The release and establishment of two biological control agents of horehound (*Marrubium vulgare* L.) in south-eastern Australia

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Summary

In 1991 a survey of the native range of horehound, *Marrubium vulgare* L. in Europe was conducted to identify potential biological control agents for Australia. Four insects were chosen for closer investigation; a plume moth, *Wheeleria spilodactylus* (Curtis), a skipper butterfly, *Carcharodus boeticus* Rambur, a clearwing moth, *Chamaesphecia mysiniformis* (Boisduval) and a seed beetle, *Meligethes rotroui* Easton. Host specificity testing of *W. spilodactylus* began in 1991 at Keith Turnbull Research Institute in Frankston, Victoria and in 1993 it was approved for release. In 1994 host specificity of *C. mysiniformis* was performed in France and approval for release was gained in 1996. Initial attempts to begin host specificity testing for *C. boeticus* and *M. rotroui* failed, and future attempts have been halted. In the spring of 1998 a redistribution program for *W. spilodactylus* was initiated. A total of 32 release sites in Victoria, New South Wales, South Australia and Tasmania were visited to determine establishment of *W. spilodactylus* and the potential for redistribution of insects from the sites. At 90% of the sites visited the biological control agent had established and of those, 41% were at a stage that the collection and redistribution of *W. spilodactylus* to new sites was possible.

Introduction

In 1990 to 1992 scientists from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Biological Control Unit in Montepellier, France surveyed horehound populations in southern France for phytophagous insects that could be used as biological control agents. Surveys were also carried out on roadside populations in Spain, Portugal, Morrocco, Italy and Yugoslavia (Sagliocco 2000). A total of 27 different insect species were found feeding on horehound and of these four were chosen to be investigated in more detail. Two of these insects have successfully established at sites throughout south-eastern Australia.

Results

Horehound skipper butterfly (Carcharodus boeticus Rambur)

Carcharodus boeticus has two generations per year and is a voracious nocturnal defoliator that specializes in the bud region of young shoots. The larvae build a nest by weaving several leaves together with silk thread, to protect themselves from predators. Larvae are nocturnal, feeding on horehound leaves and returning to the nest during the day. In 1994 and 1995 *C. boeticus* was imported into quarantine at Keith Turnbull Research Institute (KTRI) for host specificity testing. However mating conditions were difficult to replicate in quarantine and two attempts to rear F1 generation offspring failed. It was decided to concentrate time and funds on other more promising biological control agents.

Horehound seed beetle (Meligethes rotroui Easton)

Meligethes rotroui feeds on the flower and pollen of horehound and therefore reduce the number of seeds produced. In April 1997 *M. rotroui* adults collected from Morocco were imported into quarantine at KTRI to initiate a culture for host specificity testing. Unfortunately all insects were dead on arrival and no further shipments are presently planned.

Horehound plume moth (Wheeleria spilodactylus (Curtis))

Wheeleria spilodactylus (Figure 1) is a multivoltine insect that has up to four generations per year and is active from spring to autumn. The larvae (Figure 2) feed on the leaves and developing tips of horehound, which weakens the plant and reduces the numbers of flowers and seeds produced. In November 1991 *W. spilodactylus* was imported into quarantine at KTRI for host specificity testing. Over the next two years the insect was tested on 56 plant species (Weiss *et al.* in press). This testing demonstrated *W. spilodactylus* was host specific to horehound and in December 1993 it was approved for release in Australia. Preliminary releases of *W. spilodactylus* were made at Wyperfeld National Park and Swifts Creek in Victoria, Murray Bridge in South Australia and Tamworth in New South Wales. From 1993 until 1996 the rearing and releasing of *W. spilodactylus* was primarily performed in Victoria. In October 1996 the Cooperative Research Centre (CRC) for Weed Management Systems began rearing *W. spilodactylus* at Adelaide University and in September 1997 the Tasmanian Institute of Agricultural Research, Hobart, also began a rearing program. As a result

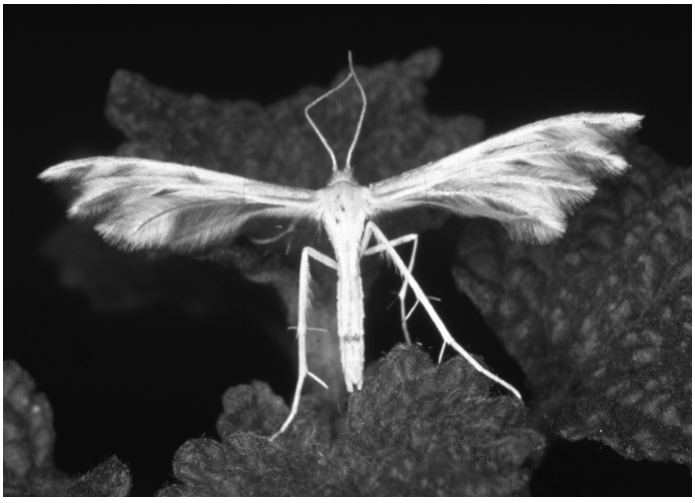

Figure 1. Adult horehound plume moth, *Wheeleria spilodactylus*.

Figure 2. Larva of the horehound plume moth, *Wheeleria spilodactylus*.

over 100 000 insects have been released at over 100 sites in the four south-eastern states of Australia.

Monitoring and redistribution of W. spilodactylus

In spring 1998 a travel grant from CRC for Weed Management Systems enabled a large number of *W. spilodactylus* nursery sites in south-eastern Australia to be monitored for insect establishment, distribution and suitability for use as harvesting sites for redistribution (Figure 3).

At each site:

- The presence or absence of *W. spilodactylus* was monitored.
- The distribution and density of the insect if present, was measured.
- Site managers were trained in insect identification, monitoring and redistribution methods.

As an operational guideline, a minimum of 200 *W. spilodactylus* insects is required to set up a new site, redistribution was only recommended if it was considered that the original nursery site would not suffer from insect removal. A total of 32 sites in Victoria, South Australia, Tasmania and New South Wales were monitored in 1998. At 29 of the sites *W. spilodactylus* had established and 12 contained populations that would sustain harvesting for redistribution (Table 1).

There are several methods possible for harvesting *W. spilodactylus* from field sites.

i. Collection of larvae-infested horehound stems in early spring, when larvae have recently started to feed and develop again after overwintering as first instar larvae in the tips of the plant. During this time the population is synchronized and larvae are at roughly the same stage of development. Redistribution is recommended when the larvae reach the 4th or 5th instar, prior to pupation. Plant stems containing larvae are simply cut at the base and layed across a fresh plant at the new site. The larvae will crawl onto the new plant as the stem dies. It should be noted that stems will wilt and die very quickly after being cut so they must be stored in a cool container and released within 24 hours. This method has been used successfully in the field in Tasmania and in laboratory cultures.

ii. Collection of pupae in mid spring when the first wave of larvae have started to pupate. Leaves containing pupae are collected from the plants and sent or taken to a new site. This was the original method of distributing *W. spilodactylus* to new sites from the mass rearing cultures.

iii. Collection of larvae or pupae later in the season. This method is not as effective or time efficient because the *W. spilodactylus* population has lost the synchronization of lifecycles present at the start of the season. Pupae are more difficult to locate on the plants and stems containing large larvae also contain eggs and small larvae that will perish once the stem is cut.

Several methods of releasing *W. spilodactylus* have been trialed over the years.

- **Tent.** Pupae and adults are released inside 2 × 3 metre by 2 metre high nylon shadecloth tents. The insects build up large numbers in a small area while protected from predators and the elements. This was useful for sites with scattered horehound plants or in dry areas as the tents keep the plants greener and healthier than surrounding plants. Tents are removed after the insects have completed at least one lifecycle (four weeks later).
- **Cage.** Similar to tent releases except the cages only cover one or two plants and are used in areas where the horehound is scattered.
- **Container.** Pupae are put in a container that protects them from the elements and / or predators yet allows the adults to escape once they emerge.
- **Free.** Larvae, pupae and / or adults are placed on horehound plants in the open.

Table 2 shows the different release methods used for the 32 sites monitored in 1998 in comparison to insect establishment and density (suitability for redistribution). No particular release method was more effective for the establishment of *W. spilodactylus* however it appears that free releases produced populations with higher densities.

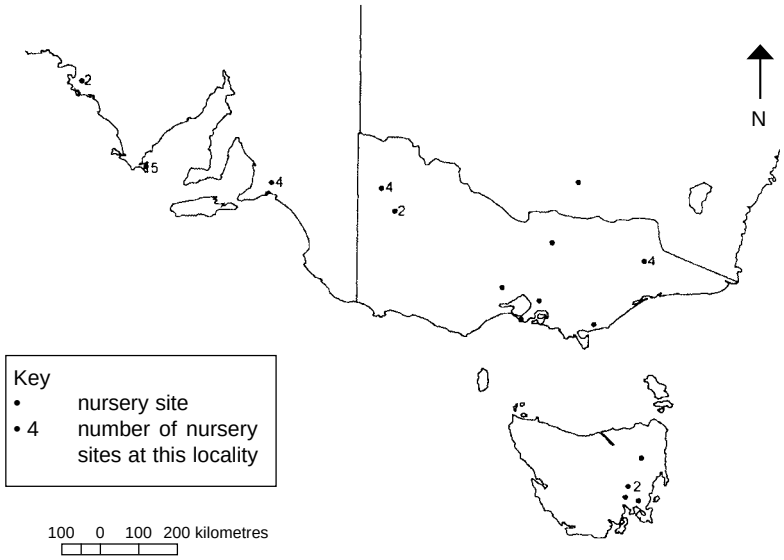

Figure 3. *Wheeleria spilodactylus* nursery sites monitored in spring 1998 in south-eastern Australia.

Table 1. Summary of evaluation of *W. spilodactylus* nursery sites monitored in spring 1998.

State	No. of sites surveyed	No. of sites with establishment	No. of sites suitable for harvesting for redistribution
Victoria	15	13	5
South Australia	11	11	5
Tasmanian	5	5	2
New South Wales	1	0	0
Total	32	29	12

Table 2. Method of *W. spilodactylus* release in relation to the establishment and suitability for harvesting for redistribution at sites monitored in spring 1998.

Release method	No. of sites	No. of sites with establishment	Establishment success (%)	No. of sites suitable for harvesting for redistribution
Tent	13	10	77	4
Cage	3	3	100	0
Container	4	4	100	0
Free	12	12	100	8

Figure 4. Adult horehound clearwing moth, *Chamaesphecia mysiniformis*.

Figure 5. Larva of the horehound clearwing moth, *Chamaesphecia mysiniformis*.

Horehound clearwing moth (Chamaesphecia mysiniformis (Boisduval))

Chamaesphecia mysiniformis (Figure 4) is a univoltine moth that attacks the roots of horehound (Figure 5). The moth can cause mortality of the plant by disrupting vascular flow and indirectly by allowing secondary infection by pathogens (Sagliocco and Coupland 1995). In April 1994 host specificity testing of *C. mysiniformis* began at the CSIRO European Biological Control Unit. After host specificity testing on 60 different plant species in 1995, it was approved for release into the Australian environment. Between 1995 and 1998 a mass rearing culture was attempted at KTRI, however, insufficient emerging adults were produced due to difficulties in maintaining prolonged plant health and vigour in quarantine conditions. In 1998 the Australian Quarantine Inspection Service granted permission for modified non-quarantine rearing of *C. mysiniformis.* In the same year, staff from the CRC for Weed Management Systems at Adelaide University also set up a *C. mysiniformis* culture. The rearing of both of these cultures proved successful and releases were made in South Australia and Victoria in 1999. However, in order for *C. mysiniformis* to establish at a site, several hundred eggs are required. As a result only four sites have been set up (Wyperfeld National Park in Victoria and Nurrung, Monarto and Wilpena in South Australia). The final laboratory generation was reared in 1999 for release in early 2000. If the opportunity for mass rearing again becomes available in the future it will be possible to collect *C. mysiniformis* adults or larvae in the roots from the release sites, rear them in the laboratory and again release as eggs.

Monitoring of C. mysiniformis

Monitoring of *C. mysiniformis* is difficult as the larvae feed in the roots of horehound for around 10 months before emerging as adults in the summer. The root of the plant must be cut open to view the larva, which kills the plant, and subsequently the larva. In January 1998 larvae were recovered from horehound roots at Wyperfeld National Park where 150 eggs had been released one year earlier. A month later 800 eggs were released at the same site and in May 1999 the progress of the site was visually monitored. Frass from larval feeding in the root was found at the base of a large number of horehound plants at the site. Destructive sampling of plants at the site is to occur in February 2000 to determine if the larvae pupated and emerged as adults to confirm establishment. A larva has also been recovered from a plant at a South Australian site in October 1999.

Discussion

Ideally, eight years since the initial survey for insects on horehound in Europe, at least three biological control agents would now be feeding on horehound populations in south-eastern Australia. Unfortunately many factors affect the success of a biological control agent, either at the collecting, transporting, testing, rearing or releasing stage. Once released in the field many more factors can affect the establishment and success of a biological control agent; including climate, predators and human influences. *W. spilodactylus* is having a significant impact on some horehound infestations in north-east Victoria and Tasmania and is showing promise in South Australia. Preliminary monitoring of *C. mysiniformis* sites indicate establishment; however, as there are so few sites, the insect has much more work to do before it makes an impact on the horehound populations in south-eastern Australia.

Acknowledgments

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