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Weed Biocontrol

WHAT'S NEW?

Contents

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COVER IMAGE:
Galls caused by *Trichilogaster acaciaelongifoliae on* Sydney golden wattle.

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Heather biochemistry differs between its native and invasive ranges

All plants have a biochemical profile, which can be altered by abiotic conditions such as light and nutrient availability and biotic factors such as herbivory and plant pathogens. In issue 99 we wrote about plant metabolomics (the study of plant biochemistry at the molecular level) and how it can affect the success of biocontrol. The gist of the article was that a plant's biochemical profile can significantly affect the performance and population dynamics of insect biocontrol agents feeding on the plant. Therefore, understanding the biochemistry of plants and their biochemical phenotypes (observable characteristics) may help us understand the success or failure of weed biocontrol programmes.

In a recent study, researchers from Massey University, AgResearch, the James Hutton Institute in Scotland, and MWLR investigated the biochemical phenotypes of heather (*Calluna vulgaris*) sourced from its native range in Scotland and its invasive range in New Zealand. The researchers were particularly interested in heather as a study system because its introduced biocontrol agent, the heather beetle (*Lochmaea suturalis*), was difficult to establish in New Zealand and initial performance was inconsistent.

To study the biochemical phenotypes, scientists analysed primary and secondary metabolites (intermediate or end products of metabolism) in heather from Scotland and from the Central Plateau in New Zealand. Foliage samples were collected in the Northern and Southern Hemisphere summers, as were soil samples. The foliage samples were analysed for their chemical compounds and soil samples for their nutrient composition.

The chemical analysis found that the biochemical profile of heather plants growing in New Zealand is significantly different from that of plants in Scotland. "We found higher levels of defensive secondary metabolites in New Zealand heather samples compared to those from Scotland, which may have implications for the performance of the heather beetle in New Zealand, in terms of its survival, reproduction and development rates. These factors are linked to intrinsic rates of increase for insect populations, and could therefore have a significant effect on their success as a biocontrol agent," said Paul Peterson, a senior technician from MWLR involved in this work.

The different biochemistry could be explained by the soil and UV intensity levels at the New Zealand heather sites: compared to the sites in Scotland, New Zealand soils have lower nutrient availability and higher UV intensity levels. Both of these have been found in other studies to lead to higher levels of chemical defence compounds in plants, although this was not directly tested in this study. Experimental tests in controlled conditions would be needed to confirm the direct effects of soil nutrients and UV intensity on heather's biochemical phenotypes.

So, what does this mean for the heather beetle in New Zealand? These findings suggest that the altered biochemical phenotype of heather in the invaded range in New Zealand could impair the success of the heather beetle. Since heather plants in New Zealand were found to have higher chemical defences, the plants could be more resistant to feeding by the beetles, which could have made it more difficult for them to successfully establish and build up high numbers. This may explain, at least in part, why the heather beetle was so difficult to establish and why its initial performance as a biocontrol agent in New Zealand was poor.

"Further research is needed to confirm the direct effects of soil nutrients and ultraviolet light on heather's biochemistry and to understand their impact on biocontrol agents," said Paul Barrett, the research leader from Massey University. "This study shows that plant

Heather invasion on the Central Plateau in 2003

biochemical analyses are a valuable tool for understanding the success or variability of biocontrol agents."

For heather beetles in New Zealand, the team at MWLR is continuing research efforts to understand why the heather beetle is now well established, with outbreaking populations that are having substantial impacts on heather over a large area of Tongariro National Park. More on this complex insect–weed system in future issues!

Further reading:

Barrett DP, Subbaraj AK, Pakeman RJ, Peterson P, Clavijo McCormack A 2024. Metabolomics reveals altered biochemical phenotype of an invasive plant with potential to impair its biocontrol agent's establishment and effectiveness. Scientific Reports 14: 27150.

Paul Barrett was funded by the QEII Technicians' Study Award; Paul Peterson was funded by MWLR's Beating Weeds Programme, which is funded by the Ministry of Business, Innovation and Employment's Strategic Science Investment Fund; and Andrea Clavijo McCormack was funded by the Royal Society Fast Start Marsden Grant 'Plant communication in times of rapid environmental change'.

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Heather beetles.

A newly established wasp unanimously welcomed

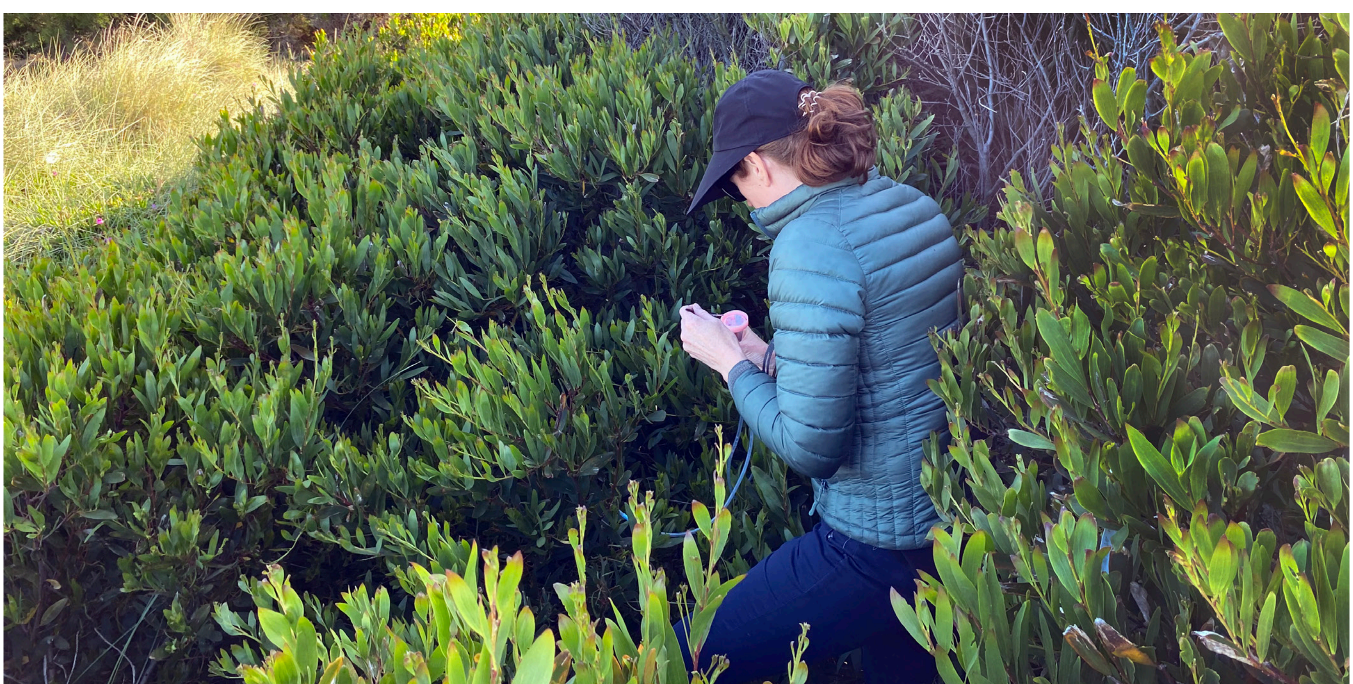
The bud-galling wasp *Trichilogaster acaciaelongifoliae* was released at two sites in the Manawatū-Whanganui region against Sydney golden wattle (*Acacia longifolia*) in December 2022 and 2023. We are now excited to report that this agent has successfully established at both sites and numbers are expected to expand quickly. Further efforts were made to import a large consignment of galls infested with immature stages of the wasp in November 2024, allowing us to release over a thousand wasps in different parts of the country.

This tiny bud-galling wasp (3–4 mm in length) lays eggs inside the immature flower buds, and sometimes the vegetative buds, of Sydney golden wattle in late spring/early summer. The eggs remain dormant and only start hatching in late winter of the following year. Then, the small larvae feeding inside the buds induce the formation of large growths, called galls, instead of flowers and shoots. This, in turn, reduces the production of seeds and diverts the plant's resources. In South Africa, where a biological programme for Sydney golden wattle was initiated in the 1980s, the production of galls reportedly weakens the trees to such an extent that they often die under severe attack by this agent. Sydney golden wattle is no longer a threat to the environment and unique biodiversity in South Africa thanks to the introduction of this wasp and a seed-feeding weevil.

As part of a collaborative project with Horizons Regional Council (HRC), HRC staff have been monitoring the two release sites at Castlecliff beach in the Whanganui District and Waitārere beach located in the Horowhenua District of Manawatū-Whanganui. Their observations of galls on Sydney golden wattle trees at these two sites in November 2023 were very encouraging and the first early signs that the wasp has successfully established.

This season these sites were monitored again, and higher numbers of galls were found by Robbie Sicely and Abi Wightman, HRC biosecurity officers, who have been closely involved with the project since the introduction of this agent. During a brief search, over a hundred galls were located at a sub-site of the first release site at Castlecliff beach. "This sub-site looks particularly promising!" enthused Robbie, who was in charge of releasing the wasps the previous years and monitoring this Castlecliff beach site. Indeed, even though the adults are short lived (living for only 3–4 days), because the vast majority of them are females due to their parthenogenetic reproduction (whereby females lay fertile eggs without the need for mating), populations have the potential to grow quickly. Each female can lay a few hundred eggs, making this wasp an ideal biocontrol agent.

Following some difficulties with exporting more galls from South Africa in 2023, the decision was made to hand-carry our next shipment. A well-timed trip home for MWLR Science Team Leader Angela Bownes, combined with the support of our collaborator Fiona Impson from the University of Cape Town, were the perfect ingredients to end up with a significant number of galls collected from South Africa in November. After a few hiccups Angela successfully managed to import over 2,000 galls into our containment facility in Lincoln. Due to biosecurity concerns the galls containing the larvae couldn't



Angela releasing wasps in Christchurch.

Imported galls from South Africa.

be directly released. Instead, they were handed over to MWLR senior technician and project leader Arnaud Cartier to look after them and collect each individual wasp emerging from the imported material. "Luckily, we were able to gather all the required documentation without any significant delays to be able to satisfy MPI, so they could grant us approval to release the wasps from containment and start planning our upcoming releases without compromising the timing of the peak of emergence," said Arnaud.

The sheer number of galls imported compared to previous years, and a better knowledge of the conditions required to keep the galls healthy, meant that plenty of wasps were successfully released in several different parts of the country. Along with HRC, new regional and district councils such as Environment Canterbury, Tasman District Council and Bay of Plenty Regional Council were very supportive of its introduction, and all welcomed the release of between 150 to 200 females at new sites. In just over a month, MWLR's biocontrol team were kept busy releasing over a thousand wasps thanks to this third shipment of galls.

The very first release in the Bay of Plenty region took place on Matakana Island in early December, with the support of Ngāi Tuwhiwhia, Ngāi Tamawhariua, Ngāti Tauaiti, Te Whānau a Tauwhao, and Te Ngare, all part of the Ngāi Te Rangi iwi. Matakana Island is accustomed to the use of biocontrol agents. "This will be the third trial we have done here in partnership with BOPRC. There are of course negative and positive aspects when using biocontrol," said Hayden Murray (Ngāi Te Rangi iwi). "Some of the positives are that they are environmentally friendly and do not add pollutants into the environment. One of the negatives is that it can be a slow process and can take a while to get the desired results. We look forward to seeing the results of this latest release," he added.

Chris McKay (pictured left) and Hayden Murray (right) releasing the bud-galling wasps on Matakana Island.

The upcoming years will be interesting to assess the establishment success of these new releases. This would equally be a great opportunity to study the population growth and spread of this new agent in more detail to allow collection and further redistribution to other places, such as Northland, which is in need of help to fight Sydney golden wattle. There is no doubt that expectations are high to control this invasive species of wattle, and promising results will hopefully be achieved in the near future.

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

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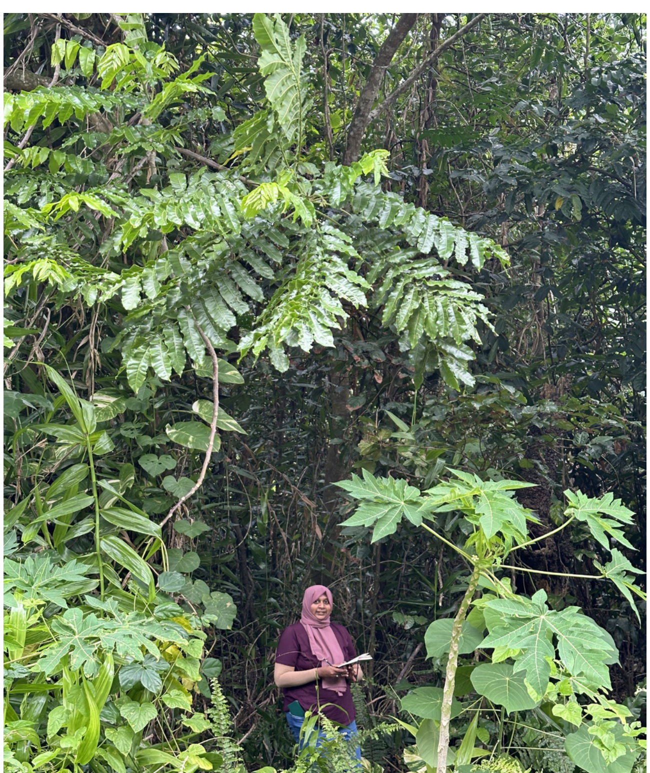
Progress for taro vine in the Pacific

MWLR's biological control programme against Devil's ivy, also known more commonly in the Pacific as taro vine (*Epipremnum pinnatum* cv. 'Aureum'), began in 2020 when the weed was chosen as a novel target for work under the Managing Invasive Species for Climate Change Adaptation in the Pacific (MISCCAP) project. With the conclusion of MISCCAP in 2024, efforts to find suitable biocontrol agents for use in the Pacific have continued under the Restoring Island Resilience project. In 2021 a feasibility study was conducted to assess if biocontrol would be a suitable option for managing taro vine in the Pacific. The study identified knowledge gaps relating to the identity of taro vine, its origins, and diversity across the Pacific. Addressing these uncertainties was critical if MWLR were to develop a sustainable, long-term solution for this highly invasive vine.

"A major problem was the taxonomic uncertainty regarding the status of *Epipremnum pinnatum* cv. 'Aureum'," said Chris McGrannachan, who led the feasibility study. It was first described in 1880 as *Pothos aureus* from a plant growing in a nursery belonging to a Belgian botanist, Jean Jules Linden, in the Solomon Islands. However, the absence of wild plants in the Solomon Islands led botanists to search elsewhere to determine its native range. A specimen, initially described as *Epipremnum mooreense*, found growing in the island of Moorea in French Polynesia in 1899 proved to be morphologically identical to the specimen from the Solomon Islands. Subsequently, botanists assumed that Moorea was the origin of this species, which was renamed *Epipremnum aureum* in 1964. However, the vine is now considered to be an invasive introduced species in French Polynesia. A botanist, Albert Smith, noted in 1978 that the only difference between *E. pinnatum* and *E. aureum* is the former only ever has green leaves while the latter is mostly variegated. This, coupled with the fact that *E. aureum* rarely flowers, is unknown in the wild, and easily reproduces vegetatively, led Smith to conclude that it should instead be considered as a cultivar of *E. pinnatum* rather than a botanical species.



Asma Bibi in Niue surveying taro vine.

Molecular studies were needed to determine the true identity of taro vine, its diversity, and native range. *Epipremnum* plants were therefore sampled in Australia, parts of Asia, and across the Pacific at the same time as natural enemy surveys were undertaken in 2022 and 2023. Microsatellite analysis was used to compare genetic variation present in the two vines. "These analyses showed that taro vine (*E. pinnatum* cv. 'Aureum') and *E. pinnatum* have little genetic difference, supporting the view that taro vine is likely a cultivar of *E. pinnatum*," concluded Caroline Mitchell, who led the molecular work. The genetic work also confirmed that populations of taro vine across the Pacific are identical. This meant that a highly damaging agent would be likely to provide effective control across the entire invaded range in the Pacific.

E. pinnatum is native to some Pacific Island countries in Melanesia, such as Fiji, Papua New Guinea, the Solomon Islands, and Vanuatu, and possibly on other Pacific islands, such as Samoa. However, it seems that *E. pinnatum* cv. 'Aureum' (henceforth called taro vine) has been extensively introduced and moved about for ornamental purposes, as it is predominantly found close to human habitation. It is still unknown where taro vine was first discovered, or if the cultivar even occurs in the wild. What we do know is that it is now a massively popular ornamental houseplant in many parts of the world for its colourful leaves, and perhaps because it is easy to grow and hard to kill.

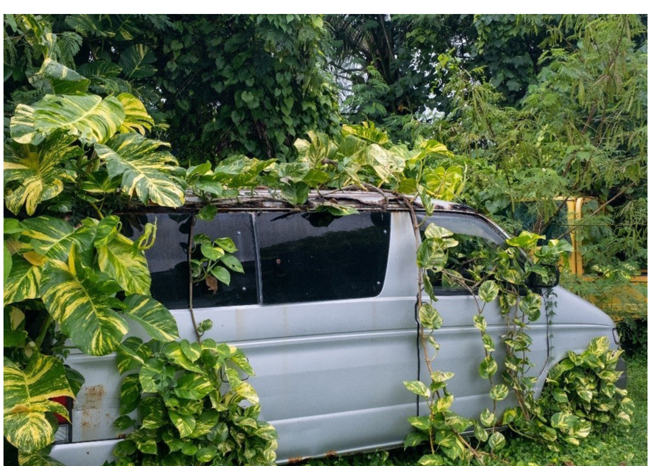
The South Pacific islands provide the perfect environment for taro vine to thrive. It does well in wet, tropical and subtropical regions, is shade-, heat- and drought-tolerant, and grows in a range of soils. Taro vine is highly invasive in Niue and French Polynesia, and is showing invasive tendencies in places such as the Cook Islands, Hawaii, Marshall Islands, Samoa, Tonga, and Wallis and Futuna. In 2023 taro vine was ranked the third-highest priority weed for which natural enemies should be developed for the Pacific region by invasive species practitioners from across the Pacific. In Niue taro vine was scored the top priority weed during a prioritisation workshop in November last year. Anyone visiting or living in Niue can understand why: you don't need to look far to see the impacts that taro vine is having. A quick drive from Hanan International Airport to the capital village, Alofi, will reveal entire coconut trees smothered by the vine, disused heritage homes completely covered, and entire stretches of forest smothered in a thick curtain of the green and yellow leaves.

The hard reality of invasive weeds such as taro vine for small island nations is that most lack the resources to keep these weeds under control, causing them to wreak havoc on the environment. "You have to rip it out and burn it, otherwise it grows back wherever you leave the cuttings. Sometimes a tree is too far gone, it's got too much taro vine on it. The only option is to cut the whole tree down and burn it," says Zack Smith, a local landscaper on Niue. Often, homeowners and growers on Niue are overwhelmed by the effort it takes to manage taro vine on their land. "People have to be aware about how they're getting rid of the weed. If you're just mowing over it, the fragments that the mower spits out will just grow into new plants. You need to know what you're doing when you're managing these weeds. It's a process," said Zack.

Using herbicides is problematic because the weed often smothers other plants, creating a risk of damage to the smothered plants underneath from herbicide spraying. Biocontrol offers the only possible sustainable, long-term option.

Fortunately, natural enemy surveys identified at least five potential biocontrol candidates for taro vine, including a stem-mining moth and beetle in Papua New Guinea, and sap-sucking lace bugs in Vanuatu and Fiji. It was noted that in some countries such as Fiji and Vanuatu, where *E. pinnatum* is native, taro vine is a well-behaved ornamental. In these places natural enemies of *E. pinnatum* appear to have moved across onto taro vine, preventing it from becoming invasive. This is also good news for the prospects of managing taro vine in places where it is problematic.

The first natural enemy to be studied is a lace bug, *Holophygdon melanesica*, from Fiji, which was imported into MWLR containment facilities in February last year. Life-cycle studies and host range testing were undertaken by Asma Bibi, a researcher from the Fijian Ministry of Agriculture, whose Master of Science degree, based at MWLR, involved investigating the lace bug as a potential biocontrol agent of taro vine. "My research confirmed that the lace bug only damages taro vine, *E. pinnatum*, and another close relative, *Monstera deliciosa*, making it a suitable agent for Pacific countries to consider," said Asma.


Taro vine covers a car in Niue.


Taro vine (with Asma for scale!).

Work is now underway to introduce the lace bug to Niue later this year. Huggard Tongatule, of Niue's Department of Environment, is excited about the release of a biocontrol agent against taro vine in Niue: "People here have been waiting for a long-term solution against taro vine for years. They can see how serious the problem is becoming, and they know we need to act quickly in order to get this under control," Huggard said.

The introduction of a biocontrol agent against taro vine in Niue would represent a significant advance in the sustainable management of this invasive species. Successful control would contribute to the restoration of native vegetation, improve land management outcomes, and serve as a model for similar biocontrol efforts across the Pacific region. Given that more than one biocontrol agent is often needed to control such a highly invasive weed, work is underway to study the stem-boring moth in Papua New Guinea.

The Restoring Island Resilience project is funded by New Zealand's Ministry of Foreign Affairs and Trade (NZMFAT) and administered by the Secretariat of the Pacific Regional Environment Programme as part of the Pacific Regional Invasive Species Management Support Service. The Managing Invasive Species for Climate Change Adaptation in the Pacific project was also funded by NZMFAT.

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The mystery of gregarines in air potato leaf beetles

When conducting a routine screening of the air potato leaf beetle for disease, MWLR technicians discovered gregarines in the intestines of the beetles. This spurred a thorough investigation of where these gregarines came from as well as a lengthy process to get rid of the gregarines in the leaf beetles.

But first, what are gregarines? Just as humans have single-celled organisms living inside them, so too do insects. Gregarines are single-celled eukaryotes that live in the gut of invertebrates. They can be symbiotic, commensal or parasitic. They are host-specific and can be transmitted to the offspring of individuals via faeces. Gregarines have a very diverse morphology, taxonomy, and genetics. There's limited research on them because they are difficult to study; they cannot live outside of their host, they have a high diversity, and standard genetic markers cannot distinguish them from other eukaryotes and their insect hosts.

Because we did not know much about the gregarines we found in the gut of the leaf beetles we had to treat them as if they were parasitic. We therefore increased our hygiene measures in our insect containment facility by sterilising the eggs of the beetles and keeping them separate from the infected adults. We also trained our staff in sample preparation and microscopy to identify the presence of gregarines.

We continued to assess the colony health of the beetles and tested to see if the infection had transferred to other insects in the containment facility. Thankfully we did not find gregarines in the other insects, indicating that they had not transmitted to other insect species in the facility. It's therefore likely that the gregarines we found in the air potato leaf beetles are host specific.

We also investigated where the gregarines came from. We contacted our collaborators in Florida from where we had imported our colony of air potato leaf beetles, and they also found gregarines in their lab colony, as well as in wild populations of the air potato leaf beetles. It is thus highly likely that the gregarines in our colony of air potato leaf beetles came from the hosts in Florida. Interestingly, both our New Zealand and Florida colonies of the air potato leaf beetles were healthy and performing well. They were free of disease symptoms such as high mortality, low fecundity, and deformities, so we have concluded that these gregarines are not having a negative effect on their host beetles.


Air potato leaf beetle larvae.


A gregarine through a microscope.

Current gregarine levels in the air potato leaf beetles are very low, but we have not been able to prove their absence from the colony yet because the infection load of gregarines may be too low to isolate enough gregarines from the host gut. From here our approach is to continue to control the gregarine infection to avoid overburdening the insects. We have also adjusted our release strategy for this biocontrol agent. In the previous newsletter issue we discussed the release of the air potato leaf beetle in Niue. To conduct this release safely we exported surface-sterilised eggs to Niue and then released the freshly hatched larvae.

Throughout this process we gathered information from our collaborators and the literature to improve our methods for clean rearing, microscopy, and molecular analysis. Because not a lot is known about gregarines, in the future we would like to study their transmission mechanisms and host range, as well as the effects of different concentrations or loads of gregarines on the host health. It would also be useful to develop specific molecular methods to identify the species of gregarines detected.

While some mysteries relating to gregarines remain unsolved, this experience has improved our safety measures for biocontrol and our understanding of the complexity of insects and their gut. The discovery of gregarines in the air potato leaf beetle taught us that routine disease screening is essential. We also learned the importance of routinely recording colony health and performance so that we can effectively monitor future populations.

This investigation of gregarines is part of MWLR's Beating Weeds Programme, funded by the Ministry of Business, Innovation and Employment's Strategic Science Investment Fund.

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