bit dodgy about this *Septoria* species," revealed Jane Barton. "It went by the name of *Septoria passiflorae* in Hawai'i, but it didn't really match the published description of that species. It was more similar in appearance to *S. passifloricola*, a species that already occurs on *Passiflora* hosts in New Zealand." With the help of Eric McKenzie (Landcare Research), Jane compared dead material of the Hawaiian *Septoria* with herbarium material of *S. passifloricola* from New Zealand and South America (including the original "type" collection of the species). While the material was all very similar, even under a microscope, there did seem to be some consistent differences. For example, the spores of the fungus from Hawai'i seemed to be just a tiny bit longer and thinner than those from New Zealand and South America.

Initial, small-scale, host-range tests conducted by Jane in Hawai'i suggested the fungus, whatever its identity, would not attack our native or commercially grown passionfruit species (see *Bring on the Passion Killers*, Issue

29). However, when Nick Waipara returned to Hawai'i and did larger-scale tests, a very different picture emerged. Nick tested seven *Passiflora* species, including five weed targets (*P. tarminiana* from New Zealand and Hawai'i, *P. antioquiensis*, *P. tripartitia* var. *mollissima*, and *P. tripartitia* var. *azuayensis*), the New Zealand endemic species (*P. tetrandra*), and the species grown commercially here (*P. edulis*). "We were surprised to find that infection and leaf disease symptoms appeared on all seven species 2–6 weeks after inoculation," said Nick. "Only slight symptoms appeared on one *P. tetrandra* plant, and it has not been possible to confirm if this was caused by the leaf fungus being tested or something else, so this species is probably not very susceptible. However, more worryingly, the commercially grown passionfruit (*P. edulis*) was shown to be highly susceptible to the fungus."

"We were surprised to find that infection and leaf disease symptoms appeared on all seven species"

Testing before the fungus was released in Hawai'i did not reveal any attack on *P. edulis*, with infection limited to *P. tarminiana* (banana-poka) and *P. foetida* (a species that is exotic and weedy in Hawai'i). So it seemed that either there was more than one fungus running around in Hawai'i (one that could attack *P. edulis*, and one that couldn't) or there was only one fungus with a broader host range than previously thought. Therefore, Helen Harman undertook some molecular studies to clarify just what we had been working with in Hawai'i, and also to check Jane and Eric's theory that it wasn't the same fungus we already have in New Zealand. Her results were the final nail in the coffin with respect to the character of our potential agent. "The *Septoria* species released in Hawai'i to control banana-poka is different to the *S. passifloricola* that occurs in New Zealand," confirmed Helen. Furthermore, Helen's work showed that both Jane and Nick had been using the same Hawaiian species when they did their host range trials in Hawai'i (we suspect the differing results were due to different environmental conditions), and most importantly, it was this same fungus that Nick reisolated from plants infected in his trials. That clearly proves that the lesions he was seeing on both target and non-target plants were caused by the fungus we thought had biocontrol potential.

The end result of all this is that while we have shown the *Septoria* species in Hawai'i (which we believe is a new, unnamed species) is a virulent pathogen with potential to damage all of the weedy *Passiflora* species in New Zealand, it is not suitable to use here because of its potential to damage a commercially cultivated species. While this result is disappointing, at least the true identity and unfaithful nature of the fungus was revealed before it all ended in tears!

We would not have been able to undertake this testing programme without the support of the Hawaiian State Department of Agriculture, Honolulu, who kindly allowed us to use their faciltites. We would in particular like to thank Eloise Killgore for her valued contribution to this work. This research was funded by a national collective of regional councils and the Department of Conservation. Jane Barton is a subcontractor to Landcare Research.

Attack on commercially grown passionfruit (P. edulis) *spelt the end of hopes to use the fungus here.*

Another Worthy Target

Over the past year we have undertaken a survey to find out what lives on Japanese honeysuckle (*Lonicera japonica*) in New Zealand. We have scrutinised populations of this rampant garden escapee at 33 sites, ranging from the Hokianga Harbour in the north to Ross on the West Coast of the South Island.

The results were what we have largely come to expect from these kinds of surveys. No specialist natural enemies of Japanese honeysuckle were present. "Although we found 112 species of invertebrates associated with the plant, overall the amount of damage that could be attributed to them was minimal, <5%," explained Chris Winks. Little or no damage was seen to the flowers or fruits. Leaves were sometimes attacked and the culprits appear to be caterpillars (especially leafrollers) plus slugs and snails. Two sap-feeders, the passionvine hopper (*Scolypopa australis*) and the green planthopper (*Siphanta acuta*), were the only invertebrates that could be classed as "abundant". Thrips (*Hercinothrips bicinctus, Heliothrips haemorrhoidalis*) occasionally produce silvery-coloured patches on the foliage. Damage caused by sap-feeders, either by removal of nutrients or possibly allowing entry of pathogens, is very difficult to quantify. Generalist predators included spiders, ladybirds, lacewings, earwigs, ants and praying mantids – nothing that might potentially throw a major spanner in the works of a biocontrol programme. "Some parasitoids were found that may interfere with the effectiveness of any potential lepidopteran agents," cautioned Chris.

On the pathogen side of things at least 35 fungal species were identified but caused more than minor and insignificant blemishes, and there is little potential to use any of them as inundative or classical biocontrol agents.

One leaf spot pathogen (*Cercospora lonicera*) was quite common. It starts with a brown circular spot at the leaf edge which gradually increases in size. Surrounding leaf tissue turns yellow and the entire leaf becomes discoloured and dry. "This leaf spot was listed as a pathogen of Japanese honeysuckle in North America and a potential candidate for classical biocontrol. However, as the New Zealand strain(s) appear to be weakly pathogenic, surveys in the native range would be needed to determine if more aggressive biotypes exist," revealed Nick Waipara.

Silver leaf fungus (*Chondrostereum purpureum*), a registered mycoherbicide for tree weeds overseas that we have been investigating for its potential use against woody weeds here (see *Bioherbicides: All in a Days Work…For a Superhero!* page 4) was found at one site. It was collected from the woody base of a vine in Northland, but there was no sign it was harming the plant in any way. This observation is a new host record for this pathogen in New Zealand, and possibly the world. Silver leaf fungus has been reported on other *Lonicera* species, including *L. tatarica* in New Zealand.

Honeysuckle leaf blight (*Insolibasidium deformans*) was found at one site in Auckland. Young leaves showed a silvery-white discolouration and older infected leaves were brownish. This observation was expected as honeysuckle leaf blight is a ubiquitous pathogen with a worldwide distribution. Most species and varieties in the *Lonicera* genus are susceptible and it has been recorded on Japanese honeysuckle here before. The blight is a major problem for the nursery and garden industry in North America. "Biocontrol potential of this disease is unfortunately low as attack on adult plants would be too sporadic because there is a relatively narrow range of climatic conditions in which it proliferates," concluded Nick.

In light of these results we recommend that a classical biological control programme for Japanese honeysuckle should proceed.

This project was funded by a national collective of regional councils and the Department of Conservation.

Nick Waipara surveying Japanese honeysuckle.

Bioherbicides: All in a Day's Work...For a Superhero!

"Pick me! Pick me!" begged Jane Barton when she was told that someone from Landcare Research should attend the International Bioherbicide Group (IBG) meeting in Italy in June this year. And Jane wasn't the only one with her hand in the air, as in the end six Kiwis, plus hangers-on, attended the meeting (see photo).

The purpose of IBG workshops is to encourage co-operation between researchers involved in developing bioherbicides to control weeds. The term "bioherbicides" is used for herbicides where the active ingredient is a living micro-organism, and includes mycoherbicides (which use fungi). The focus of this particular workshop (the seventh to date) was current and future prospects in bioherbicide research and product development. Papers presented described work underway in a wide range of countries including Australia, Canada, Egypt, Italy, Korea, France, New Zealand, Norway, Russia, and the USA, with participants representing other countries as well. A wide variety of weeds are being targeted including quite a few parasitic weeds, such as *Orobanche* species. We have one *Orobanche* species in New Zealand (broomrape, *O. minor*). It doesn't seem problematic at present, but given its wide host range, and its status as a "species of agronomic importance" in the USA, we should perhaps keep an eye on it.

There are many steps to developing a bioherbicide and the talks highlighted some of the hurdles to be overcome at each stage. "A bioherbicide is usually based on a pathogen that already occurs in the country where it will be used," explained Jane. That means the weed and the proposed agent are already interacting in the field, but something is limiting disease development and often it is these "limiting factors" that researchers must overcome. Challenges that were discussed included getting the taxonomy right for an organism with bioherbicide potential; applying the pathogen where it can do the most damage (this is particularly difficult for parasitic weeds, which need to be attacked in the soil before they damage the host plant underground); producing inoculum in large quantities without compromising the ability of the microbe to cause disease; and convincing regulators that a product will be safe. Happily, speakers reported progress had been made in areas such as identifying plant toxins produced by some fungi (in the hope of producing toxin-based products that might be more stable than whole-organism-based ones); finding weed targets that suit this approach and markets that would best benefit from it, developing better production systems; improving application methods; and demonstrating that risks of environmental damage are low.

Three of the workshop talks focused on projects currently underway in New Zealand. Graeme Bourdôt (AgResearch) talked about progress towards commercialising white soft rot (*Sclerotinia sclerotiorum*) as a mycoherbicide against giant buttercup (*Ranunculus acris*) in dairy pastures in New Zealand. There are several chemical herbicides that can be used against buttercup, but all but one of these are problematic due to either evolved herbicide resistance or pasture damage. Research done by Graeme and his team showed that their mycoherbicide would on average control giant buttercup to a level acceptable to dairy farmers (60% reduction) at a realistic application rate of 50 kg/ha. This is as good as or better than can be achieved by some chemicals and without the pasture damage. "Registration and commercialisation of a *Sclerotinia*-based product is currently under consideration by a leading New Zealand fertiliser company," said Graeme. Their product will be called "Bioshield EN64", and if all goes to plan it should be available in New Zealand in 2 years time.

Geoff Hurrell (AgResearch) presented a paper, co-authored with Graeme and Jane, on the potential of the silver leaf fungus (*Chondrostereum purpureum*) and fusarium blight (*Fusarium tumidum*) as mycoherbicides for gorse (see *Field Trials and Tribulations*, Issue 24). Results from

The Kiwi contingent at Bari. From left to right: Jane Barton, Graeme Bourdôt, Nick Waipara, Brenda Pottinger (Lincoln University) and Emmanuel Yamoah (Lincoln University). Geoff Hurrell (Agresearch) was "missing in action" when this photo was taken.

their latest field trials suggest that while both fungi are capable of reducing gorse growth and killing its tissues, they aren't "synergistic" and work independently. Also, disappointingly, neither managed to penetrate very far into the treated plants. "To kill plants with silver leaf fungus it looks like we would have to cut them off near the ground and apply the fungus to the stumps," explained Geoff.

Emmanuel Yamoah (Lincoln University) spoke about his PhD project on potential methods to increase the efficacy of fusarium blight on gorse. Emmanuel is exploring whether insects could be used to transport spores of the fungus to the target weed, and also whether insect damage (wounding) might help the fungus infect the plants. He reported experiments where he looked at whether making wounds on gorse plants increased damage caused by the fungus in a glasshouse situation. "Results were promising," said Emmanuel. "While young gorse plants can readily be killed by the fungus, even without wounding, once plants are several months old the fungus has greater impacts on plant growth and mortality when plants are wounded prior to application."

If there had been an award at the workshop for the "most promising up and coming bioherbicide" it would have gone to Professor Raghavan Charudattan (University of Florida, USA) for "Solvinix". Solvinix is unique in that it is based on a virus, tobacco mild green mosaic virus (TMGMV), which is highly effective against tropical soda apple (*Solanum viarum*) (see *A Virus Worth Sharing*? Issue 24). The main stumbling block has been the virus's ability to damage other plants in the Solanaceae family (e.g. capsicum species and tobacco), but extensive experimentation has now been done to alleviate any fears of non-target damage. "The risk to these plants is negligible and manageable because the virus has no natural vectors and does not spread except through physical contact," concluded Professor Charudattan. This product is also unusual in that the Professor is creating his own company (in collaboration with the University of Florida) to get it through registration and commercialisation. This is to avoid the risk of having the rug pulled out from under them by an organisation that is after a quick profit – the sad fate of many bioherbicides.

While it was reassuring to see so much work underway on new bioherbicides worldwide, little was said of the current status of products that have made it through the registration process and/ or to the market place. Therefore, on returning to New Zealand, Jane did a bit of research (with assistance from Professor Charudattan) to see what had happened to the trailblazers like Collego™ and DeVine® (see table). To date, 15 mycoherbicides have been registered and/or used outside of a research situation. Eight of these are still available, either commercially, or on request. For example, Stumpout™ and Hakatak are produced on a small scale to order by the researchers who developed them. These brave souls decided to produce the bioherbicides themselves, in order to ensure their long-term availability.

The five products that are definitely not available at present (Collego™, CASST™, Dr Biosedge, BioMal® and BioChon™) have all suffered from financial problems, not efficacy problems (although Dr Biosedge may have suffered from both, due to very high host specificity). Interestingly at least 11 of the 15 products are not currently being actively marketed (the possible exceptions are Lubao, Camperico™, Ecoclear™ and Myco-tech™ paste). It is very disappointing that after all the hard work that goes into developing a bioherbicide, and getting it registered, so little effort is apparently spent on promoting the final product. It is doubly unfair that the product is often then shelved due to lack of profits.

Perhaps the only way forward in the immediate future is for researchers to have the courage to create companies and market their products themselves as Professor Charudattan is doing with Solvinix, although this is perhaps asking them to go "beyond the call of duty". Indeed, a single researcher would need to master microbe taxonomy, plant pathology, mass production, risk assessment, formulation and application technology, battling bureaucracy (for registration), fund raising, and marketing and promotion as well. It's a job for a Superhero! Perhaps it needs to be recognised that developing a bioherbicide should be a team effort that includes someone with business and marketing skills, and that resources for advertising are also needed.

On the bright side, there are eight bioherbicide products that seem likely to remain available, or to become available in the near future. At least two of these are being considered for use in large-scale projects: Wageningen University (The Netherlands) are collaborating with land managers in Berlin on a project to use BioChon™ to control black cherry (*Prunus serotina*) in Berlin forests; and in South Africa, the world-famous "Working For Water" programme is planning to use Stumpout™ to prevent acacias re-sprouting after they have been cut down in watercourses. Other products are being rescued by new financial backers (e.g. BioMal®) and/or renewed interest from farmers (e.g. Collego™).

Jane Barton is a subcontractor to Landcare Research. Her attendance at this workshop was funded by the Foundation for Research, Science and Technology. Thanks to the following people for providing information for this article: Karen Bailey (Agriculture and Agri-food, Canada), Raghavan Charudattan (University of Florida, USA), Meindert de Jong (Wageningen University, The Netherlands), David Te Beest (University of Arkansas, USA), Sherman Thomson (Utah State University, USA), Gary Walker (Sylvan Bioproducts, USA), Ron Wall (retired, Canada), and Alan Wood (ARC-PPRI, South Africa).

Bioherbicides that have been registered and their current status, October 2005.

Where and When	Product and Pathogen	Target weed	Status
USA: 1960	*Acremonium diospyri*	Persimmon trees in rangelands	Status unknown
China: 1963	Lubao: *Colletotrichum gloeosporioides* f. sp. *cuscutae*	Dodder (*Cuscata* spp.) in soybeans	Probably still available
USA:1981	DeVine®: *Phytophthora palmivora*	Strangler vine (*Morrenia odorata*) in citrus orchards	Status unknown, may no longer be marketed
USA: 1982	Collego™: *Colletotrichum gloeosporioides* f. sp. aeschynomene	Northern joint vetch (*Aeschynomene virginica*) in rice & soybeans	Not produced or distributed since 2003, but rice producers are showing renewed interest
USA: 1983	CASST™: *Alternaria cassiae*	Sickle pod & coffee senna (*Cassia* spp.) in soybeans & peanuts	No longer available due to lack of commercial backing
USA: 1987	Dr BioSedge: *Puccinia canaliculata*	Yellow nutsedge (*Cyperus esculentus*) in soybeans, sugarcane, maize, potato & cotton	Registered, but product failed due to uneconomic production system and resistance in some weed biotypes
Canada: 1992	BioMal®: *Colletotrichum gloeosporioides* f. sp. *malvae*	Round-leaved mallow (*Malva pusilla*) in wheat, lentils & flax	Not commercially available at present, but recently taken on by a new financial backer who is exploring market opportunities. Can be made on request
South Africa: 1997	Stumpout™: *Cylindrobasidium leave*	*Acacia* species in native vegetation & water supplies	Still available for sale, though demand has declined due to lack of advertising. May be taken up by "Working for Water"
Netherlands: 1997	Biochon™: *Chondrostereum purpureum*	Woody weeds, e.g. black cherry (*Prunus serotina*) in plantation forests	Available until end of 2000. Marketing/ production stopped due to low sales and regulatory concerns
Japan: 1997	Camperico™: *Xanthomonas campestris* pv. *poae*	Turf grass (*Poa annua*) in golf courses	Probably commercially available
South Africa: 1999	Hakatak: C*olletotrichum acutatum*	*Hakea gummosis* & *H. sericea* in native vegetation	Never registered, but will be produced on request
USA: 2002	Woad Warrior: *Puccinia thlaspeos*	Dyers woad (*Isastis tinctoria*) in farms, rangeland, waste areas & roadsides	Registered, but never commercially available due to lack of commercial backer. Once registered, the fungus was spread by researchers
Canada: 2004	Chontrol™ = Ecoclear™: *Chondrostereum purpureum*	Alders, aspen & other hardwoods in rights of way & forests	Commercially available
Canada: 2004	Myco-Tech™ paste: *Chondrostereum purpureum*	Deciduous tree species in rights of way & forests	Commercially available
USA: 2005	Smolder: *Alternaria destruens*	*Dodder* species: in agriculture, dry bogs & ornamental nurseries	Only just registered. Company planning to do more field trials and then market it in 2007

BIOHERBICIDE

Things To Do This Summer

While your mind may be turning to thoughts of barbeques and lazing about on beaches, the summer period is a busy time for some biocontrol agents so you might need to also plan to take your new field guide for a spin and undertake some of these activities:

- Checking old man's beard sawfly (*Monophadnus spinolae*) release sites – we have still not had a confirmed sighting of these in the field so keep your eyes peeled and let us know if you see anything. Look for leaves that have semicircular incisions along the margin or that have been completely skeletonised by the white caterpillar-like larvae. Black balls of frass may also be visible where larvae have been feeding. Our colleagues overseas tell us that the adults are hard to spot, but sometimes you can see females sitting underneath the leaves or males swarming around looking for females to mate with.

- If you get a move on it might not be too late to check gorse soft shoot moth (*Agonopterix ulicetella*) release sites that have previously shown positive results from pheromone trapping. The best time to look is early December when the caterpillars are quite large but have not yet pupated.

- Checking Portuguese gorse thrips (*Sericothrips staphylinus*) release sites when the gorse isn't flowering and flower thrips (*Thrips obscuratus*) won't be around to confuse you. Look on new growth in particular. As thrips are tiny, you may need to use a hand lens if your eyes aren't as good as they used to be. If you can't see any thrips by eye try gently beating some foliage over a white sheet or piece of white cardboard. However, don't disturb the bush any more than necessary.

- Checking heather beetle (*Lochmaea suturalis*) release sites – they will be hard to find unless they are present in large numbers and have caused a lot of damage. The best way to check them is by beating heather plants with a stick over a white sheet or piece of white cardboard, or by using a sweep net.

- Checking hieracium gall midge (*Macrolabis pilosellae*) release sites for plants with the swollen and deformed leaves caused by larval feeding.

- Harvesting broom seed beetles (*Bruchidius villosus*) – beetles can be redistributed while still inside the pods but keep an eye on pod development. Harvest pods when they are brown and mature, otherwise the beetles inside may not be completely developed. Be aware that pods ripen rapidly in hot weather so don't delay once the first pods have begun to burst.

- Harvesting cinnabar moth (*Tyria jacobaeae*) – cinnabar moth is now becoming harvestable in some parts of New Zealand where previously it has been rare. It can be difficult to establish this insect in some areas and the reason why is not always obvious. If you have been unsuccessful in a particular area in the past then it's probably best to try releasing caterpillars somewhere else.

Remember to read up the relevant pages in "The Biological Control of Weeds Book" before embarking on any of these activities, and let us know how you get on!

Gorse soft shoot moth larvae are intitially dark brown but later turn greyish-green.

Will the Rot Set In?

Recently, in collaboration with AgResearch and NIWA, we assessed the potential of white soft rot (*Sclerotinia sclerotiorum*) against some aquatic weeds. This naturally occurring plant pathogen has a wide host range and is known to damage many terrestrial plant species. AgResearch has been working for a number of years to try to develop it as a product for controlling Californian thistles (*Cirsium arvense*), but have not yet been able to come up with a formulation that is reliably effective in dry conditions. While research to overcome this hurdle is continuing, AgResearch is also evaluating the fungus on weeds of wetter environments such as dairy pastures, e.g. giant buttercup (*Ranunculus acris*) (see *Bioherbicides: All in a Days Work…For a Superhero!* page 4).

Healthy water lettuce and a plant infected with white soft rot.

Weeds of aquatic ecosystems are also an obvious possibility for this moisture-requiring fungus so we looked at whether it could attack seven aquatic species. White soft rot caused severe damage to water lettuce (*Pistia stratiotes*) resulting in death. "There would be good potential to develop this fungus for water lettuce control, especially in countries where this weed is a serious problem," suggested Nick Waipara and Graeme Bourdôt. Unfortunately the fungus hardly touched alligator weed (*Alternanthera philoxeroides*), *Azolla pinnata*, giant salvinia (*Salvinia molesta*), or water hyacinth (*Eichhornia crassipes*). Disease symptoms were minor, with no significant plant damage or mortality. "The fungus has little potential for controlling these weeds, unless a more virulent strain can be sourced which may be worth pursuing," explained Nick. Bladder wort (*Utricularia gibba*) and parrot's feather (*Myrophyllum aquaticum*) were not harmed at all.

Recently we isolated this same pathogen from a sickly-looking tradescantia (*Tradescantia fluminensis*) plant in Auckland. As a consequence we had a go at inoculating tradescantia with the fungus, using AgResearch's formulation. All the plants in the trial became infected, with symptoms ranging from small leaf spots to extensive rot of the stems. Some plants were able to continue growing while others were severely damaged, so the overall impact was highly variable. "However, the results showed that it would be worth doing further testing to explore the full potential of white soft rot as an inundative biocontrol product against tradescantia," concluded Nick. "EnCoate, the AgResearch Joint Venture that is funding the formulation research, has a broad vision for the commercialisation of this pathogen that extends beyond pasture weeds to weeds of amenity turf and natural ecosystems, including waterways," revealed Graeme.

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