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Postteneral Protein Feeding may Improve Biological Control Efficiency of *Aphytis lingnanensis* and *Aphytis melinus*

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ABSTRACT. The augmentative releases of mass-reared *Aphytis* spp. (Hymenoptera: Aphelinidae) parasitoids are widely used against armored scales. The nutritional status and the initial egg load of *Aphytis* spp. females are key to their success as biological control agents. For these reasons, this work focuses on the study of providing a protein feed to *Aphytis lingnanensis* (Compere) and *A. melinus* DeBach to improve the egg load before their release. The addition of protein to a honey diet during the first 2 d after the adult parasitoid emergence increased the initial egg load in both species of parasitoids by more than five eggs. Furthermore, the addition of protein increased the total number of eggs laid by *A. lingnanensis* on oleander scale, *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae). In contrast, this effect was not observed on *A. melinus* probably because *A. nerii* is considered a suboptimal host for this parasitoid. The host-feeding activities of the two *Aphytis* species were differentially affected by the addition of protein to their diets. These results may have direct implications for augmentative biological control programs, especially during transportation from insectaries to the field, a period of time when parasitoids are deprived of hosts.

Key Words: egg load, honey, host-feeding, parasitism

The ectoparasitoids *Aphytis lingnanensis* (Compere) and *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) are among the most important natural enemies of armored scales insects. Their augmentative release is one the most environmentally safe measures against armored scales and have efficiently controlled California red scale, *Aonidiella aurantii* (Maskell), and oleander scale, *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) (Rosenheim and Rosen 1991) in those areas with insufficient natural control (Reeve and Murdoch 1985, 1986; Moreno and Luck 1992; Bedford and Cilliers 1994; Smith et al. 1997; Bedford 1998; Lucas et al. 2009; Suma et al. 2009; University of California (UC) 2012). *Aphytis* spp. are mass-reared in both public and private insectaries using a parthenogenetic strain of *A. nerii* as host (DeBach and White 1960, Rosen and DeBach 1979, Vasquez and Morse 2012).

Aphytis spp. are synovigenic parasitoid wasps; females emerge with a small fraction of their potential fecundity. Around 10–15% of their lifetime egg complement is already matured 24 h after the emergence of the adult female (Rosen and DeBach 1979, Opp and Luck 1986, Collier 1995). The additional eggs are produced from nutrients, such as protein, vitamins, or salts, gained from successive feeding activities (Jervis and Kidd 1986, Opp and Luck 1986, Collier et al. 1994, McGregor 1997, Rivero and West 2005). Among these nutrients, proteins are essential in the production and maturation of eggs, and therefore, parasitoid females look for sources rich in protein. These are present in honey, nectar, and honeydew but in very small quantities. Because host fluids are high in protein, parasitoids use them as a protein source by means of host-feeding (Heimpel and Rosenheim 1995, Heimpel et al. 1997, Jervis and Kidd 1999). *Aphytis* spp. use their ovipositor to drill through the scale cover and insert it into the scale body (Collier et al. 1994). Then they feed on the host fluids through a feeding tube (Fisher 1952, Rosenheim and Rosen 1992, Rosenheim and Heimpel 1994). Smaller scales are preferred for host-feeding, whereas larger scales for parasitism (Walde et al. 1989).

Proteins may also extend the longevity of parasitoids (Briggs et al. 1995), although the main dietary requirements are sugars (Briggs et al.

1995, Heimpel et al. 1997, Jervis and Kidd 1999). Furthermore, the interactive effects of host and sugar feeding on the fecundity and longevity of *Aphytis* spp. are of interest (Heimpel et al. 1997) and deficiencies in either of these two nutrients will result in a reduction in parasitoid performance. In fact, under circumstances where nutrients are scarce, parasitoid females are able to resorb their eggs to supply some of their metabolic needs (Jervis and Kidd 1986, 1999, Kidd and Jervis 1989, Collier 1995, Luck and Nunney 1999). Oösortion, however, incurs a metabolic cost (Jervis and Kidd 1999): total fecundity decreases and additionally, when protein is also limited, the production of males increases (Kidd and Jervis 1989). Furthermore, when mature eggs are not available for oösortion, the parasitoid death rate increases (Jervis and Kidd 1986, Briggs et al. 1995). Host feeding allows *Aphytis* spp. to mature oocytes. This activity is, therefore, related to their nutritional status and egg load. Higher nutritional reserves have been linked to lower rates of host-feeding behavior (Heimpel and Rosenheim 1995). Additionally, host-feeding is more likely to happen when egg load is low (Briggs et al. 1995, Heimpel and Rosenheim 1995).

Host-feeding is considered an important mortality factor in the field (DeBach 1943; Urbaneja et al. 2000, 2002; Stansly et al. 2005; Vanaclocha et al. 2009, 2011). However, the more time dedicated to host-feeding the less time to oviposit, since parasitoid females need to make the decision whether to oviposit and/or host-feed (Kidd and Jervis 1991). Additionally, mortality caused by host-feeding will render those potential hosts unsuitable for subsequent parasitism (Jervis and Kidd 1986, Reeve and Murdoch 1986, Collier et al. 1994, Briggs et al. 1995). For this reason, high rates of host-feeding may reduce the opportunity for establishment and increase in parasitoid populations, and consequently could increase the risk of parasitoid extinction (Jervis and Kidd 1999). Furthermore, host-feeding activity has to be adequately regulated under mass-rearing conditions to minimize its effect on the parasitoid production system (Waage and Ng 1984).

For all the aforementioned reasons, the nutritional status, as well as the initial egg load of *Aphytis* spp. females that are going to be released

as a part of a biological control program, are essential to the success of the program. These statements bring us to hypothesize that offering protein from an outside source would improve the nutritional status of the parasitoid females before their release and as a consequence their initial egg load would be increased or maintained during the transportation of parasitoids from the rearing facilities to the fields where they are going to be released. In this study, we report on the contribution of a diet rich in proteins to the oviposition and host-feeding activities of *Aphytis* spp. The studies were conducted using commercial strains of *A. lingnanensis* and *A. melinus* to better extrapolate our results to the current biological control situation of armored scale insect pests, where augmentative releases of these species are being implemented. This information assists to improve the efficiency of these releases.

Material and Methods

Plant and Insect Hosts. *Aphytis lingnanensis* and *A. melinus* pupae reared on *Aspidiotus nerii* on butternut squash (*Cucurbita moschata* Duchesne) (Cucurbitales: Cucurbitaceae) were obtained from the commercial mass-rearing facilities of Bugs for Bugs (Mundubbera, QLD, Australia) and Koppert Biological Systems S.L. (Águilas, Murcia, Spain), respectively. Butternut squash infested with *A. nerii* obtained from the same commercial facilities were used. Experiments with *A. lingnanensis* specimens were conducted in the Bugs for Bugs facilities whereas experiments with *A. melinus* were performed in the Instituto Valenciano de Investigaciones Agrarias (IVIA) (Moncada, Valencia, Spain).

Egg Load. For each *Aphytis* species, egg load was compared between parasitoids fed with two diets: “honey” Buzzy Bee Apiaries, Bundaberg, QLD, Australia for the *A. lingnanensis* experiments and 1000 flowers honey, Apisol S.A., Valencia, Spain, for the *A. melinus* experiments and honey with the addition of protein “honey+protein” (Yeast autolysate, Fruit fly Lure, Bugs for Bugs, Mundubbera, QLD, Australia) in a 3:1 (honey:protein, wt:wt) ratio. The protein insect lure is a light brown smooth paste derived from the hydrolysis of yeast, which contains nitrogen compounds, amino acids, and potassium sorbate.

Aphytis spp. pupae were isolated from beneath scale covers and placed individually in glass vials of 4.5 cm high and 1 cm in diameter. A small drop of honey or honey plus protein was provided as a food source onto the sides of the vials. The amount of food provided was always in excess to try to homogenize feeding rates. The vials were sealed with a piece of cotton. The pupae were checked daily for emergence of adult parasitoids and then they were sexed once emerged. After that, males and females were paired and left individually undisturbed for 2 d to maximize the number of females mated. These pairs were fed with the same diets that they received after the emergence. In the “honey” treatment, 23 *A. lingnanensis* females and 16 *A. melinus* females were tested, whereas 21 *A. lingnanensis* females and 15 *A. melinus* females were tested in the “honey+protein” treatment. The environmental conditions for the experiment were $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and a photoperiod of 16:8 h (L:D).

After 2 d, females were killed by freezing at -20°C and subsequently dissected to quantify their egg load. To facilitate the dissection of females, each specimen was placed onto a drop of water on a glass slide (7.5 by 2.5 cm). Dissections were conducted under a binocular stereoscope. The female abdomen was severed from the thorax, using two entomological needles (Austerlitz Insect Pins[®], Size 0, Czech Republic) and the contents of the abdomen were extracted into the drop of water. Mature eggs were counted. These eggs were elongate-oval and opaque in contrast to immature eggs that were translucent (Collier et al. 1994).

Oviposition and Host-Feeding. Oviposition and host-feeding were evaluated on *A. lingnanensis* and *A. melinus* females that were allowed to feed on the two diets described above (“honey” or “honey+protein”) and using third-nymphal instar *A. nerii* as host. These two parameters were assessed at 72-, 96-, and 120-h after parasitoid emergence.

Mated females used in this experiment were obtained using the same procedure described above. Each pair was fed according to their corresponding diet treatment. After that, each mated female was transferred to an experimental arena. In the “honey” treatment eight *A. lingnanensis* females and 14 *A. melinus* females were tested, whereas six *A. lingnanensis* females and 12 *A. melinus* females were tested in the “honey+protein” treatment. This arena consisted of a transparent plastic jar of 7 cm in diameter and 4.5 cm in height closed with a tight-fitting lid that had a 2.5-cm-diameter gauze-covered hole for ventilation. Inside the plastic jar a piece of squash (3.5 by 3.5 cm) was introduced with 60 third instars of *A. nerii* scales fixed on it. These scales were selected at random from a denser population and the remaining scales were removed. The piece of squash, except the peel containing the scales, was covered with absorbent paper to prevent parasitoids from feeding on the vegetable. A light streak of diet (“honey” or “honey+protein”) was provided on the side of the plastic jar as a food source for the parasitoids. The diet offered was the same that the females received after emerging. The slice of scale infested squash was removed daily from the experimental unit and replaced with a fresh one. For each piece of squash, all scale covers were lifted up to check the number of eggs laid in each scale body and the number of scales showing host-feeding symptoms were counted. *Aspidiotus nerii* scales upon which *Aphytis* spp. have host-fed exhibit necrotic spots (brown spots) and these were considered to be indicators of host feeding.

Parasitoid females were not replaced during the experiment. The experimental conditions were $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and a photoperiod of 16:8 h (L:D).

Data Analysis. Differences between diets on egg load per each parasitoid species were analyzed using Student’s *t*-test (STSC Inc. 1987). Differences between diets on number of eggs laid and host-feeding events per day and female parasitoid for each time tested and each parasitoid species were analyzed using two-way repeated measures analysis of variance (STSC Inc. 1987). “Time” was considered a within factor and “eggs laid” and “host-feeding” were considered between factors. Estimated marginal means of between factors were compared within each level of time.

Results

Egg Load. The number of mature eggs was significantly influenced by the presence of supplemental protein in the diet (honey+protein), for both species tested (Table 1). The addition of protein to the honey during the first 2 d after adult parasitoid emergence significantly increased by five eggs the initial egg load in both *A. lingnanensis* females and *A. melinus* females, when compared to females without the protein supplement.

Oviposition. For 3 d of evaluation, the total number of eggs laid by *A. lingnanensis* was significantly higher for females fed on honey and protein [28.17 ± 1.62 ($n = 6$)] than for those females fed only with honey [21.63 ± 1.66 ($n = 8$)] ($F = 7.57$; $df = 1, 12$; $P < 0.05$). Differences on oviposition between days were also found ($F = 47.88$; $df = 2, 24$; $P < 0.01$) as well as an interactive effect between diets and days of evaluation ($F = 3.71$; $df = 2, 24$; $P < 0.05$). In the case of *A. melinus* females, no significant effect of the diet was found in the total number of eggs laid ($F = 0.17$; $df = 1, 24$; $P = 0.68$), whereas significant differences were found on oviposition between days ($F = 58.88$; $df = 2, 48$; $P < 0.01$). The mean number of eggs laid by

Table 1. Egg load [mean \pm SE (replicates)] of 2-day-old mated *Aphytis* spp. parasitoid females allowed to feed with “honey+protein” or “honey” during the 48 h after their emergence

	Honey+Protein	Honey	Statistical values
<i>A. lingnanensis</i>	16.62 \pm 1.06 (21)	11.61 \pm 0.64 (23)	$t_{42} = 4.12$; $P = 0.0002$
<i>A. melinus</i>	17.53 \pm 1.46 (15)	12.88 \pm 0.99 (16)	$t_{29} = 2.67$; $P = 0.0122$

Table 2. Oviposition and host-feeding [mean \pm SE (replicates)] of 2-day-old mated *Aphytis* spp. parasitoid females when allowed to feed with “honey+protein” or “honey” and when a host, *Aspidiotus nerii* was offered at different periods of time (72, 96, 120 h after parasitoid emergence)

	Hours after parasitoid emergence	Honey+Protein	Honey	Statistical values
Eggs laid				
<i>A. lingnanensis</i>	72-h	16.33 \pm 1.63 (6)	11.25 \pm 1.18 (8)	$t_{12} = 2.60$; $P = 0.02$
	96-h	7.50 \pm 0.80 (6)	5.75 \pm 0.41 (8)	$t_{12} = 2.08$; $P = 0.06$
	120-h	4.33 \pm 0.92 (6)	4.63 \pm 0.73 (8)	$t_{12} = 0.25$; $P = 0.81$
<i>A. melinus</i>	72-h	10.25 \pm 1.06 (12)	9.29 \pm 1.01 (14)	$t_{24} = 0.22$; $P = 0.83$
	96-h	4.67 \pm 0.80 (12)	4.14 \pm 0.91 (14)	$t_{24} = 0.42$; $P = 0.68$
	120-h	3.33 \pm 0.75 (12)	3.00 \pm 0.60 (14)	$t_{24} = 0.35$; $P = 0.73$
Host-feeding				
<i>A. lingnanensis</i>	72-h	0.67 \pm 0.21 (6)	0.5 \pm 0.27 (8)	$t_{12} = 0.46$; $P = 0.65$
	96-h	0.00 \pm 0.00 (6)	0.12 \pm 0.12 (8)	–
	120-h	0.00 \pm 0.00 (6)	0.37 \pm 0.26 (8)	–
<i>A. melinus</i>	72-h	1.08 \pm 0.31 (12)	0.86 \pm 0.27 (14)	$t_{24} = 0.55$; $P = 0.59$
	96-h	0.00 \pm 0.00 (12)	0.43 \pm 0.14 (14)	–
	120-h	0.17 \pm 0.11 (12)	0.21 \pm 0.11 (14)	$t_{24} = 0.30$; $P = 0.77$

A. melinus females was 18.25 ± 1.96 ($n = 12$) in the “honey+protein” treatment and 17.07 ± 2.04 ($n = 14$) in the “honey” treatment.

The number of eggs laid by *A. lingnanensis* on *A. nerii* scales during the first day of evaluation (72 h after parasitoid emergence) was significantly higher for females fed with additional protein in their diet. In contrast, no significant differences were found on *A. melinus* females. On the second day of evaluation (96 h after parasitoid emergence), a numerical difference in the effect of diet approaching statistical significance at a 0.05 level, in the number of eggs laid by *A. lingnanensis* was observed with a higher number of eggs laid by females fed with the “honey+protein” diet. Again, there were no significant differences between the number of eggs laid by *A. melinus* females fed with either of the two diets. On the third day of evaluation (120 h after parasitoid emergence), no significant differences between the two diets in the number of eggs laid were found for both *Aphytis* species tested (Table 2).

Host-Feeding. There were no significant differences between diets in the total number of host-feeding events caused by *A. lingnanensis* and *A. melinus* on *A. nerii* during the 3 d of the experiment ($F = 0.49$; $df = 1, 12$; $P = 0.50$ and $F = 1.063$; $df = 1, 24$; $P = 0.31$, respectively). On the contrary, significant differences were found for the number of host-feeding events between days of evaluation for both parasitoid species (*A. lingnanensis*: $F = 3.49$; $df = 2, 24$; $P < 0.05$; *A. melinus*: $F = 9.02$; $df = 2, 48$; $P < 0.01$). A significant interaction effect was found between time and diet for *A. melinus* ($F = 4.48$; $df = 2, 48$; $P < 0.05$). The mean number of *A. nerii* scales showing host-feeding symptoms was 1.00 ± 0.38 ($n = 8$) in the “honey” treatment and 0.67 ± 0.21 ($n = 6$) in the “honey+protein” treatment for *A. lingnanensis* females, whereas the values were 1.50 ± 0.33 ($n = 14$) in the “honey” treatment and 1.25 ± 0.30 ($n = 12$) in the “honey+protein” treatment for *A. melinus* females.

On the first day of evaluation (72 h after parasitoid emergence), diet had no effect on the host-feeding activities of the two *Aphytis* species tested. For the second day of evaluation (96 h after parasitoid emergence), for both *Aphytis* species, females fed with the “honey+protein” diet did not perform any host-feeding, whereas females fed only with honey showed this feeding activity. On the third day (120 h since parasitoid emergence), *A. lingnanensis* fed with “honey+protein” again did not host-feed on *A. nerii*. The incidence of host-feeding did not differ significantly between the two diets for both parasitoid species (Table 2).

Discussion

In our study, the initial egg load obtained for 2-day-old females fed a honey only diet was similar to the one obtained in previous studies with *A. melinus* (Opp and Luck 1986, Collier 1995, Heimpel and

Rosenheim 1995, Heimpel et al. 1997) and to what is considered the approximate maximum or near maximum egg capacity of this species (approximately 12 eggs per female; Heimpel et al. 1997). By contrast, when a diet rich in protein was offered, the average number of mature eggs increased by five for both *Aphytis* species tested. Heimpel and Rosenheim (1995) in a similar experiment did not find an influence of additional protein to the egg loads of 2-day-old *A. melinus* females. The differences found between both studies could be attributed to the different concentrations of the protein extract used in the diet or to differences in their protein, and therefore, amino acids composition. In our study, the protein concentration used was six times higher than that used by Heimpel and Rosenheim (1995).

Our results demonstrated that, contrary to what has been shown in previous studies, the use of protein in the diet of recently emerged adult females can increase their initial egg load (Heimpel and Rosenheim 1995). Additionally, because the protein source used in these experiments is a commercial protein source widely used in fruit fly IPM programs, the addition of protein to parasitoid diets would be inexpensive and affordable to any rearing facility. Adjusting the amount of protein used in the diet will be essential to optimize further performance of the parasitoids.

The diet rich in protein increased female oviposition when host scales were offered in the case of *A. lingnanensis* but not with *A. melinus*. With *A. lingnanensis*, these differences were mainly observed during the first day of evaluation, corresponding to the 72 h period, after parasitoid emergence. On this day, in both treatments *A. lingnanensis* females were able to lay the same amount of eggs as in the initial egg load experiment (approximately 16 eggs in the diet rich in protein treatment and 12 eggs in the honey diet treatment). Therefore, the rest of the eggs laid on the second and third day of evaluation, when females were 96 and 120 h old, would be newly produced or matured eggs. In this case, no statistically significant differences were found between treatments probably due to action of host-feeding. Females deprived of protein in the diet performed host feeding the second and third day of evaluation, whereas no host-feeding was detected in females supplied supplemental protein in their diets, probably indicating an improvement in their nutritional status. *Aphytis melinus* females, during the first 24 h that they were in contact with the host, laid a lower number of eggs than their initial estimated egg loads. In addition, no differences for this parameter were found between treatments for all the evaluation periods. This could be related to the host used, instead of the protein, since oleander scale, *A. nerii*, is considered to be a suboptimal host for *A. melinus* (DeBach and Sundby 1963, Luck and Uygun 1986). Further studies will be needed to study the efficiency of *A. melinus* on other optimal host scales such as *A. aurantii*, and to compare the effects of this protein supplement on its oviposition and host-feeding activities.

In conclusion, the use of a diet rich in protein prior to *Aphytis* spp. augmentative releases in steps such as the transportation from the mass-rearing facilities to the field could help to increase the initial egg load and to improve the nutritional status and further performance of these parasitoids at the moment of their release in the field. The results obtained in this work may provide valuable information to improve the current *A. lingnanensis* and *A. melinus* augmentative releases in IPM citrus programs, especially in those citrus growing areas where biological control of the California red scale is inefficient. The higher initial egg load in parasitoids fed with protein previous to their release and the resulting increase in parasitism rates just after their release would help to increase biotic mortality of the target pest. Higher biotic mortality rates may help to reduce the frequency of releases or insecticide sprays needed to keep this key pest under economic injury levels. Further studies aimed to determine the optimal amounts of protein needed, as well as studies targeted to try to define more accurately the protein composition that most increase the efficiency in eggs production and maturation, such as the vitellin, the most abundant egg protein, would be needed to complete the information herein presented.

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