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Manaaki Whenua
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Weed Biocontrol

WHAT'S NEW?



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COVER IMAGE:
Old man's beard sawfly larva



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Selwyn MP, Nicola Grigg, Visits the Lincoln Team

Following on from the publication of a report by the Parliamentary Commissioner for the Environment on the issue of weeds in New Zealand, a Parliamentary Select Committee hearing was held to discuss the findings. At the hearing a question about non-chemical control options for weeds came from none other than the MP for Selwyn, Nicola Grigg. We saw this as an excellent opportunity to talk about biocontrol, and promptly invited Nicola to visit the weed biocontrol group in her constituency of Lincoln.

Nicola took us up on the invitation in late March, visiting our facilities along with Parliamentary Assistant Adam Griffin. We covered the basics of weed biocontrol practices and shared some of New Zealand's historical and emerging success stories. While learning about research funding and the regulatory process for introducing biocontrol agents, a key point of the discussion was the model of operation of the National Biocontrol Collective [NBC]. The NBC provides an example of a nationally coordinated approach to prioritising weed targets, and to pulling resources together for the greater good – two of the key issues identified by the Commissioner as currently lacking in the approach to managing environmental weeds in New Zealand.

The visit also highlighted – not for the first time – how little is known 'out there' about weed biocontrol and how successful and beneficial it can be. Nicola mentioned she had noticed how dramatically ragwort [*Jacobaea vulgaris*] had declined since her days growing up on a farm, when she had to be vigilant about preventing her horses from ingesting this toxic weed. Yet she had not been aware that biocontrol was behind this spectacular decline.

We finished up the visit at the containment facility, where Nicola and Adam got to see the tutsan beetle [*Chrysolina abchasica*] and the field horsetail weevil [*Grypus equiseti*]. As a last stop, they popped into the Honshu white admiral [*Limenitis glorifica*] rearing greenhouse for an experience of being surrounded by butterflies.

Nicola has a strong interest in the sustainability of New Zealand's primary industries, where weed biocontrol has an important role now and, possibly even more so, in the future.

Thank you for the visit, Nicola and Adam!

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Left to right: Nicola Grigg, Angela Bownes, Adam Griffin, Arnaud Cartier

Nicola and Arnaud in the Honshu white admiral rearing greenhouse

Two Recruits to Battle Old Man's Beard

Recent efforts to get two biocontrol agents established on old man's beard (*Clematis vitalba*), one of New Zealand's worst invasive weeds, have proven successful. According to Arnaud Cartier, our technician on the project, this last season was full of exciting developments with new populations of the old man's beard sawfly (*Monophadnus spinolae*) and the recently released eriophyid bud mite (*Aceria vitalbae*).

Both agents have been particularly challenging to work with, but persistent effort has paid off. The sawfly was re-released in 2018 after largely failing to establish when it was first released in the late 1990s. Following an intensive mass-rearing programme at our Lincoln site, thousands of sawfly larvae were released at one site in the Waipara District in Canterbury in the South Island. Our team have been monitoring this release site closely since early 2019, consistently noting the presence of adult males. But it wasn't until this last season that Arnaud could report back that the sawfly population is now booming.

"I visited the release site monthly from November last year until April, and each time it didn't take me long to spot heaps of males flying around in search of females, which remain hidden," he said. "Even more promising, during my last visits I easily found sawfly larvae feeding on the leaves of old man's beard, without much search effort," he enthused. A sawfly larva was a rare sight 2 years ago, but now even their damage has become obvious at the Waipara release site. These recent observations suggest that collecting and relocating part of this sawfly population may be possible in the next couple of years.

Along with the sawfly work, Arnaud was also very busy preparing releases of the old man's beard mite for regional councils. Rearing of this agent had a slow start, but last spring old man's beard plants heavily infested with the mites were sent across the country for planting out in the field. Among the multiple locations where the mite was released is a site selected by Environment Canterbury, very close to the sawfly release site in the Waipara district. "The mites were assumed to be good dispersers, being closely related to the broom gall mite (*Aceria genistae*), and we very soon found evidence of it," said Arnaud. "While observing the sawfly feeding damage to old man's beard in March, I spotted the curled and atrophied leaflets typical of the damage caused by the mite. By dissecting a few buds I was able to confirm the presence of the mites. This was about 8 months after the official release took place in August last year, and I was also able to confirm they had spread at least 800 m from the plants that were potted out in the field near old man's beard infestations," he added.

Travelling around Canterbury armed with a microscope, Arnaud expanded his search and found the mites at several new locations. One new location is at least 65 km away from the nearest known established site. In fact, Arnaud reports that it has become challenging for him to find clean plants without



Sawfly larvae and damage



Shoot tips and leaves damaged by the mite

a population of the mites throughout Canterbury as far as Hanmer Springs.

These recent findings are very encouraging, and by all accounts it seems we finally have two agents to tackle old man's beard, which has severe environmental and productive sector impacts. The establishment of both the sawfly and the mite at the Waipara sites provides an excellent opportunity to monitor populations of the agents together, and hopefully to see their combined impacts on old man's beard. The rapid dispersal of the mite over long distances and even over a range of hills (the Port Hills, Christchurch) seems to be a positive sign that we can expect big things from this tiny mite.

This project is funded by the Ministry for Primary Industries' Sustainable Food, Fibre and Futures Fund (Grant #20095) and the National Biocontrol Collective.

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Yellow Flag Iris Project Soldiers on

The yellow flag iris (*Iris pseudacorus*) biocontrol programme has been progressing at a steady pace, with activities in both New Zealand and South Africa contributing to the project's momentum. Host specificity testing of the iris flea beetle (*Aphthona nonstriata*) by the Centre for Biocontrol (CBC) at Rhodes University, South Africa, has revealed it to be a promising biocontrol agent. In New Zealand, surveys of yellow flag iris populations conducted in 2021 and 2022 revealed that no local herbivores or pathogens are significantly damaging the weed, and none from the assemblage identified could potentially hamper the successful establishment of an introduced biocontrol agent.

The CBC has led the way with host specificity testing of the iris flea beetle. So far 35 species and varieties in the Iridaceae family have been subjected to no-choice testing with the flea beetle. The preliminary results clearly demonstrated that the flea beetle is oligophagous (eating only a few closely related plant species), specialising on plant species in the *Iris* genus. Larvae of the flea beetle were not able to complete development on any plant species or varieties outside of this genus. In addition to yellow flag iris, flea beetle larvae developed on the following species: *I. domestica*, *I. confusa*, two varieties of *I. laevigata*, two varieties of *I. spuria*, *I. foetidissima* and *I. orientalis*. Four of these species (*I. laevigata*, *I. spuria*, *I. foetidissima* and *I. orientalis*) were important for host testing for New Zealand because all four are naturalised here: three are approved species, but *I. foetidissima*, or stinking iris, is regarded as a weed.

In no-choice larval development tests, the flea beetle was able to complete a full life cycle on stinking iris. Further, adult emergence on stinking iris began a week earlier and ended a month earlier than emergence on yellow flag iris. However, the total number of adults that emerged from stinking iris was 38% of those that emerged from yellow flag iris. Despite being shorter in stature and having narrower leaves compared to yellow flag iris, stinking iris has similar below-ground organ structures that are crucial for development of the flea beetle



Zane McGrath and Temo Talie sampling yellow flag iris

larvae. However, further testing will need to be conducted to determine the preference and performance of the beetle on this non-target plant in comparison to yellow flag iris. This will assist in assessing the potential host range of the beetle in New Zealand, should it be released.

Another encouraging finding by the CBC is that the flea beetle has high fecundity under controlled conditions in the laboratory. During a 3-month study between April and July 2021, the number of captive flea beetles increased five-fold when reared on their host plant, yellow flag iris. This prolific increase in beetle numbers in one generation is good news for mass-rearing efforts, and a high reproductive capacity will increase the chances of establishment and control of yellow flag iris.

While significant progress with host testing the flea beetle has been made, there have also been a couple of setbacks. Seeds of two native *Libertia* species that were sent to the CBC for host testing failed to germinate. "Despite this, we have a couple of backup plans in place," said Chris McGrannachan, who leads the project. "Our collaborators at the CBC still have plenty of *Libertia* seeds to sow, and I managed to locate a useful guide for propagating *Libertia*, which they are now using," he added. Failing this, Chris and the CBC will look into acquiring permits to send root material of the *Libertia* species to South Africa, which should make growing the plants much easier. Once the *Libertia* species have been tested, all six species pertinent to host specificity testing of the beetle in a New Zealand context will have been tested.

Covid-19 has also caused delays in importing a second candidate biocontrol agent, the iris seed weevil (*Mononychus punctumalbum*), into containment at the CBC for host specificity testing. However, with pandemic travel restrictions easing around the world, plans to import the weevil are looking set to materialise later this year. Host specificity testing of this candidate agent can then begin once a thriving colony has been established there.

The CBC has also made headway in determining the DNA profiles of southern hemisphere yellow flag iris populations. Leaf material collected throughout the distribution range of yellow flag iris in New Zealand was sent to the CBC in late 2019 for molecular analysis. This analysis will help to determine the genetic diversity of New Zealand yellow flag iris populations (the species is capable of both sexual and vegetative reproduction), and their genetic diversity and relatedness in comparison to other southern hemisphere populations, as well as the native range populations.

Meanwhile, surveys in New Zealand have been completed to identify invertebrates and fungal pathogens associated with yellow flag iris, the results of which will be presented in

a report to the National Biocontrol Collective. Surveys were completed in both the North and South Islands in May 2021 and January 2022. These surveys are important to identify any local organisms that might be attacking the weed, and to ensure that any candidate biocontrol agents are not already present. They also enable us to predict the risk of parasitism of any introduced biocontrol agents by determining the presence of 'native analogues'. Native invertebrates that are closely related to a prospective biocontrol agent may have parasitoid natural enemies capable of transferring to the agent, reducing their effectiveness. Other species may be predatory, or antagonistic through disrupting feeding by a candidate biocontrol agent, so it is important to determine their presence.

Results of these surveys revealed a total of 81 invertebrate species or taxonomic units (where identification to species level was not feasible), of which 38 are herbivores. However, most species were only found rarely or occasionally, and any damage caused to the roots, leaves and stems of yellow flag iris was minimal. The most common herbivore recorded was the introduced meadow spittlebug [*Philaenus spumarius*], which feeds on a broad range of plants. The lack of feeding damage to yellow flag iris means there is unlikely to be intense competition for the iris flea beetle or the iris seed weevil in New Zealand. This, coupled with the fact that no beetle parasitoids were recorded during the surveys, suggests the impact of these two natural enemies could be high.



Yellow flag iris infestation at Mangawhai in the North Island

A total of 31 fungal pathogen species from 19 genera were found in association with New Zealand populations of yellow flag iris. The most common plant pathogens associated with yellow flag iris disease symptoms have a wide host range, and none had any major impact on the plants observed. One species [*Rachicladosporium iridis*] isolated from leaf spots on yellow flag iris has only been found on plants from the genus *Iris*. However, the fungus did not seem to have a significant impact on yellow flag iris and was found at only one location. The report concludes that no fungal pathogens or invertebrates recorded in New Zealand offer potential as biocontrol agents, and we should continue to pursue the iris flea beetle and the iris seed weevil as the most promising candidates for biocontrol of yellow flag iris.

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Coming Soon! The Chilean Flame Creeper Beetle

Research on the biocontrol programme for Chilean flame creeper [*Tropaeolum speciosum*] has progressed well, continuing simultaneously in New Zealand and in the weed's native range of Chile.

As for the yellow flag iris project, teams in the North and South Islands embarked on surveys to look for herbivores and pathogens already attacking the plant here. Finding time to conduct the surveys between Covid-19 lockdowns and inter-island travel restrictions was no mean feat, but the teams managed to pull off the work in record time to complete the surveys. Although Chilean flame creeper is naturalised in parts of New Zealand from Stewart Island / Rakiura to Waikato, it is mainly a problem in Southland, where it suppresses and replaces native species through shading and its smothering growth. Currently only isolated populations are present in the North Island.

Ten sites were included in the surveys, half of which were visited in the autumn of 2021 and the other half in late summer of 2022. The autumn surveys were timed to optimise the detection of fungal pathogens, and the summer surveys to optimise detection of herbivores. "We found a variety of critters on Chilean flame creeper, but none could be considered as biocontrol agents," said Chantal Probst, who led

the North Island crew. Of the 56 species or taxonomic units of invertebrates that were collected, 23 are herbivorous and three are omnivorous. None are host specific, nor are they highly damaging to the plant. In addition, none were abundant, all being classified as either occasional (four species / taxonomic units), or rare (19 species / taxonomic units) on Chilean flame creeper in most instances. "We found no leaf-feeding beetles and, most importantly, we didn't find the chrysomelid leaf beetle [*Blaptea elquetai*] that is our prospective agent for this weed," added Robyn White, who led the South Island crew.

The team also looked for parasitoids and predators that could potentially derail biocontrol efforts. Although a few parasitoids were collected, none are parasitoids of beetles. The predators that were found are generalists, and there were only a few, which is not surprising given the low overall abundance of potential prey associated with the Chilean flame creeper plants that were sampled. Interestingly, three sites (all in the North Island) had no invertebrates at all on the Chilean flame creeper plants that were sampled!

In terms of fungal pathogens, there were hardly any disease symptoms on Chilean flame creeper plants. The few symptoms observed were leaf spots, leaf yellowing and leaf reddening. Leaves with symptoms were taken to the laboratory for

culturing, which yielded 34 species in 18 genera. Nine species are known pathogens with a wide host range. Another 12 are known from a variety of hosts, and can be either pathogenic, endophytic or saprophytic, depending on the host. The remaining species are all either endophytes or saprophytes.

Once the survey data were analysed we were able to conclude that none of the organisms associated with Chilean flame creeper in New Zealand are sufficiently specific and/or damaging to be considered a potential agent, and that the risks of predators or parasitoids disrupting a biocontrol programme is small. "Consequently, we think it is wise to proceed researching the leaf beetle to determine its suitability as a biocontrol agent," explained Chantal.

The leaf beetle in question was discovered serendipitously during surveys for a rust fungus that attacks Darwin's barberry [*Berberis darwinii*] in Chile in late 2019. Our Chilean collaborator, Dr Hernán Norambuena, immediately started work on the beetle, which continued during this last summer season. Hernán's first challenge was to find new collection sites for the beetle. Chilean flame creeper is rare in its native range, and habitat destruction has made it even scarcer. For example, some of the original sites where Hernán was initially able to collect beetles are now construction sites. "We were very relieved to hear that Hernán found new sites now that Covid-19 travel restrictions have lifted," said Ronny Groenteman, who leads the project. Hernán travelled to places where the plant has previously been recorded. At some he hit gold, at others there was no sign of the plant, and fortunately, while on the road between historical Chilean flame creeper sites, he discovered 14 new sites. This made a total of 29 Chilean flame creeper sites for surveying, which is a substantial improvement on the two sites we had to rely on in the early days!

Egg-laying adults were found and collected from 20 of these sites. An important find this season was leaf beetle eggs in the field. Hernán was perplexed last year when he could not find where the beetles lay their eggs. "They happily oviposited on a piece of paper in the laboratory, but no eggs were to be seen in the field," recalled Ronny. But this time, they were found on Chilean flame creeper leaves, on nearby vegetation, in the leaf



Adult leaf beetle

litter, and even in the soil under the plants. Eggs were found in the field from late September to early December. Hernán also found larvae at 15 sites, and their developmental stage ranged from newly hatched to nearing pupation.

Some sites were visited twice through the season, which provided a better understanding of the beetles' phenology at different latitudes. This allows us to plan the timing of collection for when we want to import these beetles into containment in New Zealand for host range testing.

Hernán's next challenge was to learn more about the biology and life cycle of the beetle. He compared egg laying and larval development between beetle populations collected from low-altitude sites earlier in the season with those collected from higher-altitude sites later in the season. He concluded that eggs laid by early active adults from lower altitudes have higher viability. This, too, is very useful information for planning where and when to collect beetles for importing to New Zealand.

Another useful observation was that newly hatched larvae did not start feeding immediately. This may be an indication that the beetle larvae search for their host plant, and is an important observation for the testing environment we use to assess host specificity.

While surveying field sites Hernán was also on the lookout for any other promising natural enemies of Chilean flame creeper. Similar to the previous season, he found a few caterpillars of a plutellid moth, but they were again found in small numbers and, based on Hernán's observations, do not inflict much damage to Chilean flame creeper. Hernán also noted that the moth was always found at sites adjacent to rapeseed [*Brassica napus* var. *napus*] fields, so it is likely that rapeseed is one of its main host plants. Armyworm larvae were found on Chilean flame creeper at a couple of sites, but these too appear to be a spillover from other vegetation growing in association with Chilean flame creeper. Further, Hernán's attempts to rear these larvae on Chilean flame creeper foliage in the laboratory were unsuccessful. Finally, another leaf beetle was found when beating Chilean flame creeper plants, but it, too, seems to be associated with a native shrub that grows in association with Chilean flame creeper and does not feed on the creeper at all.

"Thus," says Hernán, "our conclusion that the leaf beetle *Blaptea elguetai* is the key natural enemy of Chilean flame creeper, and should be pursued for biological control, remains unchanged."

"As for other work," said Ronny, "we continue to build up our stock of test plants, and all going according to plan with the borders opening, we hope to see these beetles in containment in New Zealand this coming spring for host specificity testing."

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Disappointed but not Defeated

In September 2021, more than a decade after receiving permission from the Environmental Protection Authority (EPA) to import a rust fungus [*Uromyces pencanus*] as a candidate biocontrol agent for Chilean needle grass [*Nassella neesiana*], we finally got export approval from the Argentinean government. The full story behind the 10-year delay was recently published in Issue 98 of the newsletter.

With only a 3 month timeframe to prepare and ship a starter culture of the rust (a condition of the permit), Freda Anderson [CERZOS – CONICET], our Argentinian collaborator, had her work cut out for her. The packaged spores were shipped on 23 December 2021, which felt like an early Christmas present. We knew that Covid-19 would make shipping difficult, but did not expect the package to take 3 weeks to leave Argentina, and then travel via Tennessee in the USA, before spending a couple of days at the FedEx facility in Auckland. The package was finally delivered and unpacked in the Beever Plant Pathogen Containment Facility in Tamaki on 18 January 2022.

“Within an hour of receiving the spores, Chantal Probst and I inoculated Chilean needle grass plants with a 1:1 mixture of spores and talcum powder,” explained Alana Den Breeyen, who leads this project. “In addition, we did a spore viability test, which involves placing spores on water agar plates, incubating them for 24 hours, and then counting the number that are viable,” she added. After 24 hours Alana and Chantal found less than 1% germination – in other words, only 1 out of 100 spores were viable. Despite this, they were still hopeful that some spores would be infective, waiting 2 weeks to check for yellow spots on the leaves. Unfortunately there was no happy ending: the rust fungus had not survived the long journey from Argentina to New Zealand. The loss of the starter culture was a big disappointment for all involved.

On a brighter note, the international compliance certificate and Memorandum of Understanding remain valid until the end of 2022, and the EPA application time waiver for the Chilean needle grass biological control agent [APP203314] was recently extended until 30 April 2024. This means we can try again to import the rust without too much of an administrative burden.

To ensure the next rust culture arrives in New Zealand in the best possible condition, several steps are being put in place. Firstly, the remaining spores held by Freda in Argentina will be germinated in her lab to obtain a fresh culture of the original rust isolate. Once this colony is well established and producing viable spores, Freda can again start preparations for exporting the rust.

Because Chilean needle grass grows very slowly from seed, Freda and her team started growing plants immediately after they heard the news that the imported culture had not survived. All going well, the plants should reach a size that



Rust spores in gelatin capsules

allows for inoculation by September. When establishment of the colony is confirmed, Freda will again need to apply for an export permit from the Argentinian National Service for Food Health and Quality [SENASA], hopefully by the end of October. To reduce the potential loss of spore infectivity, the spores will then be hand-carried from Argentina to New Zealand, now that this is again possible, ensuring a short transit time. Once again, Freda will have some painstaking work to do, and we remain appreciative of her and her team’s expertise and perseverance.

Despite the many setbacks experienced over the past decade with the Chilean needle grass project, we remain confident that the rust will be successfully re-imported into New Zealand. Once we have a viable culture, the additional host range testing that is required can be conducted.

The rationale behind testing an additional three grass species is based on evidence from Australia that *U. pencanus* isolate UP 27 was able to produce spores on two *Austrostipa* species. Neither of these grass species is present in New Zealand, but three of our native grasses [*Austrostipa stipoides*, *Achnatherum petriei* and *Anemanthele lessoniana*] are closely related enough to warrant further investigation. Once testing is successfully completed, if the rust proves to pose no risk to these grasses, and with permission from the EPA to release it, the first releases could be possible in the spring of 2023. That will be a cause to celebrate!

Further reading: Bownes A [2021]. Permit for rust export renews hope for biocontrol of Chilean needle grass. Weed Biocontrol: What’s New? Issue 98, pp. 4–5.

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Woolly Nightshade Still a Tough Target

The search has been on again for new biocontrol agents for woolly nightshade (*Solanum mauritianum*), and fortunately we didn't have to look far to find a candidate agent worth considering. Our plans to revive the woolly nightshade programme coincided perfectly with the same plans in South Africa. Even better, our collaborators there at Wits University managed to import a starter colony of the agent, a flowerbud-feeding weevil (*Anthonomus morticinus*) from Uruguay, just before the first 'worldwide' Covid-19 lockdown, when international travel became nearly impossible for almost 2 years.

During this time Wits has undertaken host specificity testing with the weevil on commercially important crop species or varieties in the *Solanum* genus (e.g. eggplant/aubergine, *Solanum melongena*) and the Solanaceae family (e.g. chilli pepper / capsicum, *Capsicum annum*). The fundamental host range of the weevil has so far been assessed in no-choice and paired-choice adult feeding and oviposition trials, and in larval development trials. The adult weevils feed on the leaves of woolly nightshade, and the larvae feed and develop inside the flowerbuds.

Preliminary results have shown that the weevil is able to feed and develop on some varieties of eggplant, but no development occurred on other important Solanaceous crops, such as potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*). The eggplant variety 'Black Beauty', which is available for domestic cultivation in New Zealand, was particularly susceptible to feeding and oviposition. However, the numbers of larvae on eggplant were low compared to woolly nightshade, and the reproductive risk, considering oviposition preference and larval survival of the weevil on eggplant, was also very low.

"These findings are not entirely unexpected, since host specificity testing of another closely related flowerbud weevil (*Anthonomus santacruzi*) produced similar results in the laboratory," explained Nic Venter (Wits University), who is leading this research in South Africa. "However, further testing of this weevil under multi-choice conditions, and a risk assessment, suggested that the risk to non-target *Solanum* species such as eggplant was low. This weevil was released as a biocontrol agent for woolly nightshade in South Africa in 2009, and non-target impacts have not been recorded in over a decade," he added.

This weevil was also considered for release in New Zealand in the early 2010s, but the results from no-choice tests with our native poroporo species (*Solanum aviculare* and *S. laciniatum*) were a cause for concern. Complete development occurred on *S. aviculare*, which is significant for Māori as a food, medicinal and culturally important plant. This poroporo species also has a current conservation status of 'Threatened



Flowerbud weevil adults

Nic Venter

– Nationally Vulnerable' (www.nzpcn.or.nz/flora/species/solanum-aviculare-var-aviculare).

With this in mind, seeds of both poroporo species were sent to Wits in November last year for cultivating to undergo host specificity testing with the new flowerbud weevil. "Although the host testing results with eggplant are a little concerning, it will be crucial to have a full set of results of host testing the poroporo species, and the additional testing planned with eggplant, before we can assess the safety of this weevil for release in New Zealand," said Angela Bownes, who leads this project. "Wits will soon start feeding trials with the adult weevils, and floral trials to assess oviposition and larval development will commence as soon as the plants start flowering," she added. Wits also plans to do more testing with eggplant and other commercial and South African native species closely related to woolly nightshade. Other research conducted by Wits demonstrated that the new flowerbud weevil has a lower thermal tolerance compared to its congener released in South Africa, whose distribution is restricted to the subtropical belt along the east coast of the country. So in terms of climate, the new flowerbud weevil is a better match for New Zealand, as a lower thermal tolerance suggests a higher tolerance of cooler climates.

Although having a biocontrol agent that can reduce woolly nightshade's prolific fruit production is highly desirable, the search for new agents won't stop with the flowerbud weevil. Other promising candidates include two stem weevils. Little is known about these weevils, but now that international travel is back on the cards, we hope to investigate them further in the native range as soon as possible. Woolly nightshade is still proving itself to be a tough target, but we're not done with this weed yet!

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