

INITIAL ESTABLISHMENT OF THE IRISH STRAIN OF *MICROCTONUS AETHIOPOIDES* IN NEW ZEALAND

P.J. GERARD¹, T.M. EDEN¹, S. HARDWICK², C.F. MERCER³,
M.W.A. SLAY⁴ and D.J. WILSON¹

¹AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton,
New Zealand

²AgResearch, PO Box 60, Lincoln, New Zealand

³AgResearch, Grasslands Research Centre, Private Bag 11008,
Palmerston North, New Zealand

⁴Homelea, Shanley Road, RD 9, Hastings, New Zealand

Corresponding author: pip.gerard@agresearch.co.nz

ABSTRACT

Four experimental releases of the parthenogenetic strain of *Microctonus aethiopoides* from Ireland were made in early 2006 in the Waikato, Manawatu (two sites) and Hawke's Bay. By early winter, establishment was confirmed at all sites with parasitism levels exceeding 10% in the target host *Sitona lepidus*, a serious pest of white clover in New Zealand. Subsequent monitoring revealed considerable between-site variation. At the Hawke's Bay and Manawatu Feilding sites where overwintering weevil adults were still present in October, over 30% parasitism was found in newly emerged weevils in December 2006. In contrast, at the Waikato and Manawatu Bulls sites where there was no overlap of host generations, parasitism was below detectable levels during spring and early summer but recovered subsequently. The Irish *M. aethiopoides* appears to have four generations a year and diapauses over winter as a first instar larva. Following the initial success, releases have commenced in other regions.

Keywords: *Microctonus aethiopoides*, *Sitona lepidus*, parasitism, establishment.

INTRODUCTION

The clover root weevil, *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), was first discovered in New Zealand in 1996 (Barratt et al. 1996) and has subsequently become one of New Zealand's worst white clover pests (Erens et al. 2005). A biological control programme was initiated in 1998 and approval was granted in November 2005 by the Environmental Risk Management Authority for the release of a parthenogenetic Irish strain of the European biotype of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) (Gerard et al. 2006). This parasitoid attacks adult weevils, rendering females sterile almost immediately and killing the host when the parasitoid larva emerges to pupate.

Releases commenced in early 2006 with approximately 5000 parasitised weevils released in the Waikato (5 January 2006) and Hawke's Bay (26 January 2006) and around 2500 weevils at each of the Manawatu sites (14 February 2006). This paper reports on the initial results from the four experimental release sites.

METHODS

Sites

The sites were as follows:

- (1) Springdale, Waikato. Ryegrass/white clover dairy pasture > 10 years old. Data available on weevil populations and pasture cover since 1996. September 2005 weevil population at 638 ± 95 larvae/m².
- (2) Patoka, Hawke's Bay. High altitude site (625 m a.s.l.) with annual rainfall typically 1700 mm. New ryegrass/white clover sheep and beef pasture sown in autumn 2005. Site conducive for wind dispersal of parasitoid into surrounding region. September 2005 weevil population at 198 ± 49 larvae/m².
- (3) Feilding, Manawatu. Ryegrass/white clover dairy beef pasture. Topography and prevailing wind conducive for parasitoid dispersal. September 2005 weevil population at 112 ± 30 larvae/m².
- (4) Bulls, Manawatu. Ryegrass/white clover/plantain/chicory dairy beef pasture sown autumn 2001. High biodiversity site with lucerne and the lucerne weevil *Sitona discoideus* Gyllenhal nearby. September 2005 weevil population at 258 ± 65 larvae/m².

Adult weevil sampling

Monthly sampling of adult weevils at release sites commenced 2 months following the releases to avoid collecting weevils parasitised and released from the laboratory. At Waikato, Hawke's Bay and the Manawatu site at Feilding where the primary objective was to ascertain parasitism levels in *S. lepidus* populations, four suction samples were taken by dragging a modified blower/vac along transects within the release paddocks. This rapid sampling technique, used for *S. lepidus* since the insect was first discovered in New Zealand (Barker et al. 1996), provides a sample that can be readily inspected in the field and transect length adjusted accordingly. The length of transects ranged from 30 m to 200 m as weevil abundance, and therefore the effort to get a desired minimum sample size of 20 weevils/transect for assessment of parasitism, varied with site and season. The focus of the Manawatu Bulls site was on non-target weevil populations, and when combined with the diversity of forage plants present, it was deemed that taking five sets of 15 pooled 201 cm² vortice suction samples would provide greater accuracy. A supplementary dragged transect sample was collected from the Manawatu Bulls site, and occasionally from the Waikato site, to increase the number of *S. lepidus* available for dissection. Pasture and weather conditions were recorded at time of sampling.

Assessment of parasitism

Weevils recovered were dissected under a binocular microscope and physiological state and parasitoid stages present were recorded. When weevil numbers were low, all were dissected, but when numbers were high a random sub sample of 20 weevils was taken from each suction sample for dissection; parasitoid emergence was measured in the remainder by holding them in emergence cages (Phillips et al. 2004) for approximately 1 month at 20°C in a 16:8 h light:dark regime.

Standard errors for the means were calculated where sufficient population or parasitism data were collected from individual suction samples on a given date.

RESULTS

Excellent initial establishment by the parasitoid was achieved at all sites and by early winter (June 2006) parasitism levels in "release" paddocks were $33 \pm 24\%$ at Waikato, $13 \pm 2\%$ at Hawke's Bay, and in Manawatu, $13 \pm 8\%$ at Feilding and 20% (eight of pooled sample total of 40 weevils) at Bulls (Fig. 1). Parasitism was detected in paddocks adjacent to the release paddocks in May 2006 at Waikato but not until October at Hawke's Bay and January 2007 in Manawatu. No parasitism had been detected in *S. lepidus* populations prior to release.

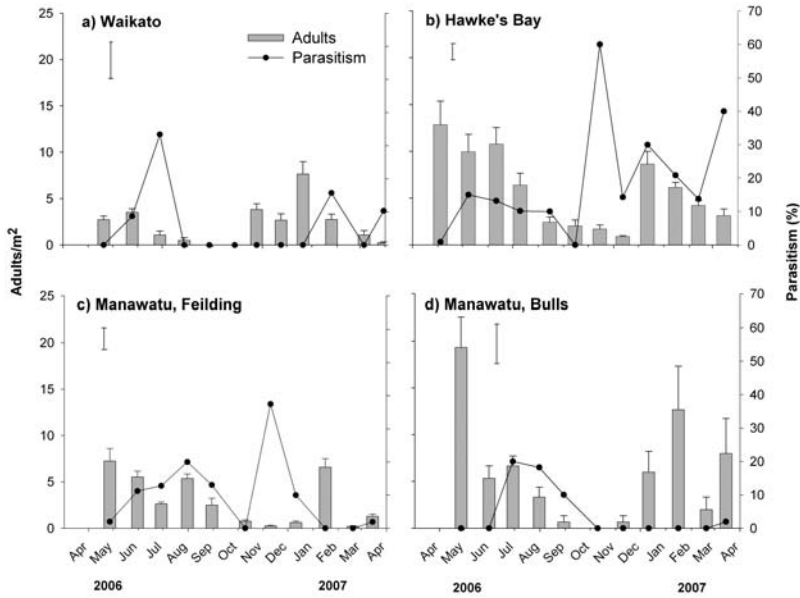


FIGURE 1: Mean *Sitona lepidus* adult populations (\pm SEM) and percentage parasitism by Irish *Microctonus aethiopoides* at four experimental release sites from April 2006-March 2007. Single error bar denotes SEM for mean parasitism over all site collection dates. Parasitoids were released at each site in January (Waikato and Hawke's Bay) and February 2006 (Manawatu: Feilding and Bulls).

Irish *M. aethiopoides* may diapause over winter within their host. For instance, all but one of the parasitoids recovered during dissections of Hawke's Bay subsamples from May-August inclusive were first instars. The parasitoid prepupae that emerged from the remainder of the May-July samples held at 20°C and 16:8 h light:dark were recovered from day 21-25 (n=36), but those from the August sample were found on day 15 (n=9).

At the Waikato and Manawatu Bulls sites, overwintering *S. lepidus* adult populations fell to below detectable levels by September. In contrast, at the Hawke's Bay and Manawatu Feilding sites physiologically old adults, as indicated by fat state and very dark colouration of cuticle on the upper abdomen, were present throughout spring and were found alongside newly-emerged adults in the November and December samples. At both the latter sites during spring, there was evidence of an absence of parasitoids followed by a sharp peak in parasitism (Figs 1b & c) consisting of a mix of eggs and newly hatched first instars. Two subsequent generations of parasitoids at the Hawke's Bay site could be inferred from further parasitoid eggs being found in December/January and March samples and later instar larvae at the intervening samples. As Waikato May 2006 samples contained parasitoid eggs and mature larvae, it would appear that Irish *M. aethiopoides* may complete four generations a year in the North Island.

Where there were no hosts available in early spring (Waikato and Manawatu Bulls) parasitism was below detectable levels in the generation of *S. lepidus* adults emerging late spring/early summer (Fig. 1). However, over 10% parasitism was found at the Waikato site in January 2007 and parasitoids were detected in the March sample at Bulls. At time of submission of this paper, raw data from dissections in progress indicated minimum parasitism levels in April of around 60% at Waikato, 70% at Hawke's Bay and 55% at Manawatu Feilding. All parasitoids were eggs or newly hatched larvae and recently-laid eggs were present in samples from all four sites, suggesting adult parasitoids were still active.

It was evident that Irish *M. aethiopoulos* commonly lays more than one egg in a host weevil in the field and that all of these progeny complete development. When the parasitism rate means were calculated and pooled over all sites for the parasitised weevils (n=176) found through host dissections (n = 1698) during the reporting period, 74 ± 8% contained one parasitoid, 17 ± 7% had two, 8 ± 9% had three and 1 ± 3% had four (SEM scaled to allow for extra binomial variability in the data). When multiple parasitoids were found within a host, all were at the same developmental stage and there was no significant variation with season or site. Comparisons of the numbers of parasitoids detected in the weevil samples by dissection or by rearing were similar, indicating multiples had similar viability to singletons.

DISCUSSION

The Irish *M. aethiopoulos* is the third *Microctonus* parasitoid to be released in New Zealand to control forage weevil pests, the first being the Moroccan *M. aethiopoulos* biotype in 1982 for *S. discoideus* (Stufkens et al. 1987), followed by *M. hyperodae* Loan in 1991 for the Argentine stem weevil *Listronotus bonariensis* (Kuschel). However, while it may have been expected that the two *M. aethiopoulos* would be most similar with respect to the pattern of establishment and phenology, in fact the Irish *M. aethiopoulos* resembles more closely that of *M. hyperodae*. Both are parthenogenetic, over winter in adult hosts as diapausing first instars, exhibit post-diapause development commencing in early spring and have 2-3 generations through the summer and autumn. The host weevil generally has two partially overlapping adult generations a year and, just as with *M. hyperodae* in the North Island (Barker & Addison 2006), asynchrony between parasitoid and host emergence in spring may determine the efficacy of Irish *M. aethiopoulos* as a biocontrol agent in any given site or year.

While initial establishment and rapid increase in parasitism levels by Irish *M. aethiopoulos* at all sites indicated the parasitoid has excellent search and attack capabilities, no parasitism was observed in spring-emerging *S. lepidus* populations at Waikato and Manawatu Bulls. One explanation is the apparent absence of available hosts at these sites in October when the overwintering parasitoid generation emerged as adults. While there are many possible variables that can influence the abundance and longevity of *S. lepidus* adults in the field, climate is likely to be a major factor. Adult longevity has been reported to be a few weeks in hot dry conditions (Gerard & Arnold 2002) and up to 2 years in cold climates as found in Finland (Markkula 1959). The Hawke's Bay site is a well-drained high altitude site with snow in winter. This fosters *S. lepidus* adult longevity and because eggs are being laid throughout the year, there is a broad overlap in generations with newly emerged immature adults present in the population from November to May. In contrast, the warmer Waikato site tends to have more synchrony in weevil development and an earlier autumn emergence than seen at the other sites. In addition, it was intensively grazed, especially when wet winter soil conditions slowed growth. These conditions have relatively little direct effects on larval population densities below ground but probably would impact on adult mortality and stimulate adults to seek more protected feeding sites, such as along fence lines and farm tracks.

Severe soil moisture deficits (>130mm) and high soil surface temperatures in Manawatu in February 2007 (National Climate Centre 2007) are likely to have contributed to the marked drop in March *S. lepidus* adult numbers at both Manawatu sites (Fig. 1) and probably also impacted negatively on parasitoid pupal and adult survival.

The most economically damaging stage of *S. lepidus* is the winter generation of larvae (Gerard et al. 2007) and thus it is the reproductive females present in autumn that are most crucial to control. Therefore, while it may be desirable to have good parasitism levels in pre-Christmas *S. lepidus* populations, it is the parasitism levels from March onwards that will determine how effective Irish *M. aethiopoides* will be as a biocontrol agent. One important attribute that should assist Irish *M. aethiopoides* persistence and rate of increase is its ability to have multiple viable offspring from a single host. This strategy gives parasitoids searching in very low density host populations a greater chance of laying their egg complement, and while the multiples will be less robust and fecund as adults compared to singletons, it would result in more daughters surviving the winter and being available to rapidly exploit the abundance of hosts available from November through to June. When combined with the fact that they have no need to find a mate, this may explain why Irish *M. aethiopoides* was able to persist and increase above 2006 levels at the Waikato release site, even though both target host and parasitoid populations were below detectable levels during spring and no possible alternative hosts were attacked (P.J. Gerard, unpubl. data).

The results from this first year indicate that Irish *M. aethiopoides* will persist even under adverse conditions and has the ability to increase rapidly. The parasitism levels achieved are comparable to the previous *Microctonus* spp. introduction in the North Island. For instance *M. hyperode* was released at Ruakura, Waikato, during winter 1991, peaked just under 40% during winter 1992 and over 80% in winter 1993 (Barker & Addison 2006). This has generated the confidence to commence distribution of parasitoids at field days and to undertake a release in Nelson in July 2006, followed by further releases in December 2006 at sites in Northland, Taranaki (establishment confirmed March 2007) and at Ruakura in Waikato (establishment confirmed April 2007). Studies will continue at the four initial release sites to follow changes in the populations of host and parasitoids and determine dispersal into surrounding farmland.

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