Forest Protection SSIF research on species other than radiata pine 2019/20

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Date: 26 June 2020

Publication No: SWP-T102

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FOREST GROWERS RESEARCH

EXECUTIVE SUMMARY

Plantation species other than *Pinus radiata* (radiata pine), such as Douglas-fir and eucalypts, form an important part of a diversified forest estate. *Eucalyptus species* are planted over approximately 27,000 ha and contribute ~$40 million pa in hardwood chip exports. Throughout New Zealand the biggest threat to *E. nitens* plantations has historically been from the *Eucalyptus* tortoise beetle, *Paropsis charybdis* (Bain and Kay 1989). As part of the SWP partnership, Forest Protection also contributes research from its core funding (SSIF) to research on ensuring sustainable growth of diversified forest species. This report summarises progress made in the last financial year on *Eucalyptus* pest management, on the safety of biological control research (through research on the safety and lack of non-target impacts of biological control and the development of risk assessment models), and on a new polyphagous ambrosia beetle in Auckland, *Xylosandrus crassiusculus*.

New Zealand eucalypt forests are now under an additional threat to their health from the eucalyptus variegated beetle (EVB *Paropsisterna variicollis*). We report the name change due to synonymy to *Paropsisterna cloelia*, and update the prospects of locating a suitable biological control agent for *Paropsisterna cloelia* (= *variicollis*) from the collaboration with international entomologists. Ryan Ridenbaugh, University of Central Florida, has concluded his thesis during which he confirmed 37 rearings of *Eadya annleckieae* from *Paropsisterna cloelia* (= *variicollis*) that he collected from infested *Eucalyptus globulus* plantations in early December in Victoria, Australia. Fortuitously he also located two sites where *Eadya daenerys* were reared from *Paropsisterna agricola* and *Paropsisterna m-fuscum* (forming a new parasitoid-host association record). These collections will provide Scion with two possible sites (Mortlake and Heywood) in Victoria, Australia where we might be able to recollect *E. daenerys* for the biocontrol of *P. charybdis* in the future as Scion seeks a disease-free population for release in New Zealand.

Research was also completed on the likely geographic origin of *P. cloelia* (= *variicollis*) based on sequences from two gene regions of beetles from throughout Australia and New Zealand. The analyses reveal the beetle is invasive in both Western Australia and New Zealand, and is likely closest genetically to mainland Australian populations, rather than Tasmania, but the identical haplotype to the New Zealand incursion was not located. Research in the field and reports from the public and NZDFI have tracked *Pst. cloelia* (= *variicollis*) spread through both islands of New Zealand, including now being established in Marlborough and having reach the central North Island plateau. Observations on pest presence in Gisborne are included as a file note.

BACKGROUND

The Forest Growing Science and Innovation Strategy 2020-2035 provides a roadmap for achieving the forest growers vision for 2050. This includes a key science and innovation theme (Theme 2) "Ensuring the long-term sustainability of commercial forestry through realising value from emerging species (exotic and indigenous) and developing new models for forestry". The strategy clearly recognises the need to grow investment into science and to take emerging tree species into mainstream forestry. Doubling the planting of eucalypt species (and cypresses and redwoods) by 2035 is a medium priority focus area for Theme 2. This will be achieved by increased confidence in their resilience, achieved by a multitude of pathways, but relying heavily upon the successful biological control for suppressing the negative impacts of pests of *Eucalyptus* species. Breeding for resistance to pests and diseases and silviculture will also contribute significantly. Theme 3 covers future proofing forest growing, and another outcome is "minimising biotic risk to trees from pests and pathogens". The forestry sector's ability to grow *E. nitens* and other Symphyomyrtus species clearly fits into this strategy as they contribute ~$40 million pa in hardwood chip exports. The ability to reduce pesticide inputs by managing the pest with biological control will also benefit forestry through maintaining its social licence to operate (NZ Forest Owners Association 2017). Throughout New Zealand the biggest threats to *E. nitens* plantations have historically been from the eucalyptus tortoise beetle, *Paropsis charybdis* (Withers and Peters 2017). Unfortunately the *Eucalyptus* variegated beetle *Paropsisterna cloelia (= variicollis)* poses even further challenges to the *Eucalyptus* forestry industry (Millen et al. 2018).

Eucalyptus and Paropsis and Paropsisterna

To manage populations of *P. charybdis*, chemical control by aerial spraying of insecticides has regularly taken place in up to a quarter of all New Zealand large plantations annually (Rolando et al. 2016). However, the costs associated with aerial spraying are prohibitive for many growers and a major barrier to increasing eucalypt plantations. Other undesirable outcomes, such as environmental and ecological harm to fresh water organisms, and risking loss of FSC certification could also result from reliance on chemical insecticides. The only viable alternatives to insecticides are using classical biological control (Rolando et al. 2016), or breeding for tree resistance to insect pest browsing (Millen et al. 2018; Westbrook et al. 2015).

In the case of *Paropsis charybdis*, four potential biological control agents have been introduced already in previous decades, but only two of these are highly effective, and generally only control the second generation (Pugh et al. submitted). The need for a more effective biological control (Withers and Peters 2017) sparked an eight year research project aiming to establish a spring-active larval parasitoid against *P. charybdis*. This year Scion sought to recollect the parasitoid *Eadya daenerys* into Containment, for mass-rearing and the first releases to take place in the field in New Zealand.

Research in the last three years has continued investigating options for future control of the *Eucalyptus* variegated beetle *Pst. cloelia (=variicollis),* in particular the origin and identity of the population in New Zealand was investigated by molecular methods. Peixoto et al. (Peixoto et al. 2018) showed the importance of integration of molecular taxonomy into the biological control program against *P. charybdis* with understanding which species of *Eadya* attack which species of beetle. Not only did we discover an undescribed cryptic species, *Eadya daenerys* (Peixoto et al. 2018) but two new non-cryptic species as well, *E. annleckieae* and *E. spitzer* (Ridenbaugh et al. 2018). A large CO1 DNA barcode gap was recovered between the three new species and *E. paropsidis* with interspecific distances ranging from 8.7% to 31.2%. No geographic isolation was recovered between the four species and the wasps overlapped in host use. All four species were found to be univoltine, attacking the spring generation of *Paropsis* and *Paropsisterna* and emerging in late December before undergoing a ten-month obligate diapause. All species were found to be oligophagous (host flexible). *Eadya annleckieae* and *E. daenerys* were each reared from four different host paropsine beetles (Peixoto et al. 2018). Interest in these complex host interactions led to Scion SSIF supporting a Masters student, Ryan Ridenbaugh, to undertake field work and further analysis on these potentially very useful biological control agents in Australia. We can now report on the results.

RESULTS

1.1.11.9 Support for *Paropsis* biocontrol

This last financial year *Eadya daenerys* was recollected from Tasmania for mass-rearing with the aim to release the offspring in 2020. Timing of the life cycle of *Eadya daenerys* (one generation per annum) means the adult females are only present to attack larvae and parasitise the next generation during the month of December. Adult females (n=61) were collected successfully in Tasmania and imported into Scion quarantine on 13/12/2019 and 17/12/2019 (this recollection was co-funded by Oji Fibre Solutions Ltd, the Speciality Wood Products Partnership, the NZ Farm Forestry Association (NZFFA) Eucalyptus Action group, the Gisborne branch of the NZFFA and a private benefactor). After arrival the first recollection funding was fully utilised and the mass-rearing was undertaken in containment with assistance of MPI (sponsoring a summer student through a Te Uru Rakau summer scholarship) and SWEL, Southwood Exports Limited and Scion funded one Toi Ohomai summer student and one University of Waikato summer student to also assist with the intensive laboratory work. In summary the whole *Eucalyptus* industry helped to fund this recollection and mass-rearing attempt.

Mass-rearing of the offspring of *E. daenerys* who were separated into 12 different groups of up to six female parasitoids began on the 15th of December 2019. We also requested and obtained permission for Go Early funding from MPI's Sustainable Food Fibre Futures fund. Each *P. charybdis* larvae was considered successfully parasitized when it had been observed being stung by one *E. daenerys* wasp for at least one second. Once stung, the larvae were transferred into a takeaway container to be raised on fresh *E. nitens* foliage until each larva had completed feeding and was ready to be put onto soil. Over a 43-day rearing period, we managed to successfully sting and raise 6070 infested *P. charybdis* larvae to begin the mass- rearing programme (B Prout, Waikato University Science Work Placement Report).

New knowledge on the method to put *Eadya daenerys* females into comas to assist with mating success, if required in the future, was also completed. This was because we were testing a hypothesis that impaired or anaesthetised adult females could be more receptive to mating in a laboratory setting. Insect anaesthesia is a well-established practice for easier handling and examination of insect specimens. We trialled different durations of chilling, as well as exposure to ethyl acetate on individual adult females within the containment facility. We concluded 2-3 mins chilling at minus 20 °C, or ethyl acetate exposure, were the most promising treatments (M. Davy, 2020).

Bad news: Apart from the Voucher Specimens to be deposited into the New Zealand Arthropod Collection, the remaining 54 *Eadya daenerys* females had their abdomens smeared onto slides when fresh. Examination of the slide smears was undertaken by Dr Peter Wigley by oil-immersion compound microscopy and dissecting microscopic examination of unstained and Giemsa-stained slides. Unfortunately all of the *E. daenerys* individuals were infected by either, or both, of a microsporidian or a sporozoan species. All but 5 of the 55 individuals were infected with the microsporidian and 9 with the sporozoan species. Dual infections were present in 42 individuals (P. Wigly Report 24/2/2020). Scion immediately contacted its project partners, and reported to MPI Risk Team on 13 March 2020 that the importation under NOR100169 was infested by secondary organisms and Scion would not be requesting their release from containment.

OUTPUTS

Mike Davy and Toni Withers (2020). File Note: *Eadya daenerys* latency to recover after exposure to chilling or ethyl acetate. Scion PAD 25688664

Bernadette Prout (2020). Mass rearing of the biological control agent *Eadya daenerys* to control *Paropsis charybdis* beetles in New Zealand. Summer research scholarship work placement report. Waikato University.

UTAS News story in the Environment, Resource & Sustainability theme news article . "Mother of Dragons wasp flying to New Zealand's aid". https://www.utas.edu.au/news/2020/6/22/1028-mother-of-dragons-wasp-flying-to-new-zealands-aid/ Scion PAD ID 26984474

University of Tasmania You-tube channel story "Mother of Dragons". https://www.youtube.com/watch?v=UYZS3m6imu0 Scion PAD ID 26984538

Milestone 1: Non-target impacts of a *Eucalyptus* biological control agent

A decade before Scion re-launched the *P. charybdis* biocontrol project, it successfully introduced a parasitoid to control the gum leaf skeletoniser *Uraba lugens*. This was achieved through introduction of the biological control agent *Cotesia urabae* (Braconidae) (Berndt and Allen 2010; Avila et al. 2013). Due to on-going interest in the safety of biological control (Avila et al. 2016), in collaboration with Gonzalo Avila, Plant and Food Research, we followed up on the establishment of *C. urabae* by undertaking field host range assessments utilising a non-target caterpillar *Nyctemera annulata*, the native magpie moth. Field assessments were undertaken in sites in Northland, Auckland, Waikato and Bay of Plenty. Data will shortly be submitted to a scientific journal but we await final molecular analysis of the species of parasitoids reared from the final replicate. The interim results are presented here from the B3 (Better Border Biosecurity) conference presentation given in May 2020. The results of the field trials are in close agreement with predictions. *Cotesia urabae* when released into an environment when both its host gum leaf skeletoniser and non-target caterpillars are present (called a choice test), only attacks the gum leaf skeletoniser. This means it has retained the predicted host specificity in the field. When *Cotesia urabae* were released into an environment devoid of eucalypts and devoid of gum leaf skeletoniser (called a no-choice test), but in the middle of a ragwort patch infested with non-target magpie moth caterpillars, these cateprillars showed attack by *C. urabae* in 0.5% of cases, a very low attack rate and a lower rate than attack by other parasitoids in the environment. This research gives us confidence that our methods of host specificity testing we undertake in quarantine, do in fact produce reliable assessments of risk for when the parasitoid is released into the New Zealand environment (Barratt et al. 2010). These reassuring results will ensure biological control continues to be trusted and used for managing pests in New Zealand.

Output:

Report title	Field assessment of non-target impacts of *C. urabae*
Authors	***GONZALO AVILA*, *FRANCES MACDONALD*, *KARINA SANTOS*, *TONI WITHERS***
Client	Better Border Biosecurity (B3) Collaboration
PAD ID number	25792951
Date completed	13 May 2020
Confidentiality requirement	CONFIDENTIAL

Summary slides from powerpoint presentation

B3 Science Solutions for BETTER BORDER BIOSECURITY www.b3nz.org

Field assessment of non-target impacts by *C. urabae*

Research update 2020

13 May 2020

Gonzalo Avila, Frances MacDonald, Karina Santos, Toni Withers

Theme 1A - Risk Assessment: Intentional Introductions

Project #A17.3

Ministry for Primary Industries · Forest Owners Association · Department of Conservation · Environmental Protection Authority · Horticulture · bee-tarb · Plant & Food Research · agresearch · scion · Manaaki Whenua Landcare Research · Bio-Protection

Summary

- *N. annulata* parasitism observed in both trials
- Predominant parasitoids *D. perniciosus* and *M. pulchicornis*
- Current results confirmed *C. urabae* in No-choice control only
  - ✓ Very low parasitism = 0.5%
  - ✓ Very similar results to previous semi-field trials
- 14 Unidentified parasitoids from Choice trials yet to be IDed
  - ✓ If *C. urabae*, parasitism to raise from 0 to 6%
  - ✓ However, this result is from a single trial – may not be the trend
- Other parasitoid species likely to outcompete *C. urabae*

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Milestone 2: Bayes net risk model for predicting risks of biological control

Classical biological control, the introduction of natural enemies to new environments to control unwanted pests or weeds, is, despite numerous successful examples, associated with rising concerns about unwanted environmental impacts such as population decline of non-target species (Barratt et al. 2018). Recognition of these biosafety risks is increasing globally, and pre-release assessments of biological control agents (BCAs) have become more rigorous in many countries (Barratt et al. 2010). Traditionally, biological control assessments focus on providing assurance about the specificity of a proposed BCA, generally via a list of suitable vs non-suitable hosts determined in the laboratory (i.e. by determining the BCA's physiological host range). However, the outcome of interactions of introduced agents in the natural environment can differ from laboratory-based predictions. Potential non-target host testing may be incomplete, additional ecological barriers under field conditions may restrict host accessibility or attack levels, and BCAs could expand to field environments beyond those used by the target species and adversely affect non-target species.

Scion entomologists Nicolas Meurisse and Toni Withers, in collaboration with colleagues from Plant and Food Research, AgResearch, Manaaki Whenua Landcare Research, USDA Forest Service and Bayesian Intelligence (Melbourne) have worked on a new tool to assess the potential negative impacts of BCA candidates on non-target species. The “Biocontrol Adverse Impact Probability Assessment” (BAIPA), uses a probabilistic, BN-based, model to combine nine evaluation components to assess the probability that an introduced BCA will reduce the population of a specified non-target species.

BAIPA integrate information on probabilities and uncertainties of multiple ecological factors influencing the potential of a BCA to disperse and establish outside of its habitat of introduction, locate and attack non-target species, and reduce their population level. Results provided by BAIPA aims to be routinely used by the Environmental Protection Agency (EPA) for guiding the decisions to release new BCA in New Zealand.

A review of risk assessment frameworks for assessing the risks of introduction of new biological control agents has been prepared by the team, assessing the advantages and drawbacks of currently used methods, and advocating for the use of ecologically-based probabilistic models. The article also provides an example of use with BAIPA. The manuscript will be submitted in a Special Issue of the journal Risk Analysis aligned with the recent joined conference of the Australasian Bayesian Network Modelling Society and the Society for Risk Analysis Australia and New Zealand (“Risk analysis as an integral part of a structured, responsible and culturally-appropriate decision-making process”, held in Wellington, 13-14 November 2019).

Milestone 3: Monitoring the risk from ambrosia beetles

Output:

File Note
PAD ID: 26856644
Date 23 June 2020

Granulate ambrosia beetle phenology and trapping methods

Xylosandrus crassiusculus, the granulate ambrosia beetle (GAB), was first identified in New Zealand through the High-Risk Site Surveillance programme on 20th February 2019 in two pinoak (*Quercus palustris*) trees in Blockhouse Bay Recreation Reserve, Auckland. On the 27th February, Biosecurity New Zealand's (BNZ) and Plant Health and Environment Laboratory (PHEL) confirmed the identity of the beetle. These trees were then removed by the Auckland council and disposed of by deep burial. Though broad scale surveillance GAB has been identified in seven sites within the Auckland Region: Blockhouse Bay, Kumeu, Huapai, Riverhead Village, Titirangi, Sunnyvale, and Henderson. There has been no record of the beetle establishing outside of the Auckland region. The Ministry for Primary Industry (MPI) undertook a pathways assessment to determine the entry point into New Zealand, but this came back inconclusive. GAB represented 0.6% of Scolytinae intercepted in New Zealand during a study by Brockerhoff et al. (Brockerhoff et al. 2006).

GAB is a minute species of bark beetle native to East Asia (Coleoptera: Curculionidae: Scolytinae). It is highly invasive and regarded a serious pest in nurseries overseas (Ranger et al. 2016). The beetle is known to attack healthy and stressed tress, as well as freshly cut timber. The beetle has been recorded attacking over 120 plant species in 40 different families (https://www.cabi.org/isc/datasheet/57235). The species of host trees that are of obvious concern to New Zealand include *Eucalyptus* (*camaldulensis* and *robusta*), *Grevillia robusta*, macadamia, avocado, *Prunus*, *Acacia*, persimmon and *Ulmus*. GAB has attacked more than 15 different species in West Auckland (7 native and 8 exotics as of August 2019, MPI unpublished data). This number has increased in 2020 and is expected to increase as the beetle establishes across the region. The female beetles are flighted and range in size (2-3 mm) whereas the males are flightless and smaller (~1.5 mm). Female beetles bore into the host tree leaving behind toothpick/ frass noodles extruding from the branch or trunk (see Fig 4B for an image of a frass noodle). The beetle creates a brood gallery which she inoculates with a symbiotic fungus *Ambrosiella roeperi,* to feed her and her offspring. Once the fungus has established the already mated female will lay numerous eggs. These eggs, larvae and pupae can all be found together in a single gallery. The female will stay with the brood until they reach maturity around 50-60 days after egg laying, during spring and summer.

Control of the spread of the beetle is complicated due to the beetle's dispersal ability and mating practices, where the adult female leaves the brood chamber already mated. In New Zealand, eradication is probably not feasible given the current infected area in the Auckland region is > 20km². MPI has not officially "closed out" the response to GAB, and are continuing to monitor the situation (J. Russell, MPI, pers. comm).

Currently there is a lack of pest management being attempted, and surveillance options are limited for detecting the beetle at low density. The most effective management tool at the moment is removal and deep burial of infected trees.

A section 53 Permission under the Biosecurity Act was granted by the Ministry of Primary Industry (MPI) on 21/10/2019 to enable Scion to transport dead beetles outside of Auckland for research purposes.

Table 1. Host species of GAB recorded in New Zealand as of August 2019.

Native hosts	**Non-native hosts**
Caprosma robusta (karamu) x 24	*Quercus palustris* (Oak) x 4
Piper excelsum (kawakawa) x 5	*Prunus* sp. x 2
Melyicytus ramiflorus (whiteywood)x 5	*Prunus campanulata* (Taiwanese cherry) x 2
Pseudopanax lessonii (houpara) x 2	*Acacia* sp. x 1
Pseudopanax crassifolius (lancewood) x 1	*Liquidambar styraciflua* (liquid amber) x 1
Pseudopanax arboreus (five finger) x 1	*Quercus* sp (red oak) x 1
Pittosporum tenuifolium (kohuhu) x1	*Albizia julibrissin* (Persian silk tree) x 1
	Cestrum nocturnum (Night-blooming jasmine) x 1

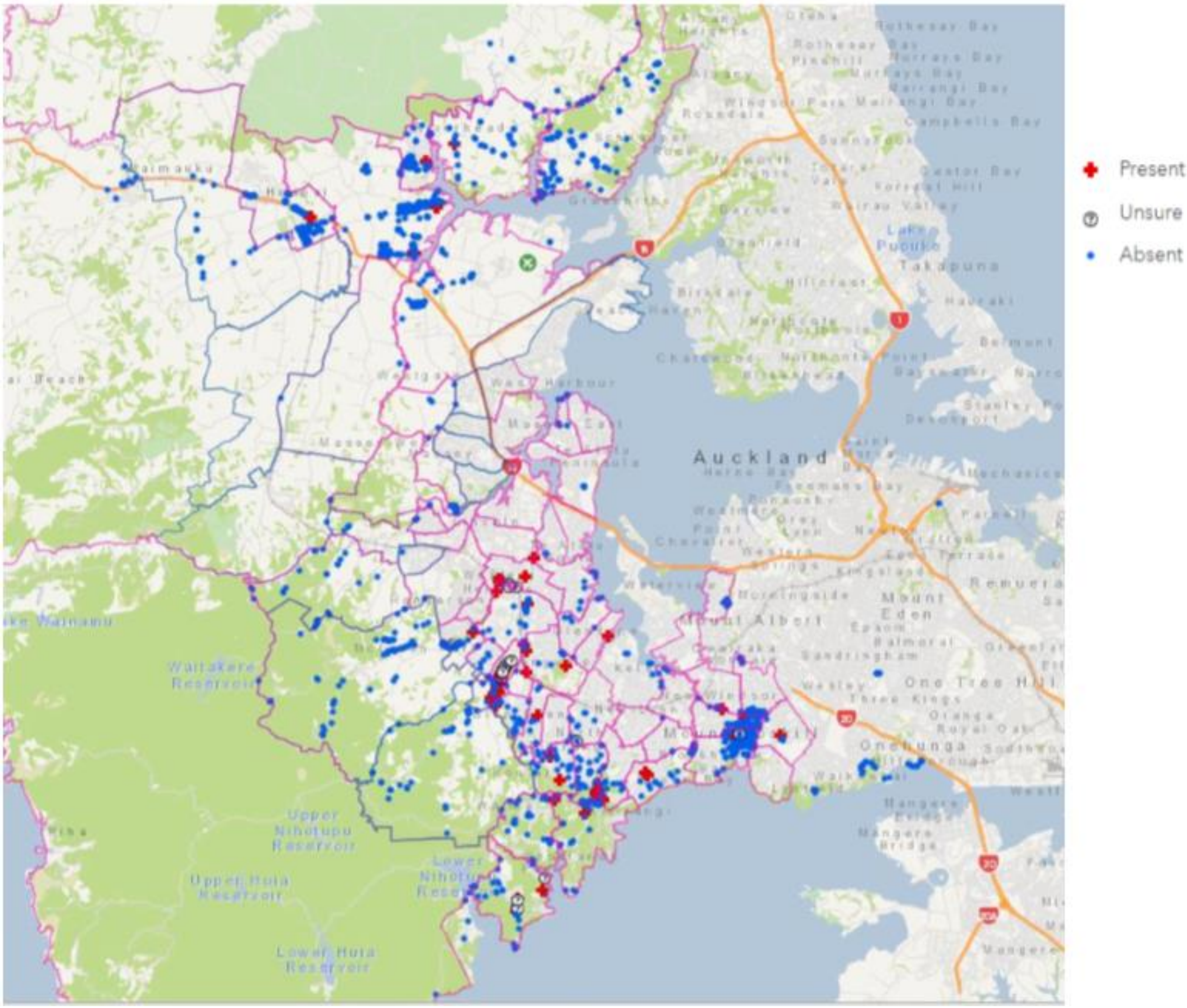

Figure 1. Granulate ambrosia beetle detection in the Auckland region as of August 2019 (used with permission of MPI).

Monitoring

Panel traps with ethanol lures

Monitoring has been undertaken fortnightly commencing October 2019-June 2020 in West Auckland. Three sites were established with insect panel traps (Figure 2) and ethanol lures, Titirangi (5 traps), Blockhouse Bay (5 traps) and Kumeu (7 traps) on private property. The panel traps had a very low catch rate at Blockhouse Bay and in Titirangi this may have been due to the very low ethanol release rates of the lures. Scion intends to undertake additional research on lure release rates next year. The traps at Kumeu were in close proximity (between 2 and 10 m) to a highly infected tree and had a much higher catch rate (Figure 3).

Figure 2. Insect panel trap with ethanol lure.

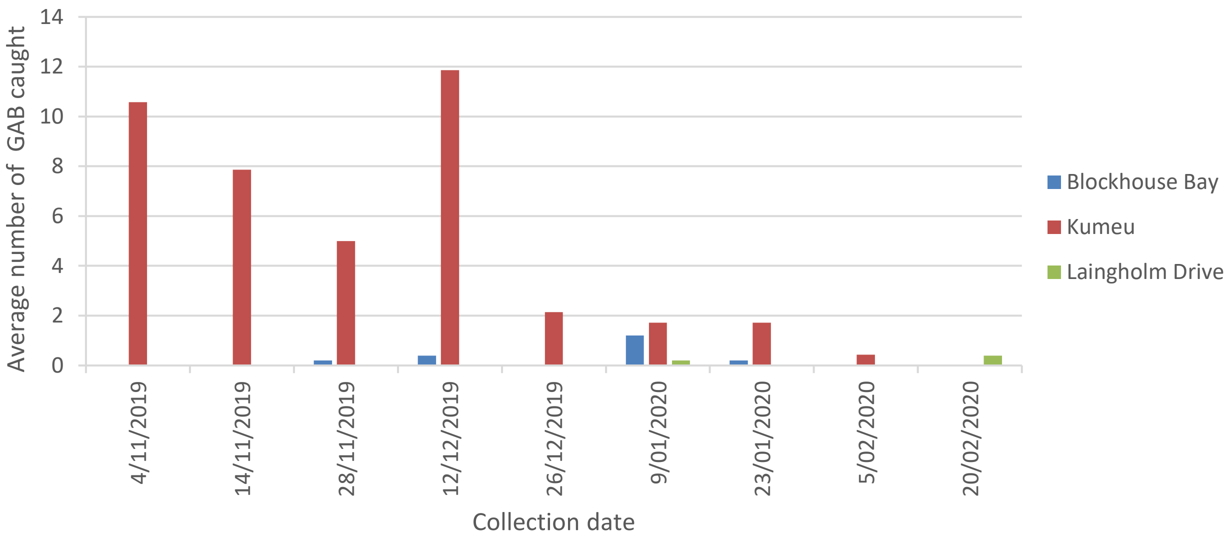

Figure 3. The average number of GAB caught in panel traps with ethanol lures over a three-month period at three sites in West Auckland from November 2019 till February 2020.

Wood bolt traps

Wood bolt traps (Figure 4) similar to those described by Ranger (Ranger et al. 2016) were trialled at two sites (Blockhouse Bay and Kumeu). Freshly cut wood bolts of known host species *Prunus campanulata* (Taiwan cherry) and *Persea americana* (Hass avocado) with diameter between (2.5-5 cm) and 30 cm long were soaked in 20% ethanol for 24 hours, transported to the field site, and set up alternatively with un-soaked controls. Only ethanol-soaked wood bolts were attacked by GAB and brood chambers established with eggs and larvae present after four weeks (Figure 4 B). Wood bolts were bagged and frozen on site at AsureQuality for two weeks before being transporting to Scions PC1 lab where they will be dissected.

Figure 4. (A) wood bolt trap using *P. campanulata,* (B) with GAB frass noodle evident. (C) GAB gallery exposed in the laboratory with adult eggs and larvae present.

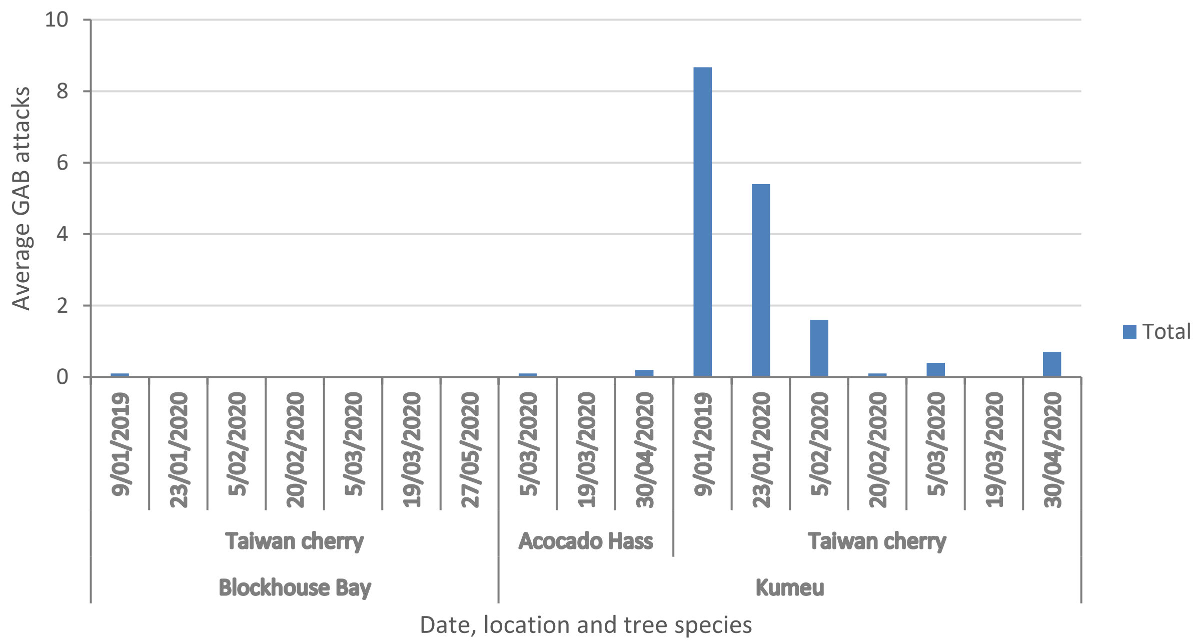

Figure 5. The average number of attacks on ethanol-soaked wood bolts at two locations in West Auckland, New Zealand.

Phenology

The lifecycle that GAB is undertaking in Auckland remains undetermined at this stage. When monitoring started in November 2019 the adult female beetles were already in flight. Primary data shows that there was one, possibly two, flight periods this last season. One flight, the spring generation, started at some stage prior to November, but possibly another occurred in December/

January which would account for the fresh attacks of the wood bolts (Figure 5) found in January 2020. The number and timing of generations may vary year to year with climatic conditions, and it is our intention to continue to investigate this pest and ascertain the lifecycle and number of generations being undertaken.

Recommendations

Xylosandrus crassiusculus is one of the most damaging non-native ambrosia beetle pests in plant nurseries in America. The wide host range of this beetle means it could threaten the ornamental, production, and forestry nurseries in New Zealand. There is currently no evidence that it will utilise *Pinus radiata*, however eucalypts and other important hardwood trees, along with natives are within its host range. With eradication ruled out by MPI, Scion intends to continue to invest SSIF funds in research on this pest to learn more about it, so that we can learn to manage it here. Future research aims include:

- Monitoring infested sites year round to ascertain the phenology in detail
- Using ethanol-soaked wood bolts to test potential host trees, both native and important agricultural species in New Zealand
- Trial new ethanol lures with higher release rates to obtain a more efficient trapping method
- Extend monitoring to a wider area

Roanne Sutherland
Senior field and Lab Technician

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Ranger CM, Reding ME, Peter B. Schultz, Jason B. Oliver, Frank SD, Addesso KM, Chong JH, Sampson B, Christopher Werle, Stanton Gill, Krause C (2016) Biology, ecology, and management of nonnative ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in ornamental plant nurseries. Journal of integrated pest management 7 (1):1-23

Milestone 4: The natural enemies of EVB, *Paropsisterna variicollis* in Australia

The prospects of locating a suitable biological control agent for the recently invaded *Eucalyptus* pest *Pst. cloelia variicollis* in its invaded region of New Zealand was strengthened through successful collaborations with international entomologists. Firstly, a collaboration agreement was drawn up between Scion and Australian entomologists Helen Nahrung and Geoff Allen. Co-funding was received from the University of the Sunshine Coast to boost the SSIF funding from Scion. Adult beetles superficially resembling *Pst. variicollis* were collected from the Hawke's Bay region in New Zealand, and from several sites throughout Tasmania and mainland Australia.

The collaboration has revealed a number of interesting results:

1. The name change (synonymy) to *Pst. cloelia* (summarised in: Forest Health News No. 299: "Eucalyptus variegated beetle renamed").

Paropsisterna cloelia was, until recently, a name only applied to a distinctively coloured species of this group: orange (normal form) to dark brown/black elytra with broad orange lateral margins (melanistic form). The recent synonymy of *Pt. variicollis* under *Pt. cloelia* by (Leschen et al. 2020) based on morphology, is supported by evidence from studies of eastern Australian populations Identified under these names. Both have the same species of sexually transmitted mite, hybrids produce viable offspring in laboratory crossing trials (Dave de Little pers. comm.) and both have a melanistic form (Nahrung and Allen 2005) in mainland Australia (Nahrung et al. 2020).

2. Following the intensive molecular research, the WA and NZ populations showed no diversity at the gene regions studied, and hence represent invasive populations. The NZ haplotype was unique, but most similar to one shared between WA, Tasmania and Victoria. The results are now publicly available in the following publication summarised below (Nahrung et al. 2020).

3. Thirdly, M.Sc student Ryan Ridenbaugh was financially supported by Scion SSIF to conduct field work for two successive years in the Australian Capital Territory, New South Wales, and Victoria. His second progress report, and his completed Master's thesis (abstract) are attached here as outputs. In summary, he has reported that the dominant parasitoid of *Pst. cloelia* (=*variicollis*) is *Eadya annleckieae*, and it can be located in *Eucalyptus globulus* juvenile plantations throughout Victoria, to add to the previous knowledge of a collecting locality at Seven Mile Beach, Hobart, Tasmania (Peixoto et al. 2018). Ryan also reports on the interesting research angle that *Eucalyptus* phytochemistry could be how *Eadya* species separate their host usage.

4. Monitoring the spread and impact of *Pst. cloelia*.

Toni Withers has continued to work with the web application iNaturalist.org to identify all *Eucalyptus* defoliating insects, and *Pst. cloelia* is relatively easy to identify from photographs. https://www.inaturalist.org/observations?place_id=6803&taxon_id=559118
As well as scion staff, NZDFI staff and students, foresters, farm foresters and members of the public have all contributed to these data points. The geographic range of *Pst. cloelia* is continuing to spread and this year has reached the NZDFI sites in Marlborough, as well as Gisborne and has entered the central north island, being located current in Poronui Station, west of Taupo (see map below, Figure 6).

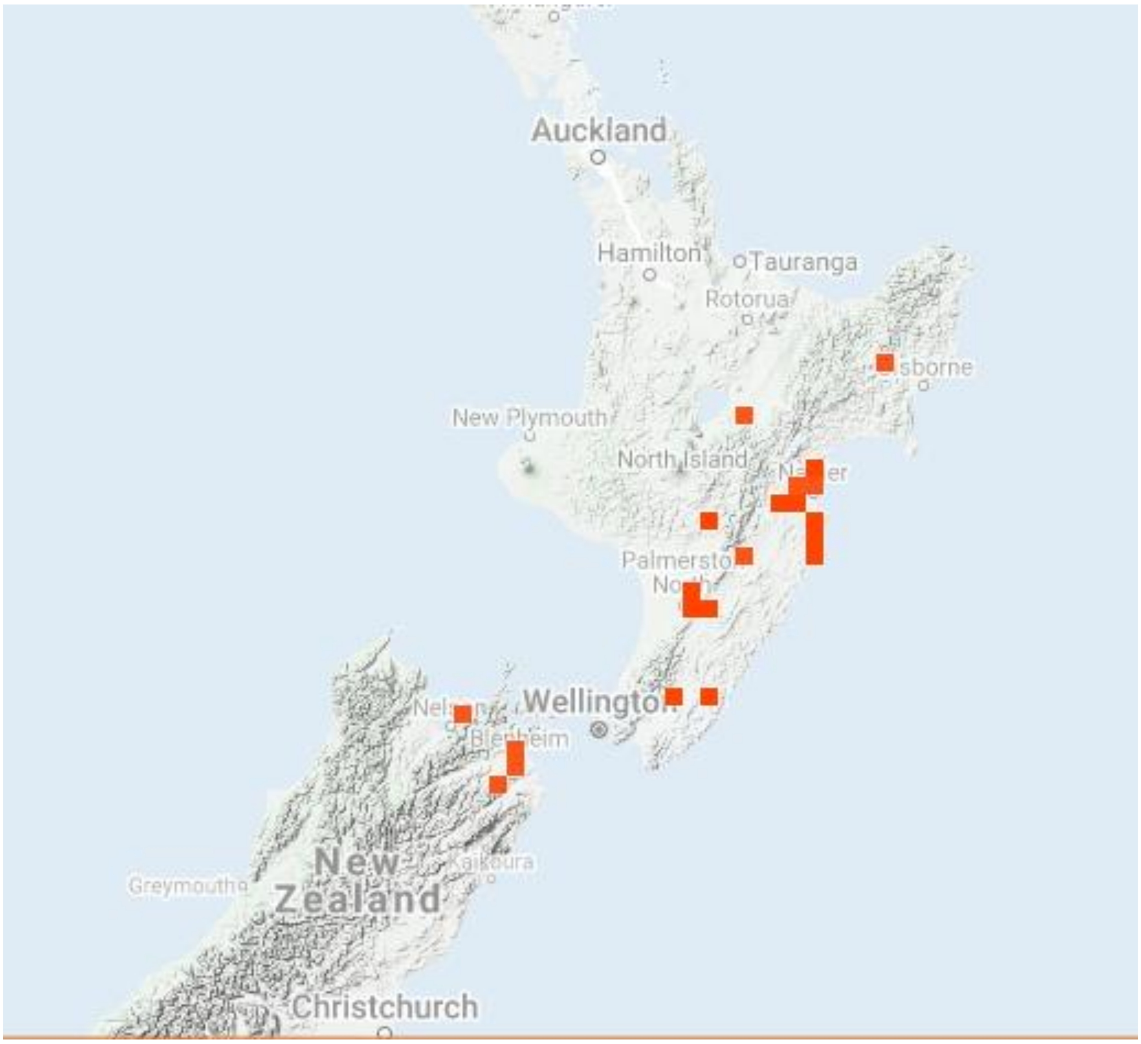

Figure 6. The distribution of *Paropsisterna cloelia* in New Zealand as at June 2020.

OUTPUT

Austral Entomology

doi: 10.1111/aen.12476

Expansion of the geographic range of the eucalypt pest *Paropsisterna cloelia* (Stål) (Coleoptera: Chrysomelidae) through synonymy and invasion

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Abstract

The accidental introduction of the eucalypt-feeding paropsine beetle *Paropsisterna cloelia* (= *P. variicollis*) into New Zealand in 2016 prompted investigation of the species complex to which the invasive species belongs. Two mitochondrial gene regions, Cytochrome c oxidase subunit 1 (CO1) and Cytochrome b (CytB), were sequenced from field-collected specimens superficially resembling *P. cloelia* collected in Queensland, the Australian Capital Territory, New South Wales, Victoria, South Australia, Tasmania, Western Australia (WA), and New Zealand (NZ). Four distinct *Paropsisterna* species groups were found and representatives from each clade were identified with named species using morphology. *Paropsisterna cloelia* was confirmed via sequencing and morphology to occur in all sampled geographic regions. The WA and NZ populations showed no diversity at the gene regions studied, and hence represent invasive populations. The invasive WA haplotype was shared with both Tasmania and Victoria, whereas the NZ haplotype was unique, but most similar to one shared between WA, Tasmania and Victoria. The melanistic colour variant of *P. cloelia* was only found in eastern mainland Australian populations and was absent from Tasmania and invasive populations. Implications for pest management, including biological control, are discussed.

Figure 7. These beetles are all the same genetically and are all *Paropsisterna cloelia* but note (a) the black form has not been seen in New Zealand. From Nahrung *et al* (2020).

OUTPUT:

PAD ID 26983224
2020 Progress Report

Name of Recipient: New Zealand Forest Research Institute Limited (SCION)
Project Title: Field work to identify natural enemies of *Paropsisterna variicollis* (Chrysomelidae) Eucalyptus Variegated Beetle, in Australia
Period Covered by Report: August 1st, 2019 to May 1st, 2020
Author: Ryan D. Ridenbaugh, University of Central Florida, USA.

Abstract

From the material collected during my 2018 field season, a total of 67 *Eadya* and 72 hosts beetles were successfully sequenced and identified using CO1 Bayesian and maximum likelihood phylogenetics, as well as CO1 DNA barcoding. Four different species of host beetle were collected: 1 *Paropsis aegrota elliotti*, 5 *Paropsisterna m-fuscum*, 17 *Paropsisterna agricola*, and 49 *Paropsisterna variicollis** (Recently synonymized by Leschen et al. (2020) under *Paropsisterna cloelia*). From these hosts the following *Eadya* were reared: 20 *Eadya daenerys*, 44 *Eadya annleckieae*, and 3 specimens referred to as *Eadya* sp. 1 which did not fall out with any of the Peixoto et al. (2018) molecular vouchers. As the three specimens referred to as *Eadya* sp. 1 were destructively sampled, I was unable to perform a morphological examination to determine whether this was a new species of *Eadya*, or a species for which no molecular records exists, such as *Eadya duncan* or *Eadya falcata*. A total of 51 wasp-beetle associations were established: 37 *Eadya annleckieae* from *Paropsisterna cloelia (variicollis)*, 8 *Eadya daenerys* from *Paropsisterna agricola*, 4 *Eadya daenerys* from *Paropsisterna m-fuscum*, and 2 *Eadya* sp. 1 from *Paropsisterna cloelia (variicollis*). These rearings, all from Victoria, represent new distribution records for *Eadya annleckieae* and *Eadya daenerys*, as well as a new host record for *Eadya daenerys* (*Paropsisterna m-fuscum*). From phytochemistry results I concluded that between the three species of *Eadya* collected, *Eucalyptus* phytochemistry indeed influences host location and use. These compounds may have potential as baits to augment biological control efforts, and have potential to reduce potential competition between *Eadya annleckieae* and *Eadya daenerys* in the control of *Paropsis charybdis*, if *Eadya annleckieae* were to be released targeting *Paropsisterna cloelia* (*variicollis*).

OUTPUT:

PAD ID 26983267
Masterate thesis co-funded by Scion:

THE EFFECTS OF PLANT PHYTOCHEMISTRY
ON PARASITOID (HYMENOPTERA: BRACONIDAE)
NICHE BREADTH
by
RYAN D. RIDENBAUGH

A thesis submitted in partial fulfilment of the requirements
for the degree of Master of Sciences
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida
Spring Term
2020

Abstract:
The definition of a species, a fundamental unit of biology, has been debated since its inception. This level of classification is vital to our ability to make meaningful comparisons across all subdisciplines of biology. Cryptic species, those which are indistinguishable from another species using morphology alone, pose a unique problem. This is especially true for biological control programs, where the control of an invasive pest is achieved through the importation of a natural enemy or parasitoid from the pest's native range. The accidental importation of a cryptic species could have long lasting negative environmental effects. Molecular taxonomy provides a solution. A recent large-scale phylogenetic study of *Eadya paropsidis*, a potential biological control agent for the invasive New Zealand pest *Paropsis charybdis*, also known as the Eucalyptus tortoise beetle, is a perfect model for the integration of molecular taxonomy into biological control research. This study not only uncovered a cryptic species, but three additional non-cryptic species as well, each of which exhibit varying degrees of host flexibility. Here I formally describe three new species of *Eadya* (*E. daenerys, E. spitzer, E. annleckieae*) using an integrative taxonomic approach, and redescribe the two previously known species of *Eadya* (*E. paropsidis* and *E. falcata*). An additional species (*E. duncan*) from the Australian National Insect Collection is described using morphology. The formal description of these host flexible species enables investigation on the influence of plant phytochemistry on parasitoid niche breadth. Using 112 compounds extracted from *Eucalyptus* leaves, I conclude that host selection is heavily influenced by infochemicals from the 1st trophic level. With this evidence, I amend the reliability- detectability hypothesis of Vet and Dick (1992) on infochemical use by natural enemies of herbivores, to include the scenario in which an oligophagous parasitoid utilizes oligophagous hosts.

OUTPUT:

PAD ID: 26807370
Date: 22 June 2020
Author: Toni Withers

File Note, Observations on *Eucalyptus* health from NZFFA field day at Makihoi Forest, Gisborne, 24 February 2020

Abstract
Brian Gibson replanted his block called Makihoi Forest in Rakauroa, Matawai (Gisborne district) in a mix of species in 2015. As well as redwoods and blackwood, he planted three eucalypt species that have remained as the top priority for the NZDFI (*E. bosistoana, E. globoidea* and *E. quadrangulata*) along with two more species that are in the secondary priority for NZDFI (*E. argophloia*, and *E. macrorhyncha*), and finally he also planted *E. camaldulensis*.

During the visit in Feburary 2020 I noted superior growth is being demonstrated by *E. globoidea* and *E. bosistoana*. The other species are either very slow growing or suffering crown damage. The most serious defoliation is apparent on *E. camaldulensis* and *E. quadrangulata*, although by February the trees were re-foliating following what appeared to have been complete defoliation in the spring. The main defoliating agent is the eucalyptus tortoise beetle *Paropsis charybdis*, but also observed on the site were the weevil *Gonipterus platensis*, and the beetles *Aporocera melanocephala* and *Paropsisterna cloelia* (=*variicollis*). Predators and parasitoids were abundant, including native New Zealand predatory pentatomid *Oechalia schellenbergii*, and the exotic ladybird *Harmonia axyridis*. The *Paropsis* parasitoids *Neopolycystus insectifurax* and *Enoggera nassaui* were infesting eggs of both paropsines at the time of the field day. It would be an interesting site to monitor whether the predators and parasitoids can reduce the defoliation by *Paropsis* to only during the spring generation, which is what the owner has been observing.

Figure 8. Mating pairs of *Paropsis charybdis* on *Eucalyptus bosistoana* at Makihoi and feeding damage evident.

Figure 9. *Oechalia schellenbergii* feeding on *P. charybdis* larvae at Makihoi, *Neopolycystus insectifurax* parasitoids guarding *P. charybdis* egg batches (left) and evidence of larvae that have been "sucked dry" (right).

Figure 10. Distinctive eggs (left) and larvae (right) of *Paropsisterna cloelia* on *Eucalyptus camaldulensis* at Makihoi. Eggs show evidence of parasitism by *Enoggera nassaui* (Pteromalidae), and two adult parasitoids are present on the egg batch in this photograph.

ACKNOWLEDGEMENTS

Eadya: The 2019 recollections were possible thanks to Geoff Allen, Raylea Rowbottom, Steve Quarrell, Roanne Sutherland, Dean Satchell, Pam Taylor, Mike Davy, Giovanni Mascarinas, Bernie Prout and Hannah Humphreys.

Funding was from the New Zealand Ministry of Business Innovation and Employment Strategic Science Investment Funding contract to Scion. Industry funding co-partners Southwood Exports Limited and Oji Fibre Solutions NZ Ltd, are gratefully acknowledged, as are the Speciality Wood Products Partnership, Go-early funding from MPI, the New Zealand Farm Forestry Association Eucalyptus Action Group, The Gisborne Branch of the NZFFA and Scion.

EVB: Dongmei Li (MPI Plant Health Environment Laboratory) contributed additional sequences from a March 2016 collection in New Zealand. New Zealand collections were undertaken by SPS Biosecurity. We are grateful for the additional collections in Australia conducted by A. Garcia, M. Schroder, N.M. de Souza, and Megan Head.

Funding was provided by the New Zealand Ministry of Business Innovation and Employment Strategic Science Investment Funding contract to Scion Forest Protection, and the University of the Sunshine Coast.

Biocontrol: Research on improving biocontrol risk assessment is being undertaken within the Better Border Biosecurity Collaboration (b3nz.org). Scion team members have included at various times Belinda Gresham, Andrew Pugh, Stephanie Kirk, Brenna Douglas, and Mike Davy, in addition to Toni Withers and Nicolas Meurisse. External organisation team members from Theme One (Risk) contributing to aspects of the research include: Jacqui Todd, Barbara Barratt, Gonzalo Avila, Frances Macdonald, Karina Santos, Quentin Paynter and Darren Ward. This research is on-going.

GAB: Scion (Roanne Sutherland, Stuart Fraser, Toni Withers, Nicolas Meurisse) is undertaking this research with the co-operation and assistance of Brad Siebert (Avocados NZ), Jess Russell and John Brightwell (Biosecurity Response MPI), Rachel Langman, Phil Brown (AC), Kerry King AsureQuality, Sophie Melles (SPS) and landowners Gerald Collett and Darryn Harrell, and orchardists Denise McBrydie and Gilbert Haine.

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