What's New In Biological Control Of Weeds?

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What's Inside:

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Adventures in Japan

Our staff have now been to Japan three times to look for potential biocontrol agents for Japanese honeysuckle (*Lonicera japonica*), as no-one had ever looked seriously into the natural enemies of this plant in its native range before. "Japanese honeysuckle is a well-behaved plant in its homeland and not the rampant weed we see here in New Zealand," confirmed Quentin Paynter. Our base for the surveys has been the National Institute for Agro-Environmental Sciences in Tsukuba, where our collaborator Dr Akihiro Konuma is based. Akihiro is studying the genetic diversity and origins of Japanese honeysuckle. He has already discovered that the plant in New Zealand appears to be more diverse than populations in its native range, and it would appear that Japanese honeysuckle has been introduced to New Zealand from China and Korea as well as Japan. Akihiro arrived in October an OECD fellowship for 6 months on to continue his honeysuckle study.

We have tried to leave no stone unturned in our quest to find potential agents for this rampant climber. Using Tsukuba as a base we have travelled north-west to subalpine areas in Nikkō National Park and north-east to lowland coastal regions east of Ishinomaki. The latter region has relatively cool summers and mild winters and is climatically about as close to New Zealand as you can get (most regions where Japanese honeysuckle grows have much hotter summers or much colder winters than here). These surveys have covered a range of seasons and environmental conditions, from high-altitude inland sites to low-altitude coastal areas, both in relatively natural forest habitats and on plants growing along fences and embankments in open agricultural areas.

A white admiral butterfly that feeds on Japanese honeysuckle.

During the first survey we identified a number of potential insect candidates, but few signs of fungal pathogen damage. The second survey, conducted slightly later in the year, found a variety of fungal pathogens that were beginning to infect plants, as well as additional insect species. The third survey revisited infected plants to see how they had fared in the interim and to get some idea of how damaging the pathogens might be. "Unfortunately a number of these plants had been removed, possibly because they had become untidy looking, which was really frustrating," reported Olimpia Timudo. Our pathologists are still working through the numerous specimens collected that were showing disease symptoms to figure out what the associated pathogens are. So far a number of different species of the well-known genera *Colletotrichum, Fusarium, Pestiloti, Phomopsis* and *Phoma* have been found associated with leaf and stem lesions. There were also plants found exhibiting typical viral symptoms. Of particular interest so far is a *Phoma* sp. that was associated with "herbicide-like" dieback at one site. "Once all the pathogens have been identified, we will need to check if they can infect New Zealand Japanese honeysuckle plants and what sort of impact they have," explained Sarah Dodd.

On the entomological side of things we have now identified most of the insects found and have begun to prioritise them. There are quite a few butterflies and moths feeding on Japanese honeysuckle that show promise, including two attractive white admiral butterflies (*Limenitis camilla* and *L. glorifica*). The latter is reputed to only attack Japanese honeysuckle, and is quite common, so is a good contender for further study. Another top prospect was a moth (*Bhadorcosma lonicerae*), which has only been recorded feeding on two *Lonicera* species, whose larvae destroy stem tips in early spring. This moth was present at nearly all the sites visited and quite damaging. Tips that survive this attack were often hit later in the season by another, yet to be identified, leaf-tying moth which was also extremely common and damaging. A fifth moth (*Apha aequalis*), which has very large fluffy larvae that consume a lot of vegetation, is also of interest. "They were really cute and despite the hairs you can safely stroke them and they are lovely and soft," said Quent.

These unusual beasts eat a lot of foliage and are safe to touch.

Overall the prospects for finding a line-up that can do some serious damage to Japanese honeysuckle are looking very good

Amongst the myriad of other creatures found two further species stand out. The larvae of a longhorn beetle (*Oberea mixta*) bore into the woody stems, and were present at many disturbed lowland sites, but were not found in forested and inland sites. Sawfly larvae (*Zaraea lewisii,* a Japanese honeysuckle specialist) were also found defoliating the leaves at many sites we visited. So overall the prospects for finding a line-up that can do some serious damage to Japanese honeysuckle are looking very good, and Quent is now working on an application to import the most promising species into containment for further testing. A key issue that remains to be resolved is what level of attack to other ornamental *Lonicera* species might be acceptable and whether this might compromise the success of the programme.

This project is funded by the National Biocontrol Collective. We are grateful to the National Institute for Agro-Environmental Sciences in Japan for their assistance with this project, which would not have been possible without their help. Olimpia Tumido has spent 7 months with us as a pathology technician and is moving to the USA in December.

Fascinating Fungal Findings

As part of our comprehensive studies to check for any non-target damage caused by weed biocontrol agents we needed to check if the old man's beard leaf fungus (*Phoma clematidina*) is behaving itself. We isolated *P. clematidina* from native *Clematis* plants, but that was the easy part. A fungus by the name of *P. clematidina* was known to be present in New Zealand when the old man's beard leaf fungus was released. It was believed to cause mainly only cosmetic damage to old man's beard (*Clematis vitalba*) in the autumn, and the introduced strain was thought to be capable of causing much more damage earlier in the season. We discovered that we could not reliably tell *P. clematidina* strains apart in the good old fashioned way by studying morphological features under a microscope. We therefore needed to resort to modern molecular techniques to confirm which strain (or strains) was responsible for attacking native *Clematis*.

We therefore undertook DNA studies, which revealed that the situation was more complex than we thought and that we actually have multiple strains of *P. clematidina* present in New Zealand as well as several other *Phoma* species on *Clematis*. Our results also suggest that various fungi currently called *P. clematidina* may in fact be different species. It is only in recent times that we have been able to study DNA and clarify relationships between organisms, so there are still plenty out there whose taxonomy needs to be checked and if necessary revised. "Our results are of interest to researchers in the Netherlands who are currently attempting to sort out the taxonomy of *Phoma*," explained Nick Waipara.

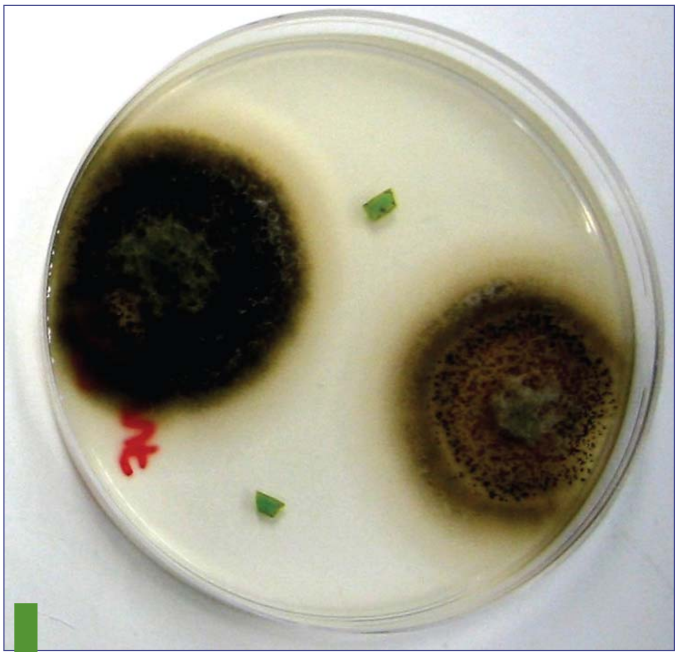
One of the unexpected strains of *P. clematidina* that we found exists in the leaves as an endophyte without causing any disease symptoms. All terrestrial plants have fungal endophytes and usually both gain from the arrangement. The endophytes get protection and nutrients, and the plant gets additional resistance to pests and diseases. How plants obtain endophytes is not well understood. We found *Clematis* seeds that were infected with the *P. clematidina* endophyte. "Because the seed had been surface-sterilised the only way they could have become infected with the endophyte would be by inheriting it from their parent plant. This form of fungus transmission has only been demonstrated in dicotyledonous plant species a few times before," said Helen Harman.

We mentioned the endophyte release hypothesis in issue 41 of this newsletter. This hypothesis suggests that weeds with lots of endophytes can be more difficult targets for biocontrol, and old man's beard is certainly proving to be just that. It is nearly a decade since "the superior strain" of the old man's beard leaf fungus was introduced as a biocontrol agent, and after some good results early on, more recent reports have suggested that it is no longer doing much. Once the DNA studies clarified what we had found during the non-target surveys, we were nevertheless surprised that we had not picked up any sign of the introduced strain at all. If the endophyte hypothesis is correct, it may be that endophytes are the reason the introduced strain has become rare or possibly even died out, but further research would be needed to confirm this. On a positive note the introduced strain of *P. clematidina* would also seem to be in the clear with respect to native *Clematis*.

The introduced strain of *P. clematidina* would seem to be in the clear with respect to native *Clematis*

This project has highlighted the difficulties we face when trying to track the movements and behaviour of introduced fungal biocontrol agents. However, we have been involved in some research to see if it would be possible to

Typical symptoms of *Phoma clematidina* infection.



Examples of fungi isolated from fragments of old man's beard leaves.

mark fungi to make it is easier to track them, and we have shown that this can be done.

We developed mutant strains of *P. clematidina* that were marked by the absence of a particular enzyme. The presence or absence of this enzyme does not affect the fungus's ability to infect or damage plants, or its host range. We then inoculated old man's beard plants with the mutant strains. Once symptoms of disease became apparent, we collected samples of infected leaves and surface-sterilised them to remove contaminants. These pieces of leaf tissue were then grown on agar plates to firstly confirm that *P. clematidina* was present, and secondly to check if they were the mutant strains. "We successfully re-isolated the mutant strains off infected old man's beard plants and we were able to distinguish them from each other and from non-mutant isolates of *P. clematidina*," said Helen.

The proof-of-concept study means that in future we may be able to mark fungi before we release them and be better placed to keep tabs on them. We are also hoping in future to be able study endophytes and more fully understand their implications for biocontrol projects.

But where does this leave us right now with respect to old man's beard, which continues to be a major problem in many parts of New Zealand? As well as the fungal disappearing act, the old man's beard sawfly (*Monophadnus spinolae*) seems unlikely to have established, and the old man's beard leaf miner (*Phytomyza vitalbae*) seems hampered by parasites. Hopes are currently pinned on trials underway in the UK to see if a beetle (*Xylocleptes bispinus*) might be suitable to release. It may also be worth having another crack at establishing the sawfly. A third possibility is to use the old man's beard fungus in a different way. If the superior strain is not able to persist at high levels or in the long term as the result of a single release, then it might be feasible to repeatedly mass-produce and apply it in bulk as often as needed in order to provide control.

Old man's beard sawfly adult.

The non-target and tracking fungi projects were funded by the Foundation for Research, Science and Technology. Hilary Kitchen spent a year with us in 2006 working on the latter as a Royal Society Teaching Fellow. Nick Waipara is now with the Auckland Regional Council.

Advanced Biocontrol Workshop

If there is sufficient interest we will hold an advanced biocontrol workshop at either Lincoln or Auckland in autumn 2009. The aim of this workshop is to give people the skills and confidence to manage their own biocontrol programmes. This workshop is ideal for people who have a reasonable knowledge of weed biocontrol and ideally will have undertaken our basic training workshop two or more years ago. We build on existing knowledge and bring people up to speed with new developments. If your organisation contributes to, or supports, our research in some way then there is no charge for this course. If not you may still be able to attend, if there are places available, for a small fee. If you are interested in this workshop please contact Lynley Hayes (hayesl@landcareresearch.co.nz or Ph 03 321 9694).

Rust Progresses Slowly

We are pleased to report that boneseed leafroller caterpillars (*Tortrix* s.l. sp. "chrysanthemoides") have been sighted this spring, and now that they have survived their first New Zealand winter they can be deemed to be established. Now we just need to wait and see how quickly they can multiply and start to cause damage. While we are quietly hopeful of big things to come from the leafroller, experience tells us that successful biocontrol of a widespread weed normally requires more than one agent. A few years back we decided that a rust fungus (*Endophyllum osteospermi*) might be just the thing to complement the leafroller. Our Australian colleagues are also keen to see if the rust might be suitable to release there, as insect agents for boneseed have struggled in Australia, at least in part due to the aggressive ants which give them a hard time.

On the plus side the rust can be highly damaging to boneseed (*Chrysanthemoides monilifera monilifera*) reducing the plant's ability to grow and reproduce by causing extensive deformities known as witches' brooms. Also, being a rust, it is likely to be highly specific. The downside is that this particular rust has an extremely long life cycle so it can take from 1 to 3 years before symptoms of infection show up. This makes undertaking host-range testing more challenging than usual especially if you need to test annuals that may not survive long enough!

Alan Wood, of the Plant Protection Research Institute in South Africa, has been helping both countries to test the rust. Alan developed a technique where plants can be assessed microscopically soon after inoculation to determine if the rust fungus can penetrate the plant tissue, and results can be obtained very quickly. If there are no signs that penetration through the epidermis has occurred then the plant is unlikely to be at risk. However, while most of the test plants could be quickly discounted as unsuitable hosts using this method, unfortunately three New Zealand and six Australian test plants were penetrated. "For these species it is necessary to go a step further and check if penetration actually leads to the subsequent colonisation of plant tissue by the rust, because the plant's defences may still be able to prevent an infection from actually developing, "confirmed Sarah Dodd, our plant pathologist overseeing this project.

Our Australian colleagues have managed to develop a PCR-based molecular tool that can detect the presence of the rust in minute quantities in infected tissue. "This tool will be extremely useful for checking for early establishment in the field, if permission to release the rust is granted, explained Louise Morin of CSIRO. But the tool is not able to speed up the testing regime because of the possibility of false negatives, i.e. rust is present but not actually causing infection.

Attempts to use various staining techniques to reveal the presence of fungal hyphae growing within the leaves, before the appearance of visible symptoms, have also not produced any short cuts. So unfortunately the only way to resolve the matter now for both countries is to inoculate test plants this spring in South Africa and wait for up to 3 years to see if they develop any visible symptoms.

This project is funded by the National Biocontrol Collective. The Australian project began first so we have been able to benefit from their research and test fewer species.

Boneseed plant severely infected by the boneseed rust near Stellenbosch, South Africa.

Exposing the Lag Phase in Woody Weed Invasions

Weed invasions tend to start covertly, with barely noticeable spread initially before a sudden change to rapid population growth. One of the greatest challenges in managing weeds is identifying which of the many small "sleeper" weed populations present in New Zealand are likely to explode first, and what triggers them to awaken from their "lag phase". A study of hawthorn (*Crataegus monogyna*) in Porters Pass, Canterbury, is shedding light on the factors governing the lag phase of bird-dispersed woody weeds in montane environments.

Peter Williams and Rowan Buxton of Landcare Research recently resampled the 350-ha hawthorn population at Porters Pass that they first measured 25 years ago. By estimating the ages of a sample of the mature hawthorns present, they were able to reconstruct the invasion from its source, a 100-year-old tree growing next to a roadside shelter. John Kean of AgResearch helped to analyse the data, which showed that the rate of population increase had apparently changed dramatically in the late 1950s and again in the mid-1970s.

Up until 1959, hawthorn at Porters Pass spread slowly, with only a 3% increase in numbers each year. Historical photographs show that during this lag phase the area was grassland with scattered short matagouri (*Discaria toumatou*), which was kept trimmed by abundant rabbit populations. However, in the late 1950s the rate of hawthorn spread accelerated by more than six times to 21%. This coincided with heavy rabbit control, allowing the matagouri to grow in stature, thereby providing roosting and nesting sites for the blackbirds which hawthorn relies on to spread its seeds. Matagouri growth was also promoted further by the instigation of aerial topdressing in the 1950s.

However, the spread of hawthorn was not even across the landscape. The plant was initially restricted to the steeper hillsides and scarps, with the flat river terraces only lightly occupied, and only since topdressing began. Once again this reflects the influence of grazing, this time by sheep, and the preference of matagouri for the rubbly soils of scarps and gullies. It also reinforces the role that providing suitable perches for blackbirds has in the spread of hawthorn. Peter and Rowan found that 70% of hawthorn seedlings and juveniles grew within 50 cm of a blackbird perch, and 99% grew within 2 m.

The complex interactions between landform, soil fertility, sheep, rabbits, native scrub and blackbirds seem to govern the invasion rates of hawthorn, and probably other bird-dispersed woody weeds too. "You can see exactly the same thing happening with rowan trees spreading over Jack's Pass in Hanmer and from plantings in Tekapo Village," commented Peter. "We believe that it is really important that founder trees are removed from all settlements, and from montane land before grazing pressure is released following land retirement."

But what of the future for the Porters Pass site? Since the mid-1970s suitable habitat there has largely become saturated with hawthorn, resulting in much slower spread, at least until grazing pressure eases further, or hawthorn manages to colonise other valleys in the area. Meanwhile the montane environment is dotted with numerous other populations of bird-dispersed woody weeds. Hawthorn, rowan (*Sorbus acuparia*), *Prunus* and *Cotoneaster* were often left behind when high country homesteads and huts were abandoned. Unless we take action there they wait, biding their time until conditions are right for them, too, to awaken from their lag phases and march across the landscape.

This research was funded by the Foundation for Research, Science and Technology as part of the Beating Weeds programme.

John Kean

Rowan Buxton fells the original source of the hawthorn invasion at Porters Pass.

Summer Activities

With temperatures rising and days getting longer many of our biocontrol agents will be in full swing so it will be a good time to check for establishment and even shift some around. Some activities that you might need to schedule in are:

Boneseed leafroller (*Tortrix* s.l. sp. "chrysanthemoides")

- Check release sites. Look for the feeding shelters the caterpillars make by webbing together the tips of two or more neighbouring leaves. Leaves will have "windows" where the caterpillars have eaten the green tissue away and may be turning brown. Small caterpillars are olive-green in colour and become darker as they get older and develop rows of white spots along the length of their bodies. If you see severe damage to the foliage, we would be very interested to hear about this.
- Unless you find enormous numbers of caterpillars it is probably best to hold off harvesting and shifting them around until we have a better idea of the minimum number you need for a release.

Broom leaf beetles (*Gonioctena olivacea*)

- Although it is early days yet, you might be tempted to check the sites where you released these beetles a year ago, but don't be too disappointed if you can't find them just yet! If you are lucky you may be able to see the smallish (2–5 mm long) adult beetles. Females are goldish-brown and males have an orangey-red tinge, but colouration can be quite variable. You might be able to see the larvae feeding on the leaves and shoot tips. If you can't see adults or larvae then perhaps try gently beating some foliage over a white sheet.
- We would not expect you to find enough beetles to be able to begin harvesting and redistribution yet.

Broom seed beetle (*Bruchidius villosus*)

- If you missed out during flowering time you can still collect and shift broom seed beetles around inside mature pods. Pods should be brown and just beginning to burst open. Avoid green pods as the beetles will not be completely developed inside.

Boneseed leafroller feeding shelter.

Gorse soft shoot moth (*Agonopterix ulicetella*)

- If you hurry you can still check release sites. By late November or early December the caterpillars are usually about half-grown. Look for webbed or deformed growing tips with a dark brown or greyish-green caterpillar inside. Please let us know if you find an outbreak or caterpillars anywhere that you didn't expect. We are especially interested to hear how they are doing in the North Island and lower South Island.
- If the moths are not yet widespread, you can help to move them around. Shift the caterpillars by harvesting branches or even whole bushes.

Green thistle beetles (*Cassida rubiginosa*)

- Again this one has only just been released and is likely to be hard to find. If you are desperate for an early look you may be able to see the larvae, which are extremely distinctive with prominent lateral and tail spines and a protective covering of old moulted skins and excrement. You many also find feeding damage which looks like windows have been made in the leaves.
- We would not expect you to find enough beetles to be able to begin harvesting and redistribution just yet.

Gorse thrips (*Sericothrips staphylinus*)

- It is a good idea to check gorse thrips when gorse isn't flowering so you won't be confused with flower thrips (*Thrips obscuratus*). In particular look closely on soft new growth. If you can't see any thrips try gently beating some foliage over a white sheet.
- If thrips are present in good numbers you can harvest them by cutting infested material and wedging it in uninfested bushes.

Heather beetle (*Lochmaea suturalis*)

- Check release sites for the greyish larvae. Last summer there were several outbreaks with huge numbers of beetles causing noticeable damage. If numbers are low the best way to find the larvae is by beating plants with a stick over a white sheet or by using a sweep net. Harvesting is best left to autumn when new adults have emerged.

Hieracium gall midge (*Macrolabis pilosellae*)

- Check release sites for plants with swollen and deformed leaves caused by larval feeding. The only way to shift this agent around is by transplanting infected plants, so summer is not likely to be a good time to do this unless you can keep them well watered.

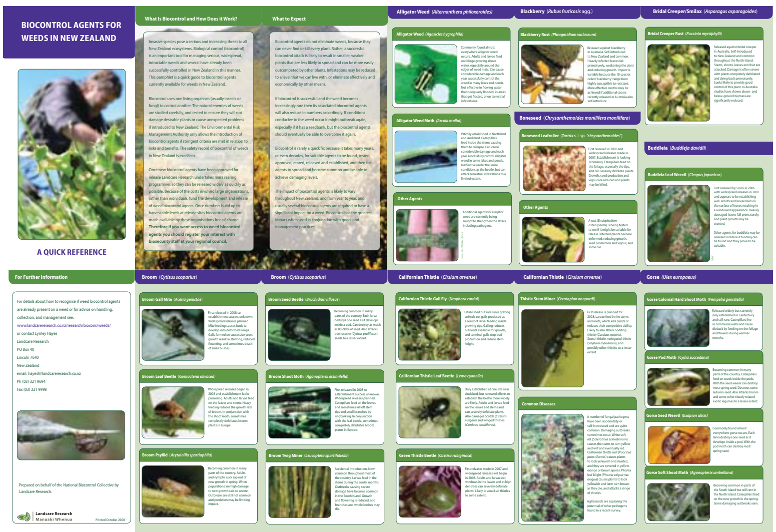
Old man's beard sawfly (*Monophadnus spinolae*)

- Before we write this one off completely perhaps check saw fly release sites one last time. Look for leaves with semicircular incisions along the margin or which have been completely skeletonised, and for black balls of frass, both of which are produced by the white caterpillar-like larvae.

Send any reports of interesting, new or unusual sightings to Lynley Hayes (hayesl@landcareresearch.co.nz, Ph 03 321 9694). Monitoring forms for most species can be downloaded from www.landcareresearch.co.nz/research/biocons/weeds/book/ under Release and Monitoring Forms.

New Biocontrol Pamphlet

We have prepared a new pamphlet that explains biocontrol in a nutshell and illustrates all the biocontrol agents currently available for weeds in New Zealand. The pamphlet is designed with the general public in mind and we hope in the future it can be updated every few years. The pamphlet is printed in full colour on A2-sized paper, but can be folded up like a map to fit in your back pocket. Copies of the pamphlet are being distributed with hard copies of this newsletter, and each member of the National Biocontrol Collective (regional councils nationwide and DOC) have been supplied with some to give away at their discretion. If you would like one or more copies of the pamphlet please contact Lynley Hayes (hayesl@landcareresearch.co.nz or Ph 03 321 9694).



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