THE EFFECT OF PARASITISM ON CLOVER ROOT WEEVIL FLIGHT CAPABILITY

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ABSTRACT

The Irish strain of *Microctonus aethiopoides* was released in New Zealand in 2006 to help suppress populations of the clover pest, clover root weevil (*Sitona lepidus*). A study was undertaken to determine if this parasitoid will be passively dispersed through flight activity by parasitized hosts. In the laboratory, Irish *M. aethiopoides* parasitized equally hosts with or without flight muscles and subsequent presence of parasitoid eggs or first instar larvae had no effect on the propensity for *S. lepidus* to prepare to take flight during laboratory observations. In the field, significantly fewer clover root weevil with flight muscles were found to be parasitized compared to those without flight muscles, and those that were parasitized contained predominantly eggs and first instar larvae. The results were compared with other *Microctonus* biocontrol agents released in New Zealand and it was concluded that passive dispersal should play a major role in dispersing Irish *M. aethiopoides* in New Zealand, especially in warm, dry summers.

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

INTRODUCTION

Experimental releases of the Irish strain of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), a biocontrol agent introduced to control the clover root weevil (*Sitona lepidus*), commenced in New Zealand in late summer 2006 (Gerard et al. 2007). This parasitoid attacks adult weevils, rendering females sterile upon parasitism, and killing the weevil upon emergence of the parasitoid larva (Gerard et al. 2007). Results during the first year following release showed that Irish *M. aethiopoides* had the ability to establish and multiply rapidly, and that it could persist under adverse conditions (Gerard et al. 2007). Therefore work commenced on a nationwide dispersal strategy.

One factor essential to planning a dispersal strategy is knowledge of whether Irish *M. aethiopoides* will be passively carried to distant sites through flight activity by parasitized *S. lepidus*. When the Moroccan biotype of *M. aethiopoides* was introduced to control *Sitona discoideus* Gyllenhal in Australia and New Zealand, dispersal from release sites was rapid through the passive dispersal of larvae inside adult hosts on obligatory flights to and from aestivation sites (Aeschlimann 1983). However, *S. lepidus* does not aestivate and flight capability is facultative, with flight muscle development predominantly associated with warm, dry summer conditions and limited clover (Gerard & Arnold 2002). Therefore it was of concern that in the second winter following release, Irish *M. aethiopoides* had been detected only 2 km at most from any of the release sites. Similar slow dispersal was observed with *Microctonus hyperodae* Loan, which following release in Canterbury in 1991 to control *Listronotus bonariensis* (Kuschel), dispersed on average under 2 km/year (Goldson et al. 1999a) and still had limited South Island coverage in 2002 (McNeill et al. 2002).

As a slow dispersal rate would increase markedly the effort required to establish the biocontrol agent in all *S. lepidus* affected areas, further investigations on flight capability of parasitized *S. lepidus* were undertaken in 2008. This paper reports on a laboratory experiment designed to determine if *S. lepidus* are able to fly when parasitized by Irish *M. aethiopoides*, and compares results with field observations.

METHODS

Laboratory experiment

Sitona lepidus adults were collected from the field in January and February 2008 and randomly sorted into batches of 100. Each batch was caged with two *M. aethiopoides* adults and held for 7–18 days at 20°C in 16:8 h light:dark. Fresh clover, water and 20% sugar solution was provided in the cage at all times. This was replicated seven times.

After 7–18 days exposure, individual cages were placed on the lab bench in bright sunlight with their lids removed. Temperatures were generally above 25°C. All weevils that responded to the sunlight by fanning their elytra during a 30 min observation period were collected, labelled and frozen at -20°C for later dissection. A sample of the same number of weevils from the same cages comprising those that did not fan their elytra was also collected and frozen.

To confirm that fanning of elytra was a behaviour that strongly correlated to the ability of *S. lepidus* to fly, 50 field-collected *S. lepidus* were placed in a 10 litre container on the same laboratory bench in full sun with the lid removed. Ten weevils fanning their elytra were collected, then individually observed under the same conditions but for a longer period to see whether they achieved lift-off. A cut-off time of 1 h was arbitrarily imposed. All weevils were recovered and frozen for later dissection.

Each weevil was dissected under a binocular microscope at 20 x magnification and presence, number and developmental stage of any endo-parasitoids present was recorded. The degree of flight muscle development was assigned a score from 0 = no flight muscles present through to 3 = flight muscles fully developed.

Field data

As part of the *S. lepidus* biocontrol programme, *S. lepidus* adult populations from experimental release sites in Waikato, Hawke's Bay and Manawatu are sampled at monthly intervals and dissected as above (Gerard et al. 2007). To ascertain if there was a difference in the field in parasitism levels between adult weevils with or without flight capability, data from all samples containing both parasitized weevils and adults with fully developed flight muscles (score of 3) were collated and analysed. Weevils with partially developed flight muscles were excluded from the analyses.

Statistical analyses

Experimental data were analysed using binomial regression analysis to allow for the varying parasitoid exposure periods. Field data were analysed using binomial regression to take into account site × replicate × date interactions.

RESULTS

Laboratory experiment

Of the 10 individual weevils observed for flight activity, seven achieved lift-off during the 1 hour observation period. Dissection revealed that all 10 weevils had fully developed wing muscles, thus providing evidence that fanning of elytra is a behaviour that strongly correlates to flight capability.

In total 102 weevils were dissected from the seven exposure cages, including 51 that exhibited fanning of their elytra and an equivalent 51 that did not. All weevils that were observed to fan their elytra had the maximum flight muscle score of 3. Although equal numbers of fanning and non-fanning weevils were collected, dissection revealed that 17% of the non-fanning weevils had a maximum flight muscle score of 3, and should therefore have been physically capable of flight. These nine weevils were transferred to the “fully-developed flight muscles” data set for analysis of parasitism. As only one weevil had a flight muscle score between 0 and 3, it was excluded from analyses. The results of the dissection of weevils exposed to parasitoids in the laboratory are presented in Figure 1. There was no significant difference ($P=0.52$) in parasitism levels in weevils with fully developed flight muscles ($38 \pm 8\%$) and those without flight muscles ($30 \pm 9\%$). Although most weevils had been exposed to *M. aethiopoides* for up to 18 days at time of dissection, only two parasitoids had reached the second instar (both in adults capable of flight).

FIGURE 1: Parasitism by Irish *M. aethiopoides* (%) of *S. lepidus* adults with none or fully developed muscles in laboratory experiments and field collections.

Field observations

A total of 25 samples from all four experimental release sites were taken from December to February, averaging 17 *S. lepidus* adults/sample. Overall, 28% of weevils in these samples had flight capability. A significantly higher percentage ($P=0.011$) of weevils with no flight muscles were parasitized ($21.3 \pm 0.3\%$) compared to those with fully developed muscles ($9.7 \pm 0.3\%$). While a range of larval instars was present in weevils with no flight muscles, only one second instar parasitoid larva was found in weevils with fully-developed flight muscles, the rest being first instars or eggs.

DISCUSSION

The laboratory experiment showed that Irish *M. aethiopoides* females parasitised hosts both with and without fully-developed flight muscles and that the subsequent presence of parasitoid eggs or first instar larvae had no apparent effect on these muscles during the period observed. However, in the field where all larval instars were present, parasitism was lowest in *S. lepidus* with flight muscles, and when parasitized, the weevils with flight muscles almost all contained either eggs or first instars. No weevils containing 3rd or later instar parasitoid larvae had wing muscles. The most likely explanation is that as the larva matures and draws on more resources from the host, the weevil absorbs its flight muscles. Flight capability in *S. lepidus* is facultative and stress-induced changes in adult physiology have been reported previously (Gerard & Arnold 2002).

These results are similar to published observations on the other two *Microctonus* weevil biocontrol agents previously released in New Zealand. In the case of the Moroccan *M. aethiopoides* and *S. discoideus*, Goldson et al. (1990) found parasitized adults trapped in flight going to aestivation sites all contained first instar larvae in sympathetic diapause, but 10.7% of those trapped on return flights contained larvae with development beyond first instar. Barratt & Johnstone (2001) carried out a laboratory experiment showing that when a full range of parasitoid developmental stages were present, there were significantly fewer parasitized than unparasitized *S. discoideus* with flight muscles. They suggested that flight muscles of *S. discoideus* are absorbed as the parasitoid develops so as to maximise resources available to the developing parasitoid.

The field observations reported match those of Goldson et al. (1999b) who showed that *L. bonariensis* adults trapped in flight appeared to have much lower levels of parasitism by *M. hyperodae* than ground populations during the same period and that only eggs and first instar were observed in trapped adults. Therefore, as with *S. lepidus*, one can infer *L. bonariensis* adults containing later instars do not fly. Goldson et al. (1999b) concluded that the low levels of parasitism in flying adults may have contributed to the slow dispersal rate of *M. hyperodae* following release.

Laboratory studies have shown *S. lepidus* physiology changes in response to environmental conditions with few adults having flight muscles in cool moist conditions and a high proportion of the population having flight muscles in hot, drought conditions (Gerard & Arnold 2002). Therefore passive parasitoid dispersal by host flight is more likely during warm, dry summers, such as was experienced in 2007/08. This has been demonstrated in the field by the collection of parasitized *S. lepidus* adults this autumn at a site at Rissington, 12 km from the Hawke's Bay release site that has been monitored regularly in anticipation of parasitoid arrival. Conversely, cool, moist conditions in December and early January, such as in summer 2006/07, would hamper passive dispersal by not only reducing the proportion of weevils with flight capability, but also restricting the number of warm sunny days when flight occurs. This would be compounded if poor weather occurred during the window of opportunity when the mid-summer Irish *M. aethiopoides* generation are predominantly eggs and first instars.

In conclusion, Irish *M. aethiopoides* does parasitize *S. lepidus* adults with flight capability and it is predicted that these weevils will retain flight capability while the parasitoids are eggs or first instars. Therefore, passive dispersal from release sites inside host weevils should play a role in dispersing Irish *M. aethiopoides* in New Zealand, especially in warm, dry summers.

ACKNOWLEDGEMENTS

The authors sincerely thank Catherine Cameron for data analysis, and the rest of our clover root weevil biocontrol team, in particular Derrick Wilson, Mike Slay, Chris Mercer and Kieran Miller, who undertook the weevil sampling and collections. The clover root weevil biocontrol programme is funded by New Zealand Foundation for Research, Science and Technology (Contract number LINX0304), DairyNZ and Meat & Wool New Zealand.

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