

WHAT'S NEW IN

# Biological Control of Weeds?

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*Buddleia leaf weevil*  
Scion

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Landcare Research  
Manaaki Whenua

# Field Horsetail Project Forges Ahead

Last year, the Lower Rangitikei Horsetail Control Group successfully applied to the Sustainable Farming Fund for a grant to investigate biological control options for field horsetail (*Equisetum arvense*). Field horsetail is an ancient fern-like vascular plant that is a significant weed in New Zealand as well as other Southern Hemisphere countries including Madagascar, South Africa, South America, and Australia. It made its way to New Zealand in the early 1900s from Eurasia (possibly as a passenger with iris root stock from Japan). Like many of New Zealand's weeds, it is toxic and unpalatable to stock, reducing pasture quality. The stems contain silica, which is not digestible, but more serious is the condition of 'equisetosis', which is brought on by grazing the plant, leading to acute thiamine deficiency in horses and cattle. Field horsetail also prevents recruitment of native seedlings and it grows well around river margins, blocking waterways and causing flooding. The plant reproduces and spreads by producing spores (as other ferns

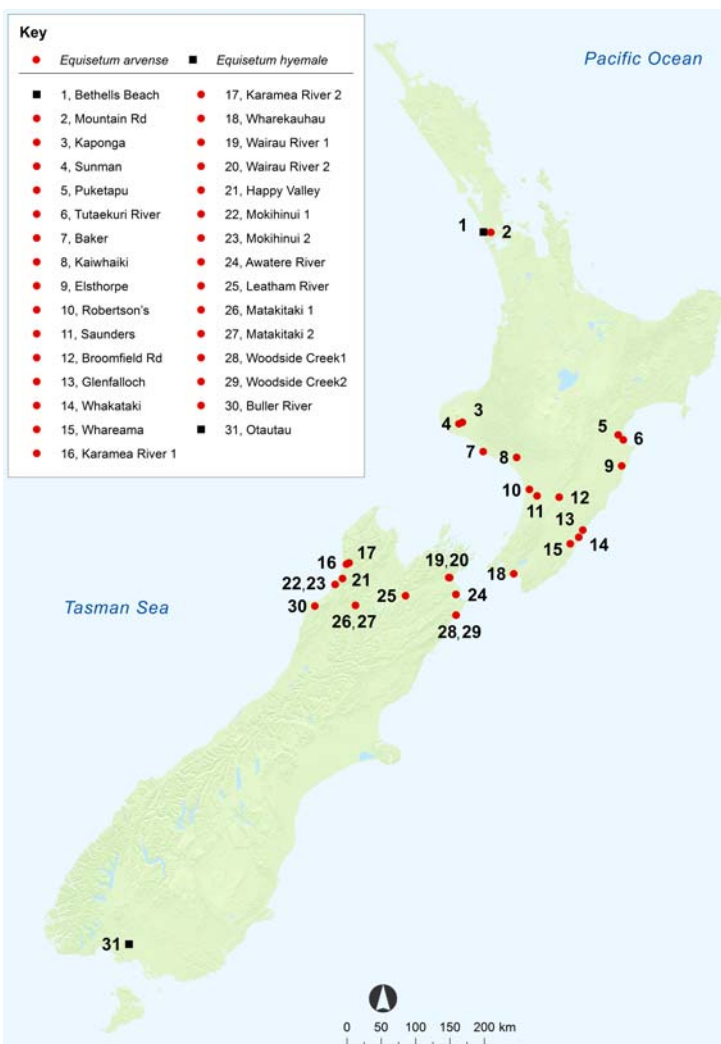
do), but also vegetatively via stolons and tubers. In some areas field horsetail has been unwittingly spread around in gravel extracted from infested areas. Now designated an unwanted organism, it is illegal to knowingly grow or transport the plant within New Zealand. Two other closely-related species have also found their way here – *E. hyemale* (rough horsetail) and *E. fluviatile*. Rough horsetail has not shown the invasive tendencies seen by field horsetail and *E. fluviatile* has been successfully eradicated.

Field horsetail prefers the wetter regions of New Zealand and is now widespread in Whanganui, Rangitikei, Taranaki, parts of Wellington and the West Coast of the South Island. But it has also been recorded on the east coast in Havelock North, Marlborough, Canterbury and Otago. Traditional control measures using herbicide are costly and not always successful. "It is not a plant that can be controlled easily by applying herbicide,"

explained Lindsay Smith, who is leading the project. "Because the plant has a deep root system, it is difficult to find chemicals that penetrate deep enough into the roots to be effective," he added. As a result, field horsetail is not able to be controlled adequately and biocontrol might be the only hope of managing the plant and preventing further spread. New Zealand is the first country to investigate the use of biocontrol agents to manage this plant.

Recently we have completed an important first step, a survey to see what natural enemies are already attacking field horsetail in New Zealand. "It is important to get an idea of what is already here, what niches are already occupied and which organisms may compete with, parasitise or predate on any introduced agents," explained Lindsay. The survey mainly focused on field horsetail but two rough horsetail sites were also sampled (see map).

The invertebrate samples were taken by beating field horsetail plants over a sheet and collecting what was dislodged from the plant. The samples produced only 19 generalist herbivorous species and there were extremely low levels of damage seen on the plants. It was a similar story with the pathogens collected, with most being generalist species that occur commonly in commercial crops (e.g. *Phoma* spp.). A total of 38 pathogens were recovered by collecting plants with disease symptoms and then cultivating the fungal colonies on potato dextrose agar plates so they could be identified using DNA sequence analysis. Two species found might have some potential to be developed into mycoherbicides, but the size of the market for such a product is likely to be too small for this



Field horsetail (*Equisetum arvense*) and rough horsetail (*E. hyemale*) sites sampled for invertebrates and plant pathogens.

to be economic; and so classical biocontrol options look more promising at this stage and are being investigated first.

It was clear from the survey, that compared with other weeds in New Zealand, the invertebrate fauna associated with field horsetail is depauperate. This was supported by the lack of damage seen on the plants, with <1% of the foliage showing any signs of feeding by insects. There were few predatory insects but an abundance of spiders, which were likely to be using the field horsetail plants as a convenient habitat. Therefore there is considerable scope for the introduction of host-specific invertebrate biocontrol agents that could markedly reduce the vigour of field horsetail in New Zealand.

Four potential insect agents from the UK are currently undergoing host-range testing at the Lincoln containment facility. They include a flea beetle (*Hippuriphila modeeri*), a weevil (*Grypus equiseti*), and two sawfly species (*Dolerus germanicus* and *D. vestigialis*). Despite their misleading name, sawflies are in fact a type of herbivorous wasp, and are notoriously difficult to rear in captivity. As adults the males and females look quite different and can easily be told apart. They hibernate over winter and can emerge from this diapause at different times depending on environmental factors. As if this isn't enough, they have quite elaborate courtship rituals requiring the right environment to get the females "in the mood"! Figuring out these requirements and replicating them in a containment facility can be a big challenge. "So far, though, we have managed to produce enough sawflies to get host-testing underway, and the larvae appear to be capable of inflicting severe damage to above-ground foliage, grazing it down to ground level," said Lindsay.

The flea beetle is easier to manage in containment but there are questions about whether it would be an effective agent. The larvae mine the thin needle-like fronds of the plant, which stunts plant growth but doesn't compare favourably to the damage caused by the other agents.

The weevil appears to be the most promising of all. Adults lay their eggs near the top of the plant and the larvae then bore into and down the central stem, killing all of the above-ground material. Larvae continue to bore down into the root system, causing complete collapse of the stem. "Initial host-testing results are promising and the weevil is so effective that we are beginning to wonder whether any other agents would be needed; however, it is good to have the other species as a back-up. Also, depending on the phenology and emergence rates of the weevil, the sawfly larvae could inadvertently eat the weevil eggs or disrupt the larvae, which would be counterproductive," said Simon Fowler. All of these agents co-exist in the UK but the environmental conditions are slightly different here and subtle differences in the climate or the niche-overlap might constrain their ability to colonise the plants. We plan to study likely interactions further.



Sawfly (*Dolerus germanicus*) laying eggs on horsetail in containment.

Two fungal pathogens, *Stammaria persoonii* and *Ascochyta equiseti*, available overseas appear to be specific to field horsetail and look like promising agents, and could also be considered later if needed.

To conclude, the prospect of finding agents to manage field horsetail looks very encouraging. There aren't any plants even closely related to field horsetail in New Zealand's indigenous flora, which makes the logistics of host-range testing simpler and the chance of non-target attack minimal. Although the plant has been grown for medicinal properties overseas, it hasn't been used here for this purpose, which will reduce the hurdles required to gain approval to release biocontrol agents. The agents that have been selected are likely to be host-specific and all have been shown to damage field horsetail to varying degrees. With host-testing proceeding at a good pace, it is likely that an application will be made to EPA to release field horsetail agents in 2015.

*The Lower Rangitikei Horsetail Control Group represents a diverse group of landowners and managers who have a significant problem with field horsetail, and who have come together to try to find a better solution. Alastair Robertson chairs the group, which also includes other arable and pastoral farmers, and representatives from the aggregate extraction industry, district and regional councils, the New Zealand Landcare Trust, and Landcare Research as the science advisor.*

CONTACT: Lindsay Smith  
smithl@landcareresearch.co.nz

# Promising Pathogen for Cruel Climber

The invasive climber *Araujia hortorum* is pollinated by insects, especially moths, and so is usually referred to as moth plant in New Zealand. However, a secretion inside the flowers sometimes traps and kills these winged visitors, so it is also known as cruel plant. It isn't just the insect-murdering reputation of this vine that makes it unpopular in New Zealand. Moth plant grows quickly, smothering desirable vegetation, and its milky sap is a poisonous skin irritant.

Towards the end of 2011 we were granted permission to release the first biocontrol agent for moth plant, a beetle (*Colapsis argentinensis*). Unfortunately releases of the beetle have not yet begun due to increased restrictions in South America around the exportation of native biodiversity. Confirming and meeting the new requirements is proving to be a protracted business. The earliest that beetle releases could begin would be spring 2015 and, paperwork permitting, a second agent might also be ready for release at that time.

A rust (*Puccinia araujiae*) was identified by Rolf Delhey and Mirta Kiehr, from Universidad Nacional del Sur in Bahía Blanca, Argentina, as the most promising pathogen found during surveys in the native range. More recently Freda Anderson at CERZOS, also in Bahía Blanca, has built on their work by confirming their findings about its life cycle. "The rust is microcyclic and autoecious, which means it only has two types of spores (some have as many as five) and it completes its life cycle on moth plant, without the need for any other host," confirmed Freda. However, unfortunately the rust is commonly attacked in the field by another fungus, a hyperparasite. It has proven quite challenging to get rid of this unwanted parasite, which can quickly overgrow and kill rust colonies.



Freda Anderson

Moth plant rust with hyperparasite (*Cladosporium*) inset.

Fortunately Freda is no stranger to difficult lab work and is now well on the way to obtaining a clean colony of the rust and is confident of success. The rust only grows on living plants, so Freda took plant material with rust pustules on it from the field and surface-sterilised the tissue to get rid of other organisms. She then cut small (2.5 mm) discs of infected tissue and put these onto agar plates. Once telia (resting spores) had germinated on the plates the cleanest ones were selected and suspended over moth plant for 24 hours in an environment favourable for infection. This allowed healthy plants to be inoculated with basidiospores, and in due course new pustules would develop. Meanwhile, Freda kept the agar plates, and if the hyperparasite developed on them then plants infected with material from those plates were assumed to be contaminated and were destroyed. The remaining plants provided material to repeat the process, gradually reducing the hyperparasite infection. "But wouldn't you know it, the hyperparasite was still there in my fourth generation!" reported Freda. "So I started all over again and I think I now have several new fourth-generation plants free of the hyperparasite."

Meanwhile, Freda also completed the work begun by Rolf and Mirta to establish the host range of the rust. "We knew this pathogen would not be specific to just moth plant because it had been reported from several hosts in the field," reported Jane Barton (contractor to Landcare Research). However, the reported hosts were close relatives (belonging to the same subtribe as moth plant, Oxypetalinae) not present in New Zealand. Not many plants in the same family as moth plant (Apocynaceae) are present and valued in New Zealand. Notable exceptions are native jasmine (*Parsonsia* spp.) and the favourite food of monarch caterpillars: blood flower (*Asclepias curassavica*) and swan plant (*Gomphocarpus* spp.). Luckily, testing showed these are not at risk. One plant that was not able to be tested is the minor ornamental tweedia (*Oxypetalum caeruleum*). Since tweedia is in the Oxypetalinae subtribe it is likely to be a host to the rust. The trade-off between protecting this plant and bringing a serious weed potentially under control will need to be weighed up as part of the EPA process. An EPA application is currently being prepared, which will be submitted by Northland Regional Council, on behalf of the National Biocontrol Collective, before Christmas.

*This project is funded by the National Biocontrol Collective. More information about the moth plant rust application can be found at: <http://www.landcareresearch.co.nz/science/plants-animals-fungi/plants/weeds/biocontrol/approvals/current-applications/moth-plant>.*

**CONTACT:** Jane Barton  
Jane.Barton@ihug.co.nz

# Pampas Proves To Be a Difficult Target

When we first considered the feasibility of biocontrol for pampas (*Cortaderia* spp.) back in 2000 we concluded that it would likely be a difficult target. No potential agents were known and agents with a high level of specificity would be needed to avoid harming our closely-related native toetoe (*Austroderia* spp.). After three years of searching for potential biocontrol agents pampas has indeed proven to be a difficult target, but not always for the reasons we expected!

We initially planned to look for agents in Argentina, the only place in South America where both the problem species in New Zealand, *Cortaderia jubata* and *C. selloana*, were reported to occur. “That was when we discovered that *Cortaderia* taxonomy doesn’t resolve the genetic complexity in the group, with molecular studies quickly showing that *C. jubata* and *C. selloana* from Argentina were not the same as material in New Zealand of the same name,” explained Gary Houliston. We were able to quickly match our *C. jubata* with material in southern Ecuador, but a match for *C. selloana* proved much trickier. With the help of a number of South American collaborators we sourced and genotyped more material from Argentina plus from Uruguay, Brazil and Chile. When we had almost given up hope, a perfect match for New Zealand material turned up in a handful of plants deliberately planted next to a soccer field in Chile. Tracing the source of these plants, and further sampling around the hotspots, enabled more matches to be identified.

Another revelation, thanks to molecular techniques, was that some New Zealand pampas was neither *C. jubata* nor *C. selloana*, but an entity we commonly encountered when sequencing South American material – we can’t put an accurate name on it because of the state of *Cortaderia* taxonomy. Initially we recommended these populations be eradicated, but the more we looked the more we found, and so the project scope has widened to seek biocontrol agents for this entity as well.

The next challenge was that surveys in South America turned up only two potential control agents worthy of further study, a fungus and an insect. A black smut that damages the flower-heads, reducing seeding, was found in Ecuador on *C. jubata* and in Chile on another *Cortaderia* species. Sequencing has shown the Ecuadorian smut is a 100% match with the published strain of *Ustilago quitensis*, and the Chilean smut is a 98% match. So the two smuts are likely to be the same species, but it is unclear whether they are different strains with different host preferences. “Dr Charlie Barnes (Pontificia Universidad Católica del Ecuador) is undertaking further studies of the Ecuadorian smut for us, and with the help of Dr Hernan Norambuena we are planning to import the Chilean smut into containment later this year for further study,” explained Lynley Hayes, who is leading the project.



Hernan Norambuena

Black floral smut found in Chile.

The other potential agent is a delphacid planthopper that attacks the leaves. Our Chilean collaborators suspected they had found two similar planthopper species and sent us specimens for sequencing. We confirmed the identity of the more common of the two as *Saccharosydne subandina*, which has no potential as a biocontrol agent as its host range is too wide. The less commonly found planthopper is a novel, un-named species so no information about it is available. With Hernan’s help again we plan to explore the host range of this novel planthopper, and investigate whether, like *S. subandina*, it is associated with a phytoplasma disease. Relatively little is known about phytoplasma diseases in New Zealand except where they have caused serious problems like cabbage tree decline and flax yellows. A lot more research will be needed to explore whether a potential biocontrol agent that can vector phytoplasma diseases would provide a double-whammy advantage or be total unsuitable.

In case a classical biocontrol approach does not work out, Stan Bellgard has been exploring whether the utility of synthetic and organic herbicides can be increased through co-formulation with a plant pathogen (*Nigrospora oryzae*) recovered from pampas in New Zealand. “Results to date have been a bit mixed, but work is continuing this year on co-formulation trials using 25% Gallant (haloxyfop) and also pine-oil and pine-oil/fatty acid commercial formulations, and if we can improve the current spore formulation that could provide a breakthrough,” said Stan.

*This project is funded by the National Pampas Biocontrol Initiative through a grant from the Ministry for Primary Industries’ Sustainable Farming Fund (11/049), supported by a number of co-funders, including the National Biocontrol Collective.*

**CONTACT:** Lynley Hayes

hayesl@landcareresearch.co.nz

# How Many Replicates Are Enough?

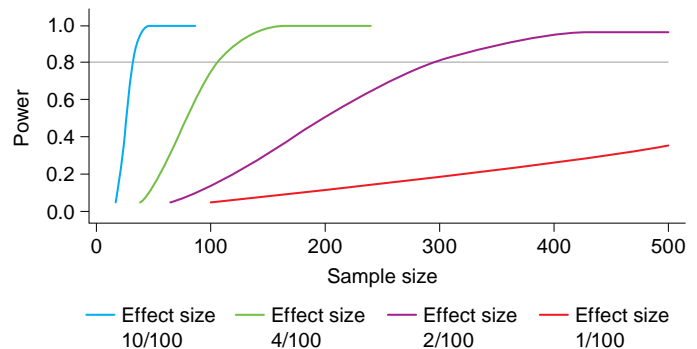
The buddleia leaf weevil (*Cleopus japonicus*) is now a well-established biocontrol agent for buddleia (*Buddleja davidii*). Since its first release in 2006 it has spread throughout both the North and South Islands. First identified as a possible control agent in the early 1990s, testing by Scion showed it could suppress buddleia growth and even kill the plants.

An essential step in the introduction of a new biocontrol agent is an assessment of the risk it poses to non-target species. Thoughtful experimental design and statistical analyses are needed to ensure valid results are produced and that their meaning can be interpreted correctly so that suitable agents are not rejected unnecessarily or unsuitable agents released.

“Of special concern in the buddleia weevil testing was the potential threat to *Hebe speciosa*, an endangered plant and a taonga quite closely related to buddleia,” explained Toni Withers of Scion. The risk to *H. speciosa* was initially assessed by placing five larvae on leaves and monitoring their progress. In one of six replicates, one of the five larvae continued to feed and develop into a pupa, long after all the others died, but the ultimate fate of this pupa was lost. A second trial with double the number of replicates was then carried out. No larvae survived to pupation and approval was then given by the Environmental Risk Management Authority to release the weevil.

Plant species that do not support development of a candidate biocontrol agent during host-range testing are considered to be outside the fundamental host-range of that agent and not hosts. However, when a biocontrol agent is capable of rearing through to adult on a test plant it can be difficult to determine the risk of non-target attack. Clearly, if only a tiny proportion of individuals rear through on a test plant, compared to the target weed (as in the case of the buddleia weevil), then the test plant is unlikely to be a suitable host, but how much replication do you need before you can be certain that you aren't missing or dismissing a potentially important result? The more replication undertaken, the more the testing costs and the longer it takes – so it is important to find the right balance here.

Ideally, the number of replicates needed for any experiment should be established during the design phase. “One way to do this is to carry out a power analysis – a statistical technique to determine the sample size required for the results of an experiment to be statistically valid,” explained Toni. Selecting a biologically relevant effect size (in this case the “effect” is percentage survival to pupation) can be a major limitation to power analysis. An alternative is to use a range of effect sizes to better understand the relationship between effect and



Comparison of sample sizes needed to be 80% confident of detecting a rare event (horizontal line).

sample sizes. Figure 1 provides an example showing that to confidently detect something that occurs often, or 10 times in 100, only 30 replicates may be necessary. Detecting a rarer event, something that occurs twice in 100, would require 300 replicates, while the detection of extremely rare events would require thousands of replicates.

Buddleia weevil on *H. speciosa* provides a useful retrospective case study to test the power analysis approach. The first trial could be viewed as providing a preliminary look for effects and effect size. With development to pupation observed in one out of six replicates, the effect could be assumed to be large. A second trial could then be designed using power analysis to ensure the inclusion of enough replicates (20–30) to ascertain whether the first trial had returned a false positive and whether pupation on the non-target was not a frequent event. If doubt still remained, a third trial using a higher sample size, say 100 replicates, could be run to confirm that pupation was indeed a rare event.

Other retrospective analyses have shown that unexpected non-target damage from weed biocontrol agents is extremely rare. However, given that with host specificity testing there is always a danger of false positive and false negative results, it is good to have additional tools that increase confidence in our predictions, which in turn help to maintain this safety record.

*This research was supported by the Better Border Biosecurity collaboration (B3) [www.b3nz.org](http://www.b3nz.org). For further information see Withers TM, Carlson CA, Gresham BA 2013. Statistical tools to interpret risks that arise from rare events in host specificity testing. Biological Control 64: 177–185.*

**CONTACT:** Toni Withers  
[toni.withers@scionresearch.com](mailto:toni.withers@scionresearch.com)

# Summer Activities

Summer is a busy time in the world of biocontrol. Some activities you may need to schedule over the next few months are listed below.

## Boneseed leafroller (*Tortrix* s.l. sp. “chrysanthemoides”)

- Check release sites for feeding shelters made by caterpillars webbing together leaves at the tips of stems. Also look for “windows” in the leaves and sprinkles of black frass. Small caterpillars are olive-green in colour and become darker as they mature.
- Caterpillars can be harvested if you find them in good numbers. Cut off infested tips and wedge them into plants at new sites. Aim to shift at least 500 caterpillars to sites where scale insects and invasive ants are not present.

## Broom gall mite (*Aceria genistae*)

- Check release sites for galls, which look like deformed lumps and range in size from 5 to 30 mm across.
- If galls are present in good numbers, late spring to early summer is the best time to undertake harvesting and redistribution. Aim to shift at least 50 galls to each site and tie them onto plants so the tiny mites can shift across.

## Broom leaf beetle (*Gonioctena olivacea*)

- Check release sites by beating plants over a tray. Look for the adults, which are 2–5 mm long and goldish-brown (females) through to orangey-red (males) with stripes on their backs. Look also for greyish-brown larvae that may also be seen feeding on leaves and shoot tips.
- It is probably still a bit soon to begin harvesting and redistribution.

## Green thistle beetle (*Cassida rubiginosa*)

- Check release sites for windows eaten into the leaves made by the adults and larvae. Adults are well camouflaged, being green, so it may be easier to spot the larvae, which have a distinctive protective covering of old moulted skins and excrement, and prominent lateral and tail spines.
- It should be possible to begin harvesting and redistribution at some sites. Use a garden-leaf vacuum machine and aim to shift at least 50 adults from spring throughout summer and into autumn. Be careful to separate the beetles from other material collected, which may include pasture pests.

## Tradescantia leaf beetle (*Neolema ogloblini*)

- Check release sites, especially the older ones. Look for notches in the edges of leaves caused by adult feeding or leaves that have been skeletonised by larvae grazing off the green tissue. You may see the dark metallic bronze adults, but they tend to drop or fly away when disturbed. It may be easier to spot the larvae, which have a



Green thistle beetle adult.

distinctive protective covering over their backs. The white, star-shaped pupal cocoons may be visible on damaged foliage.

- We would not expect you to find enough beetles to be able to begin harvesting and redistribution just yet.

## Tradescantia stem beetle (*Lema basicostata*)

- Check release sites, especially the older ones. The black knobby adults also tend to drop when disturbed, but look for their feeding damage, which consists of elongated windows in the upper surfaces of leaves or sometimes whole leaves consumed. The larvae inside the stems will also be difficult to spot. Look for stems showing signs of necrosis or collapse and brown frass.
- We would not expect you to find enough beetles to be able to begin harvesting and redistribution just yet.

## Tradescantia tip beetle (*Neolema abbreviata*)

- Releases only began in 2013, but there is no harm in checking release sites. The adults are mostly black with yellow wing cases, but like the other tradescantia beetles tend to drop when disturbed. Larvae will also be difficult to see when they are feeding inside the tips, but brown frass may be visible. When tips are in short supply, the slug-like larvae feed externally on the leaves.
- We would not expect you to find enough beetles to be able to begin harvesting and redistribution just yet.

## Other agents

You might also need to check or distribute the following this summer (for further details see <http://www.landcareresearch.co.nz/publications/books/biocontrol-of-weeds-book>):

- Broom seed beetle (*Bruchidius villosus*)
- Gorse colonial hard shoot moth (*Pempelia genistella*)
- Gorse soft shoot moth (*Agonopterix umbellana*)
- Gorse thrips (*Sericothrips staphylinus*)
- Ragwort crown-boring moth (*Cochylis atricapitana*)
- Woolly nightshade lace bug (*Gargaphia decoris*)

**CONTACT:** Lynley Hayes

hayesl@landcareresearch.co.nz

# Which Insects Pose the Greatest Risks?

Our expertise in determining which insects attack which plants has recently been put to a slightly different use, i.e. helping to predict risks to native plants. Quentin Paynter assisted Nick Martin (Plant & Food Research) in looking at the risk that exotic herbivores pose to our indigenous plants. In particular, they were interested in teasing out whether the taxonomic group the insects belonged to was important.

Despite the best efforts of border security, there is a constant influx of herbivorous insects arriving here either accidentally on imported goods or arriving under their own steam and one of the tasks of the Ministry for Primary Industries (MPI) is to assess the risk of any new incursions to our indigenous flora. This assessment is based on an estimate of the likelihood that a new herbivore will feed on an indigenous plant.

Although some taxa may be more likely to attack indigenous plants than others, until now nobody has looked into what has actually happened in New Zealand. Nick and Quent searched the literature and talked to experts in the field to document known associations between adventive species and indigenous plants. They produced a summary showing the number of adventive mites and insects known to have arrived in New Zealand and which plants they are hosted by. Seven major arthropod orders were used in the analysis with

the largest group being Hemiptera (true bugs). The other six comprised Thysanoptera (thrips), Lepidoptera (moths and butterflies), Hymenoptera (wasps, bees and ants), Diptera (flies), Coleoptera (beetles and weevils) and Acari (mites), with a total of 624 species included in the assessment (other orders, such as Dermaptera (earwigs), were omitted from the analysis because there were insufficient data on their host associations in New Zealand).

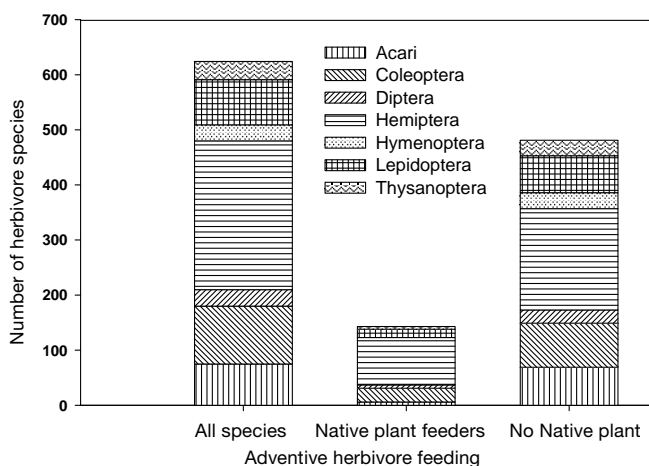
“Hemiptera were the main group found attacking our indigenous plants, with 32% of the adventive species caught in the act,” commented Quent (see graph). One order, Hymenoptera, and some families within orders, e.g. gall mites (Acari: Eriophyoidea) and gall-flies (Diptera: Cecidomyiidae), are a low risk since they are not known to attack indigenous plants.

“This may reflect the degree of host-specificity exhibited by different insect groups,” said Quent. “The New Zealand flora has evolved in isolation and contains a high proportion of endemic plants found nowhere else in the world, so arthropod groups with a high proportion of specialist herbivores are less likely to attack endemic native plants. Plants that are most at risk of attack by specialist herbivores tend to be those that are not confined to New Zealand, such as mānuka (*Leptospermum scoparium*) which is also native to Australia.”

In general the more polyphagous species (able to feed on many plant species) there are in an arthropod family, the more likelihood there is that any particular adventive species will feed on our native plants. Risk assessments should therefore factor in the taxonomic group insects belong to as this will give a clue as to the likely risks they pose and which plants are likely to be on their menu.

For more information see Martin NA, Paynter Q 2013. Predicting risk from adventive herbivores to New Zealand indigenous plants. *New Zealand Entomologist* 37: 21–28. Available online: <http://doi.org/10.1080/00779962.2012.759308>

CONTACT: Quentin Paynter  
paynterq@landcareresearch.co.nz



The number of adventive herbivores in New Zealand, and those that feed, or do not feed, on New Zealand indigenous (native) plants.

