**CIRCADIAN PATTERNS OF OVIPOSITION AND FEEDING
IN *LISTRONOTUS BONARIENSIS* (KUSCHEL)
(COLEOPTERA: CURCULIONIDAE) IN THE LABORATORY**

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ABSTRACT

A laboratory experiment compared the daily patterns of oviposition and feeding of *Listronotus bonariensis* (Kuschel) exposed to either a 12L:12D or 16L:8D photoperiod. Eggs and weevil feeding scars on ryegrass plants were recorded every 2 hours for 8 days, with 2 days between each assessment when weevils were not disturbed. Females exposed to the 16L:8D photoperiod produced significantly more eggs than those subjected to a 12L:12D regime, but the circadian pattern of oviposition was consistent throughout for both photoperiod treatments. There were three distinct peaks of oviposition over the 24 hour period, one of which was synchronised with the onset of darkness, and the other two occurred during the light period. There was no difference in feeding activity in relation to photoperiod, and the circadian feeding pattern was consistent for both treatments with a single peak occurring at the beginning of the dark period and lasting for 4-6 hours

Keywords: *Listronotus bonariensis* (Kuschel), photoperiod, circadian rhythm, feeding, oviposition

INTRODUCTION

Listronotus bonariensis (Kuschel) (Argentine stem weevil) is an important introduced pest of pasture grass species (Prestidge *et al.* 1991). While it has been reported that *L. bonariensis* is more easily collected by sweeping vegetation at dusk than during daylight hours, and therefore thought to be more 'active' at this time (Goldson 1979; Goldson and Proffitt 1991), there is little information available about the daily pattern of activity for the species. Since the introduction of the adult parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) for biological control of *L. bonariensis* (Goldson *et al.* 1993), it has become important to examine in detail aspects of the biology and behaviour of the adult weevil in order to better understand factors which might influence success of the biological control programme. It is similarly important to compare the activity patterns of *L. bonariensis* with potential non-target hosts, to provide some information on the probability of interactions occurring with *M. hyperodae* in the field. As an initial approach to this study, the daily pattern of *L. bonariensis* oviposition and feeding was investigated in the laboratory for two different photoperiods. These were used to represent extreme daylengths experienced by adult weevils in the field over the main spring to autumn period of seasonal activity.

METHODS

Twenty adult *L. bonariensis* were collected from the field near Middlesmarch, Otago, by vacuum sampling in early November 1994 when there was approximately 14.5 h light and 9.5 h dark. They were placed in each of twenty cages 160 x 180 x 75 mm deep with a fine gauze lid. The experiments were conducted in a temperature controlled room maintained at $20 \pm 2^\circ\text{C}$, with humidity ranging from 40-52%. Ten replicate cages were exposed to a 16 h light: 8 h dark photoperiod (16L:8D) and another 10 replicates were exposed to a 12 h light: 12 h dark (12L:12D) regime. In both

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cases lights went off at 12.30 am and on at 8.30 am and 12.30 pm in the 16L:8D and 12L:12D treatments respectively. Cages were exposed to the photoperiod to which they had been assigned for 36 h before the experiment started.

Food plants, Grasslands Manawa ryegrass (*Lolium (perenne x multiflorum) x perenne* L.) were grown in commercial seed raising mix in glasshouse trays with 27 x 27 x 45 mm deep cells with 6-10 plants per cell. They were grown to a height of about 100 mm, removed with the roots and soil intact, and placed in a 100 x 75 mm plastic bag secured firmly at the base of the plants using a plastic cable clip to prevent weevil access into the bags. Approximately 0.3 g of pollen granules collected from a honeybee hive were provided in each cage since this has been shown to increase oviposition rate (Evans and Barratt 1995). The plant species from which the pollen was derived was mixed and undetermined. Water was supplied in saturated cotton dental wicks, which were resoaked when necessary at food changes.

The experiment was conducted over a 24 day period, during which every third day, two-hourly assessments of oviposition and feeding were carried out. This was done by providing fresh food at 6.30 am, removing it at 8.30 am and immediately replacing it with fresh food. This process was repeated every 2 hours for 24 hours. When food changes occurred during dark periods, a red light was used. The ryegrass bunches removed were examined for eggs, inserted between the upper and lower leaf cuticle, and feeding scars. Both of these were counted for each cage. *L. bonariensis* feeds by removing the upper cuticle and mesophyll of ryegrass leaf blades leaving the lower cuticle as a 'window' which is usually a regular rectangular shape.

To determine the extent to which disturbance on the days when 2-hourly food changes were carried out affected feeding and oviposition of weevils, ryegrass bunches that were left in the cages during the 2-day periods between the assessment dates were also examined for eggs and feeding scars. Weevil counts were carried out on these 'interim' days, and dead weevils removed.

Weevils that died during the experiment, and those that survived were dissected at the conclusion of the experiment to determine the sex ratio of weevils in the cages, presence of sperm in the spermatheca, and the number of females with eggs in the calyces.

Data were analysed using generalised linear models by fitting parsimonious models with binomial error distributions and logit link functions (Nelder and Wedderburn 1972).

RESULTS AND DISCUSSION

Oviposition

Adult weevils were collected from the field in early November when ovary length, and mature egg numbers in females would have been near their maximum (Goldson 1981). In the laboratory, egg production of female *L. bonariensis* exposed to a 12L:12D photoperiod was significantly reduced by about 24% compared with those exposed to a 16L:8D photoperiod (Table 1). Goldson *et al.* (1981) reported that the critical photoperiod below which *L. bonariensis* entered reproductive diapause was 13.3L:10.7D. Consequently, the females in this study exposed to the short photoperiod were probably entering reproductive diapause and egg production was declining in comparison with the 16L:8D photoperiod. Dissections showed that all females had mated, and that the percentage of females with eggs in the calyces was significantly higher in the 12L:12D treatment (69.2 ± 7.32 (SE)) than in the 16L:8D treatment (55.4 ± 5.09 (SE)). This could indicate that females exposed to the short photoperiod were retaining eggs rather than ovipositing.

Over the 24 h assessment periods, three peaks in egg laying were apparent, starting from 12.30 pm, 6.30 pm, 12.30 am (Fig. 1), which was consistent for both photoperiods. Between 4.30 am and 12.30 pm, oviposition rates were relatively low. The peak in egg-laying starting at 12.30 am coincided with the onset of the dark period, which may have triggered the pattern since the oviposition model was not significantly different for the two photoperiod treatments. The peaks observed during the light period may have been caused by endogenous rhythms governed or limited by the rate of egg maturation

in females, but synchronised, under laboratory conditions, by the unnaturally abrupt onset of darkness. Correcting for the number of females remaining in each cage did not improve the model.

TABLE 1: Mean number of eggs and feeding scars (both $\pm$ SE) produced per cage over 24 hours for each photoperiod.

	n	Photoperiod	
		12L:12D	16L:8D
No. eggs/ cage/ day	6.7 ¹	5.64 $\pm$ 0.242	7.43 $\pm$ 0.278
No. feeding scars/ cage/ day	15.8 ²	5.95 $\pm$ 0.297	6.60 $\pm$ 0.596

¹ Mean number females per cage surviving at end of experiment

² Mean number weevils per cage present at each date

FIGURE 1: Circadian pattern of oviposition for *L. bonariensis* averaged for both photoperiods, using GLIM predicted data. The bars represent 2SE.

Oviposition during the 48 h before each assessment period (excluding the initial one) was found to be close to twice the 24 h value (Table 2) suggesting that disturbance during food changes on assessment days had little impact upon oviposition.

Feeding

Feeding as measured by the number of feeding scars on the ryegrass plants provided did not differ significantly between the 12L:12D and 16L:8D photoperiods (Table 1). While feeding scars varied in size, the assumption was made that size-frequency distribution would be similar for each treatment. Correcting for the number of weevils in each cage did not improve the model. Feeding appeared to be typical of a single phase circadian rhythm (Danilevsky *et al.* 1970) with one peak synchronised by the beginning of the dark period starting at 12.30 am (Fig. 2). Activity then declined steadily during the following 4 h to a level which was fairly constant from about 6.30 am onwards (Fig. 2).

The number of feeding scars recorded during the 48 h before each assessment period (excluding the initial one) were 5-6 times higher than those found during the 24 h assessment periods (Table 2) suggesting that disturbance during food changes on assessment days had a substantial impact upon feeding. In the field, disturbance during

FIGURE 2: Circadian pattern of feeding activity of *L. bonariensis* averaged for both photoperiods, using GLIM predicted data. The bars represent 2SE.

feeding on the distal parts of the plant may trigger a defence response to avoid predation. Field observations (unpubl.) of a number of curculionid species suggest that they typically drop from foliage when disturbed, so continuation of feeding may be temporarily inhibited. Eggs, however, are usually inserted at the tiller bases, where disturbance is less likely to occur, so that the same defence response might not be stimulated.

TABLE 2: Mean number of eggs and feeding scars produced per cage during 24 hour assessments (dates 2-8) compared with each preceding 48 hour interim period. Feeding data are $\log_e$ transformed with back-transformed values in brackets.

	12L:12D		16L:8D		SE
	48 h interim	24 h assessment	48 h interim	24 h assessment	
No. eggs/ cage	12.80	6.42	17.87	8.26	2.36
No. feeding scars/ cage	4.41 (82.43)	2.70 (14.94)	4.62 (101)	2.75 (15.56)	0.084

The results suggest that the main period of *L. bonariensis* activity in the upper parts of the pasture sward may occur during hours of darkness, possibly beginning at dusk, concurring with previous observations related to weevil trapping (eg. Goldson 1979). Oviposition, which is generally restricted to the base of tillers also took place after dark but also during daylight.

Little is known at this stage about the comparative activity patterns of New Zealand native brachycerine weevils which have been identified as potential hosts for *Microctonus* spp. (Ferguson *et al.* 1994). However, Bremner (1988) measured surface activity of weevils in an area where a complex of brachycerine species were present on the East Otago Plateau, and found that they were strictly diurnal for most of the year, but active throughout the 24 hour period in summer during the peak of their seasonal activity, when surface temperature remained above 6°C.

Further research will investigate the diurnal pattern of *M. hyperodae* oviposition in *L. bonariensis* and non-target species to determine whether this corresponds with periods of feeding, or periods of relative inactivity at the base of the sward.

ACKNOWLEDGEMENTS

We would like to thank Peter Johnstone for data analysis, and those who had to tolerate our irregular activity patterns for their patience while this experiment was in progress.

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