FIELD CAGE AND LABORATORY PARASITISM OF *NICAENA CERVINA* BY *MICROCTONUS AETHIOPOIDES*

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ABSTRACT

A study was carried out to compare parasitism of the New Zealand native weevil *Nicaena cervina* Broun (Coleoptera: Curculionidae) by *Microctonus aethioides* Loan (Hymenoptera: Braconidae) in field cage versus laboratory conditions. Total parasitism was 40–55% and 15% in laboratory and field cages, respectively. The level of parasitism obtained in the field cages was similar to that recorded in a natural population nearby. Proportions of failed parasitism and superparasitism in the field cages were similar to that in laboratory cages. Laboratory cages gave an adequate representation of the effectiveness of the parasitoid in the field.

Keywords: *Microctonus aethioides*, *Nicaena cervina*, non-target host, laboratory parasitism, field parasitism

INTRODUCTION

Safety testing of new organisms before release in New Zealand for the purpose of biological control is a contentious issue. Despite thorough laboratory testing, the fate of a new organism in the environment can be difficult to predict. The assessment of host ranges for potential biological control agents prior to release is, of necessity, undertaken under laboratory conditions. Extrapolating from these to field situations can be difficult and conclusions reached can be controversial. Sands (1993) believed that the effects of confinement could give unreliable results and that information gleaned from a study of a parasitoid in its natural environment was sufficient to determine its host range. Caging a parasitoid with a non-target but potential host could conceivably result in parasitism when in a more natural environment an interaction is unlikely to occur (Goldson *et al.* 1992). Alternatively, artificial conditions might alter parasitoid behaviour and misrepresent what could occur in the field.

In 1982 the parasitoid *Microctonus aethioides* Loan was introduced to New Zealand to control the lucerne pest *Sitona discoideus* Gyllenhal (Stufkens *et al.* 1987). Subsequently *M. aethioides* has been found parasitising a wide range of endemic and introduced weevils in different habitats (Ferguson *et al.* 1994; Barratt *et al.* 1997). *M. aethioides* is being used as a model parasitoid by AgResearch to investigate potential versus realised parasitism, with the objective of improving quarantine protocols for future introductions.

The aim of this study was to investigate the similarities between parasitism of *Nicaena cervina* Broun, a known non-target host of *M. aethioides*, in field cages and the laboratory.

METHODS

Laboratory experiments

Three laboratory experiments were carried out where the native broad-nosed weevil *N. cervina* was exposed to the introduced parasitoid *M. aethioides*. All experiments were conducted in an insect rearing room maintained at 20±2°C with a relative humidity of 40–60%, and a 16 hour light: 8 hour dark photoperiod.

Groups of either 20 or 40 *N. cervina* (Table 1) were caged in two tier acrylic cages (160 x 150 mm x 75 mm deep) fitted with fine gauze lids. The floor of the upper container was of 1 x 1 mm plastic mesh and was inserted into the top of another similar container

having a paper towel lining the base. The paper towel served as a substrate for parasitoid pupation after they emerged from their host and passed through the floor mesh of the top cage. Each group of weevils were exposed to 2 or 3 mated female *M. aethioides* for 48 or 72 hours (Table 1). After the parasitoids were removed, the cages were checked daily for dead weevils and parasitoid pupae. Dead weevils were placed in 70% ethanol for dissection. Parasitoid pupae were placed in a Petri dish until parasitoid emergence.

N. cervina were provided with pollen, collected from a bee hive, and a mixture of ryegrass, ((*Lolium perenne* x *L. multiflorum*) x *L. perenne* cv. 'Manawa'), and lucerne (*Medicago sativa* cv. 'Wairau') seedlings as food. Fresh seedlings were provided every three days. Water was provided in two moistened dental rolls in each cage.

After parasitoid emergence was completed and no further parasitoids had emerged for two days (usually 25 days after parasitoid exposure), surviving weevils were placed in 70% ethanol for dissection.

Field experiment

A field experiment was conducted in December 1996 in established pasture near Sutton on the Strath Taieri, where *N. cervina* was common. The pasture had been recently grazed and was approximately 40 mm tall. A Jonsered blower-vac was used to remove as many of the resident weevils as possible from the areas where the field cages were to be set up. The litter collected was not returned to the cage area.

Six metal-framed cages (450 x 900 mm x 500 mm high) covered with nylon mesh were placed in the pasture and the bottom was pushed into the soil. *N. cervina* collected from the same site in early November and maintained in the laboratory to purge them of parasitoids were added to each cage (40 per cage). In one cage, each of the weevils were marked with a spot of white acrylic paint on the pronotum. These weevils were intended to provide an estimate of weevil recovery from the cages.

Three female *M. aethioides*, reared from *S. discoideus*, were added to five of the cages immediately after the *N. cervina*, leaving the paint-marked weevils unexposed to parasitoids. The *M. aethioides* were all less than 72 hours old and had been fed with a honey water solution (approximately 20% w/v) prior to their release.

The density of weevils and parasitoids used in the field cages was representative of naturally occurring densities in the area. During spring the density of *N. cervina* often peaks at approximately 110/m² and this density can persist for some time (B.I.P Barratt unpubl. data). The number of weevils introduced to the field cages (40) produced a density of 98.8/m² introduced *N. cervina*.

Weevils were recovered from the cages after 72 hours using a combination of blower-vac as above, and an adapted household vacuum cleaner, both fitted with fine mesh bags, to recover as many weevils as possible from within the cages. This extraction was carried out initially through the side window of the cage before the cage was lifted and the area re-vacuumed. No attempt was made to recover the parasitoids.

The collected litter was first hand sorted, then placed in Berlese funnels. Weevils from each field cage were placed in laboratory rearing cages and were maintained in the laboratory for 11 days, then preserved in 70% ethanol prior to dissection. Dead weevils were removed daily from the cages and similarly preserved.

RESULTS AND DISCUSSION

Laboratory cages

Laboratory parasitism levels of *N. cervina* by *M. aethioides* were consistently within the range 40-55% (Table 1). These levels are generally lower than those for parasitism of the target host, *S. discoideus* (53-71%) (Barratt *et al.* 1997).

Parasitism of *N. cervina* by *M. aethioides* in the laboratory often results in dead 1st instar larvae being found on dissection some time after exposure (Barratt *et al.* 1997; Barratt unpubl. data). These larvae are very small (<500 µm) and often only the slightly melanised head capsule of the larva is visible. High levels of parasitoid mortality resulting from an immune response from the host are often attributed to artificial laboratory conditions, where host/parasitoid ratios are unnaturally high. Goldson *et al.* (1992) argued that high levels of *Microctonus hyperodae* larval mortality in non-target hosts in

the laboratory “indicate a mismatch between the parasitoid and its host” suggesting that successful parasitism in field populations may be reduced. Goldson *et al.* (1992) considered that the experimental design used in their study, which was similar to the design in this study, contributed to an overestimation of host range. Loan and Holdaway (1961) reported that *Sitona cylindricollis* Fahraeus was a suitable host of *Microctonus aethiops* Nees (= *aethiopoidea* Loan) when caged in laboratory experiments, despite failed parasitism occurring. However, *M. aethiopoidea* was not recovered from *S. cylindricollis* in the field, in either Europe or North America.

In these experiments, the proportion of parasitised *N. cervina* containing dead 1st instar parasitoid larvae on dissection (failed parasitism) and the proportion found superparasitised (more than one larvae found in a host) was similar for all laboratory experiments (Table 1). This suggested that the parasitism behaviour of *M. aethiopoidea* did not change in response to host density and level of exposure in the laboratory.

Field cages

The recovery rate of paint-marked weevils from the field cage was 43% (17/40). However, 7 of the 24 *N. cervina* recovered from this cage were unmarked, indicating that the initial removal of weevils from the areas covered by the cages were not entirely successful. No teneral *N. cervina* were found suggesting that adult emergence did not account for the unmarked weevils. Two other weevil species were recovered in small numbers from the cages, *Listronotus bonariensis* Kuschel and *Irenimus stolidus* Broun. Numbers of *N. cervina* recovered from each exposed cage ranged from 16–24.

Parasitism of recovered *N. cervina* by *M. aethiopoidea* ranged from 5–36%. Parasitism of *N. cervina* in only one case was further advanced (4th instar) on dissection than could have been expected after 11 days in the laboratory (1st–2nd instar) and this was not included in the following results. The proportion of parasitised hosts containing dead first instar larvae and that were superparasitised is similar to that in the laboratory experiments.

TABLE 1: Parasitism status of *Nicaeana cervina* after exposure to *Microctonus aethiopoidea* in the three laboratory experiments and the field cage trial.

Trial	r:w:p ¹	Exposure period (h)	% Parasitism (total) ±SEM	Failed parasitism ²	Superparasitism ³
Laboratory					
1	5:20:3	48	55.1 ± 9.00	0.17 ± 0.06	0.05 ± 0.03
2	15:20:3	48	39.5 ± 6.39	0.24 ± 0.04	0.03 ± 0.03
3	10:40:2	72	52.7 ± 7.03	0.37 ± 0.05	0.07 ± 0.02
Field cages					
	5:40:3	72	14.7 ± 5.67	0.56 ± 0.16	0.09 ± 0.06

¹Number of replicate cages: weevils per cage: parasitoids per cage

²Proportion of parasitised hosts containing dead 1st instar parasitoid larvae

³Proportion of superparasitised hosts found

Field cage parasitism of 15% (Table 1) was similar to that recorded in field populations. *N. cervina* parasitism of up to 17% has been recorded regularly at a nearby site and parasitism over 20% is regularly recorded in other populations of *N. cervina* (B.I.P. Barratt unpubl. data). However, considering the 72 hour exposure time, the large area of the field cage (c.f. laboratory cages), and that the *M. aethiopoidea* had been reared from *S. discoideus* and had not been exposed to *N. cervina* previously, total parasitism was unexpectedly high.

Comparison of laboratory and field studies

The density of the hosts and parasitoids in the laboratory cages used were much

higher than would be under field conditions and the environment is very artificial. However, commonly observed aspects of laboratory parasitism, such as failed parasitism and superparasitism occurred in the field cages in similar proportions as in laboratory cages (Table 1). This would suggest that the laboratory cages provided an environment for parasitism that was similar to the field cages and that while parasitism may be lower in the field cages and field, *M. aethiopoides* performance is similar. Failed parasitism is currently detected in field populations of *N. cervina* at a level of 11% (B.I.P. Barratt unpubl. data).

In this particular case, aspects of parasitism found in laboratory conditions are present in similar levels in field conditions, suggesting that laboratory cages may give an adequate representation of parasitoid field effectiveness.

ACKNOWLEDGEMENTS

The authors would like to thank Ray Taylor for the use of his property for both collection and field cage sites, Ann Cresswell for technical assistance and Bruce Chapman and Colin Ferguson for valuable input and support. This work was conducted as part of AAE's MSc and was funded by the Foundation for Science, Research and Technology.

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