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Ecological mechanisms for non-target parasitism by the Moroccan ecotype of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) in native grassland

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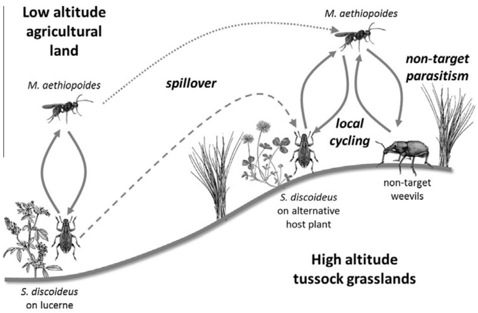
HIGHLIGHTS

- *Microctonus aethiopoides* is a biocontrol agent of *Sitona discoideus*.
- A range of non-target weevils are attacked.
- Attack in native grassland may result from BCA imigration or establishment.
- BCA overwintering in non-target weevils is possible.
- Low temperatures limit BCA generations/year.

GRAPHICAL ABSTRACT



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ABSTRACT

The Moroccan ecotype of the braconid parasitoid *Microctonus aethiopoides* was introduced into New Zealand for biological control of the lucerne pest *Sitona discoideus*. The parasitoid also attacks several non-target native weevil species found in pasture and also to a lesser extent in native tussock grassland. We carried out a series of laboratory and field experiments, and population modelling to investigate whether the parasitoids were established at low levels on native weevils in tussock grassland, whether *S. discoideus* was able to survive and support parasitoid development away from lucerne, its normal host plant, or whether parasitism was occurring as a result of spillover from agricultural environments. We found that *S. discoideus* was able to survive and support parasitoid development on white clover which is commonly found in native grassland. However, the levels of parasitism in weevil species in tussock grassland appeared to be constrained, at least in part, by low temperatures limiting the number of parasitoid generations possible per year and by the frequency of sub-zero temperatures that caused pupal mortality. Projected climate change might reduce this constraint and the implications of this are discussed.

1. Introduction

The Moroccan ecotype of the solitary koinobiont endoparasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae: Euphorinae) was introduced into New Zealand in 1982 as a biological control agent for the adult stage of *Sitona discoideus* Gyllenhal

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(Coleoptera: Curculionidae) (Stufkens et al., 1987). The target weevil had been first recorded in New Zealand in 1974, although by then it was well established (Esson, 1975), and was soon recognised as a serious pest of lucerne (*Medicago sativa*) (Goldson et al., 1984). Lucerne is an important forage crop in New Zealand agricultural systems, especially in summer-dry areas where it is an integral part of summer grazing, hay production and livestock feed pellet production. *S. discoideus* adults damage foliage, but the root nodule and root feeding larvae can markedly reduce crop production. Both stages are present in lucerne crops from early autumn until early summer at which time overwintering adults die as the next generation of adults emerges from pupal cocoons formed in the litter layer. New generation adults fly to aestivation sites away from lucerne crops before returning 3–4 months later. The introduction of *M. aethiopoides* was successful in suppressing *S. discoideus* populations and providing an economic benefit for farmers (Goldson et al., 1993). This parasitoid oviposits, usually, a single egg within the adult weevil causing it to rapidly become reproductively sterile and eventually killing the weevil when the parasitoid pre-pupa exits its host to pupate. *M. aethiopoides* is capable of several generations per year but *S. discoideus* is univoltine. Adult *S. discoideus* are present for most of the year but become scarce in mid-summer when overwintered weevils die and before new generation adults emerge from the soil. They aestivate soon after emergence over the remainder of the summer and early autumn.

It is often assumed that biological control agents will establish only in the receiving environment where the target pest occurs, most commonly productive systems, and not move into natural ecosystems (Howarth, 1991). However, there are several examples of introduced biological control agents establishing beyond the habitats into which they were released (e.g. Murray et al., 1988; Roberts, 1986; Simberloff and Stiling, 1996), and this is also true of *M. aethiopoides* (Barratt et al., 2010). In addition, it has been shown since its release that the host range of *M. aethiopoides* in New Zealand is not restricted to *S. discoideus*; at least 14 species of New Zealand native weevils are parasitised in pasture (Barratt et al., 2001, 2007, 2010) and native tussock grasslands which occur at higher elevations (Barratt et al., 2007).

Non-target attack of native weevils in native grassland by *M. aethiopoides* might come about as a result of either 'spillover' from productive environments or establishment of the parasitoid in hosts in native tussock grassland (Fig. 1). Spillover could occur during parasitoid dispersal each year from target host populations in nearby lucerne crops. Although weak fliers, adult parasitoids could be blown into native grasslands (dotted arrow in Fig. 1), or transported there as early instar larvae in dispersing parasitised weevils (dashed arrow). The establishment of local populations of the parasitoid, which we refer here to as 'local cycling' would require that self-sustaining parasitoid populations have established in native grasslands, cycling within resident *S. discoideus* (which does occur in tussock grassland) or non-target weevils (solid arrows in Fig. 1). Possibly a combination of both spillover and local cycling occur. A better understanding of these mechanisms would contribute both to our ability to predict the extent of non-target impacts, and decision support for regulatory agencies. For example, the knowledge gained about the Moroccan ecotype of *M. aethiopoides* was an important component in the decision making process relating to the introduction of an Irish ecotype of the same species as a biocontrol agent for *Sitona obsoletus obsoletus* (Gmelin) (formerly *Sitona lepidus* Gyllenhal) (clover root weevil) when imported to New Zealand in 2006 (Gerard et al., 2007).

Policy agencies responsible for regulation of biological control introductions value information that can assist with and inform future decision-making. While *M. aethiopoides* was approved for release in New Zealand more than 30 years ago under less rigorous environmental safety legislation, the finding that several indigenous weevil species were subject to attack from the parasitoid raised interest in a retrospective study of non-target impact, its mechanisms and implications. To this end, our objective in a series of laboratory and field studies was to examine the relative importance of spillover and local cycling on non-target parasitism. We investigated aspects of the ecology of non-target parasitism by *M. aethiopoides* in native tussock grasslands to understand the factors which enable or constrain non-target parasitism in this natural ecosystem. To achieve this we needed to determine whether *S. discoideus* can survive and successfully host parasitoids on an alternative plant such as *Trifolium repens* (white clover) which is common in some tussock grasslands; whether *M. aethiopoides* could establish from a founder population introduced experimentally into a tussock grassland environment; the impact of low winter temperature on survival of *M. aethiopoides* larvae in hosts, and pupae in cocoons in the litter. Phenological modelling was used to predict seasonality and likelihood of survival of *M. aethiopoides* over several generations.

2. Methods

2.1. Insect collection and rearing

Adult *S. discoideus* used in the investigation were collected from lucerne crops. Laboratory rearing of weevil species and *M. aethiopoides* was carried out using cages (160 × 180 mm × 75 mm deep) with a fine-gauze lid (e.g. Barratt, 2004) in a controlled environment room at 18 °C and with a 14 h light:10 h dark photoperiod. For experiments requiring parasitism-free weevils, field-collected individuals were maintained for 4 weeks to allow naturally occurring parasitoids to develop and emerge and weevils surviving after this period were assumed to be unparasitised. *Microctonus aethiopoides* were sourced from field collected weevils or weevils parasitised in captivity. Food plants (lucerne or white clover) were glasshouse grown in commercial potting mix in cell-trays. Plants were removed from the trays when approximately 50–100 mm high, and the roots and soil enclosed in a plastic bag which was sealed using a cable clip at the base of the stems. The plants were replaced twice weekly to ensure a constant supply of fresh foliage for the weevils. Water was supplied in soaked cotton dental wicks and these were remoistened or replaced every 1–2 days.

2.2. Weevil feeding and parasitoid survival and development on white clover vs. lucerne

2.2.1. Experiment 1

In order to compare *S. discoideus* development from egg to adult on their usual host plant, lucerne, with an alternative host, white clover, adult *S. discoideus* collected in October 2008 were caged and eggs collected. Fifty eggs were then scattered on the soil surface of 100 pots (100 mm diameter × 120 mm deep) in which either white clover (var. Grasslands Huia) (50 pots) or lucerne (var. Wairau) (50 pots) was growing in an unheated outdoor screenhouse. The pots were filled with silt loam and lined internally with fine nylon mesh to prevent weevil larvae escaping via drainage holes at the base of the pots. Gauze bags were secured over each pot to prevent adult *S. discoideus* escaping following eclosion and the pots housed in the screenhouse for 136 days. The pots were assessed after 60, 77 and 136 days when 10, 15 and 25 pots respectively were searched and numbers of larvae, pupae and adult *S. discoideus* recorded and larvae weighed. Adult weevils were kept in cages for four weeks after which they were killed and dissected to assess gender, reproductive status and development of wing muscles. The relative size of weevils was assessed by measuring