Biocontrol Science and Technology (1995) 5, 509–515

Biology and Host Specificity of *Chamaesphecia mysiniformis* (Lepidoptera: Sesiidae), a Potential Biological Control Agent of *Marrubium vulgare* (Lamiaceae) in Australia

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(*Received for publication 9 March 1995; revised manuscript accepted 29 July 1995*)

The biology of the rhizophagous clearwing moth Chamaesphecia mysiniformis *Rambur and its specificity to* Marrubium vulgare *L. (Lamiaceae) (horehound), a serious introduced weed in southern Australia, were studied in France. Adults emerged in late spring during the morning and began mating on the same day, usually in the mid to late afternoon. Eggs were laid among flower clusters, with females laying an average of 96 ± 2.41 (range, 1–268) with an overall hatch success of 79%. In no-choice, host-specificity tests, first instar larvae attacked only four species of* Marrubium*, along with* Ballota nigra *L. and* Stachys arvensis *L. This high level of specificity and the high mortality of the target plant in its native range make this moth a promising biological control agent of horehound in Australia.*

Keywords: *biological control, horehound,* Marrubium vulgare*, Australia, Sesiidae*

INTRODUCTION

Marrubium vulgare L. (Lamiaceae) is an erect perennial herb. It originates from central and western Europe and north Africa (Parsons & Cuthbertson, 1992), where it occurs in well-drained calcareous soils, and is most common where grazing occurs. Originally introduced into Australia by settlers for medicinal purposes, *M. vulgare* rapidly spread throughout the southern states to become a major weed of pastures. Its spread was particularly augmented by sheep transporting burrs and seeds in their fleece. In undisturbed natural habitats, the weed also quickly established and invaded new areas due to dissemination of its seeds by native mammals and introduced rabbits. In Victoria, this weed has been estimated to infest 6 million ha, with dense infestations covering up to 100 000 ha (Lane *et al*., 1980). Although seedlings are outcompeted by other plants, they establish rapidly when overgrazing and/or drought reduces the density of competing vegetation. Some herbicides, such as 2,4-D and MCPA, are effective when used against *M. vulgare* (Carter, 1990; McMillan, 1990), especially if used in conjunction with pasture improvement programmes. However, chemical methods are ineffective and undesirable in conservation areas where the weed is a serious threat. Biological control was seen as the most economic

0958–3157/95/040509–07

alternative to other forms of control, and a research project investigating potential biocontrol agents against horehound was initiated jointly by the CSIRO Division of Entomology and the Keith Turnbull Research Institute in Europe in 1991.

The clearwing moth *Chamaesphecia mysiniformis* Rambur (Lepidoptera: Sesiidae) is a rhizophagous univoltine moth that attacks *M. vulgare* in the Iberian peninsula. The insect was also recorded in Algeria by Bartel (1912). The moth causes mortality both directly, by disrupting vascular flow, and indirectly, through secondary infection by pathogens. This study examines aspects of its biology, in particular mating, oviposition, fecundity and host specificity, to determine the potential of *C. mysiniformis* as a possible candidate to control *M. vulgare* in Australia.

MATERIALS AND METHODS

In April 1994, 800 *M. vulgare* plants were collected from three sites near Zaragoza (Spain) at 41°39′N, 0°56′W and transported to the CSIRO Biological Control Unit at Montferrier, France. After pruning, the roots were stored in $40 \times 20 \times 60$-cm plastic containers in 1:1 vermiculite:perlite, covered with $40 \times 60 \times 80$-cm screen cages and kept in a glasshouse at 25°C under semi-controlled sunlight (provided by an electrically controlled screen).

Emergence and Rearing

Cages were checked daily for adult emergence. Newly emerged *C. mysiniformis* were transferred to a $40 \times 40 \times 40$-cm clear Perspex cage, which contained a small jar of 7% sucrose solution and a bouquet of flowering *M. vulgare*. Moths were observed continuously during the day for coupling. When matings were observed, both temperature and light intensity (W m^{-2}) were recorded. Paired adults were carefully transferred to plastic tubes covered with nylon mesh, and the duration of mating was recorded. When copulation ceased, the males were transferred back into the Perspex cage, and each female was kept individually in a glass cage made from a hurricane lamp glass bulb, 14 cm in diameter and 17 cm high, covered with nylon mesh. A small jar containing 7% sucrose solution provided an additional food source, and a bouquet of flowering *M. vulgare* clusters maintained in distilled water was provided as an oviposition site. Every second day, the bouquet was removed and replaced, eggs were then counted, removed from the bouquet, stored in Petri dishes and checked daily for larval emergence.

Host-specificity Studies

Test plants were selected using the centrifugal phylogenetic method described by Wapshere (1974). Those used included a number of plants of economic importance in Australia and plants taxonomically related to the weed. The taxonomic relationships between species were assessed using the classification system described by Cronquist (1981) and generic position within the family Lamiaceae was based on Engler (1964).

Host-specificity tests were carried out using newly emerged larvae. Five larvae were transferred on to each test plant with a fine camel-hair brush. Normally, five replicates of each plant species were used, though 10 were made on *Ajuga australis* and four on several others due to reduced test-plant availability (Table 1). Test plants were maintained in a glasshouse between 22 and 28°C, and dissected after 2–3 weeks, when the numbers of initial attacks and of surviving larvae were recorded.

RESULTS

C. mysiniformis adults emerged between 08.00 and 15.00 from 19 May until 20 June 1994 (Figure 1), with a peak in emergence for both males and females between 26 and 28 May. A total of 351 adults emerged between 3 May and 21 June, with a sex ratio not significantly different from 1:1 (Figure 1). Mating occurred only under specific conditions of both light and temperature. Of the 135 matings that were observed, all occurred when the light intensity fell below

141 W m^{-2}, with a peak in frequency at 114 ± 3 W m^{-2} ($n = 109$). Mating occurred between 22 and 37°C, with the frequency peaking at about 30°C (Figure 2). Mating took a relatively long time, on average 70.1 ± 2.6 min (range 5–150 min, $n = 132$). Egg production averaged 96.0 ± 5.58 eggs/female (range 1–268, $n = 127$). The total number of eggs produced during the study was 12 195, while the number of hatching larvae was 9664, giving an overall hatching success of 79%.

Hatching and Specificity Tests

Eggs were black and oval, with a fine reticulum mesh on the chorion. They measured 0.73 ± 0.008 mm in length and 0.47 ± 0.004 mm in width ($n = 30$). The eggs hatched in 10–14 days at 24–28°C. Larval hatching began just before dawn and finished before noon. The photophobic first instar larvae crawled to the base of the plant, where they began to feed on the outer cambium to make a gallery prior to entering the root. All subsequent development, including a winter diapause, occurred in the root before completion the following year. Normally only one larva/root developed. The last instar larva burrowed an exit hole at the collar level or at the base of an erect stem prior to pupating, which took place lower within the root. Pupation lasted between 2 and 4 weeks, and adults emerged in May–June.

In the host-specificity tests, larvae of *C. mysiniformis* were found to attack and survive on all four species of horehound tested. The highest survivorship of larvae was on *M. vulgare, M. supinum, M. alysson* and *M. leonuroides*, as well as the black horehound, *Ballota nigra* and stagger weed, *Stachys arvensis*. Small initial attacks were recorded on other plants within the families Lamiaceae, Asteraceae (one species), Boraginaceae (one species), Caricaceae (one species), Gramineae (one species) and Scrophulariaceae (two species), but no development occurred, and larvae died at the first instar (Table 1).

DISCUSSION

The biology of *C. mysiniformis* is similar to other sesiids occurring in the western European region, including two which have already been introduced into Australia as biological control agents (Scott & Sagliocco, 1991a,b). While the biology of many species of *Chamaesphecia* is unknown, the results of the few studies that are available indicate a long life cycle (1 or 2 years). The univoltine life history and the spring or early summer flight period of May–June are common for species occurring in southern Europe (Tosevski, 1986; Lastuvka & Lastuvka, 1980), though development times may be plastic and are probably determined by climatic conditions, as evidenced by varying developmental times within northern and central European species (Lastuvka, 1983; Spatenka *et al.*, 1993). Furthermore, most species have been recorded emerging in the morning (Lastuvka & Lastuvka, 1980; Tosevski, 1986; Scott & Sagliocco, 1991a,b), with mating occurring during the same day. The relatively high temperature at which most mating occurred in *C. mysiniformis* is comparable to that observed in *Bembecia chrysidiformis* Esper and *Chamaesphecia doryliformis* Ochsenheimer, where mating occurred between 28 and 34°C for the former and 28°C for the latter (Scott & Sagliocco, 1991a,b). While the fecundity was lower for *C. mysiniformis* (96 ± 5.5 eggs/female) than either *B. chrysidiformis* (up to 368 ± 25 eggs/female) or *C. doryliformis* (330 ± 28 eggs/female), the overall egg viability was much greater at 80%, compared with 42% for *B. chrysidiformis* and 53% for *C. doryliformis*.

Previous studies on clearwing moths (Sesiidae) have shown that these moths can be extremely host specific (Pussard, 1961; Králicek, 1975; Lastuvka & Lastuvka, 1980; Tosevski, 1986; Lastuvka, 1989; Scott & Sagliocco, 1991a,b). The species, *C. doryliformis* and *B. chrysidiformis*, released for control of *Rumex* spp. (chiefly *Rumex pulcher* L.), attacked only plants within the genus *Rumex* (Scott & Sagliocco, 1991a, b). In the present study, no larval development was observed to occur on plants other than *Marrubium* spp., *B. nigra* L. and *S. arvensis* L. In Australia, both *B. nigra* and *S. arvensis* have been introduced, and the latter is classified as a minor weed (Parsons & Cuthbertson, 1992). In France, *B. nigra* L. (black horehound) is the known host plant of another sesiid, *Chamaesphecia annellata* (Zeller)

TABLE 1. Plant species tested with *C. mysiniformis*, showing the initial number of larvae tested, surviving larvae and the instar to which larvae were found when plants were dissected

Family and species	Common name	Initial no. of larvae	Surviving larvae	Instar
Lamiaceae				
Marrubium vulgare L.	Horehound	25	21	2
M. supinum L.		25	9	2
M. alysson L.		25	7	1
M. incanum Desr.		25	2	2
M. leonuroides Desr.		25	2	2
M. friwaldskyanum Boiss.		20	—	—
M. velutinum Sibth. & Sm.		25	—	—
M. anisodon C. Koch		25	—	—
Ballota nigra L.	Black horehound	25	3	2
Stachys arvensis L.	Stagger weed	25	1	2
S. alopecuros (L.) Benth.		25	—	—
S. densiflora Benth.		25	—	—
Leonotis leonurus (L.) R. Br.		25	—	—
L. oxymifolia (Burm. f.) Iwarsson		25	—	—
Lamium amplexicaule L.	Deadnettle	25	—	—
Prunella vulgaris L.	Self-heal	25	—	—
Salvia officinalis L.	Sage	25	—	—
S. haematodes L.	Austral sage	25	—	—
Scutellaria humilis R. Br.	Dwarf skullcap	25	—	—
Mentha diemenica Spreng.	Wild mint	25	—	—
M. australis R. Br.	Australian mint	25	—	—
M. spicata L.	Spearmint	25	—	—
Lycopus australis R. Br.	Water horehound	25	—	—
Thymus vulgaris L.	Thyme	25	—	—
Origanum vulgare L.	Wild marjoram	25	—	—
Dracocephalum ruyschiana L.		20	—	—
Lavandula latifolia Medicus	Lavender	25	—	—
Ocimum basilicum L.	Basil	25	—	—
Plecthranthus parviflorus Vill.	Cockspur flower	25	—	—
Rosmarinus officinalis L.	Rosemary	25	—	—
Ajuga australis R. Br.	Australian bugle	50	—	—
Teucrium racemosum R. Br.	Grey germander	30	—	—
Westringia fruticosa (Wild.) Druce	Coastal rosemary	25	—	—
Hemiandra pungens R. Br.	Snake bush	25	—	—
Prostanthera ovalifolia R. Br.		25	—	—
Lauraceae				
Persea americana Miller	Avocado	25	—	—
Casuarinaceae				
Allocasuarina littoralis (Salisb.) Johnson		25	—	—
Theaeceae				
Camellia sinensis (L.) Kuntze	Tea	—	—	
Actinidiaceae				
Actinidia chinensis Planch.	Kiwi fruit	25	—	—
Passifloraceae				
Passiflora caerula L.	Passionfruit	25	—	—
Caricaceae				
Carica papaya L.	Paw-paw	25	—	—
Mimosaceae				
Acacia melanoxylon R. Br.	Wattle	25	—	—
Fabaceae				
Glycine max (L.) Merr.	Soybean	25	—	—

TABLE 1. Continued

Family and species	Common name	Initial no. of larvae	Surviving larvae	Instar
Proteaceae				
Macadamia integrifolia Maiden & Betche	Macadamia nut	20	—	—
Myrtaceae				
Eucalyptus nitens Maiden	Gum tree	25	—	—
Asteraceae				
Helichrysum bracteatum (Vent.) Andrews		25	—	—
Olearia axilaris D. C. (Benth.)	Daisy bush	25	—	—
Stemmacantha australis (Gaudich.)	Australian cornflower	25	—	—
Anacardiaceae				
Mangifera indica L.	Mango	10	—	—
Boraginaceae				
Borago officinalis L.	Borage	20	—	—
Cynoglossum australe R. Br.	Hound's tongue	25	—	—
Verbenaceae				
Gmelina leichhardtii (F. Muell.) Benth.	White beech	25	—	—
Premna lignum-vitae (Schauer) Pieper	Queensland lignum	25	—	—
Vitex trifolia L.		25	—	—
Scrophulariaceae				
Morgania floribunda Benth.	Blue rod	25	—	—
Verbascum thapsus L.	Mullein	25	—	—
Graminae				
Lolium perenne L.	Perenial ryegrass	25	—	—
Saccharum officinarum L.	Sugar cane	25	—	—
Musaceae				
Musa x sapientum L.	Banana	25	—	—
Zingiberaceae				
Zingiber officinale Rosc.	Ginger	25	—	—
Pinaceae				
Pinus radiata D. Don	Monterey pine	25	—	—

(Lastuvka, 1989). However, in the field, *C. mysiniformis* has never been recorded from a plant other than *M. vulgare*. Finally, within the genus *Marrubium*, there are no economically important plants, and four of the *Marrubium* spp. it attacked during testing do not occur in Australia. These results indicate both the high specificity of *C. mysiniformis* to plants of the genus *Marrubium* and its potential safety to indigenous Australian plants.

Results of previous introductions of biologically similar clearwing moths into Australia for the control of *Rumex* spp. have confirmed that mass rearing is feasible, that populations of the insect established without difficulty in regions with matching climates, and that they were able to disperse and colonize new weed populations within a few years (Fisher, 1992). Damage by the insect in its native range indicates that the insect attacks 50–90% of 2–3-year-old plants. This latter result, along with the organism's high specificity, makes *C. mysiniformis* a potentially efficient and safe agent for the biological control of *M. vulgare* in Australia.

ACKNOWLEDGEMENTS

This work was funded by Australian wool growers through the International Wool Secretariat (project DCV14). John Weiss (KTRI Frankston, Victoria) established the host-specificity test

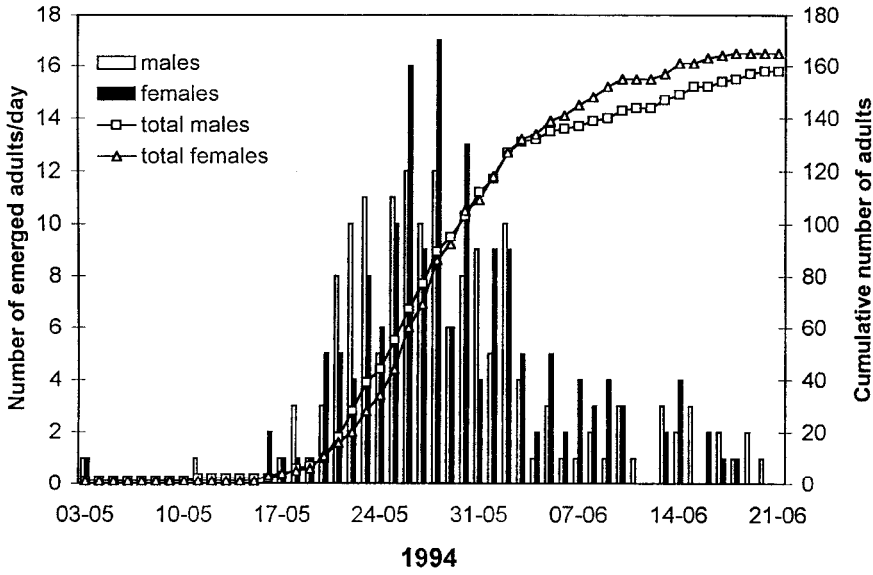

FIGURE 1. Patterns of emergence of *C. mysiniformis* from field-collected *M. vulgare* roots. Emergence peaked in the last week of May. The sex ratio was not significantly different to 1:1.

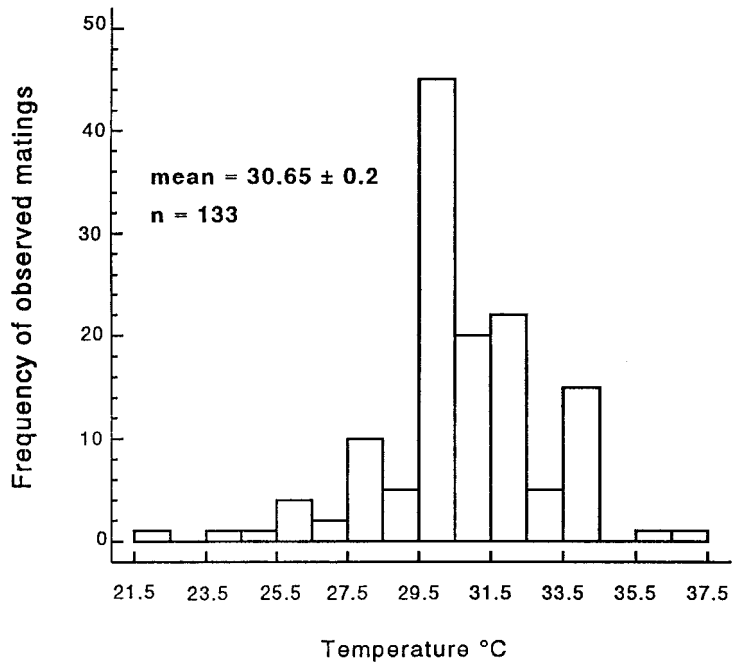

FIGURE 2. Frequency of observed matings of *C. mysiniformis*, showing the large peak at 30°C.

plant list. Andi Walker (CSIRO Division of Entomology, Canberra) grew and provided most of the tests plants. Dr Karel Spatenka (Piscne, Czech Republic) determined specimens of *C. mysiniformis*. Drs David Briese, John Scott and Andy Sheppard kindly provided useful comments on the manuscript.

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