NEGATIVE EFFECTS OF STRAIN HYBRIDISATION ON THE BIOCONTROL AGENT *MICROCTONUS AETHIOPOIDES*

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ABSTRACT

Clover root weevil, *Sitona lepidus*, remains a threat to New Zealand's white-clover based pastoral production system. Unfortunately, the New Zealand-established strain of *M. aethiopoides* (originally from Morocco) active against *Sitona discoideus*, has been found to have no potential as a control agent against *S. lepidus*. It was therefore unexpected that a European strain of *M. aethiopoides* was found to be highly active against the weevil. This contribution examined the effect of Moroccan x European strain crosses on the value of *M. aethiopoides* as a control agent of *S. discoideus* and *S. lepidus*. It was found that such strain hybridisation led to significant reductions in the impact of this parasitoid species as a control agent against either *S. lepidus* or *S. discoideus*. The widespread implications of this finding for biological control are discussed.

Keywords: parasitoid strains, strain hybridisation, *Sitona discoideus*, *Sitona lepidus*, control efficacy.

INTRODUCTION

The genus *Sitona* Germar comprises over 100 species worldwide (Bright 1994). The clover root weevil, *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae) is distributed throughout Europe (Dieckmann 1980), where it is thought to be endemic (Campbell et al. 1989). *Sitona lepidus* was first recognised in New Zealand in March 1996 by Barratt et al. (1996).

Sitona lepidus strongly prefers white clover (*Trifolium repens* L.) (Murray & Clements 1994). Like other *Sitona* spp., adults feed on the foliage and the larvae develop on the root nodules and roots. Larvae cause the most damage (e.g. Care et al. 2000) with each surviving larva destroying about 10 nodules (Gerard 2001). Soon after the weevil's discovery in New Zealand, the larvae were found at far higher densities than those measured in Europe. Typical densities of 300/m² have been noted by Addison et al. (1998), in contrast with maximum populations of 34-90 larvae/m² in England (Murray & Clements 1994). Such large New Zealand populations cause severe pasture nitrogen stress and general loss of vigour.

With the virtual certainty of *S. lepidus* spreading throughout New Zealand's clover-based pastures, it was recognised that biological control offered the only effective opportunity for widespread suppression. There had been success in New Zealand in suppressing *S. discoideus* Gyllenhal in lucerne with a Moroccan strain of *Microctonus aethiopoides* Loan (e.g. Goldson et al. 1990) and there was initial optimism that the same parasitoid would be useful in suppressing *S. lepidus*. However, Barratt et al. (1997) demonstrated that the Moroccan strain (Aeschlimann 1983) has no potential at all for the control of *S. lepidus*. Consequently, an intensive search for suitable agents was commenced in the United States and Europe in 1998 (e.g. Goldson et al. 2003).

As the Moroccan strain of *M. aethiopoides* had been shown to be ineffective against *S. lepidus*, it was with surprise that the most promising parasitoid species against *S. lepidus* in Europe was found to be another strain of *M. aethiopoides* (Goldson et al. 2003). This discovery raised the possibility that should the European strain of

M. aethiopoides be introduced into New Zealand in an attempt to control *S. lepidus*, there could be the danger that it would cross with the Moroccan strain already present and lose efficacy through strain hybridisation. Conversely, and just as bad, such crossing of strains could also lead to the loss of existing suppression of *S. discoideus* in lucerne. This contribution presents an analysis of data collected to ascertain whether either or both unfortunate outcomes could be possible.

METHODS AND MATERIALS

The data for this analysis were obtained from experiments investigating the biocontrol efficacy of *M. aethiopoides* Moroccan female and European male strain crosses, European female and Moroccan male strain crosses and 'hybrid' progeny of European female and Moroccan male strain crosses. The performance of these strain crosses and strain hybrids was compared directly to that of 'pure' European and Moroccan strains when attacking *S. lepidus* and *S. discoideus* respectively.

All parasitoids used in this study were less than three days old and maintained under ambient laboratory conditions. Parasitoid pairs were mated in 2.0 ml plastic tubes. The time taken until the onset of mating (pre-mating time) and the duration of mating (mating time) were recorded. After mating, the females were maintained in 5.0 ml transparent polycarbonate tubes and fed using 1 mm² pieces of filter paper soaked in a honey and water solution.

Host *S. lepidus* were field-collected from ryegrass-clover pasture near the Ruakura Research Centre, Hamilton (37°46'S, 172°30'E) and sent to Lincoln (43°36'S, 172°30'E) where they were maintained in cool storage until use. Host *S. discoideus* were collected from lucerne close to the Lincoln campus. *Sitona discoideus* were purged of any naturally occurring *M. aethiopoides* through storage in ambient laboratory conditions for ca 4 weeks.

In all experiments 30–40 weevils were exposed to a single parasitoid for ca 48 h in a 220 mm x 130 mm x 75 mm transparent polycarbonate cage fitted with a mesh lid. For measurement of parasitoid attack and development in both *S. lepidus*, and *S. discoideus*, a single mated *M. aethiopoides* female was placed with the weevils. Females were from (i) pure European strains, (ii) pure Moroccan strains, (iii) Moroccan female by European male, (iv) European female by Moroccan male or (v) hybrids arising from the European by Moroccan crosses. For the latter, hybrids were mated with hybrids. The cages were also provided with two bouquets of either glasshouse-grown white clover or lucerne as food for *S. lepidus* and *S. discoideus* respectively. In all cases the parasitoids were removed after 48 h and the weevils thereafter maintained under ambient laboratory conditions until all parasitoid prepupae had emerged. These pupae were collected using 220 mm x 135 mm x 75 mm deep transparent polycarbonate holding cages that had been divided into an upper and lower chamber. The weevils were maintained in the upper chamber along with two moist cotton wicks to provide water and favourable humidity. Two bunches of either white clover or lucerne were also provided as food; these were replaced 2–3 times per week. The upper chamber was fitted with a gauze floor (0.5 mm mesh) to allow emergent prepupal parasitoids to drop and pupate under strips of paper towel in the lower chamber. The lower chambers were checked 2–3 times per week for prepupae and pupae. Cumulative counts of emergence were made. The emergent pupae were maintained in Petri dishes under laboratory conditions in order to determine the sex ratio of the progeny. These experiments were replicated as indicated in Table 1.

Pre-mating time, sex-ratio of progeny and percentage parasitism, as indicated by prepupal emergence, were analysed using an analysis of deviance based on a binomial distribution.

TABLE 1: Number of experiment replicates, where M = Moroccan *Microctonus aethioides* strain and E = European *Microctonus aethioides* strain. An experimental replicate consisted of a single mated female exposed to 30-40 weevils for 48 h.

Host species	Parasitoid strain combination tested ¹				Hybrid ²
	M x M	M x E	E x E	E x M	
<i>Sitona lepidus</i>	-	-	89	16	16
<i>Sitona discoideus</i>	8	12	8	-	-

¹First letter of each cross denotes the female of that particular strain.

²hybrid x hybrid cross.

RESULTS

Crosses between strains resulted in significant ($P<0.05$) reduction of impact on parasitoid efficacy against both *Sitona* spp. (Fig. 1). When the Moroccan *M. aethioides* strain was crossed with the European strain, its efficacy as a control agent against *S. discoideus* in lucerne was effectively halved (Fig. 1). When the European strain was crossed with that from Morocco, its efficacy against *S. lepidus* did not differ significantly from the pure European strain. However, hybrid cross efficacy against *S. lepidus* was reduced by about half compared to that of both the pure European and European by Moroccan strains (Fig. 1). The attack rate of the pure European strain against *S. discoideus* was even lower at 16%.

The pre-mating and mating times of the Moroccan x European crosses were not significantly different from those of the pure Moroccan and European strains. Similarly, *M. aethioides* strain hybridisation had no significant impact on progeny sex ratios.

FIGURE 1: Histogram showing parasitism levels of *Sitona discoideus* and *Sitona lepidus*. M x M = Moroccan strain, M x E = Moroccan European strain cross, E x E = European strain and Hybrid = offspring of E x M, hybrid by hybrid cross. First letter of each cross denotes the female. No overlap of confidence limits denotes significant difference at $P<0.05$.

DISCUSSION

The results presented in this preliminary study highlight the importance of exploiting the genetic diversity within a biological control species by choosing the right strain or ecotype. Just as important, but less obvious is the need to understand the potential impact of biocontrol agent strain interaction on control efficacy before any decision to release is made. This finding has important implications for biocontrol theory and raises some interesting biogeographical and population genetics questions beyond the scope of this contribution.

When an inappropriate strain is chosen, its impacts on the target pest may be negligible. This has been highlighted in this study by the ca 16% of European *M. aethiopoidea*s development in *S. discoideus*. Similarly, Barratt et al. (1997; pers.comm.) found that the Moroccan *M. aethiopoidea*s strain develops in only ca 4% of *S. lepidus* (Fig. 1). These results also showed a progressive decline in efficacy between the initial crossing of European females with Moroccan males and ensuing strain hybrids. Whether this decline reflected reduced oviposition effort by the female or a host physiological response was not ascertained in this study. Irrespective of the mechanism, strain crosses result in reduced efficacy in the target weevil. With escalating costs for biological control, it is now more important than ever that funding is well spent through optimising the choice of biocontrol ecotype or strain. Fortunately, DNA-based methodologies now offer opportunities with which to better understand subspecific variation and thereby increase the chance of such optimal exploitation.

While notable host preference differences have been observed between the Moroccan and European strains of *M. aethiopoidea*s, the lack of strain-related barriers to reproduction and the absence of any effect on progeny sex ratios, indicate an absence of reproductive isolation often associated with speciation.

Of particular significance, this study shows how the introduction of inappropriate biocontrol strains can destroy the potential for a useful biological control opportunity, even if subsequently, more appropriate strains are introduced. Figure 1 indicates that should the European strain of *M. aethiopoidea*s become genetically mixed with the Moroccan strain in New Zealand there would probably be irreversible loss of efficiency in the suppression of *S. discoideus* in lucerne (Goldson et al. 1990). Likewise, and for the same reasons, it would seem that the crossing of the Moroccan and European parasitoid strains could severely and permanently compromise the chances of *M. aethiopoidea*s ever effectively reducing the impact of *S. lepidus* on white clover.

These observations highlight the paramount importance of the need to understand the ecological context of any biological control initiatives. Integral to this, a grasp of the likely interplay of strains on biological control attack rates and host range is required. Such work should also include systematic experimentation to examine any effects of ecotypic crossing on parasitoid population reproductivity and vigour.

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