What's New In Biological Control Of Weeds?

What's Inside:

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Manaaki Whenua
Landcare Research

Scaling Down an Unwanted Climber

Moth plant (*Araujia sericifera*) has been causing concern now for a number of years and recently it was finally deemed serious and intractable enough to warrant investigating biological control for it. Last year we completed a survey of the insects and fungi already present on the plant here (See *Not Much Menacing Moth Plant,* Issue 30) and a report on the feasibility of using viruses against this target. Coincidentally towards the end of these investigations an Auckland Regional Council staff member discovered a strange and stunted-looking moth specimen at Awhitu Regional Park.

"The plant was exhibiting symptoms that are often commonly associated with a plant virus disease (mottling, yellowing, misshapen and stunted leaves)," described Nick Waipara. MAF virologist Dr Francisco Ochoa-Corona has tried in vain to identify a transmissible infectious agent in the plant's sap, so to date the organism's identity remains elusive. There is a possibility that the culprit is the Araujia mosaic virus (AjMV), a disease of the weed in its native range of Argentina. AjMV has been studied by our American colleague, Professor Raghavan Charudattan, as a possible biocontrol for the closely related strangler vine (*Morrenia odorata*). This virus has also been of interest to us as a possible biocontrol agent, hence the recent virus feasibility study. "There is currently no genetic identification tool available for AjMV, as it was first identified in the early 1980s before this technology had been developed," explained Nick. This means that molecular identification methods (which are now routinely used to identify plant viruses) cannot be used on the Awhitu specimens. Progress now relies on a genetic identification of AjMV being completed overseas, and arrangements are being made to allow this to happen.

Alistair MacArthur

Diseased moth plant from Awhitu Regional Park

Whatever the virus turns out to be it is likely to be a new record for New Zealand, but whether or not it could be used to fight moth plant remains to be seen. In the past we have not surveyed for viruses as they were not considered to be suitable for biocontrol, but recent advances mean this might no longer be the case. However, a lot more research needs to be done before using these tiny plant pathogens becomes standard practice.

A classical biological control programme against moth plant has been initiated in collaboration with the University of Bahia Blanca, Argentina. Surveys of more than 50 different populations of the plant are now underway in the plant's native range. The first aim of the project is to clarify the taxonomic classification of the plant. Although there are currently thought to be two closely related species, A. *sericifera* and A. *hortorum*, some local botanists are convinced they are the same plant. The distribution of moth plant in its native range is also being recorded so that extensive surveys for potential biocontrol agents can be conducted. Botanist Dr Carlos Villamil is helping us with both these matters.

A preliminary survey to identify any fungal pathogens on the plant has been undertaken with the help of plant pathologist Dr Ralph Delhy. Seven fungal diseases have so far been recorded including a rust, *Puccinia araujia*. Nearly all the populations of moth plant surveyed had spots on the leaves and fruit that were generally associated with species of *Ascochyta* and/or *Septoria*. "These fungi might be able to subdue moth plant through applying continuous disease pressure, but their pathogenicity needs to be assessed in the lab to confirm or otherwise these anecdotal field observations," cautioned Nick. Three viruses have also been encountered, two of which may be vectored by aphids.

These early results are positive as they confirm that moth plant is susceptible to a wide range of plant pathogens in its native range, so hopefully at least one of them will prove to be a prospective biocontrol agent. Surveys are now being expanded to include insects. We will let you know what turns up and the identity of the mystery virus affecting moth plant at Awhitu Regional Park in due course.

This project is funded by a national collective of regional councils and the Department of Conservation. A report on the feasibility of using viruses against moth plant was funded by the Auckland Regional Council.

Winter Activities

There is not much work to be done on the biological control front at this time of the year as most control agents hide away or become dormant. However, you can still:

- Check nodding thistle crown weevil (*Trichosirocalus* spp.) release sites. Although some weevils lay eggs all year round, most begin to lay in the autumn and the damage to the rosettes becomes more noticeable as the winter progresses. Look for leaves that have lost their prickliness and for black frass in the crown. Although nodding thistle (*Carduus nutans*) is the preferred host you may also find other species of thistles are attacked too. Crown weevil adults can often be successfully harvested and shifted around as late as June. To see the adults you will need to look carefully on the undersides of the leaves.
- Shift ragwort flea beetles (*Longitarsus jacobaeae*) around, provided you can find them in good numbers.
- Make sure all the paperwork relating to release sites is up to date. If you have been shifting agents around then we would be interested to know about this (send information to Lynley Hayes).

International Bioherbicide Workshop

The International Bioherbicide Group is a collection of scientists with an interest in weed control using microbes. They organise workshops from time to time in association with major international conferences and also produce a newsletter (see http://ibg.ba.cnr.it/). The group is holding its next workshop in Italy in June, immediately before the 13th European Weed Research Society Symposium. The theme will be "Current status and future prospects in bioherbicide research and product development" (see http://www.ewrs-symposium.com/workshops.php.). Jane Barton will be attending the workshop and will report on it for the November issue of this newsletter.

Hot Gossip

In mid-February we received word that the **Environmental Risk Management Authority** had granted approval for the **boneseed leaf roller** (*Tortrix s.l.* sp."*chrysanthemoides*") to be released in New Zealand. We are now organising for a shipment of the moth to be sent from South Africa to form a nucleus rearing colony. The moths will have to spend some time in quarantine until they have been officially identified and cleared of any unwanted associated organisms. If everything goes smoothly the first releases may be able to be made this summer.

The **old man's beard saw fly** (*Monophadnus spinolae*) has always been a difficult insect to mass rear and recently we have decided **to abandon our rearing programme**. It appeared that our rearing colony had become diseased as numbers were decreasing each generation, not increasing. At this stage it does not appear to be worth the expense of sourcing more saw flies from Switzerland and we will instead **carefully monitor the existing release sites** (see table) for establishment.

Region	No. of release sites
Bay of Plenty	2
Hawke's Bay	1
Taranaki	1
Manawatu -Wanganui	2
Wellington	2
Tasman	2
Marlborough	1
Canterbury	5
Otago	1
Total	17

Ronny Groenteman

No evidence of establishment has been seen yet, but it is not unusual for it to take several years before new agents are detectable in the field. At least three of the release sites have been destroyed by flooding or slipping, so we will be hoping that no further calamities befall any of the others.

Ronny Groenteman is a new PhD student who has come to New Zealand from Israel to help us **outsmart thistles**. Simon Fowler, Graeme Bourdôt (AgResearch), and Dave Kelly (University of Canterbury) are supervising her work in the AgResearch-led "Outsmarting Weeds" programme, funded by the Foundation for Research, Science and Technology. Ronny is studying the **effectiveness of the three nodding thistle agents**, including their impacts on other thistle species – some work that is long overdue. She plans to use this thistle group to develop a model to look at whether the effectiveness of a biocontrol agent could be determined before it is introduced into New Zealand. She will be exploring factors such as potential competition with other agents, using the two seed feeders, the nodding thistle receptacle weevil (*Rhinocyllus conicus*) and gall fly (*Urophora solstitialis*), as her example. Ronny will also **investigate the potential usefulness of using biocontrol agents that have a wider host range** than just one species of thistle. The benefit of this approach is that one agent could possibly target several weeds, for example all *Carduus* and *Cirsium* species. Ronny suspects this approach could help stop other closely related plants that are not yet weedy from becoming so in the future.

A half buried sign marks the now unrecognisable sawfly site at Ohakea – a result of the 2004 floods.

Working Out Which Ones Are the Bad Eggs

In the previous issue of this newsletter we told you about the decision-making process that was used to decide if the boneseed leaf roller (*Tortrix* s.l. sp."*chrysanthemoides*") was suitable to introduce to New Zealand (See *Weighing Up the Risk*, Issue 31). This was probably the most complicated case we have ever had to prepare in support of a biocontrol agent, and it is worth explaining in more detail how anyone in their right mind could possibly propose or approve the introduction of a moth whose larvae in lab tests fed on no fewer than 36 plant species from 11 plant families!

What the boneseed leaf roller can do to boneseed in its homeland.

"Fundamental" versus "field" host-range

Host-specificity testing is used to discard potential agents that are likely to cause unacceptable non-target damage to desirable plants. The simplest tests are "no-choice" ones where the potential agent is given a test plant and either feeds on it or starves to death. As you can imagine the results are extremely robust and they can be used to define the fundamental or physiological host-range of a particular species, that is, all the plant species that it can survive on if push comes to shove. If the fundamental host range of a particular agent is restricted to the target weed, then the risk of non-target damage is negligible.

However, no-choice tests can often lead to "false positives" when insects are forced to accept a plant species they would never utilise in nature. Specialist insect herbivores commonly show a broader host-range in no-choice tests than in choice tests, where the normal host plant is presented along with the test plant. Usually this increase in host-range is small, for example, in a no-choice situation the lantana leaf-beetle (*Uroplata girardi*) fed and developed on several plants in the same family as lantana (*Verbenaceae*) but only fed on lantana in choice tests.

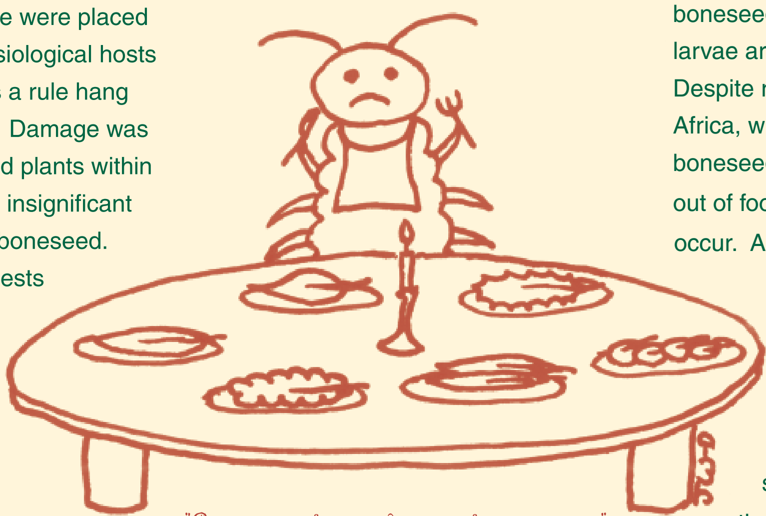
A few species, including the boneseed leafroller, have broad fundamental host-ranges. The water lettuce weevil (*Neohydronomus affinis*) attacked plants from five families (*Araceae, Lemnaceae, Hydrocharitaceae, Azollaceae* and *Salviniaceae*) in no-choice tests, but in choice tests only bothered with water lettuce (*Pistia stratoites*) (*Araceae*). Likewise two puncture vine weevils (*Microlarinus lareynii* and *M. lypriformis*) went for plants from 17 and 12 families respectively in no-choice tests, but in choice tests restricted themselves to puncture vine (*Tribulus terrestris*). If the results of no-choice had been used alone then all of these insects would have been discarded, and the USA would have missed out on three safe and effective agents.

Obtaining more meaningful results

The problem of false positives has led biological control scientists to develop more natural tests to determine field host range that give more reliable results. This kind of testing is more elaborate and costly than no-choice tests, and is not always necessary for straightforward cut and dried agents. However, it was considered essential for the boneseed leafroller. "Extensive field surveys in South Africa indicated that the boneseed leafroller only feeds on boneseed (*Chrysanthemoides monilifera monilifera*) and bitou bush (*Chrysanthemoides monilifera rotundata*), so we were fairly confident that results from no-choice tests our Australian colleagues had carried out, where the larvae appeared to be fairly indiscriminate, were leading us up the garden path," confided Chris Winks. However, there was still the problem that many of the plants the leafroller might encounter if it were to be introduced into New Zealand or Australia do not occur in South Africa, so field data could not be relied upon alone to make a case for importing the leaf roller.

As a consequence field tests were

carried out in South Africa to see if the adult moths would lay eggs on a selection of plants, of importance to Australia, had not encountered before. Large numbers of moths were released from a central point and allowed to naturally colonise these plants. The moths only laid on boneseed and bitou bush so Australian researchers concluded that the risk of eggs being laid on non-target species was low. In addition some transfer tests conducted in the field demonstrated that when boneseed leafroller larvae were placed on plants, which are physiological hosts in the lab, they did not as a rule hang around on them for long. Damage was confined to closely related plants within the Asteroideae and was insignificant compared to damage to boneseed. However, these transfer tests did also provided some false positives, as some larvae did survive on plants that are known to not be field hosts in South Africa.



"So many choices but nothing to eat"

Selection of New Zealand test plants

Landcare Research scientists utilised the field-testing technique that had been developed by their Australian colleagues to test additional plant species of importance to New Zealand. The most appropriate species to test were chosen according to phylogenetic relatedness to boneseed, which belongs to the tribe Calenduleae of the family Asteraceae. There are no native Calenduleae in New Zealand, but a number of genera within the subfamily Asteroideae, to which boneseed belongs, are native to New Zealand. We therefore endeavoured to test representatives of each of these genera. "Unfortunately, not all could be sourced in South Africa and could not be imported because of quarantine restrictions," explained Chris. Therefore, we used "surrogates" for genera that could not be sourced in South Africa. For example, *Sonchus oleraceus* was used instead of the endemic *Sonchus kirkii*. "While this is not ideal, the use of surrogates, selected according to biogeographic overlap and ecological similarity, is a common, internationally accepted practice in host-specificity testing," confirmed Quentin Paynter. It is often necessary to use surrogates because the cost of testing all the representatives of large plant families or genera would be prohibitive, and both theory and practice support this approach. As before, eggs were only laid on boneseed and bitou bush and we concurred with CSIRO researchers that the risk of eggs being laid on non-target species is low. "Whilst there is a risk that some untested plants might be more acceptable hosts than the representatives we selected, the risk is likely to be small, given the robustness and track record of host-range testing procedures to date," suggested Quent.

Relative risk

The field specificity tests indicated that the moths would only lay eggs on boneseed. Therefore, the only potential scenario where non-target damage may occur would be from "spill-over" due to larvae that have defoliated boneseed and run out of food. Such damage, if it occurs, is likely to be transient, because prolonged defoliation should rapidly lead to successful control of boneseed and therefore a reduction in both boneseed and the leafroller. Furthermore, such damage should only occur within close proximity to boneseed bushes because wandering larvae are unlikely to disperse far. Despite regular outbreaks in South Africa, where the leafroller larvae strip boneseed plants and must at times run out of food, spillover damage does not occur. Against a backdrop of boneseed continuing to invade, with detrimental consequences to native biodiversity and coastal amenity values, the low risk of minor spillover damage posed by the introduction of the boneseed leafroller was deemed acceptable by ERMA, especially considering the potential benefits that should arise from successful biological control of boneseed.

Further reading

Marohasy J. 1998. **The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour.** *Biocontrol News and Information* 19 (1): 13N-20N.

The introduction of the boneseed leafroller is being funded by a national collective of regional councils and the Department of Conservation.

No Surprise that Aussie Rust Misbehaves

"@#$%&!" exclaimed Jane Barton when she looked down the microscope and saw the distinctive black resting spores of blackberry rust (*Phragmidium violaceum*) on a bush lawyer (*Rubus cissoides*) leaf. Up until that moment, the results of her survey looking for non-target effects from the rust had been beautifully clear-cut. She had found plenty of blackberry rust on blackberry (*Rubus fruticosus* agg.), and none on the three New Zealand native *Rubus* species, or the three commercially grown *Rubus* species, that she had collected from 35 sites all over the North Island.

Still, finding blackberry rust on *R. cissoides* was not unexpected. The rust went through vigorous host-range testing when it was being considered as a potential biocontrol agent for blackberry in Australia, and in those tests a number of *Rubus* species, including some species native to New Zealand, were found to be susceptible to it. Supporters of the biological control project in Australia argued successfully that the rust should be released anyway because "although the effect of the rust on populations of the susceptible species of Australian and New Zealand native *Rubus* is not known, these are not economic plants, nor are they listed as endangered species." It probably didn't help that these species are covered in spines and have few, if any, redeeming features.

Blackberry rust was officially released in Australia in 1991, but had appeared there earlier, in 1984, presumably as the result of one or more "illegal" introductions. The rust first showed up in New Zealand in 1990, and recent DNA analysis has confirmed that our populations are derived from the illegal Australian introduction (see *Confirming Our Suspicions*, Issue 27).

Happily, blackberry rust did not appear to be causing significant damage to the couple of *R. cissoides* plants on which it was found. "Very few of the leaves had lesions attributable to the rust, and there were only one or two rust pustules per leaf," explained Jane. Also, infection proved to be an extremely rare event. More than 132 individual *R. cissoides* plants growing at 26 sites scattered across the North Island were carefully examined for disease symptoms, and only two plants, both at the same site (a clump of roadside vegetation beside State Highway 1, North of Taupo, near Mt Maroanui), yielded blackberry rust. There were blackberry plants heavily infected with the rust growing directly below the lightly infected *R. cissoides* plants, and this close proximity between target and non-target plants probably contributed to this result. However, at 14 other sites infected blackberry was found very close to *R. cissoides* but there was no sign of any cross infection occurring. Perhaps some individuals of this bush lawyer species are more resistant to the rust than others? In any case, it seems that to date the strain/s of blackberry rust present in New Zealand are having a negligible impact on this native species.

The other good news is that blackberry rust was not found on either of the other two native bush lawyer species included in the survey: *Rubus australis* (31 plants from five sites checked) and *R. schmidelioides* (over 70 plants from 16 sites checked). Both of these species were found to be susceptible to the rust in host-range tests, especially the latter. The three commercially grown *Rubus* species that were checked, raspberry (*R. idaeus*), boysenberry (*R. ursinus*) and loganberry (*R. ursinus*), were found to be free of blackberry rust. This result was also expected, as Australian tests had shown these species were resistant to the rust.

Two other Rubus species are native to New Zealand: *R. squarrosus* (leafless lawyer) and *R. parvus* (creeping

Jane carefully inspecting bush lawyer.

lawyer). These were not included in the survey because they were classed as "resistant" in Australian host-range tests and also because *R. squarrosus* has highly reduced leaves (the rust usually attacks leaves) and *R. parvus* only grows in the South Island, which was beyond the scope of the survey.

This project on blackberry rust is part of a much bigger survey for non-target effects of weed biocontrol agents (See *Staying on Target*, Issue 29). "The aim of the project is to improve our ability to predict non-target impacts, by checking in the field to see how accurate past predictions were," explained Quentin Paynter. While surveys have already been performed to assess the safety of 20 arthropod biocontrol agents, the blackberry rust survey was the first completed on a pathogen. Surveys to look for non-target damage from old man's beard leaf fungus (*Phoma clematidina*), hieracium rust (*Puccinia hieracii* var. *piloselloidarum*), and mist flower fungus (*Entyloma ageratinae*) are currently underway.

In early 2004, Biosecurity Australia approved the importation of eight additional strains of blackberry rust from Europe to Australia. The common name "blackberry" does not apply to a single plant species, but rather to a collection of closely related *Rubus* species (there are 22 naturalised *Rubus* species and hybrids referred to as "blackberry" in New Zealand). The additional rust strains were released in Australia in April 2004, because the strains already present there were not having sufficient impact on some of their weediest "blackberry" taxa. Also, the legally introduced strain (F15) was released again, as DNA results suggest it may not have established. To date releases have been made in three states: Western Australia, New South Wales and Victoria.

The release of the new blackberry rust strains in Australia is good news for New Zealand, because we also have plenty of blackberry species that are not sufficiently damaged by the rust strain/s that we already have here. While it took 6 years for the illegally introduced strains of the rust to blow across from Australia last time, other rusts (e.g. euphorbia rust, poplar rusts and willow rust) have reached New Zealand within about a year of being first recorded in New South Wales or southern Queensland. Fingers crossed that at least some of the new strains establish in Australia and then rapidly use the trans-Tasman westerlies to get to New Zealand.

Blackberry rust

The eight new rust strains were not tested on any of the New Zealand bush lawyer species because initial host-range testing (before the release of strain F15) was done using a pool of 15 different isolates, and the Aussies argued that they were therefore representative of the species as a whole. They were probably right, and there is no real reason to believe the new strains will be any more damaging to bush lawyer than the one/s we already have (and the illegally introduced strain we have here wasn't tested at all). Still, the timing of the just-completed survey was fortuitous, as initial monitoring in Australia suggests the distribution of the new strains is still limited, so they are unlikely to have reached New Zealand yet. "That means that if blackberry rust suddenly starts appearing on bush lawyer from now on, we can be pretty certain one of the new strains is to blame," suggested Jane. The DNA work that has been done both here and in Australia could ultimately allow the origin of any misbehaving strains to be clearly identified.

So, what should you do if you come across rust symptoms on bush lawyer? The first thing is to make sure you're looking at blackberry rust, and not either of the other rusts that occur on native *Rubus* species, *Kuehneola uredinis* or *Hamaspora australis* (see box). If you still think you have found blackberry rust, please collect about 10 leaves with rust pustules, press them in newspaper underneath something heavy (e.g. a phone book) for a day or two, and then post them to Jane at 353 Pungarehu Rd, RD 5, Te Kuiti. We would also be interested in hearing from you if you notice blackberry suddenly being attacked in areas where you have not seen it before email Jane at jane.barton@ihug.co.nz).

So to conclude: this non-target impact on bush lawyer was predicted, and rightly or wrongly a decision was made in Australia that some collateral damage was an acceptable price to pay for bringing blackberry under biological control. It also emphasises the importance of good communication between neighbouring countries especially when highly mobile biocontrol agents are being considered.

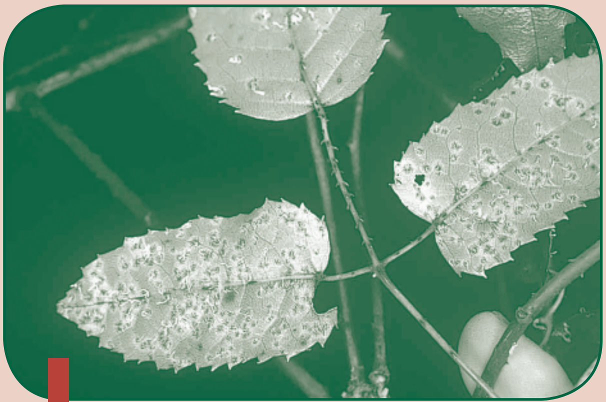
This work was funded by the Foundation for Research, Science and Technology. Thank you to all the people who assisted Jane, and her long-suffering husband Tony, with their quest to find populations of bush lawyer across the country. They couldn't have done it without you!

Further reading

Bruzzese, E.; Hasan, S. 1986b: **Infection of Australian and New Zealand *Rubus subgenera Dalibarda* and *Lampobatus* by the European blackberry rust fungus *Phragmidium violaceum***. *Plant Pathology* 35: 413–416.

Rusts on exotic and native Rubus species

Blackberry cane and leaf rust (*Kuehneola uredinis*) has small, closely spaced, lemony-yellow-coloured spore pustules. Blackberry rust (*Phragmidium violaceum*) pustules are usually larger, further apart, and yellowy-orange in colour. Blackberry rust also produces distinctive pustules of black spores on the underside of the leaves towards the end of the growing season, and blackberry cane and leaf rust does not. Blackberry rust causes distinctive purplish-brown spots on the upper surface of the leaves that correspond with pustules on the underside. Blackberry cane and leaf rust does not do this although areas of discolouration may occasionally be present. Septoria leaf spot (*Septoria rubi*) also causes similar purplish-brown spots on the leaves, but they never have corresponding pustules underneath. Another rust (*Hamaspora australis*), which is common on native *Rubus* species here, causes reddish-purple spots on the upper surface of leaves, but is easy to identify because it often produces characteristic "horns" of spores that stick out from the spots on the under surface of the leaves.



Hamaspara australis on a native Rubus sp.

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