Manaaki Whenua
Landcare Research

The horehound clearwing moth in New Zealand – new developments and insights

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The horehound clearwing moth in New Zealand – new developments and insights

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Summary

Project and Client

- An interim report about the status of the horehound clearwing moth, *Chamaesphecia mysiniformis*, and the discovery of other insects inhabiting horehound roots, was prepared for the Horehound Biocontrol Action Group. This report fulfils Milestone 1 of SFF Futures project 'Bringing horehound under control faster'.

Objectives

- Collate the current best knowledge about the insects found in roots of horehound *Marrubium vulgare* L., at two sites in New Zealand.
- Recommend next steps for use of the horehound clearwing moth, *Chamaesphecia mysiniformis* (Boisduval) in New Zealand.

Methods

- Field-collected horehound roots were kept in a controlled rearing environment or dissected to expose larvae.
- We used molecular tools to try to identify the larvae.

Results

- Presence of the horehound clearwing moth was confirmed at one site.
- Horehound clearwing moth abundance at the confirmed site is far lower than originally thought. Their developmental state was out of synchrony with the stage expected for the time of year they were detected.
- Two other species were discovered as populating the roots of horehound at higher abundance than the clearwing moth (one other species per site).
- The Molecular tools identified one of the species to family level and the other species to subfamily level.

Recommendations

- Confirm presence of the horehound clearwing moth at the north Canterbury site in early-mid spring of 2022.
- Rear the 'other' insects to maturity. This will be required for morphological identification in spring 2022.
- Survey the abundance and developmental stages of the clearwing moth at the north Canterbury and Mackenzie sites during spring and summer 2022/23.
- Make recommendations for the next steps in the biocontrol programme for horehound based on the outcomes of the current study.

1 Background

The horehound clearwing moth, *Chamaesphecia mysiniformis* (Boisduval), was released at five sites in New Zealand in December 2018 as part of a biological control programme against white horehound, *Marrubium vulgare* L. Establishment was confirmed two years later (January 2021) based on observations of larvae inside the roots of horehound at two release sites, one in the Mackenzie Basin and one in north Canterbury. Before the release of the clearwing moth, no other insects were recorded from the roots of horehound in New Zealand (Winks et al. 2018).

Impact from the moth appeared to be high: horehound cover declined dramatically, and grazing grasses were infilling the space vacated. This effect can be seen in the photos from the north Canterbury release site (Fig. 1): the top photo is from the time of release (December 2018). The bottom photo is from November 2021. In the photo from 2021 horehound is still present, but far less abundant.

A plan was initiated to redistribute the moth to new sites by intensive egg-collection under controlled conditions in the spring of 2021. The plan included collecting roots of horehound with pupating moths, transferring them to a controlled environment for emergence, mating and egg-laying, and distributing the eggs to new release sites at participating catchments.

Figure 1. North Canterbury release site at the time of release (Top: December 2018) and 3 years later (Bottom: November 2021).

2 Methods & Results

The first collection of pupating larvae inside roots was from the North Canterbury site, in November 2021 (Fig. 2).

Figure 2 (continued on following page). North Canterbury collection event.

Figure 2 (continued).

Given the developmental stage of the larvae/pupae, we were expecting adults to begin emerging within 10–14 days. When no emergence occurred, we went back to check development of the immature stages of the moth at the site. We continued to check on the site, but no signs of progress with immature development were detected either at the site or at our controlled environment.

Due to the unseasonal cool and wet conditions we decided to:

1 raise the temperature of our controlled environment

2 continue to follow up on larval/pupal development at the north Canterbury site for signs of progress in larval/pupal development

3 delay root-digging at the more southern Mackenzie Basin site, but check regularly for larval development there, so not to miss the optimal developmental stage (pupae).

The cool and wet conditions meant the soil temperature would have been lower than normal for the season. We hypothesised that this would slow the development of the larvae/pupae. This meant it would have been even more crucial to bring those pupae into the controlled environment for emergence and mating. Otherwise, if they emerged under natural field conditions that are below the threshold for the moth's requirement for successful reproduction, we could risk local extinction of the population.

When the team at the Mackenzie site provide photos for the science team at Lincoln to assess larval development, it became apparent that the larvae occupying the roots at the

two sites were different from one another. A sample of roots was sent from the Mackenzie site to Lincoln, so that the larvae from both sites could be compared. In Figure 3 below, the larva on the right is from north Canterbury horehound roots and the larva on the left is from the Mackenzie horehound roots.

Figure 3. two types of larvae found at the two sites where horehound clearwing moth was thought to have established: Mackenzie site (L) and North Canterbury (R).

This realisation raised a red flag, sparking an investigation. The steps and findings are detailed below.

Step 1: What do clearwing moth larvae look like? We did not have the opportunity to see clearwing moth larvae during the initial releases in 2018. When the moth was introduced to New Zealand we brought pupae over from Australia, allowed the adults to emerge and mate in containment, and then released the new generation in the form of eggs. We could not find any reliable photos of the clearwing moth larvae online. So, we had no point of comparison.

Step 2: Contact the collaborators in Australia who guided us through the introduction of the biocontrol agents to find out which is the true horehound clearwing moth. We sent photos of the two types of larvae and the tunnels they created in roots of horehound to the experts in Australia. To our great surprise, their assessment of the photos was that neither larva looked like the clearwing moth. The frass and sawdust in the root tunnels also didn't look right to them.

Step 3: Molecular analysis of the larvae found in horehound roots at the two release sites: Four larvae from the north Canterbury site (coded as NC1 and NC2) and two larvae from the Mackenzie site (coded as Ohau) were sent for molecular analysis to try to identify what these insects are.

Methods: Insect larvae (~2 mm^3 of insect tissue) were first crushed with a plastic pestle. DNA was extracted using the MN kit NucleoSpin Tissue kit, REF 740952.250, with the following modifications: initial incubation at +56°C for 1.5 h and elution in 100 uL BE buffer. PCR was performed using the KAPA3G Plant PCR Kit (KK7252) using 0.45 µM of each primer (M255/M256 – insect COI region, (Folmer et al.); modified in Astrin and Stüben (2008), 0.3 U of polymerase and 1µL of DNA in 15 µL volume and amplified according to the manufacturer's recommendations. PCR products were sequenced with M13F/R sequencing primers. Edited DNA sequences were then compared against sequences from GenBank, administered by The National Center for Biotechnology Information (NCBI). The search settings were as follows: nucleotide collection (nr/nt), max target sequences 500, expected threshold 1e-6.

DNA extractions were stored in -20°C. The M255/M256 primers are detailed in Figure 4 below:

M255	M13F-Bw-LCOI490-JJ	TGTAAAACGACGGCCAGT	CHACWAAYCATAAAGATATYGG
M256	M13R-Bw-HCO2198-JJ	caggaaacagctatgacc	AWACTTCVGGRTGVCCAAARAATCA

Figure 4. Primers M255/M265

Results: DNA was successfully extracted from all six samples (Fig. 5):

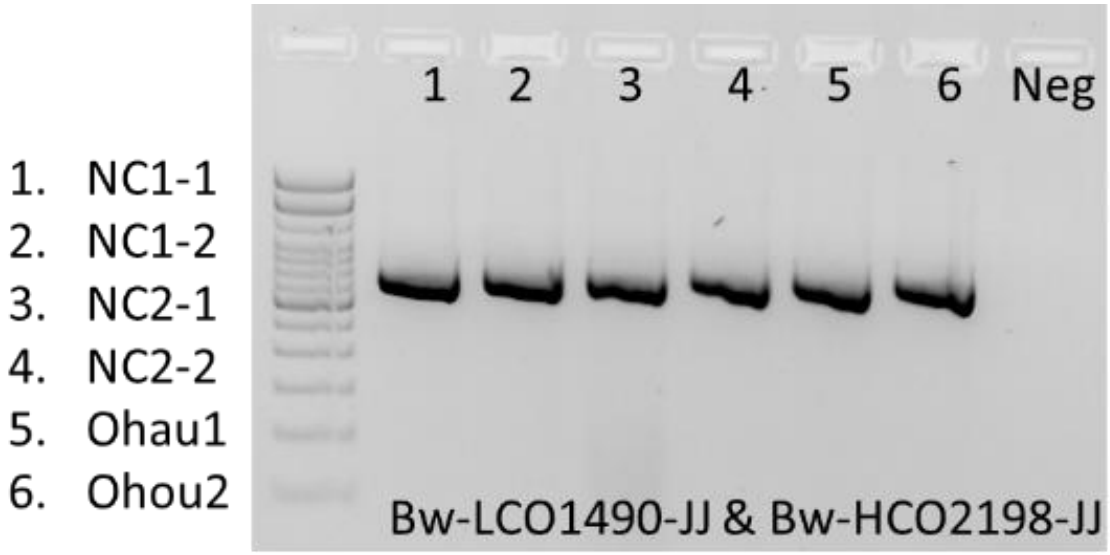


Figure 5. DNA extraction results.

Sequencing results:

1 North Canterbury COI sequences were identical for all four samples (i.e., they are all from a single species). They gave a best match of 84.32% to species in the weevil subfamily Cryptorhynchinae.

2 Mackenzie COI sequences were identical for the two samples (i.e., they are all from a single species), but different to the north Canterbury samples. They gave a best match of 85.21% to species in the family Scraptiidae.

Step 4: Verify whether we even have the clearwing moth established. We dug up more roots from the Mackenzie site and brought them to the controlled environment for careful dissection. In some of those roots we found a third type of larva, at a much lower abundance (Fig. 6). Photos of these larvae and their tunnels were sent to the experts in Australia, who confirmed they did look like the larvae of the horehound clearwing moth. This provisional confirmation was encouraging, and we sent these larvae for molecular analysis. Five larvae were analysed using the same protocol as above. All came back as a match with the clearwing moth, *Chamaesphecia mysiniformis* (96.45–96.81% match).

We have not dug up any more plants at the north Canterbury site after learning to identify the moth larvae. Therefore, we have not yet confirmed if the clearwing moth is in fact established at that site.

Figure 6. Tunnel with larva inside (top) and the larva extracted (bottom) from roots from the Mackenzie site.

Step 5: What does the size distribution mean? We were concerned about the variability of developmental stages of the larvae we found from the Mackenzie site. At that time of year, all individuals should have been at a late stage of larval development, or even pupae. But instead, we found a mix of younger larval stages (Fig. 7).

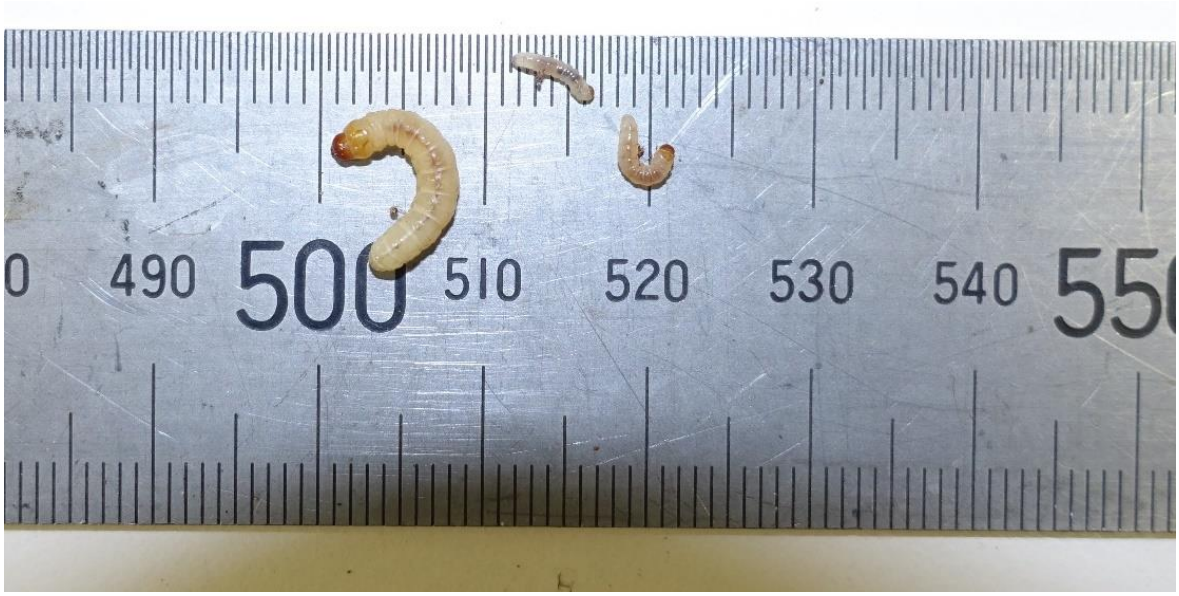
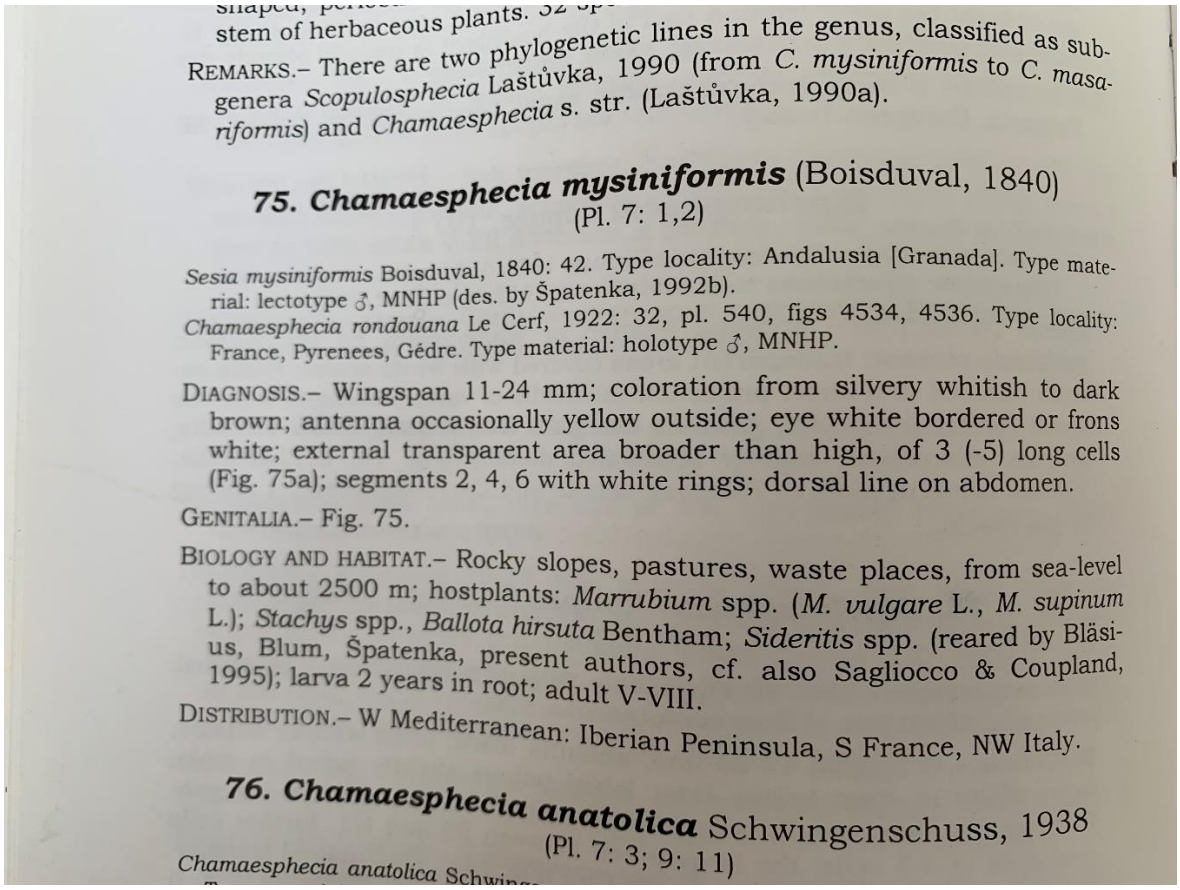


Figure 7. A mix of larval stages found at the Mackenzie site, all earlier stages than would be expected at the time of year they were collected.

This point is important for the clearwing moth with its particular climatic requirements for mating. To mate successfully, the clearwing moth adults require 2–3 days in a row of temperatures above 30°C and calm conditions. Adults normally emerge from late November to mid-January, ensuring at least some adults encounter the required conditions for mating. However, the larvae we found appeared slower in their development than we would expect at this time in the season and have likely missed the opportunity to emerge as adults when the conditions would be suitable for mating in that current season. This could mean the population is heading for extinction. Alternatively, one of the experts from Australia has indicated he read that the clear wing moth can have a life cycle of 2 years in the native range (Laštůvka & Laštůvka 2001). A photo of that book entry is in Figure 8 below. This expert has never experienced a 2-year life cycle in horehound in the population from Spain that he had studied closely, and which is the source population of the moths that ended up in New Zealand (from Australia). He is sceptical about this book entry for a variety of reasons. However, under cooler conditions in New Zealand, we should not dismiss the possibility that the larvae may undergo a 2-year life cycle and that the small larvae that were observed will only mature and pupate to emerge as adults next spring.



stem of herbaceous plants. 32 sp

REMARKS.– There are two phylogenetic lines in the genus, classified as subgenera *Scopulosphecia* Laštůvka, 1990 (from *C. mysiniformis* to *C. masariformis*) and *Chamaesphecia* s. str. (Laštůvka, 1990a).

75. *Chamaesphecia mysiniformis* (Boisduval, 1840)
(Pl. 7: 1,2)

Sesia mysiniformis Boisduval, 1840: 42. Type locality: Andalusia [Granada]. Type material: lectotype ♂, MNHP (des. by Špatenka, 1992b).
Chamaesphecia rondouana Le Cerf, 1922: 32, pl. 540, figs 4534, 4536. Type locality: France, Pyrenees, Gédre. Type material: holotype ♂, MNHP.

DIAGNOSIS.– Wingspan 11-24 mm; coloration from silvery whitish to dark brown; antenna occasionally yellow outside; eye white bordered or frons white; external transparent area broader than high, of 3 (-5) long cells (Fig. 75a); segments 2, 4, 6 with white rings; dorsal line on abdomen.

GENITALIA.– Fig. 75.

BIOLOGY AND HABITAT.– Rocky slopes, pastures, waste places, from sea-level to about 2500 m; hostplants: *Marrubium* spp. (*M. vulgare* L., *M. supinum* L.); *Stachys* spp., *Ballota hirsuta* Bentham; *Sideritis* spp. (reared by Bläsius, Blum, Špatenka, present authors, cf. also Sagliocco & Coupland, 1995); larva 2 years in root; adult V-VIII.

DISTRIBUTION.– W Mediterranean: Iberian Peninsula, S France, NW Italy.

76. *Chamaesphecia anatolica* Schwingenschuss, 1938
(Pl. 7: 3; 9: 11)

Chamaesphecia anatolica Schwing

Figure 8. An excerpt from the book on Sesiidae of Europe indicating that *C. mysiniformis* spends two years as a larva inside the roots

Step 6: Relative abundance of clearwing moth larvae. In mid-February 2022 we collected a further 43 roots from the Mackenzie site for dissection in the laboratory. The aim was to get an initial understanding of the abundance of the clearwing moth larvae compared with the 'other' larvae. We collected horehound roots within the release area, and ~100 m away from the release area, to get an indication of the moth's spread. The results (Table 1) are telling us that:

1 the moth is probably still mainly confined to the area where it had been released, and has not moved further out

2 within the release area there are at least twice as many larvae of the 'other' insect as there are larvae of the clearwing moth

3 both species can be found inside the same root – in parallel tunnels

4 the larvae of the 'other' insect may not require the clearwing moth to attack first (since no signs of clearwing moth attack were found away from the release area)

Table 1. abundance of clearwing moth larvae and larvae of the 'other' type at the Mackenzie site

Collection area	N roots collected	N roots with a clearwing moth larva	N roots with a larva of 'other'	N roots with both species (one of each)	N roots with none
Release area	30	1	5	2	22
~100m away from release area	13	0	4	0	9

3 Discussion

What is known about the taxonomic groups to which the 'other' root-borers belong?

The larvae in North Canterbury roots best matched the weevil family Curculionidae, and subfamily Cryptorhynchinae. This is the largest subfamily of weevils, and in New Zealand there are at least 250 known species and many others assumed undiscovered (Lyal 1993). Most Cryptorhynchinae in New Zealand are thought to feed on dead wood. It is not known if they feed on the dead wood itself or on fungi growing on the wood. Large species can tunnel in quite thick branches and tree trunks (Lyal 1993). One genus, *Psepholax*, is known to make tunnels in dead and dying wood of both indigenous and exotic species (Lyal 1993).

This subfamily was recently the focus of a large-scale molecular phylogenectic study, including the New Zealand clade (Letsch et al. 2020).

The larvae from the Mackenzie site best matched the beetle family Scraptiidae, which has two genera in New Zealand, including four described indigenous species, a number of indigenous undescribed species and no exotic species (Klimaszewski & Watt 1997). Larvae have been recorded from underneath bark from decaying woody fibres of dead logs, and from lichens (Young 1991).

Action: It will be crucial to get species level identification of these insects in order to find out their biology and association with horehound and whether they may be biocontrol agents in their own right.

What may be the relationships between the other insects and the clearwing moth?

We do not yet know the exact identity of the species found in the roots at the two sites. Our best current hypothesis from what is known about the higher classification of their taxonomic groups is that they may be attracted to horehound plants that may be decaying following attack by the clearwing moth.

If this hypothesis is correct, then these insects are not in their own right able to suppress horehound. Rather they may be taking advantage of the weakening of the plants following clearwing moth attack.

Questions that need to be addressed about the relationships:

1 Does attack by the other insects hasten the collapse and death of horehound following clearwing moth attack?

2 Does the high abundance of the other insects compared with the abundance of the clearwing moth suggest that they displace the clearwing moth?

3 Do plants die too soon for the clearwing moth to complete development following secondary attack by the other insects?

4 Can the other insects attack live intact plants?

5 Do the other insects interfere with the clearwing moth population build-up or even establishment in the first place?

These questions are likely to require longer-term study of the insects and their relationships, beyond the resources available in the current project.

4 Recommendations

1 Confirm presence of the horehound clearwing moth at the north Canterbury site. Plants should be dug up in early-mid spring 2022, when larvae should be actively feeding. It is possible that when we dug up plants in late spring 2021 (for pupae), clearwings had already been displaced by the weevil. Earlier in spring it should be easy to see the early greying of plants under attack, and it may be easier to locate the insects.

2 Rear the weevil and beetle to maturity. This will be required for morphological identification. Molecular tools were not able to identify these insects to species level, so we will have to dig plants in spring 2022 and rear the insects under controlled environment to be able to relate the emerging adults to the larvae we have already attempted to identify with molecular methods.

3 Survey the abundance and developmental stages of the clearwing moth at both sites. Depending on the number of attacked plants in spring 2022, decide if enough plants can be surveyed systematically to assess the abundance of the moth at least at the Mackenzie site, and preferably at the north Canterbury site as well (if enough plants are available there). Compare the abundance of clearwing moth to the 'other' insects during spring and also assess the developmental stage of the clearwing moth, to assess whether it may be exhibiting a 2-year life cycle. Note that surveying the clearwing moth is destructive – once the larva is out of its tunnel it can no longer complete its development. Therefore, frequent surveys at low abundance can be detrimental to the population and should be avoided.

4 Make recommendations for the next steps in the biocontrol programme for horehound based on the outcomes of the current study.

5 Acknowledgements

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