Manaaki Whenua
Landcare Research

Invertebrates and fungal pathogens associated with horehound, *Marrubium vulgare* L. (Lamiaceae), in New Zealand

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Invertebrates and fungal pathogens associated with horehound, *Marrubium vulgare* L. (Lamiaceae), in New Zealand

Contract Report: LC3266

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Summary

Project and Client

- A survey of the invertebrate fauna and fungal pathogens associated with horehound, *Marrubium vulgare* L., in New Zealand was carried out during the spring of 2017 and the autumn of 2018 by Manaaki Whenua – Landcare Research for the Horehound Biocontrol Group.

Objectives

- To survey the invertebrate fauna and plant disease symptoms associated with horehound, *Marrubium vulgare*, in New Zealand, and to identify the herbivores (and their associated predators and parasitoids) and plant pathogens present.

Methods

- The invertebrate fauna and plant pathogens associated with *Marrubium vulgare* L. were sampled at 11 New Zealand sites, ranging from Hawke's Bay in the North Island to Central Otago in the South Island, during spring 2017 and autumn 2018.
- Invertebrates were collected, reared and identified.
- The causal pathogens were isolated from surface-disinfected symptomatic material.
- ITS-based DNA sequences of pure fungal cultures were analysed.
- Literature review of information on life histories of plant pathogens isolated

Results

- A total of 12 collections of invertebrates and pathogens were made from 11 sites (one site was sampled twice).
- A total of 39 herbivorous invertebrate species, or groups of taxonomically related taxonomic units, were recorded from horehound. None were horehound specialists.
- Only one herbivorous species, the sage leafhopper (*Eupteryx melissae*), was classed as 'Abundant' on horehound. One herbivorous taxonomic unit, unidentified Dermaptera (earwigs), was classed as 'Common', 13 herbivorous species or taxonomic units were 'Occasional' and 24 were 'Rare'.
- By far the most obvious damage to horehound was attributed to the sage leafhopper, *E. melissae*, and damage caused by all other herbivorous species combined was considered to be minimal.
- 122 pure fungal cultures were recovered from symptomatic tissues, comprising 29 species, from 17 genera.
- The predominant genera isolated were *Phoma* and *Alternaria*.
- Only species belonging to the genera *Phoma*, *Alternaria*, *Fusarium*, *Colletotrichum*, *Diaporthe*, *Stemphylium*, *Leptosphaeria* and *Plectosphaerella* were considered as potential pathogens
- None of the primary plant pathogens found to be associated with disease symptoms of horehound are host-specific, and none had any major impact. The symptoms

observed in the field were mainly "superficial" without any chronic disease symptoms observed killing whole plants.

- The remaining species identified were considered either/or saprophytes, endophytes or secondary pathogens.

Conclusions

- No specialised horehound-feeding invertebrates were collected from horehound during this survey.
- The two Lepidopteran biocontrol agents currently being considered for introduction into New Zealand are unlikely to meet with any significant competition from resident herbivores, since both agents feed in niches that are currently under-utilised by existing herbivores.
- The combined effect of generalist predators and parasitoids has minor potential to inhibit the effectiveness of some potential invertebrate biocontrol agents for horehound.
- None of the primary pathogens found on horehound in the surveys is suitable as a classical biocontrol agent.

Recommendations

- Given that no specialised horehound-feeding invertebrates or pathogens have been recorded in New Zealand, we recommend that a classical biological control programme for horehound should proceed.

1 Introduction

A survey of the invertebrate fauna and fungal pathogens associated with horehound, *Marrubium vulgare* L., in New Zealand was carried out during the spring of 2017 and the autumn of 2018 by Manaaki Whenua – Landcare Research for the Horehound Biocontrol Group.

2 Background

Horehound, *Marrubium vulgare* L., is an aromatic perennial herb in the mint family (Lamiaceae). It is a small bushy plant that produces numerous quadrangular stems that grow to 25–50 centimetres tall (Fig. 1). The silvery-green leaves, arranged in opposite pairs, are 2–5 cm long with a densely-crinkled surface and are covered in downy hairs. The small white flowers are arranged in dense clusters, and the fruit forms brown burrs with small hooked spines (Fig. 2). Each burr contains up to four seeds that are about 1–2 mm long, and one plant can produce thousands of seeds per year.

Figure 1 Horehound at the Eskdale survey site, Hawke's Bay (November 2017).

Figure 2 ***Marrubium vulgare*** **L. (Franz Eugen Köhler, 1897. Köhler's Medizinal-Pflanzen).**

Horehound is native to temperate Eurasia, Europe, the Middle East, and the Mediterranean region, including North Africa (Ohtera et al. 2013; Rodriguez Villanueva & Martin Esteban 2016). It is a weed in parts of North America (California, Texas), in South America (Argentina, Chile, Peru, Uruguay), in Australia and in New Zealand (Weiss & Sagliocco 2000, 2012; Fig. 3).

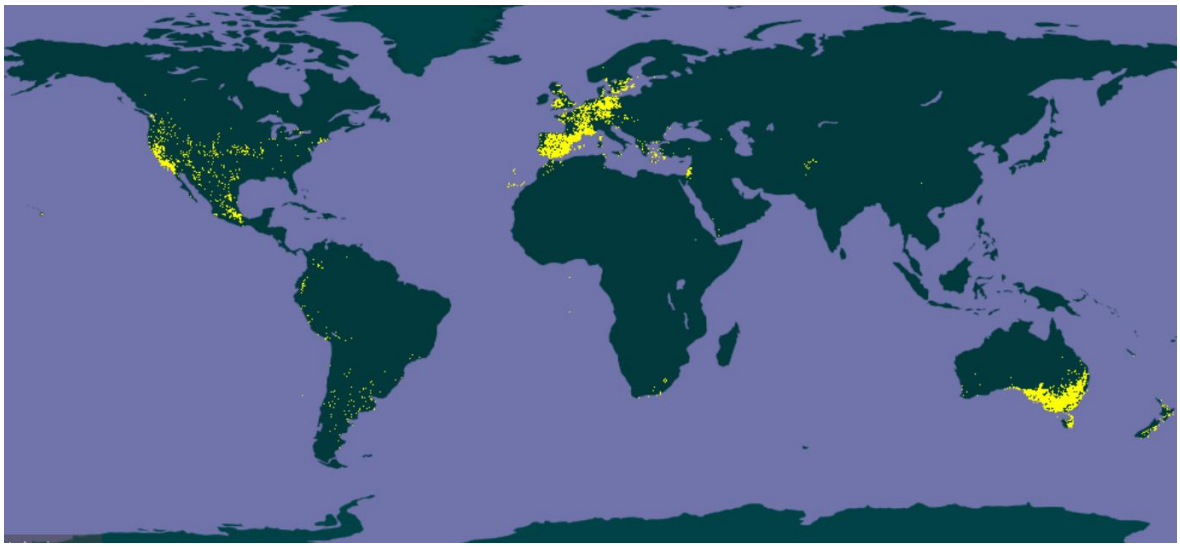

Figure 3 Global distribution of *Marrubium vulgare* (from GBIF https://www.gbif.org/species/2927069).

Horehound is presumed to have been introduced to New Zealand deliberately for its medicinal properties and was first recorded as naturalised here in 1867 (Webb et al. 1988). It was noted as abundant in New Zealand by 1906 (Cheeseman 1906) and can now be found in most of the low-rainfall parts of New Zealand, forming particularly dense stands in the high country of the South Island (Fig. 4). It grows well in alkaline and poor soils and is often an early coloniser of eroded areas, sheep camps, rabbit warrens, and other disturbed sites.

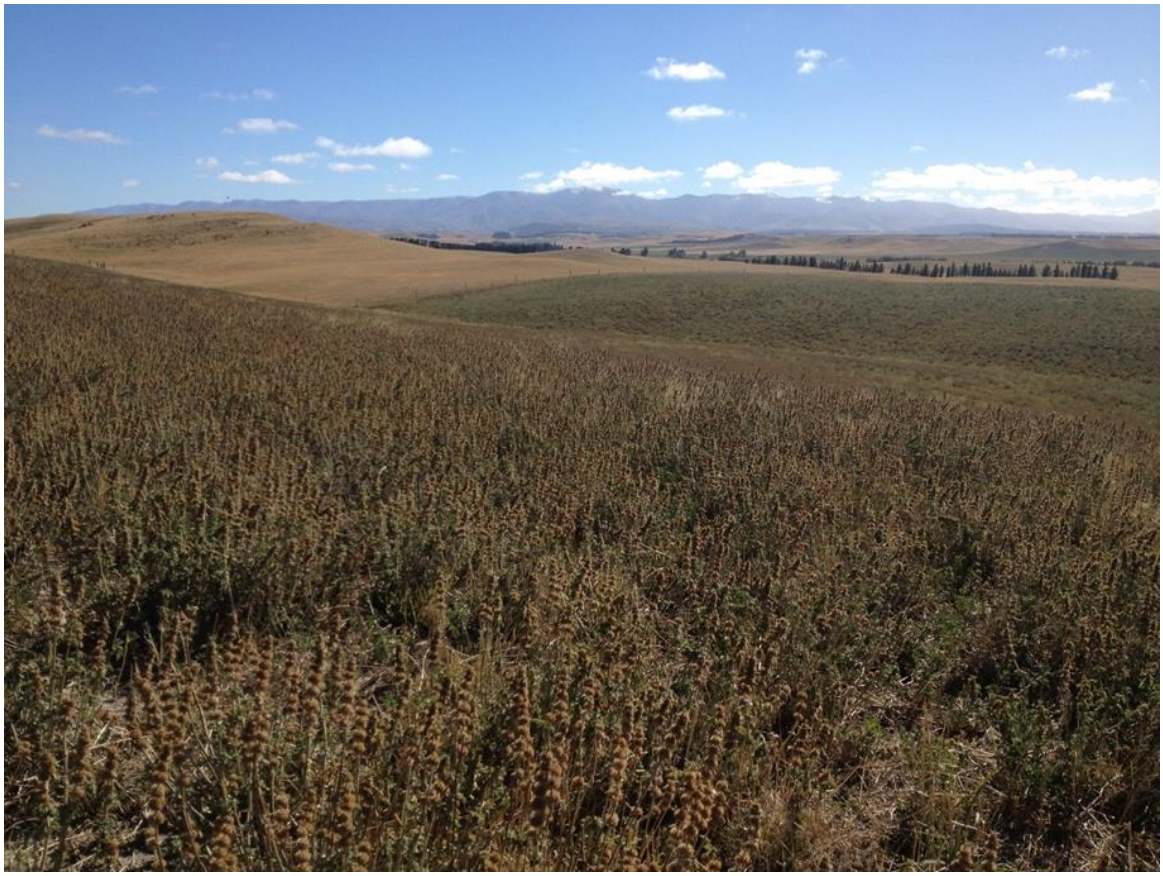

Figure 4 Dense infestation of horehound in Otago.

Horehound fruit form burrs with small hooked spines that attach easily to fleece, which more than doubles the cost of processing the wool – and grazing animals contribute to seed dispersal via movement of burrs and seeds attached to their fleece. Although horehound is rich in protein and metabolites (Carter 1990) it also contains an alkaloid called marrubiin that makes the plant bitter and unpalatable to stock and when animals are forced to eat it their meat becomes tainted (Parsons & Cuthbertson 2000). The problem is exacerbated by the push to convert an increasing area of dryland pastures into lucerne, which facilitates the invasion of horehound as chemical control is proving to be ineffective in lucerne crops.

In New Zealand, control of horehound is mainly chemical (mostly using metsulfuron-methyl) but chemical control is proving ineffective for a number of reasons: the waxy coating on the leaves protects them from herbicide; the most cost-effective chemical, metsulfuron-methyl, has a long residual period of 2–3 years; young lucerne stands are susceptible to chemicals in their first 3–4 years so if horehound becomes established in newly sown lucerne crops, chemical control is not an option; and horehound may be developing resistance to metsulfuron-methyl. Areas treated chemically become bare, resulting in soil erosion, and horehound tends to be the first plant to regenerate following chemical control. Mechanical control is an option but it is used more rarely because of the challenging terrain and vast areas of infestation (Groenteman et al. 2017).

Biological control could offer many advantages over current control methods for horehound. Use of host-specific biocontrol agents would reduce herbicide impacts on desirable flora, and biological control also offers continuous action and self-dispersal that current control methods do not offer. Two candidate biocontrol agents are currently being considered for introduction into New Zealand – the horehound plume moth, *Wheeleria spilodactylus* (Curtis) (Lepidoptera: Pterophoridae), and the horehound clearwing moth, *Chamaesphecia mysiniformis* (Boisduval) (Lepidoptera: Sesiidae). Both of these moths have been released as biocontrol agents for horehound in Southern Australia and both have successfully established there (Weiss & Sagliocco 2012).

Horehound is valued in New Zealand for its medicinal properties and is harvested from the wild by medical herbalists. Opposition from medical herbalists to horehound biocontrol will need to be addressed, and a consultation process is already underway (Groenteman et al. 2017). Horehound is used medicinally for a wide range of ailments including inflammatory disorders, gastroenteric disorders, indigestion, liver disorders, and respiratory disorders such as bronchitis, colds, and asthma. Horehound is also used by the brewing industry as a substitute for hops (Fig. 5).

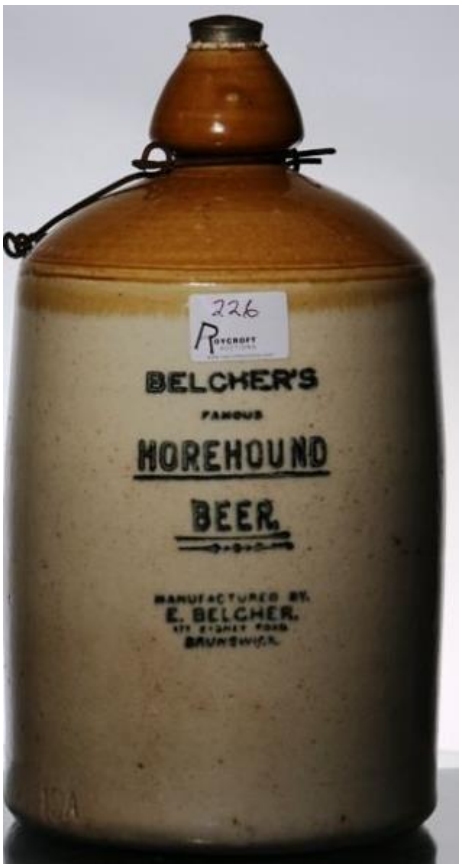

Figure 5 Horehound beer.

This report describes the results of a survey of the invertebrate fauna and fungal pathogens associated with horehound in New Zealand. The main aims of the survey were to determine:

1 Whether any specialist horehound invertebrates or pathogens are already present in New Zealand.

2 Whether any generalist invertebrate herbivores or plant pathogens are exerting a significant adverse impact on horehound in New Zealand.

3 To record the invertebrate parasitoids and predators associated with the herbivorous invertebrates on horehound in New Zealand.

4 To record utilisation of horehound as a resource by indigenous invertebrates and pathogens.

Such information is useful at the early stages of a biological control programme to avoid wasting resources on importing something that is already here. Also, knowing whether a candidate agent already has similar 'analogue' native arthropod herbivores utilising the exotic weed target in NZ helps predict whether natural enemies of the 'analogue' could potentially interfere with the efficacy of any future agent released here (Paynter et al. 2010).

3 Objectives

To survey the invertebrate fauna and plant disease symptoms associated with horehound, *Marrubium vulgare*, in New Zealand, and identify the herbivores (and their associated predators and parasitoids) and plant pathogens present.

4 Methods

4.1 Invertebrates

The invertebrate fauna of horehound, *Marrubium vulgare*, was surveyed at 11 New Zealand sites, spread from Eskdale in the North Island to Armidale in the South Island, between October 2017 and April 2018 (Fig. 6 and Appendix 1). One site, Martinborough, was surveyed twice, once in the spring and again in the autumn, making a total of twelve collecting events. At each site, 10 collection locations were selected. A collecting tray, 80 × 80 cm, was placed under suitable parts of selected plants, and the foliage above the tray was hit five times with a stick. Most invertebrates that fell onto the tray were collected with an aspirator, and preserved in 95% alcohol. Caterpillars (Lepidoptera) were collected live, and placed, along with horehound foliage, in ventilated containers to rear through to adult for identification. Any parasitoids emerging from the caterpillars were identified. An additional 10 plants per site were dug up and their roots cut open lengthwise in search for root feeders.

A rapid (generally less than a minute for each of the 10 collection locations per site) visual inspection of foliage was made for signs of invertebrates such as gall-formers, leaf miners, scale insects and stem/shoot borers. Invertebrates found during the visual inspections were collected live, along with the plant material they were on, for identification. If burr clusters (Fig. 7) were present, approximately 100 burr clusters were collected from each site and stored in ventilated rearing containers. At each site, a visual estimate was made of the amount of herbivore-related damage, and the likely cause of the damage was noted (e.g., adult beetles, leafroller caterpillars).

The invertebrates collected were identified to species or genus level where feasible. However, some invertebrates were placed into higher-level taxonomic units (e.g. 'spiders'). They were then ranked on a scale of abundance according to the total number of individuals collected, and the number of sites at which they were present. Invertebrates were classed as rare, occasional, common or abundant according to the definitions below:

rare: fewer than 5 individuals collected in total

occasional: 5–15 individuals collected in total, **or** present at fewer than 5 sites

common: 16–99 individuals collected **and** present at 5 or more sites

abundant: 100+ individuals collected **and** present at 7 or more sites

4.2 Plant Pathogens

Plant pathogens associated with horehound were surveyed at the same 11 New Zealand sites and times as for invertebrates, with Martinborough being surveyed twice (spring and autumn). At each site, plants were inspected for signs of pathogen damage and/or presence on either diseased leaves or flowers. Samples of symptomatic tissues were placed in paper bags, and then placed into labelled zip-lock bags (with the sample number and GPS location), and kept cool in transit, then held at 4–10°C until processing. Collected material was usually processed within 5 days of collection.

In the laboratory, disease symptoms were recorded and photographed. A dissecting microscope was used to search necrotic areas for fungal reproductive structures especially, conidia and spores. Small pieces of tissue (c. 3 × 3 mm) were cut from the edge of diseased areas and surface-disinfested. Disinfestation was achieved by immersion in 70% ethanol for 30 s, 2% sodium hypochlorite for 1 min and 70% ethanol again for 30 s, followed by rinsing in two washes of sterile reverse osmosis water. The tissue fragments were air-dried, and placed on potato dextrose agar (PDA; Difco Labs, Detroit, MI, USA) amended with 0.02% streptomycin (Sigma, St Louis, MI, USA), contained in 9-cm Petri dishes. Plates were sealed with parafilm, and incubated under near-ultraviolet and white light (12-hour photoperiod) at temperatures of 18 ± 2°C.

Fungal colonies that grew out of the tissue fragments were transferred to fresh plates of PDA. Each identified isolate was given a unique number. Isolates that produced spores were identified to species level where possible. Pure mycelial isolates had their ITS ribosomal gene sequenced, using a standard PCR protocol with the fungal-specific primers ITS1F and ITS4 (White et al. 1990; Gardes & Bruns 1993). All PCR products were sequenced in both directions using standard protocols established in the EcoGene® laboratory; and DNA sequences were assembled using Geneious v 10.1.3 (http://www.geneious.com; Kearse et al. 2012). Sequences were subjected to a GenBank BLASTn search (https://blast.ncbi.nlm.nih.gov/Blast.cgi; Altschul et al. 1990) to determine the closest sequence-based match. Species identifications were confirmed using spore or cultural morphology combined with the sequence data where possible.

Taxonomic literature and fungal systematists were consulted to determine which of the identified fungi were likely to be causing the damage, and which of the agents were associative saprotrophs, and/or endophytic organisms.

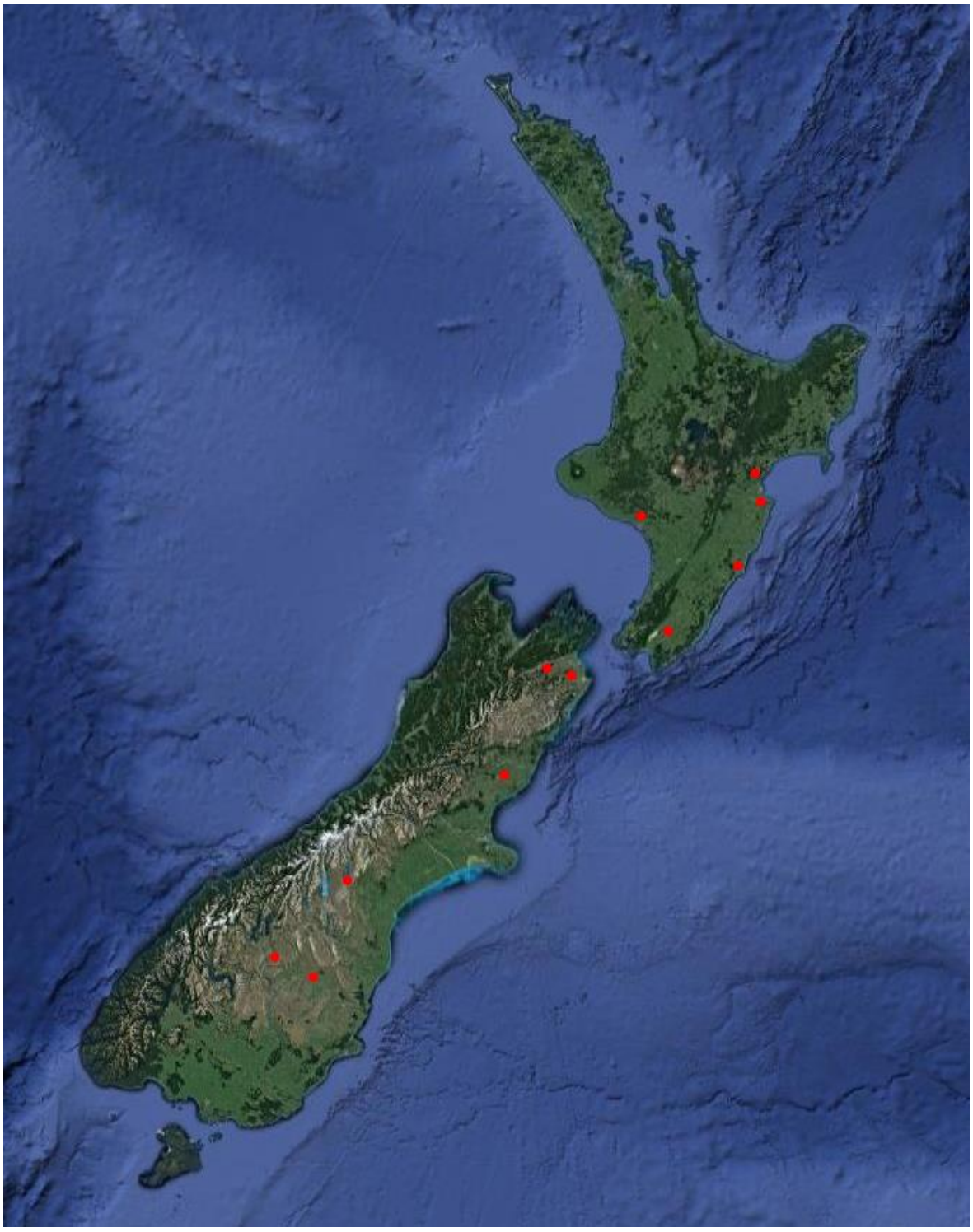

Figure 6 Map of the survey sites showing the extent of geographic spread.

Figure 7 Horehound burr clusters. Inset: close-up burr and seeds.

5 Results

5.1 Invertebrates

A total of 95 invertebrate species or taxonomic units were recorded during the survey, roughly classified as herbivores, predators, parasitoids or saprophytes/fungivores (Appendix 2).

Herbivores

A total of 39 herbivorous invertebrate species and taxonomic units (where identification to species level was not feasible) were recorded from horehound during this survey. One herbivorous species, the sage leafhopper (*Eupteryx melissae*), was classed as 'Abundant'. One herbivorous taxonomic unit, unidentified Dermaptera (earwigs), was classed as 'Common', 13 species or taxonomic units were 'Occasional' and 24 were 'Rare' (Appendix 2).

By far the most obvious damage to horehound was attributed to the sage leafhopper, *E. melissae* (Fig. 8), which is native to Europe and feeds on many herb species within the mint family (Lamiaceae), including many culinary herbs such as lavender, mint, sage, rosemary and thyme. *E. melissae* was present at every survey site. At some sites *E. melissae* was present in very large numbers (a total of 862 individuals were collected) and was causing very noticeable damage, particularly on the older leaves (Figs 9 and 10). Damage caused by all other herbivorous species combined was considered to be minimal.

Figure 8 Sage leafhopper, Eupteryx melissae (S.E. Thorpe – Public Domain Photograph).

Figure 9 Sage leafhopper feeding damage (small white spots/pale blotches).

Figure 10 Sage leafhopper damage (bleaching of foliage).

Apart from *E. melissae*, 13 other sap-feeding species, or groups of taxonomically related sap-feeding species, were collected from horehound during this survey. No other sap-feeders were classed as 'Abundant' or 'Common', seven were 'Occasional' and six were 'Rare' (Appendix 2).

Larvae from several lepidopteran families were collected from horehound during the survey but damage attributed to lepidopteran larvae was minimal. The two lepidopteran biocontrol agents currently being considered for release in New Zealand for horehound belong to the families Pterophoridae and Sesiidae, and no members of these families were found during the survey. A total of 11 Lepidopterous species were collected during the survey and 10 were classed as 'Rare' and one species, the silver Y moth (*Chrysodeixis eriosoma*), was classed as 'Occasional'. Four of the moth species were collected from the plant as adults, and may have been just visiting flowers for the nectar. For example, *Bedellia* sp. specializes on species of Convolvulaceae, and *Tebenna micalis* specializes on Asteraceae.

Eight species or taxonomic units of herbivorous adult beetles were collected during this survey but no foliage damage that could be attributed to beetles was observed. However, several beetle species may have been feeding on horehound. Fuller's rose weevil (*Asynonychus cervinus*) and the white-fringed weevil (*Naupactus leucoloma*) are relatively large weevils with wide host ranges. Similarly, the native bronze beetle, *Eucolaspis* sp., has a wide host range that could include horehound. Some of the other beetle species probably would not have fed on horehound but used the plant as shelter or as a source of nectar or pollen. For example, the weed biocontrol agent *Bruchidius villosus* (broom seed beetle) was classed as 'Occasional' during the survey and it is highly likely that the adult beetles were just using horehound flowers as a source of pollen.

At each survey site, 10 plants were dug up and their roots cut open lengthwise in search of root feeders, but none were found. This is significant as the larvae of one of the horehound candidate biocontrol agents, the clearwing moth, *Chamaesphecia mysiniformis*, attacks the roots.

If burr clusters were present, approximately 100 burr clusters were collected from each site and stored in ventilated rearing containers for several months. This was to identify any seed-feeding agents that may be utilizing horehound, but none were found. With regard to flower-feeding agents, apart from visiting pollinators (e.g. honey bees), very few invertebrates were observed to be associated with the flowers.

Garden snails (*Cantareus asperses*) were classed as 'Rare' and found at only one site during the survey and no evidence of mollusc 'slime trails' was recorded. It is likely that most places where horehound thrives are not very suitable snail or slug habitat. Earwigs (order Dermaptera), are omnivorous, and can cause damage to living plant tissue. Unidentified earwig species were classed as 'common' on horehound and the European earwig, *Forficula auricularia*, was also found but classed as 'Rare'. Slaters (order Isopoda) were classed as 'Occasional', and may have been causing some damage to living tissue, but are likely to have been feeding mostly on dead and decaying material.

One herbivorous mite species, *Tetranychus ludeni*, was identified from horehound during the survey but any mite damage may possibly be obscured by sage leafhopper damage.

Parasitoids

Parasitoids and predators were recorded to help identify factors that may inhibit introduced biological control agents. The two candidate biocontrol agents currently being considered for

introduction into New Zealand are both Lepidopterans so Lepidopteran parasitoids are of particular interest. Only two parasitoids were reared from hosts during the survey. One parasitoid, *Trigonospila brevifacies* (Diptera: Tachinidae), was reared from a Lepidopteran (Tortricidae) larva and the other parasitoid (Braconidae – Aphidiinae) was reared from an aphid. *T. brevifacies* was introduced to New Zealand as a biocontrol agent for the light brown apple moth, *Epiphyas postvittana* (Walker) (Munro 1998). This parasitoid is polyphagous and known to parasitise at least 18 species of Lepidoptera in 8 families (Wilkipedia 2018) including Pterophoridae, which is the family to which one of the candidate biocontrol agents (*Wheeleria spilodactylus*) belongs.

All the other parasitoids recorded during the survey were collected directly from the plants by beating, and none of them are Lepidopteran parasitoids: Diapriidae (parasitoid of Dipterans); Megaspilidae (parasitoid of hemipteran eggs); Braconidae – Alysiinae (parasitoid of Dipterans); Figitidae – *Anacharis zealandica* (parasitoid of Tasmanian lacewing – *Micromus tasmaniae*); Figitidae – *Phaenoglyphis villosa* (hyperparasitoid of aphidiine species); Braconidae – *Dinocampus coccinellae* (parasitoid of coccinellids).

Predators

Twenty–three species of predatory invertebrates or taxonomic units were recorded on horehound during this survey. One taxonomic grouping, spiders, was collectively classed as 'Abundant'; two predatory species or taxonomic units, *Anystis* sp. (whirlygig mites) and *Micromus tasmaniae* (Tasmanian lacewing), were classed as 'Common'; seven species were 'occasional'; and 13 were 'rare' (Appendix 2).

A very interesting mite found during the survey was a species called *Eharius chergui* (Acari: Phytoseiidae) (Athias-Henriot, C. 1960a). This was the first record of this species in New Zealand and this finding has been reported to MPI. This species was described from Algeria on *Marrubium vulgare* and it has mainly been found on plant species of the family Lamiaceae (Tixier M, Allam L, Douini M, Kreiter S 2016). Very little is known of the biology of *E. chergui*. However, the diversity of life-styles in the family Phytoseiidae have been categorized into four basic types (McMurtry & Croft 1997) and all four types involve predation to some degree, but may also include feeding on pollen, plant exudates and/or honeydew.

Saprophytic and fungivorous invertebrates

A number of saprophytic and fungivorous invertebrates were recorded during this survey (Appendix 2), and they would most probably have been associated with decaying material and not damaging living plant material.

5.2 Plant Pathogens

Very few symptoms caused by pathogens on horehound were observed in the field. These symptoms consisted of leaf spots, marginal leaf necrosis, leaf yellowing, wilting and tip dieback (Fig. 11).

From a total of pure 122 fungal cultures recovered from symptomatic tissues, 29 species were identified, belonging to 17 genera (Appendix 3). Several species were only identified to genus.

The predominant genera isolated were *Phoma* and *Alternaria.* Only species belonging to the genera *Phoma*, *Alternaria*, *Fusarium*, *Colletotrichum*, *Diaporthe*, *Stemphylium*, *Leptosphaeria* and *Plectosphaerella* sp. were considered as potential primary pathogens; the remaining species identified were considered either/or saprophytes, endophytes or secondary pathogens.

Phoma spp. are usually considered to be saprophytes or weak wound pathogens, often isolated from dead or dying plants in New Zealand (Johnston 1981). *Boeremia exigua* was recovered from wilting horehound tissue and dieback. This pathogen is known to cause leaf spots on a wide range of hosts, root rot and damping off (Plantwise 2018a). However, it is believed to be only a weak wound pathogen (Johnston 1981).

Alternaria spp. commonly attack aerial tissues but can also cause stem lesions, fruit lesions, damping off, and collar rot (Laemmlen 2001). The *Alternaria* spp. recovered from horehound were mainly isolated from leaf material, and are likely to be responsible for leaf spots and marginal leaf necrosis.

Fusarium spp. have been associated with stem base diseases, dieback, root necrosis, rot, blight, foliar yellowing and wilt. Three *Fusarium* spp. isolated from horehound were likely to have caused tip dieback; however, they are known to have a wide host range, with *Gibberella avenacea* causing blight on many crops including cereals, maize, carrots and potatoes (Plantwise 2018b), and *Gibberella baccata* causing twig and trunk canker on *Citrus* trees (Holliday 1980). *Gibberella acuminata* is considered as a weak pathogen which can occasionally cause disease (Pitt & Hocking 2009).

Diaporthe spp. have been reported as pathogens where they are responsible for root rot, dieback, canker, leaf spot, blight, fruit rot and wilt but also as endophytes and saprophytes on a wide range of hosts (Gomes et al. 2013).

*Colletotrichum s*pp. can cause stem, leaf and fruit spots on a wide host range (Plantwise 2018c). *Colletotrichum destructivum* is a known pathogen of forage and grain legumes (Damm et al. 2014), while *Colletotrichum boninense* causes fruit and leaf anthracnose on a wide range of plants (Damm et al. 2012). These species were recovered from marginal leaf necrosis and leaf yellowing on horehound.

A *Leptosphaeria* species was recovered only once from marginal leaf necrosis of horehound. Species from this genus can cause lesions on leaves, stems and seed pods (e.g. *Leptosphaeria maculans* on *Brassica*; Howlett et al. 2001).

A *Stemphylium* species was recovered from marginal leaf necrosis. Species from this genus can cause leaf spots and leaf blight on several hosts (e.g. *Stemphylium vesicarium* (Plantwise 2017); *Stemphylium solani* (Cerkauskas 2005)).

Plectosphaerella species have been associated with root and collar rots of a range of horticultural crops (e.g. melon, tomato, bell pepper, cucumber (Carlucci et al. 2012)). We used

only ITS and more gene loci are needed to be able to determine the species affinity of this isolate.

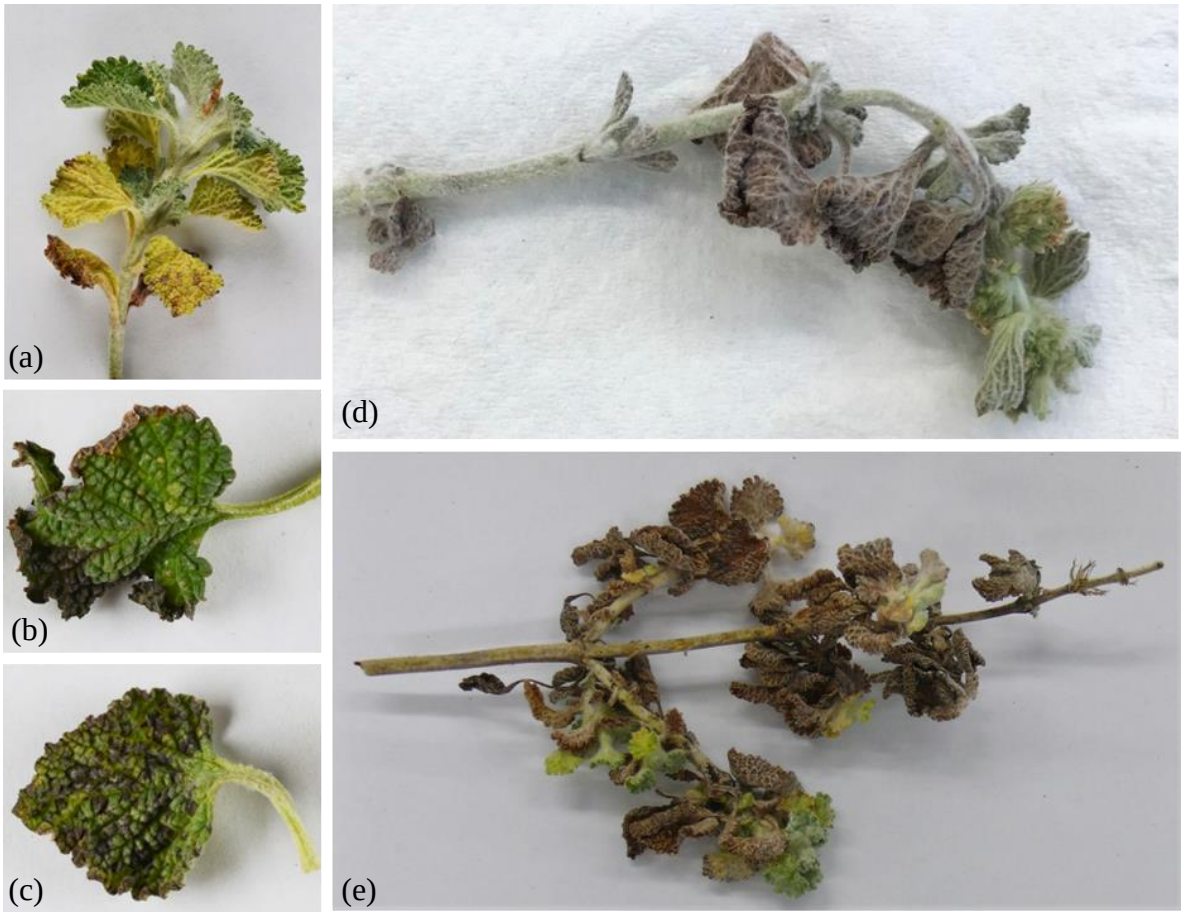

Figure 11 Horehound (Marrubium vulgare) pathogen damage symptoms: (a) leaf yellowing, (b) leaf marginal necrosis, (c) leaf spots, (d) wilting, and (e) tip dieback.

6 Conclusions and Discussion

6.1 Invertebrates

Herbivores

A range of native and introduced invertebrates are associated with horehound in New Zealand but no specialised horehound-feeding invertebrates were recorded in the New Zealand survey. Most herbivorous invertebrates recorded in our survey occurred in small numbers and the damage they caused to horehound was considered minimal. The one notable exception was the sage leafhopper, *Eupteryx melissae*.

The sage leafhopper was present in very large numbers at some survey sites and was causing obvious 'bleaching' of the foliage, particularly on the older leaves, with the shoot tip and younger leaves often appearing to be relatively unscathed. However, the effect of the

damage caused by a sap-feeding insect such as the sage leafhopper, either directly by removal of nutrients or indirectly by facilitating the entry of pathogens through puncturing the plant, can be difficult to quantify.

Damage to horehound that could be attributed to Lepidopteran larvae was minimal. The two candidate biocontrol agents currently being considered for introduction into New Zealand are both Lepidopterans. They are unlikely to meet any significant competition from other Lepidopterans in New Zealand, and they could complement the damage already being caused by the sage leafhopper.

Horehound plume moth larvae begin feeding in the developing shoot tip. When they have grown larger they move out and feed on the leaves, working their way down the shoot, progressively defoliating the stem, until ready to pupate (Agriculture Victoria 2017). The horehound clearwing moth has a root boring larva which reduces the flow of water and nutrients through the plant, reducing growth and increasing plant mortality (Agriculture Victoria 2017).

The three invertebrates, the plume moth, the clearwing moth and the sage leafhopper should complement each other by attacking different parts of the plant. However, note that the sage leafhopper, *Eupteryx melissae*, feeds on many valued herb species in the mint family (Lamiaceae) and therefore it should not be deliberately spread or encouraged.

Predators and Parasitoids

The combined effect of generalist predators found during the survey, and especially spiders that were collectively classed as 'Abundant', could potentially inhibit the effectiveness of the two moth species currently being considered for introduction as biocontrol agents into New Zealand for horehound. The very large numbers of sage leafhoppers could possibly boost the populations of generalist predators. Lepidopteran parasitoids, such as *Trigonospila brevifacies* which was reared from a Lepidopteran larva during this survey, could possibly affect lepidopteran biocontrol agents. However, *T. brevifacies* is native to Australia where the horehound plume moth is described as 'very common and performing well in areas where horehound rarely experiences extremes of summer temperature' (Weiss & Sagliocco 2012) so it doesn't appear to be badly affected by parasitism there. The horehound plume moth is possibly more vulnerable to parasitoid attack than the clearwing moth whose larvae bore into the roots of the plant (Hill & Hulley 1995).

Pathogens

Cullen (1995) and others have suggested that to predict the effectiveness of a weed control agent, both the specificity of the agent and its ability to damage the weed, need to be considered. According to these two criteria, none of the primary plant pathogens found to be associated with disease symptoms of horehound are host-specific, and none had any major impact. The symptoms observed in the field were mainly superficial without any chronic disease symptoms observed killing whole plants.

The main primary pathogens identified associated with horehound, have a broad host-range, including important horticultural species. As such, none of the plant pathogens can be recommended for further testing as biological controls for horehound.

7 Recommendations

In light of our conclusions that:

1 No specialised horehound-feeding invertebrates and no specialised pathogenic fungi have been recorded in New Zealand and;

2 the two candidate biocontrol agents currently being considered for introduction into New Zealand, the horehound plume moth (*Wheeleria spilodactylus*) and the horehound clearwing moth (*Chamaesphecia mysiniformis*), are unlikely to meet with significant levels of competition from other herbivorous species currently in New Zealand;

we recommend that a classical biological control programme for horehound should proceed.

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Appendix 1 – Horehound survey (New Zealand 2017–2018) site details

Site no.	Site name	Collection date	GPS Coordinates (NZTM)
1	Eskdale	21.11.17	1928377E 5635075N
2	Te Mata	18.4.18	1933628E 5596994N
3	Wanganui	23.11.17	1776410E 5583945N
4	Herbertville	17.4.18	1900466E 5514924N
5a	Martinborough (Spring)	22.11.17	1806711E 5433760N
5b	Martinborough (Autumn)	16.4.18	1806711E 5433760N
6	Waihopai Valley	22.4.18	1649610E 5387570N
7	Breach Oak	7.4.18	1682102E 5379545N
8	Inverlock	6.4.18	1596416E 5252578N
9	Sawdon Station	1.11.17	1401916E 5120203N
10	Ardgour Station	31.10.17	1316930E 5021971N
11	Armidale	29.3.18	1363555E 4998490N

Note: one site, Martinborough (5a and 5b), was sampled on two occasions – in spring 2017 and autumn 2018.

Appendix 2 – Invertebrates associated with horehound, *Marrubium vulgare* L., at eleven New Zealand sites (2017–2018)

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Phylum Mollusca	**Molluscs**			
Class Gastropoda	**Slugs and snails**			
Cantareus asperses Müller	Brown garden snail	Herbivorous	Rare (4)	4
Phylum Arthropoda				
Class Crustacea				
Isopoda	Slaters			
Unidentified Isopoda		Saprophytic	Occasional (5)	3,4
Class Arachnida				
Acarina	Mites and ticks		z	
Anystidae				
Anystis sp.	whirlygig mite	Predatory	Common (67)	1,2,4,5b,7,8
Erythraeidae				
Balaustium sp.		Predatory	Occasional (50+)	1
Oribatidae	Beetle mites			
Unidentified Oribatida		Saprophytic/ fungivorous	Occasional (10)	1,2,5a
Parasitidae				
Pergamasus sp.		Predatory	Occasional (50+)	1
Phytoseiidae				
Eharius chergui Athias-Henriot		Predatory	Occasional (50+)	2
Phytoseiulus persimilis Athias-Henriot		Predatory	Occasional (30+)	2
Tetranychidae	Spider mites			
Tetranychus ludeni Zacher		Herbivorous	Occasional (100+)	2
Araneida	Spiders			
Unidentified Araneida		Predatory	Abundant (199)	1,2,3,4,5a,5b ,7,8,9,1011
Opiliones	Harvestmen			
Unidentified Opiliones		Predatory	Rare (1)	7
Pseudoscorpiones	Pseudoscorpions			
Unidentified Pseudoscorpiones		Predatory	Rare (1)	3
Class Collembola				
Unidentified Collembola	Springtails	Saprophytic	Occasional (11)	5a, 8, 11
Class Insecta	**Insects**			
Blattodea	Cockroaches			

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Celatoblatta sp.		Saprophytic	Rare (1)	5b
Coleoptera	Beetles			
Anthicidae	Ant beetles			
Anthicus glaber King		Saprophytic	Occasional (5)	3
Anthribidae	Fungus weevils			
Sharpius brouni (Sharp)		Fungivorous	Rare (2)	3
Cerambycidae	Longhorn beetles			
Zorion sp.	Flower longhorn	Adults: pollen	Rare (1)	1
Chrysomelidae				
Bruchidius villosus (Fabricius)	Broom seed beetle	Herbivorous (adults pollen)	Occasional (14)	1
Eucolaspis sp.	Bronze beetle	Herbivorous	Rare (2)	5a
Longitarsus fuliginosus (Broun)		Herbivorous	Rare (1)	5a
Coccinellidae	Ladybirds			
Coccinella undecimpunctata Linnaeus	Eleven-spotted ladybird	Predatory	Occasional (17)	7,8,9,11
Coccinella leonina Fabricius	Orange-spotted ladybird	Predatory	Rare (4)	7,9
Stethorus sp.	Spidermite ladybird	Predatory	Rare (4)	1
Corylophidae	Hooded beetles			
Sericoderus sp.		Fungivorous	Occasional (7)	1,4,5b
Cryptophagidae	Cryptic beetles			
Paratomaria sp.		Pollen/fungus feeder	Common (73)	1,3,5a,7,9
Curculionidae	Weevils			
Asynonychus cervinus Boheman	Fuller's rose weevil	Herbivorous	Occasional (19)	2,4,5b,7
Mecinus pascuorum (Gyllenhal)	Plantain weevil	Herbivorous	Rare (1)	3
Irenimus sp.		Herbivorous	Rare (1)	5a
Naupactus leucoloma Boheman	White-fringed weevil	Herbivorous	Rare (1)	3
Sitona lepidus Gyllenhal	Clover root weevil	Herbivorous	Occasional (12)	7
Dermestidae	Hide beetles			
Unidentified Dermestidae		Scavanger	Rare (2)	1
Latridiidae	Mildew beetles			
Aridius bifasciatus (Reitter)		Fungivorous	Rare (4)	1,7
Cortinicara sp.		Fungivorous	Common (32)	1,2,3,4,5a,5b ,7,8,11
Melyridae	Flower beetles			
Unidentified Melyridae		Predatory	Rare (3)	1
Mycetophagidae	Fungus beetles			
Litargus vestitus (Sharp)		Fungivorous	Rare (3)	1,2,3

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Nitidulidae	Sap beetles			
Unidentified Nitidulidae		Unknown	Rare (1)	5a
Salpingidae	Bark mould beetles			
Salpingus bilunatus Pascoe		Fungivorous	Rare (1)	5b
Dermaptera	Earwigs			
Forficula auricularia Linnaeus	European earwig	Omnivorous	Rare (3)	1,7
Unidentified Dermaptera		Omnivorous	Common (22)	1,2,3,4,5a
Diptera	Flies			
Tachinidae	Bristle flies			
Trigonospila brevifacies (Hardy) (reared from a Tortricid larva)		Parasitoid (of Lepidoptera)	Rare (1)	4
Unidentified Diptera		Unknown	Common (16)	1,3,5a,6,9,10
Hemiptera	Bugs			
Anthocoridae	Minute pirate bugs			
Unidentified Anthocoridae		Predatory	Rare (2)	1,3
Aphididae	Aphids			
Unidentified Aphididae		Sap feeder	Rare (2)	3,7
Aphrophoridae	Spittle bugs			
Philaenus spumarius (Linnaeus)	Meadow spittlebug	Sap feeder	Occasional (7)	1,3,6,7
Unidentified juvenile Aphrophoridae		Sap feeder	Occasional (14)	1,3,10
Cicadellidae	Leafhoppers			
Anzygina sp.		Sap feeder	(Rare) 3	4
Eupteryx melissae Curtis	Sage leafopper	Sap feeder	Abundant (862)	1,2,3,4,5a,5b,6,7,8,9,10,11
Cixiidae				
Oliarus oppositus (Walker)		Sap feeder	Occasional (5)	1,3
Lygaeidae	Seed bugs			
Nysius huttoni White	Wheat bug	Sap/seed feeder	Occasional (29)	5b,8,9,11
Rhypodes sp.		Sap/seed feeder	Rare (2)	5a,11
Margarodidae				
Icerya purchasi Maskell	Cottony cushion scale	Sap feeder	Rare (1)	7
Miridae	Mirid bugs			
Chinamiris sp.		Sap feeder	Occasional (5)	5a,5b
Closterotomus norwegicus (Gmelin)	Potato capsid	Sap feeder	Rare (2)	5a
Sejanus albisignatus (Knight)		Predatory	Rare (4)	5a
Unidentified juvenile Miridae		Unknown	Occasional (10)	1
Nabidae	Damsel bugs			

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Unidentified nabidae			Occasional (13)	4,5b,7,8
Pentatomidae	Shield bugs			
Cuspicona simplex Walker	Green potato bug	Sap feeder	Rare (1)	3
Dictyotus caenosus (Westwood)	Brown shield bug	Sap feeder	Occasional (10)	7,9,10,11
Nezara viridula (Linnaeus)	Green vegetable bug	Sap feeder	Occasional (12)	1,2,6,7
Oechalia schellenbergii (Guérin)	Schellenberg's soldier bug	Predatory	Rare (1)	5a
juvenile Pentatomidae		Unknown	Occasional (17)	5b,9
Unidentified Hemipteran juveniles		Unknown	Occasional (14)	3,9
Hymenoptera	Bees, wasps, ants			
Apidae	Social bees			
Apis mellifera Linnaeus	Honey bee	Pollen/nectar feeder	Rare (1)	1
Bombus sp.	Bumble bee	Pollen/nectar feeder	Rare (2)	1,7
Braconidae	Parasitic wasps			
Dinocampus coccinellae (Schrank)		Parasitoid (of coccinellidae)	Rare (1)	9
Alysiinae				
Unidentified Alysiinae		Parasitoid (of diptera)	Rare (2)	5a
Aphidiinae				
Unidentified Aphidiinae		Parasitoid (of aphids)	Rare (2)	7
Diapriidae				
Unidentified Diapriidae		Parasitoid (of diptera)	Rare (3)	1
Figitidae				
Anacharis zealandica Ashmead		Parasitoid (of *Micromus tasmaniae*)	Rare (1)	5a
Phaenoglyphis villosa (Hartig)		Parasitoid (hyperparasitoid of aphidiine spp.)	Rare (1)	5a
Formicidae	Ants			
Monomorium antarcticum (F. Smith)	Southern ant	Omnivorous	Rare (1)	3
Ochetellus glaber (Mayr)		Omnivorous	Rare (2)	2
Nylanderia sp.	Garden ant	Omnivorous	Occasional (12)	1,5a
Pheidole rugosula Forel		Omnivorous	Rare (2)	1
Tetramorium grassii Emery		Omnivorous	Occasional (6)	1,5a
Megaspilidae				

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Unidentified Megaspilidae		Parasitoid (of hemipteran eggs)	Rare (2)	2
Unidentified Hymenoptera		Unknown	Rare (3)	7
Lepidoptera	Moths and butterflies			
Bedelliidae				
Bedellia sp. (adult collected)		Herbivorous (on Convolvulaceae)	Rare (1)	10
Choreutidae				
Tebenna micalis (Mann) (adult collected)	Small thistle moth	Herbivorous (on Asteraceae)	Rare (1)	7
Crambidae				
Orocrambus sp. (adult collected)		Herbivorous	Rare (1)	11
Gelechioidea				
Unidentified Gelechioidea (adult collected)		Herbivorous	Rare (1)	9
Geometridae	Looper moths			
Unidentified Geometridae (larva collected)		Herbivorous	Rare (3)	1,5a,5b
Noctuidae	Armyworms, cutworms			
Chrysodeixis eriosoma (Doubleday) (larva collected)	Green looper or silver Y moth	Herbivorous	Occasional (7)	1,2,4,5a,5b
Leucania stenographa Lower (larva collected)	Sugarcane Armyworm	Herbivorous	Rare (1)	7
Thysanoplusia orichalcea (Fabricius) (larva collected)	Burnished brass moth	Herbivorous	Rare (1)	4
Unidentified noctuidae (larva collected)		Herbivorous	Rare (1)	8
Tortricidae	Leafroller moths			
Merophyas sp. (larva collected)		Herbivorous	Rare (1)	7
Unidentified Tortricidae (larva collected)		Herbivorous	Rare (2)	4
Mantodea	Praying mantids			
Unidentified juvenile Mantodea		Predatory	Rare (1)	10
Neuroptera	Lacewings			
Micromus tasmaniae (Walker)	Tasmanian lacewing	Predatory	Common (49)	1,3,4,5a,5b
Psectra nakaharai New		Predatory	Rare (1)	3
Psocoptera	Book lice			
Unidentified Psocoptera	Saprophytic/Fungivorous			2,5b,11
Orthoptera	Grasshoppers, crickets, weta			

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Tettigoniidae	Long-horned grasshoppers			
Conocephalus sp.	Field grasshopper	Herbivorous	Rare (1)	4
Thysanoptera	Thrips			
Thripidae				
Thrips obscuratus (Crawford)	New Zealand flower thrips	Herbivorous	Occasional (10)	3,4,5a
Unidentified Thysanoptera		Unknown	Common (63)	2,3,4,5a,5b,6,7,8,9

[1]Abundance categories: **Rare**: fewer than 5 individuals collected in total; **Occasional**: 5–15 individuals collected, or present at fewer than 5 sites; **Common**: 16–99 individuals collected and present at 5 or more sites; **Abundant**: 100+ individuals collected and present at 7 or more sites

[2]Site codes are detailed in Appendix 1 above

Appendix 3 – Fungal species isolated from symptomatic horehound tissues in New Zealand (2017–2018)

Species recovered	Symptoms associated	Plant material isolated from	Location
Phoma Sacc.	marginal leaf necrosis, leaf yellowing, leaf spots, wilting, tip dieback, lesions	leaves, petioles, stems	Armidale station, Martinborough, Whanganui, Inverlock station, Breach oak station, Herbertville, Tekapo, Eksdale, Te Mata, Waihopai Valley
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley (= *Phoma exigua* Desm.)	wilting, dieback	roots, leaves, stems	Martinborough, Waihopai valley
Alternaria Nees sp. 1	marginal leaf necrosis, leaf spots, leaf yellowing, tip dieback	leaves, stems	Breach oak station, Martinborough, Eksdale, Waihopai Valley
Alternaria Nees sp. 2	marginal leaf necrosis	leaves, petioles	Inverlock station
Lewia infectoria (Fuckel) M.E. Barr & E.G. Simmons (= *Alternaria infectoria* E.G. Simmons)	marginal leaf necrosis	leaves	Inverlock station
Gibberella avenacea R.J. Cook (=*Fusarium avenaceum* (Fr.) Sacc.)	tip dieback	stems	Te Mata, Martinborough
Gibberella baccata (Wallr.) Sacc. (= *Fusarium lateritium* Nees)	tip dieback, leaf spots, leaf yellowing	stem, leaves	Herbertville

Gibberella acuminata Wollenw. (= *Fusarium acuminatum* Ellis & Everh.)	marginal leaf necrosis, wilting	leaves, roots	Amidale station, Martinborough
Fusarium Link	leaf spots	leaves	Herbertville
Diaporthe Nitschke sp. 1	marginal leaf necrosis, leaf yellowing, tip dieback	leaves, stems	Martinborough, Whanganui, Breach oak station, Te Mata
Diaporthe Nitschke sp. 2	leaf yellowing	leaves	Inverlock station
Colletotrichum boninense Moriwaki, Toy. Sato & Tsukib.	marginal leaf necrosis	leaves	Martinborough
Colletotrichum destructivum O'Gara	marginal leaf necrosis	leaves	Martinborough, Whanganui
Colletotrichum Corda	leaf yellowing	leaves	Herbertville
Leptosphaeria Ces. & De Not. sp. 1	marginal leaf necrosis	stems	Armidale station
Stemphylium Wallr.	marginal leaf necrosis	Leaves	Whanganui, Martinborough
Epicoccum purpurascens Ehrenb. (= *Epicoccum nigrum* Link)	marginal leaf necrosis, leaf yellowing, leaf spots, tip dieback	leaves, stems	Armidale station, Martinborough, Breach oak station, Tekapo, Te Mata, Herberville, Waihopai valley
Epicoccum plurivorum (P.R. Johnston) Q. Chen & L. Cai	leaf spots, tip dieback	leaves, stems	Herbertville
Preussia Fuckel	marginal leaf necrosis	leaves	Inverlock station
Sporormiella australis (Speg.) S.I. Ahmed & Cain (= *Preussia australis* (Speg.) Arx)	leaf spots, wilting	leaves	Inverlock station
Sordaria Ces. & De Not. sp. 1	tip dieback	stems	Te Mata, Herbertville
Sordaria Ces. & De Not. sp. 2	tip dieback	stems	Te Mata
Xylaria Hill ex Schrank	leaf yellowing	leaves	Inverlock station
Plectosphaerella Kleb.	wilting	roots	Martinborough
Trichoderma Pers.	marginal leaf necrosis	leaves	Martinborough
Cercophora Fuckel	wilting	roots	Martinborough
Penicillium Link	marginal Leaf Necrosis	leaves	Whanganui
Cladosporium Link	dieback, marginal leaf necrosis	leaves, stems	Waihopai valley, Whanganui, Martinborough, Eskdale
Periconia Tode	dieback	leaves, stems	Martinborough, Whanganui, Waihopai valley