

Improving the knowledge of *Aphytis melinus* biology to optimize its mass production: influence of food source, host and parasitoid densities

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Abstract

The purpose of this work is to study the influence of several factors on fecundity and proportion of female parasitoids in the mass rearing of *Aphytis melinus* DeBach (Hymenoptera Aphelinidae). Its mass-rearing can be influenced by both host and parasitoid densities and by the available food source. In this study, host density did not influence the number of observed offspring per female per day (1.14 ± 0.15) or the sex ratio (0.32 ± 0.07 ♂) of *A. melinus*. Parasitoid density, on the other hand, did influence offspring production, with the higher parasitoid densities resulting in the lowest number of offspring (0.41 ± 0.07 per female per day). Medium and low parasitoid densities, meanwhile, produced similar numbers of offspring (0.83 ± 0.18 and 0.77 ± 0.13 per female per day, respectively). Sex ratio (0.43 ± 0.03 ♂) was not influenced by parasitoid density. The highest survival for *A. melinus* was achieved with honey (14.1 ± 1.2 days), but no statistical difference was observed with 10% honey. A mixture of honey, sugar and agar was not as good food source (only 3.0 ± 0.6 days of survival). Observed parasitoid host feeding was not continuous in the honey and 10% honey treatments, occurring only during 11.9 and 20.4% of the life-span, respectively. Maximum efficiency in offspring production per female was achieved when the host/parasitoid ratio was 5 to 10 hosts per female parasitoid per day. To maintain the lowest male bias of the offspring, female parasitoids should be in contact with the host for a period of no more than 3-4 days.

Key words: *Aphytis melinus*, *Aonidiella aurantii*, host density, parasitoid density, honey source.

Introduction

Biological control of many crop pests relies on the introduction of natural enemies reared in insectaries, mainly through augmentative biological control programs where large numbers of individuals are mass-reared and released (van Lenteren, 2012). This is the case for *Aphytis melinus* DeBach (Hymenoptera Aphelinidae), which is commercially reared for release in citrus orchards to control *Aonidiella aurantii* (Maskell) (Hemiptera Diaspididae) (California red scale), a key pest of citrus (Grafton-Cardwell *et al.*, 2011). Such augmentative biological control through *A. melinus* releases is used in many citrus-growing regions (Mazih, 2008; Zappalà, 2010; Grafton-Cardwell *et al.*, 2011; Olivas *et al.*, 2011; Zappalà *et al.*, 2012), with success in maintaining the pest population at low levels in California (Moreno and Luck, 1992). Biological control of *A. aurantii* can be complemented with other parasitoids such as *Aphytis chrysomphali* (Mercet), *Aphytis lingnanensis* Compere, *Encarsia perniciosi* (Tower), or *Comperiella bifasciata* (Howard), depending on the biological and environmental characteristics of the particular situation (Sorribas and García-Marí 2010; Sorribas *et al.*, 2012).

Biological control based on augmentative releases requires the reliable, inexpensive production of the necessary natural enemies by insectaries (van Lenteren, 2003; Warner and Getz, 2008). As demand for such products increases, commercial insectaries must optimize production to meet these requirements. Good practices should consider all the knowledge generated in this field, such as the influence that host size has on parasitoid sex allocation, the food needed for maximum lifetime fertility,

and other aspects like priming and the use of uniparental (female only) strains of the natural enemies (Mills and Wajnberg, 2008), and also the effect of temperature on sex ratio and progeny production of *A. melinus* (Abdelrahman, 1974a; 1974b; Kfir and Luck, 1979).

Females are more important to produce, especially with regard to parasitoids, because they are responsible for attacking the host (via host feeding and oviposition) and, by extension, actually controlling pests. This is the case for *A. melinus*, where a great deal of research has been carried out to understand the environmental conditions required to maximize female production (see Ode and Hardy, 2008 for a review). Commercially reared aphelinids generally have a female-biased sex ratio, in contrast to many species of Ichneumonidea, in which male-biased production is more common, leading even to the extinction of rearing colonies (Heimpel and Lundgren, 2000). *A. melinus* is a facultative gregarious wasp, with arrhenotokous reproduction, and its commercial production can be improved in several different ways. The first of these is host size, where female eggs are allocated to larger, higher-quality hosts and male eggs to smaller, poorer-quality hosts; this is known as the host quality model or Charnov's theory (Charnov *et al.*, 1981, see a review in Luck *et al.*, 1999; Ode and Hardy, 2008). A second way is through the number of foraging females present in a patch: when one or a few females are present, the best evolutionary stable strategy is to produce only as many sons as are needed to mate with all of the daughters. Because males are usually capable of polygyny, this enhances the female-biased sex ratio of the offspring. However, as the number of females increases the progeny production becomes pro-

gressively less female-biased, due to local mate competition (LMC) (Hamilton, 1967, see a review in Luck *et al.*, 1999; Ode and Hardy, 2008). Both influences can be combined, especially in gregarious parasitoids, because females will lay more eggs with a higher proportion of daughters in the presence of larger hosts, since fewer sons are needed to mate with the females (Ode and Hardy, 2008). Another aspect to consider is that egg limitation can occur in the parasitoid if host density is high (Heimpel and Rosenheim, 1998), which is a common situation in mass production.

Finally, the presence of sufficient supplementary food is of primary importance in obtaining maximum lifetime fertility, especially in idiobiont parasitoids with a synovigenic eggload (Strand and Casas, 2008), as it is the case with *A. melinus*. *A. melinus* host-feed, which means that females can take in nutrients needed for their own maintenance and to produce new eggs. At the same time, like most parasitoids species, *A. melinus* needs a supplementary source of food (carbohydrates) to complement the nutrients obtained from hosts, that in turn will increase the egg production (Collier, 1995; Heimpel *et al.*, 1997). This generally includes carbohydrates from plant nectar (or other plant sources), honeydew from hemipterans, and honey in the case of insect rearing (Thompson, 1999; Wäckers, 2003; Bernstein and Jervis, 2008), as it has been previously demonstrated with *A. melinus* (Pekas *et al.*, 2011; Tena *et al.*, 2013a; 2013b).

A. melinus final mass production quality has been analysed from different commercial insectaries, especially regarding the proportion of females (Heimpel and Lundgren, 2000), fitness costs due to *Wolbachia* bacterium infection (Vasquez *et al.*, 2011), and longevity, sex ratios, and size (Vasquez and Morse, 2012), but we have found no references of studies directly aiming to improve or to understand the rearing process of this parasitoid. This study focused on some aspects of the biology of *A. melinus* of special interest in its mass-rearing: a) the effect of host density on parasitoid production, b) effect of parasitoid density on production and the most appropriate ratio between host and parasitoid, and finally c) adult host feeding activity and survival on different honey sources. We have applied the methodology and environmental conditions obtained from the current practice of a commercial insectary. For this reason experiments a) and b) lasted only 6 and 3 days respectively, because adult parasitoids would be released into the environment after a short period in the rearing facility. The final aim of this study was to examine the influence of these factors on fecundity and proportion of female parasitoids produced, as a way of improving the mass-rearing quality (van Lenteren, 2003).

Materials and methods

Insect rearing

The adult wasps used to start the colony were provided by Koppert Biological Systems S.L. (Aguilas, Spain). *A. melinus* adults used in the experiments were reared in the facilities of the University of Seville, following the method developed for rearing *A. lingnanensis*

(DeBach and White, 1960), which was later modified by others (Rose, 1990; Raciti *et al.*, 2003) and it is of common use in commercial insectaries that produce this insect. The procedure is based on first rearing the host (a parthenogenetic strain of *Aspidiotus nerii* Bouche) on butternut squash (*Cucurbita moschata* Duchesne ex Lamarck). When the host is in the third instar (young female), which is the preferred age for the parasitoid to oviposit and maximize progeny production, the infested squash and adult parasitoids are placed together with honey distributed on plastic dishes in a ventilated cage. Adult parasitoids emerge about 15 days later. Female parasitoids produced in this manner were used in our experiments within 1 to 2 days after emergence, which is enough time for females to mate (Rao and DeBach, 1969). Using these females for the experiments assured that they had mated, had access to honey meals, and were able to host feed.

Experiment 1: Effect of host density

This experiment was designed to study the effect of host density on the fecundity of *A. melinus*, especially under high host densities, as it can happen in commercial mass-rearing. Three densities (treatments) of the host *A. nerii* (in the young female instar), which are usual on butternut squash used in mass rearing, were used in this experiment: high (40-60 hosts per cm², equivalent to 126-190 hosts per female parasitoid), medium (16-40 hosts per cm², equivalent to 50-126 hosts per female parasitoid), and low (3-15 hosts per cm², equivalent to 9-48 hosts per female parasitoid). The experimental unit was a transparent plastic cylinder 2 cm in diameter (equivalent to a floor area of 3.14 cm²) and 1.5 cm high, with metallic mesh on one end for ventilation, a small hole (1 mm in diameter) on the side, and a foam rubber band glued onto the edge of the opposite end. This unit was placed on the surface of a half cut butternut squash with the required host density, and fastened with rubber bands. One 24 h-old adult female, which had been in contact with adult males to mate, was introduced into each plastic cylinder in each of the three treatments. A drop of honey (Ynsadiet “rosemary honey”, Leganés, Madrid, Spain) was applied to a wooden stick that was placed in the small hole in the cylinder and changed daily. Within each treatment, females were exposed to the host for 24 h and for 6 consecutive days by changing the experimental unit every day to a new squash patch with the same host density in the same squash, or in a new squash in order to assure the appropriate host stage. All the zones occupied were circled using a permanent marker (Artline[®], Shachihata). After 8-10 days, the marked zones were separated with a sharpened knife, taking care not to damage the marked zones. Each marked portion was held individually in ventilated boxes (9.5 cm diameter, 4 cm height, with a 4 cm² hole covered with a metallic mesh in the lid for ventilation) until adult emergence, about 15 days after parasitization. Emerged adults were counted and sexed. The boxes with squashes exposed to parasitoids were held in a growth chamber at 25.0 ± 0.1 °C, 65.0 ± 5.0% RH, and 24 hours of light as recommended by a commercial insectary (Mulholland Insectary, Orange

Cove, CA, USA). The number of females, considered as replicates, used in this experiment were 5, 9 and 7 for high, medium and low host density, respectively.

Experiment 2: Effect of parasitoid density

This experiment was designed to study the effect of parasitoid density on the fecundity of *A. melinus*, which is especially important in mass-rearing, where high numbers of adult parasitoids can be crowded closely together. Three parasitoid densities (treatments) were used in this experiment: high (eight adult females), medium (four adult females), and low (two adult females), each paired with a host population of 20 young female instars. Ratios host:parasitoid were 2.5, 5.0, and 10.0 respectively, values within the range of those used in different insectaries producing *A. melinus* (around 10.0 in Raciti *et al.*, 2003, and around 4.0 in Mulholland Insectary, T. Mulholland personal communication). A piece of butternut squash (10-12 cm² of surface) with the desired number of hosts was placed in a ventilated box, several drops of honey (3 to 5, of the same type as described in experiment 1) were added to the squash surface, and then the desired number of adult *A. melinus* females, aged 24 h, were introduced. The piece of squash was changed daily until day 3, when the experiment finished. Each piece of squash bearing parasitized scales was put into a ventilated box (as described above) until parasitoid emergence, when adults were counted and sexed. All boxes were held in a growth chamber at the same conditions than experiment 1: 25.0 ± 0.1 °C, 65.0 ± 5.0% RH, and 24 hours of light. Ten replicates per treatment were performed in this experiment.

Experiment 3: Effect of supplementary food on parasitoid longevity and host feeding activity

This experiment was designed to study the effect of different honey sources on *A. melinus* survival, especially the suitability of honey + agar. Four different diets (treatments) were used in this experiment: 1) pure bee honey, the same used in the experiment 1; 2) honey diluted with water to provide a concentration of 10% (volume); 3) a preparation of honey with agar (40 ml distilled water, 20 g sugar, 40 g honey, 0.2 g agar-agar, in a water bath for 20 minutes and then poured on a Petri dish for cooling), a formulation used in some insectaries (Raciti *et al.*, 2003) to feed adult parasitoids; 4) the control, with no honey or water added. In all the four treatments, two live, young female *A. nerii* scales were detached from a squash and added each day in addition to the treatment food sources, as per Heimpel *et al.* (1997).

The same plastic cylinder described in experiment 1 was used in this experiment, but the end with the foam rubber rested on a Petri glass dish, and the cylinder was fastened to the Petri dish with two elastic bands. Food [2-3 drops of honey, portions of honey + agar (0.02 cm³), and *A. nerii* bodies for all treatments] was introduced into the plastic cylinder on a small piece of Parafilm[®], separating the cylinder from the Petri dish and sliding in the Parafilm with the food. The 10% honey treatment was added by soaking a cotton swab in the solution, which was placed in the small hole at the side of the plastic cylinder. One *A. melinus* adult female aged 24 h

was introduced into each cylinder and food was changed between 1-2 days, recording whether the parasitoid was dead or alive and whether it had fed on the host body. Because the symptoms of host feeding (dark spots on the host body) can take a longer time to appear, evaluation of host feeding as done in the experiment could be underestimated. The experiment lasted until all adults were dead. The experiment was replicated four times, with five females per treatment in each replication, making a total of 20 females per treatment. Experimental units were held in a growth chamber at 25.0 ± 0.1 °C, 70.0 ± 5.0% RH, and 24 hours of light.

Statistical analysis

Repeated measures analysis was used to include time as another factor in those cases where it was needed. Similarity of variances between treatments was first checked in order to use different post-hoc tests when necessary: HSD Tukey's test if similar variances occurred, or Tamhane's T2, Dunnet's T3, and Games-Howell's test if variances were different. Two-way ANOVA (with replications and treatments as factors) was used to compare different parameters in experiment 3: survival, days of feeding activity, and percentage of days with feeding activity. It was first analysed if there were differences among replicates. If no differences were found a one-way ANOVA was used to analyse the effect of the four treatments on the previous parameters. If factors studied in the ANOVA were significant at $P < 0.05$, then the differences between the means were determined using HSD Tukey's test at a 95% confidence level. The data were transformed using the arcsine of the square root for variables recorded as proportions. A Kruskal-Wallis test was used when data did not fulfil ANOVA requirements. The Statgraphics Centurion XVI package (Stat Point Technologies, 2010) was used to perform the one- and two-way ANOVA. Survival curves of the different diets were compared using the Cox proportional hazard model, to determine possible differences between the survival curves. SPSS v15.0 for Windows (SPSS Inc., 2006) was used to perform this last analysis and the repeated measures analysis.

Results

Experiment 1: Effect of host density

The number of parasitoid progeny per day, produced over 6 days, was similar for the three levels of host density, with no statistical differences between them (repeated measures analysis: $F = 0.13$; $df = 2, 17$; $P = 0.88$, table 1, assuming equality of variances after checking them with the Levene's, Barlett's, and Cochran's test with P values equal or greater than 0.38). The average number of offspring per female per day for all treatments was 1.14 ± 0.15 . There were no differences in the sex ratio of the offspring among host densities (Kruskal-Wallis test: $\chi^2 = 1.37$; $df = 2$; $P = 0.50$, table 1), and the overall sex ratio was $0.32 \pm 0.07 \text{ ♂}$.

The average daily offspring production over the six days period was relatively similar throughout the period (around 1 offspring per female and day, figure 1a), with

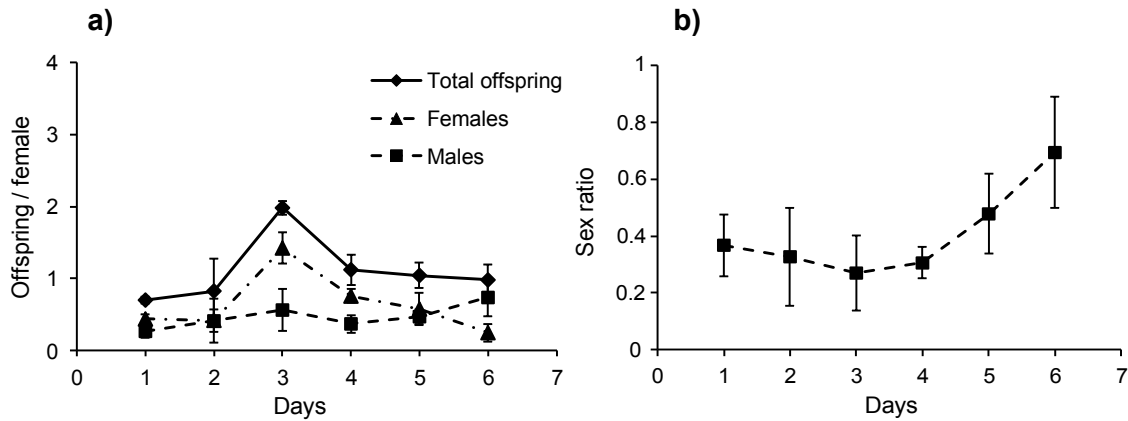


Figure 1. Effect of host density on *A. melinus* rearing: a) daily average offspring production throughout the period of study for the three treatments (High, Medium and Low host densities); b) daily average sex ratio during the period of study for a) the three treatments. Vertical bars represent standard error.

a peak on day 3 (1.98 ± 0.10 , significantly different from days 1, 2, and 5, with $P = 0.009$, $P = 0.012$, and $P = 0.05$ respectively; repeated measures analysis). Females were predominant in the first four days, and males tended to be more abundant at the end of the experiment, but no statistical difference between days was found ($F = 1.01$; $df = 5, 49$; $P = 0.42$) (figure 1b).

Experiment 2: Effect of parasitoid density

The number of offspring produced over a 3 days period was similar at medium and low parasitoid densities (0.83 ± 0.18 and 0.77 ± 0.13 offspring per female and day, respectively, table 2), but both were higher than at high parasitoid density (0.41 ± 0.07 offspring per female and day, table 2). No statistical difference was found initially between treatments using the repeated measures analysis ($F = 1.49$; $df = 2, 27$; $P = 0.24$, assuming inequality of variances after checking them with the Levene's, Barlett's, and Cochran's test, with P values of 0.087, 0.003, and 0.0001 respectively), but post-hoc tests produced differences between high and low parasitoid densities (Tamhane's T2 with $P = 0.034$, Dunnet's T3 with $P = 0.034$ and Games-Howell with $P = 0.030$, table 2). There were no differences in the sex ratio of the offspring among parasitoid densities (Kruskal-Wallis test: $\chi^2 = 1.51$; $df = 2$; $P = 0.47$, table 2). The overall sex ratio was 0.43 ± 0.03 ♂.

The number of offspring produced over a period of three days increased steadily in the low parasitoid density treatment, whereas in the high parasitoid density treatment, offspring production was rather stable (figure 2a). On average, the offspring produced on day 3 was higher than offspring produced on day 1 (0.90 ± 0.18 and 0.46 ± 0.11 offspring per female respectively, with $P = 0.027$, repeated measures analysis). An increased trend in male production was observed in the three days of the experiment, but no statistical difference between days was found ($F = 0.53$; $df = 2, 68$; $P = 0.59$) (figure 2b).

The different host:parasitoid ratios gave different offspring production (table 1 and table 2). The ratio that produced the maximum offspring was 5 to 10 hosts per parasitoid.

Table 1. *A. melinus* total offspring per female per day (mean \pm SE) and sex ratio (mean \pm SE) with different host densities, at 25.0 ± 0.1 °C, $65.0 \pm 5.0\%$ RH, and 24 hours of light.

Treatment ¹	Offspring per day	Sex ratio ²	Host/parasitoid ratio
High	1.04 ± 0.28	0.19 ± 0.09	126
Medium	1.13 ± 0.21	0.35 ± 0.08	88
Low	1.24 ± 0.29	0.43 ± 0.16	28
	$F = 0.13$	$\chi^2 = 1.37$	
	d.f. = 2, 17	d.f. = 2	
	$P = 0.88$	$P = 0.50$	

¹Treatments (host density per parasitoid female): High, 40-60 hosts/cm² (126-190 hosts per female parasitoid, $n = 5$); Medium, 16-40 hosts/cm² (50-126 hosts per female parasitoid, $n = 9$); Low 3-15 hosts/cm² (9-48 hosts per female parasitoid, $n = 7$).

²As male proportion.

Table 2. *A. melinus* total offspring per female per day (mean \pm SE) and sex ratio (mean \pm SE) with different parasitoid densities, at 25.0 ± 0.1 °C, $65.0 \pm 5.0\%$ RH, and 24 hours of light.

Treatment ¹	Offspring per day ²	Sex ratio ³	Host/parasitoid ratio
High	$0.41 \pm 0.07a$	0.39 ± 0.06	2.5
Medium	$0.83 \pm 0.18ab$	0.49 ± 0.05	5
Low	$0.77 \pm 0.13b$	0.42 ± 0.10	10
	$F = 1.49$	$\chi^2 = 1.51$	
	d.f. = 2, 27	d.f. = 2	
	$P = 0.24$	$P = 0.47$	

¹Treatments: High, 8 parasitoids; Medium, 4 parasitoids; Low, 2 parasitoids. 20 hosts per day were offered in each treatment.

²The differences between treatments High and Low are statistically significant with Tamhane's T2 ($P = 0.034$), Dunnet's T3 ($P = 0.034$) and Games-Howell ($P = 0.030$) post-hoc tests, as determined by repeated measures analysis assuming that variances are not equal.

³As male proportion.

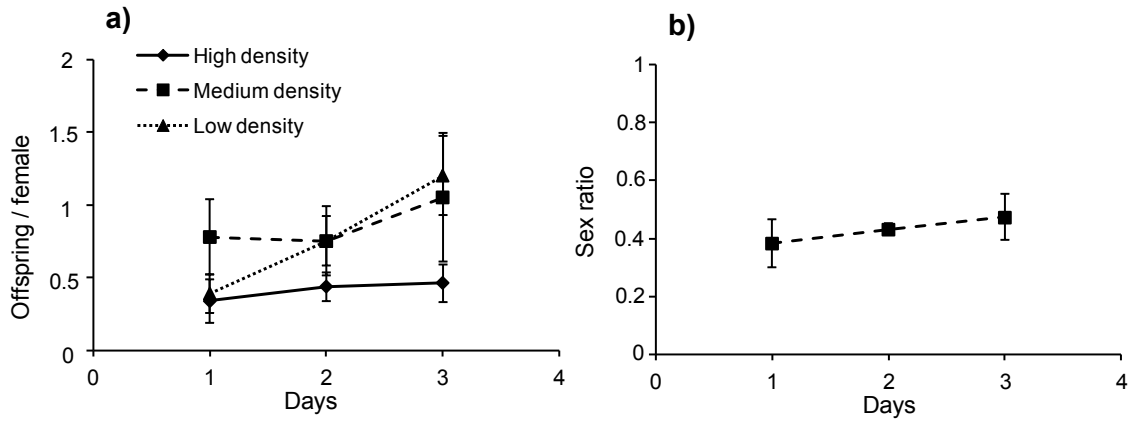


Figure 2. Effect of parasitoid densities on *A. melinus* rearing: a) daily offspring production for the three treatments throughout the period of study (High, Medium and Low parasitoid densities); b) daily average sex ratio during the period of study for the three treatments. Vertical bars represent standard error.

Experiment 3: Effect of supplementary food on parasitoid longevity and host feeding activity

No significant differences were found between the four replicates in the three parameters studied: survival ($F = 0.86$; $df = 3, 9$; $P = 0.497$), days of feeding activity ($F = 1.18$; $df = 3, 9$; $P = 0.372$), and percentage of days with feeding activity ($F = 2.23$; $df = 3, 9$; $P = 0.154$). Adult survival was higher in the honey and 10% honey treatments (14.1 ± 1.2 and 10.6 ± 1.2 days, respectively) than with the honey/sugar/agar mixture and control treatments (3.0 ± 0.6 and 2.1 ± 0.2 days, respectively) ($F = 55.7$; $df = 3, 12$; $P < 0.0001$, table 3). There were differences between survival curves (figure 3) ($\chi^2 = 59.0$; $df = 3$; $P < 0.0001$), with the honey and 10% honey treatments showing similar hazard ratios (0.035 [0.012-0.097], and 0.062 [0.023-0.162], respectively, with confidence intervals at 95% between brackets), while honey/sugar/agar mixture had a hazard ratio of 0.521 (confidence interval of 0.265-1.021 at 95%), which includes the unity value (1), indicating it did not differ from the control.

Similarly, the number of days in which adult parasitoids fed on *A. neri* bodies was higher in the honey and 10% honey treatments (1.7 ± 0.1 and 2.2 ± 0.5 days respectively), and very low in the honey/sugar/agar mixture and control treatments (0.2 ± 0.1 and 0.0 ± 0.0 re-

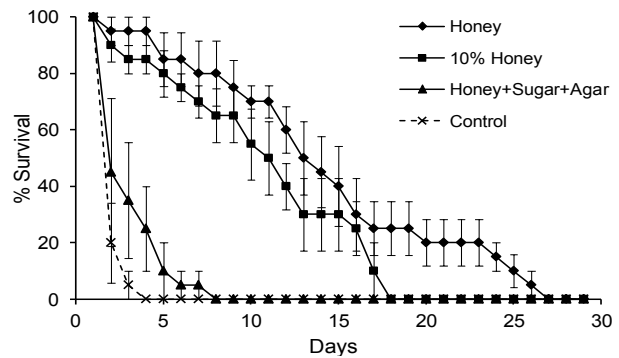


Figure 3. Survival (as a percentage) of adult females of *A. melinus* with different honey sources and control (no honey source). Vertical bars represent standard error.

spectively) ($F = 37.7$; $df = 3, 12$; $P < 0.0001$, table 3). While the number of days on which host feeding occurred was not very high, the 10% honey treatment had the highest percentage over total life-span ($20.4 \pm 4.1\%$), followed by the honey treatment ($11.9 \pm 0.8\%$) ($F = 9.9$; $df = 3, 12$; $P = 0.001$, table 3). Finally, feeding on host bodies was significantly more frequent for adults in the honey and 10% honey treatments than in the honey/sugar/agar mixture and control treatments

Table 3. Survival (mean \pm SE) of adult females of *A. melinus*, their host feeding activity (mean \pm SE), expressed in the number of days on which host feeding was observed, and its percentage over total survival (mean \pm SE), with different diets, at 25.0 ± 0.1 °C, $70.0 \pm 5.0\%$ RH, and 24 hours of light.

Treatments (diets)	Survival (days)	Feeding activity on <i>A. neri</i>	
		(days)	%
Honey	14.1 ± 1.2 b	1.7 ± 0.1 b	11.9 ± 0.8 bc
10% Honey	10.6 ± 1.2 b	2.2 ± 0.5 b	20.4 ± 4.1 c
Honey+sugar+agar	3.0 ± 0.6 a	0.2 ± 0.1 a	7.2 ± 4.7 ab
Control	2.1 ± 0.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a
<i>F</i> =	55.7	37.7	9.9
<i>d.f.</i> =	3, 12	3, 12	3, 12
<i>P</i> =	<0.0001	<0.0001	0.001

Different letters in the same column mean significant differences between treatments at $P < 0.05$ with HDS Tukey test.

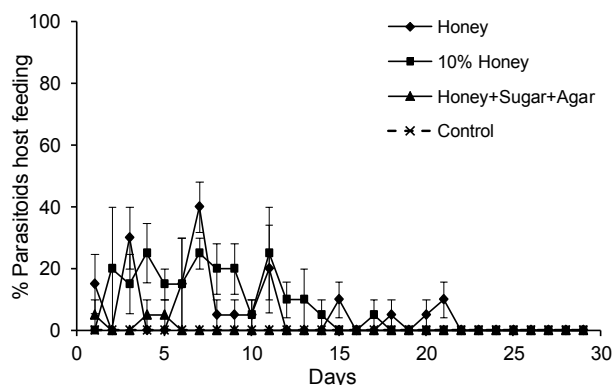


Figure 4. Percentage of adult females of *A. melinus* host feeding with different honey sources and control (no honey source). Host feeding evaluation was made through observation of the host bodies. Vertical bars represent standard error.

along the experiment ($F = 24.6$; $df = 3, 12$; $P = 0.0001$), using repeated measures analysis with different variances (inequality of variance between treatments was detected with Levene's, Barlett's, and Cochran's test, all of them showing P values much below of 0.0001), with Dunnett's T3 and Games-Howell's post-hoc tests showing $P \leq 0.05$ (figure 4).

Discussion and conclusions

The production of offspring by *A. melinus* was not affected by host density in the interval studied, between 9 and 50 hosts per cm^2 (or between 28 and 158 hosts available per female), with an average number of offspring of 1.14 ± 0.15 individuals per female per day. The estimated average fertility of *A. melinus* is around 2-4 eggs per female per day in the presence of honey and/or hosts (Heimpel *et al.*, 1997; Casas *et al.*, 2000), and although the offspring rate obtained in our study is far from the potential value, it is congruent with offspring values obtained by other authors (Vasquez *et al.*, 2011, with average values ranging between 1.0-1.3 descendants per female per day). In field experiments, researchers neither observed any density-dependent parasitism by *A. melinus* in response to scale density over time (Reeve and Murdoch, 1986, see a review in Heimpel and Casas, 2008).

Offspring production peaked on day 3 and then decreased. This situation is compatible with the behaviour to avoid egg load limitation. Egg load limitation can happen with high host density or availability (Heimpel and Rosenheim, 1998), as the three host densities used in the first experiment (with mean values of 28, 88 and 158 hosts per parasitoid female) can be considered. Egg load limitation has been observed in field populations of *A. melinus* (Casas *et al.*, 2000). Meanwhile, sex ratio was female-biased in general, although in the last two days of the experiment males were more abundant. Altogether, females were prevalent (sex ratio 0.32 ± 0.07 ♂), a finding similar to that of other studies (Heimpel and Lundgren, 2000). However, variation in this parameter

can be found in commercial insectaries (Vasquez and Morse, 2012).

Parasitoid density had a clear effect on offspring production. The treatment with higher density of adult parasitoids produced the lowest number of offspring, probably because the fixed number of hosts available was a limiting factor for the number of adult parasitoid females present in this treatment. As *A. melinus* female also feed on hosts (one host for host feeding per parasitoid per day as much, Heimpel *et al.*, 1997), this behaviour probably reduced the availability of hosts for oviposition. Parasitoid density had no effect on sex ratio, with no statistical differences between the three treatments, contrary to the prediction of the LMC theory and other models that have included the effect of the density of foraging females on sex allocation (Murdoch *et al.*, 2003; Ode and Hardy, 2008), or to that observed with other parasitoids (Irvin and Hoddle, 2006). Offspring production increased until the last day of the experiment, and the sex ratio changed throughout the experiment, starting with more females and ending with an equal ratio, which is a similar trend to that observed in the experiment with different host densities.

The availability of honey is fundamental for a longer life span of *A. melinus* adult females. Different authors have noted that the life-span of sugar-fed *Aphytis* females varies between 2 and 6 weeks (DeBach and White, 1960; Avidov *et al.*, 1970; Collier, 1995; Heimpel *et al.*, 1997). In contrast, the lifespan of sugar-deprived females rarely exceeds three days (Avidov *et al.*, 1970), whether or not host feeding is allowed (DeBach and White, 1960; Heimpel *et al.*, 1997). The results of many studies suggest that host feeding may increase survival in *A. melinus* only when females have access to sugar. The honey/sugar/agar mixture did not have a significant effect on adult longevity, which was very similar to the control without any source of honey, even though this food is used in at least one insectary (Raciti *et al.*, 2003). A possible explanation could be that the small portion of honey/sugar/agar mixture introduced in the experimental arena was insufficient for the adult parasitoids to feed on, and they were unable to obtain enough honey from the substance. Our personal experience with mass rearing indicated that honey/sugar/agar mixture (as was used at the beginning of the rearing) produced a poor adult survival rate. Survival rates found in our work with honey (and also with 10% honey) were very similar to the values found by Heimpel *et al.* (1997) when adult females were not allowed to feed on the host. Indeed, the presence of host bodies in our study seemed not to increase adult survival to the extent observed by other authors (Heimpel *et al.*, 1997). This discrepancy could be due to the number of days (and its proportion) in which host feeding occurred that was very low, far from the values obtained by Heimpel *et al.* (1997). The daily proportion of adult females feeding on the host was only around 20%, but it was always more significant in the honey and 10% honey treatments than in the other two treatments.

We have not found any detrimental effects of higher host density on offspring production over a period of six days. The change in sex ratio in the later days is worth being noted, and although not statistically significant, it

can have implications in commercial rearing. Parasitoid density had an effect on offspring due to host availability, but no effect was observed on sex allocation. Therefore, it can be concluded that within the values tested, no male increment would be expected with the highest parasitoid density. Another matter is the shift toward male production that occurs over the course of the experiment, similar to what happened in the second experiment, but again not statistically significant. Honey as a supplemental food has been shown to be crucial for increasing adult survival (with or without host feeding), allowing *A. melinus* females to reach their maximum lifetime fertility.

In general, the relationship between hosts and parasitoids should continue to be examined to improve the mass-rearing. Our results suggest that a daily host:parasitoid ratio of around 5 to 10 allows maximum efficiency in parasitoid production, in terms of number of progeny per female per day (table 1). As no clear effect of parasitoid density has been observed in our experiments, it could be concluded that, given a squash with a certain host density (which has an optimum density between 20 and 40 hosts per cm², as obtained in a previous study, González-Zamora *et al.*, 2012) the best procedure would be to add the number of parasitoids that would create the above ratio (on a daily basis), which includes hosts used for oviposition and those used for host feeding plus some extra for security. The number of days parasitoids should be in contact with the squash should be not higher than 3-4 days, to keep the male bias of the offspring at its lowest values.

Acknowledgements

This work was only possible with the help of Saray Marín. We would also like to thank Jean-Claude Malausa and Nicolas Ris from the INRA Vallbonne (France) for the uniparental strain of *Aspidiotus nerii*. We acknowledge all the help and support given by the Servicio de Sanidad Vegetal (Conselleria de Agricultura of the Generalitat Valenciana, Spain), with special thanks to José Luis Porcuna, Alberto García and Carmen Laurin. This work was funded by RioTinto Fruits S.A. (El Campillo, Huelva, Spain), with the help of the regional government of Andalucía (Junta de Andalucía, Spain), within the research project “Cría de *Aphytis melinus* y *Comperiella bifasciata* para el control biológico del piojo rojo de California en cítricos”. We would also like to thank J. J. Sorribas for his comments in the preparation of this manuscript.

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Received September 29, 2014. Accepted February 16, 2015.