PARASITISM BY *MICROCTONUS AETHIOPOIDES* ON A NOVEL HOST, *LISTRONOTUS BONARIENSIS*, IN CANTERBURY PASTURES

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ABSTRACT

The parasitoids *Microctonus aethiopoides* and *M. hyperodae* have been introduced as biological control agents against *Sitona discoideus* and Argentine stem weevil *Listronotus bonariensis* respectively. Collections from ryegrass/white clover pastures in mid-Canterbury found that *M. aethiopoides* was widespread in *L. bonariensis,* despite the weevil being a novel host. *Microctonus aethiopoides* was recovered from 83% of the 65 sites sampled in Canterbury, with parasitism rates of 0.4-19%. Studies in lucerne crops showed that *M. aethiopoides* preferentially parasitised its natural host *S. discoideus*, although in some cases up to 10% of *L. bonariensis* found in the lucerne were parasitised. A possible reason for the prevalence of *M. aethiopoides* in Canterbury pasture was that the widely-dispersive flights of *S. discoideus* from aestivation sites provided a source for re-infection of the parasitoid into ryegrass pasture. Analysis of field parasitism suggested that parasitism of *L. bonariensis* by *M. aethiopoides* was negatively correlated with parasitism by *M. hyperodae*.

Keywords: Argentine stem weevil, *Sitona discoideus*, novel host-association, biological control, parasitoid competition.

INTRODUCTION

Two *Microctonus* species have been introduced to New Zealand in classical biological control programmes. Both are solitary endoparasitoids that attack the adult weevil. *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) was released in 1982 (Stufkens et al. 1987) against *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), a Mediterranean pest of *Medicago* L. spp. *Sitona discoideus* is found throughout New Zealand where lucerne (*Medicago sativa* L.) is grown. In Canterbury, the weevil was shown to be a significant pest of lucerne on light alluvial soils with the larvae causing significant short and long-term losses in dry matter yield (Goldson & Muscroft-Taylor 1988). Research subsequently showed a significant suppression of *S. discoideus* populations due to parasitism by *M. aethiopoides* (Goldson et al. 1990; Barlow & Goldson 1993), with atypical summer build-up in parasitism being a key to the success of the parasitoid (Barlow & Goldson 1993). While this result can be considered a 'successful' classical biological control programme, *M. aethiopoides* has also been found to parasitize 13 native and introduced weevil species including *L. bonariensis* (Goldson & Proffitt 1991; Barratt et al. 1997).

Microctonus hyperodae Loan (Hymenoptera: Braconidae) was released in 1991 against Argentine stem weevil *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) (Goldson et al. 1993). *Listronotus bonariensis* is of South American origin and causes significant damage to ryegrass pasture in New Zealand (Prestidge et al. 1991). The successful establishment and spread of *M. hyperodae* has been documented in detail (e.g. Goldson et al. 1993, 1999), and its build-up in Canterbury coincided with a reduction in the size of *L. bonariensis* first summer generation egg and larval peaks (e.g. Goldson et al. 1998).

Between 1992 and 2000, several projects provided the opportunity to collect adult *L. bonariensis* from ryegrass pastures in Canterbury. This included a study to follow the dispersal of *M. hyperodae* from the Lincoln research release site (Goldson et al. 1999), a study to assess non-target impacts of *Microctonus* spp. on native weevil species (M.R. McNeill, unpubl. data) and commercial contracts that required pre- and post-parasitoid establishment sampling of paddocks (McNeill et al. 2002). *Listronotus bonariensis* adults were also collected as part of the *S. discoideus* biological control programme (Kean & Barlow 2000). The results of these studies as they relate to the incidence of *M. aethiopoides* in the novel host *L. bonariensis,* and its interaction with *M. hyperodae,* are reported in this paper.

MATERIALS AND METHODS

Field sampling from ryegrass pasture

Listronotus bonariensis adults were collected using either a blower vacuum (Homelite[R] 180V) with a net fitted to the inlet or a modified electric blanket that used heat generated from the blanket to attract the weevils out of the litter (Goldson & Proffitt 1991). Weevils were then held in cages that consisted of an upper 220 mm x 130 mm x 75 mm transparent cage with a mesh floor, placed on top of a similar container, the floor of which was lined with paper towelling. Parasitoid prepupae emerged from weevils, crawled through the mesh and pupated under the paper towelling in the lower chamber. Pupae were placed in 9 cm diameter plastic Petri dishes for the development and emergence of the parasitoid adult. The two introduced *Microctonus* species were identified by morphological characteristics (McNeill et al. 1993).

The number of sites sampled from year to year varied from 1 in 1995 to 42 in 1997. While a total of 65 unique sites were sampled between 1992 and 2000 (Fig. 1), several sites were sampled repeatedly so that parasitoid recoveries were available for a total of 139 sampling occasions. This was particularly so at the Lincoln *M. hyperodae* release site (43°63′ S, 172°47' E), which was sampled 18 times at irregular intervals between 1992 and 2000. Multiple collections both within and between years were also made

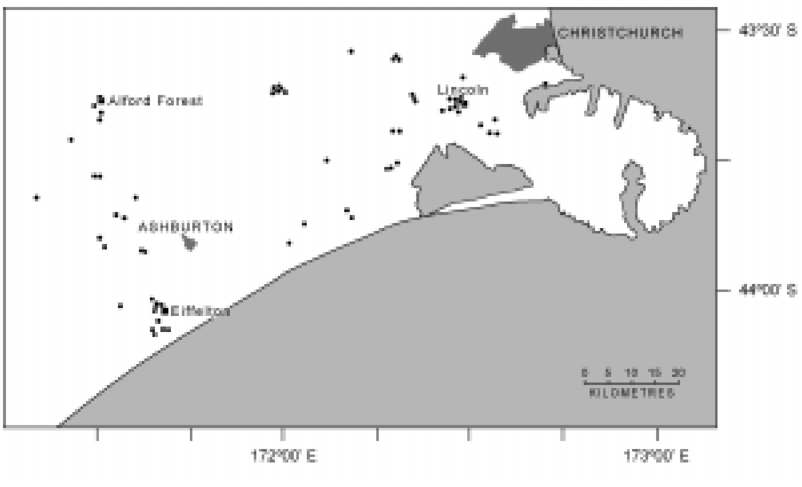

FIGURE 1: Collection sites for *L. bonariensis* made between 1992 and 2000 in mid-Canterbury. The two sites at Culverden are not shown.

from pasture near Eiffelton, Alford Forest and Culverden. The northern-most site was near Culverden (42°75′ S, 172°92' E) and the southern-most site was near Eiffelton (44°09′ S, 171°68' E).

The relationship between *M. aethiopoides* and *M. hyperodae* was analysed by comparing the relative levels of parasitism of the two species in *L. bonariensis*. Analysis was conducted on the whole data set, which included collections when only one of the parasitoid species was recovered, and on a data set limited to collections when both species were recovered. Results were analysed by generalised linear modelling with a binomial distribution using Genstat.

Field collections from lucerne

Adult *Sitona discoideus* and *L. bonariensis* were collected from 13 lucerne stands in mid-Canterbury in August 1996 and 19 stands between 20 May and 17 July 1997 as part of a *M. aethiopoides* post-release survey (Kean & Barlow 2000). Ten of the 1996 sites were resampled in 1997. Because the majority of these stands contained volunteer grass species, *L. bonariensis* was also present in the collections. Weevils were sampled by taking 15 randomly chosen 0.2 m^2 quadrats and removing the litter and insects using a modified Nilfisk Tellus vacuum cleaner powered by a portable generator. This provided density estimates for both weevil species. Weevils removed from the litter were separated into the two species, frozen and dissected to determine parasitism by *M. aethiopoides.* This survey was undertaken prior to *M. hyperodae* reaching the mid-Canterbury region thereby simplifying the interpretation of the data. At the time the samples were taken, *S. discoideus* was feeding on lucerne and reproductively mature (Goldson et al. 1984), while *L. bonariensis* was also feeding but in reproductive diapause (Goldson 1981). The relative densities of the two species and parasitism were analysed using the Generalised Linear Model (GLIM) procedure.

RESULTS

In ryegrass pasture, *M. aethiopoides* was recovered from *L. bonariensis* at 83% of the 65 unique sites and on ca 78% of all 139 sampling occasions in Canterbury (Table 1). Levels of parasitism varied widely with a maximum parasitism of 18% recorded at Eiffelton. In comparison, the maximum level of parasitism by *M. hyperodae* measured during this study was 56%, with parasitism of over 20% recorded at 19 sites. There were also district differences, for example, parasitism of *L. bonariensis* collected in 21 samples at sites near Eiffelton averaged 10% compared to 2.1 % parasitism in 36 samples at sites near Lincoln. Overall, the male: female ratio of *M. aethiopoides* emerging from *L. bonariensis* was 1.1: 1.0.

TABLE 1: The number of collections of *L. bonariensis* by year, as well as the incidence and rates of parasitism by *M. aethiopoides* in Canterbury.

Year	Collection periods	No. of samples collected[1]	Range in no. of *L. bonariensis* collected	No. of times *M. aethiopoides* recovered	Mean (range) parasitism (%)
1992	Feb – May	7 (4)	188-1650	6	4.7 (1.6-8.6)
1993	Feb – Aug	17 (6)	67-801	15	1.9 (0.4-7.5)
1994	Feb and Jun	3 (0)	130-209	2	1.5 (1.4 - 1.5)
1995	April	1 (0)	200	1	2.5
1996	Mar-Oct	23 (18)	43-1200	22	8.0 (1.0-17.0)
1997	Mar-Dec	52 (26)	14-760	41	6.7 (0.4-15.7)
1998	Jan-Nov	28 (11)	23-510	20	3.7 (0.2-18.7)
2000	Jun, Jul, Oct	8 (0)	43-190	2	2.5 (2.1 - 3.1)

[1]Value in parenthesis is the number of unique sites from which collections were made.

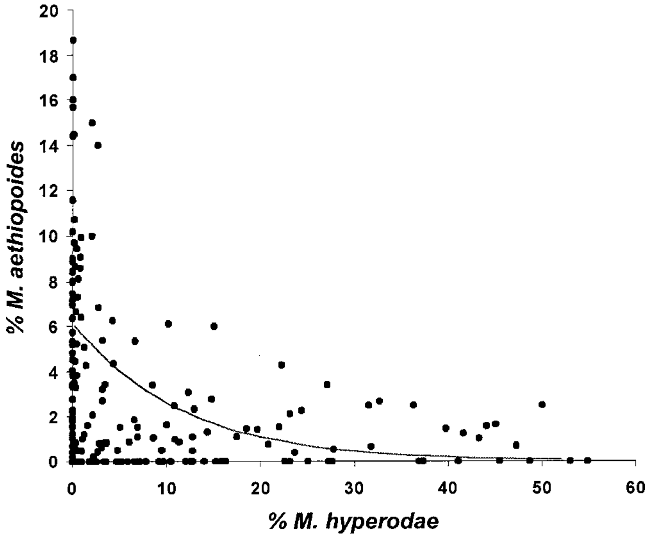

FIGURE 2: Relationship between percentage parasitism of *M. aethiopoides* and *M. hyperodae* reared from *L. bonariensis* collected in ryegrass pasture. Line is given by the equation: log (*p*/1-*p*) = -2.72-0.09* %*M. hyperodae*, where *p* is the % of *L. bonariensis* parasitised by *M. aethiopoides*.

There was a significant negative binomial relationship between the percentage parasitism of *L. bonariensis* by *M. aethiopoides* and *M. hyperodae* (Fig. 2). This relationship held true for both the whole data set ($P<0.001$) and for sites where both *M. aethiopoides* and *M. hyperodae* were recovered ($P<0.001$) (Fig. 2).

In 1996, 10 lucerne sites had similar or substantially higher densities of *L. bonariensis* than *S. discoideus*. However, none of the *L. bonariensis* were parasitized, while parasitised *S. discoideus* were found at all sites with a mean 19.0% parasitism by *M. aethiopoides*. In 1997, eight sites supported similar or higher densities of *L. bonariensis* than *S. discoideus* (Table 2). Parasitism of both *L. bonariensis* and *S. discoideus* by *M. aethiopoides* was found at six sites with a maximum parasitism rate of 10% for *L. bonariensis* against 58% for *S. discoideus*. Overall, parasitism of *L. bonariensis* and *S. discoideus* by *M. aethiopoides* averaged ca 5% and 29% respectively (Table 2). There was no interaction between the relative densities of the two weevil species and parasitism of *L. bonariensis* by *M. aethiopoides* ($P>0.05$), although the percentage of *L. bonariensis* parasitised declined with increasing density of this weevil.

DISCUSSION

Two features apparent from the Canterbury ryegrass results were the spatial and temporal frequency with which *M. aethiopoides* was found to be parasitising *L. bonariensis*. Not only was *M. aethiopoides* consistently recovered from disparate sites ranging from Culverden in north Canterbury to Eiffelton in mid Canterbury, invariably *L. bonariensis* populations collected from adjacent paddocks or paddocks within a locality (e.g. Eiffelton) were found to support *M. aethiopoides*. Furthermore, where sites were sampled at intervals over a year, *M. aethiopoides* was present in the weevil population from February through to December.

TABLE 2: Relative density of *S. discoideus* and *L. bonariensis* recovered from 10 Canterbury lucerne crops in 1997, and rates of parasitism by *M. aethiopoides*.

Site no.	*S. discoideus*		*L. bonariensis*	
	No./m^2	Parasitism (%)	No./m^2	Parasitism (%)
1	10.0	23.3 (30)[1]	1.7	0.0 (5 [1])
2	18.3	34.5 (55)	6.7	0.0 (19)
3	0.7	0.0 (2)	2.3	0.0 (7)
4	27.7	8.5 (82)	1.7	0.0 (5)
5	13.7	15.0 (40)	7.3	0.0 (22)
6	12.3	5.4 (37)	12.0	0.0 (36)
7	6.7	25.0 (20)	6.0	0.0 (17)
8	12.3	58.3 (36)	1.3	0.0 (4)
9	20.3	12.1 (58)	0.7	0.0 (2)
10	14.0	50.0 (42)	3.3	0.0 (10)
11	2.0	36.4 (11)	2.7	0.0 (11)
12	11.0	28.1 (32)	2.3	0.0 (7)
13	26.3	48.0 (75)	0.3	0.0 (1)
14	1.7	20.0 (5)	3.7	10.0 (10)
15	4.7	14.3 (14)	7.0	9.5 (21)
16	9.0	18.5 (27)	3.7	9.1 (11)
17	12.7	52.6 (38)	5.0	6.7 (15)
18	9.3	25.9 (27)	10.0	3.3 (30)
19	12.0	34.3 (35)	14.7	2.3 (44)

[1]Value in parenthesis is the number of weevils dissected.

In all cases, the pastures from which *M. aethiopoides* were recovered were not immediately adjacent to lucerne stands. Movement of adult parasitoids from nearby lucerne can therefore be discounted. However, there are two components that may explain why *M. aethiopoides* was so prevalent in ryegrass pasture. Firstly, the post-aestivatory flights of *S. discoideus* in autumn (late March - April), involve a general dispersal from the aestivation sites (Goldson et al. 1984). During this phase of the life cycle, a proportion of the *S. discoideus* fail to reach lucerne crops and some arrive in ryegrass pasture. As a proportion of these returning weevils are parasitised (Goldson et al. 1990), they provide a means whereby *M. aethiopoides* are reintroduced into the pasture each year. After pupation and emergence of the adult, some parasitoids would then attack further *L. bonariensis*. Secondly, the continued presence of *M. aethiopoides* in *L. bonariensis* over a year, largely in the absence of *S. discoideus*, indicates that once established in the pasture the parasitoid was able to maintain itself on *L. bonariensis*.

In laboratory studies, parasitism of *L. bonariensis* by *M. aethiopoides* has been found to be ca 40% (M.R. McNeill, unpubl. data). The same study also showed that when *L. bonariensis* was exposed to both *M. aethiopoides* and *M. hyperodae,* levels of parasitism were not significantly different. This demonstrated that *M. aethiopoides* is potentially able to compete with *M. hyperodae* for *L. bonariensis* in the field. However, the comparatively low levels of parasitism of *L. bonariensis* by *M. aethiopoides* suggest that behavioural acceptability may be low. This is perhaps due to a disparity in circadian activity patterns between the parasitoid and the novel host *L. bonariensis* (Armstrong et al. 1996) which is expressed strongly in the field but attenuated under laboratory conditions.

The results from the lucerne collections suggest that while *L. bonariensis* is a suitable host, given the choice, the parasitoid selectively attacks *S. discoideus*. *Sitona discoideus*, at 4-6 mm long, is considerably longer than *L. bonariensis*, which is 3-4 mm long. Field studies have found that the size of *I. egens* Broun can influence the level of parasitism by *M. aethiopoides*, with larger weevils preferred to smaller individuals (M.R. McNeill, unpubl. data). The same study also found that *I. egens* was preferred to *L. bonariensis* for oviposition. Alternatively, the low levels of parasitism of *L. bonariensis* may reflect a *M. aethiopoides* oviposition pattern that is matched to the diurnal activity of its natural host *S. discoideus* (e.g. Armstrong et al. 1996).

The Canterbury results contrast with collections made from several regions in the North Island between 1992 and 1999 as part of commercial releases of *M. hyperodae* (McNeill et al. 2002). Of 209 collections of *L. bonariensis* adults from 136 unique sites, *M. aethiopoides* was only recovered on 20 occasions from 10 sites. These sites were from Waikato, Bay of Plenty, Taranaki, Manawatu and Northland. Parasitism of *L. bonariensis* at one site was recorded at 8.4%, but the average across all sites where *M. aethiopoides* was found was 2.2%. Recent surveys conducted in Wairarapa between 1998 and 2000 have also shown that *M. aethiopoides* is rare, with the parasitoid recovered only twice from 29 collections with a mean parasitism of 0.4%.

The simple explanation for the difference between Canterbury and North Island sites might relate to the area of lucerne grown in Canterbury. Statistical information on the area grown in lucerne by region were last available in 1983 (Agricultural Statistics 1984), when 948, 8997 and 265 ha of lucerne was grown in Waikato, Bay of Plenty and Taranaki respectively, while 43 738 ha of lucerne was grown in Canterbury. While not directly related to the period of this research, it is indicative of the regional differences in lucerne production. By implication, the relatively high prevalence of *M. aethiopoides* in *L. bonariensis* from Canterbury may be due to higher local populations of *S. discoideus* and its natural enemy, which provides a greater reservoir from which the parasitoid can invade ryegrass pasture.

In conclusion, the results showed that in Canterbury ryegrass pasture, *L. bonariensis* was parasitised by *M. aethiopoides*. Prior to the introduction of *M. hyperodae, M. aethiopoides* was the only significant entomophagous natural enemy of *L. bonariensis*, albeit one that would have had minimal impact on reducing the damage caused by the weevil. Parasitism of *L. bonariensis* by *M. aethiopoides* has declined as levels of parasitism by *M. hyperodae* have increased. This negative relationship may simply indicate that *M. aethiopoides* is less competitive than *M. hyperodae* but further investigation is required to explain the mechanism for this relationship. In lucerne, it was clear that *M. aethiopoides* preferentially attacks its natural host, *S. discoideus.*

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