Summary of tests to determine the host-ranges of *Wheeleria spilodactyus* and *Chamaesphecia myriniformis*, two control agents for the weed horehound, *Marrubium vulgare*.

Introduction

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. It 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 crops. *Marrubium vulgare* is not palatable to stock. It taints the meat of animals that are forced to graze on it, it displaces preferred pasture species. It downgrades wool quality, and it is difficult to control chemically and mechanically.

Horehound is also valued as a medicinal herb. It is wild-harvested 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 (Groenteman et al. 2017). Flowers are foraged by bees. The actual value of horehound to New Zealand is yet to be determined.

Biological control of horehound has been successful, or partially successful in Australia. Larvae of the horehound plume moth (*Wheeleria spilodactylus*) attack the above ground vegetation, and larvae of the horehound clearwing moth (*Chamaesphecia mysiniformis*) attack the roots. Extensive host-range tests were conducted to ensure that these agents were safe before they were released in Australia over 20 years ago. There have been no reports of adverse effects to valued non-target plants or ecological values.

This is a summary of the host-range tests conducted in Australia, and an evaluation of how well this research addresses potential risks in New Zealand.

Selection of test plants

Horehound (*Marrubium vulgare*) belongs to the mint family, the Labiatae or Lamiaceae. A recent phylogenetic reconstruction of the family (Li et al., 2016) established 4 moderately- to highly-supported clades. Horehound belonged to the sub-family Lamioideae, which fell into clade 4 together with the sub-family Ajugoideae. Less closely-related was the sub-family Nepetoideae which made up clade 3. The sub-families Viticoideae and Prostantheroideae are even less-closely-related. Li et al. (2016) found that, within the sub-family Lamioideae, the closest tribes to the Marrubieae (the horehound tribe) were the Lamieae and the Leucadeae. Plants belonging to the tribe Stachydeae were less-closely-related.

Species of the genus *Wheeleria* occur worldwide. The host range is typically restricted to one or two plant species (VDCNR, 1993). In Europe *Wheeleria spilodatylus* has only been recorded on horehound and occasionally on *Ballota nigra*, and in field surveys in France and Spain the moth was only found on horehound (VDCNR, 1993).

Plants to be tested against the two agents in Australia were selected according to the universally accepted centrifugal phylogenetic method of Wapshere (1974). This protocol is based on the premise that insects with a specialised diet are more likely to attack plants that are closely-related to the observed host than plants that are less-closely related. Plant selection centred on those species closely-related to horehound, but also included many unrelated plants of commercial and domestic importance. The list of plants to be tested on each control agent varied slightly because of the taxonomic framework in place at the time, but this had no influence on the conclusions drawn

from tests. The sub-family Nepetoideae contains a wide range of species that are of culinary and medicinal importance. Many of these were tested.

There are five native New Zealand plant species that belong to the family Labiatae. None of the natives belong to the same sub-family as *Marrubium* (Lamioideae) and so are only distantly-related to horehound. One of these species is also present in Australia, and was tested. The other four have not been tested, but related species were included in the tests conducted in Australia before the agents were released there. These test plants can be regarded as surrogates for the New Zealand native species (Table 1).

NZ native species	**Sub-family**	**Surrogate species tested in Australia against**	
		Wheeleria spilodactylus	***Chamaesphecia mysiniformis***
Mentha cunninghamii	Nepetoideae	*Mentha diemenica*	*Mentha diemenica*
		Mentha spicata	*Mentha spicata*
			Mentha australis
Plectranthus parviflorus	Nepetoideae	Tested	Tested
Teucridium parvifolium	Ajugoideae	*Teucrium racemosum*	*Teucrium racemosum*
		Teucrium corymbosum	*Ajuga australis*
		Ajuga australis	
Scutellaria novae-zelandiae	Scutellariodeae	*Scutellaria humilis*	*Scutellaria humilis*
Vitex lucens	Viticoideae	*Vitex trifolia*	*Vitex trifolia*

Table 1. Surrogates for New Zealand native plant species included in host-range tests conducted in Australia

The plants tested against *Wheeleria spilodactylus* are listed in Table 2, while those test against *Chamaesphecia mysiniformis* can be found in Table 3.

Testing of *Wheeleria spilodactylus*

Larval starvation tests were conducted in quarantine at the Keith Turnbull Research Institute at Frankston, Australia in 1992-93. Ten first instar larvae were placed on the growing shoots of test plants. This was replicated 8 times. Plants were examined after 10, 20 and 30 days and the number of larvae remaining was recorded. Development on horehound controls was completed by 30 days. A summary of the results can be found in Table 2.

FAMILY Sub-family	**Tribe**	**Test species**	**Common name**	**Larval feeding observed**	**Mean % survival to 30 days**	**Complete development?**
LABIATAE						
Lamioideae	Marrubieae	*Marrubium vulgare* (Control)	Horehound	Yes	49	100% by 30 days
		M. supinum		Yes	21	76% by 30 days
		Ballota nigra		Yes	10	0% by 30 days
	?	*Leonotis nepetaefolia*		No	0	No
Nepetoideae	Salveae	*Salvia officinalis*	Sage	No	0	No
		S. uliginosa		No	0	No
		S. 'purple velvet'	Ornam. sage	No	0	No
	Mentheae	*Mentha diemenica***	Wild mint	No	0	No
		*M. spicata***	spearmint	No	0	No

		Origanum vulgare	Oregano	No	0	No
		Prunella vulgaris		No	0	No
		Thymus vulgaris	Thyme	No	0	No
	Ocimieae	*Ociminum basilicum*	Basil	No	0	No
		*Plectranthus parviflorus***		No	0	No
	Rosmarineae	*Rosmarinus officinalis*	Rosemary	No	0	No
	Lavanduleae	*Lavandula spicalis*	Lavender	No	0	No
Ajugoideae		*Ajuga australis***		No	0	No
		*Teucrium racemosum***		No	0	No
		*T. corymbosum***		No	0	No
		*Scutellaria humilis***		No	0	No
Prostantheroideae		*Prostanthera ovalifolia*		No	0	No
		P. rotundifolia		No	0	No
		Hemiandra pungens		No	0	No
		Westringia fruticosa		No	0	No
Viticoideae		*Vitex trifolia***		No	0	No
OTHER FAMILIES						
22 families		30 species		No	0	No

Table 2. Survival and development of first instar *Wheeleria spilodactyla* larvae after 30 days, and damage to test plants (from VDCNR, 1993) (** = New Zealand native species or surrogate for New Zealand native species).

Larval feeding was observed only on horehound controls and two of the test plants, both of which were closely-related to *Marrubium vulgare* within the tribe Marrubieae. One other member of the tribe was not attacked. An average of 4.9 of the 10 larvae placed on horehound controls completed development at day 30 (range 2-10; n=18). Development took longer on the closely-related *M. supinum*, and only an average 2.1 larvae of the ten applied completed development (range 0-7; n=8). Mortality on black horehound, *Ballota nigra*, was high, with only 10% of larvae alive after 30 days. None had developed beyond third instar at the end of 30 days. Plants belonging to other sub-families of the Labiatae were immune from attack, as were 30 test species belonging to 22 other plant families. There was no survival of larvae on plants in the sub-family Nepetoideae, which contains the important culinary herbs such as thyme, sage, mint, oregano etc. There was no attack on the native *Plectranthus parviflorus* or on any of the plants designated as surrogates for other New Zealand native species.

Conclusion

Wheeleria splodactylus has only been reported on horehound and on *Ballota nigra* in its native Europe. The test results confirmed this narrow host range. Larvae did not feed on plants outside the sub-family Lamioideae. One plant in the sub-family Lamioideae that was tested (*Leonotis*) was not damaged. This suggests that the moth is restricted to the tribe Marrubieae. Further, the relative performances of larvae on controls and two other species in the tribe indicate that the risk of colonisation ability of species plants other than *Marrubium* species is negligible.

Testing of *Chamaesphecia mysiniformis*

Five newly-emerged larvae were transferred onto each test plant using a fine brush. There were usually at least five replicates (25 larvae) for each plant species though availability of plants limited some tests to four replicates. Test plants and controls were maintained in a glasshouse at 22-28°. Root crowns were dissected after 2-3 weeks when the number of initial attacks and the survival of larvae were recorded (Table 3).

Larvae of *C. mysiniformis* were found to attack and survive on four of the seven species of *Marrubium* tested. Of the larvae placed on *M. vulgare*, 84% were still alive after 2-3 weeks. *M. supinum* is sufficiently closely-related to *M. vulgare* that the two species can hybridise. Survival on this species was 36%. Lower survival was recorded on the other *Marrubium* species (Table 3). Three of the 25 larvae survived on black horehound, *Ballota nigra*. All of these plants belong to the Tribe Marrubieae in the sub-family Lamioideae. A single larva was still alive on *Stachys arvensis* (4%) which belongs to the Stachydeae, a related tribe. The larvae live inside root crowns and test plants were destroyed during analysis. At the time of examination larvae on all attacked plant species had reached second instar at best (only first instar on one of the test species). It is not known whether larvae found in plants other than *M. vulgare* would have completed development.

Small initial attacks were recorded on other plants as larvae tried to establish, but no larvae survived on plants of any other sub-families of the Labiatae, or in plants from other families (Table 3.; Sagliocco and Coupland, 1995). There was no survival on any species in the sub-family Nepetoideae, which includes most of the important culinary herbs such as thyme, sage, mint, oregano etc (Table 3.). There was no survival of larvae on the plants designated as surrogates for New Zealand native species.

Family Sub-family	Tribe	Test species	Common name	initial larvae	% larval survival to observation at 14-21 days
LABIATAE					
Lamioideae	Marrubieae	*Marrubium vulgare* (Control)	Horehound	25	84
		M. supinum		25	36
		M. alysson		25	28
		M. incanum		25	8
		M. leonuroides		25	8
		M. friwaldskyanum		20	0
		M. velutinum		25	0
		M. anisodon		25	0
		Ballota nigra	Black horehound	25	12
	Stachydeae	*Stachys arvensis*		25	4
		S. alopecurus		25	0
		S. densiflora		25	0
	Leucadeae	*Leonotis oxymifolia*		25	0
		L. leonurus		25	0
	Lamieae	*Lamium amplexicaule*		25	0
Nepetoideae		*Salvia officinalis*	Sage	25	0
		S. haematodes		25	0
		*Mentha australis***		25	0
		*M. diemenica***	Wild mint	25	0
		*M. spicata***	spearmint	25	0
		Lycopus australis		25	0
		Origanum vulgare	Oregano	25	0
		Prunella vulgaris		25	0
		Thymus vulgaris	Thyme	25	0
		Dracocephalum ruyschiana		20	0
		Ociminum basilicum	Basil	25	0

	*Plectranthus parviflorus***		25	0
	Rosmarinus officinalis	Rosemary	25	0
	Lavandula latifolia	Lavender	25	0
Ajugoideae	*Ajuga australis***		50	0
	Teucrium racemosum		30	0
	*Scutellaria humilis***		25	0
Prostantheroideae	*Prostanthera ovalifolia*		25	0
	Hemiandra pungens		25	0
	Westringia fruticosa		25	0
Viticoideae	*Vitex trifolia***		25	0
OTHER FAMILIES				
19 families	25 species		10-25	0

Table 2. Survival and development of first instar *Chamaesphecia mysiniformis* larvae after 14-21 days on a range of test plants (from Sagliocco and Coupland, 1995) (* = New Zealand native or surrogate for New Zealand native species).

Conclusion

Sagliocco & Coupland, 1995) reported *Chamaesphecia mysiniformis* only from horehound in its native Europe. The test results confirmed this narrow host range. In tests, larvae only survived on plant species in the tribe Marrubieae of the subfamily Lamioideae. There are no native species or other species of value growing in New Zealand that belong to that tribe. With the exception of one out 25 larvae still alive on *Stachys arvensis* after 14-21 days, species belonging to other tribes of the sub-family were not susceptible to *C. mysiniformis*, nor were species belonging to other sub-families or families. Survival was much higher on *Marrubium vulgare* than on the other horehound relatives tested. Tests therefore indicate that the host range of *C. mysiniformis* is restricted to the horehound tribe and, functionally, may well be restricted to the species *M. vulgare* (as observed in its native range).

References

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