CIRCADIAN PATTERN OF OVIPOSITION IN THE PARASITOIDS *MICROCTONUS AETHIOPOIDES* LOAN AND *M. HYPERODAE* LOAN (HYMENOPTERA: BRACONIDAE), IN RELATION TO HOST ACTIVITY

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ABSTRACT

Laboratory experiments were carried out to investigate the circadian patterns of oviposition of the parasitoids *Microctonus aethiopoides* Loan and *M. hyperodae* Loan in their respective hosts *Sitona discoideus* Gyllenhal and *Listronotus bonariensis* (Kuschel). Successful oviposition by *M. aethiopoides* in *S. discoideus* occurred more frequently during light than during dark periods, but *M. hyperodae* achieved higher parasitism levels in *L. bonariensis* during hours of darkness than during light. Circadian parasitoid oviposition activity was related to host activity, measured by feeding and oviposition. *S. discoideus* was found to feed and lay eggs mostly during the latter part of the light period, whereas *L. bonariensis* feeds mainly after dark.

Keywords: *Microctonus aethiopoides*, *M. hyperodae*, oviposition, circadian rhythms, host activity

INTRODUCTION

Argentine stem weevil, *Listronotus bonariensis* (Kuschel) and *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) are significant forage pests in New Zealand. *L. bonariensis* is one of New Zealand's most damaging ryegrass (*Lolium perenne* L.) pests (Prestidge *et al.* 1991), and *S. discoideus* is a significant pest of lucerne (*Medicago sativa* L.) (Goldson *et al.* 1985). Biological control programmes have led to the release and successful establishment of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae: Euphorinae) to control *S. discoideus* (Stufkens *et al.* 1987) and *M. hyperodae* to control *L. bonariensis* (Goldson *et al.* 1994). Both parasitoid species have established successfully in New Zealand (Goldson *et al.* 1993; Goldson *et al.* 1994).

Recent research has shown that *M. aethiopoides* successfully parasitises at least six native and two other non-target species in the field (Barratt *et al.* 1995a). Identification of non-target species at risk from introduced parasitoid attack could be enhanced if parasitoid behaviour in relation to host behaviour was better understood. Previous work (Barratt *et al.* 1995b) has determined the circadian pattern of oviposition and feeding in *L. bonariensis* Kuschel, where peak feeding occurs at the beginning of the dark period, lasting 4-6 hours. Oviposition fluctuated over the 24 hour period with one peak also synchronised with the onset of darkness.

The objectives of this laboratory study were to determine the circadian pattern of oviposition of *M. aethiopoides* and *M. hyperodae* in their respective hosts, and to investigate the circadian pattern of activity in *S. discoideus* measured by oviposition and feeding.

METHODS

Adult *M. aethiopoides* reared from field collected *S. discoideus* weevils were used in the investigation. *M. hyperodae* was supplied from a rearing programme at AgResearch, Lincoln. For the investigation, adult *S. discoideus* were collected from

a lucerne stand at Sutton, Strath-Taieri, in spring 1995. Adult *L. bonariensis* were collected from a ryegrass pasture at Sutton and on the Taieri Plain in January 1996.

For the experiments, weevils were held in plastic cages 160 x 180 x 75 mm deep with a plastic-coated mesh floor inserted into the top of another similar container with textured absorbent paper towel covering the base. This served as a substrate for pupation of emergent pre-pupal parasitoids which moved down through the mesh from the upper to the lower cage. *L. bonariensis* was fed Grasslands cv. Manawa ryegrass (*L. perenne* x *L. multiflorum*) x *L. perenne*), and *S. discoideus*, Grasslands cv. Wairau lucerne (*Medicago sativa* L.). Plants were grown in commercial seed raising mix to a height of about 100 mm, and when required, the roots and soil were enclosed in 100 x 75 mm plastic bag secured firmly at the base of the plants using a plastic cable clip. Fresh plants were supplied every 3-4 days, and water was provided in the form of 2-3 saturated cotton dental wicks placed in each cage.

Parasitoid oviposition

Fifteen cages, each with 20 *S. discoideus* were held at $20 \pm 2^\circ\text{C}$ in a 16L: 8D photoperiod for 48 hours, to acclimatise weevils. *M. aethiopoides*, which had newly emerged from field collected *S. discoideus* were similarly held for 36 hours, allowing an opportunity to mate. Three female parasitoids were introduced into each cage and five cages were assigned to each of the following treatments:

1. 48 hours continuous exposure to parasitoids (2 cycles of 16L : 8D)
2. 48 hours of light (3 cycles of 16L:8D); parasitoids removed during dark periods
3. 48 hours of dark (6 cycles of 16L:8D); parasitoids removed during light periods.

A similar experiment was carried out with *M. hyperodae* and its host *L. bonariensis*. For both experiments, parasitoids removed from the hosts cages between exposure periods were held at the same photoperiod as the weevils and supplied water and honey solution to maintain maximum reproductive potential (Hodgson 1993). The parasitoids were returned to the same cages for each exposure period. Parasitoids that died during the course of the experiment were replaced with similarly aged, acclimatised wasps.

On completion of the 48 hour exposure periods for each treatment, the parasitoids were removed and the weevils maintained for up to 30 days until all resulting parasitoid pupae had emerged. Weevils that died, and those that survived, were dissected and examined for parasitoids.

S. discoideus oviposition and feeding

The experiment was conducted in two rooms, both $20 \pm 2^\circ\text{C}$ and 16L: 8D photoperiod, but with different on:off times, so that oviposition and feeding could be more conveniently measured over the 24 hour period. The weevils were acclimatised to their allocated rooms for 48 hours before the investigation began. Five cages each with 20 *S. discoideus* adults were placed in each room. The experiment was conducted over 12 days and measurements of oviposition and feeding were carried out at 4 hour intervals on every second day (6 days in total).

S. discoideus eggs which passed through the mesh floor of the upper cage were collected in the lower cage, from where they were removed every 4 hours and counted.

S. discoideus feeding was measured by placing the lucerne leaves on graph paper, and estimating the amount removed in mm^2 . Fresh lucerne was provided for each 4 hour sampling period. All weevils which died during the experiment, and those which survived, were dissected at the conclusion of the experiment to establish the sex ratio of weevils in the cages. Survival was recorded every second day.

Data analysis

Total percent parasitism was calculated from the sum of those parasitoids which emerged successfully from their hosts and those which were found during dissection. The data were analysed using a Generalised Linear Model, with a binomial distribution and logit link functions (Dobson 1990). *S. discoideus* oviposition and feeding data were analysed using a Generalised Linear Model, adjusted for the covariates survival, and number of females present (Nelder and Wedderburn 1972).

Mean egg production and feeding values were calculated for each of the 6 days of measurement, and for the two rooms where the experiment was conducted. There were no significant differences in *S. discoideus* oviposition between the two rooms, but

there were consistent differences in weevil feeding activity between rooms 1 and 2. Consequently, the higher data values of room 1 were corrected, by deducting from them the difference in mean feeding between the rooms. Data were further corrected using the number of surviving weevils, and the number of females (for oviposition) as covariates.

RESULTS

Parasitoid oviposition

There was no significant difference between the percent parasitism achieved by *M. aethiopoides* during 48 hours continuous exposure to *S. discoideus* and that achieved during light periods only, but parasitism levels resulting from exposure during dark periods were significantly reduced (Table 1). A significantly higher percentage of successful oviposition by *M. hyperodae* occurred during the dark exposure periods compared with the light periods and continuous exposure (Table 1). The latter two treatments were not significantly different.

TABLE 1: Percentage parasitism of *S. discoideus* exposed to *M. aethiopoides* and *L. bonariensis* exposed to *M. hyperodae* during periods of light (L), dark (D) or the normal light : dark cycle.

Parasitoid exposure	Total % parasitism $\pm$ SE	
	<i>M. aethiopoides</i> (ex <i>S. discoideus</i>)	<i>M. hyperodae</i> (ex <i>L. bonariensis</i>)
2 x 16L:8D (continuous)	36.6 $\pm$ 4.79	48.5 $\pm$ 5.02
3 x 16L	32.0 $\pm$ 4.66	39.2 $\pm$ 4.96
6 x 8D	3.0 $\pm$ 1.72*	69.1 $\pm$ 4.69*

*significantly different (P < 0.01)

FIGURE 1: Mean number of eggs (dashed line) and leaf area removed by feeding (solid line) in *S. discoideus* measured every 4 hours for 6 days. Error bars represent 2SE.

Parasitoid mortality was higher in the dark period exposure treatments (33% and 27% for *M. aethioides* and *M. hyperodae* respectively) compared with the continuous exposure (no mortality) and light period exposure treatments (13% and 7% respectively). Mortality was almost certainly related to the amount of handling required in the respective treatments.

***S. discoideus* oviposition and feeding**

Oviposition reached a peak 8–12 hours after the beginning of the light period, and then declined throughout the remainder of the light period and entire dark period (Fig. 1). Feeding varied between 1.9 and 2.7 mm² of lucerne leaf consumed per weevil per 4 hour period. Feeding activity was relatively low for the first 4–8 hours of light, and then increased rapidly to a peak 12–16 hours from the beginning of the light period, and falling off after the onset of the dark period (Fig. 1).

DISCUSSION AND CONCLUSIONS

M. aethioides oviposited successfully in *S. discoideus* mainly during periods of light, with little parasitism occurring during darkness. This corresponded with *S. discoideus* feeding and oviposition activity which occurred mainly during light periods. Previous studies, (e.g. Loan and Holdaway 1961), indicate that oviposition by *M. aethioides* occurs only while the host is active, immobile weevils being ignored. Goldson *et al.* (1990) showed that aestivating *S. discoideus* were not attacked by *M. aethioides*, again indicating host movement is an essential cue to initiate stalking by the parasitoid, and eventually oviposition. Mityakina *et al.* (1993) and Idoine and Ferro (1990) showed a similar diurnal pattern of oviposition by the egg parasitoid *Edovum putleri* in the Colorado potato beetle, where no oviposition occurred during the dark period or early light period.

In the case of *M. hyperodae*, the results indicated a less distinct adherence to a light : dark activity pattern, but significantly more parasitism occurred during the dark periods than during light. Barratt *et al.* (1995b) showed that *L. bonariensis* feeding was triggered by the onset of the dark period, but that there were three peaks of egg-laying, one coinciding with the peak in feeding activity after dark. As with *M. aethioides* it appears that *M. hyperodae* oviposition activity corresponds with host activity, particularly feeding, which occurs on ryegrass foliage, whereas during oviposition, weevils would be located near the base of ryegrass tillers.

Twilight periods were not included in the treatments in this study, and these may be important periods, or triggers, for host and parasitoid activity which require further investigation.

New Zealand native broad-nosed weevils have been shown to be more susceptible to parasitism by *M. aethioides* than by *M. hyperodae* (Barratt *et al.* 1995a). Bremner (1988) showed that surface activity in sub-alpine native broad-nosed weevils correlated well with temperature in spring and autumn and hence they were mainly diurnal, while in summer, some activity continued during the hours of darkness. The importance of circadian activity patterns of non-target species, in relation to that of introduced parasitoids, requires further investigation.

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