

Feasibility for biological control of horehound, *Marrubium vulgare* L.

Feasibility for biological control of horehound,	Marrubium
<i>vulgare</i> L.	

Ronny Groenteman	, Chantal	Probst, Stanle	ey Bellg	gard, Jess	ie Prek	bl	le
------------------	-----------	----------------	----------	------------	---------	----	----

Manaaki Whenua – Landcare Research

Prepared for:

Horehound Biocontrol Group

Sawdon Station PO Box 9 Lake Tekapo 7945

November 2017

Manaaki Whenua – Landcare Research, 54 Gerald Street, PO Box 69040, Lincoln 7640, New Zealand, Ph +64 3 321 9999, Fax +64 3 321 9998, www.landcareresearch.co.nz

Reviewed by:	Approved for release by:
Dead Determine	Chair Lance
Paul Peterson	Chris Jones
Research Technician	Portfolio Leader – Managing Invasives
Manaaki Whenua – Landcare Research	Landcare Research
Contract Report:	LC3040

Disclaimer

This report has been prepared by Manaaki Whenua – Landcare Research for Horehound Biocontrol Group. If used by other parties, no warranty or representation is given as to its accuracy and no liability is accepted for loss or damage arising directly or indirectly from reliance on the information in it.

Contents

Sum	nmary .		۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰۰
1	Intro	duction	1
2	Back	ground	1
	2.1	Global distribution and biology of <i>Marrubium vulgare</i>	
	2.2	Distribution and pest status in New Zealand	3
	2.3	Beneficial uses	6
	2.4	Phylogeny	8
	2.5	Potential for opposition to biocontrol	11
	2.6	Control options	11
	2.7	Potential advantages and disadvantages of biological control	12
	2.8	Predicting establishment of biocontrol agents	13
	2.9	Predicting impact of biocontrol agents	14
	2.10	Biological control of <i>M. vulgare</i> in Australia	15
3	Obje	ectives	16
4	Metl	nods	17
	4.1	Identifying fungal pathogens of Marrubium vulgare	17
	4.2	Identifying arthropod biocontrol agents for Marrubium vulgare	17
5	Resu	lts	18
	5.1	Pathogens attacking <i>Marrubium vulgare</i>	18
	5.2	Arthropods attacking Marrubium vulgare	19
6	Con	clusions	20
	6.1	Prospects for achieving biological control of <i>Marrubium vulgare</i> in New Zealand	20
7	Reco	ommendations	21
8	Ackr	owledgements	22
9	Refe	rences	22
App	endix	1 – Pharmacological uses of <i>M. vulgare</i> vs. evidence to support these uses	37
		2 – Letters of opposition to the biocontrol programme against <i>M. vulgare</i>	
		3 – Records of pathogens associated with <i>Marrubium vulgare</i>	
App	endix	4 – Records of invertebrates feeding on <i>Marrubium vulgare</i>	54
App	endix	5 – Steps in a biocontrol project	70

Summary

Project and Client

 The prospects of developing a biocontrol programme against horehound, Marrubium vulgare L., in New Zealand were assessed by Landcare Research for the Horehound Biocontrol Group.

Objectives

- Review the literature to identify potential biocontrol agents for *M. vulgare* and assess the feasibility of their release in New Zealand.
- Assess the prospects of achieving successful biological control of *M. vulgare* in New Zealand.
- Estimate the cost of the programme for biological control of *M. vulgare* in New Zealand.

Results

- Horehound, Marrubium vulgare, is an aromatic herb native to temperate Eurasia, Europe, the Middle East and the Mediterranean region, including North Africa. It is naturalised in Australia, Japan, the United States, South America, and New Zealand.
- *Marrubium vulgare* is widespread in low rainfall parts of New Zealand, especially in the high country of the South Island, and is a growing problem in lucerne fields.
- Marrubium vulgare is not palatable to stock. It taints the meat of animals that are
 forced to graze on it, it displaces lucerne and other pasture species, it downgrades
 wool quality, and it is difficult to control chemically and mechanically. Negative
 economic impacts to farmers are currently estimated at \$29m to \$39m annually.
- Biological control of *M. vulgare* has been successful in Australia.
- *Marrubium vulgare* is tolerant of drought, and benefits from low temperatures for seed germination. It relies on seeds for reproduction.
- Beneficial uses of *M. vulgare* are mainly as a medicinal herb, for which it is valued in New Zealand. The plant has also been studied for its potential to rehabilitate soils from salinity and heavy metal contamination, as a pesticide, as an improver for cereal crops, as a food preservative, as an ingredient in beer brewing, and for inhibiting corrosion.
- Popular culinary and medicinal herbs in the mint family are grown/wild-harvested in New Zealand. Sufficiently host specific agents have been found to restrict any damage to *M. vulgare*, but there may still be some opposition from growers, nurseries, and home-gardeners to biocontrol of this species. Medical herbalists wishing to continue to harvest *M. vulgare* could protect the plant either chemically or by IPM methods. The value of *M. vulgare* to New Zealand is yet to be determined, and a cost-benefit analysis would be useful to inform the existing conflicts with herbalists.

- *Marrubium vulgare* is in the mint family (Lamiaceae). The family is represented in New Zealand by five plant species in five genera, all relatively distant from *Marrubium*. Two species are endemic and their conservation status is either declining or threatened. The other three species are indigenous but non-endemic, and are not threatened.
- Marrubium vulgare makes a good target in terms of predicting biocontrol impact and is expected to be a relatively inexpensive target, given that a biocontrol programme would build on the successful programme in Australia. For such projects the cost of developing biocontrol agents is historically on average \$203,000 per agent, and generally 2–3 agents are needed to provide control.
- Detailed surveys of the natural enemies of *M. vulgare* in its native range recorded a large number of host-specific damaging candidate arthropod agents. Two of those have been established in Australia and are contributing to control.

Conclusions & Recommendations

Biological control is a prudent and viable option to manage populations of *Marrubium vulgare*. The extensive work done in Australia and the resulting successful suppression of *M. vulgare* there provides a head-start for a New Zealand programme, and potential for significant savings in developing agents for this country. Open and ongoing consultation with medical herbalists should offer viable options for mitigating herbalists' concerns.

- Undertake a survey of the natural enemies of *M. vulgare* in New Zealand and look for any potential biocontrol agents and any other species living on the plant (such as predators) that might interfere with potential biocontrol agents. Note this baseline information is vital for any subsequent application to release new biocontrol agents. Estimated cost \$50,000–\$60,000.
- Undertake DNA studies to identify whether the geographic source of *M. vulgare* in New Zealand and in Australia is similar. Estimated cost \$20,000–\$50,000.
- Analyse costs of and benefits expected from biological control in comparison to conventional control of *M. vulgare* in New Zealand. While initial information has been gathered, a clear and strong case will be important for any application to release new biocontrol agents. Estimated cost \$15,000–\$25,000.
- If required, undertake host-range testing of potential agents. Estimated cost \$60,000– \$100,000 per agent.
- If testing shows agents are suitable, apply to release them in New Zealand. Estimated cost \$50,000–\$70,000 for one or more species.
- Import into containment and gain permission to remove. Estimated cost \$25,000-\$50,000 per species.
- Mass-rear and release agents. Estimated cost \$50,000–\$100,000 per species.

Note: Estimated costs are exclusive of GST and are based on 2016/17 figures. New estimates will need to be provided if work is to be undertaken beyond those dates, and/or if complicating factors arise (e.g. disease infecting imported agents).

Page vi Landcare Research

1 Introduction

The prospects of developing a biocontrol programme against horehound, *Marrubium vulgare* L., in New Zealand were assessed by Landcare Research for the Horehound Biocontrol Group.

2 Background

2.1 Global distribution and biology of *Marrubium vulgare*

Marrubium vulgare L. 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 widespread in the Mediterranean areas of Europe and North Africa (Rodriguez Villanueva & Martin Esteban 2016; Fig. 1). It is a weed in southern parts of North America (California, Texas), in South America (Argentina, Chile, Peru, Uruguay), in Australia and in New Zealand (Weiss & Sagliocco 2000, 2012), and was introduced to Brazil during European colonisation (Schlemper et al. 1996). It was introduced to Japan, but not recorded as a weed there. In parts of its native range *M. vulgare* is cultivated (Egypt: Sabry et al. 2012; Poland: Zawislak 2012; Morocco: Ben Hmamou et al. 2013).



Figure 1: Global distribution of Marrubium vulgare (from GBIF https://www.gbif.org/species/2927069).

Marrubium vulgare L. is an erect perennial herb in the mint family, Lamiaceae, 0.3–0.75 m high and wide, with up to 200 stems. The heart-shaped and wavy-edged leaves are silvery-green, arranged in opposite pairs, are wrinkled on the upper surface, and densely covered with short, white globular hairs on the lower surface (Fig. 2). The flowers are small and white, and come in dense clusters. The fruit form brown burrs with small hooked spines. Each burr contains up to four spear-shaped seeds, 1–2 mm long. M. vulgare reproduces from seeds, and one plant can produce tens of thousands of seeds.



Figure 2: A young M. vulgare plant.

In its southern European native range populations of *M. vulgare* are scattered and low-density, occurring in warm areas, on wasteland, on well-drained calcareous soils, and on intensely grazed sites (Weiss & Sagliocco 2000; Rodriguez Villanueva & Martin Esteban 2016). In India it grows at an altitude between 5,000 and 8,000 ft (1,500–2,500 m), and can grow up to 120 cm tall (Masoodi et al. 2008). The plant shows plasticity in adaptation to tolerate droughts (Habibi & Ajory 2015).

In Australia, *M. vulgare* has spread to most pastoral areas with rainfall over 200 mm, and has become a significant weed in Victoria and South Australia, both in pastures and in natural habitats (Sagliocco 2000; Weiss & Sagliocco 2000). In Australia *M. vulgare* is found mostly on well-drained calcareous soils, and is more common on grazed lands with little or no competition from other plants (Sagliocco 2000). In national parks the plant spreads easily in areas that experience frequent tourist visitations, such as campgrounds and along creek lines (Clarke et al. 2000). *Marrubium vulgare* dominance in native habitats in Australia is considered an indicator of problems such as high rabbit densities or lack of a native seed source (Carter 1990). A comparison of plant size and reproductive output between Australia and the native range suggests that plants in the invaded range are significantly larger and form more dense stands, but that the number of seeds produced per plant is similar between the native and invaded range in Australia (Weiss & Sagliocco 2000). Seeds in two invaded ranges, Australia and North America, required different chilling temperatures to germinate (Lippai et al. 1996; Weiss & Sagliocco 2000).

Marrubium vulgare leaves contain marrubiin, a bitter alkaloid that makes the plant unpalatable to livestock (Weiss & Sagliocco 2000). Marrubiin is also the active component responsible for the medicinal properties of the plant (Rezgui et al. 2017), although other molecules have also been associated with some of its beneficial properties, e.g. flavonoids, terpenoids phenolics (Al-Bakri & Afifi 2007; Masoodi et al. 2008), diterpenoids, iridoids (Matkowski & Piotrowska 2006; Piozzi et al. 2006), and phenylpropanoids (Martin-Nizard et al. 2004). Maximum accumulation of marrubiin occurs in the glandular trichomes on fully

Page 2 Landcare Research

expanded leaves just before the plant flowers (Piccoli & Bottini 2008), and that is the time herbalists tend to harvest the plant (S. Clair, Artemis, pers. comm., 27 May. 2017).

Marrubium vulgare is tolerant of salt stress, but growth and marrubin content decrease with exposure to increasing salt concentration (Rezgui et al. 2017). Increased activity of antioxidant enzymes was insufficient to compensate for the biomass loss (Rezgui et al. 2016).

Germination is optimal when diurnal temperatures fluctuate by 15°C, and maximum germination occurs at daily maximum temperatures of 40°C (Young & Evans 1986). Cool moist conditions for 3–6 weeks pre-germination stimulated germination of New Zealand *M. vulgare* (Dastgheib & Field 1994). Germination occurs mostly in autumn and winter and the plant grows rapidly in winter and spring (Carter 1990). Germination after 10 years in cold storage (4 °C) was poor (Novak et al. 2010).

2.2 Distribution and pest status in New Zealand

Marrubium vulgare 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). The plant is now found in all of the low-rainfall parts of New Zealand, and forms especially dense stands in the high country of the South Island (Figs 3 & 4).



Figure 3: Occurrences of *Marrubium vulgare* in permanent vegetation plots in the NVS database (https://nvs.landcareresearch.co.nz/Data/Search).



Figure 4: Occurrences of *Marrubium vulgare* in Department of Conservation database (green dots) and the Australasian Virtual Herbarium (red dots).

The plant grows in alkaline and poor soils. It is an early coloniser of eroded areas, sheep camps, rabbit warrens, and other disturbed sites, from which it encroaches into adjacent farmland (Fig. 5). It is primarily a problem in high country lucerne crops, where chemical control is proving ineffective. Areas treated chemically become bare, resulting in soil erosion (Fig. 6). *Marrubium vulgare* tends to be the first plant to regenerate following chemical control. The long tap-root makes *M. vulgare* a strong competitor in dry conditions and the plant takes hold faster in dry years than in wet years. Given long-term predictions that the dry, eastern parts of the country could become even more drought-prone, *M. vulgare* populations are expected to expand dramatically. The problem is exacerbated by the push to convert an increasing area of dryland pastures into lucerne, which facilitates further *M. vulgare* invasion.



Figure 5: High-country hillside infestation by *M. vulgare*. Mckenzie country.

Page 4 Landcare Research



Figure 6: Soil erosion following herbicide treatment of *M. vulgare*. Mckenzie country.

Marrubium vulgare fruit form burrs with small hooked spines that attach easily to fleece, more than doubling the cost of processing the wool. In the absence of *M. vulgare*, New Zealand wool is globally renowned for supreme cleanliness and near-freedom from organic matter, but in the presence of *M. vulgare* this wool downgrades to the lowest grade, has to be manually separated from non-contaminated wool, and is no longer suitable for processing locally, having to be shipped to Australia for processing. Grazing animals contribute to seed dispersal via movement of burrs and seeds attached to their fleece.

Although *M. vulgare* is rich in protein and metabolites (Carter 1990), it is bitter and unpalatable to stock, and when animals are forced to eat *M. vulgare*, their meat becomes tainted (Parsons & Cuthbertson 2000).

The cost of the weed is not well documented. Preliminary results from a survey held by the Horehound Biocontrol Group in August 2016 suggest *M. vulgare* invasion makes high-country land un-economic to farm. On top of doubling wool processing costs, the main cost of *M. vulgare* is due to control actions. Chemical control costs \$55 per ha on average (up to \$180 per ha from one respondent), and mechanical control costs \$30 per ha on average. While both options provide 75–80% effective control of above-ground material, most respondents commented on the rapid return of *M. vulgare* from the seedbank following control operations, in addition to the damage to lucerne stands following chemical application (40% of lucerne killed on average). Another cost is crop replacement frequency as a result of herbicide damage: lucerne, which is normally replaced on average every 12–13 years, now gets replaced on average every 5 years in horehound infested-land, at an average cost of \$550 per ha. *M. vulgare* infestations are estimated to double in size on average every 3 years on the surveyed properties.

Given these preliminary results, and the estimates that between 150,000 and 200,000 ha of New Zealand farming sector are already planted in lucerne (and the area is growing rapidly), we can estimate the annual cost of *M. vulgare* to the sector at \$29m to \$39m in control and lucerne replacement costs alone, not including the loss on wool prices. A more widespread roll-out of survey is recommended to achieve better estimates of the cost of *M. vulgare* to the farming community.

While high-country farms are worst-affected, any dryland and drought-prone farming operation is vulnerable to *M. vulgare*.

Although horses eat *M. vulgare*, the seed remains viable passing through their guts, making horses a source for redistribution of the weed.

Marrubium vulgare has been reported as a non-symptomatic reservoir for the cucumber mosaic virus in New Zealand (Fletcher 2001).

Marrubium vulgare does not appear to be a problem in undisturbed native habitats but can become a problem in disturbed or open native grasslands (Weiss & Sagliocco 2012).

2.3 Beneficial uses

2.3.1 Pharmacological beneficial uses

Marrubium vulgare has many traditional medicinal uses both in its native range (Pieroni et al. 2002; Redzic 2007; Quave et al. 2008; Warda et al. 2009; Robles-Zepeda et al. 2011; Watkins et al. 2011; Boudjelal et al. 2012), as well as its introduced range (e.g. Meyre-Silva & Cechinel 2010; Juarez-Vazquez et al. 2013). The plant is ranked as highly important by traditional users (Juarez-Vazquez et al. 2013), with the most used parts being the leaves and flowering tops, either fresh or dried (Rodriguez Villanueva & Martin Esteban 2016).

An Italian traditional medicine proverb asserts that "the white horehound destroys every disease" (Quave et al. 2008). Indeed, uses in folk medicine in the native range include treatment of diabetes (Roman et al. 1992; Boudjelal et al. 2012; Ohtera et al. 2013; Maiti et al. 2016; Rezgui et al. 2016; Barkaoui et al. 2017), inflammatory disorders (Sahpaz et al. 2002; Stulzer et al. 2006; Thomas 2011; Ohtera et al. 2013; Rezgui et al. 2016), gastroenteric disorders (Chopra et al. 1956; Redzic 2007; de Oliveira et al. 2011; Robles-Zepeda et al. 2011; Rezgui et al. 2016), and respiratory disorders such as bronchitis, colds, and asthma (Thomas 2011; Boudjelal et al. 2012; Rezgui et al. 2016), as well as treatment of appetite loss (Chopra et al. 1956; Boudjelal et al. 2012), indigestion (dyspepsia) (Chopra et al. 1956; Redzic 2007; Boudjelal et al. 2012; Ohtera et al. 2013), gut (visceral) and liver (hepatic) conditions (Chopra et al. 1956; Rodriguez Villanueva & Martin Esteban 2016), blotch, leprosy, foot ache, loathing of food, nausea, breast pain, loin pain, 'watery congestion', and 'an enlarged body' (Robles-Zepeda et al. 2011; Thomas 2011).

In addition, the plant is reported to possess hypoglycaemic (Roman et al. 1992), hyperglycaemic (Elberry et al. 2015) and antihypertensive qualities (El Bardai et al. 2004a; Boudjelal et al. 2012; Namjoyan et al. 2015b); cholesterol reduction (Berrougui et al. 2006; Ibrahim et al. 2016), cardiovascular protection (Martin-Nizard et al. 2004; Redzic 2007; Yousefi et al. 2013), and immune system stimulator activities (Daoudi et al. 2013); analgesic (painkilling) (de Souza et al. 1998; Weel et al. 1999), antioxidant (Weel et al. 1999; Matkowski & Piotrowska 2006; Matkowski et al. 2008; Orhan et al. 2010; Kadri et al. 2011; Pukalskas et al. 2012; Perez-Cruz et al. 2013; Amessis-Ouchemoukh et al. 2014a, b; El Euch et al. 2014; Boulila et al. 2015; Brahmi et al. 2015; Yousefi et al. 2016) anti-ulcer (de Oliveira et al. 2011),

Page 6 Landcare Research

antispasmodic (smooth muscle spasm in the gastrointestinal tract, Weel et al. 1999; Boudjelal et al. 2012), antifungal (Bouterfas et al. 2016), antibacterial properties (Masoodi et al. 2008; Quave et al. 2008; Warda et al. 2009; Gonzalez & Marioli 2010; Robles-Zepeda et al. 2011; Zarai et al. 2011; Bokaeian et al. 2014; Imani et al. 2014; Khaled-Khodja et al. 2014; Brahmi et al. 2015; Saeidi et al. 2015); and is used as a general antimicrobial (Al-Bakri & Afifi 2007; Khalil et al. 2009; de Coss et al. 2011; Zarai et al. 2011; Masoodi et al. 2015), for protection of the liver from toxicity (Ettaya et al. 2016), and to treat tumours (Paunovic et al. 2016). The plant has also been studied for dementia-preventing properties (Orhan et al. 2010), and was shown to have an abortifacient effect (causing abortion) in rats (Aouni et al. 2017).

On a number of medicinal properties and activities attributed to *M. vulgare*, evidence in the literature suggests the plant or its extracts do not perform as well as extracts from other plants. Thus, out of a list of 21 Mediterranean plants, *M. vulgare* was classified in the group with moderate to low total phenolics content, low flavonoids content, moderate antioxidant capacity and low antimicrobial potential – lower than antibiotics (Stankovic et al. 2017), least antibacterial activity out of 22 plants (Kunduhoglu et al. 2011), no moderating effect on blood sugar (Alkofahi et al. 2017), moderate to relatively low antioxidant activity (Matkowski & Piotrowska 2006; Matkowski et al. 2008; Kadri et al. 2011; Pukalskas et al. 2012; Amessis-Ouchemoukh et al. 2014b; El Euch et al. 2014; Khaled-Khodja et al. 2014; Brahmi et al. 2015; Masoodi et al. 2015; Saeidi et al. 2015), and low/variable antifungal/antibacterial/antimicrobial activity (Al-Bakri & Afifi 2007; Masoodi et al. 2008; Quave et al. 2008; Khalil et al. 2009; Gonzalez & Marioli 2010; Zarai et al. 2011; Khaled-Khodja et al. 2014; Brahmi et al. 2015; Masoodi et al. 2015; Saeidi et al. 2015; Bouterfas et al. 2016).

Furthermore, recent reviews of the current evidence for pharmacological actions attributed to *M. vulgare* concluded that the flurry of testimonials on the health benefits of this herb that can be accessed online and in health magazines is not supported by clinical data in the scientific literature (Rodriguez Villanueva & Martin Esteban 2016; Rodríguez Villanueva et al. 2017, summarised in Appendix 1). The reviewers go on to say that while the number of scientific publications on *M. vulgare* is growing rapidly, the state of knowledge is unsatisfactory at best. In particular, the combination of compounds and the therapeutic dosage are not standardised and not transferable from experiments with animals to human use (Rodriguez Villanueva & Martin Esteban 2016).

Adverse side effects of using *M. vulgare* noted in traditional herbal medicine include vomiting and headache (Juarez-Vazquez et al. 2013).

The market value of *M. vulgare* in the US mainstream sales channel in 2014 was US\$106m (a drop of 1% from the previous year), which positions this herb as the top-selling herbal dietary supplement in that market, at double the sales value of the next best-selling supplement, cranberry (Izzo et al. 2016). Notably, the Izzo et al. (2016) meta-analysis demonstrates a lack of correlation between sales value and evidence to support medical properties associated with some best-selling herbs/herbal supplements.

Note: The bibliography on pharmacological uses of *M. vulgare* listed above is **not** comprehensive. The above mentioned uses have been recorded in more studies than listed

here. We have attempted to list at least one example for every pharmacological use found in the literature.

2.3.2 Non-pharmacological beneficial uses

Rezgui et al. (2016) consider that *M. vulgare* can be grown in soils with moderate salinity for rehabilitation of marginal salted land for primary production. Use of *M. vulgare* to rehabilitate mercury contamination from soils has also been suggested (Millan et al. 2011, 2013), and it has been shown that other heavy metals from polluted soils do not transfer to the essential oils, such that the plants can still be used for medicinal purposes (Moreno-Jimenez et al. 2006; Zheljazkov et al. 2008). However, other plants may be better suited for soil rehabilitation from mercury in comparison to *M. vulgare* (e.g. Moreno-Jimenez et al. 2007).

Potential use of *M. vulgare* extracts as a pesticide was tested against mosquitoes, caterpillars and molluscs (Pavela 2004; Salama et al. 2012; Amel & Selima 2015). *Marrubium vulgare* is actively planted in chicken farms in Spain to avoid lice; in addition, animals were observed scratching against *M. vulgare* plants to try worming (Rodriguez Villanueva & Martin Esteban 2016).

Tests of the potential of decoctions from 40 different herb species to improve vitality and healthiness of five species of cereal crops found no evidence for any beneficial effect by *M. vulgare* (Sas-Piotrowska & Piotrowski 2011).

Marrubium vulgare was studied for use as natural antioxidative additive in food (Amessis-Ouchemoukh et al. 2014a; Brahmi et al. 2015), as well against browning of food (Namjoyan et al. 2015a). In the latter study, M. vulgare demonstrated the lowest potential out of the four plant species examined. Ortega-Ramirez et al. (2014) note that tests for potential use of herbal compounds in food preservation are usually futile because they are **not** conducted on bacterial biofilms, ignoring the issue that most bacterial problems are caused by this biofilm-type organisation of microbial growth (and see also Quave et al. 2008).

Marrubium vulgare is cultivated for use by the brewing industry as a substitute for hops (Pukalskas et al. 2012; Rodriguez Villanueva & Martin Esteban 2016).

Marrubium vulgare was studied for use as corrosion inhibitor for steel (Ben Hmamou et al. 2013).

2.4 Phylogeny

Marrubium vulgare L. is in the family Lamiaceae. Five genera in this family have representatives in New Zealand's indigenous flora (Table 1): Scutellaria, Mentha, Plectranthus, Vitex, and Teucridium. Although in the same family, none of these 5 genera are closely related to Marrubium, and none belong to the same subfamily (Fig. 7). Marrubium is in subfamily Lamioideae, Scutellaria is in subfamily Scutellarioideae, Teucridium is in subfamily Ajugoideae, Mentha and Plectranthus are in subfamily Nepetoideae, and Vitex is in

Page 8 Landcare Research

subfamily Viticoideae. Of the native Lamiaceae genera, *Scutellaria* is therefore the most closely related to *Marrubium*, however, the subfamilies are significantly differentiated, and individuals in different subfamilies are not closely related. Each of the five genera with New Zealand indigenous species are represented by a single species (Table 1).

This considerable phylogenetic distance between *M. vulgare* and New Zealand's indigenous flora increases the prospect that potential biocontrol agents will be unlikely to pose a threat to native species.

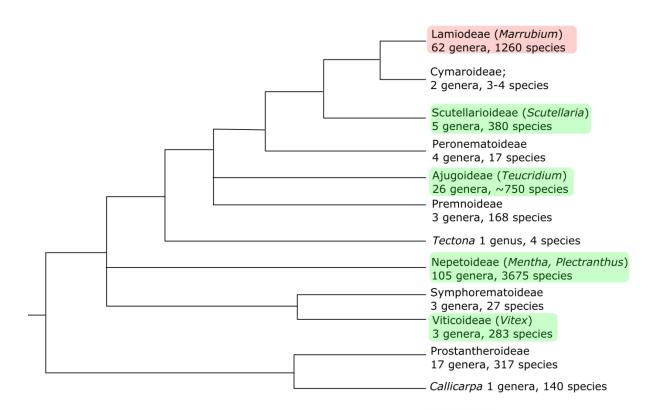


Figure 7: Phylogenetic relationships between subfamilies in the family Lamiaceae. The subfamily that contains *Marrubium vulgare* is highlighted in red, while the subfamilies that contain NZ native Lamiaceae genera are highlighted in green. The subfamilies are significantly differentiated, and individuals in different subfamilies are only distantly related. Phylogeny adapted from (Li et al. 2016); subfamily placements for each genus were taken from http://www.mobot.org/MOBOT/Research/APweb/genera/lamiaceaegen.html

 Table 1: New Zealand indigenous representatives in the family Lamiaceae

Species & growth form	Endemic / Indigenous	Phylogenetic distance from Marrubium (subfamily Lamioideae)	Conservation Status ¹	NZ distribution ¹
Scutellaria novae-zealandiae herb	Endemic	Most closely related to Marrubium of the native genera, but in a different subfamily (Scutellarioideae) and therefore not closely related.	Conservation Network	To the state of th
<i>Mentha cunninghamii</i> herb	Endemic	In subfamily Nepetoideae, not closely related to the genus <i>Marrubium</i> .	At risk – declining (New Zealand Plant Conservation Network 2014a)	
Plectranthus parviflorus herb	Indigenous	In subfamily Nepetoideae, not closely related to the genus <i>Marrubium</i> .	Non Resident Native – Coloniser (New Zealand Plant Conservation Network 2014b)	Confined to the North Island, where it is known locally from Tangihua and Mimiwhanga in Northland, and from the Waikato near Kawhia Harbour (Pukenoi, Awaroa Valley, Pirorua Valley), and at Whenuakite, on the Coromandel Peninsula.
<i>Teucridium parvifolium</i> herb	Endemic	In subfamily Ajugoideae, not closely related to the genus <i>Marrubium</i> .	At Risk – Declining (New Zealand Plant Conservation Network 2014c)	Occurring sporadically from Northland to Southland, but more common in the east of both islands.
Vitex lucens (Puriri) Large tree	Endemic	In subfamily Viticoideae, not closely related to the genus <i>Marrubium</i> .	Not threatened (New Zealand Plant Conservation Network 2014e)	Three Kings Islands and North Island from Te Paki to Taranaki, Mahia Peninsula and the northern Hawke's Bay. Scarce south of about Opotiki and Kawhia.

¹Conservation status and NZ distribution taken from NZ Plant Conservation Network (http://www.nzpcn.org.nz/)

2.5 Potential for opposition to biocontrol

Marrubium vulgare is harvested from wild populations by medical herbalists, who have already expressed opposition to the biocontrol programme (Appendix 2). The main concerns raised by medical herbalists so far include: the plant will become eradicated; following control, the plant would be replaced by another opportunistic plant; the biocontrol agents would impact on other medicinal/culinary herbs in the family Lamiaceae once the populations of the target host had declined; targeting other weeds would make better use of resources; accessibility to wild-crafted *M. vulgare* will be reduced due to the irreversible nature of biocontrol; the presence of medicinally valued plants on a farming property should be viewed as an additional source of income to the farmer; and that removal of *M. vulgare* from the environment undermines indigenous rights as well as national obligations under the WHO Traditional Medicine Strategy and the Convention on Biological Diversity. These concerns are possible to alleviate or mitigate, and consultation with this group is already under way via the New Zealand Association of Medical Herbalists and the Herb Federation of New Zealand.

In some parts of the world *M. vulgare* is valued by apiarists as an early season resource for honeybees. Initial conversation with Apiculture New Zealand indicates that *M. vulgare* is not a highly valued resource for apiarists in this country.

Beer brewers may also value *M. vulgare* for craft beer.

2.6 Control options

In New Zealand control is mainly chemical, most commonly using metsulfuron-methyl, and other chemicals to a lesser extent. Chemical control is proving ineffective for a number of reasons:

- 1. The waxy coating on the leaves protects them from the spray
- 2. The most cost-effective chemical, metsulfuron-methyl, has a long residual period, and a waiting period of 2–3 years is needed before any legumes can be sown in the sprayed area. During this period, *M. vulgare* regrows from the seedbank
- 3. Young lucerne stands are susceptible to the chemicals in their first 3–4 years, so if *M. vulgare* becomes established in newly sown lucerne, chemical control is not an option
- 4. *Marrubium vulgare* may be developing resistance to metsulfuron-methyl.

Mechanical control is used more rarely, mainly due to the challenging terrain and vast areas of infestation. Although frequent cultivation can remove *M. vulgare* (Parsons & Cuthbertson 2000), this practice is not economically viable (in low-intensity farming) and/or technically prohibitive on much of the *M. vulgare*-infested steep terrain in New Zealand.

In fire-adapted ecosystems, such as parts of Victoria, Australia, the integration of fire and herbicide has been tested (Weiss & Shepherd 1996; Weiss & Wills 2000a). Initial controlled burn removed the above-ground plants and close to 80% of the seed bank (still leaving 2,000 seeds per m², while a drop to 1,000 seeds per m² for a number of years is required for effective control; Weiss & Shepherd 1996); and the effects of burning last for about 3 years. Emerging seedlings can be treated with herbicide before they get a chance to contribute new seeds to the depleting seedbank. A 3-year fire cycle is, however, too frequent in these systems, and following spraying, colonisation of more *M. vulgare* is a likely scenario (Weiss & Wills 2000a). Pressure from grazing animals can help reduce further the re-establishment of *M. vulgare* (Weiss & Wills 2000a). However, *M. vulgare* is also encouraged by both heavy and selective/intermittent grazing (Carter 1990). Repeated burning is not compatible with biocontrol agents which may struggle to re-colonise the burnt site (Weiss & Wills 2000a).

Integrated control has been suggested whereby fire, herbicide and/or mechanical removal can be used to remove mature plants and stimulate germination of young plants, which would have fewer reserves to overcome repeated defoliation by the horehound plume moth, and may also be more susceptible to the clearwing moth (Clarke et al. 2000). For more details on the plume moth and the clearwing moth refer to the section 'Biological control of *M. vulgare* in Australia' below.

Integration of herbicide use with biological control by the plume moth is suggested for situations where these two practices can be compatible, including where herbicide would otherwise be considered not feasible due to the difficulty of repeat application and the rapid recovery of *M. vulgare* following application (Ainsworth & Morris 2000). Under Australian conditions, early autumn herbicide application was the optimal timing, enabling adult moths to avoid oviposition on herbicide-treated plants (providing sections of untreated plants are left available). Populations are then expected to build up to keep any new germination of *M. vulgare* in check. Alternatively, herbicide application can be timed for spring, when the overwintering moths are still in the pupal stage, which is less susceptible to herbicide than the adult stage. The emerging adults can then migrate to untreated areas to lay eggs (Ainsworth & Morris 2000). An untested suggestion was also made to treat *M. vulgare* with sub-lethal herbicide application, to stress the plant and yet to enable the moth to build up high population that can control the plant (Ainsworth & Morris 2000).

2.7 Potential advantages and disadvantages of biological control

Biological control could offer many advantages over current control methods for the management of *M. vulgare*, given the difficulty of controlling this weed by conventional means and the increasing infestations. Classical biological control, if successful, is more costeffective than other forms of control as it offers continuous action and self-dispersal to areas that are not likely to be targeted by other control programmes. Biological control is an attractive option for a target weed such as *M. vulgare* that is increasing in density and has yet to reach its full weedy potential. While biocontrol is often used as the last-resort management option, once all else has failed, it may be even more effective when the pest is targeted at the earlier stages of invasion (Henderson 1999; Delfosse 2005).

Page 12 Landcare Research

Despite its advantages, biological control may not be a 'silver bullet', although multiple analyses have indicated the success rate of weed biocontrol programmes has been greater than previously supposed (Hoffmann 1995; McFadyen 1998; Briese 2000; Fowler et al. 2000). Complete successes, where biological control is so dramatic that no other control methods are required, only account for approximately one-third of all completed programmes (McFadyen 1998). Furthermore, although biological control is often perceived as an environmentally benign alternative to chemicals, some cases of predictable damage to nontarget plants have been reported (e.g. Louda et al. 2003). Nevertheless, the risk of failure and the impacts of non-target attack are likely to be minor compared with the potential benefits. For example, Paynter and Flanagan (2004) showed that, of the weed biocontrol programmes that did not deliver complete control, most resulted in substantial or partial control (i.e. biocontrol contributes to management, but other control methods are still required to achieve adequate control). An example of partial control in New Zealand (listed by Fowler et al. 2000) is the control of alligator weed Alternanthera philoxeroides, in which biocontrol of floating weed mats is often successful on static water bodies, but agents do not attack terrestrial infestations. Failure (i.e. an inability to find or establish control organisms, or an absence of agent impact) is rarely an outcome of weed biocontrol programmes (Paynter & Flanagan 2004), and often is the result of funding running out before all options have been exhausted.

Studies on host use by arthropod biocontrol agents indicated that virtually all risk of non-target attack is borne by plant species closely related to the target weeds, and that the risk to native and beneficial flora can be judged reliably before introduction (Pemberton 2000; Suckling & Sforza 2014). Similarly, a survey in New Zealand indicated that host-range testing in past weed biocontrol programmes was highly reliable overall. Only two cases of non-target attack were recorded on native plant species; both examples were of plants very closely related to the target weeds that were predictable from host-range testing, and resulted in minor impacts (Paynter et al. 2004; Paynter et al. 2008).

An economic analysis from Australia indicated that weed biocontrol programmes provided a strongly positive return on investment overall, with benefits provided by the programmes far outweighing the total costs: for every dollar invested in weed biocontrol in Australia, a benefit of \$23.10 is generated (Page & Lacey 2006). No such detailed analysis is available for New Zealand, but Suckling (2013) suggests that the net benefits to New Zealand from weed biocontrol range between \$11 m and \$217 m per annum.

2.8 Predicting establishment of biocontrol agents

Reliably predicting the likelihood of establishment and impacts of introduced arthropods and pathogens on plant populations has long been a goal of weed biological control programmes. Research has been conducted to determine the importance of both release size (e.g. Memmott et al. 1998, 2005) and climate matching (e.g. van Klinken et al. 2003).

The best current predictor of establishment success of new organisms is the number and size of releases. In New Zealand, weed biocontrol agent establishment rates are very high, largely due to the extensive technology transfer programme that Landcare Research operates with

regional councils, community and farmer groups, the Department of Conservation, and forestry companies (Fowler et al. 2000, 2010; Hayes 2000). This approach allows numerous releases of biocontrol agents to be made quickly at many sites.

In a broad sense, climate matching should rarely be problematic because co-evolved weed biocontrol agents and their host plants should, generally, be adapted to the same climatic conditions. However, van Klinken et al. (2003) noted that climate became an issue when agents were collected from a restricted part of the range of a plant species that occurs over a wide range of climatic and ecological conditions (a similar scenario could also occur if the native distribution of a plant was restricted, compared to its introduced range). Problems may also arise if the target country has an unusual climate that the plant can tolerate better than the agent. Problems may also arise if an agent's sensitivity to climate is confounded with photoperiodism, which can result in shifts (either up or down) to the number of generations per year in the new climate (Grevstad & Coop 2015). Given the Mediterranean distribution of *M. vulgare*, Northern Spain may provide the best climatic match between New Zealand and the native range.

2.9 Predicting impact of biocontrol agents

Predicting the impact of an exotic organism in a new environment is more challenging than predicting the likelihood of its establishment (e.g. Cock et al. 2015). In addition to climate matching, an organism will face a host of other factors, such as predation, parasitism and competition, that might affect its ability to thrive in a new environment, as well as the ability of its host plant to compensate for attack. Information regarding the growth of *M. vulgare* in New Zealand compared with the native range is not available, but a comparison between Australia and the native range (Weiss & Sagliocco 2000) can be used as a benchmark, and ample information regarding the impact of invertebrate herbivory on *M. vulgare* in the native range as well as in the biocontrol programme in Australia is also available (Sagliocco 2000; Weiss & Sagliocco 2012). This level of information is unusual at the beginning of a biological-control programme of weeds, and puts the programme at a significant advantage.

Denoth et al. (2002) found that the success of biological control against weeds increased with the number of agents released, although they argued that this result might be because of the increased likelihood of the most damaging species being released with the greater number of agents released (lottery model), rather than the cumulative impact of multiple natural enemies. Certainly, spectacular biocontrol successes have been achieved with only one agent (Denoth et al. 2002), and a challenge for biocontrol practitioners is to identify the agent(s) most likely to impact on weed populations.

Crawley (1989) and Charudattan (2005) reviewed the use of insect and plant pathogenic fungi, respectively, in weed biological control. Crawley showed that certain insect groups have proved more successful at reducing host plant abundance than others; for example, c. 50% of releases of Dactylopiidae (cochineal insects), Curculionidae (weevils) and Chrysomelidae (leaf beetles) that established resulted in marked or complete control of the target weed.

Page 14 Landcare Research

Weed biocontrol agents, especially rust fungi, can be so specialised that they show host specificity within a given plant species. Matching the target host's susceptibility with the candidate pathogen's virulence is of utmost importance for biocontrol success with pathogen agents. Host-pathogen interactions at the species and subspecies levels are often governed by single-gene differences in rusts (e.g. varietal specificity, Charudattan 2005). For example, efficacy of the skeletonweed rust fungus Puccinia chondrillina varies from low to high, depending on the form of the weed (Burdon et al. 1981). Eriophyid mites can show similar levels of specificity: at least one biotype of St John's wort, Hypericum perforatum, appears to be resistant to the eriophyid mite Aculus hyperici in Australia (Mayo & Roush 1997). For certain candidate agents, it is prudent to determine which subspecies and forms of a weed are present in the introduced range, and if possible survey for potential agents on similar material, if it can be identified in the native range, to facilitate finding the correct agent biotype(s) and reduce the potential for incompatibility. Molecular techniques can be used to determine the diversity within and origin of a weed species. Some studies have used a cheaper method of directly testing the susceptibility of a weed to candidate agent species by planting out 'trap plants' collected in the weeds' introduced range and monitoring damage to them in the native range. Logistics and biosecurity regulations can sometimes prohibit the use of trap gardens. In recent years the cost of conducting molecular tests has decreased markedly and their use in determining the origin of weeds is becoming an integral component of biocontrol programmes.

Tapping into multiple experts' deep functional understanding of the system in question has been suggested as a mechanism to assess the likelihood of success of biocontrol programmes (van Klinken et al. 2016).

Finally, (Charudattan 2005) and (Seastedt 2015) also concluded that the stakeholders' perceptions of the effectiveness of a biological control programme can be unpredictable, leading to conflicting views of 'success'. Therefore, the aims of a biological control programme against *M. vulgare* should be clearly defined from the outset, so that success or failure can be assessed objectively against a well-defined goal.

2.10 Biological control of *M. vulgare* in Australia

Australia is the only country where biological control for *M. vulgare* has been attempted.

Initial assessment suggested that biocontrol for *M. vulgare* would not be beneficial because other alien plants may fill the vacated gaps (Carter 1990). Nevertheless, given the difficulty of managing *M. vulgare* using standard methods in national parks and natural habitats, Australia launched on a biocontrol programme in 1990 (Clarke et al. 2000). Native range surveys of 55 *M. vulgare* populations in France, Spain, Portugal, Italy, former Yugoslavia, and Morocco between 1991 and 1997 yielded a list of 27 insect species associated with this host plant, five of which were prioritised as candidate biocontrol agents (Sagliocco 2000). Finally, two species of moths – the horehound plume moth, *Wheeleria spilodactylus* (Curtis) (Lepidoptera: Pterophoridae), and the horehound clearwing moth, *Chamaesphecia mysiniformis* (Boisduval) (Lepidoptera: Sesiidae) – were tested for host specificity, introduced to Australia and subsequently approved and released (Wills 2000). Other high priority

candidates proved too difficult to rear or test in containment and were abandoned for these technical reasons (Sagliocco 2000; Wills 2000). *Wheeleria* releases began in 1993 and ramped up in 1996—997, with establishment confirmed in 1998 (Wills 2000). Releases were made initially from a French population, which did not establish well in areas with rainfall below 450 ml per year, had low fecundity, and was a poor disperser (Clarke et al. 2000). Further releases from another close-by French population and from a Spanish population were expected to perform better in more drought-prone areas, resulting in successful establishment (Clarke et al. 2000). Failure of the original population to establish is attributed to inbreeding depression due to continuous breeding under laboratory conditions during the host-specificity tests and mass rearing programme (Clarke et al. 2000). Several methods for re-distribution were compared and the simple method of transferring late-instar larvae on cut shoots directly onto plants at their designated new site provided the best rate of establishment (Wills 2000). *Chamaesphecia* was first released in 1997 with establishment confirmed already in 1998; the main releases took place in 1999 (Wills 2000), after a method for mass rearing and redistribution was successfully developed (Clarke et al. 2000).

A workshop on *M. vulgare* management in Australia in the year 2000 suggested that biological control alone will not control the plant, and integration with grazing, herbicide and fire regimes would be required (Weiss & Wills 2000b). However, concerns were raised that in Tasmania biocontrol may not integrate well with winter grazing by sheep that often graze the young growing tips of *M. vulgare*, where overwintering young larvae of *Wheeleria* take refuge (Ireson et al. 2000).

By 2008 the biocontrol programme had not yet delivered measurable economic benefits (Sheppard et al. 2008), but by 2012 it appeared that the existing agents may suffice and no further agents would be required (Weiss & Sagliocco 2012).

3 Objectives

- Review the literature to identify potential biocontrol agents for *M. vulgare* and assess the feasibility of their release in New Zealand
- Assess the prospects of achieving successful biological control of *M. vulgare* in New Zealand
- Estimate the cost of the programme for biological control of *M. vulgare* in New Zealand.

Page 16 Landcare Research

4 Methods

4.1 Identifying fungal pathogens of *Marrubium vulgare*

A table was compiled of the fungi that have been reported associated with *M. vulgare*. The information was obtained by searching online databases and Internet sites as well as through personal communication with overseas experts. Online databases searched included:

- USDA Fungus-host database or FDSM (which includes most New Zealand plant disease records, Farr & Rossman 2017): http://nt.ars-grin.gov/fungaldatabases/fungushost/FungusHost.cfm
- Fungal Records Database of Britain and Ireland or FRDBI (Cooper 2006): http://www.fieldmycology.net/FRDBI/assoc.asp
- Kew Royal Botanic Garden Plants and Fungi species browser (previously IMI fungal herbarium):
 http://www.kew.org/science-conservation/plants-fungi/species-browser
- New Zealand fungi and bacteria database or NZFUNGI (Landcare Research 2009): http://nzfungi2.landcareresearch.co.nz/
- Plant parasites of Europe, leaf miners, galls and fungi: http://bladmineerders.nl/
- Ecological Flora of the British Isles (Fitter & Peat 1994): http://ecoflora.org.uk/

In addition, CAB abstracts, Current Contents, PubMed, Ingenta, Web of Science, Agricola, Science Direct, and Google were searched, using the terms 'Marrubium vulgare or horehound or white horehound' and sub-searched using the terms 'pathogen* or fung*'. Once a list had been created, further information about each fungus was sought in the published literature as well as in the following online databases:

- Index Fungorum database (Index Fungorum 2004): http://www.indexfungorum.org/Names/Names.asp
- Global Biodiversity Information Facility or GBIF (Global Biodiversity Information Facility 2017): http://data.gbif.org/species/
- MycoBank database: http://www.mycobank.org/quicksearch.aspx

4.2 Identifying arthropod biocontrol agents for Marrubium vulgare

Unlike for fungal pathogens, comprehensive online databases for all arthropod herbivores do not exist. However, the following databases were searched:

 HOSTS – a Database of the World's Lepidopteran Hostplants, the Natural History Museum's world listing (Natural History Museum London 2007): http://www.nhm.ac.uk/jdsml/research-curation/research/projects/hostplants/

- Database of Insects and Their Food Plants Biological Records Centre (UK)
 (Biological Records Centre (BRC) 2009):
 http://www.brc.ac.uk/dbif/Interpreting_foodplant_records.aspx
- Plant parasites of Europe, leaf miners, galls and fungi: http://bladmineerders.nl/
- Plant-SyNZ™: http://plant-synz.landcareresearch.co.nz/SearchForm.aspx

In addition, CAB abstracts, Current Contents, Web of Science, Agricola, Science Direct, Google, and Google Scholar were searched using the terms 'Marrubium vulgare or horehound or white horehound' and sub-searched using the terms 'invertebrate* or herbivor*'. Checklists of New Zealand fauna were referred to, to determine whether any of the species recorded feeding on/infecting M. vulgare already occurs in New Zealand.

5 Results

5.1 Pathogens attacking Marrubium vulgare

Several fungi that cause leaf disorders have been reported (Appendix 3). These are:

- leaf spots caused by *Cercospora marrubii* (Horst 2013), *Ramularia marrubii* and *Phyllosticta marrubii* (Oudemans 1923)
- powdery mildews caused by *Neoerysiphe galeopsidis*, *Leveillula taurica* and *Leveillula duriaei* (Amano 1986)
- rust on leaves caused by *Uredo marrubii* (Rabenhorst 1850)
- a leaf gall caused by *Synchytrium marrubii* (Tobler 1913)

The leaf spots have not been assessed, and may only be responsible for aesthetic damage. None of the species was specific to *Marrubium vulgare* alone, and infects other hosts in the family Lamiaceae (Andrianova & Minter 2016; Farr & Rossman 2017).

The powdery mildews recorded on *M. vulgare* have all also been reported on a wide host range, with *Neoerysiphe galeopsidis* and *Leveillula taurica* present in New Zealand (Landcare Research 2009).

The rust *Uredo marrubii* was only ever reported once, on *Marrubium vulgare* leaves. Rust fungi can be highly host-specific and damaging but not enough information is available about this species and further investigations would be required to determine: i) if this rust is specific to *Marrubium vulgare* and, ii) if it requires an alternate host. The rust *Puccinia marrubii* has been recently described on the related *Marrubium globosum* subsp. *globosum* in Turkey (Kabaktepe et al. 2016). It may be worthwhile exploring whether its host range also includes *M. vulgare*.

Synchytrium marrubii, which causes a leaf gall, has been isolated from *M. vulgare* in the USA (Crops Research Division Agricultural Research Service 1960); however, not much information is available about this organism. Species in the genus *Synchytrium* can be pathogenic to

Page 18 Landcare Research

plants, and their use as weed biocontrol agents has been explored (e.g. Eskandari et al. 2011).

Stemphylium vesicarium was isolated from *Marrubium vulgare* stems and is responsible for onion leaf blight (Plantwise 2017). This pathogen has a wide host range, and is found in New Zealand, so is unsuitable to pursue as a biocontrol agent.

A range of fungal species has been identified on dead stems and have been listed in Appendix 3; however, they do not constitute potential biocontrol agents. The Ecological Flora of the British Isles lists 56 additional fungal species associated with *Marrubium vulgare*. These species all had a wide host range, and were mainly found on dead stems and leaves. They are considered to be saprophytes or secondary pathogens.

Native range surveys for potential biocontrol agents for the Australian programme only found one unidentified soil pathogen associated with this plant (Weiss et al. 1999).

Overall, very limited information is available on the impacts of fungal pathogens on *M. vulgare*. The leaf spots and powdery mildews do not represent viable candidates for biological control as they have a broad host range. The single historical record of the rust *Uredo marrubii* (Rabenhorst 1850) will require some validation to ascertain if the pathogen was correctly identified, and some clarification of its life history. The more recent discovery and description of *Puccinia marrubii* in Turkey on *M. globosum* subsp. *globosum* provides the first evidence of rust fungi associated with *Marrubium*. This research suggests the possibility of undescribed rusts being associated with *M. vulgare* in its native range. Given that no plant diseases were recorded in the extensive native range surveys performed as part of the Australian biocontrol programme, any field surveys for plant diseases should focus on parts of the native range that form the origin of *M. vulgare* in New Zealand. This part of the native range has not yet been determined.

5.2 Arthropods attacking Marrubium vulgare

Native range surveys by the Australian programme found specialist insects attacking all parts of the plant (Sagliocco 2000, Appendix 4). Furthermore, the cumulative number of different species of specialised insects increased as the number of *M. vulgare* populations surveyed increased (Sagliocco 2000), suggesting that the resource has not been exhausted, and that more specialist insect species may exist unrecorded in parts of the native range that have not been covered in the surveys.

Overall, 45 species of insects/mites have been recorded on *M. vulgare*, 27 of them found during the native range surveys performed for the Australian programme. Of the 45 species, 23 are potentially sufficiently host specific, and a subset of five highly damaging candidates was prioritised by the Australian programme, resulting in the introduction and establishment of two species, the plume moth *Wheeleria spilodactylus* and the clearwing moth *Chamaesphecia mysiniformis*, both of which contribute to *M. vulgare* control over a range of environmental conditions in Australia.

The species prioritised highest in the Australian programme were abandoned due to technical issues that caused difficulty in testing/importation; although they remain high priority. Nevertheless, as the plume moth and the clearwing moth are expanding their hold over larger areas, there is a perception that their impact may be sufficient, so that no further agents would be necessary (Weiss & Sagliocco 2012). It is recommended that these two moths should be the highest priority agents to introduce to New Zealand, because this country can benefit from the host-specificity tests already carried out as part of the Australian programme, and from the fact the these agents have already been phased from northern to southern hemisphere, thus removing a major technical challenge.

A single record of a root knot nematode from the genus *Meloidogyne* in the United States (Crops Research Division Agricultural Research Service 1960) is not recommended for pursuing as a biocontrol agent since these nematodes are major pests of crop plants worldwide.

6 Conclusions

6.1 Prospects for achieving biological control of *Marrubium vulgare* in New Zealand

A large number of arthropod and several pathogen records from *M. vulgare* appear in the literature, with many of the arthropods being host-specific, at least at the genus if not at the species level. Valuable information about damage levels in the native range is available for a number of the herbivorous arthropod species that were assessed for the Australian programme. In addition, a comparison of the plant performance in the native range vs in the invaded range in Australia before the introduction of biocontrol agents provides a useful benchmark for comparison.

Pathogens have not been well-explored as biocontrol agents for *M. vulgare*. Our literature search revealed one rust fungus and several other leaf disorders caused by fungal pathogens that appear to be specific to the family Lamiaceae and may be worthy of pursuing. Their level of damage to *M. vulgare* is not well known. Furthermore, no pathogens were recorded in the extensive native range surveys conducted for the Australian biocontrol programme other than one unidentified species of soil pathogen.

Biological control of *M. vulgare* has been attempted successfully in Australia. A biocontrol programme against *M. vulgare* in New Zealand would therefore be a repeat programme and can benefit greatly from the work done in the Australian programme, including the native range surveys, host-specificity testing, re-phasing of agents from northern to southern hemisphere, and mass rearing and re-distribution techniques. For repeat projects the cost of developing biocontrol agents is historically on average \$203,000 per agent, and generally two to three agents are needed to provide control (Paynter et al. 2015).

There are five indigenous species in the family Lamiaceae in New Zealand. Two are endemic and three are non-endemic. Four are herbs and one is a tall tree (Puriri). Their conservation

Page 20 Landcare Research

status ranges from non-threatened to nationally critical. Phylogenetically, all are relatively distant from *Marrubium*. Representatives of these five genera were included in host-specificity tests in Australia (Sagliocco & Coupland 1995; J Weiss, pers. comm., 20 Sep 2017) and have been determined to not be at risk from the biocontrol agents. The tests done in Australia may satisfy the requirements to demonstrate safety for New Zealand. However, if additional tests are deemed necessary, then at least some of the herbaceous indigenous species will need to be included in host range tests. With the two endemic species being relatively rare, it would be advisable to begin searching for propagation material in nurseries specialising in native flora immediately.

Marrubium vulgare is valued in New Zealand for its medicinal property and is wild-harvested by practicing medical herbalists. Opposition from medical herbalists to *M. vulgare* biocontrol is already being voiced, and a consultation process is already taking place. Whether *M. vulgare* is valued by craft beer makers is yet to be established. No other opposition is anticipated. While grazing animals eat *M. vulgare* if pushed, it is not valued as a fodder plant, nor is it valued by New Zealand apiarists as a pollen/nectar source.

Marrubium vulgare is possibly already occupying much of its range in New Zealand, but is increasing in density, especially where land management practices adopt lucerne as a more drought-tolerant fodder. *Marrubium vulgare* is proving impractical to control chemically or mechanically, whereas biological control has proven successful in Australia. While chemical and mechanical control will continue to provide short-term management, the pursuit of a biological control solution is recommended as a long-term approach.

There is a risk that the agents introduced to suit the climate in Australia may not perform well in New Zealand. Alternatively, it is also possible that at least one of these agents, the plume moth, which struggled in extremely low rainfall areas in Australia, will perform better in New Zealand. If the agents that have established in Australia do not provide sufficient control in New Zealand, other agents that have been shortlisted but not tested could then be pursued, albeit at a greater cost.

7 Recommendations

- Undertake a survey of the natural enemies of *M. vulgare* in New Zealand and look for any potential biocontrol agents and any other species living on the plant (such as predators) that might interfere with potential biocontrol agents. Note this baseline information is vital for any subsequent application to release new biocontrol agents. Estimated cost \$50,000–\$60,000.
- Undertake DNA studies to identify whether the geographic source of *M. vulgare* in New Zealand and in Australia is similar. Estimated cost \$20,000–\$50,000.
- Analyse costs of and benefits expected from biological control in comparison to conventional control of *M. vulgare* in New Zealand. While initial information has been gathered, a clear and strong case will be important for any application to release new biocontrol agents. Estimated cost \$15,000–\$25,000.

- If required, undertake host-range testing of potential agents. Estimated cost \$60,000–\$100,000 per agent.
- If testing shows agents are suitable, apply to release them in New Zealand. Estimated cost \$50,000–\$70,000 for one or more species.
- Import into containment and gain permission to remove. Estimated cost \$25,000-\$50,000 per species.
- Mass-rear and release agents. Estimated cost \$50,000–\$100,000 per species.

Note: Estimated costs are exclusive of GST and are based on 2016/17 figures. New estimates will need to be provided if work is to be undertaken beyond those dates, and/or if complicating factors arise (e.g. disease infecting imported agents).

8 Acknowledgements

We thank AGMARDT for funding this work, John Weiss for ample advice and for sharing the wealth of unpublished information collected as part of the biocontrol programme in Australia, Dave Havell for generating a map from DOC database, and Paul Peterson for helpful comments on this report.

9 References

- Ainsworth N, Morris C 2000. Integration of herbicide treatments with the plume moth for horehound control. Plant Protection Quarterly 15(1): 37–39.
- Akther N, Shawl A, Sultana S, Chandan B, Akhter M 2013. Hepatoprotective activity of Marrubium vulgare against paracetamol induced toxicity. Journal of Pharmacy Research 7(7): 565–570.
- Al-Bakri AG, Afifi FU 2007. Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. Journal of Microbiological Methods 68(1): 19–25.
- Alkofahi AS, Abdul-Razzak KK, Alzoubi KH, Khabour OF 2017. Screening of the Antihyperglycemic activity of some medicinal plants of Jordan. Pakistan Journal of Pharmaceutical Sciences 30(3): 907–912.
- Amano K 1986. Host range and geographical distribution of the powdery mildew fungi. Tokyo, Japan Scientific Societies Press. 741 p.
- Amel A, Selima B 2015. Larvicidal effect of *Marrubium vulgare* on *Culex pipiens* in eastern Algeria. In: Salame C, Aillerie M, Papageorgas P ed. International Conference on Technologies and Materials for Renewable Energy, Environment and Sustainability Tmrees15. Amsterdam, Elsevier Science. Pp. 1026–1031.
- Amessis-Ouchemoukh N, Abu-Reidah IM, Quirantes-Pine R, Madani K, Segura-Carretero A 2014a. Phytochemical profiling, in vitro evaluation of total phenolic contents and antioxidant properties of *Marrubium vulgare* (horehound) leaves of plants growing in Algeria. Industrial Crops and Products 61: 120–129.

Page 22 Landcare Research

- Amessis-Ouchemoukh N, Madani K, Fale PLV, Serralheiro ML, Araujo MEM 2014b.

 Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities. Industrial Crops and Products 53: 6–15.
- Andrianova TV, Minter DW 2016. *Ramularia marrubii*. IMI Descriptions of Fungi and Bacteria. Wallingford, CABI. Pp. Sheet 2067.
- Aouni R, Ben Attia M, Jaafoura MH, Bibi-Derbel A, Haouari M 2017. Effects of the hydroethanolic extract of *Marrubium vulgare* in female rats. Asian Pacific Journal of Tropical Medicine 10(2): 154–158.
- Audisio P 2011. The Nitidulidae and Kateretidae of Sardinia: recent data and updated checklist (Coleoptera). In: Nardi G, Whitmore D, Bardiani M, Birtele D, Mason F, Spada L, Cerretti P ed. Biodiversity of Marganai and Montimannu (Sardinia). Research in the framework of the ICP Forests network. Conservazione Habitat Invertebrati 5. Pp. 447–460.
- Barkaoui M, Katiri A, Boubaker H, Msande F 2017. Ethnobotanical survey of medicinal plants used in the traditional treatment of diabetes in Chtouka Ait Baha and Tiznit (Western Anti-Atlas), Morocco. Journal of Ethnopharmacology 198: 338–350.
- Ben Hmamou D, Salghi R, Zarrouk A, Zarrok H, Benali O, Errami M, Hammouti B 2013. Inhibition effect of horehound (*Marrubium vulgare* L.) extract towards C38 steel corrosion in HCl solution. Research on Chemical Intermediates 39(7): 3291–3302.
- Berlese AN 1888. Monografia dei generi *Pleospora, Clathrospora* e *Pyrenophora*. Firenze, Italy, Bullettino della societa Botanica Italiana. 100 p.
- Berrougui H, Isabelle M, Cherki M, Khalil A 2006. *Marrubium vulgare* extract inhibits human-LDL oxidation and enhances HDL-mediated cholesterol efflux in THP-1 macrophage. Life Sciences 80(2): 105–112.
- Biological Records Centre (BRC) 2009. Database of Insects and their Food Plants. Retrieved 25 Aug 2017 http://www.brc.ac.uk/dbif/Interpreting_foodplant_records.aspx
- Bokaeian M, Saboori E, Saeidi S, Niazi AA, Amini-Borojeni N, Khaje H, Bazi S 2014. Phytochemical Analysis, Antibacterial Activity of *Marrubium vulgare* L against *Staphylococcus aureus* in vitro. Zahedan Journal of Research in Medical Sciences 16(10): 60–64.
- Boudjelal A, Henchiri C, Siracusa L, Sari M, Ruberto G 2012. Compositional analysis and in vivo anti-diabetic activity of wild Algerian *Marrubium vulgare* L. infusion. Fitoterapia 83(2): 286–292.
- Boulila A, Sanaa A, Ben Salem I, Rokbeni N, M'Rabet Y, Hosni K, Fernandez X 2015.

 Antioxidant properties and phenolic variation in wild populations of Marrubium vulgare L. (Lamiaceae). Industrial Crops and Products 76: 616–622.
- Bouterfas K, Mehdadi Z, Aouad L, Elaoufi MM, Khaled MB, Latreche A, Benchiha W 2016. Does the sampling locality influence on the antifungal activity of the flavonoids of *Marrubium vulgare* against *Aspergillus niger* and *Candida albicans*? Journal De Mycologie Medicale 26(3): 201–211.
- Brahmi N, Scognamiglio M, Pacifico S, Mekhoukhe A, Madani K, Fiorentino A, Monaco P 2015. H-1 NMR based metabolic profiling of eleven Algerian aromatic plants and

- evaluation of their antioxidant and cytotoxic properties. Food Research International 76: 334–341.
- Braun U 1995. The Powdery Mildews (Erysiphales) of Europe. Stuttgart, VEB Gustav Fischer Verlag. 337 p.
- Briese DT 2000. Classical biological control. In: Sindel BM ed. Australian weed management systems. Melbourn, Australia, RG & FJ Richardson. Pp. 161–186.
- Brunaud P 1889. Champignons a ajouter a la flore mycologique des environs de Saintes (3-e serie). Bulletin de la Societe botanique de France 36: 335–340.
- Burdon JJ, Groves RH, Cullen JM 1981. The impact of biological-control on the distribution and abundance of *Chondrilla juncea* in southeastern Australia. Journal of Applied Ecology 18(3): 957–966.
- Carter R 1990. Biology and control of horehound, *Marrubium vulgare*. Proceeding of the 9th Australian Weeds Conference. Pp. 382–386.
- Charudattan R 2005. Ecological, practical, and political inputs into selection of weed targets: What makes a good biological control target? Biological Control 35(3): 183–196.
- Cheeseman TF 1906. Manual of the New Zealand flora. Wellington, New Zealand, J. Mackay, government printer. 1199 p.
- Chopra RN, Nayar SL, Chopra IC 1956. Glossary of Indian medicinal plants. New Delhi, Council of Scientific and Industrial Research. 329 p.
- Clarke C, Baker J, Keller M, Roush R 2000. Biological control of horehound: lessons from South Australia. Plant Protection Quarterly 15(1): 29–32.
- Cock MJW, Day RK, Hinz HL, Pollard KM, Thomas SE, Williams FE, Witt ABR, Shaw RH 2015. The impacts of some classical biological control successes. CAB Reviews 10(042): 1–58.
- Cooper J 2006. The fungal records database of Britain and Ireland (FRDBI). Retrieved 25 Aug 2017 http://www.fieldmycology.net/FRDBI/assoc.asp
- Cooper J 2013. The Status of names of powdery mildews in New Zealand. Mycological Notes 25, Fungal Network of New Zealand.
- Correll JC, Gordon TR, Elliott VJ 1987. Host range, specificity, and biometrical measurements of *Leveillula taurica* in California. Plant Disease 71(3): 248–251.
- Crane JL, Shearer CA 1991. A nomenclator of *Leptosphaeria* V. Cesati and G. de Notaris (Mycota- Ascomycotina-Loculoascomycetes). Illinois Natural History Survey bulletin 34(3): 195–355.
- Crawley MJ 1989. The successes and failures of weed biocontrol using insects. Biocontrol News and Information 10(3): 213–223.
- Crops Research Division Agricultural Research Service 1960. Index of plant diseases in the United States. Washington, United States Department of Agriculture. 531 p.
- Daoudi A, Aarab L, Abdel-Sattar E 2013. Screening of immunomodulatory activity of total and protein extracts of some Moroccan medicinal plants. Toxicology and Industrial Health 29(3): 245–253.
- Dastgheib F, Field RJ 1994. Seed germination of horehound. In: Popay AJ ed. Proceedings of the Forty Seventh New Zealand Plant Protection Conference. Rotorua, New Zealand Plant Protection Society. Pp. 93–94.

Page 24 Landcare Research

- de Coss AL, Tinajero JJM, Agreda FJM, Castillo CGG, Valdez ODM, Medina EG 2011.

 Assessment in vitro of the desfaunant capacity of the plant extract in the rumen.

 Revista Cientifica-Facultad De Ciencias Veterinarias 21(5): 414–420.
- De Jesus RAP, Cechinel V, Oliveira AE, Schlemper V 2000. Analysis of the antinociceptive properties of marrubiin isolated from *Marrubium vulgare*. Phytomedicine 7(2): 111–115.
- de Oliveira AP, Santin JR, Lemos M, Klein LC, Couto AG, Bittencourt CMD, Cechinel V, de Andrade SF 2011. Gastroprotective activity of methanol extract and marrubiin obtained from leaves of *Marrubium vulgare* L. (Lamiaceae). Journal of Pharmacy and Pharmacology 63(9): 1230–1237.
- de Souza MM, de Jesus RAP, Cechinel V, Schlemper V 1998. Analgesic profile of hydroalcoholic extract obtained from *Marrubium vulgare*. Phytomedicine 5(2): 103–107.
- Delfosse ES 2005. Risk and ethics in biological control. Biological Control 35(3): 319–329.
- Denoth M, Frid L, Myers JH 2002. Multiple agents in biological control: improving the odds? Biological Control 24(1): 20–30.
- Easton A 1952. A review of the *Meligethes* (Col. Nitidulidae) associated with the Labiate *Marrubium vulgare* L. Bulletin de la Société des Sciences Naturelles du Maroc 32: 181–192.
- El Bardai S, Lyoussi B, Wibo M, Morel N 2004a. Comparative study of the antihypertensive activity of *Marrubium vulgare* and of the dihydropyridine calcium antagonist amlodipine in spontaneously hypertensive rat. Clinical and Experimental Hypertension 26(6): 465–474.
- El Bardai S, Hamaide MC, Lyoussi B, Quetin-Leclercq JL, Morel N, Wibo M 2004b. Marrubenol interacts with the phenylalkylamine binding site of the L-type calcium channel. European Journal of Pharmacology 492(2-3): 269–272.
- El Bardai S, Morel N, Wibo M, Fabre N, Llabres G, Lyoussi B, Quetin-Leclercq J 2003. The vasorelaxant activity of marrubenol and marrubiin from *Marrubium vulgare*. Planta Medica 69(1): 75–77.
- El Euch SK, Ciesla L, Bouzouita N 2014. Free radical scavenging fingerprints of selected aromatic and medicinal tunisian plants assessed by means of TLC-DPPH test and image processing. Journal of AOAC International 97(5): 1291–1298.
- Elberry AA, Harraz FM, Ghareib SA, Gabr SA, Nagy AA, Abdel-Sattar E 2015. Methanolic extract of *Marrubium vulgare* ameliorates hyperglycemia and dyslipidemia in streptozotocin-induced diabetic rats. International Journal of Diabetes Mellitus 3(1): 37–44.
- Ellis MB, Ellis JP 1997. Microfungi on Land Plants: An Identification Handbook. 2nd ed. Slough, England, Richmond Publishing. 868 p.
- Ellis WN 2017. Plant Parasites of Europe leafminers, galls and fungi. Retrieved 26 Sep 2017 http://bladmineerders.nl/
- Eskandari FM, Bruckart WL, Widmer TL 2011. Field damage to yellow starthistle infected by *Synchytrium solstitiale,* and greenhouse maintenance and host range of the fungus. Plant Disease 95(8): 907–912.

- Ettaya A, Dhibi S, Samout N, Elfeki A, Hfaiedh N 2016. Hepatoprotective activity of white horehound (*Marrubium vulgare*) extract against cyclophosphamide toxicity in male rats. Canadian Journal of Physiology and Pharmacology 94(4): 441–447.
- Farr D, Rossman A 2017. Fungal databases, U.S. National Fungus Collectios, ARS, USDA. Retrieved 25 Aug 2017 https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm
- Fitter AH, Peat HJ 1994. The ecological flora database. Journal of Ecology 82: 415-425.
- Fletcher JD 2001. New hosts of Alfalfa mosaic virus, Cucumber mosaic virus, Potato virus Y, Soybean dwarf virus, and, Tomato spotted wilt virus in New Zealand. New Zealand Journal of Crop and Horticultural Science 29(3): 213–217.
- Fowler S, Paynter Q, Hayes L, Dodd S, Groenteman R 2010. Biocontrol of weeds in New Zealand: an overview of nearly 85 years. In: Zydenbos S, James T ed. Proceedings of the 17th Australasian Weeds Conference, Christchurch, New Zealand. Pp. 211–214.
- Fowler SV, Syrett P, Hill RL 2000. Success and safety in biological control of environmental weeds in New Zealand. Austral Ecology 25: 553–562.
- Ginns JH 1986. Compendium of plant disease and decay fungi in Canada, 1960-1980. Ottawa, Canadian Government Publishing Centre. 416 p.
- Global Biodiversity Information Facility 2017. GBIF. Retrieved 10 Oct 2017 http://data.gbif.org/species/
- Gonzalez Fragoso R 1914. Contribucion a la flora micologica del Guadarrama.

 Deuteromicetos. Trabajos del Museo Nacional de Sciencias Naturales. Serie Botanica 5: 36.
- Gonzalez Fragoso R 1916. Bosquejo de una florula hispalense de Micromicetos. Trabajos del Museo Nacional de Ciencias Naturales. Serie botánica No. 10. Madrid, Junta para Ampliación de Estudios e Investigaciones Cientificas (Imprenta Clásica Española. Cardenal Cisneros, 10). 221 p.
- Gonzalez MJ, Marioli JM 2010. Antibacterial activity of water extracts and essential oils of various aromatic plants against *Paenibacillus* larvae, the causative agent of American Foulbrood. Journal of Invertebrate Pathology 104(3): 209–213.
- Govindu HC, Thirumalachar MJ 1956/1957. Notes on some Cercosporae of India VIII. Sydowia 10: 271–277.
- Grevstad FS, Coop LB 2015. The consequences of photoperiodism for organisms in new climates. Ecological Applications 25(6): 1506–1517.
- Grove WB 1919. Species placed by Saccardo in the genus *Phoma*. Part II. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) 1919(10): 425–445.
- Grove WB 1935. British Stem- and Leaf-Fungi (Coelmycetes) / A Contribution to our Knowledge of the Fungi Imperfecti Belonging to the Sphaeropsidales and the Melanconiales / British Coelomycetes. Cambridge, Cambridge University Press. 488 p.
- Groves R, Sheppard AW, Julien M, McFadyen R, Cullen J 2012. *Carduus pycnocephalus* L.– slender thistle *Carduus tenuiflorus* Curt.–slender thistle. Biological Control of Weeds in Australia: 131–138.

Page 26 Landcare Research

- Habibi G, Ajory N 2015. The effect of drought on photosynthetic plasticity in *Marrubium vulgare* plants growing at low and high altitudes. Journal of Plant Research 128(6): 987–994.
- Hayes LM 2000. Technology transfer programs for biological control of weeds the New Zealand experience. In: Spencer NR ed. Proceedings of the X International Symposium on Biological Control of Weeds, Bozeman, Montana, USA, 4–14 July, 1999. Pp. 719–727.
- Heluta VP, Minter DW 1998. *Leveillula duriaei*. Descriptions of Fungi and Bacteria. IMI Descriptions of Fungi and Bacteria(138): Sheet 1374.
- Henderson L 1999. The Southern African Plant Invaders Atlas (SAPIA) and its contribution to biological weed control. In: Olckers T, Hill MP ed. Biological control of weeds in South Africa. Pretoria, South Africa, Entomological Society of Southern Africa. Pp. 159–163.
- Herrera-Arellano A, Aguilar-Santamaria L, Garcia-Hernandez B, Nicasio-Torres P, Tortoriello J 2004. Clinical trial of *Cecropia obtusifolia* and *Marrubium vulgare* leaf extracts on blood glucose and serum lipids in type 2 diabetics. Phytomedicine 11(7–8): 561–566.
- Higgins LG, Riley ND 1970. A Field Guide to the Butterflies of Britain and Europe, Houghton Mifflin.
- Hoffmann JH 1995. Biological control of weeds: the way forward, a South African perspective. In: Waage JK ed. Proceedings of the British Crop Protection Council Symposium. Pp. 77–98.
- Horst K 2013. Field Manual of Diseases on Grasses and Native Plants, Springer Netherlands. 179 p.
- Ibrahim AY, Hendawy SF, Elsayed AAA, Omer EA 2016. Evaluation of hypolipidemic *Marrubium vulgare* effect in Triton WR-1339 induced hyperlipidemia in mice. Asian Pacific Journal of Tropical Medicine 9(5): 439–445.
- Imani E, Saeedi S, Sahraei S, Azizi A, Khaje H 2014. Phytochemical analysis, antibacterial activity of *Marrubium vulgare* I against antibiotic resistance *Escherichia coli*. Iranian Journal of Public Health 43: 105–105.
- Index Fungorum 2004. World database of fungal names. Retrieved 02 Oct 2017 http://www.indexfungorum.org/Names/Names.asp
- Ireson J, Holloway R, Chatterton W 2000. Progress on the rearing, release and establishment of the horehound plume moth, *Wheeleria spilodactylus*. Plant Protection Quarterly 15(1): 33.
- Ivory S 2012. The establishment of the clearwing moth for horehound control in South Australia. In: Developing solutions to evolving weed problems. Proceedings of the 18th Australasian Weeds Conference, Melbourne, Victoria, Australia, 8–11 October 2012. Pp. 349–350.
- Izzo AA, Hoon-Kim S, Radhakrishnan R, Williamson EM 2016. A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies. Phytotherapy Research 30(5): 691–700.
- Juarez-Vazquez MD, Carranza-Alvarez C, Alonso-Castro AJ, Gonzalez-Alcaraz VF, Bravo-Acevedo E, Chamarro-Tinajero FJ, Solano E 2013. Ethnobotany of medicinal plants used in Xalpatlahuac, Guerrero, Mexico. Journal of Ethnopharmacology 148(2): 521–527.

- Kabaktepe S, Mutlu B, Karakus S, Akata I 2016. *Puccinia marrubii* (Pucciniaceae), a new rust species on *Marrubium globosum s*ubsp globosum from Nigde and Malatya in Turkey. Phytotaxa 272(4): 277–286.
- Kadri A, Zarai Z, Ben Chobba I, Bekir A, Gharsallah N, Damak M, Gdoura R 2011. Chemical composition and antioxidant activity of *Marrubium vulgare* L. essential oil from Tunisia. African Journal of Biotechnology 10(19): 3908–3914.
- Kanyonga P, Faouzi M, Meddah B, Mpona M, Essassi E, Cherrah Y 2011. Assessment of methanolic extract of *Marrubium vulgare* for anti-inflammatory, analgesic and anti-microbiologic activities. Journal of Chemical and Pharmaceutical Research 3(1): 199–204.
- Karadjova O, Krumov V 2015. Thysanoptera of Bulgaria. ZooKeys(504): 93-131.
- Khaled-Khodja N, Boulekbache-Makhlouf L, Madani K 2014. Phytochemical screening of antioxidant and antibacterial activities of methanolic extracts of some Lamiaceae. Industrial Crops and Products 61: 41–48.
- Khalil A, Dababneh BF, Al-Gabbiesh AH 2009. Antimicrobial activity against pathogenic microorganisms by extracts from herbal Jordanian plants. Journal of Food Agriculture & Environment 7(2): 103–106.
- Kunduhoglu B, Pilatin S, Caliskan F 2011. Antimicrobial screening of some medicinal plants collected from Eskisehir, Turkey. Fresenius Environmental Bulletin 20(4): 945–952.
- Landcare Research 2009. NZFUNGI New Zealand Fungi (and Bacteria). Retrieved 25 Aug 2017 http://nzfungi.landcareresearch.co.nz/html/search_hosts.asp
- Laštuvka Z, Laštuvka A 1995. An Illustrated Key to European Sesiidae (Lepidoptera). Brno, Association for Scientific and Technical Development, Faculty of Agriculture, Mendel University of Agriculture and Forestry. 173 p.
- Li B, Cantino PD, Olmstead RG, Bramley GL, Xiang CL, Ma ZH, Tan YH, Zhang DX 2016. A large-scale chloroplast phylogeny of the Lamiaceae sheds new light on its subfamilial classification. Scientific Reports 6: 34343.
- Lippai A, Smith PA, Price TV, Weiss J, Lloyd CJ 1996. Effects of temperature and water potential on germination of horehound (*Marrubium vulgare*) seeds from two Australian localities. Weed Science 44(1): 91–99.
- Louda SM, Arnett AE, Rand TA, Russell FL 2003. Invasiveness of some biological control insects and adequacy of their ecological risk assessment and regulation. Conservation Biology 17(1): 73–82.
- Maiti R, Rodriguez HG, Kumari CA, Sarkar NC 2016. Macro and micro-nutrient contents of 18 medicinal plants used traditionally to alleviate diabetes in Nuevo Leon, northeast of Mexico. Pakistan Journal of Botany 48(1): 271–276.
- Martin-Nizard F, Sahpaz S, Kandoussi A, Carpentier M, Fruchart JC, Duriez P, Bailleul F 2004. Natural phenylpropanoids inhibit lipoprotein-induced endothelin-1 secretion by endothelial cells. Journal of Pharmacy and Pharmacology 56(12): 1607–1611.
- Masoodi M, Ali Z, Liang S, Yin HQ, Wang W, Khan IA 2015. Labdane diterpenoids from *Marrubium vulgare*. Phytochemistry Letters 13: 275–279.

Page 28 Landcare Research

- Masoodi MH, Ahmed B, Zargar IM, Khan SA, Khan S, Singh P 2008. Antibacterial activity of whole plant extract of *Marrubium vulgare*. African Journal of Biotechnology 7(2): 86–87.
- Matkowski A, Piotrowska M 2006. Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. Fitoterapia 77(5): 346–353.
- Matkowski A, Tasarz P, Szypula E 2008. Antioxidant activity of herb extracts from five medicinal plants from Lamiaceae, subfamily Lamioideae. Journal of Medicinal Plants Research 2(11): 321–330.
- Mayo GM, Roush RT 1997. Genetic variability of *Hypericum perforatum* L. (Clusiaceae) and the detection of resistance to the biological control agent *Aculus hyperici* Liro (Eriophyidae). Plant Protection Quarterly 12(2): 70–72.
- McAlpine D 1904. Australian fungi, new or unrecorded. Decades VII-VIII. Proceedings of the Linnean Society of New South Wales 29: 125.
- McFadyen REC 1998. Biological control of weeds. Annual Review of Entomology 43: 369–393.
- Memmott J, Fowler SV, Hill RL 1998. The effect of release size on the probability of establishment of biological control agents: Gorse thrips (*Sericothrips staphylinus*) released against gorse (*Ulex europaeus*) in New Zealand. Biocontrol Science and Technology 8(1): 103–115.
- Memmott J, Craze PG, Harman HM, Syrett P, Fowler SV 2005. The effect of propagule size on the invasion of an alien insect. Journal of Animal Ecology 74(1): 50–62.
- Menendez R, Thomas CD 2000. Metapopulation structure depends on spatial scale in the host-specific moth *Wheeleria spilodactylus* (Lepidoptera: Pterophoridae). Journal of Animal Ecology 69(6): 935–951.
- Menéndez R, Thomas CD 2006. Can occupancy patterns be used to predict distributions in widely separated geographic regions? Oecologia 149(3): 396-405.
- Meyre-Silva C, Cechinel V 2010. A review of the chemical and pharmacological aspects of the genus *Marrubium*. Current Pharmaceutical Design 16(31): 3503–3518.
- Meyre-Silva C, Yunes R, Schlemper V, Campos-Buzzi F, Cechinel-Filho V 2005. Analgesic potential of marrubiin derivatives, a bioactive diterpene present in *Marrubium vulgare* (Lamiaceae). Il Farmaco 60(4): 321–326.
- Millan R, Sierra MJ, Schmid T, Gil SC, Ledesma DMS, Puent FJD 2013. Mercury distribution in an abandoned metallurgical plant. In: Pirrone N ed. Proceedings of the 16th International Conference on Heavy Metals in the Environment. le Ulis, France, EDP Sciences.
- Millan R, Schmid T, Sierra MJ, Carrasco-Gil S, Villadoniga M, Rico C, Ledesma DMS, Puente FJD 2011. Spatial variation of biological and pedological properties in an area affected by a metallurgical mercury plant: Almadenejos (Spain). Applied Geochemistry 26(2): 174–181.
- Millspaugh CF, Nuttall LW 1923. Flora of Santa Catalina island (California). Chicago, Field Museum of Natural History. 413 p.
- Moreno-Jimenez E, Penalosa JM, Esteban E, Carpena-Ruiz RO 2007. Mercury accumulation and resistance to mercury stress in *Rumex induratus* and *Marrubium vulgare* grown in

- perlite. Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 170(4): 485–494.
- Moreno-Jimenez E, Gamarra R, Carpena-Ruiz RO, Millan R, Penalosa JM, Esteban E 2006. Mercury bioaccumulation and phytotoxicity in two wild plant species of Almaden area. Chemosphere 63(11): 1969–1973.
- Namjoyan F, Jahangiri A, Azemi ME, Arkian E, Mousavi H 2015a. Inhibitory Effects of *Physalis alkekengi* L., *Alcea rosea* L., *Bunium persicum B. Fedtsch.* and *Marrubium vulgare* L on mushroom Tyrosinase. Jundishapur Journal of Natural Pharmaceutical Products 10(1): 6.
- Namjoyan F, Azemi ME, Abdollahi E, Goudarzi N, Nikan K 2015b. Angiotensin I converting enzyme inhibitory activities of hydroalcoholic extract of *Nardostachys jatamansi*, *Prangos ferulacea* and *Marrubium vulgare*. Jundishapur Journal of Natural Pharmaceutical Products 10(2): 5.
- Natural History Museum London 2007. HOSTS a Database of the World's Lepidopteran Hostplants. Retrieved 25 Aug 2017 http://www.nhm.ac.uk/our-science/data/hostplants/search/
- New Zealand Plant Conservation Network 2014a. *Mentha cunninghamii*. Retrieved 13 Aug 2017 http://www.nzpcn.org.nz/flora_details.aspx?ID=974
- New Zealand Plant Conservation Network 2014b. *Plectranthus parviflorus*. Retrieved 13 Aug 2017 http://www.nzpcn.org.nz/flora_details.aspx?ID=729
- New Zealand Plant Conservation Network 2014c. *Teucridium parvifolium*. Retrieved 13 Aug 2017 http://www.nzpcn.org.nz/flora_details.aspx?ID=207
- New Zealand Plant Conservation Network 2014d. *Scutellaria novae-zealandiae*. Retrieved 13 Aug 2017 http://www.nzpcn.org.nz/flora_search.aspx?scfSubmit=1&scfLatin_Name=Scutellaria+novae-zelandiae
- New Zealand Plant Conservation Network 2014e. *Vitex lucens*. Retrieved 13 Aug 2017 http://www.nzpcn.org.nz/flora_details.aspx?ID=1359
- Novak I, Riba Z, Kalisko A, Ferenczy A, Sarosi S 2010. Investigation of the germination capacity of some medicinal plant species after ten years of cold-storage. Zeitschrift Fur Arznei- & Gewurzpflanzen 15(2): 76–80.
- Ohtera A, Miyamae Y, Nakai N, Kawachi A, Kawada K, Han J, Isoda H, Neffati M, Akita T, Maejima K and others 2013. Identification of 6-octadecynoic acid from a methanol extract of *Marrubium vulgare* L. as a peroxisome proliferator-activated receptor gamma agonist. Biochemical and Biophysical Research Communications 440(2): 204–209.
- Orhan IE, Belhattab R, Senol FS, Gulpinar AR, Hosbas S, Kartal M 2010. Profiling of cholinesterase inhibitory and antioxidant activities of *Artemisia absinthium*, *A. herba-alba*, *A. fragrans*, *Marrubium vulgare*, *M. astranicum*, *Origanum vulgare* subsp *glandulossum* and essential oil analysis of two *Artemisia* species. Industrial Crops and Products 32(3): 566–571.
- Ortega-Ramirez LA, Rodriguez-Garcia I, Leyva JM, Cruz-Valenzuela MR, Silva-Espinoza BA, Gonzalez-Aguilar GA, Siddiqui MW, Ayala-Zavala JF 2014. Potential of medicinal plants

Page 30 Landcare Research

- as antimicrobial and antioxidant agents in food industry: A hypothesis. Journal of Food Science 79(2): R129–R137.
- Oudemans CAJA 1923. Enumeratio systematica fungorum. Divisio XIII: subdivio II angiospermae, classis: Dicotyledoneae. Subclassis: Archichlamydeae Series Malvales. ser. Umbelliflorae et subclassis: Metachlamydeae. Supplementum: additamenta ad vol. I–III. The Hague, Netherlands, Hagae Comitum: Apud Martinum Nijhoff. 1231 p.
- Page AR, Lacey KL 2006. Economic impact assessment of Australian weed biological control. Technical Series 10. 150 p.
- Parsons WT, Cuthbertson EG 2000. Noxious weeds of Australia. 2nd ed. Melbourn, Inkata Press. 692 p.
- Pastircakova K, Ivanova H, Bernadovicova S 2008. *Neoerysiphe galeopsidis* on *Stachys* species in Slovakia and the Czech Republic based on a re-examination of herbarium collections. Czech Mycology 60(2): 251–264.
- Paunovic V, Kosic M, Djordjevic S, Zugic A, Djalinac N, Gasic U, Trajkovic V, Harhaji-Trajkovic J 2016. *Marrubium vulgare* ethanolic extract induces proliferation block, apoptosis, and cytoprotective autophagy in cancer cells in vitro. Cellular and Molecular Biology 62(11): 108–114.
- Pavela R 2004. Insecticidal activity of certain medicinal plants. Fitoterapia 75(7-8): 745–749.
- Paynter Q, Flanagan GJ 2004. Integrating herbicide and mechanical control treatments with fire and biological control to manage an invasive wetland shrub, *Mimosa pigra*. Journal of Applied Ecology 41(4): 615–629.
- Paynter Q, Fowler SV, Hayes L, Hill RL 2015. Factors affecting the cost of weed biocontrol programs in New Zealand. Biological Control 80: 119–127.
- Paynter Q, Martin N, Berry J, Hona S, Peterson P, Gourlay AH, Wilson-Davey J, Smith L, Winks C, Fowler SV 2008. Non-target impacts of *Phytomyza vitalbae* a biological control agent of the European weed *Clematis vitalba* in New Zealand. Biological Control 44(2): 248–258.
- Paynter QE, Fowler SV, Gourlay AH, Haines ML, Harman HM, Hona SR, Peterson PJ, Smith LA, Wilson-Davey JRA, Winks CJ and others 2004. Safety in New Zealand weed biocontrol: a nationwide survey for impacts on non-target plants. New Zealand Plant Protection 57: 102–107.
- Pemberton RW 2000. Predictable risk to native plants in weed biological control. Oecologia 125(4): 489–494.
- Perez-Cruz F, Cortes C, Atala E, Bohle P, Valenzuela F, Olea-Azar C, Speisky H, Aspee A, Lissi E, Lopez-Alarcon C and others 2013. Use of Pyrogallol Red and Pyranine as probes to evaluate antioxidant capacities towards Hypochlorite. Molecules 18(2): 1638–1652.
- Piccoli PN, Bottini R 2008. Accumulation of the labdane diterpene Marrubiin in glandular trichome cells along the ontogeny of *Marrubium vulgare* plants. Plant Growth Regulation 56(1): 71–76.
- Pieroni A, Quave C, Nebel S, Heinrich M 2002. Ethnopharmacy of the ethnic Albanians (Arbereshe) of northern Basilicata, Italy. Fitoterapia 73(3): 217–241.
- Piozzi F, Bruno M, Rosselli S, Maggio A 2006. The diterpenoids of the genus *Marrubium* (Lamiaceae). Natural Product Communications 1(7): 585–592.

- Pitkin B, Ellis WN, Plant C, Edmunds R 2016. The leaf and stem mines of British flies and other insects (Coleoptera, Diptera, Hymenoptera and Lepidoptera). Retrieved 27 Sep 2017 http://www.ukflymines.co.uk/
- Plantwise 2017. Plantwise Technical Factsheet. Onion leaf blight (*Stemphylium vesicarium*). Retrieved 21 Aug 2017 http://www.plantwise.org/KnowledgeBank/Datasheet.aspx?dsid=42014
- Pukalskas A, Venskutonis PR, Salido S, de Waard P, van Beek TA 2012. Isolation, identification and activity of natural antioxidants from horehound (*Marrubium vulgare* L.) cultivated in Lithuania. Food Chemistry 130(3): 695–701.
- Quave CL, Plano LRW, Pantuso T, Bennett BC 2008. Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. Journal of Ethnopharmacology 118(3): 418–428.
- Rabenhorst L 1850. Systematische uebersicht der auf meiner Italienischen reise beobachteten Kryptogamen. Flora 33: 626.
- Redzic SS 2007. The ecological aspect of ethnobotany and ethnopharmacology of population in Bosnia and Herzegovina. Collegium Antropologicum 31(3): 869–890.
- Rezgui M, Hosni K, Majdoub N, Ben Kaab S, Marzouk B, Gouia H, Ben Kaab LB 2016. Effect of long-term salinity on compatible solutes and antioxidant status of horehound (*Marrubium vulgare* L.). Agrochimica 60(3): 161–172.
- Rezgui M, Majdoub N, Ben-Kaab S, Marzouk B, Gouia H, Araujo MEM, Ben-Kaab LB 2017. How salt stress represses the biosynthesis of marrubiin and disturbs the antioxidant activity of *Marrubium vulgare* L. Polish Journal of Environmental Studies 26(1): 267–277.
- Robles-Zepeda RE, Velazquez-Contreras CA, Garibay-Escobar A, Galvez-Ruiz JC, Ruiz-Bustos E 2011. Antimicrobial activity of northwestern Mexican plants against *Helicobacter pylori*. Journal of Medicinal Food 14(10): 1280–1283.
- Rodriguez Villanueva J, Martin Esteban J 2016. An insight into a blockbuster phytomedicine; *Marrubium vulgare* L. Herb. More of a myth than a reality? Phytotherapy Research 30(10): 1551–1558.
- Rodríguez Villanueva J, Martín Esteban J, Rodríguez Villanueva L 2017. A reassessment of the *Marrubium vulgare* L. herb's potential role in diabetes mellitus type 2: First results guide the investigation toward new horizons. Medicines 4(3): 57.
- Roman RR, Alarcon-Aguilar F, Lara-Lemus A, Flores-Saenz J 1992. Hypoglycemic effect of plants used in Mexico as antidiabetics. Archives of medical research 23(1): 59–64.
- Sabry R, Salama A, Sharaf-Eldin M 2012. Herbage growth, yield and oil content of *Marrubium vulgare* grown in Egypt as affected by plant spacing and nitrogen fertilization.

 Zeitschrift Fur Arznei- & Gewurzpflanzen 17(4): 164–168.
- Saeidi S, Boroujeni NA, Ahmadi H, Hassanshahian M 2015. Antibacterial activity of some plant extracts against extended-spectrum beta-lactamase producing *Escherichia coli* isolates. Jundishapur Journal of Microbiology 8(2): 6.
- Sagliocco J-L, Coupland JB 1995. Biology and host specificity of *Chamaesphecia mysiniformis* (Lepidoptera: Sesiidae), a potential biological control agent of *Marrubium vulgare* (Lamiaceae) in Australia. Biocontrol Science and Technology 5(4): 509–516.

Page 32 Landcare Research

- Sagliocco J 2000. The insect fauna associated with horehound (*Marrubium vulgare* L.) in western Mediterranean Europe and Morocco: potential for biological control in Australia. Plant Protection Quarterly 15(1): 21–25.
- Sahpaz S, Garbacki N, Tits M, Bailleul F 2002. Isolation and pharmacological activity of phenylpropanoid esters from *Marrubium vulgare*. Journal of Ethnopharmacology 79(3): 389–392.
- Salama MM, Taher EE, El-Bahy MM 2012. Molluscicidal and mosquitocidal activities of the essential oils of *Thymus capitatus* Hoff. Et Link. and *Marrubium vulgare* L. Revista Do Instituto De Medicina Tropical De Sao Paulo 54(5): 281–286.
- Sas-Piotrowska B, Piotrowski W 2011. Vitality and healthiness of cereal grains treated with plant decoctions. Rocznik Ochrona Srodowiska 13: 571–595.
- Savele M 1999. Lepidoptera and some other life forms. Retrieved 25 Aug 2017
- http://www.nic.funet.fi/pub/sci/bio/life/insecta/lepidoptera/ditrysia/gelechioidea/coleophori dae/coleophora/#lineolea
- Schlemper V, Ribas A, Nicolau M, Cechinel V 1996. Antispasmodic effects of hydroalcoholic extract of *Marrubium vulgare* on isolated tissues. Phytomedicine 3(2): 211–216.
- Seastedt TR 2015. Biological control of invasive plant species: a reassessment for the Anthropocene. New Phytologist 205(2): 490–502.
- Sheppard A, Briese D, Cullen J, Groves R, Julien M, Lonsdale W, Scott J, Wapshere A 2008. Fortieth anniversary review of the CSIRO European Laboratory: does native range research provide good return on investment? Proceedings of the XIIth International Symposium on Biological Control of Weeds. Wallingford, UK, CAB International. Pp. 91–98.
- Shoemaker RA 1976. Canadian and some extralimital *Ophiobolus* species. Canadian Journal of Botany 54(20): 2365–2404.
- Somerfield K 1977. Insects of economic significance recently recorded in New Zealand. New Zealand journal of agricultural research 20(3): 421–428.
- Sopow SL, Bain J 2017. A checklist of New Zealand Cerambycidae (Insecta: Coleoptera), excluding Lamiinae. New Zealand Entomologist: 1-17.
- Stankovic MS, Radic ZS, Blanco-Salas J, Vazquez-Pardo FM, Ruiz-Tellez T 2017. Screening of selected species from Spanish flora as a source of bioactive substances. Industrial Crops and Products 95: 493–501.
- Stulzer HK, Tagliari MP, Zampirolo JA, Cechinel V, Schlemper V 2006. Antioedematogenic effect of marrubiin obtained from *Marrubium vulgare*. Journal of Ethnopharmacology 108(3): 379–384.
- Suckling DM 2013. Benefits from biological control of weeds in New Zealand range from negligible to massive: A retrospective analysis. Biological Control 66(1): 27–32.
- Suckling DM, Sforza RFH 2014. What magnitude are observed non-target impacts from weed biocontrol? Plos One 9(1): e84847.
- Tharp BC 1917. Texas Parasitic Fungi. Mycologia 9(2): 105–124.
- Thomas DB, Eger JE, Jones W, Ortega-Leon G 2003. The African cluster bug, *Agonoscelis puberula* (Heteroptera : Pentatomidae), established in the new world. Florida Entomologist 86(2): 151–153.

- Thomas V 2011. Do modern-day medical herbalists have anything to learn from Anglo-Saxon medical writings? Journal of Herbal Medicine 1(2): 42–52.
- Tobler G 1913. Die Synchytrien. Archiv für Protistenkunde 28: 81–82.
- van Klinken RD, Fichera G, Cordo H 2003. Targeting biological control across diverse landscapes: the release, establishment, and early success of two insects on mesquite (*Prosopis* spp.) insects in Australian rangelands. Biological Control 26(1): 8–20.
- van Klinken RD, Morin L, Sheppard A, Raghu S 2016. Experts know more than just facts: eliciting functional understanding to help prioritise weed biological control targets. Biological Invasions 18(10): 2853–2870.
- van Swaay C, Wynhoff I, Verovnik R, Wiemers M, López Munguira M, Maes D, Sasic M, Verstrael T, Warren M, Settele J 2010. *Carcharodus boeticus*. The IUCN Red List of Threatened Species 2010. Retrieved 20 September 2017 http://www.iucnredlist.org/details/173217/0
- Warda K, Markouk M, Bekkouche K, Larhsini M, Abbad A, Romane A, Bouskraoui M 2009. Antibacterial evaluation of selected Moroccan medicinal plants against *Streptococcus pneumoniae*. African Journal of Pharmacy and Pharmacology 3(3): 101–104.
- Watkins F, Pendry B, Corcoran O, Sanchez-Medina A 2011. Anglo-Saxon pharmacopoeia revisited: a potential treasure in drug discovery. Drug Discovery Today 16(23–24): 1069–1075.
- Webb CJ, Sykes WR, Garnock-Jones PJ, Given DR 1988. Flora of New Zealand. Volume IV, Naturalised Pteridophytes, Gymnosperms, Dicotyledons. Christchurch, N.Z., Botany Division D.S.I.R. 1365 p.
- Weel KGC, Venskutonis PR, Pukalskas A, Gruzdiene D, Linssen JPH 1999. Antioxidant activity of horehound (*Marrubium vulgare* L.) grown in Lithuania. Fett-Lipid 101(10): 395–400.
- Weiss J, Shepherd R 1996. Control of horehound, *Marrubium vulgare* I., in Wyperfeld National Park, Victoria. Proceedings of the 11th Australian Weeds Conference, Melbourne, Australia, 30 September-3 October 1996. Pp. 417–419.
- Weiss J, Wills E 2000a. Integrated management of horehound (*Marrubium vulgare* L.) in Wyperfeld National Park. Plant Protection Quarterly 15(1): 40–42.
- Weiss J, Sagliocco J 2000. Horehound (*Marrubium vulgare*): a comparison between European and Australian populations. Plant Protection Quarterly 15(1): 18–20.
- Weiss J, Wills E 2000b. Outcomes from the horehound workshop. Plant Protection Quarterly 15(1): 43–43.
- Weiss J, Sagliocco J-L 2012. *Marrubium vulgare* L.–horehound. In: Julien M, McFadyen R, Cullen J ed. Biological Control of Weeds in Australia. Pp. 360–367.
- Weiss J, Sagliocco J-L, Wills E 1999. A comparison between European and Australian populations of Horehound, (*Marrubium vulgare* L.). 12th Australian Weeds Conference, Papers and Proceedings, Hobart, Tasmania, Australia, 12–16 September 1999: Weed management into the 21st century: do we know where we're going? Pp. 596–600.
- Wills E 2000. The release and establishment of two biological control agents of horehound (*Marrubium vulgare* L.) in south-eastern Australia. Plant Protection Quarterly 15(1): 26–28.

Page 34 Landcare Research

- Xue XF, Sadeghi H, Honarmand A 2016. Three eriophyoid mite species (Acari: Eriophyoidea: Eriophyidae) from Iran. Zootaxa 4132(3): 403–412.
- Young JA, Evans RA 1986. Germination of white horehound (*Marrubium vulgare*) seeds. Weed Science 34(2): 266–270.
- Yousefi K, Hamedeyazdan S, Torbati M, Fathiazad F 2016. Chromatographic fingerprint analysis of marrubiin in *Marrubium vulgare* L. via HPTLC technique. Advanced Pharmaceutical Bulletin 6(1): 131–136.
- Yousefi K, Soraya H, Fathiazad F, Khorrami A, Hamedeyazdan S, Maleki-Dizaji N, Garjani A 2013. Cardioprotective effect of methanolic extract of *Marrubium vulgare* L. on isoproterenol-induced acute myocardial infarction in rats. Indian Journal of Experimental Biology 51(8): 653–660.
- Zarai Z, Kadri A, Ben Chobba I, Ben Mansour R, Bekir A, Mejdoub H, Gharsallah N 2011. The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of *Marrubium vulgare* L. essential oil grown in Tunisia. Lipids in Health and Disease 10: 8.
- Zawislak G 2012. The chemical composition of the essential oil of *Marrubium vulgare* L. From Poland. Farmacia 60(2): 287–292.
- Zheljazkov VD, Jeliazkova EA, Kovacheva N, Dzhurmanski A 2008. Metal uptake by medicinal plant species grown in soils contaminated by a smelter. Environmental and Experimental Botany 64(3): 207–216.

Appendix 1 – Pharmacological uses of *M. vulgare* vs. evidence to support these uses

Based on Rodriguez Villanueva and Martin Esteban (2016)

Pharmacological action	Evidence
Anti-inflammatory & analgesic (pain reliever)	Administration in the form of marrubiinic acid was shown to be more bioavailable, but less potent than marrubiin (Meyre-Silva et al. 2005)
Antispasmodic (muscle spasm reliever) & vasorelaxant (reduce tension in the walls of blood vessels)	A high dose is required to gain antispasmodic effect (Schlemper et al. 1996; De Jesus et al. 2000; Kanyonga et al. 2011). Vasorelaxant activity is less potent than the medicine verapamil (El Bardai et al. 2003).
Gastroprotective	It is possible that the bitterness of the essential oil marrubiin stimulates gastric secretion and bile by interacting with receptors on the taste buds, stomach and intestine. Larger trials are required to confirm the gastroprotective activity (Rodriguez Villanueva & Martin Esteban 2016).
Hepatoprotective (protecting from damage to the liver)	Akther et al. (2013) confirmed liver tissue repair of damage from chronically-administered high doses of paracetamol.
Antidiabetic & hypocholesterolemic	Studies in rats achieved lowered blood glucose, increased insulin in plasma and boosted glycogen synthesis in the liver and muscles (Elberry et al. 2015), cholesterol levels were improved in a culture of macrophages (Berrougui et al. 2006) and in mice (Ibrahim et al. 2016), but none of these effects was replicated in a clinical study with type II diabetic patients, who showed only mild improvements in cholesterol and triglyceride levels, and no improvement in blood glucose levels (Herrera-Arellano et al. 2004).
Antihypertensive	Reduced aortic hypertrophy achieved in rats (El Bardai et al. 2004b; El Bardai et al. 2004a) was not possible to replicate in clinical trials in humans (Herrera-Arellano et al. 2004).
Antioxidant	While one study found high antioxidant activity (Amessis-Ouchemoukh et al. 2014a), a later study found only moderate antioxidant activity (Stankovic et al. 2017).

Antimicrobial & antifungal	For the most part, studies on antimicrobial and antifungal activities of M. vulgare lack in details on methodology and produce contradictory results. Only one study (Robles-Zepeda et al. 2011) which recorded antiseptic activity against <i>Helicobacter pylori</i> is considered reliable.
Antiasthmatic & expectorant (for treatment of coughs)	While these uses are common in traditional herbal medicine, they have not been subjected to clinical trials (Rodriguez Villanueva & Martin Esteban 2016).
Neuroprotection	A single study (Orhan et al. 2010) showed in vitro inhibition of acetylcholinesterase and butylcholinesterase (associated with Alzheimer's Disease). No other studies are available (Rodriguez Villanueva & Martin Esteban 2016).
Toxicity	Trials in rats have not shown acute toxicity (citations in Rodriguez Villanueva & Martin Esteban 2016). No adequate trials to test teratogenicity (disturbed development of embryo or foetus) have been published (Rodriguez Villanueva & Martin Esteban 2016).
Adverse effects, contradictions and precautions	No clinical trials have been carried out on children, older adults and pregnant & breastfeeding women, so use is not recommended for these groups (Rodriguez Villanueva & Martin Esteban 2016). High tannin content in M. vulgare may lead to decrease in absorption of other medication, minerals, and thiamine (Rodriguez Villanueva & Martin Esteban 2016).

Appendix 2 – Letters of opposition to the biocontrol programme against *M. vulgare*

Hi my name is Stephen Parker, a PhD candidate at Otago University. I am also an endangered species Biodiversity ranger for the Department of Conservation, a Herbal Medicine making tutor at Otago University, and the co-owner of a small, Dunedin based herbal tonic company, Wild Dispensary. I was just wondering if I could make an objection to the proposed introduction of a moth to eradicate Horehound.

The issue at play here seems to be what, we as a culture, term to be a weed or pest species and what other introduced species we view as not being pests. As a conservationist, I may view sheep as a pest species, a species that is eating native plants, and stopping the regeneration of native forest, but to a farmer these sheep are his/her livelihood, they are a "good" introduced pest. As a herbalist, I view Horehound not as a pest species, or a weed, but as a valuable economic and also culturally significant medicinal plant, a plant that my European ancestors have being using for medicine for thousands of years. Harvesting wild horehound, to me, is also important to my livelihood. But because I will not make as much money from either teaching people how to use this medicinal plant as medicine, or through selling herbal tonics, my view of horehound as a "good" introduced species will likely not have as much weight as the farming voice trying to protect their introduced species from getting the seeds of this introduced species in its wool!

Before rushing in to introduce another bio control, one that I see got \$285,450 funding from the National Government, I am curious about what other alternatives to sustainably control horehound have been explored? Would it be cheaper, more sustainable and also provide employment opportunities to have students from Otago manually grub out horehound instead of the current spray regime or the introduction of a bio control? This option would connect urban people with rural people and perhaps foster some understanding into each other's beliefs. Also how do we know what the future economic benefits of horehound may be? The demand for quality wild harvested medicinal plants is increasing; while at the same time all around the world the places to gather these wild plants are under pressure from pollution and also urban sprawl. I am in no position to provide an economic cost benefit analysis of horehound as I am immersed in the middle of a PhD that actually addresses rural masculinity in New Zealand and male health and well-being. But all I can really say that is our small herb company hopes to expand, and to build on New Zealand's somewhat quickly diminishing clean green image, and to eventually export tonics containing horehound overseas. I believe horehound is the best wild grown expectorant herb we have in New Zealand, it is possibly the best, most potent horehound in the world. It is the herb I first look to for making tonics to expel phlegm and also as a bitter tonic, it is amazing! I am also interested if craft brewers have been consulted? Horehound has a long history of traditional use as beer bittering agent, and in my opinion it is a superior ingredient than the current trend of using hops, that as we know, is high in phytoestrogens and is definitely something, that ageing New Zealand men especially, should be limiting their intake of.

I am also worried how far the bio control will travel. If the moths were released in the McKenzie country would they eventually make it to places like Central Otago Banks Peninsula or the Otago Peninsula? I really want to be assured that there will still be places to wild harvest this valuable medicinal plant. Others points of view need to be included in this

argument, we need to consider why we prioritise the beliefs of a certain group over an others, does it simply just come down to a matter of economics?

Horehound, much like Elder, St Johns Wort, Thyme, California poppy, Rosehip, Mullein, are all taonga to me; they are valuable medicinal plants. They are part of my heritage as a New Zealander of European origin, as a seventh generation Pakeha. I, like horehound, am an introduced species. I believe there has to be some point in time that we decide we need to adapt to the ecology of the places we live in, to work with the plants and animals that we have, to stop waging war on the environment. I believe all these medicinal plants are here for a reason; maybe it is time to slow down, to take time to learn about these plants, to become grateful for the gifts that they bestow.

Stephen Parker		
Dear Dr Groenteman	 	

Re. Biocontrol of Marrubian vulgare

I am opposed to the introduction of the moth to control the *Marrubian vulgare* (Horehound).

I live in Upper Moutere, Nelson Tasman.

I am a practicing Herbal Medicine Practitioner, employed as a Medical Herbalist and have been a member of the NZAMH since 2002.

Marrubian vulgare grows wild on our property however it is not overly abundant and I do harvest any that I find both here and when out in surrounding areas. It is the main herb in my approach to treating adults, child and infants with coughs, colds, congestion and post nasal drip.

I use it as an infusion, in herbal tinctures, and syrups.

Nationally it is a commonly used herb in health shop preparations and supplements. Globally it has been the number one seller for the third year in a row. (Ref American Botanical Council (2016)).

These plants are the basis of traditional medicine and have been used for many hundreds or even thousands of years. It is an extremely safe plant to use thus it's popular use in modern day remedies.

Please reconsider the control of this plant and I would suggest that control of *Coriaria arborea* (tutu) would be of better resource use.

Kind regards

Krista Eaton Medical Herbalist Nutritionist Massage Therapist

Page 40 Landcare Research

Member NZAMH

October 30th, 2016

Dear Ronny,

Re. Biocontrol of Marrubian vulgare.

I am opposed to the introduction of the moth to control the *Marrubian vulgare* (Horehound).

My name is Crystal Epps, I work as a Herbal Medicine Practitioner and Educator in Waikino, Waihi

It is a valuable, go to, herb in the treatment of people of all ages with coughs and pulmonary congestion and post nasal drip.

Infused in Otago Thyme honey it is second to none!

Globally in the 'mainstream market it has been the number one seller for the third year in a row. (Ref American Botanical Council (2016)).

These wild plants are invaluable, it often takes a 'mindshift' to see a problem as a gain. And then managing it appropriately, to benefit ALL.

It really seems a terrible and tragic thing to eradicate such a wonderful, useful, available herb, that hopefully in future, can be entrenched in the public mindset, as free, available, and valuable medicine – because BY GOD, we need it NOW and will in the future to come. Please SAVE all the wild HERBS for everyones good.

The nasty Roundup ad on TV, where the amazing Dandelion gets poisoned, is sadly indicative of Public knowledge. That all needs to change, if CANCER is to remain at 1 in 3 or 4 deaths, as it is at the moment, and not climb to 1 in 2. - Where the Roundup causes cancer, and Dandelion helps to cure it.

Good ole Dandelion is a magic herb for Cancer, liver, kidneys, cleansing the blood, lowering Blood Pressure, and much more.

Sorry, thats my rant.

With thanks,

Crystal Epps. Dip Herb Med, NZAMH



October 18th, 2016

Re. Biocontrol of Marrubian vulgare

I am opposed to the introduction of the moth to control the *Marrubian vulgare* (Horehound).

I live in the lower Pisa Range off the Luggate-Cromwell Highway. I work as a Herbal Medicine Practitioner and Educator.

Marrubian vulgare grows wild on my property. It is the mainstay of my approach to treating people of all ages with coughs and pulmonary congestion and post nasal drip. Infused in local Thyme honey it is second to none.

Globally in the 'mainstream market it has been the number one seller for the third year in a row. (Ref American Botanical Council (2016)).

The land we live on is dry land, has been impacted on by rabbits and by reducing one opportunistic plant another in my experience will si[m]ply take its place.

These wild plants are invaluable, it often takes a 'mindshift' to see a problem as a gain.

With thanks,

Isla M Burgess (MSc)

Director www.HerbCollege.com

P.O. Box 103, Wanaka, 9343, New Zealand

Tel 0210552400

www.HerbCollege.com

Page 42 Landcare Research



8th February, 2017

Artemis

16 Fingall Street Dunedin 9012 New Zealand

Ph: +64 3 456 1551 Free Fax: 0508 278 364 Fax International: +64 3 456 1552 Email: info@artemis.co.nz Website: www.artemis.co.nz

Dear Ronny,

We have been made aware of a proposal for the possible introduction of a Horehound moth to control spread of *Marrubium vulgare* (Horehound) throughout the country. As a traditional plant medicine company, this is a concern for us.

Artemis is a leading New Zealand traditional medicine company. Our business model is based on using New Zealand naturalized and native plants wherever possible. Our plant medicine products are sought after which is reflected in our double-digit growth year on year. We won the Deloitte Fast50 award as the fastest growing manufacturer in the lower South Island in 2012 and were named as a top10 fastest growing business in New Zealand in the 2016 Westpac awards.

Our key area for harvest medicinal plants is Central Otago, although we harvest in other areas as well. Horehound has featured heavily in our growth projectory curve as a key ingredient in one of our hero products. We have other products under development that will also use Central Otago Horehound. We harvest on private land that is deemed to be otherwise unproductive (not because of the Horehound). The access agreements are of a commercial nature.

Horehound is highly valued for its expectorant and spasmolytic properties, and is particularly useful in respiratory health formulas. As an important medical plant it has significant economic value worldwide. In 2016, the American Botanical Council in 2016 confirmed that Horehound (Marrubium vulgare) was the top-selling herbal dietary supplement in mainstream retail outlets for the third year in a row with sales totalling almost \$115 million and continued growth from 2014 to 2015 of over 8%1. This is separate to selling this medicinal plants via other channels.

¹ (http://cms.herbalgram.org/herbalgram/issue111/hg111-mktrpt.html)

As previously mentioned, Horehound has been used since ancient times to relieve upper respiratory symptoms. Today, Horehound commonly is incorporated into lozenges and cough medicines for this same purpose.

Horehound is listed in various official international pharmacopoeias which underpins its significance as a medicinally important plant. Plants listed in these pharmacopoeias are deemed as essential medicines. Horehound is also featured in the newly released and legally binding European Community Monographs as well as in the ESCOP and Commission E monographs which further strengthens its importance as a medicinal plant.

When harvesting, most medical plant experts seek to source un-polluted, wild-crafted plants. We do this by looking in the very same areas that farmers are wishing to limit the existence of this plant.

We appreciate that farmers do have different agendas to our own, and are pleased to see that alternative methods to insecticides and other poisonous agents are explored. However, we do not wish to see Horehound completely removed from all areas or made inaccessible from the environment. Once bio-controls are released into the environment, they cannot be recalled. This has long lasting consequences for the ability to crop medicinal plants.

We have seen the effects of bio-control on St John's Wort, another worldwide top selling medicinal plant, via the St. John's Wort beetle. As harvesters of wild St. John's Wort, we have witnessed the results of this form of bio-control and the implications are of deep concern to us. This highly effective and valuable medicinal herb is now much less present in Central Otago. This serves to restrict the potential for development of new areas of plant medicine, that would not only be of benefit to users of these medicines but as a potential new source of income for farmers.

We would not like to see this same situation replicated with Horehound. We vigorously oppose the release of the Horehound moth without taking into consideration the wider impact this has on other commercial enterprises and on the ability to sustainably source medicines for primary healthcare in our communities.

To this end, we would appreciate if you could provide any information about the proposed introduction of the moth(s) to control Horehound, the anticipated impact of the this form of bio-control, proposed future plans for use and areas involved in the trial.

We would also seek to ascertain what consultation has been undertaken with local iwi and Rongōa practitioners as Horehound is an important plant in traditional Maori medicine and any decimation of this source of medicine will impact on their ability to care for patients. Removal of this medicine from the environment would be juxtapoints to indigenous rights and also to the

Page 44 Landcare Research

objectives of the WHO Traditonal Medicine Strategy to which New Zealand is a signatory.

Lastly, we would like to know how the release of the Horehound moth sits with New Zealand's obligations as a signatory to the international *Convention on Biological Diversity* (CBD), an international agreement of the UN Convention on Biodiversity. The objectives of CBD are spelled out in Article 1:

"The objectives or the Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of appropriate technologies, taking into account all rights over those resources and to technologies, and by appropriate funding"².

Whilst the convention recognises the sovereign rights of countries to control utilisation of their natural resources and genetic materials, we would like to see what analysis you have undertaken to fulfil the objectives of this convention, and to quantify the impact of the proposed release of the Horehound moth on the ability to harvest this essential medicinal plant for medicinal and commercial purposes.

Once we have reviewed the information we will be better placed to fully understand the situation and make further comment on the proposal.

Υοι	ırs	fc	tir	hf	ιıΙ	lν

Sandra Clair

M.A., Post. Grad. Dip. Health Science, PhD Candidate

ARTEMIS Founder & Director

² Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med, 27(1), 1-93, p.86. doi: 10.1016/j.mam.2005.07.008



HERB FEDERATION OF NEW ZEALAND P.O. Box 546, Feilding 4740

Contact: Christine Tuffnell, President. (03) 546 7907,

email:Christine.tuffnell@xtra.co.nz

SUBMISSION TO NEW ZEALAND LANDCARE RESEARCH RE PLAN TO CONTROL HOREHOUND VIA BIOCONTROL.

SUBMITTED 12 JUNE 2017

1. THE REQUEST FARMERS SEEK BIOCONTROL FOR SMELLY WEED Monday 15 Aug 2016



Horehound.

A high country sheep farmer Gavin Loxton, on his 8000 hectare property, Sawdon Station, in Lake Tekapo – has formed the Horehound Biocontrol Group, which is working with Landcare Research, in a bid to introduce to New Zealand two moths that are proving successful biocontrol agents against the weed in Australia. Moths were introduced as biocontrol agents in Australia in 1994. He asserts that Horehound reduces lucerne yields, wool quality(due to seed burrs) and taints meat. Loxton believes a few decent droughts over recent years had assisted the weed. With New Zealand drylands predicted to become even drier he's concerned the weed will become worse.

The Horehound Biocontrol Group has applied and been granted funds for a Biocontrol Feasibility Study from the Ministry for Primary Industries' Sustainable Farming Fund.

He says to date use of herbicides leave residual chemicals that stunt the lucerne and significantly reduce yields as much as 30 per cent. If it happens to coincide with a dry year you can also lose a lot of lucerne plants. The herbicides were also difficult and costly to apply in high terrain, Loxton said.

Dr Groenteman of Biocontrol in Landcare Research NZ said introducing the moths, one which feeds on the weed's foliage and the other the roots, should prove be a "relatively straight-forward undertaking" given the extensive safety testing carried out in Australia before it was introduced there The first biocontrol agent could be introduced in as little as two years, with the cost estimated to be around \$400,000.

Page 46 Landcare Research

2. HERB FERDERATION OF NEW ZEALAND

The Herb Federation of New Zealand Incorporated is the umbrella entity for sixteen Herb Societies throughout New Zealand, seven Herb Groups in the Auckland region and 136 individual members in various parts of New Zealand.

The objects of the Federation are:

- (1) To promote education in the cultivation and use of herbs amongst the general public through the production of written educational materials, coordination of national programmes, presentation of public workshops and any other relevant activities.
- (2) To promote co-operation and encourage unity among herb groups throughout the country and to assemble and disseminate information of interest to members from sources within and beyond New Zealand using appropriate means of communication.
- (3) To speak to Government through a national body and to promote or oppose legislative bills or other measures or programmes relating to herbs and allied fields.

2. HERB FERDERATION OF **NEW ZEALAND'S OPPO**SITION TO BIOCONTROL OF HOREHOUND

(1) HOREHOUND (MARRUBIUM VULGARE) – LONG HISTORY OF MEDICINALUSE

Marrubium vulgare (White Horehound) is a member of the mint family (Labiatae) and is native to Europe and now naturalized in North and South America. It is a square stemmed perennial growing to about $50 \,\mathrm{cm}$ in height. It has toothed downy leaves and double-lipped white flowers. It contains a volatile oil 0.6% in the leaves which give the plant a smell. It also contains diterpenes marrubiin (0.3-1.0%). Other diterpenoids include marrubenol. Peregrinol, and vulgarol. The plant also contains flavoinoids, and alkaloids (including betonicine and stachidrine) Marrubiin is considered to be the main expectorant principle in this plant but the volatile oil also contributes to the spasmolytic, expectorant, and vasodilatory activities.

Reference:

Ben-Erik van Wyk and Michael Wink. (2012) Medicinal Plants of the World. Briza Publications, South Africa. Pp198.

Marrubium vulgare has been used medicinally since ancient times. The Greek physician Dioscorides (AD 40-90) recommended a decoction of the leaves for tuberculosis, asthma, and coughs.

In 1597 the herbalist John Gerard praised white horehound as "a most singular remedy against cough and wheezing". (Andrew Chevallier (1997) The Encyclopedia of Medicinal Plants. Dorling Kindersley, London.pp 231.) White horehound's bitter constituents stimulate the flow of bile from the gall bladder and thus aid digestion. (David Hoffmann (1996) The Completed Illustrated Holistic Herbal. Element Books, Brisbane. Pp112.) In New Zealand, family doctor James Neil, in 1891, first published his guide to recipes and herbal remedies and regarding hoarhound states: "This well-known herb is a great favorite with herbalists. It has several well-recognised virtues, in addition to its expectorant, which is the chief. We need not describe it as most people are familiar with it.As is said of other common mercies, "their abundance causes them to be despised." So with hoarhound. It is growing by the acre in some places in nearly every land under the heavens. There are some people who have it growing at their doors, and yet are so ignorant of its virtues that they go about with sore throat or cold, or they will give money for medicine that is not so good, when a judicious use of this useful herb would cure them soon.

For a sore throat get 20z of the green or 10z of the dry. Simmer 10 minutes in a pint of water; strain through a cloth; sweeten with honey, and take in a wineglassful three to six times a day. This way it is good for hoarseness, coughs and colds. As a tonic (and it is a good one), omit the honey. In our business we gather in yearly about half a ton of this plant, and generally we find it is gone before the following season. We make a saturated tincture of the green herb, which preserves its virtues and flavor even better than the dried herb. Hoarhound is also one

Feasibility for biological control of horehound, Marrubium vulgare L.

of the ingredients in our Balm of Gilead, Herb Beer extract, Stomach Tonic, &c."

Reference:

James Neil (1998 edition) The New Zealand Family Herb Doctor. Senate Publishers, UK. Pp 92/93.

Marrubium vulgare is wildcrafted by our Herb Federation of New Zealand members for use medicinally.

(2) ECONOMICS OF HOREHOUND

Marrubium vulgare grows well in dry climate and land with low productivity.

There is a history of its use commercially in New Zealand.i.e. wildcrafting and use in medicines, particularly those to treat respiratory conditions.

It is easy to grow (self seeds in the wild) and requires no use of herbicides, thus easily meets organic requirements.

Harvesting is uncomplicated as it is the aerial parts of the plant that are used medicinally.

At present two of the main suppliers of herbal tinctures to the New Zealand market wholesale price of 1:2 200ml tincture of Marrubium vulgare is \$23.90 and \$25.70 respectively.

In the above examples 1 kg of herb is used in 2 litres of menstruum, part of which uses alcohol as a solvent. So to make a 200ml bottle of herbal extract/ tincture 100grams of herb is used.

Each healthy horehound plant, harvested at the right time, is likely to provide at least 200 grams of herb.

Two horehound plants can easily come to harvest in an area of one square foot.

Minimal harvest volume of herb in this square foot would be 400 grams and worth say half the tincture price i.e. say \$12.00 x 4 = \$48.00 for 400 grams

There are 107639 sq. feet in 1 hectare so price per harvest could be \$5,166,672 per hectare.

So why would Mr Loxton persist in growing lucerne and running sheep and cattle on all of his 8000 hectares? Why not use some of this land to grow the medicinal herb – Marrubium vulgare?

(3) INDUSTRY GROWTH POTENTIAL

There is huge potential for the Ministry of Primary Industries to help New Zealand farmers create wealth through growing medicinal herbs. It is called diversification and it's about growing something markets both within New Zealand and internationally want.

(4) POTENTIAL LOSSES IF HOREHOUND SUBJECTED TO BIOCONTROL

Loss of an incredibly useful medicinal herb to New Zealand and one which may well play a future role in treatment when antibiotic resistance results in increasing death from infections.

Loss of potential for a new and thriving business – medicinal herbs, given that other growing areas of the World have become contaminated or use unsafe growing and harvesting methods.

ENDS

Appendix 3 – Records of pathogens associated with *Marrubium vulgare*

Phylum Order Family	Species (and other names) ¹	Type of pathogen	Geographic range on <i>M.</i> <i>vulgare</i> ²	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging to <i>M.</i> <i>vulgare</i> ?
Ascomycota Capnodiales Mycosphaerellaceae	Cercospora marrubii Tharp (Cercosporina marrubii (Tharp) Sacc.; Cercospora marrubii Savul. & Sandu; Cercospora marrubiicola Vassiljevsky)	Leaf spots	India (Govindu & Thirumalachar 1956/1957), USA (Tharp 1917)	Found on <i>Marrubium</i> spp. and <i>Prunella vulgaris</i> (Lamiaceae) (Farr & Rossman 2017)	Absent Damage level unknown.
Ascomycota Capnodiales Mycosphaerellaceae	Ramularia marrubii C. Massal. (Cylindrospora marrubii (C. Massal.) J. Schröt., Cylindrosporium marrubii (C. Massal.) J. Schröt., Ramularia sideritidis Hollós)	Leaf spots	Europe, central Asia (Andrianova & Minter 2016)	Marrubium spp., Lagopsis supina, Sideritis spp. (Andrianova & Minter 2016)	Absent Damage level unknown.
Ascomycota Botryosphaeriales Botryosphaeriaceae	Phyllosticta marrubii McAlpine	Leaf spots	Australia (McAlpine 1904)	<i>Marrubium</i> spp.	Absent Damage level unknown.
Basidiomycota Pucciniales	<i>Uredo marrubii</i> Rabenh.	Rust on leaves	Italy (Rabenhorst 1850; Oudemans 1923)	Only reported on <i>Marrubium</i> spp.	Absent Damage level unknown.

Phylum Order Family	Species (and other names) ¹	Type of pathogen	Geographic range on <i>M.</i> vulgare ²	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging to <i>M.</i> vulgare?
Ascomycota Erysiphales Erysiphaceae	Neoerysiphe galeopsidis (DC.) U. Braun (Erysiphe galeopsidis DC., Golovinomyces galeopsidis (DC.) V.P. Heluta, Erysiphe communis var. labiatarum Link, Erysiphe lamprocarpa var. galeopsidis Link)	Powdery mildew on leaves	Europe, China (Amano 1986; Braun 1995).	Wide range of hosts: recorded on plants from 12 families, mainly in the Lamiaceae (Pastircakova et al. 2008; Ellis 2017).	Present. Recorded on plants from the Lamiaceae family in New Zealand (Cooper 2013). Damage level unknown.
Ascomycota Erysiphales Erysiphaceae	Leveillula taurica (Lév.) G. Arnaud (<i>Erysiphe taurica</i> Lév., <i>Tigria taurica</i> (Lév.) Trevis., <i>Oidiopsis taurica</i> (Lév.) E.S. Salmon)	Powdery mildew on leaves	Europe, Russia (Amano 1986; Braun 1995).	Wide host range. Is pathogenic to onion, capsicum, chili, tomato, artichoke, cotton (Correll et al. 1987).	Present. Has been isolated from capsicum in New Zealand (Cooper 2013). Damage level unknown.
Ascomycota Erysiphales Erysiphaceae	Leveillula duriaei (Lév.) U. Braun (<i>Erysiphe duriaei</i> Lév., <i>Tigria duriaei</i> (Lév.) Trevis., <i>Tigria durieui</i> (Lév.) Trevis.)	Powdery mildew on leaves	Europe (Amano 1986)	Reported on Lamiaceae (Heluta & Minter 1998; Ellis 2017).	Absent Damage level unknown.
Ascomycota Pleosporales Leptosphaeriaceae	<i>Coniothyrium marrubii</i> Fairm.	Could be either a secondary pathogen or an endophyte	California (Millspaugh & Nuttall 1923)	Only reported once on Marrubium vulgare	Absent Found on dead stems.
Ascomycota Botryosphaeriales Botryosphaeriaceae	<i>Diplodia herbarum</i> var. <i>marrubii</i> Brunaud	Could be either a secondary pathogen or an endophyte	USA (Crops Research Division Agricultural Research Service 1960), Spain (Gonzalez Fragoso 1914), France (Brunaud 1889)	Only reported on <i>Marrubium vulgare</i>	Absent Found on dead stems.

Phylum Order Family	Species (and other names) ¹	Type of pathogen	Geographic range on <i>M.</i> vulgare ²	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging to <i>M.</i> <i>vulgare</i> ?
Ascomycota Pleosporales Leptosphaeriaceae	<i>Leptosphaeria dumetorum</i> subsp. <i>marrubii</i> Sacc.	On stems	France (Crane & Shearer 1991)	Only reported once on Marrubium vulgare	Absent Damage level unknown.
Ascomycota Pleosporales Leptosphaeriaceae	Ophiobolus erythrosporus (Riess) G. Winter (Sphaeria erythrospora Riess, Nodulosphaeria erythrospora (Riess) L. Holm, Rhaphidospora erythrospora Riess, Rhaphidophora urticae Rabenh)	Could be either a secondary pathogen or an endophyte	Canada (Shoemaker 1976; Ginns 1986)	Wide host range (Shoemaker 1976)	Present. Has been isolated from <i>Clematis vitalba</i> in New Zealand (Landcare Research 2009) Found on dead stems (Ellis & Ellis 1997).
Ascomycota Pleosporales Didymellaceae	<i>Phoma herbarum</i> f. <i>marrubii</i> Sacc.	Could be either a secondary pathogen or an endophyte	Spain (Gonzalez Fragoso 1916), Poland, France (Brunaud 1889)	Only reported on <i>Marrubium vulgare</i>	Absent Found on dead stems (Gonzalez Fragoso 1916).
Ascomycota Pleosporales Didymellaceae	Phomopsis marrubii (Durieu & Mont.) Grove (Phoma marrubii (Durieu & Mont.) Sacc., Sphaeronaema marrubii Durieu & Mont., Phoma labiatarum Cooke)		France (Brunaud 1889; Grove 1919), UK (Grove 1935)	Only reported on Marrubium vulgare	Absent Found on dead stems (Grove 1919).
Ascomycota Pleosporales Didymellaceae	Phoma lanuginis Fairm.	On stems	USA (Crops Research Division Agricultural Research Service 1960)	Only reported on <i>Marrubium vulgare</i>	Absent Damage level unknown.

Phylum Order Family	Species (and other names) ¹	Type of pathogen	Geographic range on <i>M.</i> <i>vulgare</i> ²	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging to <i>M.</i> <i>vulgare</i> ?
Ascomycota Pleosporales Pleosporaceae	Stemphylium vesicarium (Wallr.) E.G. Simmons (Stemphylium herbarum E.G. Simmons, Pleospora herbarum (Pers.) Rabenh. ex Ces. & De Not., Sphaeria herbarum Pers., Exormatostoma herbarum (Pers.) Gray, Cryptosphaeria herbarum (Pers.) Grev., Pleospora herbarum (Pers.) Rabenh., Ampullina herbarum (Pers.) Quél., Sphaeria leguminum Wallr., Pleospora labiatarum Cooke & Harkn.)	Stem blight	Spain (Gonzalez Fragoso 1916), Italy (Berlese 1888), France (Brunaud 1889), USA (Crops Research Division Agricultural Research Service 1960)	Wide host range	Present. Found on multiple hosts in New Zealand including onion, alfalfa, tomato, asparagus. Damage level unknown.
Ascomycota Pleosporales Pleosporaceae	<i>Pleospora vulgaris</i> Niessl	Could be either a secondary pathogen or an endophyte	Spain (Gonzalez Fragoso 1916)	Wide host range	Absent Found on dead stems (Gonzalez Fragoso 1916).
Ascomycota Ostropales Stictidaceae	<i>Stictis lanuginicincta</i> Fairm.	Could be either a secondary pathogen or an endophyte	California (Millspaugh & Nuttall 1923)	Described on <i>Marrubium</i> vulgare (Millspaugh & Nuttall 1923)	Absent Found on dead stems.
Ascomycota Pleosporales Phaeosphaeriaceae	Hendersonia marrubii Brunaud (Hendersonulina marrubii (Brunaud) Tassi)	Could be either a secondary pathogen or an endophyte	France (Brunaud 1889)	Only reported once <i>Marrubium vulgare</i>	Absent Found on dead stems (Brunaud 1889).

Phylum Order Family	Species (and other names) ¹	Type of pathogen	Geographic range on <i>M.</i> <i>vulgare</i> ²	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging to <i>M.</i> <i>vulgare</i> ?
Chytridiomycota Chytridiales Synchytriaceae	<i>Synchytrium marrubii</i> G. Tobler	Leaf gall (Tobler 1913)	USA (Crops Research Division Agricultural Research Service 1960)	Only reported on Marrubium vulgare	Absent Damage level unknown.

¹ Many fungi have more than one Latin name because they can produce more than one type of spore. The name given when they are producing 'sexual' spores is called the teleomorph, whereas the stage producing 'asexual' spores is called the anamorph. The two stages often look completely different. Fungi are classified according to their 'teleomorph' name, unless the 'anamorph' is the only form known. Thus, Appendix 3 gives the taxonomy of the teleomorph, but column 2 uses whichever names were recorded when the fungus was found on *M. vulgare*. If a fungus was listed under an out-of-date name (synonym) this is also stated in column 2.

²Only the places where the organism was found associated with *M. vulgare* are listed here. The pathogen may also be found elsewhere on other hosts.

Page 54

Appendix 4 – Records of invertebrates feeding on *Marrubium vulgare*

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> vulgare	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
ACARI: TROME	BIDIFORMES				
Eriophyidae		Gall mite	Iran (Xue et al. 2016).	Yes. Only known from a single collecting event, only from <i>M. vulgare</i> (Xue et al. 2016). Eriophyid mites can be highly host specific.	Not known from NZ. Eriophyid mites can be highly damaging to their host plant, and several are known as pests while others have been used successfully as biocontrol agents. Xue et al. (2016) observed no damage to the host plant <i>M. vulgare</i> . They recorded the mite from the leaf surface, not from galls. It is worthwhile exploring further the life cycle of this mite to reveal if it is damaging to <i>M. vulgare</i> , or if <i>M. vulgare</i> is not its true host.
COLEOPTERA					
Buprestidae	<i>Trachys goberti</i> (des Gozis)	Wood borer	France, Spain (Sagliocco 2000).	Yes. Oligophagous restricted to a small number of plants in the family Lamiaceae (Ellis 2017).	Not known from NZ. Larvae feed internally in roots, but it was rare in the native range (Sagliocco 2000).

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> <i>vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Buprestidae	<i>Trachys</i> scrobiculatus Kiesenwetter	Leaf miner		Possibly yes. Oligophagous, appears to be restricted to the family Lamieacea, although there is one record of adults on a plant in the Solanaceae (Levey, B.(1977) Handbk Ident. Br. Insects V:1B 1-8:6 in Biological Records Centre (BRC) 2009). One record on <i>Calamintha nepeta</i> L. (Biological Records Centre (BRC) 2009), which is an emerging weed in the Hawke's Bay region of New Zealand.	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.
Cerambycidae	Pytoecia melanocephala Fabricius	Stem and root borer	Morocco (Sagliocco 2000)	Yes. Field surveys in the native range suggested <i>P. melanocephala</i> is restricted to <i>M. vulgare</i> (Sagliocco 2000).	Not known from NZ. Although <i>P. melanocephala</i> is highly damaging in its native range (41% attack rate), this beetle is apparently adapted to high summer temperatures rising over 40°C (Sagliocco 2000), which is likely to limit its distribution in NZ. This beetle was prioritised for introduction to Australia, but technical difficulties associated with its biology and ecology prevented host-range testing and importation to Australia (Sagliocco 2000). Larvae feed internally in the roots, eventually killing the plant (Sagliocco 2000). Its life cycle probably takes one year to complete, and it was locally common in the native range (Sagliocco 2000).

Page 56

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> <i>vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Cerambycidae	Phytoecia virgule (Charpentier)	Wood borer	France (Sagliocco 2000)	No. Polyphagous (Sagliocco 2000).	Not known from NZ (Sopow & Bain 2017). Larvae feed internally in the roots, but this beetle was rare in the native range (Sagliocco 2000).
Chrysomelidae	<i>Chrysomela</i> <i>banksi</i> Fabricius	Leaf beetle	France, Spain, Former Yugoslavia (Sagliocco 2000)	Possibly yes. Oligophagous restricted to plants in the family Lamiaceae (Sagliocco 2000), although records on plants from other families also exist (Biological Records Centre (BRC) 2009).	Not known from NZ. Both larvae and adults feed externally on leaves, and this beetle was locally common in native range surveys (Sagliocco 2000). Was not prioritised as a candidate agent for the Australian programme (Sagliocco 2000).
Chrysomelidae	<i>Dibolia</i> <i>cynoglossi</i> (Koch)	Flea beetle	Poland (Mohr, K.H. (1981) Polskie Pismo Ent. 51:3 393-469:435 in Biological Records Centre (BRC) 2009) <i>M. vulgare</i> is listed along with one other host plant from Spain and Britain to the Ukraine (Ellis 2017). It is not clear which country is associated with which host.	Yes. Oligophagous restricted to a small number of host plants in the family Lamiaceae (Biological Records Centre (BRC) 2009; Ellis 2017).	Not known from NZ. Larvae are leaf-miners. Has one generation per year and hibernates as adult (Ellis 2017). Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> <i>vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Chrysomelidae	Longitarsus ballotae (Marsh.)	Flea beetle	France, Spain, Italy, Morocco (Sagliocco 2000)	Yes. Oligophagous restricted to plants in the family Lamiaceae (Sagliocco 2000; Biological Records Centre (BRC) 2009).	Not known from NZ. Larvae feed internally in roots of seedlings and young plants, and adults feed externally on leaves causing minimal damage (Sagliocco 2000). This beetle was considered insufficiently damaging and was not prioritised as a candidate agent for the Australian programme (Sagliocco 2000). If seedling survival turns out to be important for the population dynamics of <i>M. vulgare</i> in New Zealand, this beetle should be reassessed as a potential candidate.
Mordellidae	<i>Mordellistena</i> sp.	Flower beetle	France, Spain, Italy (Sagliocco 2000)	No. Polyphagous (Sagliocco 2000).	Not known from NZ. The larvae feed internally on seeds (Sagliocco 2000). Damage level unknown.
Nitidulidae	Stachygethes ruficornis (Marsham) (syn. Meligethes flavipes Sturm)	Ovary feeder	Europe (Biological Records Centre (BRC) 2009).	Unclear. Oligophagous on plants in the family Lamiaceae, and appears to be mainly associated with <i>Ballota foetida</i> Lam. (Easton 1952) and <i>Ballota nigra</i> (Audisio 2011), but with several records of adults on plants in the Asteraceae (Biological Records Centre (BRC) 2009).	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.

Page 58

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> vulgare	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Nitidulidae	Stachygethes nanus (Erichson) (syn. <i>Meligethes</i> nanus Erichson)	Ovary feeder	France, Spain (Sagliocco 2000).	Yes. Considered monophagous (Easton 1952; Sagliocco 2000). Adults have been recorded from Cruciferae and Salicaceae (Biological Records Centre (BRC) 2009).	Not known from NZ. Both larvae and adults destroy a significant number of seeds through feeding on pollen and ovaries, but this beetle was rare in the native range (Sagliocco 2000). This species is heavily parasitised in the native range by an encyrtid wasp (Easton 1952) and could have greater impact if released from parasitism pressure.
Nitidulidae	Stachygethes nigerrimus (Rosenhaue) (syn. Meligethes tropicus Reitter)	Ovary feeder	France, Spain, former Yugoslavia, Morocco (Sagliocco 2000). Sardinia (Audisio 2011).	Yes. Considered monophagous (Easton 1952; Sagliocco 2000).	Not known from NZ. Both larvae and adults destroy a significant number of seeds through feeding on pollen and ovaries, and this beetle was locally common in the native range (Sagliocco 2000).
Nitidulidae	Stachygethes villosus (Brisout) (syn. Meligethes villosus Brisout)	Ovary feeder	France, Spain, former Yugoslavia, Morocco (Sagliocco 2000).	Yes. Considered monophagous (Easton 1952; Sagliocco 2000).	Not known from NZ. Both larvae and adults destroy a significant number of seeds through feeding on pollen and ovaries, and this beetle was locally common in the native range (Sagliocco 2000).

Order and Family	Species	Type of organism	Geographic range on <i>M. vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Nitidulidae	Stachygethes rotroui (Easton) (syn. Meligethes rotroui Easton)	·	Morocco (Sagliocco 2000).	Yes. Considered monophagous (Easton 1952; Sagliocco 2000).	Not known from NZ. Both larvae and adults destroy a significant number of seeds through feeding on poller and ovaries (Sagliocco 2000). This species was thought to be the best suited for Australia's climate out of the four <i>Stachygethes</i> (<i>Meligethes</i>) spp. (Sagliocco 2000), and remains the most promising candidate agent (Weiss & Sagliocco 2012). One shipment was made to Australia but the insects were dead on arrival (Sagliocco 2000). No additional shipments were attempted (Wills 2000).
Phalacridae	<i>Olibrus</i> <i>bisignatus</i> (Ménétriès)	Seed predator	France, Morocco (Sagliocco 2000)	No. Polyphagous (Sagliocco 2000).	Not known from NZ. Larvae feed externally on seeds (Sagliocco 2000). Can reach high attack levels per plant where present but low general attack rates (Groves et al. 2012), and was rare in the native range (Sagliocco 2000).

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> <i>vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
DIPTERA					
Agromyzidae	Amauromyza labiatarum (Hendel) (Syn. Dizygomyza, Phytobia labiatarum, probably also Phytobia semigyrans Rydén)	Leaf miner	UK (Pitkin et al. 2016). <i>M. vulgare</i> mentioned along with a list of hosts and countries associated with this leaf miner, including from Scandinavia to France, Italy, Albania and Romania, and from Ireland to the Baltics, Poland, and Bulgaria (Ellis 2017).	No. Broadly oligophagous, attacking many genera in the family Lamiaceae, including genera represented in NZ's indigenous flora. The main host genera are <i>Ballota, Galeopsis, Glechoma</i> and <i>Lamium</i> (Ellis 2017).	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme. Considered the most common species of <i>Amauromyza</i> on Lamiaceae (Ellis 2017).
Agromyzidae	Amauromyza lamii (Kaltenbach) (Syn. Dizygomyza, Phytobia lamii)	Leaf miner	M. vulgare mentioned along with a list of hosts and countries associated with this leaf miner, including from Belgium to Poland, Moldavia, Romania, and possibly the UK (Ellis 2017).	Possibly yes. Oligophagous restricted to plants in the family Lamiaceae (Ellis 2017).	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.
Agromyzidae	Amauromyza morionella (Zetterstedt) (Syn. Dizygomyza, Phytobia morionella, Amauromyza novakii (Strobl))	Leaf miner	UK (Biological Records Centre (BRC) 2009). <i>M. vulgare</i> mentioned along with a list of hosts and countries associated with this leaf miner, including from Scandinavia to Iberian Peninsula, Italy, Thrace, Romania, and from the UK to the Baltics (Ellis 2017).	Possibly yes. Oligophagous restricted to plants in the family Lamiaceae (Biological Records Centre (BRC) 2009; Ellis 2017).	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> vulgare	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Anthomyiidae	<i>Delia platura</i> (Meigen)	Seed fly	France, Spain, Former Yugoslavia (Sagliocco 2000).	No. Polyphagous (Sagliocco 2000).	Present in NZ (adventive). Can be highly damaging – considered a pest of beans, peas and maize. Was locally common in the native range (Sagliocco 2000).
Cecidomyiidae	Unidentified (possibly undescribed) species	Gall midge	France, Morocco (Sagliocco 2000).	Host specificity status unknown but considered oligophagous restricted to plants in the family Lamiaceae (Sagliocco 2000). Most plant-feeding cecidomyiid gallmidges are monophagous.	Presence in NZ is unlikely but cannot be determined in the absence of taxon name. Larvae feed externally on seeds. Was locally common in the native range (Sagliocco 2000).
Tephritidae	Aciura coryli (Rossi)	Seed fly	France, Spain, Former Yugoslavia (Sagliocco 2000).	No. Polyphagous (Sagliocco 2000).	Not known from NZ. Larvae feed externally on seeds, and this fly was rare in the native range (Sagliocco 2000).
EMBIOPTERA					
Embiidae	<i>Embia ramburi</i> Rimsky- Korsakow	Webspinner	France, Spain (Sagliocco 2000)	Host specificity status unknown but considered polyphagous (Sagliocco 2000).	Not known from NZ!). Both larvae and adults feed externally on roots, and this species was rare in the native range (Sagliocco 2000). Damage level unknown.
HOMOPTERA					
Aphididae	Aphis ballotae (Passerini)	Aphid	France, Spain (Sagliocco 2000).	Yes. Monophagous (Sagliocco 2000).	Not known from NZ. Did not appear to be highly damaging during native range surveys (Sagliocco 2000). Nymphs feed externally on leaves and stems, and this species was locally common in the native range (Sagliocco 2000).

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> vulgare	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Cicadellidae	Eupteryx melissae (Curtis)	Leaf hopper	France, Spain, Morocco (Sagliocco 2000)	No. Polyphagous (Sagliocco 2000).	Present in New Zealand, adventive and considered a pest of herbs in the family Lamiaceae (Somerfield 1977). Both adults and nymphs are sap-suckers on leaves. Was locally common in the native range (Sagliocco 2000).
HETEROPTERA					
Pentatomidae	<i>Agonoscelis</i> <i>puberula</i> Stål	Stink bug	Arizona (Thomas et al. 2003)	No. Probably a generalist.	Not known from NZ. Damage levels unknown.
Pentatomidae	Agonoscelis rutile Fabricius	Stink bug	Australia (Weiss & Sagliocco 2012)	No. This bug has been associated with plants from a variety of plant families (Weiss & Sagliocco 2012). Can become an occasional pest of grains, legumes, and fruit trees.	Not known from NZ. Unlikely to be sufficiently damaging. Although this Australian-native bug can be seen swarming in large numbers on <i>M. vulgare</i> , and is known by the common name of horehound bug, it does not provide control (Weiss & Sagliocco 2012).
Tingidae	Tingis (Neolasiotropis) marrubii Vallot	Lace bug	France, Spain, Morroco (Sagliocco 2000).	Yes. Monophagous (Sagliocco 2000).	Not known from NZ. Adults and larvae suck sap from leaf-cells. Was locally abundant and only caused localised leaf depigmentation in the native range so was not prioritised as biocontrol agent in the Australian programme (Sagliocco 2000). In New Zealand, lace bugs are used successfully as biocontrol agents against the woody weeds woolly nightshade, <i>Solanum mauritianum</i> , and Chinese Privet, <i>Ligustrum sinense</i> , causing severe defoliation.

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> <i>vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
HYMENOPTERA					
Formicidae	<i>Messor Barbarus</i> (L.)	Harvester ant	France, Spain (Sagliocco 2000).	No. Polyphagous (Sagliocco 2000).	Not known from NZ. Adults harvest seeds. Was locally common in the native range (Sagliocco 2000). Damage levels unknown.
Formicidae	<i>Aphaenogaster</i> <i>senilis</i> Mayer	Ant	Portugal (Sagliocco 2000).	No. Polyphagous (Sagliocco 2000).	Not known from NZ. Adults harvest seeds. Was rare in the native range (Sagliocco 2000). Damage levels unknown.
Formicidae	Camponotus cruentatus Latreille	Ant	France, Portugal (Sagliocco 2000).	No. Polyphagous (Sagliocco 2000).	Not known from NZ. Adults harvest seeds. Was rare in the native range (Sagliocco 2000). Damage levels unknown.
Formicidae	<i>Tapinoma</i> <i>nigerrimum</i> (Nyl.)	Ant	France (Sagliocco 2000).	No. Polyphagous (Sagliocco 2000).	Not known from NZ. Adults harvest seeds. Was rare in the native range (Sagliocco 2000). Damage levels unknown.
LEPIDOPTERA					
Coleophoridae	Coleophora ballotella (Fischer von Röslerstamm)	Case moth	M. vulgare mentioned along with a list of hosts and countries associated with this moth, including Germany, Poland and the Baltic Statea to the Iberian Peninsula, Italy, Greece, northern and southern Russia (Ellis 2017).	Possibly yes. Oligophagous restricted to plants in the family Lamiaceae (Ellis 2017).	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.

Page 64

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> <i>vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Coleophoridae	Coleophora lineolea (Haworth) (syn. Porrectaria lineolea Haworth)	Case moth	UK (Pitkin et al. 2016). <i>M. vulgare</i> mentioned along with a list of hosts and countries associated with this moth, including Europe, south-west Siberia (Savele 1999).	Possibly yes. Recorded from <i>M. vulgare, Ballota nigra, Stachys</i> spp., and <i>Lamium</i> spp. (Savele 1999; Biological Records Centre (BRC) 2009; Ellis 2017).	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.
Coleophoridae	<i>Coleophora</i> <i>onopordiella</i> Zeller	Case moth	M. vulgare mentioned along with a list of hosts and countries associated with this moth, including France, Itally, Poland, Greece and Thrace (in south eastern Europe) (Ellis 2017).	No. Plyphagous (Ellis 2017)	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.
Hesperiidae	Carcharodes boeticus Rambur	Skipper butterfly	France, Spain, Portugal (Sagliocco 2000).	Yes. Oligophagous restricted to plants in the family Lamiaceae (Sagliocco 2000; van Swaay et al. 2010). Only recorded from <i>M. vulgare</i> in natural history Museum records (Natural History Museum London 2007). Black horehound, <i>Ballota foetida</i> also mentioned as a host (Higgins & Riley 1970).	Not known from NZ. Larvae feed externally on leaves and the species was found at many sites but not in high abundance in the native range (Sagliocco 2000). Has two to three generations per year in warmer parts of its native range, where it is widespread, but probably only one generation per year in colder parts, where it is now reported extinct (van Swaay et al. 2010). This leaftying butterfly was prioritised as a candidate agent for the Australian programme, but failure to obtain mating in containment resulted in this species being abandoned (Sagliocco 2000; Wills 2000).

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> vulgare	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Hesperiidae	<i>Carcharodus</i> <i>floccifera</i> (Zeller)	Skipper butterfly	M. vulgare mentioned along with a list of hosts and countries associated with this butterfly, including southern Europe, Bulgaria, Siberia and Asia Minor (Savele 1999).	Yes. Larval food plants considered restricted to <i>Marrubium</i> and <i>Stachys</i> (Higgins & Riley 1970). Was also recorded from <i>Abutilon theophrasti</i> in the family Malvaceae (Natural History Museum London 2007), but it is not clear if this record is for adult visitation or larval feeding.	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.
Hesperiidae	<i>Carcharodus</i> <i>stauderi</i> Reverdin		Algeria (Savele 1999).	Possibly yes. Larvae only recorded on <i>M. vulgare</i> and black horehound <i>Ballota foetida</i> (Savele 1999).	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.
Psychidae	<i>Apterona</i> <i>crenulella</i> Siebold	Bagworm	Germany (Natural History Museum London 2007)	No. Polyphagous (Natural History Museum London 2007).	Not known from NZ. Damage levels unknown. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.

Page 66

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> vulgare	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Pterophoridae	Wheeleria spilodactylus (Curtis) (syns, Pterophorus spilodactyla Curtis, Aciptilus confuses Merrifieldia spilodactylus Curtis)	Plume moth	France, Spain, Portugal, Italy, former Yugoslavia, Morocco (Sagliocco 2000). UK (Menéndez & Thomas 2006).	Yes. Oligophagous restricted to plants in the family Lamiaceae (Sagliocco 2000). <i>M. vulgare</i> is the only host recorded on the Biological Records Centre database (2009), and was shown to be the preferred host (J. Weiss, pers. comm.). <i>M. vulgare</i> and <i>Ballota nigra</i> are listed as larval plant hosts (Savele 1999).	Not known from NZ. Introduced to Australia as a biocontrol

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> <i>vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Pyralidae	<i>Anania hortulata</i> L. (syn. <i>Eurrhypara</i> <i>hortulata</i>)	moth	British Isles (as Eurrhypara hortulata Natural History Museum London 2007).	No. Polyphagous (Natural History Museum London 2007).	Not known from NZ. Not recorded from <i>M. vulgare</i> during native range surveys associated with the Australian programme.
Sesiidae	Chamaesphecia mysiniformis (Boisduval)	Clearwing moth	Spain (Sagliocco 2000; Natural History Museum London 2007).	Yes. Laboratory host-specificity tests in Australia observed strong larval development on <i>M. vulgare</i> , a lesser degree of development on other species in the genus <i>Marrubium</i> and minor development on the closely-related <i>Ballota nigra</i> L. (black horehound) and <i>Stachys arvensis</i> L. (stagger weed), but no field observations have ever been recorded from a plant other than <i>M. vulgare</i> (Sagliocco & Coupland 1995). <i>Ballota hirsuta</i> , three species of <i>Stachys</i> and <i>Sideritis montana</i> L. have been listed as larval food plants (Savele 1999; Natural History Museum London 2007).	Not known from NZ. Introduced to Australia as a biocontrol agent and is highly damaging in dry environments. Larvae seem to survive best in young plants (Clarke et al. 2000). Larvae feed internally in the roots. Found in high abundance in the native range (Sagliocco 2000). Has one generation per year (Wills 2000). In the native range 50-90% of 2-3 years old plants are attacked (Sagliocco & Coupland 1995), resulting in plant death within the following year (Sagliocco 2000). This species was originally not going to be released in Tasmania because it requires summer temperatures exceeding 30°C (Ireson et al. 2000). A release was made in Tasmania in 2008, and a pupa was recovered at the release site in 2009, suggesting this moth is able to complete its life cycle in this colder environment (Ivory 2012; Weiss & Sagliocco 2012). This moth continues to establish over larger areas in Australia, and it is now hoped that no further agents will be required (Weiss & Sagliocco 2012).

Order and Family	Species	Type of organism	Geographic range on <i>M.</i> <i>vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Sesiidae	Chamaesphecia oxybeliformis (Herrich- Schäffer)	Clearwing moth	Recorded on <i>M. vulgare</i> and <i>M. peregrinum</i> in Macedonia, Greece, The Balkan, Asia Minor (Laštuvka & Laštuvka 1995; Savele 1999).	Yes. Only reported host plants are <i>M. vulgare</i> and <i>M. peregrinum</i> (Savele 1999; Natural History Museum London 2007).	Not known from NZ. Potentially damaging. Was not found in the native range surveys for the Australian programme, otherwise would have been considered as a candidate agent (Sagliocco 2000).
Tortricidae	Endothenia pauperculana Staudinger (syn. Enarmonia nougatana Chrétien).	Leaf roller	France, Spain (Sagliocco 2000).	Yes. Oligophagous restricted to plants in the family Lamiaceae (Sagliocco 2000). Recorded from <i>M. vulgare</i> and <i>Sideritis hirsuta</i> L. in the native range (Sagliocco 2000).	Not known from NZ. Larvae feed externally on buds, flowers, and seeds, locally common in the native range (Sagliocco 2000).
THYSANOPTERA					
Phlaeothripidae	<i>Haplothrips</i> <i>reuteri</i> (Karny)	Thrips	France, Spain (Sagliocco 2000).	No. Polyphagous (Sagliocco 2000).	Not known from NZ. Both nymphs and adults feed externally on leaves (Sagliocco 2000). Not recorded as highly damaging in native range surveys (Sagliocco 2000).
Phlaeothripidae	<i>Haplothrips</i> <i>marrubiicola</i> Bagnall	Thrips	UK (Biological Records Centre (BRC) 2009).	Unclear. Described from <i>M. vulgare</i> , but has been collected also from <i>Onobrychis sativa</i> (family: Fabaceae) (Karadjova & Krumov 2015) and from <i>Scirpus maritimus</i> (family: Cyperaceae) (Biological Records Centre (BRC) 2009).	Not known from NZ. Not recorded from <i>M. vulgare</i> during native range surveys associated as part of the Australian programme.

Order and Family	Species	Type of organism	Geographic range on <i>M. vulgare</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
TYLENCHIDA					
Meloidogynidae	<i>Meloydogynes</i> sp.	Root knot nematode	USA (Crops Research Division Agricultural Research Service 1960)	Unclear. Root knot nematodes can be polyphagous.	Presence in New Zealand cannot be determined in the absence of taxon name. There are a number of <i>Meloydogyne</i> species in New Zealand, most of them indigenous. Damage level to <i>M. vulgare</i> unknown. Root knot nematodes are major pests of crops globally.

Appendix 5 – Steps in a biocontrol project

A classical biocontrol programme typically works through the following steps. This is usually done in a sequential manner, but some activities may occur concurrently.

- Explore the feasibility of project. If project looks feasible, proceed.
- Survey weed in places where biocontrol is desired. If any potential agents are found, explore ways to maximise them. If any likely impediments are found, look for ways to mitigate them.
- Undertake molecular studies of the weed to help narrow down the best place in the native range to find natural enemies.
- Unless natural enemies are already well known, survey weed in native range. Identify and study life cycles of natural enemies found.
- Determine host range for potential agents. Abandon any species that do not appear to be safe or effective enough.
- Apply to authorities for permission to release agents.
- If permission is granted, import, clear through containment, and develop rearing techniques for new agents (if not already known).
- Mass-rear and release agents over several years.
- Monitor establishment success and dispersal of agents over several years.
- Harvest and redistribute agents.
- Evaluate success of project. Decide if further agents are needed.