

Abundance and parasitism of clover root weevil (*Sitona lepidus*) and Argentine stem weevil (*Listronotus bonariensis*) in pastures

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Abstract The period from 2006 to 2010 in the North Island encompasses the widespread establishment of Irish *Microctonus aethiopoulos* for the biocontrol of clover root weevil (*Sitona lepidus*) and a severe drought in 2008 that greatly reduced host availability. This parasitoid occupies the same pasture habitat as *Microctonus hyperodae*, a parasitoid with a similar life cycle introduced to control Argentine stem weevil (*Listronotus bonariensis*). A retrospective study was carried out on *L. bonariensis* data and stored samples collected during *S. lepidus* studies. It was found that at all four initial *M. aethiopoulos* release sites, *L. bonariensis* populations were lowest in 2007 and 2008 while *S. lepidus* populations were lowest in 2008 and 2009. *Microctonus hyperodae* parasitism levels were lowest in 2009, with overall regional means ranging from 5% in Manawatu to 11% in Waikato, whereas corresponding Irish *M. aethiopoulos* parasitism levels were 43% and 72% respectively.

Keywords *Microctonus aethiopoulos*, *Microctonus hyperodae*, parasitoid, weevil, drought.

INTRODUCTION

In late summer 2006, a parthenogenetic Irish biotype of *Microctonus aethiopoulos* Loan (Hymenoptera: Braconidae) was released from containment in the North Island for the biocontrol of clover root weevil, *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), a major clover pest (Gerard et al. 2011). Subsequently, the parasitoid became widely established throughout the North Island through a combination of natural dispersal and deliberate distribution (Gerard et al. 2010). No other parasitoids attack *S. lepidus* in New Zealand.

This host: parasitoid combination shares the same pasture habitat as Argentine stem weevil (*Listronotus bonariensis*) and its parthenogenetic biocontrol agent *Microctonus hyperodae* Loan

(Hymenoptera: Braconidae), which was successfully introduced into New Zealand in 1991 (Goldson et al. 1993). Both *Microctonus* spp. overwinter as diapausing first instar larvae inside their adult hosts with at least three generations per year. Irish *M. aethiopoulos* can use *L. bonariensis* as an alternative host (McNeill et al. 2002) although parasitism rates are well below that of *M. hyperodae*. DNA sequencing has shown that around 93% of parasitoid larvae in *L. bonariensis* collected from release sites in 2009 and 2010 were *M. hyperodae*, the rest being Irish *M. aethiopoulos* (L.M. Winder, AgResearch, unpublished data).

A major drought affected parts of the North Island from summer into autumn 2008 resulting

in severe soil moisture deficits (>130 mm below field capacity) in Waikato and Manawatu in early autumn (NIWA 2008). The Waikato region recorded the driest January in over 100 years. In winter 2010, in the course of assessing non-target parasitism by Irish *M. aethiopoides*, it was observed that Waikato winter parasitism in *L. bonariensis* was at much lower levels than in *S. lepidus*. Was this the aftermath of the drought, a response to the presence of Irish *M. aethiopoides* or the typical parasitism levels for *M. hyperodae* in the region?

During the monitoring of Irish *M. aethiopoides* establishment at the four initial release sites in the North Island from 2006-2010, records had been kept of other weevil species found and *L. bonariensis* adult samples were frozen, along with other non-target species. This paper reports on a retrospective study of the data and stored *L. bonariensis* samples. It was undertaken to gain insights into how the abundance of both weevil species in pasture in Hawke's Bay, Manawatu and Waikato regions fluctuated during this period, and discusses the factors that may have contributed to the low *M. hyperodae* overwintering levels of parasitism.

METHODS

The initial Irish *M. aethiopoides* release sites were on farms at Springdale (Waikato), Patoka (Hawke's Bay), and Bulls and Feilding (both Manawatu). Full details of the releases sites and sampling methods are in Gerard et al. (2011). Sites were sampled for adult weevils at monthly intervals following the releases in early 2006. In addition, winter samples of adult *L. bonariensis* had been kept from other sites in the above regions, either where the parasitoid had been released subsequent to the above releases, or in 2009 and 2010, from farms where Irish *M. aethiopoides* was confirmed to be well established through survey work by the team.

All sites were sampled by suction sampling. At most release sites, the mouth of a modified blower vac was dragged through the pasture at a slow walk along four separate random transects. Insects were collected in a sleeve bag for each replicate separately and emptied into a labelled,

vented plastic container. The length of transects varied from 30 to 200 m depending on visual adult *S. lepidus* abundance. The Bulls site was sampled using a Vortis insect suction sampler (5 × 15 sucks). Where farms were being surveyed solely to determine parasitism levels, clover patches were suctioned for approximately 10 min with the aim of obtaining a sample size of 20 or more *S. lepidus*. Adult weevils were hand sorted from the suction samples in the laboratory and counted, placed in small resealable plastic bags with a sample label, and frozen. The weevils were subsequently thawed and dissected under a binocular microscope to establish whether parasitoids were present.

Sitona lepidus release site data were available from previous research (Gerard et al. 2011). *Listronotus bonariensis* adult samples were available for retrospective assessments of parasitism from 2006, 2008, 2009 and 2010. The 2007 samples had been discarded already after the other weevils in the sample had been assessed. As percent parasitism by Irish *M. aethiopoides* and *M. hyperodae* peaks within their respective host populations when overwintering first instar larvae are in diapause (Barker & Addison 2006, Gerard et al. 2011), the retrospective assessments of the levels of parasitism were made on weevil samples taken from May to August (autumn-winter) since these data would give the best indication of annual parasitism intensity. Regional mean winter parasitism rates for both weevil species were calculated using data from all sites sampled during that period. Where there were monthly release site collections, a pooled winter mean was used and farm survey site data used were restricted to those sites where both weevil species were present in the sample collected. Possible relationships between the levels of parasitism for *L. bonariensis* and *S. lepidus* were explored using regression analysis and differences between years and sites examined by ANOVA. As suction sampling efficacy varies with suction method and weevil species (McNeill & van Koten 2011), the relative changes in weevil populations were compared by expressing the data as percentages of the number of weevils in the first sample taken at each site.

RESULTS

Listronotus bonariensis numbers were relatively low compared to *S. lepidus* throughout the period monitored, except at the Bulls site (Figure 1). The numbers of weevils of both species sampled from the Waikato and Manawatu sites were very low during the 2008 drought with almost none being collected in February-April that year. The Springdale site was only sampled intermittently from 2009 after clover and *S. lepidus* populations failed to recover under the post-drought pasture management practices implemented at that site.

Figure 2 plots the peak summer and autumn adult abundance each year, corresponding to the first and second adult generations of the two weevil species, expressed as a percentage of the

number of weevils in the first season assessed. This shows clearly that *L. bonariensis* populations were lowest at all sites during 2007 in relation to initial numbers recorded, prior to the drought, while *S. lepidus* was low for the year following the drought, including at Patoka, which had normal rainfall in 2008.

Sitona lepidus parasitism by Irish *M. aethiopoulos* increased from an overall 15% in the year of release in 2006 to 75% in 2010 ($P < 0.001$) (Figure 3a). From 2008 to 2010 parasitism tended to be highest in Waikato compared to the other two regions. Parasitism rates in *L. bonariensis* by *Microctonus* spp. tended to be highest in Hawke's Bay and lowest in Waikato (Figure 3b), but the difference was not significant. Parasitism in *L. bonariensis* varied

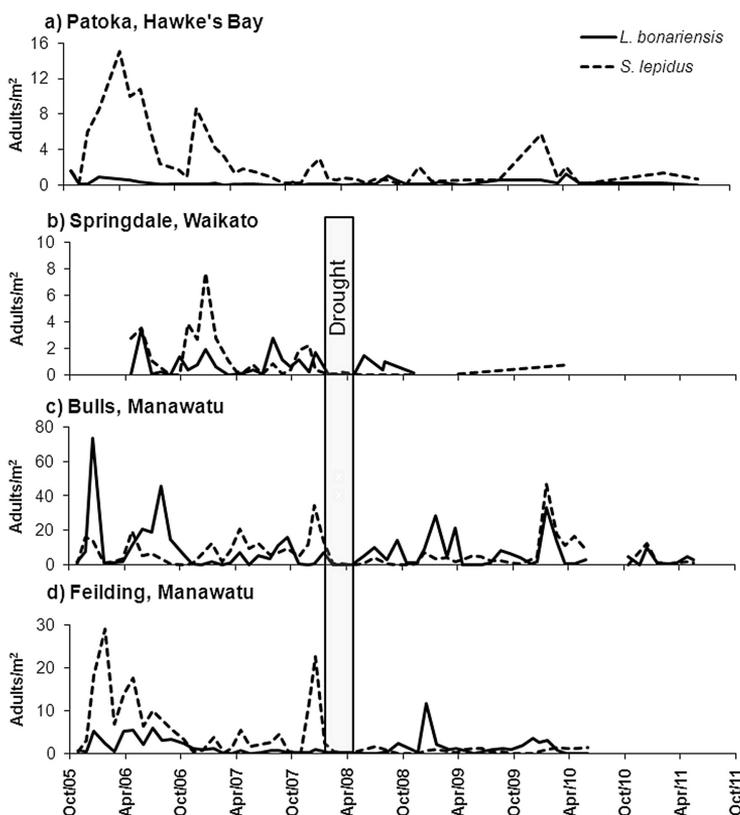


Figure 1 Mean *S. lepidus* and *L. bonariensis* adult suction sample densities (numbers/m²) at four North Island sites monitored during 2005-2011. Measurements commenced shortly before release of Irish *M. aethiopoulos* in Jan-Feb 2006. Bar denotes the period when sites b-d were affected by drought in 2008. Note different scales on Y axes.

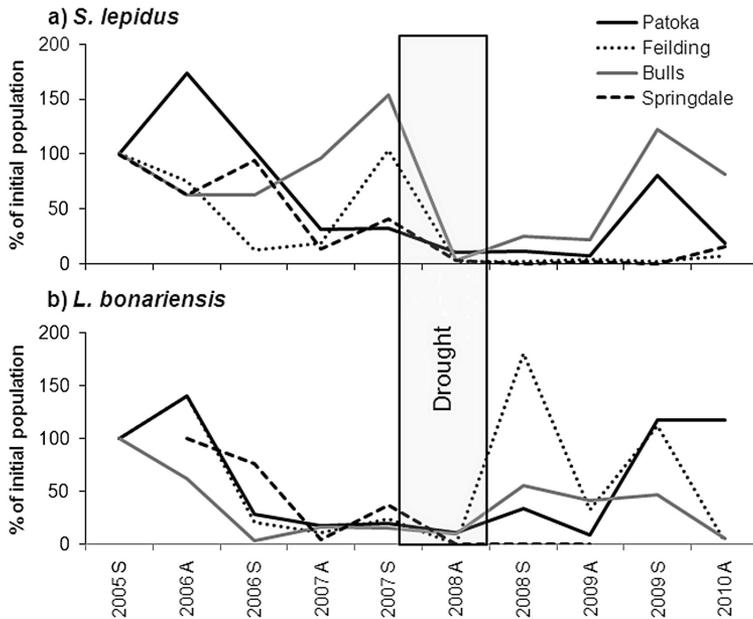


Figure 2 Relative mean population densities of adult *S. lepidus* and *L. bonariensis* at four North Island Irish *M. aethiopoidea* release sites at the time of 1st generation (S) and 2nd generation (A) adults expressed in terms of the percentage of the population in 2005 (S).

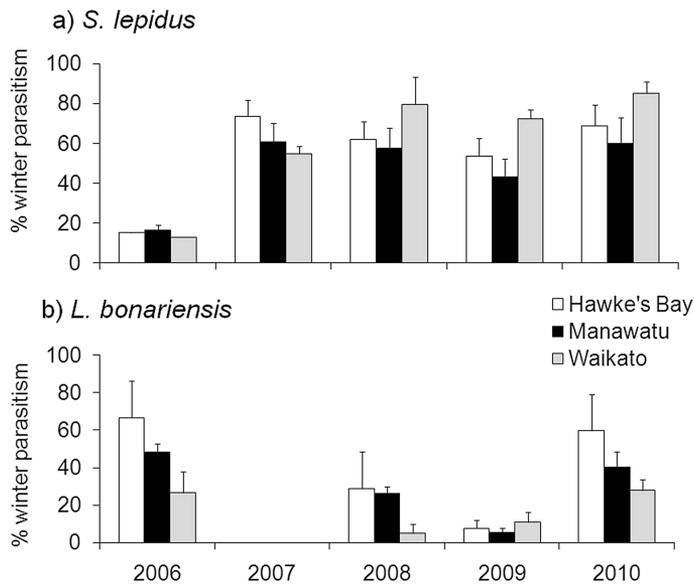


Figure 3 Mean winter parasitism (%) of (a) *S. lepidus* and (b) *L. bonariensis* at sites in Hawke's Bay, Manawatu and Waikato during 2006-2010. Error bars denote SEM where more than one release site was sampled in a region.

significantly between years ($P < 0.001$) with 2009 having lowest parasitism of all years, followed by 2008.

DISCUSSION

Although both *Microctonus* spp. occupy the same pasture habitat and have similar life histories, within 4 years following release Irish *M. aethiopoulos* appeared to reach higher levels of parasitism in its weevil host, particularly in Waikato. At the initial release sites, Irish *M. aethiopoulos* recovered rapidly from the 2008 drought providing hosts were available (Gerard et al. 2011), and Figure 3a shows the drought had little impact on regional parasitism rates. Conversely, parasitism by *M. hyperodae* showed more variation between years and was very low in 2009. This might be a delayed density dependent response to the low host populations through 2007 and into 2008. With peak end of season *M. hyperodae* parasitism levels below 50% in the Waikato and Manawatu regions, parasitism levels would have been even lower in summer, and this probably contributed to the major damage levels reported in the North Island during 2006–2011 (Popay et al. 2011).

Factors contributing to the rapid establishment and high parasitism levels of Irish *M. aethiopoulos* relative to *M. hyperodae* include its ability to produce more progeny per host as availability declines (Gerard et al. 2011) and to use *L. bonariensis* as an alternative host (McNeill et al. 2002). The overwintering generation of Irish *M. aethiopoulos* pupates in September and the adults lay eggs into the remnants of the overwintered *S. lepidus* adult generation in early October (Gerard et al. 2011). In contrast, *M. hyperodae* adults emerge from mid November onwards and lay eggs in the first summer host generation (Phillips et al. 1998). If there is high parasitism and/or adverse winter conditions, there can be a severe shortage of *S. lepidus* adults, so that non-parasitised *L. bonariensis* adults may help bridge the gap in host availability until the major *S. lepidus* adult emergence commences in November. Irish *M. aethiopoulos* is unlikely to impact on host availability for *M. hyperodae* as there is usually a surplus of both hosts over summer.

The high Irish *M. aethiopoulos* parasitism level at the initial Waikato release site was accentuated by the lack of weevil hosts in 2008 following loss of clover from the drought (Gerard et al. 2011). The high parasitism/low host availability relationship is also reflected in the regional data (Figure 3a), and at three of the four Waikato sites sampled in 2008, parasitism was 100%. As parasitism by *Microctonus* spp. sterilises female weevils, larval recruitment of the next generation would have been severely limited, giving rise to a very small spring emergence in late 2008. In addition, the combination of dry soil, high temperatures and absence of clover would greatly reduce numbers of clover root pests and therefore pathogens in the soil, while black beetle had destroyed the grass component of pastures in many northern North Island pastures (Bell et al. 2011). This led to excellent clover growth when good growing conditions returned in autumn 2009 and as *S. lepidus* females are long lived and highly fecund, the weevil populations were able to build up at Patoka and Bulls in summer 2009/10 (Figure 3).

The *L. bonariensis* adult populations at all four sites appear relatively low compared to post-parasitoid release Waikato populations reported by Barker & Addison (2006) but this may reflect the different sampling methods used: wet sieving and flotation from soil cores or turves (Barker & Addison 1989; Goldson et al. 1998) is a much more labour-intensive but more accurate method to ascertain actual densities of this small species. In addition, while every *L. bonariensis* adult had been counted when numbers were low, the research focus had not been on this species and high counts were commonly recorded as 100+ or 200+, so underestimated peak abundance.

The decline in the relative abundance of *L. bonariensis* at all four Irish *M. aethiopoulos* release sites to low levels in 2007 was unexpected (Figure 2). It is possible that the level of Irish *M. aethiopoulos* attack on non-parasitised overwintering *L. bonariensis* reduced the size of the next generation. To date only very low levels of Irish *M. aethiopoulos* have been detected in field-collected *L. bonariensis* but perhaps sterilisation may be occurring even when a viable parasitoid larva is not produced. Detailed laboratory

experiments could determine this. The recovery of *L. bonariensis* abundance after the drought, especially at Feilding, may be the interplay of a delayed density dependent response to very low parasitoid abundance and the increased availability of favourable host plants in the drought-affected regions. The severity of the drought would have encouraged the weedy summer grasses that can host *L. bonariensis*, and increased the managed use of susceptible short rotation ryegrasses once rain returned, in order to get a quick increase in pasture production.

This paper highlights the complex interactions taking place in New Zealand pasture that drive pest abundance. With changes in climate and farm practices (e.g. more frequent pasture renovation) and their potential to disrupt host:parasitoid interactions, the need for good population dynamics analyses of plant:pest:parasitoid systems is probably now more important than ever.

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