Summary  Horehound invades pastures and dry open natural habitats in Australia south of 26°S and is a serious problem in South Australia and Victoria. In western Victoria large infestations of the weed threaten natural areas such as Wyperfeld National Park (NP). In the early 1990s a research project focused on the weed’s ecology in Australia and Europe. Surveys for potential biological control agents conducted in western-Mediterranean Europe identified the arthropod fauna associated with the weed and four species were selected for host-specificity testing including Chamaesphecia mysiniformis (Boisduval).

Releases of C. mysiniformis occurred in 1999 at Wyperfeld NP, Victoria and three locations in South Australia. Surveys conducted at Wyperfeld NP in 2001 and 2003 indicate that the insect has established and dispersed over several kilometres.

Keywords Marrubium vulgare, Chamaesphecia mysiniformis, Sesiidae, biological control, Australia.

INTRODUCTION
Horehound, Marrubium vulgare L. (Lamiaceae) is an erect, perennial herb originating from Eurasia. In western Mediterranean Europe it grows mostly on poor alkaline soils where regular or occasional sheep grazing occurs. This helps its establishment by reducing competition from other plant species, contributes to horehound seed dispersal and increases local nutrient levels.

Horehound was introduced into Australia in the first half of the 19th century and has since become a major weed of pastoral lands in the southern states. In 1980, 6 million ha were affected by the weed in Victoria and 20 million ha in South Australia (Carter 1990). Horehound is also an invader of natural ecosystems especially in western Victoria, threatening some conservation areas. In natural situations, seeds are dispersed by native or introduced animals. Recent studies have shown that biological control of horehound can take place in a broad weed management approach (Ainsworth 2000, 2003).

In 1989, a research program focusing on the plant’s biology and demography started in Victoria and was extended to investigate horehound ecology and evaluate potential biological control agents. Extensive surveys were also conducted on 55 horehound populations in western Mediterranean Europe and identified 27 phytophagous insect species (Sagliocco 2000). The first insect collected for biological control, Wheeleria spilodactylus (Curtis) (Lepidoptera: Pterophoridae) was imported into Australia in 1991 and approved for release in 1993. Two biotypes were introduced from France and Spain and the moth has been widely redistributed throughout the southern states of Australia (Wills 2000).

The second biological control agent released was the moth, Chamaesphecia mysiniformis (Boisduval) (Lepidoptera: Sesiidae) which was common on horehound in Spain, especially in the northern province of Zaragoza. The insect is univoltine and larvae feed and develop in the roots until late spring with adults emerging over a five weeks period in May-June (Sagliocco and Coupland 1995).

MATERIALS AND METHODS
Eggs of C. mysiniformis were imported into quarantine at Frankston, Australia in June 1997 but failed to produce enough adults to establish a viable colony. In 1998, the direct release of larvae from imported eggs was approved by the Australian Quarantine and Inspection Service (AQIS). In April, horehound roots with larvae were collected at Zaragoza and maintained in the CSIRO laboratory, Baillarguet, France. Emerging C. mysiniformis adults mated and females produced eggs in May-June 1998. Freshly laid eggs were surface sterilised with a 0.5% sodium hypochlorite solution, then rinsed in distilled water and shipped to Frankston. On arrival eggs were surface sterilised a second time in the same manner and kept in Petri dishes at 20–25°C in an insectary glasshouse. Each day, newly emerged larvae were transferred with a fine paint-brush at the base of mature horehound plants kept in 15 cm pots in the same insectary glasshouse. Average temperatures during larval development ranged between 17.5°C and 26°C. In January 1999 C. mysiniformis adults began emerging. Adult emergence and matings were monitored and eggs produced were collected daily. Eggs of C. mysiniformis were glued singly to wooden
toothpicks that were then used for inoculation of mature plants in the field (one egg attached by toothpick to the base of a mature horehound plant). Releases of *C. mysiniformis* were undertaken at Black Flat, Wyperfeld National Park (NP), Victoria (Lat. 35.5788°S, Long. 142.0184°E.) (Figure 1) between the 9 and 18 February 1999 and on 20 December 1999, with 818 and 682 mature horehound plants being inoculated respectively.

To assess *C. mysiniformis* establishment and dispersal, monitoring began at Wyperfeld NP in November 2001. Four transects 30 m long × 2 m wide, radiating in every cardinal direction from the centre of the area where the original 1999 releases were made were established. Each transect was sub-divided into 5 × 2 m blocks. In each block the number of immature (seedlings), mature plants (bearing fruits) and dead horehound plants was recorded. To assess *C. mysiniformis* larval presence in roots, all dead plants present in the blocks were collected as well as 100 mature plants randomly selected. Roots were dissected and the insects present were recorded. Roots showing evidence of live *C. mysiniformis* larvae were kept in cages with vermiculite until adults emerged in November-December 2003. Dispersal of *C. mysiniformis* was assessed in November 2003 at 1–2 km in each cardinal direction from the original 1999 release point. Plants were collected and roots cut open to check larval or pupae presence.

**RESULTS**

The number of horehound plants at Wyperfeld NP in 2001 and 2003 are presented in Table 1.

The number of *C. mysiniformis* in horehound at Wyperfeld NP are presented in Table 2. There was a significant increase in *C. mysiniformis* from 29.0% in 2001 to 50.3% in 2003 (*P*=0.0002).

*C. mysiniformis* dispersed up to 1.6 km from the 1999 release point (Table 3).

**DISCUSSION**

Due to the destructive nature of the sampling technique, the sampling sites differed from 2001 to 2003. The 2001 sample area was flat and away from trees while the 2003 area was slightly uneven and protected
by river red gum trees (Eucalyptus camaldulensis Dehnh.). This difference in habitats may explain the higher density of seedlings and lower density of mature plants observed in 2003. As females of C. mysiniformis lay eggs on mature plants the reduction in numbers of mature plants can play a role in the overall insect’s density. However, the increase in the proportion of mature plants showing presence of C. mysiniformis suggests establishment of the insect and an expanding population. The population of C. mysiniformis introduced into Australia originates from a region in Spain with a continental climate (winter frosts and high summer temperatures) and the insect was considered able to withstand Australian summer conditions. Newly emerged C. mysiniformis females release a sexual pheromone to attract males and generally mate within five hours after emergence at a temperature above 26°C (Sagliocco and Coupland 1995). In the laboratory, male competition was observed in the presence of a female releasing pheromone and some males have been observed to mate more than one time over consecutive days. Information on the insect’s biology was used to build a model to calculate the number of eggs required for a single release. This was done to avoid any Allee effect due to males not being able to mate because of their relatively short lifespan and to take into account the high larval mortality (up to 75%) inside roots (Sagliocco, unpublished data). This model showed that a minimum of 700 eggs was required to optimise the simultaneous emergence of males and females at one location. In 2003, C. mysiniformis larvae were present on plants up to 1.6 km from the original release point. However the number, size and health conditions of horehound populations remaining at Wyperfeld NP are concerns. If horehound populations decrease further due to drought conditions, this may compromise the survival of C. mysiniformis through the lack of healthy plants and reduced insect density.

In Spain, a reduction in number and size of horehound populations harbouring C. mysiniformis was observed around Zaragoza between 1994 and 1998 due to urban expansion. The loss of suitable sites and the reduction of pastoral practices were critical in seed dispersal and plant recruitment. Currently there is no information available on the status of the releases made in South Australia. In Victoria, if C. mysiniformis population disappear from Wyperfeld, a new collection of the insect in Spain may be difficult. Collection of C. mysiniformis at Wyperfeld NP and re-distribution in other areas less severely affected by drought could be envisaged but with the insect densities recently observed and the low number of attacked plants, substantial effort would be required to collect sufficient insects to establish a new colony.

ACKNOWLEDGMENTS
This work was co-funded by the Department of Sustainability and Environment (DSE), Victoria, Australian Wool Innovation Limited (AWI) and Meat and Livestock Australia Limited (MLA). The technical assistance of Emma Wills (DPI) in rearing and releases of the insect is acknowledged.

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