MASS-EMERGENCE DEVICES TO IMPROVE SYNCHRONY BETWEEN \textit{LISTRONOTUS BONARIENSIS} AND \textit{MICROCTONUS HYPERODAE}

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ABSTRACT

A novel approach to conserving and augmenting parasitoids involves storing large numbers of parasitised hosts in mass-emergence devices. After eclosing inside the device, parasitoid adults, which are generally smaller than their hosts, are allowed to escape through a mesh screen, while any unparasitised hosts remain trapped. The goal of this study was to assess the potential of mass-emergence devices both for augmenting populations of the parasitoid \textit{Microctonus hyperodae} and for manipulating its synchrony with its host the Argentine stem weevil, \textit{Listronotus bonariensis}. The experiments demonstrated that a screen with a 900 \(\mu\)m by 900 \(\mu\)m mesh is appropriate to separate parasitoid adults from their hosts, and that a device of very simple design could be effective. However, the survival of \textit{L. bonariensis} and \textit{M. hyperodae} was reduced by cool storage and food deprivation, and further development is required to overcome these limitations.

Keywords: classical biological control, conservation biological control, parasitoid host synchrony, habitat manipulation, pasture pest management, Hymenoptera, Braconidae, Euphorinae, Curculionidae.

INTRODUCTION

Natural enemies can be important regulators of pest populations, but are sometimes ineffective for reasons which range from the antagonistic effects of hyperparasites to poor temporal synchrony with host populations (DeBach & Rosen 1991). The performance of a natural enemy can often be enhanced using approaches that increase its abundance relative to the pest (Berryman 1999). One such approach involves the use of mass-emergence devices that collect and store parasitised hosts in closed devices from which only parasitoids can emerge (Kehrli et al. 2005). The selective exits needed to enable parasitoids to leave the devices, while retaining their hosts, may exploit the generally smaller size of parasitoids compared to their hosts (Quicke 1997) by employing appropriately sized mesh screens (e.g. Bonsall & Hassell 1998). Kehrli et al. (2005) described the development of a mass-emergence device for parasitoids of the horse chestnut leafminer in Europe, and demonstrated in field experiments that their devices increased parasitism of the targeted pest by 2.5 times. In addition to augmenting parasitoid populations, mass-emergence devices enable biological control practitioners to manipulate when and where parasitoids are released. Many species of parasitised hosts can be stored for short periods at low temperatures to retard parasitoid development, thus delaying parasitoid emergence (Goldson et al. 1992; Kehrli et al. 2005). Storing parasitised hosts at low temperatures before transferring them to mass-emergence devices
can therefore help parasitoids to depart the devices in synchrony with the emergence of host populations in the field.

At Lincoln, the summer onset of emergence of the adult stage of the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) pre-empts that of its host the Argentine stem weevil, *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae), by one month (Phillips et al. 1998; C.B. Phillips, unpubl. data). The first parasitoids that emerge in mid November lay few eggs due to the lack of hosts, and it is only the later emerging parasitoids that coincide with the mid December emergence of hosts to lay most of their eggs (Phillips et al. 1998; C.B. Phillips, unpubl. data). This impedes the growth of *M. hyperodae* populations every summer, and recent research by Vattala (2005) has clearly demonstrated the value to biological control of manipulating *M. hyperodae* to overcome this impediment. In field experiments, the longevity of *M. hyperodae* adults present during November-December was extended by providing them with access to floral nectar (Vattala 2005). This increased parasitoid abundance at the onset of host emergence in mid December, and led to a doubling of parasitism in January-February compared to plots without floral nectar (Vattala 2005).

In view of these exciting results, other approaches to improving synchrony between *M. hyperodae* and *L. bonariensis* may also be very valuable. This contribution makes a preliminary assessment of the potential of mass-emergence devices for improving biological suppression of *L. bonariensis*. The general concept is that large numbers of parasitised weevils could be collected during winter then maintained at low temperatures in a manner that retards the development of *M. hyperodae* by about one month. The parasitised weevils could thereafter be returned within mass emergence devices to the field to enable *M. hyperodae* adults to emerge from the devices as hosts are becoming abundant in mid December. Here, the mesh size and design of a device suitable for use with *M. hyperodae* and *L. bonariensis* are investigated and the effect of cool storage on delaying adult parasitoid emergence is examined.

**MATERIALS AND METHODS**

**Experiment 1**

This experiment investigated two aspects of the design of the mass-emergence device. Firstly, it tested if 900 μm mesh screens would enable *M. hyperodae* adults to leave containers, while preventing the escape of *L. bonariensis* adults. Secondly, it examined whether *M. hyperodae* could successfully undergo pupation while confined in the same chamber as the hosts (‘double chamber’ treatment), or if it was necessary to allow them to pupate in a separate chamber (‘triple chamber’ treatment) by adopting an approach similar to that used by Goldson et al. (1993a) for culturing *M. hyperodae*.

In the double chamber treatment, parasitised weevils were maintained and emergent *M. hyperodae* larvae also pupated within a cylindrical transparent plastic container (11 cm diameter, 6 cm height) with a solid base and an open top. Over the chamber’s open top was positioned another plastic cylinder (also 11 cm diameter, 6 cm height) that had a 900 μm mesh screen covering its base. This arrangement was designed to test if adults of *M. hyperodae*, but not *L. bonariensis*, could escape from the lower chamber via the mesh screen to the upper chamber. The top of the upper chamber was covered with fine gauze to prevent any insects from escaping, while allowing aeration.

A triple chamber device was constructed in the same way as the double chamber device described above, except that the solid base of the chamber housing the parasitised weevils was substituted with a 900 μm mesh screen, and another chamber with a solid base was positioned underneath it. This was designed to test if weevils could be confined in the middle chamber, while allowing parasitoids to drop through the 900 μm mesh screen as larvae to pupate in the lower chamber, then to move freely as adults between all three chambers.

*Listronotus bonariensis* adults were swept from Canterbury roadsides on 15 June 2004, and maintained without ryegrass at 10°C and 14:10 h light: dark pending the start of the experiments. An unknown proportion of these weevils, probably 20-50%
Parasitoids and Predators

(C.B. Phillips, unpubl. data), was naturally parasitised by *M. hyperodae*, which overwinters as a 1st instar larva. The first trial began on 24 June 2004 and the second on 12 August 2004, both involving four devices of each type. Seventy *L. bonariensis* adults were placed in the bottom unit of each double chamber device and in the middle unit of each triple chamber device. A 5-10 mm layer of dry pasture litter was placed in the chambers housing the weevils. The devices were maintained at 20°C and 14:10 h light:dark, and water was provided via wet dental wicks, which were re-moistened as necessary. After each trial had been underway for 1 month, the numbers of living and dead adults of *M. hyperodae* and *L. bonariensis* were recorded, as were their positions in the devices.

**Experiment 2**

A second experiment examined the effect of food and cold storage on *L. bonariensis* survival and *M. hyperodae* emergence. *Listronotus bonariensis* were swept from Canterbury roadsides on 8 November 2004. A no-storage trial was set up on 10 November 2004 in which 25 *L. bonariensis* were allocated to each of eight double chamber devices, then maintained at 20°C, 14:10 h light:dark. Weevils in four of these devices were regularly provided with water and bouquets of ryegrass, whereas weevils in the other four were not. A storage trial involved maintaining 25 *L. bonariensis* in each of eight containers in the dark at 5°C from 10 November until 10 December 2004. Weevils from each storage container were then transferred to separate double chamber devices. Weevils in four of these devices were regularly provided with water and ryegrass, whereas weevils in the other four were not. All eight devices were maintained at 20°C, 14:10 h light:dark. Weevil survival and parasitoid emergence in the no-storage and storage trials were assessed on 10 December 2004 and 10 January 2005, respectively.

**Data analysis**

Weevil survival and parasitoid emergence were treated as response variables whereas chamber type, storage and food supply were treated as independent variables. Data from both experiments were analysed by Mann-Whitney U Test using the SPSS 13.

**RESULTS**

**Experiment 1**

All *M. hyperodae* adults that emerged in the devices were discovered in the upper chambers, thus demonstrating they had passed through the 900 μm mesh, whereas all weevils had been retained in their original chambers. Similar numbers of parasitoid adults emerged in the double and triple chamber devices (*P*=0.89, Fig. 1), indicating that pupal survival was not increased by physical separation from the hosts. More parasitoid adults emerged in the trial that began on 24 June 2004 than in the trial that began on 12 August 2004 (*P*=0.03, Fig. 1). Fifty-five (10%) of the 560 weevils survived until the end of the first trial, but all of the weevils died during the second trial.

**Experiment 2**

In the storage trial, only 70 (35%) of 200 weevils survived 1 month of storage in the dark at 5°C. However, following their removal from cold storage and subsequent maintenance at 20°C and 14:10 h light:dark, the stored weevils exhibited mortality rates similar to those observed in the no-storage trial (*P*=0.91, Fig. 2). Overall, about 60% of ryegrass-fed weevils survived the trials, whereas no starved individuals did (*P*<0.001, Fig. 2). Unfortunately, only two *M. hyperodae* adults emerged in experiment 2, thus the impacts of cold storage and host diet on parasitoid survival could not be assessed.
Parasitoids and Predators

FIGURE 1: Total number of *Microctonus hyperodae* cocoons and adults that emerged in two- and three-chamber devices in the early and late trials of experiment 1.

FIGURE 2: Fate of *Listronotus bonariensis* adults after 1 month of exposure to the feeding treatments in experiment 2. Weevils in the storage trial had been maintained for one month at 5°C in the dark prior to the start of the feeding treatments. ‘Missing’ *L. bonariensis* were lost during the experiment.
DISCUSSION

The results of experiment 1 demonstrated that a 900 μm mesh screen does not restrict the movement of *M. hyperodae* adults, but does confine *L. bonariensis*. The absolute separation of *M. hyperodae* and *L. bonariensis* observed in the present study compares favourably with Kehrli et al. (2005) where about 1% of host individuals and 70% of parasitoid adults were able to pass through the selective exit. Thus, the need to separate the natural enemy and pest should not impede the design of a mass-emergence device for *M. hyperodae*. The observation that *M. hyperodae* is able to pupate and reach the adult stage while confined in the same chamber as *L. bonariensis* should also simplify the design process.

The high mortality of *L. bonariensis* observed in the experiments may have been partly attributable to natural senescence of the overwintering population. Nevertheless, the results generally indicate that the survival of *L. bonariensis* and *M. hyperodae* is severely compromised by extended periods of cool temperatures and food deprivation. The negative effect of cool storage of parasitised *L. bonariensis* on the emergence of *M. hyperodae* pre-pupae has previously been documented by Goldson et al. (1992). After removal from storage at 6°C and transfer to 20°C, there was significantly higher mortality of parasitised *L. bonariensis* compared to unparasitised weevils, irrespective of the time they had earlier spent in cool storage (Goldson et al. 1992). Therefore, significant challenges remain in finding ways to minimise mortality of *L. bonariensis* and *M. hyperodae* in a mass emergence device. It may be that slightly higher storage temperatures of 10-12°C would help to reduce insect mortality, while still serving to retard the development of *M. hyperodae* larvae. Unfortunately, this approach would probably be impractical since the equipment needed to maintain such temperatures is unlikely to be readily and cheaply available to biological control practitioners using mass-emergence devices.

An alternative avenue of research aimed at retarding the development of *M. hyperodae* larvae in a mass emergence device could involve manipulating photoperiod, rather than temperature. *Microctonus hyperodae* enters photoperiodically induced diapause in the 1st instar stage at a critical photoperiod of 13.6 hours (Goldson et al. 1993b). This photoperiod occurs during mid March in New Zealand, but the environmental conditions that lead to the subsequent termination of *M. hyperodae* diapause during winter remain unexplored (Goldson et al. 1993b). Therefore, it would be useful to investigate if it is possible to delay the date at which *M. hyperodae* larvae resume their post-diapause development in spring through the imposition of artificially short photoperiods. This might be easily achieved by covering the device to keep the enclosed insects in darkness until a pre-determined date in late winter or spring. Although this approach would eliminate the need to maintain artificially cool temperatures, it would not circumvent the challenge of providing food to *L. bonariensis* during storage, for which possible solutions are yet to be identified.

Practical methods for improving synchrony between *L. bonariensis* and *M. hyperodae* in early summer have enormous potential value, and recent studies both on the use of mass emergence devices for delaying parasitoid emergence and on the provision of floral nectar to extend parasitoid adult longevity (Vattala 2005) represent exciting new perspectives on management of *L. bonariensis*. Recognition of the potential to improve synchrony between *M. hyperodae* and *L. bonariensis* stemmed from the development of detailed knowledge of the population dynamics of *L. bonariensis* and *M. hyperodae*, thus emphasising the value of population dynamics studies in biological control programmes.

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