Effect of black legume aphid *Aphis craccivora* honeydew on survival of the parasitoid *Microctonus aethioides*

P.J. Gerard, K.R. Carswell and T.M. Eden

AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
Corresponding author: pip.gerard@agresearch.co.nz

Abstract The introduced parasitoid *Microctonus aethioides* (Hymenoptera: Braconidae) appears to be an effective biocontrol agent for clover root weevil *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae) in New Zealand pastures. As these pastures lack accessible nectar sources, hemipteran honeydew may be the most readily available energy source for the parasitoid. Experiments were undertaken to compare longevity of newly emerged *M. aethioides* adults caged on white clover, firstly with and without black legume aphid *Aphis craccivora* Kock (Hemiptera: Aphididae) and then with additional water and sucrose solution treatments. Parasitoids with access to aphid honeydew lived significantly longer (mean longevity 8.1 days) than those with water or no liquid (both 6.8 days) but not as long as those fed sucrose solution (10.9 days). Therefore the abundance of aphids commonly present on clovers may enhance *M. aethioides* longevity and therefore efficacy, in the field, especially in mid spring when host availability is very low.

Keywords parasitoid longevity, honeydew.

INTRODUCTION

Two *Microctonus* spp. (Hymenoptera: Braconidae) have been successfully introduced into New Zealand and shown to have the ability to be effective biocontrol agents against their target weevil pests in pasture, namely *M. hyperodae* Loan against Argentine stem weevil *Listronotus bonariensis* (Coleoptera: Curculionidae) (Goldson et al. 1993; Goldson et al. 1998; Barker & Addison 2006) and the Irish biotype of *M. aethioides* Loan against clover root weevil *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae) (Gerard et al. 2010; Gerard et al. 2011). However, for both species, a proportion of adults from the overwintering larval generation emerge before the new host generation (Phillips et al. 1998; Gerard et al. 2011). For these individuals to

realise their potential fecundity, they must have sufficient longevity to locate hosts at a time of very low density and/or survive until the new host generation commences. Alternate hosts are not thought to play a role as both parasitoids have a strong preference for and better survival in their target hosts than non-target weevil species (Barratt et al. 1997; Goldson et al. 2005) and, at least in the North Island pastures, other weevils are very scarce at this time of year (P.J. Gerard, unpublished data).

Adult *Microctonus* spp. longevity and fecundity, and therefore efficacy against pasture pests, is greatly enhanced by access to nectar (Phillips 1998). However, white clover (*Trifolium repens*) flowers are both too narrow and too deep to

permit access to these parasitoids (Vattala et al. 2006). Therefore, in high-quality pastures in New Zealand, these parasitoids must rely on other food sources.

Previous research has shown aphid honeydew is beneficial to parasitoids. The ichneumonid *Bathyplectes curculionis*, a parasitoid of *Hypera postica* (Coleoptera: Curculionidae), caged with lucerne and *Acyrtosiphon pisum* lived around 50% longer than when caged with alfalfa alone (England & Evans 1997). In addition, egg production was significantly greater in females with access to *A. pisum* honeydew than in females with access to water only (England & Evans 1997). Similarly, *Trichogramma ostrinae* (Hymenoptera: Trichogrammatidae) adults confined in cages on corn (*Zea mays*) leaves with corn leaf aphids (*Rhopalosiphum maidis*) survived over seven times longer and had higher fecundity than adults confined with only a corn leaf or with nothing (Fuchsberg et al. 2007).

Little research has been undertaken on the abundance of aphids in New Zealand pastures although lush pastures are known as important reservoirs for cereal aphids (Mulholland 1964). However, high abundance of aphids is frequently observed when sampling dairy pastures in spring at the time when *Microctonus* spp. adults from the overwintering larval generation would be present.

This paper reports on two assays undertaken to assess if access to honeydew from black legume aphid *Aphis craccivora* Kock (Hemiptera: Aphididae) feeding on white clover enhances the longevity of the Irish *M. aethiopoulos*.

MATERIALS AND METHODS

The cage design used in the assays consisted of two 100 ml plastic specimen vials, one inverted over another. The lids of the two vials were taped together and a 2–3 mm diameter hole was drilled through the centre of both. The size of the hole depended on the size of the test plant material, which was threaded through so that the foliage was in the upper vial and the leaf petiole or roots were in the water present in the lower vial. The upper vial had a 20 mm mesh-covered vent cut in the former base.

The black legume aphid colony was set up using aphids collected from a natural infestation

on white clover plants in a glasshouse at Ruakura Research Centre, Hamilton, New Zealand. The aphids proliferated on well-watered potted clover plants held in a 55 × 65 × 55 cm cage in a glasshouse.

Newly emerged Irish *M. aethiopoulos* were obtained using a method based on McNeill et al. (2002). The parasitoid prepupae formed cocoons on paper towels in the base of cages in which parasitized *S. lepidus* were held. Paper segments bearing the cocoons were cut and placed on damp filter paper in labelled Petri dishes. The temperature the cocoons were held at depended on when the adults were required, with 10°C used to slow development when numbers needed to be bulked up (Irish *M. aethiopoulos* pupae continue development and emerge as adults at 10°C). As adults became available over several days, care was taken to set up the same number of replicates of each treatment on any given day in each assay.

Assay 1

This assay consisted of two treatments: white clover or white clover with aphids. White clover leaves (cv. Kopu II) were used and 10 aphids and three newly emerged parasitoid adults were introduced into each cage. There were six replicates of both treatments. The cages were placed into a 20°C controlled environment room with 16:8 h light:dark. Parasitoid survival was assessed on days 3–7 and 10–11 and data analysed by binomial regression.

Assay 2

This assay had four treatments: clover alone; clover + water; clover + aphids; and clover + 2M sucrose. White clover seedlings (cv. Huia) and three newly emerged parasitoid adults were introduced into each cage with or without 10 aphids. The water and sugar treatments were provided in small 4.4 ml glass vials bunged with cotton wool. There were 10 replicates of all treatments. The vials were placed into a 20°C controlled environment room with 16:8 h light:dark. Parasitoid survival was assessed daily until all parasitoids were dead. The data were analysed by Randomised Block ANOVA using the data for the individual adults (df=107).

RESULTS

Assay 1

Irish *M. aethiopoides* adults had significantly better survival in the presence of black legume aphids and clover than with clover alone on five of the inspection days (Figure 1). On day 7, 56% of adults with aphids were still alive whereas only 11% of those with clover alone were alive. All adults with clover alone were dead by day 10 while 17% of adults with aphids were alive on day 11.

Figure 1 Mean percentage survival of Irish *M. aethiopoides* adults caged with white clover leaves in the presence or absence of *Aphis craccivora*. Asterisks denote differences between means of $P < 0.05$ (*) and $P < 0.01$ (**).

Assay 2

While the addition of water had no effect (mean longevity for control and water both 6.8 days), longevity was significantly greater than the control in the presence of *A. craccivora* (8.1 days; $P < 0.05$) and sucrose solution (10.9 days; $P < 0.001$) (SED=0.61, LSD (5%)=1.2). The last adult with sucrose solution died on day 18.

DISCUSSION

The results of these assays indicate that *A. craccivora* honeydew does enhance Irish *M. aethiopoides* adult survival but it may not be as good as other energy sources. The lack of difference between the control and water indicates hydration was not a factor in Assay 2. Lee et al. (2004) found that for *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae) buckwheat nectar was superior (longevity in excess of 2 weeks) to soybean aphid *Aphis glycines* Matsumura honeydew (6-7 days), which in turn was better than the longevity of 2 days

in control treatments of water or clean soybean leaves. Possible explanations were (i) parasitoids ingest more sugars from floral sources, (ii) oligosaccharides in honeydew have a lower nutritional value than nectar sugars, and (iii) honeydew has antagonistic compounds. Also, in both Lee et al. (2004) and the present study (Assay 1), excised leaves were used, which would have influenced nutrients taken up by aphids. Faria et al. (2008) showed support for the first of these possibilities in their study on the impact of honeydew feeding on the longevity of the larval endoparasitoids *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) and *Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae) caged on barley plants. They found that the difference between sucrose solutions and honeydew was because of the high viscosity of honeydew, not its nutritional value.

In the present experiments, small honeydew droplets (0.2-0.5 mm) became increasingly visible on the sides of the test vial as the assay progressed. They did not coalesce and, at trial completion, were firm to touch. Therefore it is likely that honeydew from *A. craccivora* in the field would dry rapidly and not be readily available to foraging parasitoids. However, larger aphids that attack pasture legumes, such as bluegreen lucerne aphid (*Acyrtosiphon kondoi* Shinji), produce bigger droplets. As aphids are often found clustered, and humidity would be high within lush spring pastures, honeydew from these larger species is more likely to coalesce and remain available to spring-emerging *Microctonus* spp. adults.

Aphids such as *A. kondoi* and *A. craccivora* spread many viral diseases to susceptible pasture legumes and heavy infestations can lead to significant loss in herbage production. However, insecticides are not a viable option for aphid control. Instead, an integrated approach, combining pest-resistant forages with management practices that foster good clover levels in spring pastures, is likely to maintain the diversity of invertebrates in pasture, including the larger aphid species. This may in turn aid the efficacy of the biocontrol agents that control the more serious economically-damaging target pests.

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