

## POTENTIAL FOR BIOCONTROL OF *SITONA LEPIDUS* GYLLENHAL BY *MICROCTONUS* SPP.

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### ABSTRACT

Laboratory experiments were carried out to determine whether either of the two braconid parasitoids *Microctonus aethiopoidea* Loan or *Microctonus hyperodae* Loan, already established in New Zealand, would parasitise the recently introduced clover root weevil, *Sitona lepidus* Gyllenhal. No parasitism by either parasitoid was recorded in two no-choice experiments. In a third experiment, *M. aethiopoidea* was exposed to *S. lepidus* and *S. discoideus* in either choice or no-choice cages. In this case, a very low level of parasitism of *S. lepidus* (1%) occurred in no-choice cages, but this was increased to about 6% in cages where both weevil species were present. It was concluded that neither parasitoid was likely to be a suitable biological control agent for *S. lepidus*.

**Keywords:** *Sitona lepidus* Gyllenhal, *Sitona discoideus*, *Listronotus bonariensis*, parasitism, *Microctonus aethiopoidea*, *Microctonus hyperodae*

### INTRODUCTION

The clover root weevil, *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), occurs throughout much of Europe and the USA (Bright 1994), but is absent from Australia. The establishment of this species in New Zealand was first recorded in 1996 (Barratt *et al.* 1996), but examination of stored samples revealed that it was present in the Waikato in 1995 (Barker *et al.* 1996). The origin of the weevils, and the actual date and means of their introduction is unknown.

Adult weevils feed on foliage, and larvae on the roots and root nodules of *Trifolium* spp. (Clements and Murray 1993). In the USA *S. lepidus* appears to be a minor pest, but in the UK it has been recorded as one of the most important pests of white clover (*Trifolium repens* L.) in pasture (Clements and Murray 1991; Murray 1991). High population densities of *S. lepidus* (up to 600/m<sup>2</sup>) have been recorded in the northern North Island (Addison and Willoughby pers. comm.), and, because of the importance of white clover to the New Zealand pastoral industry, it has the potential to become an important pasture pest throughout New Zealand.

Little is known about potential parasitoids for biological control of *S. lepidus*; however, a low incidence of parasitism by *Microctonus aethiopoidea* Loan (Hymenoptera: Braconidae), a parasitoid already present in New Zealand, has been recorded in northern Germany (Muller 1963). The objective of this research was to determine whether *M. aethiopoidea* and *M. hyperodae* Loan (also present in New Zealand) introduced to New Zealand to control *Sitona discoideus* Gyllenhal and *Listronotus bonariensis* (Kuschel) respectively, were potential biological control agents for *S. lepidus*. The former species in particular was considered a strong possible candidate since it has been shown to be polyphagous (Barratt *et al.* 1997) and effective in controlling the congeneric *S. discoideus* (Goldson *et al.* 1994). Both parasitoids are solitary endoparasitoids which attack the adult stage of their hosts.

## METHODS

### Insect rearing

Tests were conducted in an insect rearing room maintained at  $20 \pm 2^\circ\text{C}$ , with a relative humidity of 40-60%, and a 16 hours light, 8 hours dark photoperiod. Weevils were held in plastic cages 160 x 180 x 75 mm deep fitted with a fine gauze lid. The cage was fitted with a plastic-coated mesh floor (holes 1 x 1 mm), and it was inserted into the top of another similar container in which textured absorbent paper towel covered the base. Parasitoid prepupae moved down through the mesh from the upper to the lower cage and pupated on the underside of the paper towel.

Food plants, Grasslands Manawai ryegrass (*Lolium perenne* x *L. multiflorum*) x *L. perrene*) for *L. bonariensis* and Grasslands Wairau lucerne (*Medicago sativa* L.) for *S. discoideus* were grown in commercial seed raising mix in glasshouse trays with 27 x 27 x 45mm deep cells sown with 6-10 seeds per cell. The plants were grown to a height of about 100 mm, removed intact and the roots and soil enclosed in a 100 x 75mm plastic bag secured firmly at the base of the plants using a plastic cable clip to prevent weevil entry into the bags. One bag of plants was placed in each cage and replaced every 3-4 days. White clover foliage from pasture at Invermay was collected and supplied to *S. lepidus* every second day, in addition to lucerne as above.

### Experiments

Three laboratory experiments were carried out. For each of these, *S. lepidus* was field collected from dairy pasture near Hamilton in the Waikato; *S. discoideus* was field collected from lucerne near Middlemarch, Otago; *L. bonariensis* was field collected from pasture near Middlemarch, Otago. *M. aethiopoidea*s was reared from field collected *S. discoideus* and *M. hyperodae* was reared from *L. bonariensis* in laboratory culture at AgResearch, Lincoln. All weevils used in the experiments except for the *L. bonariensis*, were held for 17-20 days in the laboratory to allow any parasitoids to emerge.

Because *S. lepidus* is not currently present in the South Island of New Zealand, weevils received from Hamilton were handled in quarantine-like conditions. Weevils were counted on arrival, those not required for the experiment were immediately killed by immersion in 70% ethyl alcohol, and all weevils used in the experiment were accounted for and immersed in alcohol at the end of the experiment. Food and debris that was removed from the cages was placed in plastic bags and stored at  $-18^\circ\text{C}$  until the end of the experiment, and then incinerated.

In Experiment 1, five cages of 20 *S. lepidus* and five similar cages of *S. discoideus* were each exposed for 48 h to three female *M. aethiopoidea*s. Five cages of *S. lepidus* and two cages of *S. discoideus* were left unexposed.

In Experiment 2, five cages of 20 *S. lepidus* and two similar cages of *L. bonariensis* were each exposed for 48 h to three *M. hyperodae*. Five cages of 20 *S. lepidus* and two cages of *L. bonariensis* were left unexposed.

In Experiment 3, there were three treatments, each of which was exposed for 48h to three *M. aethiopoidea*s: five cages of 20 *S. lepidus*, five cages 20 *S. discoideus*, and five cages of 10 *S. lepidus* and 10 *S. discoideus* mixed. The latter was designed to determine whether given a choice of the two *Sitona* species in cages, enhanced parasitism of *S. lepidus* by *M. aethiopoidea*s might occur, in comparison with no-choice cages of these two species. During the period of exposure to parasitoids, both lucerne and white clover was supplied in all cages to remove any effects on parasitoid behaviour arising from visual or olfactory cues from the presence of different plant species. After the 48 h period of exposure to the parasitoids, the weevil species in the mixed cages were separated.

In all three experiments, weevils were maintained in cages after parasitoid removal, and pupae emerging from the weevils were removed daily and kept in petri dishes until the adult wasps emerged. Once no further parasitoids emerged for 2-3 days, all surviving weevils were preserved in 70% ethanol, until dissected and examined to determine weevil gender and parasitism. Weevils that died during the experiment were immediately preserved for dissection. If no pupae emerged from the weevils, surviving weevils were preserved for dissection after 30 days.

## RESULTS

In Experiments 1 and 2, no parasitism of *S. lepidus* was recorded either from rearing

or dissection of weevils (Table 1). In Experiment 1, 25% of *S. discoideus* exposed to *M. aethiopoidea* were parasitised and a single melanised parasitoid was found in one of the surviving unexposed weevils during dissection. In Experiment 2, parasitism of *L. bonariensis* by *M. hyperodae* was 81% (Table 1), but field parasitism of the weevils used was 18.6% suggesting that the experimental exposure accounted for approximately 61%.

In Experiment 3, 1.1% (a single individual) of *S. lepidus* exposed to *M. aethiopoidea* was parasitised in no-choice cages compared with 27% of *S. discoideus* (Table 1). In the mixed species cages, there was a similar proportion of *S. discoideus* parasitised, but a significantly greater proportion of *S. lepidus* ( $P < 0.01$ ) was parasitised (Table 1).

**TABLE 1: Percentage parasitism of weevils ( $\pm$ SE) exposed to parasitoids in choice and non-choice experiments.**

Species exposed:	<i>M. aethiopoidea</i>		<i>M. hyperodae</i>	
	Exposed	Unexposed	Exposed	Unexposed
Experiment 1				
<i>S. lepidus</i>	0	0	-	-
<i>S. discoideus</i>	25.4 $\pm$ 6.4	1.7 $\pm$ 1.7	-	-
Experiment 2				
<i>S. lepidus</i>	-	-	0	0
<i>L. bonariensis</i>	-	-	81.1 $\pm$ 2.2	18.6 $\pm$ 3.6
Experiment 3				
<i>S. lepidus</i> (no choice)	1.1 $\pm$ 1.1	-	-	-
<i>S. discoideus</i> (no choice)	27.0 $\pm$ 7.1	-	-	-
<i>S. lepidus</i> (choice)	6.0 $\pm$ 2.5	-	-	-
<i>S. discoideus</i> (choice)	21.6 $\pm$ 7.2	-	-	-

## DISCUSSION

Previous research has shown that *M. aethiopoidea* is not a host-specific parasitoid, indeed it has been shown to successfully attack 11 non-target species, including seven native species in the laboratory, and 13 (including 10 native) species in the field (Barratt *et al.* 1997). Consequently, there was hopeful anticipation that *S. lepidus*, a species in the same genus as the target host, *S. discoideus*, would be attacked by *M. aethiopoidea*. This has not proved to be the case for reasons not yet identified.

The choice experiment described in Experiment 3, which resulted in slightly increased parasitism in *S. lepidus*, suggests that oviposition attempts may have increased in the presence of the target host. Field and Darby (1991) found that the parasitoid *Sphecophaga vesparum* (Curtis) (Hymenoptera: Ichneumonidae) oviposited in two non-target species when in the presence of the target host, but not in its absence. However, the lack of evidence of an immune response in the *S. lepidus* suggests that either successful attempts still remained infrequent, or that a host response occurred rapidly and any deposited parasitoid eggs were rejected and broken down leaving no evidence of melanisation. The ability of *M. aethiopoidea* to oviposit in *S. lepidus* will be tested in future work using parasitoids coated with the bacterial pathogen *Serratia marcescens* Bizio AgRB363, isolated from *Paropsis charybdis* Stål (Coleoptera; Chrysomelidae). Rapid weevil mortality would give an indication of successful ovipositor penetration by the parasitoid (M. McNeill pers. comm.).

*M. hyperodae* has been shown in other studies to be much more host specific than *M. aethiopoidea* and was not expected to attack *S. lepidus*. Surprisingly, the only record of parasitism of *S. lepidus* in the field in New Zealand was a single specimen of *M. hyperodae* reared from a sample of 250 *S. lepidus* collected from the Waikato (Barratt *et al.* 1997).

In conclusion it appears that the introduced *Microctonus* species currently available in New Zealand are unlikely to be effective as biological control agents for *S. lepidus*.

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